ABSTRACTS OF THE THIRTY-THIRD ANNUAL
MIDWINTER RESEARCH MEETING

ASSOCIATION FOR RESEARCH IN OTOLARYNGOLOGY

February 6 – 10, 2010

Disneyland Hotel, Anaheim, CA

Abstracts of the Thirty-Third Annual MidWinter Research Meeting of the

Association for Research in Otolaryngology

February 6 – 10, 2010
Anaheim, California, USA

Peter A. Santi, PhD
Editor

Association for Research in Otolaryngology
19 Mantua Road, Mt. Royal, NJ 08061 USA

CONFERENCE OBJECTIVES

After attending the Scientific Meeting participants should be better able to:

1. Understand current concepts of the function of normal and diseased ears and other head and neck structures.
2. Understand current controversies in research methods and findings that bear on this understanding.
3. Understand what are considered to be the key research questions and promising areas of research in otolaryngology.

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The *Abstracts of the Association for Research in Otolaryngology* is published annually and consists of abstracts presented at the Annual MidWinter Research Meeting. A limited number of copies of this CD and previous books of abstracts (1978-2009) are available.

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This book was prepared from abstracts that were entered electronically by the authors. Authors submitted abstracts over the World Wide Web using Mira Digital Publishing's PaperCutterTM Online Abstract Management System. Any mistakes in spelling and grammar in the abstracts are the responsibility of the authors. The Program Committee performed the difficult task of reviewing and organizing the abstracts into sessions. The Program Committee Chair, Dr. Sharon Kujawa and the President, Dr. Steven Rauch constructed the final program. Mira electronically scheduled the abstracts and prepared Adobe Acrobat pdf files of the Program and Abstract Books. These abstracts and previous years' abstracts are available at: http://www.aro.org.

Citation of these abstracts in publications should be as follows: **Authors, year, title, Assoc. Res. Otolaryngol. Abs.: page number.**

For Example:
Russell, Ian, 2009, Humming in Tune: Sex Recognition by Mosquitoes on the Wing Through Acoustic Distortion, University of Sussex. Abs.: **973.**

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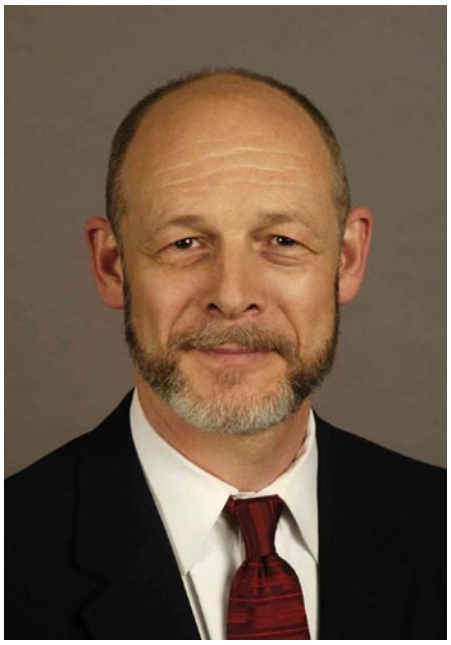

Welcome to Anaheim! The ARO Council leadership is excited about this new locale. It promises a first rate hotel, many options for dining, and a long list of other recreational activities for those of you who can tear yourselves away from the research meeting. This is ARO's first visit to Anaheim. We have only had one previous western venue for an ARO Midwinter Meeting, in Phoenix, 2008. With this year's MWM we inaugurate a new tradition of west coast venues on alternate years. We are tentatively scheduled here in Anaheim on the "even" years through 2014. We very much need your feedback about your experiences here at the Anaheim site. Hopefully we will continue this new bi-coastal tradition for many years to come.

This year's exciting list of symposia includes Migraine: from neurobiology to clinic and back; Synaptic and intrinsic plasticity in the auditory system: mechanisms and functional significance; Human otopathology and basic science: partners in translational research; Auditory stream segregation and selection; New developments in understanding hair-cell transduction; Signal processing in first and second order vestibular neurons; Stem cell applications for cochlear repair – from proof of principle to therapy; and Modeling neural responses and perceptions of complex sounds. In addition to the symposia, we will be able to attend workshops sponsored by the Media Relations Committee, the NIDCD, the Minority and Diversity Affairs Committee, and the Patient Advocacy Committee. The 2010 Award of Merit recipient, Ian Russell, FRS, will speak on "Humming in tune: sex recognition by mosquitoes on the wing through acoustic distortion." Of course, there will also be a number of official and unofficial social events to round out the schedule.

Remember to attend the Business Meeting Sunday evening at 6PM. In addition to an update of the Association's affairs, new members of the nominating committee are chosen and other issues of concern are highlighted. This year we will reprise the Exhibitors' Scavenger Hunt that was so popular last year. The Hunt's winners will be drawn at the Business Meeting. Prizes include popular gizmos such as iPod, Wii, etc. So, please attend to play your part in Association business (and for the possibility of scoring cool toys).

Our Midwinter Meeting could not occur without the continued hard work and effective administration of Talley Management. Also, many members of the ARO dedicate hours of their time to program organization, symposium and workshop development, short courses and more. We are indebted to AAO-HNSF, DRF, AAAF, NIDCD, and the Collegium Oto-Rhino Laryngologicum Amicitiae Sacrum - US Group, Inc, for their donations of travel funds for students and fellows. The collected efforts and generosity of all these deserve our recognition and thanks.

The Association for Research in Otolaryngology MidWinter Meeting continues to be the highlight of the yearly conference calendar for me and for many of you. ARO is our community. The MWM is a chance to catch up with old friends, make new friends, and see and hear the best science in the world. This organization fills a unique niche in our personal and professional lives. It does so because of the active engagement of you, its members. I hope you will all enjoy this year's meeting and I hope you will all look for opportunities to make your own contributions to this wonderful society.

Steven D. Rauch
President

Ian Russell

2010 Award of Merit Recipient

Ian Russell

2010 Recipient of the Award of Merit

Ian Russell has been an active research scientist for more than 35 years. Unlike many recipients of this award, whose magnificent achievements have resulted from concentrated intellectual investment in a single challenging area, Russell is an intellectual butterfly. His agile mind alights on problems and issues through broad ranging interests and wide reading and he pursues whatever engages him. This has allowed him to make considerable contributions to several areas, not just those in mammalian hearing, for which he is rightly being recognized. His earliest publications concerned the function of the lateral line system in fish. He demonstrated that these receptor systems were suppressed by inhibitory efferents during a variety of activities including swimming. He further demonstrated, using state-of-the-art ion-sensitive electrodes, that the cupula of lateral line organs contains high concentrations of potassium ions making it a typical hair cell system. It is surprising to many that this was one of the first publications with Peter Sellick. The interest in lateral line was maintained over many years with various collaborators such as Åke Flock, Barry Roberts and David Lowe. From the hair cells of the lateral line system it was a short intellectual jump to consider the function of cochlear hair cells. However, in the late 1970's, and probably still today, it was easy to think about recording from cochlear hair cells, but actually succeeding in doing so was another matter. Indeed, when Russell and Sellick applied for the first grant to support this work it was rejected as being impossible. Following Ted Evans's advice, they went ahead and actually recorded from hair cells and subsequently won the grant funding, which has continued uninterrupted throughout Russell's career. Bizarrely, the first recordings were funded by Readers Digest. Colin Blakemore, visiting during the inner hair cell recordings, put them in touch with Readers Digest, who give away money not collected in Prizes. They went to Mayfair in London to pick up the £20,000 check that bought the microscope, microphones and amplifiers needed for the experiments. These pioneering recordings from cochlear hair cells *in vivo* were a considerable *tour de force*. Even today the number of laboratories with such expertise can be counted on one hand. It was these early hair cell recordings that established Russell and Sellick's reputations within the auditory community and contributed to Russell's election as a Fellow of the Royal Society in 1989. What could be clearly seen in these first recordings was that outer and inner hair cells are exquisitely sharply tuned. This alone required a re-evaluation of the many hypotheses and models of how the sharp tuning, evident in auditory nerve fibre recordings and psychophysical measurements, came about. Direct measurements of the basilar membrane mechanics have subsequently demonstrated that this sharp tuning is present in the vibration patterns, which has lead on to our understanding of the active processes in the cochlea. About this time the young Jonathan Ashmore worked with Russell on transduction in frog sacculus before going on to establish himself at Bristol and subsequently in London. Further hair cell recordings with Alan Cody revealed the effects of noise damage at the level of the receptors.

The hair cell recordings led directly on to further ambitious projects towards understand the working of the mammalian cochlea. Three areas of research are particularly notable. The first required the development of a laser interferometer. Commercial interferometers were just beginning to become available and several laboratories world-wide started using these to re-examine the vibration of the basilar membrane, another technically demanding endeavor. With his own design laser interferometer Russell made extensive measurements of basilar and tectorial membrane vibration and published some of the first measurements made both across *and* along the basilar membrane rather than at a single locus, revealing complex transverse vibration patterns. In another study, with Euan Murugasu, Russell measured basilar membrane vibration while stimulating the efferent system and infusing acetyl choline via the round window, thus demonstrating for the first time the direct effect of the olivo-cochlear system on basilar membrane mechanics! The third major contribution to the understanding of cochlear function has been in collaboration with Guy Richardson and Corné Kros. Together they have developed innovative electrophysiological and molecular techniques to probe the structure and function of the tectorial membrane and the nature of mechano-electrical transducer currents of mammalian hair cells, utilizing specially designed knock-outs and an isolated mouse cochlea preparation, the organotypic cochlear culture. Following the discovery by Dallos's group of the motile protein prestin in the wall of outer hair cells Russell has made measurements of the basilar membrane vibration in a prestin knock-out mouse. The results are not quite what many expected, but data that departs from the received wisdom has never prevented Russell from publishing his results! Russell's laboratory currently employs otoacoustic emission measurements, isolated cochleas, interferometer measurements and hair cell recordings to continue the quest for a complete understanding of auditory transduction.

While pursuing these fundamental questions about cochlear function, Russell has also found time to collaborate with Marianna Vater and Manfred Kössl to investigate cochlear function and its development in the bat. To study development they had to locate bat caves in Cuba and develop portable recording equipment that they could operate deep in the humid and hot caves where the various developmental stages of the bats were all accessible. Field work in the raw!

Most recently one of Russell's great enthusiasms has been unraveling the manner in which mosquitoes choose their mates. In collaboration with Gay Gibson and Ben Warren he has shown that they adjust and synchronize their flight tones as part of the mating duet even, in some species, using non-linearly generated difference tones between inaudible higher harmonics as an error signal.

While running this creative and productive research team Russell has maintained a full undergraduate teaching and mentoring load within the Biological Sciences department of the University of Sussex for whom he has worked for the last 3 decades. As an inspirational teacher and mentor he has been instrumental in the launching or furtherance of many auspicious careers: Jonathan Ashmore, Alan Cody, Corné Kros, Andrei Lukashin, Euan Murugasu, Alan Palmer, Guy Richardson, Alfons Rüsch, Peter Sellick, to name but a few.

Russell has never been a regular contributor to the larger international conferences preferring to get on with his research and allow his publications to speak for him. What he has been and continues to be is an inordinately creative researcher who not only achieved pioneering measurements of cochlear function, but also employed cutting-edge electrophysiological, biophysical and molecular techniques to the elucidation of auditory function. For these exceptional contributions to the field of hearing he is an excellent and deserving recipient of the ARO Award of Merit.

ALAN PALMER

CORNÉ KROS

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1 Neurobiology of Migraine Symptoms: The Role of Dura-Sensitive Thalamic Neurons in Photophobia and Allodynia

Rami Burstein[1]
[1]*Harvard Medical School*

Photophobia during migraine, exacerbation of headache by exposure to ambient light, is experienced by nearly 90% of migraineurs during an acute attack. The underlying neural substrate of such photophobia is unknown. We hypothesize that it is driven by retinal signals conveyed through non-image forming pathways to thalamic dura-sensitive neurons that underlie migraine pain. We interviewed legally-blind migraine patients and found that exacerbation of migraine headache by light is experienced by blind subjects with damaged image-forming pathways who maintain light perception, but not by those devoid of visual and non-visual light perception. Electrophysiological, anatomical and immunohistological techniques were used to test our hypothesis in the rat. Ongoing activity of thalamic dura-sensitive neurons increased 2X under ambient light and 4X under bright light shone on the contralateral eye. Most dura/light-sensitive thalamic neurons were located in the lateral posterior nucleus (LP), or in the posterior nucleus (Po)/LP border, in Po, and in ventral posteromedial nucleus (VPM). Retinal projections make connections to dura-sensitive neurons in the dorsocaudal thalamus. Cortical projections of individual dura/light-sensitive thalamic neurons were mapped within the primary somatosensory, motor, retrosplenial, parietal association, primary and secondary visual cortices. We concluded that photic information is integrated by dura-sensitive thalamic neurons that receive direct input from retinal ganglion cells and project extensively to cortical areas involved in nociceptive, visual, cognitive and motor functions, providing a means for photomodulation of dura-sensitive thalamic neurons and, thus, the severity of migraine headache. We have shown clinically that the throbbing headache of migraine is commonly associated with cephalic allodynia confined ipsilaterally to the referred pain area around the eye and, often, extracephalic allodynia that extends to the arms and legs. Cephalic allodynia develops 20-60 min after onset of migraine but extracephalic allodynia does not start until 2-3 hrs later, suggesting mediation by different neuronal mechanisms. Using single-unit recording techniques in our rat model of intracranial pain we studied dura-sensitive thalamic neurons receiving convergent sensory input from cephalic and extracephalic skin.

Following a brief exposure of the dura to inflammatory soup (IS) (1) cutaneous receptive fields expanded, (2) ongoing activity increased and/or changed from occasional bursts to prolonged bursts, (3) quantitative mechanical and thermal skin stimulation evoked larger and longer neuronal responses, and (4) response thresholds decreased for innocuous skin stimuli. These findings suggest that the spread of cutaneous allodynia from the referred pain area to other parts of the head and body is mediated by sensitization in thalamic neurons that process sensory information from the dura, cephalic, and extracephalic skin.

2 A Primary Sensory Innervation Connecting Headache, Meniere's Disease and Inner Ear Dysfunction

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Trigeminal neurogenic inflammation is one explanation for the development of vascular headaches. The migraine-related inner ear symptoms of phonopobia, tinnitus, fluctuation in hearing perception, and increased noise sensitivity provide indirect evidence for a connection to basilar artery migraine. Meniere's disease is also characterized by vertiginous episodes, sensorineural hearing loss and decreased speech discrimination scores. Our studies have been directed toward determining if a physiological basis for neurogenic inflammation exists between cochlear and the basilar artery.

Capsaicin applied to the cochlea could induce a concentration dependent neurogenic inflammation as indicated by plasma extravasation from the basilar artery and anterior inferior cerebellar artery (AICA). Capsaicin apparently activates primary sensory fibers innervating vessels supplying the cochlea. In further work provided direct evidence for such a population of fibers that originates from the trigmeninal ganglion.

These results characterize a functional connection between the cochlea and vertebro-basilar system through the capsaicin sensitive primary sensory neurons. We propose that vertigo, tinnitus, and hearing deficits associated with migraine could arise by excitation of the trigeminal ganglion leading to vascular tone and permeability change. In homeostatic diseases of the inner ear (e.g. Meniere's diseases), trigemino-sensory innervation may also play a role. Moreover it is possible that cochlear dysfunction may also be able to trigger basilar and cluster headache. Supported by NIH NIDCD DC 00105

3 Epidemiology of Migraine, Vertigo and Vestibular Migraine

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Both headache and dizziness/vertigo rank among the most common complaints in the general population. Worldwide, the 1-year prevalence for headache is about 50% and ranges for migraine between 11% and 14%. Likewise, dizziness and vertigo is one of the most common complaints in medicine, affecting approximately 20% to 30% of the general population.

With respect to the high prevalence of vertigo and migraine in the general population it is not surprising that many patients suffer from both symptoms. Nonetheless, in the last decade epidemiological arguments have progressively accumulated to strengthen the hypothesis that vertigo is linked to migraine beyond a mere chance concurrence. Several studies with selected patient groups have shown that the prevalence of vertigo is increased in patients with migraine. Vice versa, patients presenting to a dizziness

clinic more often have a history of migraine than would be expected by chance. The epidemiological link between vertigo and migraine has recently been confirmed on the population level.
The relation between vertigo and migraine is intricate and the pathophysiological basis of the link between both symptoms is poorly understood. In vestibular migraine, vestibular symptoms are conceptualized as a symptom of migraine. Vestibular migraine is probably the most common cause for recurrent spontaneous vertigo with a lifetime-prevalence in the general population of about 1%. Other vestibular disorders that display an increased prevalence of migraine are benign paroxysmal positional vertigo and Menière disease. In both disorders the prevalence of migraine is about twice compared to age and sex matched controls. Furthermore, migraine is epidemiologically linked to motion sickness, several ataxia disorders and psychiatric syndromes that can also manifest with vertigo and dizziness.

4 Clinical Clues About a Dizzying Headache

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Recent years have seen a tremendous upsurge of clinical and research interest into the proposition that migraine causes dizziness. From 1970s case reports linking migraine to childhood vertigo spells, migraine has become an increasingly common diagnosis in neurotology practices worldwide. Various clinical syndromes of migraine-related dizziness (MRD) have been described and formal diagnostic criteria proposed. Epidemiologic data have been collected, mechanistic investigations performed, and neurophysiologic models developed. Yet, the burgeoning popularity of idea that migraine causes dizziness rests on a surprisingly thin research database. There is no consensus about the definition of MRD. Only one well-controlled medication trial has been published. Opinion pieces and review papers on this subject outnumber rigorous research reports. As a result, clinical practice is operating in a greater void than many realize. The field of neurotology has embraced the concept that migraine causes dizziness, but much about this notion remains unknown and speculative.
This presentation will pose three questions about future clinical investigations into the relationship between migraine and dizziness: (1) What variables should be measured? (2) What patients should be studied? and (3) How might clinical trials be designed to yield both clinically useful results and greater insights into pathophysiologic processes? The implications of these three questions will be illustrated with recent data demonstrating fundamental gaps in current clinical knowledge. Ambitious, but practical and necessary, near-term goals for clinical research will be suggested, including production of (a) well-validated, diagnostic schema to guide clinicians through the differential diagnosis of headache and dizziness, (b) treatment guidelines based on adequately powered, well-controlled intervention trials, and (c) results that inform and benefit from advances in neurosciences across all relevant disciplines.

5 Abnormal Motion Perception in Migraine Associated Dizziness

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Migraine associated dizziness (MAD) is a frequently diagnosed but poorly understood entity. The pathophysiology underlying this disorder is unclear and no diagnostic test is available for MAD. We hypothesized that psychophysical measurements of motion perception may be abnormal in MAD patients and could help clarify the pathophysiology of MAD. Motion perception was measured in four patients with MAD and four age-matched controls during movements that activated the semicircular canals (roll rotation at 0.1 and 1.0 Hz), the otolith organs (static roll tilt), or the canals and otoliths in tandem (roll tilt at 0.1 and 1.0 Hz). Perceptual thresholds were determined using a standard staircase paradigm, whereby the peak acceleration of the motion was decreased or increased based on accurate or inaccurate percepts of movement direction.Perceptual thresholds in MAD patients did not differ from normal subjects when the canals or otolith organs were activated in isolation (roll rotation, static roll tilt). When the canals and otoliths were activated in tandem, thresholds were normal in MAD subjects during high frequency (1.0 Hz) roll tilts but were dramatically lower than normal during mid-frequency (0.1 Hz) roll tilts.
Our results suggest that an abnormal synthesis of canal and otolith information by the brain may be responsible for the reduced perceptual thresholds in MAD patients during mid-frequency roll tilts. Since perceptual and eye movement responses mediated by vestibular inputs may be generated by different mechanisms, this abnormality may be evident with psychophysical testing but not eye movement measurements.

6 Migraine and the Ear: Where Do We Go from Here?

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Migraine is an extraordinarily common neurological disorder with manifestations that frequently present to otolaryngologists. Migraine has been linked to Ménière's disease, benign positional vertigo, drop attacks, and sudden sensorineural hearing loss. Yet the NIDCD portfolio lists only one grant that is presently investigating mechanisms of migraine! This talk will discuss several specific areas of potential research related to migraine that need to be pursued by auditory and vestibular neuroscientists. Cortical spreading depression has been linked to visual disturbances that occur in migraine. Could spreading disturbance of neuronal activity also occur in vestibular and auditory centers, or even in the periphery? What would be its physiological manifestation? Trigeminal efferent activation and release of peptide neurotransmitters have been linked to plasma extravasation in the inner ear. Is this just a laboratory artifact or a real phenomenon that underlies hearing and/or vestibular dysfunction? The sensitization of central sensory nuclei has been linked to pain and cutaneous hypersensitivity in migraine. Are the

auditory and vestibular nuclei also susceptible to hypersensitization, and can this be readily measured? Migraine affects hearing and balance, and there are many opportunities for our community to contribute to its understanding and amelioration.

7 $GABA_B$ Adjusts the Balance of Excitation and Inhibition in Binaural Brainstem Neurons

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Stimulus dependent adaptation of response properties of single neurons are a well known phenomenon in sensory systems. Yet, its significance for processing spatial acoustic information at the initial stages of binaural interactions (superior olivary complex, SOC) is, at best, circumstantial. We found an unexpected stimulus dependent adaptation in the responses of lateral superior olive (LSO) neurons in response to changing interaural level difference (ILD; Park et al. 2008, Hearing Res 238:58). In vivo and in vitro pharmacology showed that these effects can, to a large extent, be explained by $GABA_B$ receptor mediated modulation of the synaptic inputs to a given LSO neuron. Interestingly, this modulation is controlled by activity of the postsynaptic LSO neuron itself via dendritic release of GABA that activates presynaptic $GABA_B$ receptors mainly of excitatory inputs (Magnusson et al. 2008, Neuron 59:125).

Our most recent experiments show that synaptic inputs to interaural time difference (ITD) processing neurons in the medial superior olive (MSO) are also regulated by presynaptic $GABA_B$ receptors. In contrast to LSO, $GABA_B$ receptors in the MSO mainly regulate the inhibitory and not the excitatory inputs. Moreover, in the MSO GABA is not retrogradely released from the principle neurons themselves, but rather from GABAergic fibers of unknown origin that terminate in the region of MSO somata and proximal dendrites.

Both findings, in LSO and MSO, clearly show that the balance of excitation and inhibition is not only a crucial but also a dynamic feature in adjusting binaural sensitivity in the mammalian brain.

8 Nicotinic Regulation of Auditory Cortex: From Cells to Cognition

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In this talk I will describe recent studies to determine how nicotinic acetylcholine receptors, activated by either the neurotransmitter acetylcholine or the drug nicotine, regulate auditory cortex mechanisms and function. We use the auditory thalamocortical slice preparation to identify cellular mechanisms by which nicotinic acetylcholine receptors regulate cortical responses to thalamic inputs. We perform parallel in vivo studies to determine functional implications for cortical processing of sound. And finally, we examine how these regulatory mechanisms contribute to auditory-cued behavior. The results suggest that nicotinic cholinergic enhancement of cortical processing contributes to optimal auditory-cognitive performance.

9 Activity-Dependent Mechanisms Regulating Transmission and Integration in the Superior-Olivary Complex

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The auditory brainstem must transmit and integrate information with high fidelity over a broad range of activity levels. Transmission across the relay synapse of the calyx of Held can be highly reliable at low frequencies, but as transmission rates rise above 100Hz, short term depression of the synaptic current vastly reduces the security of transmission and postsynaptic failures are observed. Long-term changes in transmitter release or changes in synaptic strength have not been reported at the calyx (or endbulb) of Held. Alternatively, activity-dependent modulation of target neuron excitability mediated via voltage-gated ion channels, could control threshold excitability, action potential waveform and integration of sustained or high frequency synaptic inputs.

Neuronal nitric oxide synthase (nNOS) is highly expressed in neurons of the medial nucleus of the trapezoid body (MNTB) and nitric oxide (NO) is generated on stimulation of the calyx of Held, via calcium influx through postsynaptic glutamate receptors. The ability of NO to diffuse unimpeded through cell membranes allows it to act as a 'volume transmitter', providing a local indicator of on-going activity for both active and inactive neurons. Rather than a presynaptic action, the predominant mechanism is via phosphorylation of postsynaptic voltage-gated potassium channels (Kv). NO signaling mediates an activity-dependent suppression of Kv3.1 currents, so increasing action potential duration and reducing firing rates in response to auditory input, perhaps serving as a gain control. These observations demonstrate that postsynaptic excitability is an important means of activity-dependent regulation, which would complement adaptation of synaptic strength as a mechanism of neural computation in auditory processing.

10 Altered GAP Coding and Glycinergic Neurotransmission in the DCN of an Animal Model of Tinnitus: Pathologic Plasticity

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Tinnitus affects 15–35% of individuals in the United States and up to 10% of these individuals report severe and disabling symptoms. Sound-exposure animal models of tinnitus can be used to assess functional and neurochemical tinnitus-related plastic changes. The dorsal cochlear nucleus (DCN) displays changes consistent with altered pre- and postsynaptic glycinergic inhibition in animals with behavioral evidence of tinnitus. Tinnitus was

induced in adult rats using a unilateral, one-hour, 17 kHz-centered octave-band noise (116 dB SPL) and assessed using a gap-startle protocol. Three months following sound exposure, the rats showed no significant ABR threshold elevation, and a subpopulation of animals displayed impaired gap coding, indicative of tinnitus. There was a significant down-regulation of the α_1 glycine receptor (GlyR) subunit protein in fusiform cells located in the middle and high frequency regions of the DCN in rats with behavioral evidence of tinnitus. The anchoring/trafficking protein, gephyrin, displayed tinnitus-related increased protein levels across DCN frequency regions. Consistent with decreased α_1 subunit protein levels, strychnine binding showed significant tinnitus-related decreases in the number of GlyR binding sites in the fusiform cell area. Single unit studies found that DCN fusiform cells from sound-exposed rats displayed significantly increased spontaneous activity and altered gap coding, especially near the frequency limits of the units excitatory response area. These findings establish that loud sound-exposure produces tinnitus in an animal model that is associated with significant functional and glycinergic pathologic plasticity in the DCN.

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11 Homeostatic Control of Temporal Coding by Coordinated LTP and LTD in an Auditory Circuit

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Optimal neural coding over the dynamic range of natural sensory environments requires plastic neural circuits. However, the neural representation of temporal features of sensory stimuli must remain invariant for accurate stimulus identification and discrimination. The circuit mechanisms that modulate neural coding to accommodate changes in input statistics, while establishing homeostatic control of temporal coding, remain to be revealed. Here we show that coordinated long-term potentiation (LTP) and long-term depression (LTD) in a feedforward inhibitory, auditory brainstem circuit modulate sensory response threshold, while preserving spike response timing. Our findings establish a novel from of circuit plasticity that allows for modulation of response statistics to match changing input statistics, while establishing homeostatic control of a temporal code necessary for accurate sound localization.

12 Insulin-Like Growth Factor-1 Protects Mouse Cochlear Hair Cells from Aminoglycoside Ex Vivo

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Insulin-like growth factor-1 (IGF-1) has been considered as an important factor in inner ear sensory epithelial development. Previously, local IGF-1 application has been demonstrated to attenuate noise- and ischemia-induced hearing loss and hair cell damage. In addition, a phase I-IIa clinical trial to investigate the efficacy of local IGF-1 treatment for acute sensorineural hearing loss has been performed. However, molecular mechanisms of hair cell protection by IGF-1 have not been fully elucidated. To investigate molecular mechanisms of IGF-1 actions on cochlear sensory epithelia, we aimed to establish an ex vivo model using explant cultures of mouse cochleae as a basis for molecular analyses in the present study. Explants of cochleae obtained from post-natal day 2 mice were used. After 24-h incubation, the explants were cultured with the medium containing 2mM neomycin with or without supplementation of IGF-1 for 24 h. After fixation, the specimens were provided for histological analyses using immunohistochemistry for myosinVIIa and phalloidin staining. Quantitative analyses of the surviving hair cells in explants revealed a significant increase of hair cell numbers in IGF-1-treated specimens, indicating that hair cell protection by IGF-1 also occurs in our explant culture system similarly to previous in vivo experiments. The present findings demonstrate further evidence for the potential of IGF-1 as a cochlear protectant, and the validity of our ex vivo model to examine molecular mechanisms of IGF-1 actions for cochlear protection. This works was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan and by a Grant-in-Aid for Research on Sensory and Communicative Disorders from the Ministry of Health, Labour and Welfare of Japan.

13 Little Siblings with a Big Attitude: Rbl1 (P107) and Rbl2 (P130) in Inner Ear Hair Cell Regeneration

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Unlike lower vertebrates, adult mammalian HCs do not proliferate and HC death leads to irreversible neurosensory hearing loss and balance impairment. Recent advances have provided proof of principle for two sets of therapies: the use of the cyclin system or the retinoblastoma gene Rb1, to promote proliferation, and the effectiveness of Atoh1 to induce transdifferentiation of supporting cells (SCs) into HC. Combined, these two approaches can mimic the ability of lower vertebrates to regenerate HC. However, beyond the proof of principle, attempts to regulate cell cycle through ablation of Rb1 are not likely to safely repopulate lost HCs. The retinoblastoma (herein called pRB) family of cell cycle regulators, composed of Rb1, Rbl1 (p107), and Rbl2 (p130), constitutes a central node controlling G1 to S phase transition in proliferating cells. Unlike other tissues, the biochemical and molecular pathways of the pRBs, particularly Rbl1 and Rbl2 in the inner ear are relatively unexplored. Here, we discuss the potential of Rbl1 and Rbl2 in SC proliferation and HC regeneration. Knockout (KO) mice lacking either Rbl1 or Rbl2 genes have been analyzed for their inner ear phenotype. Contrasting with the drastic effects of Rb1 ablation, deletion of either gene causes the appearance of supernumerary rows of HCs

and SCs, in the apical and upper middle turns of the cochlea; while only extra SCs but not HCs are found on the basal turn. Interestingly, functional analyses of P30 mice ears show that Rbl1 and Rbl2 KO mice have near normal hearing in spite of their organ of Corti (OC) phenotype. Furthermore, quantitative RT-PCR (Q-PCR) analyses of both Rbl1 and Rbl2 expression in the OC of the KO mice point to a possible compensation mechanism taking place between these two pocket proteins. Altogether, our current results demonstrate a role for Rbl1 and/or Rbl2 in HC and SC proliferation and advocate in favor of their modulation in future therapeutic plans to repair damage in the OC.

14 Evaluation of Noise Deafened Guinea Pigs Injected with SiRNA Targeting P27kip1 Five Weeks After Noise Exposure

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Improved hearing following ototoxic drug or noise exposure is linked to supporting cell proliferation and hair cell regeneration in birds. In the mammalian cochlea, p27Kip1 (p27) inhibits cell cycle progression by functioning as a cyclin dependent kinase inhibitor in supporting cells following its expression at embryonic day 13.5. Targeted deletion of p27 in mice induces ongoing supporting cell proliferation resulting in supporting cell hyperplasia and supernumerary hair cells in postnatal and mature mouse cochlea (Chen and Segil, 1999; Lowenheim et al., 1999).The induction of supporting cell proliferation in developing mouse cochlea and mature Guinea pig cochlea has previously been reported using antisense oligonucleotides against p27 mRNA (Gu ARO 2001, Kil ARO 2001), Adenovirus encoded p27 shRNA (Kil ARO 2004, Gu ARO 2005, Yamasoba ARO 2006), and siRNA (Gu ARO 2008). Inhibitors to p27 were typically injected 5 days after ototoxic insult. We now describe the results experiments were the p27 inhibitors were injected 5 weeks after intense noise exposure.

Scala media delivery of short inhibitory RNAs (siRNAs) that bind and degrade p27 mRNA results in a decrease of p27 protein and an increase in cellular proliferation. In this Guinea pig model of noise induced hearing loss, p27 siRNAs induce cellular proliferation and regeneration in and around the organ of Corti relative to vehicle and random sense siRNA control animals. Audiologic and histologic correlations in a large series of Guinea pigs treated with scala media delivered p27 siRNA along with appropriate controls will be presented. Correlation to changes in behavior (photobeam activity assay and startle assay) will be discussed as we further elucidate the role of supporting cell proliferation in the improvement of hearing following noise induced hearing loss in the mature mammalian cochlea.

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15 Changes in Sox2 and Atoh1 Protein Expression During Supporting Cell Direct Transdifferentiation and Mitosis in the Regenerating Chick Cochlea

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We have examined changes in Sox2 and Atoh1 expression in supporting cells of the chick cochlea during regeneration. In the adult avian cochlea, Sox2 is seen only in supporting cell nuclei. Atoh1, a transcription factor needed for hair cell differentiation, appears only in embryonic hair cells. After a single gentamicin injection, some supporting cells exhibit Atoh1 protein in their nuclei as early as 9h after the gentamicin injection (AI), while still retaining Sox2 expression in these same nuclei. By 15-24h AI, Atoh1 labeling is seen in supporting cells throughout the region of hair cell damage. Both Atoh1 and Sox2 continue to be present in the nuclei of supporting cells undergoing direct transdifferentiation; labeling for both is lost by 10 days AI, as they mature into functional hair cells. Meanwhile, supporting cells undergoing mitosis take up EdU during the peak of S phase (72-96h AI). These cells are a separate population from those expressing Atoh1. Dividing supporting cells express Sox2 throughout the cell cycle until metaphase, when the nuclear membrane breaks down and chromosomes segregate to opposite spindle poles. Once metaphase begins, Sox2 labeling in the nuclei is lost. However, the daughter cells quickly resume labeling for Sox2 following mitosis. By 78h AI (6h post-EdU), >50% of the daughter cells label for Sox2. By 80h AI (8h post-EdU), >80% of the daughter cells have Sox2-positive nuclei. In contrast, Atoh1 labeling of the daughter cell nuclei is rare until 96h AI. At 96h AI, some pairs of daughter cells exhibit symmetric Atoh1 labeling, some have only one daughter of the pair labeled, while in others both daughters are negative for Atoh1. The presumptive hair cells continue to express Atoh1 and Sox2 as they mature, but labeling for both is lost by 10 days AI. Thus, for both direct transdifferentiation and mitosis, both Sox2 and Atoh1 are expressed simultaneously throughout hair cell maturation.

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16 Comprehensive Genetic Regulatory Networks During Utricle Hair Cell Regeneration

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Lower vertebrates such as birds are able to regenerate sensory hair cells in response to injury whereas mammals cannot. By utilizing Next Generation DNA sequencing technology, we are deriving a comprehensive description of the mRNA and miRNA transcriptomes as the avian utricle regenerates across a 120 hour regeneration time course after aminoglycoside ablation of hair cells. We are

also identifying the majority of enhancer elements that are activated during regeneration by ChIP-Seq with an antibody to the enhancer-associated co-factor P300. Five chicken miRNAs, including gga-miR-183, show significant changes in expression during regeneration (> 2-fold and p values < 0.05). In addition, a novel chicken miRNA predicted by sequence alignments to be the ortholog of has-miR-96 is also differentially expressed during regeneration. Previous studies have shown that both miR-183 and miR-96 play important roles during the development of the mouse inner ear and mutations in the seed region of miR-96 are associated with inherited deafness in humans. The effect of all of these miRNAs upon proliferation and differentiation of hair cells is being tested by knock-in and knock-down strategies. These three databases (avian mRNA, miRNA and enhancer elements) are being compared to comprehensive transcriptome data from postnatal mouse utricles in order to identify similarities and differences in regenerative programs between birds and mammals.

17 Culture and Characterisation of Cells from the Human Utricle

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Background: The ability to *in vitro* culture cells from various tissues has been an important research tool for many years. Benefits include the amplification of cell types that are either rare or difficult to obtain from the host organism. This enables the scientist to conduct numerous and varied controlled experiments providing data on aspects such as cellular metabolism and genetics without continued harvesting of cells from the host. This is particularly true for cells within the human cochlea, which are rare in comparison to other cells types, and extremely difficult to obtain given its location. However, before cultured cells can be used for research, they must be characterised for correct phenotype. Here we describe for the first time the culture and characterisation of cells derived from a human utricle obtained following surgery.

Methods: Cells from human utricle explants were cultured in DMEM using standard cell culture techniques. Cells from passage numbers 1 through 6 were initially examined using phase contrast microscopy followed by immunohistochemistry using several antibodies specific for various cell types including supporting and hair cells. In addition, real time PCR (RT-PCR) was used to determine genetic changes between the passage numbers.

Results: Data from early passage numbers indicate that cultured cells appear to be all of the same phenotype. However, immunohistochemistry shows that the cells are expressing markers specific for both supporting and hairs cells, also confirmed using RT-PCR. Later passage numbers are to be examined.

Conclusion: Early results indicate that the cells are most likely to be supporting cells in origin. However, it would seem that gene expression becomes altered *in vitro*. The expression of multiple cells markers provides some evidence that gene expression of cells within the cochlea are controlled by cell-cell interactions between different cell types.

18 Reciprocal Effect of BMP4 on Differentiation of Hair Cells and Neurons from Inner Ear Stem Cells

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Inner ear stem cells offer a possible source for regeneration of inner ear hair cells and neurons and might provide a future tool for treatment of sensorineural hearing loss. The inner ear develops from the hindbrain adjacent to the neural tube, and signals from the neural tube are required for inner ear development. Bone morphogenetic protein 4 (BMP4) is one such signal that influences inner ear morphogenesis. However, the role of BMP4 on the differentiation of specific cell types is still unclear.

Inner ear stem cells can be used to reconstruct the maturation of the inner ear and its cell types. We used an in vitro approach to investigate the role of BMP4 on mouse stem cells from either the spiral ganglion or the organ of Corti. We found that different concentrations of BMP4 had distinct but reproducible effects on the differentiation of inner ear stem cells to hair cells and neurons. BMP4 at low concentration increased expression of transcription factor, Pax2, in the stem cells, which coincided with an increase in Atoh1 expression. Increased Pax2 expression was also observed upon addition of retinoic acid, which may act by regulating the BMP4 level. Increased Atoh1 led to an increased percentage of hair cells (based on myosin VIIa expression). Higher concentrations of BMP4 inhibited expression of Pax2 and were accompanied by decreased Atoh1. The higher BMP4 concentration increased the percentage of neurons upon differentiation of the stem cells (based on neuronal morphology and β-III tubulin expression). Expression of Pax2 and differentiation of neurons or hair cells was distinct for differentiating stem cells from spiral ganglion and organ of Corti and was also sensitive to the stage of differentiation at which the BMP4 was added. Thus, immunocytochemistry and gene expression analysis reveal a reciprocal impact of BMP4 concentration on the development of neurons and hair cells.

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19 Functional Recovery Obtained by Human ESCs-Derived Otic NeuroProgenitor Cells (ONPs) Transplanted Into the Deafened Gerbil Cochlea

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The lack of regenerative capacity of the mammalian cochlea prevents the restoration of any sensory cell loss, making the damage irreversible and the ensuing deafness permanent. A potential therapeutic strategy would lie in the

surgical delivery of exogenous stem cells, aiming to repopulate the damaged cochlea. Although the replacement of hair cells is still impaired by substantial technical challenges, the restoration of auditory neurons is a more realistic goal. The usefulness of this approach is enhanced by the possibility to combine it with cochlear implants.

Our laboratory has previously developed a protocol to generate otic progenitor cells from hESCs by activating the FGF signalling pathway. In the work presented here, we explored the ability of hESC-derived otic neuroprogenitors (hONPs) to graft and differentiate when transplanted into an animal model of deafness. After initial induction using FGF3 and 10, hONPs were defined by the expression of auditory markers PAX8, PAX2 and GATA3 together SOX2 and NESTIN. The lineage potential of hONP cells was validated in vitro by inducing auditory neuronal differentiation with culture conditions established previously by working with human fetal auditory stem cells (hFASCs, Chen et al Stem Cells, 27:1196-1204, 2009). Neuronal characteristics were confirmed by the expression of lineage markers. Deafness was induced in young adult gerbils by a localised application of ouabain. When applied directly into the round window niche of the ear, it damages the spiral ganglion neurons while leaving hair cells intact, generating a model of neuropathic hearing loss. hONPs were harvested and transplanted into the cochlear modiolus via the round window. Cochleae were processed at different period intervals for up to twelve weeks post transplantation. Analysis after 3 weeks showed that the transplanted cells have survived and have grafted into the modiolar region, forming a coherent ectopic ganglion. After 12 weeks, exogenous cells have repopulated the Rosenthal canal and produced neural projections towards the organ of Corti. More important, improvement of the ABR thresholds was obtained, ranging from a modest 10dB to a full 60 dB recovery. The development of protocols to manipulate human stem cells should greatly facilitate the translation into a clinical application.

20 Investigating Inner Ear-Derived Neural Progenitors for Hearing Regeneration

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There are two critical unsolved issues in stem cell-based replacement therapy for the inner ear. Firstly, the appropriate donor cell type has not been determined. Secondly, the survival and differentiation of implanted stem cells have not been thoroughly explored. Compared to embryonic stem cell and neural stem cell (NSC) derived from the other tissue, inner ear-derived stem cell might be more ready to adopt an inner ear cell fate. To regenerate degenerated spiral ganglion neurons, we recently identify NSCs from the inner ear of mice. These inner ear-derived NSCs (ieNSCs) could proliferate and differentiate into cells expressing not only universal neuronal protein such as class III beta tubulin and neurofilament 200 but also glutamatergic neuronal proteins. We identified the neurotrophin that could stimulate the survival and glutamatergic differentiation of ieNSCs. In implantation of ieNSCs into the adult mammalian inner ear, we found 10-fold more stem cells survived in the neurotrophin-supplied inner ears. This will significantly enhance inner ear regenerative ability and provide clues for the stem cell-based biological and translational research that would explore strategies for treating hearing loss, tinnitus, balance disorder and other neurodegenerative diseases.

21 Differentiation of Human CD34+ Hematopoietic Stem Cells Into Glia-Like Cells in the Injured Cochlear Nerve of a Humanized Mouse Model

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Human-mouse transplantation models (humanized mice) based on immunodeficient mice have been widely used to study human stem cells *in vivo*. The NOD/SCID IL-2R γ^{null} mouse, a newly developed immunodeficient model, is deficient in mature lymphocytes and has a longer life-span than other immunodeficient models. Application of ouabain to the cochleas of NOD/SCID IL-2R γ^{null} mice selectively causes the death of most spiral ganglion neurons and induces glial hyperplasia. The pathologic changes in the injured cochlear nerve in this model were similar to those previously reported in gerbils and CBA mice. Here, we investigate if $CD34^+$ hematopoietic stem cells (HSCs) isolated from human cord blood can engraft and differentiate into neuronal cells in the injured cochlear nerves of NOD/SCID IL-2R γ^{null} mice. Flow cytometric analysis revealed high engraftment levels of human cells in recipient mice 5 months after HSC transplantation. The percentage of human $CD45^+$ cells in the bone marrow of recipient mice ranged from 63% to 71%. The percentages of the B-cell, T-cell, and granulocyte/macrophage lineages in the human $CD45^+$ cells were 25%, 1%, and 19%, respectively. The percentage of human $CD45^+$ cells in the peripheral blood of the recipient mice ranged from 23% to 30%. Numerous human-derived cells were present in the injured cochlear nerves in both peripheral and central processes 7 days after ouabain exposure. In contrast, few human-derived cells were seen in the untreated cochlea. Specifically, histological and immunohistochemical studies demonstrated that a portion of the human derived cells in the cochlear nerve differentiated into glia-like cells. These findings suggest that 1) NOD/SCID IL-2R γ^{null} mice are an excellent platform for supporting engraftment of human HSCs; and 2) cells derived from $CD34^+$ HSCs may be involved in the regeneration of neuronal cells in the injured cochlear nerve after exposure to ototoxins. NIHDC7506, NIHDC0422, NIHDC0713 and AAO-HNS CORE136165

22 A New Concept for Hair Cell Regeneration: Implantation of an Artificial Sensory Epithelium

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The sensitivity of the human ear to sounds depends on the function of mechanosensory hair cells that transduce mechanical stimuli into electrical signals. The signals are transmitted to spiral ganglion neurons, which convey the sensory information to the brain. The loss of hair cells causes permanent hearing loss. Hence, many efforts have been paid to realize hair cell regeneration in mammals, which helds the promise for restoration hearing by cell and /or gene therapy. However, practical application may be distant. At present, the cochlear implant, of which electrode is implanted into the cochlea and electrically stimulate spiral ganglion neurons, is only applicable for deaf patients. After loss of hair cells, sound-frequency sensitive architectures still remain in the cochlea, although less sensitivity than healthy cochleae. We highlighted such remaining sensitivity to sounds, and designed a piezoelectric device that can utilize the vibrations of the cochlear basilar membrane to generate electricity to stimulate spiral ganglion neurons without use of a battery. This is a process mimicking the function of inner hair cells, and this new device is named an artificial sensory epithelium. A prototype of ASE was made to test its sensitivity to sound stimuli and its potential for generation of electrical stimuli in vitro. The results demonstrate that a prototype of ASE has the sensitivity to sound frequency and generates electrical stimuli in response to sound exposure. Based on these findings, implantable devices for guinea pig cochleae were made in a nano scale using microelectromechanical systems. Now we are investigating the efficacy of such implantable devices for generation of compound action potential in deafened guinea pigs.

23 In Vitro and in Vivo Low Level Laser Therapy in the Gentamycin Induced Ototoxicity of the Rat Cochlea

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Low Level Laser (LLL) can alter the cellular behavior to favor regeneration. Although many studies point out a positive effect of LLL in neural cell survival, there has been no study in the cochlea. We aimed to elucidate the effects of LLL on hair cell survival following gentamycin exposure in the organotypic culture of cochlea and in the in vivo hearing loss model of the rat.

The organotypic culture of cochlea was allowed to grow continuously for 17 days (C group, n=8). In L group (n=8), the organotypic culture was irradiated with 808 nm laser, 8 mW/cm^2, 60 min/day for 10 days. The cochlea were exposed to 1 mM of gentamicin for 48 hr and allowed to recover (G group, n=8) or allowed to recover with daily irradiation (GL group, n=7). The hair cells were stained with FM1-43 and counted every 3 days.

For the in vivo study, adult rats (200g, n=8) were injected with intravenous gentamycin (100mg/kg) and furosemide (90mg/kg) for 2 consecutive days. After confirming a bilateral hearing loss, only the right ear was irradiated with 830 nm laser, 200 mW/cm^2, 60 min/day for 10 days. Hearing was check before and after laser irradiation. After the 10th day of irradiation, the animals were sacrificed for SEM evaluation of the remaining hair cells.

As for the in vitro study, the number of hair cells was significantly larger in the L group (p=0.05) and GL group (p=0.01) compared to the C group and G group respectively. As for the in vivo study, hearing thresholds were similar on both ears after 2 dyas of GM injection (Lt 55.0±10.7 dB, Rt 53.8±11.9 dB), but Rt side hearing was significantly better than the control ear after 10 days of irradiation (p=0.02; Lt 57.5±16.7 dB, Rt 45.0±12.0 dB). The number of hair cells was also significantly larger in the Rt irradiated ear by SEM (p=0.03; Rt 129.4±34.3, Lt 97.1¡¾±40.9).

These results may suggest that low level laser promotes hair cell survival following gentamycin damage in the cochlea.

24 FGF Signaling in OTIC Morphogenesis

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Development of the inner ear proceeds through phases of placode induction, axis specification, otocyst formation, morphogenesis and cell type specification. FGF signaling plays dosage sensitive roles in all of these processes. *Fgf3, Fgf10* and *Fgf8* are all expressed in the developing otic epithelium during morphogenesis and differentiation. *Fgf3* and *Fgf10* global null mutants each show distinct otic morphogenesis defects. Morphogenesis of *Fgf3* null inner ears is highly variable; with the most severe phenotypes being similar to those caused by mutations in genes that function from the hindbrain to direct dorsal patterning of the otic vesicle and obscuring assessment of its function in otic epithelium. *Fgf10* null inner ear phenotypes are more consistent; showing abnormalities of semicircular canal formation. These are consistent with proposals that *Fgf10* expression in the developing sensory tissues is required to induce non-sensory development. To determine the otic epithelial functions of *Fgf3* and *Fgf10*, without disrupting otic induction, we generated double conditional mutants using Tg-*Pax2Cre,* and found that as expected, both genes play roles in semicircular canal morphogenesis. Unexpectedly, these genes also appear to have redundant

roles in cochlear morphogenesis. Initial results show that the FGF signaling indicator *Erm* is affected as early as E9.5, but the first effects on morphogenesis and patterning markers occur at E10.5. We will describe progress in determining the molecular and cellular mechanisms by which these FGFs control morphogenesis, as well as a new approach to controlling *Fgf3* expression with doxycyline administration that should enable detailed analysis of otic morphogenesis, both in vivo and in culture.

25 Mycn Partially Segregates Histogenesis from Morphogenesis in Ear Development

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Mycn is a member of the Myc family of transcription factors that encodes a bHLH protein. Deregulation of Mycn is known to cause a number of tumors, most notably neuroblastomas. Another member of the myc family, Myc is deregulated in 30% of all tumors. Importantly, Mycn binds with Myc Associated Factor X (MAX) to its E-box with the sequence CACGTG, causing cell proliferation. Homozygous KOs of Mycn are early embryonic lethal precluding assessment of ear function. We generated conditional knockout for Mycn using Pax2-cre. We describe here a phenotype that links Mycn function to histogenesis and morphogenesis of the developing inner ear. Mycn CKOs reveal a fused utricle, saccule, and cochlea, similar to recent reports on Lmx1a mutants. The horizontal canal crista is present but the canal is absent. Absence of the horizontal canal but presence of a normal anterior and posterior canal suggests a different morphogenetic requirement between the canals; this is consistent with previous findings in Otx1 mutants. In contrast to Otx1 mutants, where only a partially horizontal canal crista forms, Mycn CKOs have a near normal neurosensory development of the horizontal canal crista. Unique to Mycn, the cochlear apex is shortened with a disorganized and partially segregated Organ of Corti implying a topographically distinct function of Mycn. Aberrant innervations include vestibular as well as cochlear innervations of the cochlea and disorganized neuronal path finding to the apex. In conclusion, our Mycn CKO reveals that this factor plays not only a role in proliferation, but interacts in a yet to be determined way with Otx1 and Lmx1a to determine histogenesis and morphogenesis of the developing ear. Hair cell disorganization could relate to E-box sequence similarities of Mycn and Atoh1 E-boxes and related in cross signaling. We are currently assessing Foxg1 expression in Otx1 and Mycn mutants to understand differences in their horizontal canal crista formation.

26 Involvement of the MIF Pathway in the Development of Zebrafish Inner Ear

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Macrophage migratory inhibitory factor (MIF) was first identified as a factor that inhibits the migration of normal guinea pig peritoneal exudate cells, comprised mainly of macrophages. The roles of MIF in the immune system, in tumorigenesis, and in the neuroendocrine axis have been extensively studied. However, in more recent studies, MIF has been found to be expressed in neurons, in astrocytes, in Schwann cells, and in the eye and inner ear. Furthermore, MIF expression has been detected in embryos, including human, mouse, chick, and Xenopus. In Xenopus, the knockdown of Mif expression using antisense morpholino oligonucleotides (MO) resulted in a complete lack of neural tissues, including brain, spinal cord, eye, and otic vesicle (Suzuki et al., 2004). This dramatic effect of Mif MOs was the first indication of the importance of Mif in Xenopus nervous system development. Our previous studies have shown that knockdown of Zebrafish mif and its related protein mif-like caused increased cell death in the brain, resulting in smaller anterior brain and eyes as well as smaller statoacoustic and other head ganglia and a decreased number of hair cells (HC) in the macular HC patch. These results indicated that mif plays a role in inner ear development, both in the statoacoustic ganglion and the sensory HC patch. However, due to the effect of mif MO on the brain, it was hard to determine if the ear phenotype was the result of a direct effect of the MO on the ear, or a secondary effect of the brain defect. To overcome this problem, we injected mif MO directly into the otic vesicle at 24 hours post fertilization (hpf) and performed electroporation to facilitate the uptake of MO into the otocyst. Our preliminary data showed that mif MO caused smaller numbers of HC in the HC patch, indicating that mif is important for ear development.

27 Cytokine Macrophage Migration Inhibitory Factor (MIF) Is an Essential Bioactive Component for Inner Ear Neuronal Development

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Spiral ganglion neuron (SGN) loss, either dependent or independent of sensory hair cell (HC) loss, is a major

cause of deafness, particularly in the ageing population. Cochlear implants (CI) are presently the only known "cure" for many forms of deafness. Nevertheless, successful function of a CI depends on the preservation of SGNs. In the early developing inner ear, the otocyst secretes a factor called Otocyst Derived Factor (ODF)[1,2,3]. ODF promotes directional neurite outgrowth and neuronal survival[1,2,3] of the statoacoustic ganglion (SAG), the precursor of SG.

In a previous study, we demonstrated that the bioactive components of ODF include Macrophage Migration Inhibitory Factor (MIF), which is known for its roles both in the immune system and in neuronal development and regeneration. We found that recombinant MIF alone supports both SAG directional neurite outgrowth and neuronal survival, and evokes a neuronal phenotype from mouse embryonic stem cells. We also found that MIF is expressed in supporting cells (SC) and its receptor, CD74, on both SAG/SGN. In MIF knock-out (KO) mice, abnormal development of both SC and HCs as well as a significant hearing impairment in the high frequency region of the cochlea is seen with concomitant loss of SGN in this region of the cochlea.

In the present study, we examined the role of MIF in inner ear neuronal development using a mouse model *ex vivo*. Freshly excised Organs of Corti (OC) from wild-type (WT) and MIF KO mice were cultured with SG from WT mice. Directional SG neurite outgrowth was seen toward the WT OC, while only random neurite extension that did not reach the OC was seen toward MIF KO mice. The present experiments present further evidence that MIF functions as an essential component of normal inner ear neuronal development and innervation and could potentially be used for SGN retention or regrowth as well as to potentiate the function of a cochlear implant.

28 The Role of Zic1 and Zic2 in Cell Fate Specification During Inner Ear Development

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The formation of the inner ear is one of the most intricate and well-studied of the processes that occur during organogenesis in vertebrates [reviewed in (1)]. Still, very little is known about the basic morphological and cellular development of the inner ear. One of the most basic questions is what genes are responsible for the initially uncommitted cells in the otocyst to become neuroblasts that form the statoacoustic ganglion (SAG), prosensory cells that differentiate into hair cells (HC) and supporting cells (SC), or non-sensory cells that form the other structures within the inner ear. The *Zic* genes, especially *Zic1* and *Zic2*, are involved in many different phases of development, most notably as part of regulatory networks during neural development[2-4]. However, involvement of these genes during development of the inner ear has only been described at the level of gene expression[4], so functional studies are critically needed. A previous study showed that Zic2 expression was upregulated in the sensory epithelium of the chick following noise trauma, indicating that *Zic2* may be involved in regeneration of HCs[5]. More recent work from our lab demonstrated that Zic1 and Zic2 are both expressed in regions of the developing inner ear, including in the sensory epithelium[4]. Work from the Aruga lab suggests that Zic1 and Zic2 may specify either neuronal or sensory tissue, but, depending on the species, there is no consistency in which gene specifies which cell type[2]. Therefore, the experiments in this project examine the role of *Zic1* and *Zic2* in cell fate specification during development of the inner ear and will test the hypothesis that Zic1 helps specify a neuronal fate and Zic2 a sensory cell fate in the inner ear.

29 Transgenic Rescued-GATA3 Mutant Mice Unveiled the Essential Function of GATA3 in Normal Morphogenesis of Inner Ear

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GATA3 is a zinc finger type transcription factor expressed in embryonic otic vesicles. However, the physiological roles GATA3 plays in inner ear development in the later embryonic stages have been largely unknown, because of the mid-gestational embryonic lethality (e10.5) of *Gata3* homozygous knockout mice (*Gata3*-/-) from adrenaline bio-synthesis deficiency. In order to circumvent this difficulty, we utilized a transgenic rescue system. The 5.8kbp human dopamine-β-hydroxylase (hDBH) promotor directed GATA3 transgene expression in the sympathetic nervous system, and then rescued the lethality of *Gata3*-/- embryos up to E18.5 (*Gata3*-/-:Tg$^{hDBH\text{-}G3}$). The inner ear structure of E18.5 wild-type embryo is normally composed of the three distinct parts, i.e., a cochlea, vestibules and semi-circular canals. Interestingly, the stereoscopic observation demonstrated that the inner ear region of *Gata3*-/-:Tg$^{hDBH\text{-}G3}$. E18.5 embryo is extremely shrunken. The histological observation of the serial sections demonstrated that the inner ear of *Gata3*-/-:Tg$^{hDBH\text{-}G3}$ E18.5 mutant mouse exhibited only a single cyst-like region, showing an isthmus which failed to separate otic vesicle into the three parts. These observations suggest that GATA3 is essential for the normal regionalization of inner ear into the three functionally distinct parts.

30 Transcription Factor GATA3 Is Necessary for Normal Inner Ear Neurosensory Development and Associated Morphogenesis

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Inner ear development and morphogenesis requires the coordination of many transcription factors, including zinc finger proteins. The zinc finger protein GATA3 has been shown to be expressed in most sensory epithelia of the developing otocyst as early as embryonic day 8.5 and mutations of GATA3 cause hearing loss in humans. Previous work assessing the function of GATA3 in the

inner ear has been hindered by the early lethality of these mice around embryonic day 11.5 (E11.5). We now show that older null mutant mice (E 16.5) develop a cyst-like inner ear of the size of a E10.5 mouse embryo with only an extension of the endolymphatic duct and an occasional formation of a single sensory patch. To further analyze *Gata3* function in the ear we used floxed *Gata3* and the inner ear specific Cre lines, *Foxg1*-cre and *Pax2*-cre to generate two conditional *Gata3* lines. Conditionally deleted *Gata3*$^{f/f}$*/Pax2*-cre mice have a finger like projection of the cochlea with discontinuous and deformed hair cell patches. Some spiral ganglion neurons develop, extend neurites toward these patches, but fibers may overshoot to the lateral wall. Some vestibular sensory patches develop in these mice, however, they are morphologically distorted, continuous with each other and show overshooting innervation. In contrast, conditionally deleted *Gata3*$^{f/f}$*/Foxg1*-cre mice harvested thus far show a complete loss of cochlea formation, closer matching the null phenotype. *Gata3*$^{f/f}$*/Foxg1*-cre mice show formation of a saccule, utricle, and morphologically ambiguous horizontal/anterior canal crista, and loss of the posterior canal. In conclusion, GATA3 plays a crucial role in inner ear development in particular those sensory organs that show high level of early expression. In addition, morphological development associated with these sensory organs is proportionally disrupted. We suggest that loss of cochlea formation reflects a dose dependency on GATA3 protein of sensory development.

31 A Dysmorphic Basal Cochlea Is Associated with a Failure of Canonical Wnt Signaling in Lmx1a (DreherJ) Mutant Mice

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We (Nichols et al., '08, Cell Tissue Res 334: 339-358) and others (Koo et al.'09, Dev Biol 333: 14-25) showed that the basal organ of Corti of Lmx1a (Dreher^{J}) mutant mouse embryos had up to 14 hair cell rows but lacked pillar and Deiter's cells. In contrast, the apical organ appeared histologically near normal, with 1+3 rows of hair cell rows, pillar and Deiter's cells. An abrupt transition separated the two regions and array data suggested changes in Wnt signaling pathways. To further determine the role of Wnt signaling, we generated Lmx1a wildtype and mutant mice carrying lacZ tracers for the *Atoh1* (*Math1*) gene and canonical Wnt signaling (Topgal). In the cochlea, Atoh1-lacZ stains only hair cells, while Topgal stains only capsular mesenchyme, its derivatives and adjacent cartilage. In wildtype embryos between E12.5 and E17.5 a band of first mesenchyme then mesenchyme+cartilage was closely associated with the posterior non-sensory epithelium of the duct. By P7, intensely stained mesenchyme derived cells were located in the mesothelium on the tympanic face of the basilar membrane and scattered in the basal layer of the stria vascularis. In *Lmx1a* mutants, no Wnt signal was observed at any time (E12.5-P7) basal to the transition, while a Wnt signal was present apical to the transition. In addition, both the basilar membrane and the stria vascularis were absent in the base at P7. The site of the apical transition to a normal organ of Corti was further correlated with its interaction with the posterior wall of the capsule. In summary, these results suggest that an interaction between the cochlear epithelium and the posterior (and only the posterior) capsule initiates canonical Wnt signaling in the capsule that is essential for the capsule's contribution to the cochlea and is correlated with the proper organization and differentiation of the epithelium (sensory and non-sensory) as well.

32 Smad4 Gene Plays Important Role on Inner Ear Development in Mice

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Smad4 is the central intracellular mediator of transforming growth factor-beta (TGF- beta) signaling, which plays crucial roles in tissue regeneration, cell differentiation, embryonic development, and regulation of the immune system. Conventional *Smad4* gene knockout results in embryonic lethality, precluding its use in studies of the role of *Smad4* in inner ear development. We used chondrocyte-specific *Smad4* knockout mice (*Smad4Co/Co*) to investigate the function of *Smad4* in inner ear development. *Smad4Co/Co* mice were characterized by a smaller cochlear volume, bone malformation, and abnormalities of the osseous spiral lamina and basilar membrane. The development of the hair cells was also abnormal, as evidenced by the disorganized stereocilia and reduced density of the neuronal processes beneath the hair cells. The magnitude of CM was significantly reduced, reflecting abnormal mechanotransduction in the stereocilia. ABR and CAP measurements showed that the homozygous *Smad4Co/Co* mice had severe hearing loss. However, nonlinear capacitance (reflecting motility) of outer hair cells was not statistically different from the wild type mice. Our results suggest that *Smad4* is required for inner ear development (Supported by National Natural Science Foundation of China grants No. 30871398 and 30730040 to SY and by NIH grant R01 DC 004696 to DH).

33 Pou3f4 Is Required for Normal Development of the Spiral Ligament and Stria Vascularis of the Cochlea

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Pou3f4, encoding a POU domain transcription factor, is the major gene responsible for DFN3, an X-chromosome linked nonsyndromic deafness in human. Previous studies using mice with targeted deletion of *Pou3f4* locus have shown that the lack of Pou3f4 causes abnormalities in the

temporal bone and otic fibrocytes. To better understand the role of Pou3f4 in inner ear development, we examined the inner ears of *Pou3f4*$^{del-J}$ mouse, a spontaneous mutant with a deletion in the *Pou3f4* locus (Jackson Laboratory, Bar Harbor, ME, USA). Our detailed genomic analyses of *Pou3f4*$^{del-J}$ mutants revealed a 530-570 kb deletion in the X chromosome that includes the *Pou3f4* and three hypothetical genes. The deletion breakpoints are approximately 470 kb upstream and 70 kb downstream of the *Pou3f4* single exon. *Pou3f4*$^{del-J}$ mice display head shaking and circling behavior and were profoundly deaf at 3 weeks of age assessed by auditory brainstem response assay. At the cellular level, inner ears of *Pou3f4*$^{del-J}$ mutants showed severe defects in the modiolus and spiral fibrocytes. During development, some *Pou3f4*-positive mesenchymal cells in the cochlear lateral wall undergo condensation and mesenchymal-epithelial transition (MET) to form the basal cells of the stria vascularis (SV), while other *Pou3f4*-positive mesenchymal cells in the region develop into various types of fibrocytes in the spiral ligament. In *Pou3f4*$^{del-J}$ mutants, the process of MET to form the basal cells of the SV seemed to be delayed, whereas formation of fibrocytes in the spiral ligament was severely disrupted. Type I, II, IV, and V fibrocytes were almost completely absent, and type III fibrocytes that are normally restricted to the junction of spiral ligament and the otic capsule were dispersed throughout the spiral ligament. Our observations suggest that although *Pou3f4* is expressed in the entire mesenchymal area of the cochlear lateral wall, specific role of Pou3f4 is different in fibrocytes of the spiral ligament and basal cells of the SV during development.

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34 Neural Crest Contributions to the Mammalian Inner Ear

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The mammalian inner ear develops from a specialized region of the ectoderm located on either side of the caudal hindbrain known as the otic placode. During development, the otic placode invaginates to form the otic cup, from which some cells delaminate and migrate into neighboring mesenchyme to form neurons of the cochleo-vestibular ganglion. The otic cup deepens further and pinches off from the ectoderm to form the otocyst, which over time develops into the membranous labyrinth of the inner ear. While a majority of the cells in the membranous labyrinth are derived from the otic placode, some of the cells in the labyrinth are derived from the neural crest. Neural crest originated from the junction between the epidermis and dorsal region of the neural tube gives rise to a variety of cell types in the embryo such as neurons, glia, melanocytes, bones and cartilages.

To identify the neural crest contribution to the mouse inner ear, we genetically fate mapped progeny of neural crest in the inner ear using *Pax3-Cre* mice. Pax3 is a member of the Pax family of transcription factors, and it is important for various aspects of embryogenesis including neural crest differentiation. In human, mutations in *PAX3* cause Waardenburg's syndrome Type I, characterized by neurosensory hearing loss. *Pax3* is expressed in the dorsal neural tube that includes the neural crest, but it is not expressed in the otic epithelium. Crossing *Pax3-Cre* with *R26R* reporter mice, we identified descendants of *Pax3*-expressing cells in the inner ear of *Pax-3; R26R* compound heterozygotes using β-gal histochemistry. At E17.5, β-gal positive cells are detected in various regions of the inner ear including endolymphatic duct, stria vascularis, and ganglia. In addition, β-gal positive cells are present in some parts of the otic capsule and in all three middle ear ossicles. Furthermore, analyses of *Pax3-Cre* homozygous mutant embryos showed that *Pax3* is required for melanocyte formation in the stria vascularis but not other neural crest-derived cell types associated with the middle ear and cochleo-vestibular ganglion.

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35 Differences in Right and Left SCC Diameters May Account for Circling Behavior in EphB Deficient Mice

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Previous data indicate that bidirectional signaling mediated by B-subclass Ephs and ephrins control the production and ionic homeostasis of endolymph fluid. EphB2 is expressed in K+ secreting vestibular dark cells and ephrin-B2 in the adjacent transitional cells of the inner ear. Mice lacking the EphB2 receptor tyrosine kinase demonstrate reduced endolymphatic lumenal volume in the semicircular canals (SCC). Consequently, the membranous component of these semicircular canals become collapsed and distorted such that mice exhibit a hyperactive circling locomotion. Digitalized photographs of histologic sections of EphB mutated mice and age matched controls were produced from postnatal day 14 animals. From the digital images, mean diameters were measured for both right and left lateral SCC using Image J software (NIH). The mean SCC diameter for wild type and EphB2 mutant mice were 185 ± 5.24 μm and 109 ± 21.4 μm ($p<0.0001$) respectively, demonstrating that, consistent with our previous findings, mutant mice have significantly smaller SCCs. Furthermore, the mean difference between left and right SCC diameter for wild-type mice was 6.0 ± 5.09 μm and for EphB mutants was 29.6 ± 8.85 μm ($p = 0.002$). These data suggest that the circling behavior in EphB mutated mice is due, at least in part, to an imbalance in vestibular input between right and left SCCs.

36 Kir4.1 (*KCNJ10*) Gene Is Not Down-Regulated in the Cochlea in the Cx30 Knockout Mice

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Connexin 30 (Cx30) is a predominant isoform of gap junction gene in the cochlea. Cx30 mutation can induce hearing loss. An apparent pathophysiological change in the Cx30 knockout mouse cochlea is lack of endolymph potential (EP). However, its mechanism remains unclear. *KCNJ10* encodes a member (Kir4.1) of inward rectifier-type K channel family and is involved in generation of the positive EP in the cochlea. Knockout of *KCNJ10* (Kir4.1) abolishes the EP and causes deafness in Pendred syndrome mouse model. In this study, the Kir4.1 expression and developmental changes in the cochlea in the Cx30 knockout mice were studied by quantitative real-time RT-PCR. In the postnatal development, the EP starts to increase at P8 and rapidly increases between P10 and P14. After P18, the EP reaches the normal adult level. We found that *KCNJ10* expression in the cochlea at the transcription level was down-regulated in the normal postnatal development. The mRNA level of *KCNJ10* was high at postnatal day 3 (P3), and reduced by 4-fold at P30. In the Cx30 KO mice, the expression of *KCNJ10* at P3 was similar to that in the wide-type mice. However, in contrast to the wide-type mice, the mRNA level of *KCNJ10* at P30 retained at a high level, and was not down-regulated. This indicates that Cx30 knockout hinders the normal downregulation of Kir4.1 at the transcriptional level in the postnatal development in the cochlea. The data also suggest that Cx30 deficiency may not be due to the downregulation of *KCNJ10* (Kir4.1) expression in the cochlea to cause hearing loss.

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37 The Mouse Model of SLC26A4-Related Syndromic and Non-Syndromic Deafness Develops Cochlear Bone Malformations and an Enlarged Vestibular Aqueduct

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Pendred syndrome and non-syndromic deafness due to mutations of *SLC26A4*, are characterized by an enlarged vestibular aquaduct (EVA) and by cochlea bone malformations. *Slc26a4*$^{-/-}$ mice, a model of the human disease, develop an enlargement of the membranous labyrinth and fail to hear. The goal of the present study was to determine whether EVA is present in the mouse model and whether cochlea bone malformations are due to failure or delays in the expression of bone-related extracellular matrix proteins. Cochlear and vestibular bone morphology was evaluated by confocal microscopy of cryosections and gene expression was analyzed by qRT-PCR and immunohistochemistry during early postnatal development of *Slc26a4*$^{+/-}$ and *Slc26a4*$^{-/-}$ mice. Between P1 and P10, calcification of the extracellular matrix and subsequent bone formation progressed from the vestibular labyrinth to the cochlea. The primary spongiosum, generated during endochondrial bone formation, was found in the lateral wall of the cochlea to be compacted into two layers of bone in *Slc26a4*$^{+/-}$ mice and only into a single layer of bone in *Slc26a4*$^{-/-}$ mice. Membranous bone formation of the modiolus was delayed in *Slc26a4*$^{-/-}$ mice compared to *Slc26a4*$^{+/-}$ littermates. The cross-sectional diameter of the vestibular aqueduct, which encloses the common crus and the endolymphatic duct, was enlarged 2-fold in *Slc26a4*$^{-/-}$ compared to *Slc26a4*$^{+/-}$ mice. Developmental up-regulation in the expression of *Bglap1, Dmp1, Ibsp, Mepe* and down-regulation of *Col10a1* were delayed in the cochlea of *Slc26a4*$^{-/-}$ mice. In conclusion, these data demonstrate that *Slc26a4*$^{-/-}$ mice develop a compromised cochlear bone formation and an enlarged endolymphatic duct similar to human patient.

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38 Bone Marrow Cell Migration in Early Postnatal Cochlea in a Mouse Model of SLC26A4-Related Syndromic and Non-Syndromic Deafness

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Malformation of the otic capsule has recently been reported in a mouse model of *SLC26A4*-related syndromic and non-syndromic deafness (Wangemann et al., 2009). The primary spongiosum of the otic capsule in the lateral wall of the cochlea compacted into only one layer of bone in *Slc26a4*$^{-/-}$ mice, whereas two layers of bone were found in *Slc26a4*$^{+/-}$ mice. The space between the two layers of bone was filled with bone marrow cells. This observation raised the question how bone marrow cells colonize the otic capsule and the bone of the vestibular labyrinth. Macrophages, which present a minority population among bone marrow cells in the cochlea, were visualized by confocal immunocytochemistry using anti-CD68 antibodies. Macrophages were observed to migrate from the vestibular labyrinth to the cochlea during postnatal age P1 and P5. At P3, amoeboid-shaped CD68-positive macrophages were found to migrate along the border between the spiral ligament and adjacent chondrocytes of the otic capsule. At P4 and P5 amoeboid-shaped CD68-positive macrophages were found at the border between chondrocytes and the forming bone. At P10 macrophages concentrated in *Slc26a4*$^{+/-}$ in bone marrow cavities. At P30, macrophages in *Slc26a4*$^{+/-}$ mice were round-shaped and found exclusively in bone-marrow cavities. In contrast, macrophages in *Slc26a4*$^{-/-}$ mice were found at P30 both in bone-marrow cavities and inside stria vascularis. In conclusion, these data suggest that macrophages play a role in the ossification of the primary spongiosum in the cochlea and the vestibular labyrinth. Macrophages in *Slc26a4*$^{-/-}$ mice appear to be partially redirected from entering bone to entering stria vascularis. The mechanism by which macrophages cross the basal cell border into stria vascularis remains to be determined.

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39 Enlargement of the Membranous Labyrinth in *Slc26a4*$^{-/-}$ Mice Coincides with the Onset of Pendrin Protein Expression in *Slc26a4*$^{+/-}$ Mice During Development

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Mutations of *SLC26A4* are one of the most prevalent forms of childhood deafness. Acidification and enlargement of endolymphatic spaces are key events responsible for the failure to develop hearing in *Slc26a4*$^{-/-}$ mice (Wangemann et al., 2004, 2007, 2009). The enlargement has been demonstrated by ink injection in *Slc26a4*$^{-/-}$ mice at E15.5, which roughly coincides with the onset of pendrin mRNA expression in *Slc26a4*$^{+/+}$ mice at E13 in the endolymphatic sac and at E15 in the cochlea (Everett et al 1999). The goal of the present study was to determine with greater precision whether the onset of pendrin expression in *Slc26a4*$^{+/-}$ mice coincides with the onset of the enlargement in *Slc26a4*$^{-/-}$ mice. Cryosections were prepared from embryonic and postnatal otocysts. Protein expression of pendrin was detected by confocal immunocytochemistry using a rabbit anti-pendrin antibody kindly provided by Dr. S. Nielsen, Aarhus University, Denmark. Pendrin expression was found in the apical membrane of the endolymphatic sac and duct, in the apical membrane of vestibular transitional cells, and in the apical membrane of cochlear outer sulcus and spiral prominence cells. The onset of pendrin expression was between E12.5 and E13.5 in the endolymphatic sac, at E14.5 in the vestibular labyrinth and at E14.5 in the cochlea. Cochlear expression began in the most basal portion at E14.5 and reached the apex at E17.5. Prior to E13.5 scala media of the cochlea was closed in *Slc26a4*$^{+/-}$ and *Slc26a4*$^{-/-}$ mice. Between E13.5 and E14.5 scala media began to open at base of the cochlea. Already at E14.5 scala media as well as the endolymphatic sac were enlarged in *Slc26a4*$^{-/-}$ mice. In conclusion, these data demonstrate a close relationship between the onset of pendrin expression and the formation of an enlargement of the membranous labyrinth in mice lacking pendrin expression.

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40 A Critical Test of Alternate Stimulus Level Measures for the Human Ear

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Determining how to control the input level to the human ear has been a thorny problem, especially at high frequencies. We have compared depth-compensated calibration in an ear simulator (Siegel, ARO Abst., 32:11-12, 2009), a method to predict the eardrum SPL (Siegel, 2009) and measures of sound intensity (SIL) (Neely & Gorga, JASA 104:2925-2934, 1998), forward pressure (FPL) (Scheperle, et al., JASA 124:288-300, 2008), transmitted pressure (TPL) (Withnell, et al., JASA 125:1605-1611, 2009) and the "integrated" eardrum pressure (the sum of the forward and reflected pressure waves) (IPL) (Neely, Pers. Corresp., 2009). The six quantities were compared using behavioral thresholds measured in each ear with two different insertion depths, under the assumption that the best input level measure would be the one with the least dependence on insertion depth (Neely & Gorga, 1998). All measures except the simulator and prediction methods required calibrating the Thévenin equivalent source characteristics of the ER 10B+ otoacoustic emission probe (Scheperle, et al., 2008).

Békésy tracking thresholds were measured twice (for the deepest possible and the shallowest possible insertion with a good seal using a foam ear-tip) from 125Hz-20kHz in 24 individual subjects (42 ears). The prediction method, simulator calibration, FPL and IPL were least dependent on insertion depth throughout the frequency range. The greatest dependence on insertion depth was for TPL, followed by SIL, with the largest differences below 1 kHz and above 6 kHz. Threshold TPL at 125 Hz was, on average 9 dB higher for shallow *vs* deep insertions, with correspondingly higher reflectance (lower transmitted pressure) at low frequencies for shallower insertions. A similar dependence of reflectance on insertion depth has been ascribed to the non-cylindrical geometry of the human ear canal (Farmer-Fedor and Rabbit, JASA, 112:600-620, 2002).

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41 Quantification of Human Middle-Ear Function with High-Frequency Bone Conduction Measures

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Our understanding of human middle-ear function and reconstructive surgery efficacy for high frequencies (6 – 16 kHz) is limited primarily by the insufficient high-frequency output of the standard electrodynamic transducers used to measure bone conduction sensitivity. Magnetostrictive technology has the potential for increased vibratory output in the high-frequency range. Two new commercially-available bone conduction headphones (TEAC HP-F100 and TEAC HP-F200), that use this alternative technology, were evaluated in the laboratory with laser vibrometry and under clinical conditions with threshold measures in a variety of subjects with normal hearing or sensorineural hearing loss, but with presumably normal middle-ear function. The HP-F200 transducer was modified to provide control over static coupling force, in a manner similar to the conventional electrodynamic transducer (Radioear B-71), and allow measures at several skull locations. Results indicated that harmonic distortion and acoustic radiation were both sufficiently low to allow accurate threshold measurements for high frequencies. Bone conduction thresholds were accurate under conventional clinical conditions for high frequencies, at levels up to 85 dB HL in 9 subjects (HP-F100). These measures, when combined with standard air-conduction measures for high frequencies, can accurately characterize middle-ear function and reconstructive surgery efficacy for frequencies higher than 6 kHz.

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42 Relationships Between Passive Middle-Ear Properties as Measured by Wideband Middle Ear Power Analysis (MEPA) and Distortion-Product Otoacoustic Emission (DPOAE) Response Properties in Healthy Newborns

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Wideband middle-ear immittance measures (e.g., power reflectance, transmittance, admittance and impedance) represent passive, linear transmission properties of the outer and middle ear. Collectively, such measures are referred to as Middle Ear Power Analysis (MEPA), which can be measured in human ears with the HearID system (developed by Mimosa Acoustics, Champaign, IL). This system also measures distortion product otoacoustic emissions (DPOAE) using the same probe assembly. A strong correlation between middle-ear transmission and DPOAE amplitude as a function of frequency, would be expected, due to forward and reverse transmission filtering by the middle and outer ear. In this study, wideband middle-ear transmission was compared with DPOAE stimulus and responses in ears of healthy newborns aged 1-3 days (DPOAE F2 frequencies: 2, 3, 4, and 6 kHz,) using the same probe assembly and ear tip within the same test session (n=252 ears). In our preliminary analysis, correlations for each frequency measured by MEPA which corresponded closely to the F1, F2, and/or 2F1-F2 DPOAE frequency were determined, and all reached significance ($p < .05$). However, in contrast to our expectation, the highest correlations did not occur when the MEPA frequency matched one or more of the DPOAE frequencies. Instead, the highest correlation always occurred at 2 kHz for the F1, F2 and the 2F1-F2 frequency. The DPOAE at 2 kHz had a lower negative correlation than at the three other test frequencies (poorer middle-ear transmission correlated with a weaker DPOAE). All four DPOAE frequencies showed a frequency region of largest correlation: 2 kHz DPOAE from 1-1.5 kHz, the other three from 1.5-2 kHz. These results suggest factors other than direct linear flow of power, that influence the DPOAE responses in newborn middle-ear transmission. Related to the strong correlations with 2 kHz, this region also showed the highest positive predictive value for absent DPOAEs in newborns, with an area under the ROC curve of .90. Based on these findings, a brief clinical test for newborn middle-ear function at 2 kHz is feasible.

43 Effect of Middle Ear Fluid on Sound Transmission and Auditory Brainstem Response in Guinea Pigs

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Combination of the measurements on middle ear transfer function and auditory brainstem response (ABR) in live guinea pigs with middle ear effusion is reported in this paper. The otitis media with effusion model was simulated in guinea pigs by injection of saline solution into the middle ear cavity. Vibrations of tympanic membrane (TM), incudo-stapedial joint (IS joint) and round window membrane (RWM) were measured with a laser vibrometer at frequency range of 0.2-40 kHz when the middle ear fluid increased from 0 to 0.2 ml (i.e., full fill of the cavity). The click evoked ABR was also recorded as the middle ear fluid increased. The results demonstrate that the middle ear fluid reduced the mobility of TM, IS joint and RWM mainly at high frequencies ($f > 1$ kHz). The reduction of the movements of the TM, IS joint and RWM was proportional to the volume of fluid in the middle ear. The displacement transmission ratio (DTR) of the TM to IS joint which represents the transfer function of the middle ear, varied with the frequency and amount of fluid in the middle ear. The DTR of IS joint to RW was almost constant around 1.0 over frequencies and did not vary much with the volume of fluid in the middle ear. This observation indicates that the cochlear fluid is generally incompressible and the present of middle ear fluid may not have much effect on cochlear mechanical function. The ABR thresholds elevated from 20 to 27 and 33 dB when the fluid volume in the cavity increased from 0 to 0.1, and 0.2 ml. The prolongation of the ABR latencies at wave I and wave III was observed as the fluid level elevated. The combined measurements of laser and ABR provide a solid evidence on the change of sound transmission efficiency induced by the middle ear effusion. (Supported by OCAST and NIH/NIDCD)

44 Sound Energy Transmission Simulated in 3D Finite Element Model of the Human Ear

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Finite element (FE) model of the human ear for analysis of sound conduction through the ear has been improved with advanced technologies in 3D reconstruction and multi-field FE coupled analysis. A 3D FE model including the ear canal, middle ear and cochlea (uncoiled) published by Gan et al. (JASA, 2009) has shown the potential application for simulating middle ear diseases such as the tympanic membrane (TM) perforation, and predicting the effect of perforation on sound energy transmission through the ear. However, the discrepancies between the modeling results and measurements suggest that the behavior of the model for acoustic energy transmission needs to be improved. One critical factor on modeling results is the mechanical properties of ear tissues. The measurements on TM and other middle ear ligaments have indicated that the ear tissues are typical viscoelastic materials, showing time-dependent behavior. This paper reports our efforts on employing viscoelasctic properties for ear tissues in the FE model instead of elastic properties used in the published model. First, the FE model reported by Gan and Wang (JASA, 2007) was modified by attaching the uncoiled cochlea to the middle ear at the oval and round windows. Five middle ear tissues (e.g., TM, TM annulus, incus-malleus joint, incus-stapes joint, and stapedial annular

ligament) were assumed as linear viscoelastic materials characterized by complex moduli over the frequency domain. Then, the material parameters were determined using the measurements and cross-calibration process based on frequency response behavior of the TM and stapes footplate displacement in human temporal bones. Finally, the middle ear transfer function, input impedance of the middle ear, and sound energy transmission through the ear (e.g., energy reflectance and admittance) were generated using the improved FE model of human ear. The results were compared with the temporal bone and clinical measurements. (Supported by NIH/NIDCD)

45 Middle Ear Disease and Conductive Hearing Loss in Mice

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The influence of middle ear disease on hearing in mice is not well understood. We investigated middle-ear function and structure in a small group of mice, some of which had developed hearing loss. The suspect mice were of a genetic background that is known to be susceptible to middle ear disease. We measured ABR and DPOAE thresholds and sound-induced umbo velocity, and prepared the ears using standard histological techniques. Histological analyses included reconstruction of middle ear volume, observation of TM and ossicular structures, and estimation of the volume of lightly or darkly stained middle-ear fluid. Lightly stained fluid in diseased ears was located throughout the middle ear, whereas darkly stained fluid was usually seen near the round window. The ABR and DPOAE thresholds and the umbo velocity produced by lower- and middle-frequency sound stimuli were strongly correlated with middle-ear air volume. The volume of darkly stained fluid was correlated with the DPOAE and ABR thresholds for lower- and middle-frequency sounds. The volume of lightly stained fluid was correlated with the umbo velocity in the middle and upper frequencies. Regression analyses between the thresholds and velocity show the DPOAE threshold about twice the ABR threshold in the middle frequencies, while the ABR threshold was roughly inversely proportional to the umbo velocity at lower stimulus frequencies. The correlations between histological and functional results are consistent with a decrease in middle-ear air volume and the presence of lightly stained fluid affecting the motion of the TM, while the darkly stained fluid near the round window acts to reduce the middle-ear vibrations that reach the cochlea. The inverse relationship between the ABR threshold and the umbo velocity is consistent with a conductive hearing loss produced by reduced middle-ear air volume. [Work supported by NIDCD]

46 Anatomical Survey of the Ear of a Retinoic Acid Receptor Alpha Knockout Mouse

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The effects of genetic mutations on the auditory system can be varied. Retinoic acid receptors play key roles in the development of the mammalian fetus, including the development of bony structures in the middle and inner ear. We previously examined an adult RARalpha knockout mouse that exhibited a 30-40 dB increase in ABR thresholds (Romand et al. unpublished observations). We measured sound-induced umbo velocity using laser-Doppler vibrometry and found that the velocities in control (RARalpha+/+) mice were similar to velocities measured in other strains of similar aged mice and that the RARalpha knockout (RARalpha-/-) population had velocities that were 10-15 dB lower than controls. Now we present an analysis of histological sections of the ears of these mice based on moderate resolution digital photographs of every fifth section of the mouse ear in two control mice and three RARalpha knockout mice. We used AMIRA software and the techniques described by Wang et al. (Otol & Neurotol 27:452-457, 2006) to generate 3-D reconstructions of the ears of both RARalpha+/+ and RARalpha-/- mice. In the RARalpha-/-, we find structural abnormalities in the ossicular chain, including size and shape; also, the middle-ear air spaces are flatter with significant volume reduction (about half of the RARalpha+/+). We also see size and shape differences in the bony cochlea and point fixations of the stapes. We have begun the process of higher-resolution reconstructions in more control and knockout mice to define the ossicular and inner-ear abnormalities better and to test the generality of the observed differences in the normal and control populations.
[Work Supported by NIDCD]

47 Wave Motion on the Surface of the Tympanic Membrane from Stroboscopic Holograph and Its Clinical Application

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Opto-electronic stroboscopic holography interferometry is used to study wave motion on the surface of the tympanic membrane (TM) in human temporal bones. Tones from 0.2 to 10 kHz with levels from 60 to 120 dB SPL are used as stimuli and the displacement amplitude and phase at more than 40000 nodes on the TM surface are recorded in stroboscopic holograms. The velocity of the stapes is measured by Laser-Doppler vibrometry (LDV) near simultaneously using continuous tone stimuli. Spatial maps of wave velocity on the TM surface are quantified from stroboscopic holograph data at each measured frequency. Wave delays are then computed based on wave velocities and dimensions of the TM. The results are compared with

ossicular group delays derived from phase gradients of stapes velocities measured by LDV. Such information helps us better understand the relation between wave motion on the TM surface and delays in middle ear sound transfer. Our previous studies show that an imposed local suppression of wave motion within a quadrant of TM region has little effect on stapes velocity. In this study, we continue investigating the relation between surface motion of the TM and ossicular chain vibration by applying a large piece of thin cartilage sheet to the medial side of the TM to suppress motion of the majority of the TM. The resultant significant changes of wave motion on the TM surface are then compared with simultaneously measured stapes velocities. Moreover, changes of wave motion on the TM surface caused by various ossicular chain manipulations are also evaluated with related stapes velocities. Such results will have meaningful applications in clinical tympanoplasty procedures as well as in planning treatments for patients with conductive hearing loss.
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48 Simulating Large Deformations of the Gerbil Pars Flaccida to Determine Its Material Properties

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The gerbil is a popular animal in hearing research and many aspects of its middle-ear function have been studied. Various papers have reported on studies of its pars flaccida in the last decade. For example, the role of the pars flaccida in low-frequency hearing (Teoh et al., Hear. Res. 1997; Rosowski et al., Audiol. Neuro-Otol. 1999) was investigated and the deformation of the pars flaccida with static pressure in healthy animals and in animals with otitis media (Dirckx et al., Hear. Res. 1997, 1998) was studied. The interpretation of these results and hence a better understanding of the fine details of the functioning of the hearing organ can benefit greatly from mathematical models in which structural elements of the ear are represented in a geometrically correct way and with correct physical characteristics. Finite-element analysis is often used for such mathematical models but there has been relatively little modeling of middle-ear deformations that exceed the linear (infinitesimal) range. The pars flaccida in gerbil has a flat and nearly circular shape, and in recent studies in our laboratory the thickness has been determined (Kuypers et al., JARO 2005). This makes the design of a realistic finite-element model for the gerbil pars flaccida relatively straightforward, but the material properties are not known. We have used both linear elastic and hyperelastic formulations for the constitutive equations and adjusted their parameters to find simulations that replicate well the large 3D deformations of the gerbil pars flaccida measured in our lab.
Keywords: gerbil, pars flaccida, static pressure, deformation, non-linear finite-element modeling

49 Reverse Transmission Along the Ossicular Chain in Gerbil

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In a healthy cochlea stimulated with two tones f1 and f2, combination tones are generated by cochlear nonlinearity, for example the 2f1-f2 and the 2f2-f1. These distortion tones travel "in reverse" through the middle ear. They can be detected with a sensitive microphone in the ear canal (EC) and are known as distortion product otoacoustic emissions (DPOAEs). Gerbil middle ear ossicles (malleus, incus and stapes) were accessed through an opening of the pars flaccida, and their motion was measured along a single axis in line with the stapes piston motion using a laser interferometer. The stimuli were two equal-intensity tones with fixed f2:f1 ratio of 1.05 or 1.25. Comparison between ossicular velocity and the ear canal pressure responses at distortion product frequencies allowed us to evaluate the middle ear transmission in the reverse direction along the ossicular chain. Middle ear delay was defined as the slope of the phase-frequency response. We found: (1) at frequencies above 10 kHz, the middle ear delay in the reverse direction was similar to that in the forward direction, consistent with our previous studies in which intracochlear pressure right behind the stapes and EC pressure were compared. (2) In the forward direction we have previously noted a systematic delay along the chain from the umbo to the body of the malleus and to the lenticular process of the incus, and in the reverse direction a similar delay was present between the body of the malleus and umbo but seemed less pronounced between the incus and malleus. (2) Below 10 kHz, the opening of the pars flaccida appeared to affect the sound traveling out more than the sound going in.

50 Estimation of the Quasi-Static Young's Modulus of the Rat Eardrum Using Fourier Transform Profilometry and Inverse Finite-Element Analysis

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Background: Accurate estimates of the quasi-static Young's modulus of the eardrum are important for finite-element (FE) modeling of clinical procedures such as tympanometry in which the acoustic admittance of the middle ear is measured at the eardrum as a function of quasi-static pressure applied to the eardrum. Although a few authors have reported estimates of the quasi-static Young's modulus, simplifying assumptions in the analytical approaches may raise questions as to the accuracy of the various methodologies.
Objective: To develop a method for estimating the quasi-static Young's modulus of the rat eardrum from pressurized shape measurements made using Fourier transform profilometry and optimization of a FE model.

Methods and Materials: Measurements were made on six rat eardrums with immobilized ossicular chains. A pressurization system was used to apply quasi-static pressures up to 4 kPa to each eardrum. The resting and deformed shapes of each eardrum were measured using a Fourier transform profilometer, a non-contacting optical device for surface shape measurement. A FE model was constructed for each eardrum from the resting shape data to simulate the pressurization experiment, and the Golden-Section optimization technique was used to automatically find the Young's modulus of the model eardrum that caused the simulated deformed shape to match the measured shape.
Results: The average estimated Young's modulus was 22.8MPa +/- 1.5 MPa, which is comparable to values found in the literature for human eardrums.
Conclusion: The estimation technique proposed in this work yields Young's moduli values that are comparable to those reported in the literature for other species. Moreover, the results are repeatable as indicated by the low standard deviation.

51 Specialisation for Underwater Hearing in the Red-Eared Slider Turtle, *Trachemys Scripta Elegans*

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The inner-ear of the red-eared slider has been extensively studied previously, primarily because of the tolerance of turtle tissue to low oxygen tension and the resulting viability of *in vitro* preparations of inner ear and brain. In comparison, very few studies have addressed the general sensitivity of this turtle ear *in vivo*.

Here, we report on an *in-vivo* study using auditory brainstem responses (ABR). The turtles were lightly anesthetized with ketamine and xylazine, and ABRs were measured using three needle electrodes inserted subcutaneously above the ear, at the brainstem and into the foreleg.

We used four different modes of stimulation: 1) a closed coupler sealed over the eardrum; 2) underwater sound 3) dorso-ventral vibrations and 4) direct motion of the tympanic disk.

The ABR audiogram is V-shaped with best sensitivity to airborne sound (50 dB SPL) at 300-500 Hz. Although the turtle ear looks like a normal tympanic ear, comparison of audiograms before and after removing the 'tympanum', i.e. the skin covering the cartilaginous tympanic disk shows unchanged sensitivity , indicating that the tympanic disk, not the skin, is the key sound-receiving structure. The tympanic disk is framed by a delicate membrane and very compliant. The head of the middle ear bone is firmly attached to the disk. Behind the disk is a large, air-filled cavity (volume about 0.95 ml, corresponding to a resonance frequency of approximately 500 Hz).

A comparison of the audiograms shows that the ear is 10-20 dB less sensitive to underwater sound pressure than to airborne sound. If the thresholds are compared in terms of sound intensity, however, thresholds in water are approximately 40-50 dB lower than in air, showing that the ear is clearly specialized for underwater hearing. Given the specialized structure of the tympanic disk and the large middle ear cavity, we hypothesize that pulsations of the air in the middle ear cavity drive the tympanic disk underwater.

52 Postnatal Development of the Middle Ear in New Zealand White Rabbits

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We studied the postnatal development of the middle ear (ME) in New Zealand White rabbits. Bullae (ages:0, 1, 2, 4, 6, 9, 13, 20, 30, 40, 50, 133, 180 days) were scanned using a desktop x-ray microtomograph. Reconstructed slices were segmented and 3D models of the 3 ME ossicles as well as the tympanic ring (TR) were prepared. In 0,1,2 days old rabbits the ossification process was incomplete. Thus, we present here quantitative data obtained from older rabbits (ages:4-180 days). The length of the malleus increased rapidly up to the 40th postnatal day. It varied between 1.73mm and 4.08mm. Afterwards almost no changes were observed. On day 180 it was 3.79mm. The distance between the tip of the malleus and the TR increased rapidly until day 40. It varied between 0.01mm and 1.40mm. Afterwards, the values obtained approached a plateau. On day 180 the distance was 1.21mm. The surface area of the tympanic membrane (TM) varied between 25.63mm^2 and 33.31mm^2 until day 50 (on day 180: 31.50mm^2). The area within the TR varied between 24.38mm^2 and 30.90mm^2 up to day 50. No significant changes were observed at older ages (on day 180: 28.83mm^2). The ratio: [TM area] / [TR area] increased until day 40. It varied between 1.00 and 1.11. The area of the stapes footplate (FP) increased rapidly until day 40 and varied between 0.72mm^2 and 1.49mm^2 (on day 180:1.28mm^2). The ratio: [TM area] / [stapes FP area] decreased until day 40 and varied between 36.01 and 21.15 (on day 180: 24.65). The distance between the tip of the malleus and the rotation axis increased rapidly until day 20 and varied between 3.47mm and 5.00mm (on day 180: 4.91mm). The distance between the tip of the incus and the rotation axis increased until day 133. It varied between 1.39mm and 1.69mm (on day 180: 1.64mm). This study shows that in rabbits the ME is underdeveloped at birth and that the conical shape of the TM is formed by retraction and growth of the manubrium, mainly during the first 40 days after birth.

53 Abnormal Auditory Ossicles and Hearing Loss in Osteopetrotic Mice

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The three ossicles, the malleus, incus, and stapes form a chain, which transmits vibrations from the tympanic membrane to the inner ear. Osteoclasts are cells that resorb bone including auditory ossicles. Excessive bone resorption by osteoclasts results in osteoporotic changes and spongiosis of ossicles in mice lacking osteoprotegerin, and the progressive hearing loss is prevented by treatment with antiresorptive bisphosphonate (Kanzaki et al. 2006, 2009). However, the effects of lack of osteoclasts on auditory ossicles and hearing ability have been less well studied. Here we analyzed auditory ossicles of osteopetrotic mice lacking molecules essential for osteoclast differentiation, namely, the transcription factor c-Fos or the cytokine RANKL. These mice carried auditory ossicles, which were thicker especially at the malleus manubrium and incus body, and suffered from impaired auditory function. Since c-Fos is also expressed in other cell types beside the osteoclast lineage, such as nervous cells, we generated mice lacking c-Fos specifically in the macrophage-osteoclast lineage. These mice showed hearing loss, suggesting that the lack of osteoclasts rather than alteration in the nervous system is the cause of hearing loss. As these osteopetrotic mice had abnormal morphology of the ossicles and impaired auditory function, we are currently testing whether or not the vibration of tympanic membrane is affected in these mice. We conclude that both excess and deficiency in bone resorption lead to hearing loss mostly due to alterations in morphology and function of auditory ossicles.

54 Usefulness of the Transplantation of Isolated Middle Ear Mucosal Epithelial Cells Mixed with Hydrogel for the Promotion of Mucosal Regeneration in the Middle Ear of Wistar Rat

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Middle ear mucosa plays an important role in the maintenance of middle ear pressure. However, in most of the surgical cases the mucosal regeneration is not sufficient and mucosal transplantation would be required. In the present study, we examined the usefulness of isolated mucosal cells mixed with hydrogel (polymer scaffolds), which has been successfully used as scaffolds in tissue engineering.

Sixteen Wistar rats (4 to 5 weeks) were used as recipients. The middle ear bulla with mucosa was experitated and minced into small pieces, which were cultured in the collagen-I coated dish with culture medium (1:1 mixture of Dulbecco's modified Eagle's medium (Gibco) and Small Airway Epithelial Basal Medium (Cambrex)) in a humidified atmosphere of 5% CO_2 in air at 37℃. The outgrowth cells were subcultured up to three passages. To characterize the cells, we performed immunohistochemistry using anti-pancytokeratin (an epithelial cellular marker), anti-vimentin (a mesenchymal cellular marker) and anti-MUC1 and 2 (a functional marker of mucosal cells). pEYFP-Mito DNA was transfected into the cells by electroporation as tracers. The cells were mixed with hydrogel and transplanted into the middle ear of immuno-suppressed rats by injection. Several weeks after transplantation, the middle ears with transplanted cells were collected and performed immunohistochemistry with above anitibodies to observe the effect of transplantation.

Immunohistochemical results showed that about 70% of outgrowth cells from the tissue were positive to anti-pancytokeratin but not to anti-vimentin. pEYFP-Mito gene expression in the transplanted cells was found in the host-middle ear, showing a stable residence, and the function and morphology of the transplanted cells became to be examined by immunohistochemistry precisely. In conclusion, our results may suggest that mucosal cells mixed with hydrogel could provide the new effective methods to regenerate the mucosal tissue in the middle ear of a Wistar rat.

55 Effect of in Vivo Over-Expression of KGF by Electroporatively Transfected KGF CDNA on the Histology of External Auditory Canal in a SD Rat

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Middle ear cholesteatoma is characterized by enhanced proliferation of epithelial cells with aberrant morphologic characteristics. In our previous study, we have indicated that keratinocyte growth factor (KGF) plays an important role in cholesteatoma formation. KGF is a mesenchymal-cell-derived paracrine growth factor that specifically stimulates epithelial cell growth. In this study, we investigated the effect of over-expressed KGF in vivo by electroporatic transfection of KGF-expressed vector into external auditory canal (EAC) tissues in SD rats.

After anesthetized 9 male SD rats Flag-hKGF DNA plasmid (150 μg) driven by a CMV promoter was injected into the epithelial lesion of right (r)EAC, and electric pulses were given by CUY21 Electroporator, while the left ear with TE buffer injection was used as controls. At 1, 4 and 7 days after injection, 3 rats at each time-point were sacrificed. To detect the expression of Flag-hKGF protein, western blotting was performed. The paraffin sections of EAC tissues were used for H&E and imunstaining of KGF and Ki-67.

As a result, we found chronic inflammation in rEAC at 1 and 4 days, and keratin accumulation in rEAC at 7 days after KGF cDNA electroporated. On the other hand, in the control ear, though the inflammation change was occurred in 1 of 3 rats at 1 day, no inflammation and no keratin debris were detected at 4 and 7 days. Immunohistochemical results revealed that, KGF was positive at 1, 4 and 7 days in injected specimens, while no staining was found in control specimens at all days. Moreover, in contrast to the control specimens, larger numbers of Ki-67-positive cells were detected in KGF cDNA transfected specimens (<0.0001).
These findings indicated that a single injection of KGF cDNA expressive vector coupled with electroporation induced inflammatory reaction and increased proliferative activity of epithelial cells in EAC, and as results, single KGF injection may possibly induce a cholesteatoma formation in SD rats.

56 In Vitro Properties of Osteoblasts Cultured from Stapes of Patients with Otosclerosis: A Preliminary Report

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Otosclerosis is a disease of bone remodeling and turnover in which osteoblasts play a central role. In this study we developed in vitro osteoblast cultures grown from stapes removed during stapedectomies and assessed how the characteristics of these osteoblasts are altered with exposure to bisphosphonates, a proposed therapy for otosclerosis. Cell cultures were grown from stapes removed from four patients with otosclerosis and compared to cell cultures from healthy human peripheral bone fragments harvested during four orthopedic procedures of patients matched for age and sex. Specimens were cultured in DMEM-F-12 with 15% FBS and antibiotics. Staining for alkaline phosphatase, a marker for osteoblast differentiation, verified the osteoblast-like identity of the cells. Once cells reached confluence, 10,000 cells/cm2 were replated, and adhesion and proliferation assays were performed. For adhesion studies, cells were treated with and without alendronate (10-10 - 10-8 M) for 1 week, then trypsinized and replated. Cells were assayed after 4 hours of culture. Trypsinized plates yielded higher cell counts for stapes osteoblasts than normal human osteoblasts (NHO) (mean±SEM 22616±2455 vs. 12651±908 cells; $p < 0.005$). For proliferation studies plates of cells were treated with and without alendronate (10-10 - 10-8 M) for 2 days. At 72 hours of culture, tritiated thymidine uptake for the stapes osteoblasts was lower than NHOs (2884±391 vs. 3935±513 dpm; $p < 0.05$). In the presence of alendronate, otosclerotic osteoblast adhesion decreased, and proliferation increased to levels similar to NHO osteoblasts at baseline. The present results demonstrate distinct characteristics of otosclerotic osteoblasts. The higher adhesion and lower proliferation rate of otosclerotic osteoblasts may implicate the involvement of integrins and cyclins, respectively, in the pathogenesis of otosclerosis. Alendronate appears to have a “normalizing” effect on otosclerotic osteoblasts.

57 Angiotensine 2 Effect on Inflammation Signaling Pathways in Otosclerosis

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Introduction: Our previous studies showed a genetic association between otosclerosis and 2 genetic polymorphisms (M235T et ACE I/D). activating the renin-angiotensin-aldosterone system (RAAS) and otosclerosis in a French population. We also reported the in vitro production of a proinflammatory cytokine (IL6) in the presence of angiotensin 2 (Ang 2) in otosclerosis primary cell cultures. The aim of this study was to assess the mRNA and protein expression of molecules implicated in inflammation signaling pathways in normal and otosclerotic stpadeial cell cultures.
Materials and methods: Six otosclerotic human stapes and six control (temporal bone tumors) human stapedial samples were used to prepare primary cell cultures.Cells were incubated with Ang 2 (10-7 M) or vehicle for, 24H. Assessement of porteins related to the inflammatory pathway and liberated in the culture media was carried out by an antibody array (RayBio® Human Inflammation antibody array 3, AAH-INF-G3-8, n=6). Evaluation of mRNA expression of inflammation pathway molecules was performed by a cDNA array (cDNA Oligo GEarray® Human inflammatory cytokines and receptors micro array EHS-011, SA biosciences, Frederik, MD, n=3). In case of significant variation, results were verified by a qPCR (Taqman, Chromo 4 ®, Biorad, Hercules, CA).
Results: In basal condition and with antibody array, a higher production of several cytokines (Il-1b, IL-12p70, IL-10, PDGF-BB, I309, STNF-RII), and a lower production of TIMP-2 et STNF-RI was observed in the culture media from otosclerosis in comparison to controls. Ang 2 increased the production of IFN-gamma, IL-10, d’IL-7 and decreased IL-11, MIP 1a and TNF RII in otosclerotic cultures. In control cultures, Ang 2 stimulates IL7 et TNF-alpha but inhibits IL-11, IL-12, MCSF, MIP 1 alpha, TIMP-2, and STNF RII. Gene array studies showed a higher expression of BCl-6 in otosclerosis versus control in basal condition. BCl-6 expression is ihibited by Ang 2 only in otosclerosis. QPCR studies confirmed these observations.
Conclusions: These results indicate that otosclerosis samples produce higher quantities of proinflammtory cytokines than normal stapes in basal cell culture conditions. Ang 2 effect in vitro suggests its implication in inflammatory process during the active phase of otosclerosis. This effect appears to be different in control and otosclerotic stapes. BCl-6 mRNA expression level, influenced by Ang 2 may be one of the key steps to trigger inflammation and apoptosis by Ang 2.

58 Histopathologic Study on the Obliteration of the Temporal Dorsal Bullae in Guinea Pig Using Calcium Phosphate

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Mastoid obliteration is the technique to reduce disadvantages of canal wall down procedure. Many materials for mastoid obliteration have been devised. The aim of our study is to evaluate the histopathologic changes according to the different obliterating materials in guinea pig.We divided guinea pigs into four groups. all groups underwent removal of mastoid mucosa with 2% TCA (trichloroacetic acid). One group was control. The others had mastoid obliteration by implantation of polyphosphate (Polybone¢ç), bone wax, gelfoam.Twelve weeks after implantation, the animals were sacrificed. Histopathology study was performed to evaluate inflammation, foreign body response, new bone formation, implant material resorption.We suggest that Polybone¢ç also can be used as a obliterating material in mastoid.

59 The Healing Processes of the Tympanic Membrane

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Introduction
The healing processes after myringotomy are studied as a part of an ongoing project assessing the patophysiological mechanisms involved in the destructive inflammatory processes and the physiological repair after trauma and chronic otitis media. Thereby better prophylaxis or remedies for sequel of the diseases may be invented.
Methods
Acute tympanic membrane (TM) perforations were made in rat with laser. The TMs were studied with light and electron microscopy after different observation times. The strength of the TM was measured with moiré interferometry if the perforation was closed.
Results
After hours inflammatory cells invade the perforation region. After days a massive wave of epithelial cells migrate in peripheral direction towards the perforation. After days to weeks fibroblasts enter the scene of repair to produce new collagen fibers, and after months a reorganization of the lamina propria is going on.
The epithelial invasion produces a keratin spur that eventually spans the perforation gap and serves as a scaffold for the ingrowth of a hypertrophy of fibrous scar tissue that replaces the lamina propria in the defect. A large portion of the TM shows scar tissues in the lamina propria relatively far distant from the myringotomy site, although only in the same half of the TM as the myringotomy. The composition of different collagene types in the lamina propria, each having unique mechanical properties is assessed in normal and pathological situations.
Fourteen days after myringotomy the strength of the closed TM measured almost normal, with a hypertrophy of scar tissue at the myringotomy site. At half a year the hypertrophy was reduced and the strength slightly reduced.
Conclusion
The pars tensa has normally a low metabolism, with sparse cell activity in its major layer, the lamina propria. It reacts however promptly upon trauma such as an acute perforation with a massive cellular activity. Thus, closure and thereby restoration of the physiology of the middle ear cleft is soon achieved, as long the healing process is not interrupted. Thereby the requisite for near-normal hearing is restored as well. Normalization of the structure is however o prolonged process.

60 Histopathological Incidence of the Facial Canal Dehiscence in Otosclerosis

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Background: The incidence of facial canal dehiscence in patients with otosclerosis reported in clinical studies was lower than that in histopathological study using normal human temporal bones.
Objective: To evaluate the histopathological incidence of facial canal dehiscence in human temporal bones with otosclerosis compared with normal controls without otosclerosis.
Subjects and Methods: One hundred and thirty three human temporal bones with otosclerosis from 84 cases and 102 age-matched normal temporal bones from 70 subjects were examined under light microscopy. We evaluated the location and the invasion of otosclerosis into the facial canal and incidence of facial canal dehiscence.
Results: In the oval window area, the incidence of facial canal dehiscence in otosclerosis was significantly lower than controls ($P = 0.019$). Temporal bones with otosclerotic invasion to the thin bony canal were significantly less likely to have dehiscence compared to the otosclerotic bones without invasion ($P = 0.025$).
Conclusion: Facial nerve palsy due to facial canal dehiscence is a serious complication in stapes surgery. Our findings suggest that otosclerotic lesions might have potential to close dehiscence of the facial canal. These findings might provide a histological basis for low incidence of facial canal dehiscence observed in stapes surgery.

61 Post-Operative Hearing of the Reconstructed Ear with Soft Posterior Meatal Wall

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A number of surgical techniques have been designed to treat ears with cholesteatoma such as following: posterior canal wall reconstruction using cartilage, external auditory meatus bone, mastoid bone, and mastoid bone putty. However, the disease recurrence problem was a matter of concern at all times. In 1992, we reported tympanoplasty with soft posterior meatal wall reconstruction as one

solution to the problem. We accepted post-operative occurrence of balloon-like retraction, in this technique, in order to prevent post-operative retraction pocket formation and led to lessen the possibility of cholesteatoma recurrence.
Although we have reported there are no detrimental effect on post-operative hearing of the ears this technique were applied, some suggests possibility of larger energy loss to bring about when the posterior meatal wall is reconstructed by soft materials compared to the wall reconstructed by hard ones.
We made numerical reconstructed ear models and calculated the transmitted acoustic power in the auditory pathway with both soft and hard posterior meatal walls in order to elucidate the difference between these two in sound conduction effect.
As a result, differences in the sound power transmitted to columella among the models were no more than 1.9 dB.
These results suggest that there is no disadvantage in the post-operative hearing of the ear with soft posterior meatal wall even if it has balloon-like retraction.

62 Taste Disorders in Middle Ear Disease and After Middle Ear Surgery – Evaluation of Study Methods

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Introduction
The chorda tympani nerve (CTN) location in the middle ear predisposes for nerve trauma during middle ear surgery. It is not clearly described how its function suffers in different forms of otitis media or by ear surgery: is it better to cut the CTN than to leave it traumatized after surgery? In order to elucidate these questions a prospective study is initiated based on functional measurements of the nerve.
Methods
Two gold standard methods of taste measurements, Electrogustometry (EGM) and the Filter Paper Disc method (FPD) are evaluated on healthy staff members of our ENT dept. The methods have been in clinical use but are until now not thoroughly evaluated regarding reproducibility and possible bias.
A decay test was performed, measuring before and after eating sweet, sour, bitter, salt, a mild or hot meal, after smoking and after taking tobacco snuff as well as before and after local anesthesia of the tongue. Measurements were performed at five consecutive days as well as repeatedly during one day.
Thirty persons were measured bilaterally with booth EGM and FPD in the same séance for correlation analysis between the two methods.
A clinical study with pre- and postoperative measurements has been launched including one hundred surgical cases with otosclerosis, dry chronic otitis media or cholesteatoma. Simultaneously a taste and a quality of life questionnaire are applied.
Results
The readings of 750 measurements indicate that EGM is a reliable method to measure taste with a high degree of reproducibility. The only significant taste reductions were obtained after eating bitter or having local anesthesia of the tongue. Furthermore, it was shown a significant correlation between EGM and FPT.
Conclusion
It is important to identify the best way to handle the CTN in different surgical situations. To do so, the measuring methods first need to be thoroughly evaluated. EGM was found to be a valid (significant FPT correlation) and reliable method for taste measurements.

63 Inner Ear Hair Bundle Proteomics: Identification of Novel Stereocilia Proteins

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The stereocilia of sensory hair cells contain proteins pivotal to the mechanotransduction process, the underlying core sensory process of hearing and balance. These specialized organelles must develop and maintain not only the principal transduction machinery but also many accessory structures that are essential for proper sensory function. To identify specific proteins in these specialized organelles, we utilized a mass spectrometry protein identification approach termed multi-dimensional protein identification technology (MudPIT), which is based on two-dimensional chromatographic separation followed by tandem mass spectrometry analysis of tryptic peptides. We isolated stereocilia from utricles of embryonic chickens (Gallus gallus) using an agarose embedding technique. Tandem mass spectra of stereociliary proteins from 1,900 utricles were analyzed with three database search algorithms in combination with statistical validation. These experiments resulted in identification of 138 proteins at the highest confidence level, and almost 1,000 proteins with a confidence greater than 90%. Among the proteins identified was a significant group of known hair bundle proteins as well as a substantial number of proteins previously not associated with stereocilia. We have localized 3 new proteins to stereocilia via antibody staining, and present our emerging analysis of the function of these proteins in stereocilia.

64 Molecular Constituents of the Tip-Link Complex in Hair Cells

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Tip links are thought to gate mechanotransduction channels in hair cells. Tip links consist of a cadherin 23 (CDH23) homodimer that interacts in trans with protocadherin 15 (PCDH15) homodimer to form the upper and lower part of tip links, respectively. The distribution of CDH23 and PCDH15 at tip links suggests that the mechanotransduction machinery of hair cells is inherently asymmetric. In support of this model, recent studies suggest that the mechanotransduction channels in cochlear hair cells are localized to the lower end of tip links. To identify molecules that might be important for tip link function, we have searched for proteins that interact with the cytoplasmic domains of CDH23 and PCDH15 and are localized in proximity to tip links. We show here that

the PDZ-domain protein harmonin is a component of the upper tip-link density (UTLD) where CDH23 inserts into the stereociliary membrane. Using forward and reverse genetics approaches, we have generated mouse lines with mutations in tip-link cadherins and harmonin. Some of the mutations are similar to those that are associated with deafness in humans and affect mechanotransduction and tip-link maintenance. Collectively, our finding define essential components of the tip-link complex in hair cells and provide strong evidence that some forms of human deafness are caused by defects in tip-link function.

65 Immunolocalization of Hair Bundle Proteins During Tip Link Regeneration

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Chelating extracellular calcium breaks tip links, and in the chicken basilar papilla, tip links regenerate over 12-24 hours, coincident with the return of mechanotransduction. Using immunocytochemistry, we have characterized the location of tip link proteins cadherin 23 (CDH23) and protocadherin15 (PCDH15) during tip link regeneration. Immediately after EGTA treatment, CDH23 and PCDH15 no longer localize to the hair bundle, but instead co-localize in vesicles in a region below the cuticular plate. After several hours of recovery in culture, CDH23 staining in the hair bundle is substantially increased throughout the bundle, while PCDH15 staining is restricted to the tips of stereocilia. We have also characterized the location of hair-bundle myosin motors, included myosin-6 (MYO6), following breakage of tip links. Immediately after EGTA treatment, MYO6 immunoreactivity increases in the bundle, suggesting this motor may be important for trafficking discarded transduction components out of the bundle. These data indicate that the hair cell sends many CDH23 molecules into the bundle to replace broken tip links, and that MYO6 plays an important role in the hair bundle during early stages of regeneration.

66 Asymmetric Distribution of Protocadherin15 and Cadherin23 in the Kinociliary Links of Chick Vestibular Hair Cells

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Recent studies of guinea-pig cochlear hair cells have revealed that protocadherin15 and cadherin23 associate in trans to form the hair bundle's tip links. Protocadherin15 is located at the lower end that is associated with the stereociliary tips and cadherin23 forms the upper part that attaches to the sides of the stereocilia (Kazermierczak et al., 2007). Double immunogold labelling with a monoclonal antibody that recognises an epitope (the tip-link antigen, TLA) known to be located in the ectodomain of chick protocadherin15 (Goodyear and Richardson, 2003; Ahmed et al., 2006) and a rabbit antibody raised to a recombinant fragment encompassing the 5th and 6th cadherin repeats of avian cadherin23 were used to investigate the distribution of these proteins in the tip and kinocilial links of hair cells in the bird inner ear. As in the guinea pig, cadherin23 was preferentially located towards the upper end of the tip link, whilst protocadherin15 tended to be located towards its basal end. Within kinocilial links, protocadherin15 was located on the side of the link closest to the kinocilium whilst cadherin23 was located in closer proximity to the stereocilia. In tannic acid stained preparations, transmission electron microscopy revealed a distinct density in the 120-130 nm long kinocilial links that is located 35-40 nm from the membrane of the kinocilium. The distance of this density from the membrane is consistent with it being the site at which the opposing N-termini of cadherin23 and protocadherin15 homodimers interact.

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67 HCN1 Channel Binding to Stereociliary Tip-Link Protein Protocadherin 15 CD3

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Surface plasmon resonance (SPR) analysis has previously provided evidence of calcium-dependent binding of the amino terminus of the HCN1 channel to the carboxy terminus of stereociliary tip-link protein protocadherin 15 CD3, expressed in rat organ of Corti (Ramakrishnan et al., J. Biol. Chem. 284: 3227-3238, 2009). The binding was specific, not replicated by protocadherin 15 CD1. This molecular interaction had been predicted by yeast-two hybrid analysis of HCN1 protein-protein binding partners for a model vestibular hair cell preparation from a teleost, indicating generality between vestibular and cochlear end organs, across vertebrates. The identification of stereociliary tip-link protein protocadherin 15 CD3 as a binding partner suggested possible involvement of the HCN1 channel in mechanosensory transduction.

Molecular characterization by SPR of interactions of proteins expressed in rat organ of Corti indicates that the peptide sequence of the amino terminus conserved across HCN isoforms, underlying HCN channel formation, is not required for binding. Further, HCN4-specific amino terminus sequence does not bind protocadherin 15 CD3. We have investigated phospholipid interactions of HCN1, given that PI, PIP_2 and PIP_3 enhance HCN channel activation (Zolles et al., Neuron 52: 1027-1036, 2006) and hair cells require PIP_2 for mechanotransduction (Hirono et al., Neuron 44: 309-320, 2004). The amino terminus of HCN1 contains basic amino acids which could bind phospholipids, reminiscent of the CNG channel amino terminus. Analysis with a lipid strip (Echelon) revealed binding of the HCN1 amino terminus to PIP_3 and to a lesser extent to PIP_2. These results have been confirmed by SPR at 10-20 μM PIP_3 and PIP_2 as analytes, and the full amino terminus of HCN1 as ligand at 26.5 μM Ca^{2+}. Binding of HCN1 to PIP_3 may have an ultrastructural correlate in PIP_3 localization in hair cell stereocilia (Tachibana et al., Histochem. 81: 157-160, 1984).

68 Harmonin B: Cochlear Isoforms and Interactions with Cadherin 23 and F-Actin

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Harmonin b, a member of the Usher "interactome," has been called an actin-bundling or actin-scaffolding protein of hair cell stereocilia and is believed to associate with the upper tip link density. We examined the harmonin b isoforms in isolated mouse cochlear sensory epithelium by RT-PCR using a primer from the harmonin 3'-UTR and an upstream harmonin b-specific primer. The products were inserted into a vector and sequenced. The vast majority of the cDNA clones we analyzed (29 out of 31) corresponded to harmonin b1. Only 1 clone corresponded to harmonin b3 (GenBank NM_153677), and none corresponded to harmonin b4 (GenBank NM_001163733). Upon heterologous expression in LLC-PK1-CL4 epithelial cells (CL4 cells), GFP-harmonin b1 was not targeted to microvilli, but instead became concentrated in two other F-actin-rich structures: a belt-like accumulation at the lateral margin and a lacy cytoplasmic network. The lateral belt appeared to be an enlarged version of the F-actin-containing junctional belt normally present at this location, whereas the lacy cytoplasmic network had no obvious counterpart in cells not transfected with harmonin b1. Co-expression with a cadherin 23 (Cdh23) construct caused a large fraction of the harmonin b1 to become colocalized with Cdh23 in CL4 cell microvilli, reducing its tendency to form, and accumulate in, these other F-actin-rich structures. Deletion of the Cdh23 C-terminal PDZ-binding motif (PBM) did not reduce the level of association of harmonin b1 with CL4 cell microvilli. Our results suggest that b1 is the major harmonin b isoform in cochlear sensory epithelium, that it binds to the Cdh23 cytoplasmic tail in a PBM-independent fashion and that this binding to Cdh23 regulates harmonin b1's ability to cause the accumulation of F-actin structures in transfected cells.
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69 Do Regenerating Stereocilia Links Climb Toward the Tips of Stereocilia?

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One of the strongest arguments in favor of "tip link" theory of hair cell mechanotransduction was simultaneous disappearance of tip links and transduction in Ca^{2+}-free environment (Assad et al., 1991). Upon returning to normal extracellular medium, tip links regenerate concurrently with reappearance of hair cell mechanosensitivity (Zhao et al., 1996). It was hypothesized that nascent regenerating stereocilia links are formed around the base of the bundle and moved to the tips of stereocilia by myosin motor(s) (Zhao et al., 1996). Even though this hypothesis may be relevant to the regeneration of the tip links after acoustic overstimulation (Kurian et al, 2003), it has never been experimentally confirmed. Perhaps, this is because tip link regeneration was typically studied in very tight chicken hair bundles and it was not possible to follow nascent stereocilia links using scanning electron microscopy (SEM).

We investigated disruption and subsequent recovery of stereocilia links following application of Ca^{2+}-free medium with BAPTA in young postnatal mouse inner hair cells. In these cells, BAPTA treatment disrupts not only tip links but also all other immature side links between stereocilia. Therefore, it was possible to observe re-formation of stereocilia links "from scratch". Regenerating links re-appeared stochastically with no preferred stereocilia rank. Quantification of SEM images showed that distribution of the side links along the length of a stereocilium always peaked at 80-90% from the top at any time point of the recovery period of ~8 hours. Therefore, nascent side links either do not climb along a stereocilium or "jump up" so fast that it cannot be detected in sequential SEM "snapshots" taken ~30 minutes apart. Our preliminary data also show that regeneration of tip links may proceed slower than regeneration of side links, indicating that the different sets of stereocilia links may have different recovery dynamics.
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70 Membrane Components of the Avian Mechanosensitive Hair Bundle

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We recently described a new rapid, large-scale procedure for isolation of hair bundles (Morgan & Gillespie, 2008; Assoc. Res. Otolaryngol. Abs.: 506). This new methodology has allowed us to generate 18 new monoclonal antibodies to antigens present in hair-bundle membranes. We streamlined the procedure and are using it to identify the monoclonal antigens, focusing on three monoclonal antibodies: 1H3, which stains the tips of the stereocilia; 15G11, which stains the base of the actin core; and 2C3, which stains the bundle in discrete spots. These antigens are being identified by mass-spectrometry, and candidate genes are being cloned and expressed in Cos-7 cells to verify the antigen identity. Candidate proteins include AHNAK, LYRIC, members of the ERM family (ezrin/moesin/radixin), and thioredoxin domain containing proteins of the PDIA family. All three monoclonal antibodies were of the IgM subtype, and were useful for immunocytochemistry (effective at <0.1ug/ml), immunoblotting, and immunoprecipitation. We are now using this methodology to identify antigens for the remaining 15 monoclonal antibodies. We have also used the isolation procedure to identify protein complexes of hair-bundle membranes. For example, we found that the 2C3 antigen interacts with the lipid transfer protein NIR3, and the complex can be isolated intact from hair bundles. Using another monoclonal antibody, we have isolated a complex containing PCDH15, CDH23, and MYO1C, components of the transduction apparatus. This new isolation procedure thus permits biochemical characterization of minor membrane components of hair bundles, including the transduction complex.

71 Actin-Bundling Protein Fascin 2b Is a Constituent of Stereocilia in Zebrafish

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Extending from the apical surface of each hair cell is a single, actin-based hair bundle whose unique morphology is essential for mechanosensing in the ear. Ensembles of stereocilia that are graded in height form the hair bundle. The stereocilia are rigid rods that are mainly constituted by parallel actin bundles and associated proteins. In order to identify transcripts that encode proteins that localize to stereocilia, we scrutinized the hair-cell transcriptome of zebrafish; one promising candidate detected encodes fascin 2b, a protein that bundles filamentous actin in retinal tissue. We confirmed the presence of *fascin 2b* mRNA in adult hair cells and otocystic maculae using reverse transcription polymerase chain reactions and *in situ* hybridization studies with RNA probes, respectively. Immunolabeling of adult and larval hair cells demonstrated that fascin 2b mainly localizes to stereocilia. Furthermore, after the introduction of green fluorescent protein (GFP)-tagged fascin 2 fusion proteins into hair cells using transgenic methodologies, we observed that these chimeras localize to hair bundles. In order to determine if there is a relationship between fascin 2b and another stereociliary protein that has been shown to bundle actin, espin, we performed transfection experiments on non-auditory cells in culture. Previously, espin had been shown to induce the formation of parallel actin bundles in cultured cells, allowing for the formation of elongated microvillar-like protrusions. When espin and GFP-tagged fascin 2 proteins are present in cultured cells, the fusion proteins filled the espin-induced cellular protrusions. These studies offer the possibility that there is interplay between espin and fascin 2b proteins in the stereocilia of zebrafish hair cells that may affect the physical properties of these apical projections.

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72 Dynamic State and Compressive Nonlinearity of Coupled Hair Cells in the Frog Sacculus

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Active hair bundle mobility has been proposed as the cellular basis for amplification in auditory and vestibular organs of non-mammals. This has been extensively studied in the bullfrog sacculus, in which uncoupled hair cells exhibit spontaneous mechanical oscillations and a compressive nonlinearity that agrees with theoretical predictions. Using a high-speed CMOS camera we are able to record the motion of many hair bundles in parallel in an *in vitro* preparation of the bullfrog sacculus. Spontaneous mechanical oscillations are not observed when the hair bundles are coupled to the otolithic membrane implying that the cells are in a quiescent rather than oscillatory regime. We are exploring the compressive nonlinearity of arrays of cells under native coupling conditions.

73 Mechanical Loading of Spontaneously Oscillating Hair Cells from the Bullfrog Sacculus

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Hair cells are highly sensitive detectors and compressive non-linear amplifiers. The spontaneous oscillations of the ciliary bundles of the hair cells of the *in vitro* bullfrog sacculus constitute one of the effects of an internal amplification process and provide a way to study its properties. We demonstrate that an imposed mechanical load significantly affects spontaneous oscillations of hair cells as well as their sensitivity and tuning. We find that without any imposed load, hair bundles exhibit complex movements, with multiple periodicities/timescales. An imposed load induces increasingly metronomic behavior, selecting for a favored frequency. Further, we find that a load which just barely suppresses oscillations leads to an increased sensitivity in the responsiveness of the bundle.

74 Functional Analysis of Cochlear BK Channel-Associated Proteins Using RNA Interference

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Large conductance Ca^{2+}-activated K^+ (BK) channels play a prominent role in regulating several important physiological processes, such as neuronal excitability, signal transduction, smooth muscle contraction, metabolism, and immunity. Thus, these channels will likely partner with a number of different types of proteins. Thus far, we identified 174 BK-Associated Proteins (BKAPs) in mouse (Kathiresan et al., 2009) and more recently, 125 partners in chick, using BKα co-immunoprecipitation, two-dimensional gel electrophoresis, and shotgun proteomics analysis. Here, we selected 8 BKAPs involved in chaperonin, signal transduction and cell growth and development-related functions. These proteins were partially knocked-down by interfering with endogenous proteins using siRNA or up-regulated by over-expressing BKAPs, to determine BKα regulation in CHO cells. The chaperonins, including calrecticulin, GRP78, and HSP60, when knocked down 15 – 50% by siRNA, altered BKα expression from 25 – 30% in CHO cells. Other representative BKAPs involved in signal transduction in the cochlea included 14-3-3γ and annexin A5, which increased BKα expression by 25% – 30%, when down-regulated by ~30%. In contrast, BKα expression decreased 25% – 60% when both proteins were overexpressed by 15% – 30%, respectively. Silencing of development-regulated BKAPs like valosin-containing protein and lamin A/C by 40% and 60%, respectively decreased BKα expression by 40% and 50%. A potential node in these pathways is Akt1, because an ~20% down-regulation causes an ~15% upregulation of

BKα in CHO cells. Moreover, silencing 14-3-3γ and annexin A5 decreased Akt1 expression levels by ~19% – 23%, whereas over expression increased expression 10% – 22%. These data suggest further studies of BKα regulation through the serine/threonine kinase Akt pathway, since this protein lies downstream of various cellular stimuli, including growth factors.
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75 Transduction in the Mammalian Saccule

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The mammalian saccule is sensitive to low-frequency linear accelerations (<30 Hz) and loud sounds (500-1000 Hz). We hypothesize that frequency sensitivity varies with zone (striola vs. extrastriola) in the macula. Here we present preliminary results from striolar type I hair cells (SIs), which have large, broad bundles, and extrastriolar type IIs (ESIIs), with smaller, compact bundles. Whole-cell transduction currents were recorded from hair cells in the excised maculae of early postnatal rats (standard media, 25-37°C). Hair bundles were deflected with a rigid probe driven with sinusoidal (2-100 Hz) and step (400 ms, rise time ~1 ms) waveforms. For SIs, the mean peak-peak transduction current in response to 100-Hz sinusoids was 195±31 pA (SEM, n=6) and the operating range was 901±142 nm. Adaptation was evident in the high-pass filtering of sinusoids and the time course of decay during steps. Although in some cases two adaptation time courses could be distinguished, here we compare results from monoexponential fits to the data. For sinusoids, peak amplitudes rose with frequency, with a corner (fc) of 3±1 Hz, consistent with the time constant of decay during half-maximal steps (τ=44±4 ms). The extent of adaptation was 65±6% in 400 ms. Compared to SIs, ESIIs had larger operating ranges at 100 Hz: 1.5±0.1 µm (6), and faster adaptation: τ=28±2 ms (6); fc=9±2 Hz (5). Our preliminary adaptation data from rat saccular SIs and ESIIs resemble results from the mouse utricular and frog saccular hair cells, respectively (Vollrath & Eatock, J Neurophysiol 90:2676, 2003), which are sensitive to different frequency ranges. *Supported by NSBRI through NASA NCC 9-58.*

76 Slo Interacting Proteins and Electrical Tuning in the Chick

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In several non-mammalian species, including the chick and turtle, BK channels play a critical role in electrical tuning, a mechanism of frequency discrimination in the auditory epithelium. Hair cells in these animals, which are arranged in a tonotopic manner, have a continuously changing frequency of membrane potential oscillation along the tonotopic axis. The chief determinant of changing oscillation frequency in membrane potential along the tonotopic axis is the BK channel with its changing kinetic properties. There is a sharp correlation between a BK channel's deactivation time (measured as the exponential decay of K current following a step depolarization) and its tonotopic location.
A number of mechanisms have been proposed to underlie this change in the channels deactivation times. These include alternative splicing of the alpha subunit of the channel encoded by the Slo gene, and its association with other proteins. It is apparent that alternative splicing of Slo which generates innumerable splice variants, alone cannot explain the range of kinetics in native BK channels. Splice variants of Slo that have the largest to smallest inserts, in both the chick and turtle, are unable to produce the wide range in kinetics of native channels. To investigate the role of BK channel associated proteins we isolated 11 individual proteins that have been shown to interact with Slo (Ankra, Cerebelon, Caveolin, Cotarctin, Beta subunits 1, 2 and 4, syntaxin1a, Rack1, CDK5, and beta catenin). We determined the tonotopic distribution of these channels using quantitative PCR, and determined their effects on the kinetics of the channel using an oocyte expression system. We show here that these proteins show a widely varying distribution along the tonotopic axis. We also show that these proteins have complex effects on the kinetics of the channel. These data will require a rethinking as to how electrical tuning is brought about.
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77 Outer Hair Cell Receptor Currents and Potentials with Bundles Exposed to Endolymph

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Measurements of mechanotransduction (MT) in outer hair cells (OHC) are usually performed in an isolated cochlear coil with hair bundles bathed in a high sodium and calcium saline resembling perilymph. We have measured OHC receptor currents and potentials in apical turns of rat cochleas with the solution around the bundle changed from perilymph to endolymph (composition in mM: 152 KCl; 1 NaCl; 10 KHEPES, 0.02 CaCl2 buffered with 4 HEDTA, 8 glucose) using a closely positioned puffer pipette. MT currents in endolymph were two-fold larger than in perilymph and were further augmented, with amplitudes up to 4 nA, by addition of an 80 mV endolymphatic potential (EP). MT currents still displayed sub-millisecond fast adaptation (time constant = 0.4 – 0.8 ms). Receptor potentials in response to hair bundle deflection were slowed by the membrane time constant but had amplitudes that fully utilized the electrical driving force: up to 55 mV at a resting potential of -60 mV and 130 mV on adding an 80 mV EP. Receptor potential amplitudes without EP are similar to ones recorded in turtle cochlear hair cells (Crawford & Fettiplace, 1980) but substantially larger than those reported for OHCs in vivo. Using BAPTA rather than EGTA as the intracellular calcium buffer increased the fraction of MT current activated at rest to about 30 per cent and depolarized the resting potential. The mean resting potential in apical OHCs in hearing animals (P15 – P20) was 43 +/- 8 mV with 1 mM intracellular BAPTA and -69 +/- 5 mV with 1 mM EGTA. We suggest the fraction of MT

channels open at rest with bundles exposed to endolymph generates a standing current that depolarizes the resting potential. Our results provide a yardstick for comparison within vivo recordings. Supported by Grant RO1 DC01362.

78 Changes of Mechano-Electrical Transduction After Intense Mechanical Stimulation: A First Event in Noise-Induced Hearing Loss?

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Damage to the stereocilia bundle produced by acoustic overstimulation does not appear to be reversible and thus contribute to the permanent threshold shift (Wang et al., 2002). *In vitro*, the hair bundles become more compliant after intense stimulation with fluid-jet, with stiffness recovery observed in fifteen minutes (Adler et al., 1992; Szymko et al., 1995). It was proposed (Duncan and Saunders, 2000) that the compliance changes following *in vitro* overstimulation are due to damage to the actin filaments at the taper of stereocilia (Tilney et al., 1982) rather than tip link destruction. However, after *in vivo* exposure to damaging noise both cytoskeletal and tip link injuries were observed in chick hair cells (Duncan and Saunders, 2000). Although both of these injuries are expected to affect mechano-electrical transduction (MET), the overstimulation-induced changes of the MET responses have never been thoroughly studied.

Here we used a fluid-jet and a piezo-driven rigid probe stimulation to study how the intense hair bundle deflections affect MET responses in cochlear hair cells of young postnatal mice. We found that intense deflections can result in a temporal but fairly sustained increase of the resting current through MET channels. Surprisingly, this increase was larger when the bundle had been overstimulated in negative direction. Large positive deflections produced smaller changes of the resting MET current. Overstimulation-induced changes of the resting MET current could be due to either the development of the sustained abnormal forces within a damaged hair bundle or the damage to the MET apparatus itself. Irrespective of the nature of this sustained MET current, it may initiate a chain of events leading to cell death.

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79 Characterization of Zebrafish *trpn1* Mutants

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Several members of the TRP ion channel family have been implicated in hair cell function, either as putative mechanotransduction channels or in other roles such as osmolarity regulation (reviewed in Daman et al. Curr Biol 18, 2008). We previously identified the zebrafish ortholog of *nompc*, named *trpn1*. Disrupting *trpn1* function with morpholino oligonucleotides caused deafness and balance defects as well as defects in MET channel gating (measured as a reduction in microphonic currents or uptake of the vital dye FM1-43 through the mechanotransduction channel) (Sidi et al. Science 301, 2003).

In order to further examine the function of Trpn1 in hair cells, we are currently characterizing two *trpn1* mutant zebrafish lines isolated by TILLING of ENU-mutagenized fish. Unlike morpholino disruption of *trpn1*, neither of these mutations cause obvious deafness or balance defects. Both mutations introduce a stop codon, either following the N-terminal twenty-nine ankyrin repeats (Q1176X) or between the fourth and fifth transmembrane domain (W1358X). To confirm that these mutations lead to truncated proteins, antibodies to Trpn1 epitopes either 5' or 3' to the point mutations are being characterized. Immunolocalization of full length and truncated Trpn1 will also give insight into its function, both in hair cells and in other cell types expressing Trpn1. To further characterize the mutant lines, we are recording hair cell and afferent neuron responses in intact zebrafish larvae by mechanically deflecting the hair bundles of lateral line neuromasts. This *in vivo* preparation allows exploration of the effect of these mutations on the physiological response properties of the hair cell and the first order afferent neuron. Measuring microphonic potentials, afferent neuron responses, FM1-43 dye uptake, and calcium transients in siblings compared to homozygous mutants will determine which aspects of hair cell function are affected by genetic disruption of *trpn1*.

80 HCN Channels Are Not Required for Mechanotransduction in Sensory Hair Cells of the Mouse Inner Ear

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The molecular composition of the hair cell transduction channel has not been identified. Here we explore the novel hypothesis that hair cell transduction channels include HCN subunits. The HCN family of ion channels includes four members, HCN1-4. They were originally identified as the molecular correlates of the hyperpolarization-activated, cyclic nucleotide gated ion channels that carry currents known as If, IQ or Ih. Based on recent evidence it has been suggested that HCN subunits may also be components of the elusive hair cell transduction channel. To investigate this hypothesis we examined expression of mRNA that encodes HCN1-4 in sensory epithelia of the mouse inner ear, immunolocalization of HCN subunits 1, 2 and 4, uptake of the transduction channel permeable dye, FM1-43 and electrophysiological measurement of mechanotransduction current. Dye uptake and transduction current were assayed in cochlear and vestibular hair cells of wildtype mice exposed to HCN channel blockers or a dominant-negative form of HCN2 that contained a pore mutation and in mutant mice that lacked HCN1, HCN2 or both. We found robust expression of HCNs 1, 2 and 4 but little evidence that localized HCN subunits in hair bundles, the site of mechanotransduction. Although high concentrations of the HCN antagonist, ZD7288, blocked

50-70% of the transduction current, we found no reduction of transduction current in either cochlear or vestibular hair cells of HCN1- or HCN2- deficient mice. Furthermore, mice that lacked both HCN1 and HCN2 also had normal transduction currents. Lastly, we found that mice exposed the dominant-negative mutant form of HCN2 had normal transduction currents as well. Taken together, the evidence suggests that HCN subunits are not required for mechanotransduction in hair cells of the mouse inner ear.

81 The Outer Hair Cell Potassium Current $I_{K,n}$ Requires PIP_2 and Is Inhibited by Poly-D-Lysine and Neomycin

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The electrical behaviour of outer hair cells (OHC) is mainly determined by the voltage-dependent K^+ current, $I_{K,n}$ (Housley and Ashmore, 1992) that is mediated by the voltage-dependent K^+ channel KCNQ4 (Kv7.4) (Kharkovets et al., 2000; Kharkovets et al., 2006). Highlighting its essential role for OHC function, mutations in KCNQ4 lead to degeneration of OHCs and thereby cause progressive human hereditary deafness, DFNA2 (Kubisch et al., 1999; Nie, 2008). Activity of all KCNQ (Kv7) channels strongly depends on the membrane phospholipide phosphatidylinositol-4,5-bisphosphate (PIP_2) (Suh and Hille, 2002). Since alterations in phosphoinositide homeostasis have been implicated in OHC degeneration, we investigated a possible PIP_2 dependence of $I_{K,n}$.

Plasma membrane PIP_2 levels were experimentally changed by intracellular application via a patch pipette of PIP_2-binding polycations in OHCs while current was monitored by voltage clamp. For comparison, similar experiments were performed on CHO cells heterologously expressing KCNQ4 channels.

Intracellular application of poly-D-lysine (200 µg/ml), known to chelate phosphoinositides through electrostatic interactions, inhibited $I_{K,n}$ in OHCs and recombinant KCNQ4 channels. Polycationic aminoglycoside antibiotics, such as neomycin, have also previously been used to functionally deplete PIP_2. We found that neomycin blocked $I_{K,n}$ (IC_{50}=712 µM) and recombinant KCNQ4 (IC_{50}=225 µM) in a dose-dependent manner. Strong binding of neomycin to PIP_2 at these concentrations was confirmed by observing displacement of the GFP-tagged, genetically encoded PIP_2 binding domain PLCδ1-PH from the membrane upon introduction of the aminoglycoside.

We conclude that $I_{K,n}$ activity requires membrane PIP_2 and that this dependence may play a role in aminoglycoside ototoxicity.

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82 Cyclic Nucleotide-Gated (CNG) Ion Channels in Saccular Hair Cells

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Cyclic nucleotide-gated (CNG) channels are cation-selective channels that open and close in response to cAMP/cGMP. CNG channels mediate sensory transduction in vision, olfaction and taste. Previously, we demonstrated that mRNA is expressed in rat cochlear hair cells/organ of Corti for olfactory subunits CNGA2, CNGA4 and CNGB1b, and a CNGA3 subunit. CNGA2 and CNGA4 were immunolocalized to stereocilia (Drescher et al., Mol. Brain Res. 98: 1-14, 2002). CNGA2, together with CNGA4, code for a cAMP-gated CNG channel, whereas CNGA3 codes for a cGMP-preferring channel. Here, we report full-length sequence for three CNGA3 subunit messages that are expressed, along with CNGA2, in hair cells isolated from the trout saccule, obtained by RT-PCR with degenerate primers and cloning. The primary full-length sequence for saccular hair cell CNGA3a1 and CNGA3a2 exhibited 86% and 82% identity, respectively, to trout pineal photoreceptor CNGA3 and 76% and 75% identity to zebrafish CNGA3. CNGA3b displayed 95% identity to trout pineal photoreceptor CNGA3 and 73% identity to zebrafish CNGA3 sequence. Overall, the trout saccular hair cell CNGA3 sequence is closest to mammalian cone photoreceptor CNGA3, as opposed to olfactory/gustatory CNGA3. A custom antibody targeting the cone-specific amino terminus sequence has immunolocalized CNGA3 to stereocilia of the saccular hair cells. Protein-protein binding studies with yeast-two hybrid mating and co-transformation protocols indicate specific binding of the cytoplasmic carboxy terminus of trout hair cell CNGA3b to the carboxyl terminal C1q domain of EMILIN1, elastin microfibril interfacer 1 (77% identity to EMILIN1a, Danio rerio Accession No. NP_001025378), and a protein with homology to a RhoGTPase-activating protein. Positive results have also been obtained with co-transformation protocols for rat CNGA3 and EMILIN1. EMILIN1 is an extracellular matrix protein, a member of the elastic fiber system which interacts with integrins.

83 Exploring the Electrical Resonance's Affect on the Mechanical Oscillations of Hair Cells in the Bullfrog Sacculus

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Under *in vitro* conditions, uncoupled hair bundles of the bullfrog (*Rana catesbeiana*) sacculus have been shown to exhibit spontaneous oscillations. We used a high-speed complementary metal oxide semiconductor camera to track the movements of hundreds of cells in parallel from dozens of preparations. This work revealed that the spontaneous oscillations exhibit multiple timescales with a slow modulation on a rapid oscillation. Experiments inhibiting the electrical resonance in the cell body show a strong effect on the mechanical oscillations of the hair bundles. This indicates that the electrical oscillation is

coupled with the mechanical oscillations of the hair bundles.

84 Trans-Epithelial Electrical Stimulus on Coupled Hair Cells in Bullfrog Sacculus

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Ca2+ constitutes a feedback element that affects both fast and slow adaptation of hair cells' spontaneous oscillations. Application of trans-epithelial voltage modulates the entry of Ca2+ into the stereocilia. We applied electrical stimulation under two in vitro conditions. First, voltage offsets were used to modulate the frequency of spontaneous oscillation in decoupled saccular hair bundles. The effect of voltage was compared to that of pharmacological blockers of somatic channels, and both were shown to significantly affect both the frequency and amplitude of spontaneous oscillations. Secondly, sinusoidal currents were used to induce phase-locked oscillations in arrays of coupled cells. With the otolithic membrane left partially attached to the epithelium so as to couple bundles of comparable orientations, the phase-locked response was shown to evoke movements in the overlying membrane.

85 Osmotic Properties of Auditory Hair Cells in the Leopard Frog: Evidence for Water-Permeable Channels

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When amphibian papillar hair cells (APHCs) of the leopard frog, *Rana pipiens pipiens*, are osmotically challenged, they exhibit a characteristically asymmetric (rectifying) response: A small decrease (up to 10%) in the extracellular solution's osmolarity does not affect the cells' volume; larger decreases produce a slow, and sometimes delayed, volume increase in APHCs, while exposure to a hyperosmotic medium leads to rapid shrinking of these cells. In 17 APHCs of different sizes, the time constant of a single-exponential curve fitted to the volume increase time course (τ = 236 $\pm$ 50 s; mean $\pm$ std. err.), was 4.2 $\pm$ 0.8 times of that fitted to the volume decrease time course (62.6 $\pm$ 4.7 s). In these experiments in which osmotic challenge was applied to cells through bath perfusion, the rate of volume decrease appeared to be limited by the rate of perfusion (τ = 65.7 $\pm$ 7.5 s, n = 11; measured with the fluorescent dye 2',7'-dichlorofluorescein). Conversely, the rather slower volume increase was not perfusion-limited, and may denote these cells' innate adaptability to small decreases in the extracellular osmolarity.When hyperosmotic solutions were directly injected into the recording chamber (τ = 5.9 $\pm$ 0.8 s, n = 6, for 2 μl of 1.5 M sucrose), the volume decrease had a time constant of 8.4 $\pm$ 1.6 s (n = 8; range, 4.4 – 17.4 s). From this latter group of experiments, we estimated the osmotic permeability coefficient (P_f) for APHCs to be 4.7 $\pm$ 1.1 x 10^{-2} cm/s, suggesting that these cells express water-permeable channels. The estimated P_f appears to be cell-size independent, and TEA-, acetazolamide- and mercury-insensitive.

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86 Characterizing the Inner Face of the Mechanotransducer Channel

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Hair cell mechanotransducer channels are nonspecific cation channel with a high permeability to calcium that shows no voltage dependence and limited inward rectification. Experiments probing the external face of the channel suggest an external vestibule that is quite large and a pore diameter that can pass large molecules like, FM1-43. That permeable blockers can pass through the channel when presented from the external but not the internal face is substantiated and further investigated. Whole cell voltage-clamp experiments were performed on hair cells in the intact turtle auditory papilla. A stiff probe attached to a piezo electric stack was used to stimulate the hair bundle. Drugs including FM1-43, curare and methylene blue were ineffective at blocking the mechanotransducer channel from the inside. One possibility is that drug access is limited, however two photon imaging of fluorescently tagged dextrans suggest no diffusion barrier. Alternatively, the pore size may be different at positive potentials. Amine substitution experiments estimate the pore size as slightly larger than half that estimated from the external face of the channel. One possibility for this unusual result is more dehydration of ions from the external face than from the internal face, resulting in an apparent change in pore size. However comparisons of cations with different hydration energies (Li, Cs, Na, K) resulted in similar sensitivities whether at the internal or external face of the channel. Another possibility is that calcium permeation interferes with pore size estimates differently from inside and out. Comparisons of amine permeation when equally present internally and externally revealed the unusual finding that lowering external calcium did not shift the activation curve leftward (as expected for adaptation) and also did not result in an increase in MET current amplitude. Data suggest calcium binding within the channel pore alters pore diameter.

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87 Hearing Requires Otoferlin-Dependent Synaptic Vesicle Replenishment in Inner Hair Cells

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Transmitter release at the hair cell ribbon synapses is fast and sustained. To avoid auditory fatigue vesicle replenishment has to efficiently follow consumption (i.e. synaptic release). Here we show that a multi C_2-domain protein otoferlin is indispensable for that process. We studied deaf *pachanga* mouse mutants, where one aspartate of the C_2F domain of otoferlin is exchanged by glycine. This mutation causes subtle structural changes and the reduction in the otoferlin protein level in inner hair cells (IHCs). As revealed by confocal calcium imaging and patch-clamp recordings, calcium signaling and the fast exocytotic transmitter release in IHCs of *pachanga* mice *in vitro* are not affected. Further, postsynaptic excitatory currents and action potentials are observed. Sustained component of exocytotic release and the re-supply of vesicles into the readily releasable pool (RRP) however are severely impaired. The number of ribbon-containing synapses is only slightly decreased while the number of docked vesicles is not affected. Spontaneous and sound-evoked spiking of the auditory neurons is sparse; however the evoked spiking rate improves after longer periods of silence. We conclude that in *pachanga* mutants vesicle replenishment is slow and therefore precludes the build-up of a standing RRP of vesicles, required for continuous transmitter release and normal hearing. In the process of vesicle re-supply to the ribbon synapse, otoferlin plays a crucial role and we propose its involvement in an early step of exocytosis.

88 Structure of Otoferlin C2A and Biochemical Analysis of Otoferlin C2 Domains

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Otoferlin is expressed in auditory hair cells, and presumably targeted to synaptic vesicles. It contains 6 or 7 C_2 domains. These domains are widely known to bind phospholipids in a Ca^{2+}-dependent manner. In this study, we present the crystal structure of the most N-terminal C_2 domain (C_2A) with 1.95 angstrom resolution. The structure reveals a full C_2 domain with 8 antiparallel beta strands. Further, the surface of the molecule exhibits positive charges in the putative Ca^{2+}-binding region, suggesting that the C_2A domain is not able to coordinate Ca^{2+} ions. This was experimentally confirmed with spectroscopic methods.

Further, we examined biochemical properties of the C-terminal C_2 domain (C_2F), which bears a switch from an aspartate to a glycine in the deaf *pachanga* mouse line that originated from a random mutagenesis screen.

Using circular dichorism (CD) spectroscopy of the isolated C_2F domain, we found a slight but significant structural alteration caused by this mutation. As aspartates usually coordinate Ca^{2+} ions in the Ca^{2+} binding loops of C_2 domains we investigated the Ca^{2+} binding of both variants of the C_2F domain by CD spectroscopy and fluorimetry. Both methods indicate that the C_2F domain does not bind Ca^{2+}. Next, we analysed phospholipid binding and found that this C_2 domain does not bind phospholipids, neither in presence nor in absence of Ca^{2+} or PIP_2. Further, the aspartate to a glycine mutation did not alter the Ca^{2+} or phospholipid binding properties of the C_2F domain. However, the mutation led to lower protein levels and a slightly different sub-cellular distribution of Otoferlin in auditory hair cells.

In summary, our data indicate that neither the C_2A, nor the C_2F domain bind Ca^{2+}. Although the C_2F domain seems to be important for proper function of otoferlin, the exact role of both domains remains to be investigated.

89 Synaptotagmin-1 Cannot Functionally Replace Otoferlin, and Vice Versa

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Otoferlin, a multi C_2 domain protein shown to be essential for a late step in exocytosis of auditory hair cells, is currently discussed to function as a synaptotagmin-like Ca^{2+} sensor for vesicle fusion. This hypothesis is based on (i) otoferlin having 6 or 7 C_2 domains, of which 3 are predicted to bind Ca^{2+}, (ii) the absence of synaptotagmin 1, 2 and 3 at the first auditory synapse (Safieddine and Wenthold, 1999), (iii) the absence of fast vesicle release in $Otof^{-/-}$ hair cells (Roux et al, 2006) and (iv) the interaction of otoferlin with syntaxin 1 and SNAP-25 in immunoprecipitation assays.

In this study, we tested the functional equivalence of synaptotagmin-1 (Syt1) and otoferlin by transducing hair cells of organotypic cultures with an adeno-associated virus containing Syt1 cDNA. In contrast to wild type inner hair cells, Syt1-transduced hair cells of $Otof^{-/-}$ mice did not exhibit measurable exocytosis in patch-clamp capacitance measurements. Thus, Syt1 could not restore Ca^{2+}-triggered vesicle fusion in Otof-deficient auditory hair cells. Next, we transfected the developing otocysts of $Otof^{-/-}$ embryos at E12.5 with Syt1 and measured hearing by auditory brainstem response recordings in 4-week-old

animals. We could not detect auditory brainstem responses in either non-transfected or Syt-1 mis-expressing ears, which showed a transfection rate of inner hair cells of approximately 50%. Furthermore, we analyzed exocytosis in autaptic cultures of Syt1-deficient hippocampal neurons, but found no rescue of synchronous vesicle release when overexpressing otoferlin.
Together, this study suggests that the mechanism of otoferlin function is different from syt action.

90 Candidate Function of Otoferlin

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One of the genes underlying hearing impairment in mice and humans is the sequence coding for otoferlin. Mutations within OTOF lead to a recessive disorder called DFNB9. Several studies have indicated otoferlin's association with ribbon synapses of cochlear sensory hair cells, as well as data showing the protein's presence in neurons, nerve fibers and hair cells, suggesting a more ubiquitous function. We recently notified Otoferlin's absence despite exocytosis in hypothyroid animals questioning other Ca2+-sensing proteins to be upregulated under hypothyroid conditions. Molecular studies were therefore performed to identify possible candidates substituting for the supposed Ca2+-sensor function of otoferlin under hypothyroidism. On the other side search for otoferlin binding partner may also help to clarify otoferlin`s identity as Ca2+-sensor under hypothyroid or normal conditions. Using yeast two-hybrid screen and mass spectroscopy Otoferlin interaction partners were identified and it interaction verified upon co-expression, co-localization and co-immunoprecipitation. Results will be discussed in the context of the undoubtful essential role of otoferlin for exocytosis of hair cells.
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91 Direct Interaction of Phosphatidylinositol 4,5-Bisphosphate with Otoferlin C2F Domain

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Otoferlin is thought to be a calcium sensor in auditory hair cells and is absolutely required for evoked synaptic transmission in hair cells. Our past studies involving otoferlin molecular interactions have shown that otoferlin interacts with the SNARE proteins syntaxin 1A and SNAP-25 via the C2F domain, in a calcium-dependent manner and with strong binding affinity. SNAP-25 and syntaxin 1A are expressed in the plasma membrane near the ribbon synapse. We have also shown that otoferlin is coupled to the $Ca_v1.3$ calcium channel via the otoferlin C2D domain. Yet, the molecular events involving otoferlin in the exocytotic process are not clearly understood. In the current study, we show that the C2F domain of otoferlin interacts specifically with phosphatidylinositol 4,5-bisphosphate (PIP_2), with the amount of the lipid binding almost doubled in the presence of free calcium. In contrast, other phospholipids, such as phosphatidylinositol, phosphatidylserine, and phosphatidylcholine, manifest only minimal or no interaction with C2F, and calcium has no effect on the binding. Several studies point to the role of PIP_2 in exocytosis by calcium-dependent interaction with synaptotagmin-1 C2 domains, and PIP_2 has been localized to the basolateral regions of the hair-cell plasma membrane as well as the stereocilia, indicating a strict plasma-membrane distribution of this important phospholipid. Exocytosis requires the fusion of both vesicle and plasma membrane, and otoferlin is thought to be a vesicle protein that can interact with plasma membrane proteins. Thus, the phospholipid-binding properties of otoferlin suggest its role in membrane fusion in hair cells. Viewed in this light, our current results show important features of otoferlin C2 domains that enable them to engage in molecular interactions, leading to exocytosis. Present studies reveal the residues involved in these interactions, as well as the secondary structural changes induced by PIP_2.

92 A Presynaptic Role for Harmonin in Regulating $Ca_v1.3$ Channels in Mouse Inner Hair Cells

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Presynaptic $Ca_v1.3$ Ca^{2+} channels regulate transmission of sound information by controlling glutamate release from cochlear inner hair cells (IHCs). Harmonin is the protein product of the USH1C locus for Usher syndrome, which causes combined deafness and blindness in humans. Here, we show that $Ca_v1.3$ channels interact with harmonin and that this interaction is disrupted by a genetic alteration causing the Usher syndrome phenotype in "deaf circler" (*dfcr*) mice. In addition to its localization in hair bundles, harmonin is present at the ribbon synapse where $Ca_v1.3$ Ca^{2+} channels cluster in IHCs. Harmonin, but not the *dfcr* mutant, binds to the $Ca_v1.3$ $\alpha 1$ subunit and enhances voltage-dependent facilitation of $Ca_v1.3$ in HEK293T cells. In *dfcr* IHCs, $Ca_v1.3$ currents show weaker voltage-dependent facilitation and impaired exocytosis of the readily releasable pool than in control IHCs. Collectively, our results support a novel role for harmonin in regulating $Ca_v1.3$ Ca^{2+} channels and afferent synaptic transmission in IHCs.

93 Myosin VI Is Required for the Proper Maturation and Function of Inner Hair Cell Ribbon Synapses

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The ribbon synapses of the auditory inner hair cells (IHCs) undergo morphological and electrophysiological transitions during cochlear development. Here we report that myosin VI, an actin-based motor protein involved in genetic forms of deafness, is necessary for some of these changes to occur. By using post embedding immunogold electron microscopy, we showed that myosin VI is present at the IHC synaptic active zone. In Snell's waltzer mutant mice, which lack myosin VI, IHC ionic currents and ribbon synapse maturation proceeded normally until at least postnatal day six. In adult mutant mice, however, the IHCs displayed immature potassium currents and still fired action potentials, as normally only do immature IHCs. In addition, the number of ribbons per IHC was reduced by 30%, and 30% of the remaining ribbons were morphologically immature. Ca2+-dependent exocytosis probed by capacitance measurement was markedly reduced despite normal Ca2+ currents and the large proportion of morphologically mature synapses, which suggests additional defects, such as loose Ca2+-exocytosis coupling or inefficient vesicular supply. Finally, we provide evidence that myosin VI and otoferlin, a putative Ca2+ sensor of synaptic exocytosis also involved in a genetic form of deafness, interact at the IHC ribbon synapse, and we suggest that this interaction is involved in the recycling of synaptic vesicles. Our findings thus uncover essential roles for myosin VI at the IHC ribbon synapse, in addition to that proposed in membrane turnover and anchoring at the apical surface of the hair cells.

94 Calcium Store Agonists in Cochlear Hair Cells Enhance Transmitter Release from Efferent Terminals

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Prior to hearing onset, inner hair cells (IHCs) in the mammalian cochlea are inhibited by efferent synaptic currents (IPSCs) resulting from the sequential activation of α9/α10-containing receptors and small conductance calcium activated (SK) potassium channels, perhaps augmented by calcium release from the hair cell's postsynaptic cistern. To probe the role of cytoplasmic stores in hair cell inhibition, we infused cyclic ADP ribose (cADPR) to release calcium through ryanodine-sensitive channels. Membrane-impermeant cADPR was applied through the whole-cell patch pipette on IHCs in excised apical turns of cochleas from young (P7-9) rats. cADPR increased the amplitude and duration of the SK component of spontaneous and electrically-evoked IPSCs, consistent with enhanced release from a posysynaptic calcium store. Remarkably, post-synaptically-applied cADPR (100 μM) also increased the probability of evoked transmitter release during electrical stimulation protocols. The control quantum content of ~ 0.5 at 1 Hz (n = 12 cells) increased to ~ 2.0 with cADPR in the postsynaptic cell (n = 9). This result implies the existence of a retrograde signal from hair cell to efferent terminal. Since hair cells release glutamate, cADPR could be raising calcium to increase release of this potential actor on the efferent terminal. However, a panel of mGluR antagonists (LY367385 100 μM, E4CPG 500 μM, or LY341495, 200 μM) had no effect on the cADPR-induced increase in efferent release probability; nor did a cocktail of ionotropic and metabotropic glutamate receptor antagonists (AP-5 50 μM, MCPG 500 μM, and CNQX 50 μM). Finally, cADPR effectively raised efferent release probability onto IHCs of the VGlut3 KO mouse that have no vesicular release of glutamate (Seal *et al*., 2008, Neuron 24:263). A retrograde signal other than glutamate must act on to increase transmitter release from the efferent terminal. Supported by R01 DC001508 and P30 DC005211 from the NIDCD .

95 Properties of the Olivocochlear-Outer Hair Cell Synapse in the Mouse Cochlea

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In the adult cochlea, the function of outer hair cells (OHCs) is modulated by efferent cholinergic olivocochlear (OC) fibers projecting from the central nervous system. It has been previously shown that the firing rate of OC fibers varies according to the type of sound stimulation (noise, tones, monoaural, binaural, etc.) and that it increases with sound intensity (*Brown et al., 1998*). These changes in firing rates would presumably cause different strengths in the feedback effect exerted by the OC system.

In the present work we used the technique developed by *Goutman et al.* (2005) to study the properties of the OC synapse onto OHCs. Briefly, synaptic activity was recorded in voltage-clamped OHCs from an excised apical turn of the mouse cochlea (10-12 postnatal days) during stimulation of OC fibers with a bipolar electrode placed in the modiolar region. Activation of efferent terminals by single shocks evoked inhibitory postsynaptic currents (IPSCs) with a very low rate of success (quantal content: 0.14 ± 0.03, n = 30 cells). Paired-pulse protocols showed that this synapse facilitates with maximum efficacy at pulse intervals of 10 ms (facilitation index = 2.1 ± 0.4; n = 8). Accordingly, trains of stimuli at different frequencies (10-100 Hz) produced increasing levels of transmitter release. This phenomenon, together with summation of synaptic currents, resulted in an increase of OHC responses proportional to the stimulus frequency (normalized IPSC amplitude $I_{max}/I_{single\text{-}shock}$: 5.4 ± 1.0; 8.5 ± 3.7; 12.3 ± 0.8; 15.6 ± 1.0 for 25, 50, 60 and 80 Hz, respectively; n = 2-4). These results show that this synapse can facilitate at

intervals that correlate with the physiological frequencies at which OC fibers fire. This property could be relevant for encoding different degrees of OC fiber activity in response to variable sound stimulation.

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96 ACh Release at the Efferent-IHC Synapse Is Modulated by Presynaptic $GABA_B$ Receptors

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During development, before the onset of hearing, inner hair cells (IHCs) of the mammalian cochlea are transiently innervated by medial olivocochlear (MOC) efferent fibers. Although acetylcholine (ACh) is the main neurotransmitter released at this synapse, there is evidence showing that γ-aminobutiric acid (GABA) is also present at MOC synaptic terminals. The possibility that synaptically released GABA could modulate the cholinergic input at MOC-synapses by acting on presynaptic $GABA_B$ receptors has not been investigated yet. We have previously shown that transmitter release at this synapse is supported by both P/Q and N-type calcium channels (San Martin et al., ARO Abstracts 2008). In this work, we evaluated the effects of compounds selective for $GABA_B$ receptors on the quantal content of transmitter release at the MOC-IHC synapse. Postsynaptic currents, evoked by electrically stimulating the efferent fibers, were recorded in voltage-clamped (-90 mV) IHCs from acutely isolated mouse organs of Corti at postnatal days 9 to 11. The quantal content of evoked release was significantly increased by the $GABA_B$ antagonist CGP35348 at 1μM (55 ± 19 % $p < 0.05$) and significantly decreased by 1μM of the agonist baclofen (68 ± 8 % $p < 0.001$). Our results suggest that GABA might be exerting a negative feedback control on the release of ACh through presynaptic $GABA_B$ receptors at MOC terminals. We are currently evaluating whether this effect is through the modulation of either P/Q and/or N-type calcium channels.

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97 Synapse Quantification in Rodent Utricular Hair Cells

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As reported previously, we measured exocytosis from young (P0-P6) rat utricular hair cells using cell membrane capacitance measurements and found a fast phase of release with a time constant of ~80ms and an amplitude of 5 fF, corresponding to 135 vesicles released per hair cell. In order to compare these numbers to exocytosis in other hair cells, it is important to know the number of release sites per utricular hair cell. In mammalian auditory hair cells immunohistochemical techniques have proved useful in quantifying the number of active zones, but there are few comparable reports for vestibular hair cells. To count synaptic sites in utricular hair cells, we used a well-established antibody against the transcription factor C-terminal binding protein 2 (CtBP2) which labels hair cell nuclei as well as synaptic bodies. To determine whether non-nuclear CtBP2-positive structures correspond to synaptic sites, we double-labeled utricles with antibodies against various synapse-associated proteins including the postsynaptic AMPA receptor subunit GluR2/3, the postsynaptic density protein PSD-95, the postsynaptic scaffolding protein Shank1a and the presynaptic calcium channel $Ca_V1.3$. 3-D reconstructions of stacks of confocal sections were analyzed with the goal of finding a strategy that allows for semi-automated quantification of synapse numbers per hair cell. We find that an antibody against Shank1a gives the cleanest co-localization signal and the most reproducible number for synapses per utricular hair cell (striolar region: 13 ± 3, n = 16 utricles; extrastriolar region: 12 ± 3, n = 10 utricles). These data thus suggest that the fast exocytosis we measured was due to the release of 11 vesicles per synaptic site. Contrary to results in cochlear hair cells, the antibody to GluR2/3 did not reproducibly colocalize with anti-CtBP2 labeling, suggesting a broader repertoire of AMPA-receptor subunits in Scarpa's ganglion dendrites than in spiral ganglion dendrites.

98 Micromechanical Compressive Nonlinearity and Efferent Control in the Semicircular Canals

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The remarkable sensitivity of the mammalian cochlea is achieved largely through active mechanical amplification of sound stimuli by outer hair cells. Amplification of quiet sounds results in nonlinear compression of a wide dynamic range of acoustic signals into a smaller range of micromechanical responses. Cochlear amplification and compression are controlled by the brain through centripetal efferent projections that, when activated, reduce the gain of the amplifier and sharply attenuate the vibration of the cochlear partition. This strategy is essential to the exquisite sense of hearing in mammals. In the present study we demonstrate that a similar active process is present in the semicircular canals. We measured micromechanical motions of the semicircular canal cupula overlying hair bundles as a function of stimulus level and discovered that low-level stimuli were amplified resulting in larger cupula motions than would occur in a passive system. The amplification was eliminated by electrical activation of the efferent system via bi-polar electrodes located in the brainstem vestibular nucleus. Sensitivity to efferent activation demonstrates the hair cell origin of amplification. Vestibular hair cells lack the protein prestin and there is currently no evidence supporting cycle-by-cycle somatic motility in these cells. Present data support the hypothesis that active hair bundle motility is responsible for micromechanical amplification in the

semicircular canals, and that the hair-bundle motor is actively controlled through efferent synaptic contacts on hair cells. Recordings from hair cells showed that efferent activation opened a large basolateral conductance thus shunting the transduction current to ground and reducing the receptor potential modulation. The transduction current was not attenuated by efferent activation. These data indicate that a voltage sensitive process underlies hair bundle amplification in the semicircular canals. [Supported by NIDCD R01 DC006685]

99 Multiple Kinetic Components of Release at the Hair Cell Afferent Fiber Synapse Implicate Calcium-Dependent Vesicle Trafficking

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Ribbon synapses are specialized to respond to graded changes in receptor potential with varying levels of vesicle release. These synapses tend to show linear release with calcium, to be capable of long term release with little fatigue and to have quite rapid release. The molecular mechanisms underlying the operation of ribbon synapses remain a mystery. Direct investigation of the kinetics of release have been hampered by the capacitance method used to study them, only being valid if conductance does not change, leaving kinetics to be indirectly evaluated as before and after measurements from repetitive stimulation. Here we investigate release using a multi-sine wave approach that we design to overcome this limitation. Three distinct capacitive components were measured. The first was a rapid change insensitive to calcium and eliminated by increasing the frequency of the interrogating sine waves. This component was not a function of synaptic release. The second component changed rates linearly with calcium and had a size that varied with frequency location of the hair cell along the epithelium and stimulus paradigm; this component correlated with the vesicle pool associated with the ribbon. Increasing calcium buffering slowed the rate of release for the second component. The third component had an invariant fast release rate but an onset time that varied with calcium load. The onset time was delayed with increasing calcium buffering but the rate was unchanged. The third component represented a large capacitance change requiring recruitment of vesicles not associated with the ribbon. Although depletion of small pools of vesicles could be observed with this method, release properties measured about the hair cell's natural resting potential demonstrate a linear response with no fatigue or depletion. We hypothesize that the two calcium-dependent components of release represent vesicular trafficking that is specialized to provide the expected rapid, linear and indefatigable release properties. This work supports the idea that vesicle trafficking controls release properties and that the significance of vesicle pool distributions requires re-evaluation.

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100 Synaptic Exocytosis Associated with T-Type and L-Type Ca2+ Channels in Developing Chicken Hair Cells

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Neurotransmitter release at the hair cell ribbon synapse is well known to be stimulated by local rise of intracellular Ca2+ near voltage gated Ca2+ channels (VGCC). This Ca2+ dependent process implies an efficient coupling between the synaptic vesicle fusion machinery and the VGCC by mechanisms that are still largely unknown. Remarkably, during development, chick auditory hair cells switch progressively from being spontaneously active hair cells, firing action potentials mostly driven by T-type Ca2+ channels, to quiescent mature hair cells that mostly express L-type Ca2+ channels. The goal of the present study was to characterize the progressive changes occurring in the Ca2+ dependence of the hair cell synaptic machinery during development. Hair cell exocytosis (vesicle fusion) was recorded in the intact chick basilar papilla from E10 to P2 by monitoring changes in membrane capacitance (ΔCm) during various voltage stimulations. Exocytosis associated with Ca2+ current activation could be recorded at all developmental stages examined, with a significant increase in ΔCm amplitude (~1.5 fold) and Ca2+ efficiency after ~E12-16. Mibefradil and nickel, two potent selective blockers of T-type VGCC, largely inhibited ΔCm and ICa in developing hair cells up to E10-E16 but not in more mature hair cells (>E18). Varying intracellular Ca2+ buffering, when using 2 mM EGTA instead of 0.5 mM EGTA, showed that the fast exocytosis process (RRP) was largely affected when rising EGTA in early developing hair cells (E10–E16) but not in mature hair cells (E18 and older). Overall, our data show that exocytosis is driven with a poor Ca2+ efficiency by T-type VGCC in early developmental spontaneous active hair cells. This can be explained by a reduced Ca2+ sensitivity of the synaptic machinery at these early stages of development or/and a loose spatial association of the T-type VGCC with the synaptic machinery (microdomains) as compared to L-type VGCC in mature hair cells (nanodomains).

101 3D Anatomical Atlas of the Cochlea of the CBA/J Mouse

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The three-dimensional (3D) morphology of the cochlea has been investigated using a newly developed thin-sheet laser imaging microscope (TSLIM) in five cochleas from 4-week old mice. Cochleas were fixed, decalcified, dehydrated, cleared and serial optical sections were obtained through the complete cochlea. Major components of the cochlea were segmented, 3D rendered and morphometric measurements were obtained. The volume of the following structures was determined: scala vestibuli, media and tympani; basilar, tectorial, and round window membranes; spiral ligament and limbus, stria vascularis, organ of Corti, Rosenthal's canal and the

number of spiral ganglion neurons was estimated. Average volume of cochlear perilymph and endolymph was also estimated. Regions of fluid exchange such as the ductus reuniens and helicotrema were quantified as well. Length was determined along the centroid of several cochlear structures (i.e., stria vascularis, organ of Corti, scala media, basilar membrane, and Rosenthal's canal) and virtual, orthogonal, cross sections of some of these structures were produced. Orthogonal cross sections were used to construct graphs of the area of a structure along its length. Within Rosenthal's canal there were noticeable expansions of the canal at 10, 50, and 90% (from the base). A 3D Cartesian coordinate system was developed to relate structural features to a functional reference system, such as the basilar membrane. The goal of these efforts are to construct an interactive, 3D digital atlas of the mouse cochlea for research and teaching.

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102 Saccular-Specific Hair Cell Addition During the Breeding Season of a Vocal Teleost Fish: Do More Hair Cells Equal Increased Auditory Sensitivity?

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The plainfin midshipman fish, *Porichthys notatus*, uses vocal signals during many aspects of its life history, particularly reproduction. Nesting males produce multi-harmonic signals to attract reproductive females for spawning. The female's auditory system shows considerable plasticity in that the auditory saccular sensitivity of reproductive females becomes more sensitive to a wider range of frequencies than non-reproductive females. This seasonal increase in auditory sensitivity coincides with the dominant harmonics of the male's mate call, which are thought to be well suited to propagate in the shallow-water breeding environment. The cellular and molecular mechanisms for this seasonal auditory plasticity are still unknown. The present study examined cell proliferation, cell death, and hair cell number in the saccules of non-reproductive and reproductive female midshipman to test the hypothesis that seasonal auditory plasticity is due, at least in part, to a seasonal increase in hair cell number. We found more proliferating and fewer dying cells in saccules from reproductive females than in non-reproductive females. Quantification of phalloidin-labeled hair bundles showed a significant increase in bundle density in the saccule of reproductive fish relative to non-reproductive fish, suggesting that the increased cell addition in saccules from reproductive females was a result of a net addition of hair cells. No increase in cell addition or hair bundle density was found in the utricle or lagena of the reproductive females, indicating that the increase in hair cells is saccule-specific and not due to a general inner ear epithelial expansion during reproduction. Our data suggests that increased auditory saccular sensitivity in reproductive females could be due to an increase in hair cells, particularly if the newly added hair cells are responsive to higher frequencies. Future studies will examine the role of gonadal steroids in this novel form of auditory plasticity.

103 Labeling Patterns in the Inner Ear of the Transgenic *thy1*-YFP-H Mouse

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Transgenic mice with fluorescent marker proteins have been used to trace neuroanatomical pathways in the brain (Feng et al., 2000). One such mouse line that has been developed comprises C57BL/6 mice expressing yellow fluorescent protein (YFP) in subsets of CNS neurons, driven by the neuronal *thy-1* promoter (*thy1*-YFP-H). We wished to assess inner ear labeling in this mouse line and to examine the possibility of using it as a model for studying inner ear afferents. Vestibular and cochlear endorgan and ganglia fluorescence in postnatal day 8-100 YFP-H mice was observed with confocal microscopy. Images from utricular and cochlear whole mounts and crista sections suggest that in this mouse, vestibular afferents (including both calyces and boutons) and type I and II spiral ganglion neurons are labeled. Vestibular afferents in the central region of both cristae and maculae were more intensely labeled than peripheral afferents; no supporting cell labeling was observed. Cochlear afferents innervating inner hair cells were labeled. Nerve fibers innervating the outer hair cells were also labeled and antibody markers are being used to determine whether these are afferent or efferent fibers. Markers being used to make these distinctions are ChAT, tenascin-C, calretinin, peripherin, and CGRP. In conclusion, the *thy1*-YFP-H mouse is a useful model in which to study inner ear endorgan structure and function as it lends itself to *in vivo* imaging and enables easy identification and distinction of afferent types and regions.

104 Imaging of the Mouse Cochlea by Frequency Domain Optical Coherence Tomography

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OCT (Optical Coherence Tomography) is an optical signal acquisition and imaging method that has an extremely high signal-to-noise ratio (SNR) and resolution on the order of 10 um. These features permit 3-dimensional imaging within scattering media (e.g. biologic tissue). Importantly, it is also non-invasive, and commercial OCT devices are in clinical use for ophthalmic and dermatological applications. OCT has been used to image the cochlea for research purposes using the time domain approach. However, the frequency domain OCT approach has significant advantages because it provides higher SNR and faster imaging speeds than time domain OCT. In an effort to develop an improved technique for intracochlear imaging, we have designed a frequency domain OCT system. In tissue, near infrared light with longer wavelengths have

less scattering and thus depth penetration is improved. On the other hand, silicon-based light detectors have lower efficiency at longer wavelength. As a compromise, we chose 950 nm as the center wavelength for the applied light. Depth resolution is in inverse proportion to the bandwidth of the applied light. We created light with a bandwidth of up to 100 nm by focusing an ultrafast laser (Chameleon, Coherent Inc.) into an ultrahigh numerical aperture single mode optical fiber (UHNA3, Thorlabs). Due to the self phase modulation effect, the bandwidth of the laser extended from 10 nm to 100 nm inside the fiber. The light was collimated after it came out of the fiber with an overall conversion efficiency of roughly 50%. Then to ensure high interference efficiency, we focused this light into a 2x2 fiber-fused coupler. One output of the fiber coupler (the sample arm) was coupled into the X-Y mirror scan head of an upright microscope. The other output was used as the reference arm. The reflected light from the sample and reference arms were combined inside the fiber coupler, collimated, and processed by a custom-built spectrum analyzer created by passing the light through a diffraction grating (1292, Wasatch Photonics) and focusing the output across a linear CMOS camera. We tested the OCT device by imaging the excised mouse cochlea. Three-dimensional images were created from a series of X-Z scans. We found that the modiolar bone could be identified even when imaging through the overlying otic capsule bone. The estimated pixel resolution was ~10-15 um. Further research will be done to determine the capability of this system to visualize the cochlea in vivo, image the intracochlear soft tissue structures, and measure vibratory movements.

105 Detailed Anatomical Investigation of the Basilar Papilla of the Northern Leopard Frog

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The frog inner ear contains two dedicated auditory end organs: the amphibian papilla and the basilar papilla (BP). Both of these organs are simple compared to other vertebrate hearing organs, primarily due to the lack of a basilar membrane as a flexible substrate for the hair cells. We performed a detailed study of the anatomy of the BP in the northern leopard frog using images from light microscopy in fixated and non-fixated preparations and from scanning electron microscopy

The lumen of the BP was largely cylindrical in shape. It extended medially from the wall of the saccular space, and had a length of approximately 500μm. The cross section was oval and tapers towards the medial end of the lumen, where it ended at the contact membrane. The epithelium was located at about 50μm from the medial end of the lumen. At the location of the epithelium the lumen was on average about 270μm wide and 375μm high.

The sensory epithelium consisted of hair cells and supporting cells. It measured about 100μm along the length of the lumen and 275μm along the curved perimeter. On average 76 (range 63-95) hair cells occupied the medial half of the epithelial patch and were set in six to eight rows. The hair-bundle orientation was uniformly towards the medial end of the lumen.

We identified four types of hair cells, each occupying specific areas of the epithelium. Only the medial two to three rows of hair cells appeared to connect to the overlying tectorial membrane. The variety of hair cells and the presence of free-standing hair bundles is intriguing in light of the notion that the BP appears to function as a single-frequency, passively tuned auditory filter.

The data presented here are consistent with data from related species in literature. The extent and detail of the current data may in future provide a basis for a fluid-dynamical model of the anuran BP.

106 The Changes of Hearing and Related Histology in Vitamin a Deficient Mice

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Although numerous authors reported that the vitamin A deficiency in human subjects and animals induced a reduction in hearing ability, its role is not clear in the inner ear. In the present study, we evaluated the effect of vitamin A deficiency on changes of hearing level and histopathology of inner ear with ICR mouse. Animals were divided into the two groups: group 1 (experimental group; n=6) was fed a purified vitamin A free diet from 7 days after pregnancy; group 2 (control group; n=6) was fed a normal diet. Hearing threshold was measured by ABR until 20 weeks after birth. Cochlea was collected for the histological study and Phalloidin staining was observed. Mice which completed the hearing follow-up had their cochlear extracted for tissue confirmation. After the level of retinoic acid was reached a half of control mice, the hearing threshold of experimental group was increased compared with control group. In all vitamin A deficient mice, the damage to the outer hair cell and the Organ of Corti was noticeable. We could see the stereocilia damage in cochlea after FITC phalloidin stain and stereocilia loss was more severe in basal turn. The density of spiral ganglion cells was decreased and the vacuolization was also increased in the stria vascularis and spiral ligament.

In conclusion, the hearing threshold and its correlated histology of vitamin-A deficient mice were damaged as against control ICR mice. Approach to the mechanism of its effect should be studied in the future.

107 How Do Hair Cell Currents Shape Afferent Responses in the Frog Vestibular Organs?

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Within the vestibular system, afferents vary in their discharge properties, forming different populations which differ in gain, dynamics and ISI regularity. Moreover, most afferents display compressive nonlinearities around a resting discharge which can be modulated bidirectionally. The factors setting the zero-stimulation point, the extent of compressive nonlinearities and the dynamics of the

response are still unclear, and include properties of hair cells, afferent and efferent fibers.
The main purpose of this work is to investigate how ionic currents expressed by frog vestibular hair cells contribute to the diversity of afferent responses. For this reason we have developed a combined experimental-modelling approach, to reconstruct afferent responses of the frog crista and utricle.
In this work we recorded and modelled electrical responses from hair cells differing in morphology and epithelial position. Voltage-dependent currents were studied with the whole-cell perforated-patch method in isolated and in situ hair cells; currents were dissected out pharmacologically. Voltage responses to current steps and sinusoids were studied with current clamp experiments.
The biophysical parameters obtained from experiments and literature for hair cell currents were used to simulate utricular and crista hair cells using NEURON. Simulated ribbon synapses were added, and their number was varied in order to reproduce quantal data recorded from frog vestibular organs. Spike encoding was modeled as to reproduce experimental or literature data for resting and stimulated discharge.
In the model we explored the effects on gain, dynamics and linearity of responses due to variations of hair cell currents.

108 Spontaneous Depolarizing Activities Displayed by Supporting Cells in the Organ of Corti Are Initiated by a Calcium-Dependent Chloride Conductance

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Hair cells and supporting cells in the organ of Corti are essential for the normal cochlear functions. However, little is known about physiological roles of the supporting cells. A recent report demonstrates that inner supporting cells in the Kölliker's organ of developing mouse cochlea display spontaneous depolarizing activities (SDAs) initiated by extracellular ATP. Such SDAs are a major driving force for spontaneous firing activities in the developing peripheral and central auditory systems. Supporting cells in the organ of Corti (outer supporting cells) express purinergic receptors and focal application of ATP triggers propagating Ca^{2+} waves. However, it is unclear whether these supporting cells also display SDAs similar to those observed in the inner supporting cells.
Using the whole-cell patch clamp recording, we found that supporting cells in the developing organ of Corti of mice displayed robust SDAs. Double-electrode patch clamp recordings made simultaneously from the Kölliker's organ and the organ of Corti, however, demonstrated that the SDAs in these two regions were fundamentally different in terms of synchronicity, duration, frequency and pharmacological properties. Ionic substitution experiments revealed that the SDAs in outer supporting cells are highly depended on both external and internal Ca^{2+}. Simultaneous optical imaging with the Fluo-4 Ca^{2+} indicator and patch-clamp recording showed that SDAs in outer supporting cells were triggered by apparent spontaneous intracellular Ca^{2+} spikes. In addition, SDAs were eliminated by perfusing zero Ca^{2+} external solution and by applying thapsigargin, caffeine and cyclopiazonic acid. Pharmacological experiments suggested that a Ca^{2+}-dependent chloride conductance is the major underlying ion channels that carried the current for SDAs. One subtype of Ca^{2+}-dependent Cl^- channel (ANO1) was detected by immunolabelling in the outer supporting cells. Together, these data suggested that the functional role and underlying ionic mechanisms for SDAs in the outer supporting cells are dramatically different from those recorded in the Kölliker organ. Our data support that a novel Ca^{2+}-dependent Cl^- channel is the major contributor to the SDAs in the outer supporting cells in the developing mouse cochlea.

109 Synaptic Activity and Stimulation of Type II Cochlear Afferents

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The afferent innervation of the cochlea consists of two types of neurons, Type I and Type II spiral ganglion neurons. Type I neurons comprise 95% of the afferent neuron population, and project to single inner hair cells (IHC) in the organ of Corti. Acoustic information of sound timing and intensity is encoded at this IHC to Type I synapse, while frequency is encoded by IHC position along the tonotopic cochlear axis. Type II afferents comprise the remaining 5% of spiral ganglion neurons. They project past IHCs to contact several outer hair cells (OHC). Type II afferent projections are thin and un-myelinated, and little is known about their synaptic inputs or excitability due to difficulties recording from the neurons. We used a novel approach of performing giga-ohm seal, whole-cell electrophysiological recordings from dendrites of Type II afferents directly under OHCs in the apical turn of rat cochleas. In pre-hearing (P5-9) rats, large tetrodotoxin sensitive sodium currents and slowly accommodating action potentials were found. Excitatory post-synaptic currents (EPSCs) were recorded that reversed polarity near 0 mV and were sensitive to NBQX, an AMPA type glutamate receptor blocker. This indicates that synaptic inputs to Type II cochlear afferents are glutamatergic and mediated by post-synaptic AMPA receptors. Type II afferents were strongly stimulated by ATP, which depolarizes and induces neurotransmitter release from OHCs, resulting in EPSCs in post-synaptic Type II dendrites. Additionally, ATP directly induced a large inward current in Type II dendrites. This effect of ATP depolarized Type II neurons and could initiate action potentials. Stimulation of Type II afferents by ATP was down-regulated, but not eliminated, after the onset of hearing in rats. Further, EPSCs were still observed in hearing (P17-19) rats. Supported by NIDCD grants R01 DC000276 and R01 DC006476, T32 DC000023 and a grant from the Blaustein Pain Foundation of Johns Hopkins.

110 Expression and Regulation of NMDA Receptors at Inner Hair Cell-Auditory Nerve Ribbon Synapses

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Sound stimulations induce glutamate release at inner hair cell (IHC) ribbon synapses with the dendrites of primary auditory neurons. While these synapses predominantly use AMPA receptors, they also bear NMDA receptors (NMDARs). NMDARs are tetramers formed by NR1 and NR2A-D subunits. These receptors are calcium-permeable cation channels with a strong voltage-dependent magnesium block. Their activity in the cochlea is only unmasked in pathophysiological conditions (e.g. salicylate-induced tinnitus and in neuronal reconnexions after excitotoxicity).

In this study, we demonstrate the expression in cochlear homogenates of several NR1 splice variants (N1, C1, C2 and C2') and of NR2B and 2D, not that of NR2A and 2C. When using a biotinylation technique, we found that all the expressed subunits were present at the plasma membrane. We have then studied their up- and down-regulation, phosphorylation and glycosylation under pathophysiological conditions, i.e. sound-induced excitotoxicity and salicylate-induced tinnitus. We have also studied the influence of the activation and blockade of NMDARs by dopamine, which negatively regulates the IHC activity, on these post-translational modifications.

111 Auditory Attention and the Detection of a Signal in Noise: Effects on Human Medial Olivocochlear Efferent Activity

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Attention to a target tone embedded in noise may cause cochlear physiological changes specific to the target frequency. Medial olivocochlear (MOC) efferents may produce such changes because they receive descending inputs and innervate outer hair cells. Animal studies suggest that during background noise, detection of target level changes would be enhanced by MOC activation that inhibits excitation and adaptation to the noise. The extent of this enhancement may be influenced by attention such that MOC activation strength depends on the difficulty of detecting a target in noise. Otoacoustic emissions (OAE) are a non-invasive tool for examining MOC activation strength in humans. Previous studies explored the correlation across subjects in MOC inhibition of OAEs and the ability to detect a target in noise, with MOC inhibition and psychophysical performance measured separately. No study has measured MOC inhibition during the task of detecting a target in noise.

We have begun experiments using stimulus frequency OAEs (SFOAEs) to measure MOC effects during a three-interval forced choice task. A monaural amplitude-modulated target tone is presented in one random interval while a 40 dB SPL bilateral SFOAE probe tone and a 50 dB SPL ipsilateral (re: target ear) broadband noise are presented in all intervals. There are three task conditions: 1) no task (always done first): 40 dB SPL target present, but no listening instructions, 2) easy task: 40 dB SPL target detection, and 3) hard task: detection of a target whose level is adjusted for 60% ±10% correct detection. Preliminary results from three subjects show task dependent changes in SFOAE magnitude or phase in the target ear, indicating a change in MOC activation. We are exploring the dependence of these changes on task difficulty, subject alertness ratings, the frequency ratio between the target and the SFOAE probe tone and how attention affects MOC activity.

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112 Infrasound-Induced Cochlear Microphonics in Scala Media Include a Component from Non Hair-Cell Sources

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In guinea pigs the lower limit of hearing is approximately 50 Hz. However, large cochlear microphonics (CM) to stimulus frequencies as low as 1 Hz are present in scala media (SM). As frequency decreases, the maximum amplitude of the CM input/output function increases progressively, reaching 15-20 mV at 5 Hz. The CM amplitude in SM also becomes larger relative to that in scala tympani (ST). At 5 Hz a 17.9 dB (SD 1.9, n=4) lower stimulus level is required to evoke the same CM amplitude in SM compared to ST. For infrasonic frequencies, the CM does not saturate to the degree seen with low frequencies in the acoustic range so that Boltzmann analysis becomes less meaningful. In addition, as frequency decreases the CM phase in SM increasingly differs from both SV and ST (after allowing for the ST/SM inversion).

DC coupled CM recordings with 5 Hz biasing of a 500 Hz probe tone shows there are two components in the CM response to an infrasonic bias. One component arises from displacements of operating point on the cochlear transducer curve. In addition, there is a superimposed second component, consisting of voltage changes that displace the entire transducer curve. This component is phase delayed by approximately 72 degrees (41.7 msec @ 4.8 Hz) with respect to the bias-induced operating point change. These data suggest that a substantial portion of the infrasound-induced CM in SM is not generated directly by the cochlear transducer but by another process. As the voltage changes occur symmetrically, above and below the saturation values seen with conventional low frequency stimuli, this component is unlikely to be generated by the hair cells. Instead, it may result from changes in the potential generated by stria vascularis as current passing through the structure is modulated by the infrasound. Measurements of infrasound biased potentials in scala media may provide a useful indicator of how strial function is modulated under varying current load conditions.

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113 Hysteresis in the Cochlear Microphonic Potential in Gerbil

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The mechano-electrical transduction by hair cells is nonlinear. This nonlinear character is commonly described by a sigmoidal Boltzmann activation curve, which describes the opening and closing of mechano-electrical transduction (MET) channels.

The transduction current passing through the hair cells via the MET channels is also reflected in the cochlear microphonic response (CM), and CM recordings have been used to reconstruct instantaneous input/output function of hair cells. For this purpose, the mechanical input to the hair cells is related to the CM in the form of Lissajous figures. Often, the phase of the input is *chosen* such that it "…minimise hysteresis in the Lissajous figures." [Patuzzi et al. (1990). Hear. Res. 45: 15-32]. Effectively, the first harmonic of the (distorted) CM-signal is used as reference for the entire recorded signal, thus automatically minimizing hysteresis. Hysteresis, however, may reflect (nonlinear) mechanical properties in transduction, *e.g.*, adaptation in the MET. Eliminating or minimizing hysteresis *a priori*, prevents its systematic study.

Here, CM recordings, obtained from Mongolian gerbil by means of a silver-ball electrode near the round window, are presented. Instead of using a phase reference that minimizes hysteresis, we determined the reference phase from independent measurements. We used an additional stimulus to linearize the response of the cochlea to the stimulus component under study. These recordings then served as a phase reference for additional CM recordings in which the stimulus component was presented alone. We observed pronounced hysteresis in which the output systematically lagged the input. We interpret this as a sign of transduction-channel adaptation. Supported by NWO grants 818.02.007 (ALW) to CPCV and 863.08.003 (VENI) to SWFM.

114 Ultrastructural Localization of Cochlin in the Rat Cochlear Duct

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Cochlin, a product of the COCH gene, is associated with an autosomal dominant sensorineural hearing loss referred to as DFNA9. The symptoms of DFNA9 include not only hearing loss but also vestibular disorders (Bom et al., 1999; de Kok et al., 1999; Fransen et al., 1999; Grabski et al., 2003; Khetarpal, 1993; Manolis et al., 1996; Robertson et al., 1998; Usami et al., 2003; Verhagen et al., 1989; Verhagen et al., 1992). Robertson et al. (2006) also reported the loss of cellularity and the accumulation of an abundant homogeneous acellular eosinophilic deposit in the cochlea and vestibule of DFNA9-affected ears. They suggested that these extracellularly deposited aggregates contain mutated cochlin, and that this mutated cochlin alters the interactions between cochlin and other cochlin-associated proteins. Khetarpal (2000) compared the normal spiral ligament with that in DFNA9-affected ears at the ultrastructural level, and noted the absence of major fibrillar type II collagen bundles. We speculated that the proteins interacting with cochlin might include type II collagen and have previously reported that these two extracellular matrix (ECM) proteins coexist in the same fibrillar substance in the subepithelial area of the semicircular canal (Mizuta, et al., 2008).

We expanded our immuno-electromicroanalysis of cochlin to the cochlea in this study. The immunolabeling of cochlin was observed in the fibrillar substance in the spiral limbus, beneath the inner sulcus cells, and in the basilar membrane, the spiral prominence and the spiral ligament. Immunolabeling of type II collagen was observed in the same fibrillar substance in the extracellular matrix of the cochlear duct. This localization of cochlin is consistent with the expected localization of type II collagen. The localization of cochlin and type II collagen indicates the important roles played by these proteins in the hearing process.

115 Expression of Integrins in the Adult Mouse Vestibular System

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Integrins, a family of cell surface proteins, consist of a heterodimer formed by an alpha and a beta subunit. Integrins are able to interact with the extracellular matrix and other cell surface proteins, as well as with the intracellular cytoskeleton. To date, 25 different heterodimers have been identified, with each integrin having its own specific ligand or ligands with which it may bind. Previous work in the auditory system (Davies et al, 2002), described the presence of several integrins during mouse inner ear development, but little is known about the expression of integrins in adult vestibular tissue. We aim to identify integrins present in the adult mammalian vestibular system and investigate whether there are changes in their expression patterns as a consequence of hair cell loss. Initial work using antibodies to several integrin subunits, revealed that integrin beta 1 is expressed in the utricle near the basement membrane. Immunolabelling also suggested the presence of integrin alpha V in the vestibular system, whilst an antibody against beta 3 failed to detect the presence of this subunit.

Using bioinformatics to design a set of degenerate PCR primers we have begun experiments to detect all of the integrin subunits present in the vestibular sensory epithelia. The specificity of our primers is being tested using control tissue, before using them to investigate which integrin subunits are expressed in normal adult mouse vestibular tissue. These primers will be applied initially in cultures of utricular maculae, and we are currently optimising culture technique to allow incubation for up to 4 weeks.

116 Gene Expression Differences Along the Tonotopic Axis of the Chick Basilar Papilla

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There are known differences in the properties of hair cells along the tonotopic axis of the basilar papilla, the avian equivalent of the cochlea. Among the known differences are changes in ion channel density and properties. As yet there has not been a comprehensive comparison of gene expression between the distal and proximal chick cochlear epithelium. Auditory epithelia were isolated from 9 P0 white leghorn chicks and immediately sectioned into thirds, creating low, middle, and high frequency segments. Samples were comprised of 3 segments each, to create a total of 9 samples: 3 low frequency, 3 middle frequency, and 3 high frequency. RNA was isolated from each sample and was amplified, labeled and hybridized to Affymetrix whole-genome chicken arrays. GeneSpring software was used to identify genes differentially expressed between the two ends of the epithelium. Quantitative PCR was used to validate the microarray results. 2,663 genes were found to be differentially expressed using a fold-change cut-off of 2 and a p-value of 0.05. An enrichment analysis was performed on the microarray data and revealed 14 gene sets significantly (FDR < 0.05) enriched in the low frequency end, and 88 gene sets significantly enriched in the high frequency end. We have specifically identified many ion channels which are differentially expressed between the low and high frequency regions of the basilar papilla; some of these channels are known to have this expression pattern while others are not.

117 Assessing Stapes Surgery Results by Round Window Motion Measurements

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In stapes surgery, various prostheses and techniques are used to improve the hearing for patients with otosclerosis. In this study we present a bench test method to assess functional results after stapes surgery. Round window motions, which are closely related to pressure propagation of the travelling wave inside the cochlea and thus are closely related to hearing, are measured in fresh temporal bones. The performance of the reconstructed middle ear is quantitatively assessed by comparison of the volume displacements and motion patterns at the RW before and after the stapes surgery. Retro-reflective beads are attached to the surface of the round window to improve reflectivity of the LDI laser beam, and the whole surface area of the round window is measured using a scanning LDI system. To obtain an accurate angular position of the laser angle with respect to the round window plane, correlation between the measurement frame and anatomical-fixed frame is calculated from the location of reference wires attached to the peripheral bone, using micro-CT images. As a preliminary result, the magnitude of the volume displacement at the round window of the reconstructed ear was reduced by 10 dB at low frequencies below 2 kHz and by 3 to 7 dB at high frequencies above 3 kHz, compared to the normal ear. The amounts of the reduction are similar to the corresponding amounts from hearing test at low frequencies but smaller at the high frequencies. Discrepancy between the volume displacement at the round window and hearing test in the reconstructed ears at the high frequencies is presumed to be due to complex stapes motions in the normal ears, which do not generate net volume displacement but affect hearing.

118 Measurement of Electrically Evoked Basilar Membrane Vibration at Two Longitudinal Locations

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In order to understand the backward propagation of sound in the cochlea, the electrically evoked basilar membrane vibration was measured at two longitudinal locations in sensitive gerbil cochleae. Sinusoidal currents at different frequencies and levels were delivered to the apical turn through a bipolar electrode to stimulate the organ of Corti near the electrodes. The electrically and acoustically evoked basilar membrane vibrations were measured at two longitudinal locations in the first turn using a sensitive heterodyne laser interferometer. It was found that the electrically evoked basilar membrane vibrations showed tuning similar to that of acoustically evoked responses. For either electrically or acoustically evoked wave, the phase at a basal location led the phase at a more apical location, indicating that the wave traveled in the forward direction. The delay from the measured basal location to the more apical location increased with frequency, whereas the wave speed and wavelength decreased with frequency over the same region. Thus, these results show that the electrically evoked basilar membrane vibration is dominated by a forward traveling wave. The current finding indicates that the electrically evoked sound likely exits the cochlea through the cochlea fluid as a compression wave, driving the stapes, and resulting in emissions. The compression wave-induced stapes vibration also initiated a secondary forward traveling wave.

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119 Displacements of Footplate, Round Window Membrane and Basilar Membrane in Human Temporal Bones

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Objective: To measure displacements of stapes footplate (SFP), round window membrane (RWM) and basilar membrane (BM) response to acoustic stimulation using Laser Doppler Vibrometry (LDV) and to study correlations among them.

Methods: Four fresh human temporal bones were used in this study. An extended facial recess was created and the RW niche overhang was removed allowing complete view of the RWM. A cochleostomy was performed via promontory exposing the BM. An acoustic transducer and a probe microphone were inserted into the external ear canal. The peak-to-peak displacements from SFP, RWM and BM were obtained by LDV at a frequency of 2 KHz at 90 dB SPL. Displacements for SFP and RWM were measured with and without cochleostomy, and with the cochleostomy sealed by silicon. BM measurements were obtained with and without a sealed, glass slide covering the cochleostomy.
Results: Displacements of SFP and RWM were 1.75 and 0.8 micrometers respectively without cochleostomy. Displacements of SFP and RWM were 1.2 and 0.8 micrometers with a cochleostomy and 3.2 and 2.4 micrometers with cochleostomy sealed by silicon. Displacements of BM were 0.14 and 0.2 micrometers with and without a glass slide sealing of cochleostomy.
Conclusions: Findings indicated that the displacement of SFP was greater than that of the RWM. This difference remained either with cochleostomy or after sealing the opening. BM displacements were smaller in magnitude and no difference with or without a glass slide covering. This model can be used to increase the understanding of micro-mechanics of middle ear and cochlea.

120 Efferent Inhibition of the Apical Cochlea's Mechanical Responses to Sound

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We have measured sound-evoked motion in the apical turn(s) of the guinea-pig cochlea with and without electrical stimulation of the medial olivocochlear efferent system. We used laser interferometry to monitor the motion of the partition (usually from individual Hensen's cells), and delivered short (100-200 ms) bursts of electrical shocks at the floor of the fourth ventricle to stimulate the efferents. The shock bursts were synchronised with the acoustic stimuli that we presented to the ear. Efferent-evoked mechanical changes were then quantified by comparing the sound-evoked motion across time windows (i) just before and (ii) near the end of each "shocks-on" period.
The most notable effect of the efferent stimulation was a reduction in the baseline position shifts (or dc-shifts) evoked by the acoustic stimuli (short tone bursts, rarefaction and condensation clicks). This reduction appeared to be tuned to frequencies just above the characteristic frequency (CF) of the location being studied (e.g. to ~800Hz when the CF was ~300Hz). The largest reductions observed so far had the same effect on the dc shifts as lowering the intensity of the acoustic stimulus by ~3dB. Close inspection also revealed weak inhibition of the ac responses evoked by some of the above-CF stimuli (typically by 1dB or less). We presume that the small size of these effects will have been limited by a combination of the cochlea's physiological vulnerability and our invasive surgery. Nonetheless, our results clearly go one stage further than previous attempts to study this difficult to access region, and provide the first demonstration that at least some efferent inhibition is effected mechanically even in the apex of the mammalian cochlea. These data also provide the strongest evidence so far that the sound-evoked baseline position shifts that we have reported previously either originate, or are controlled by, the outer hair cells.
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121 The Effects of Intense Sound Stimulation on the Inner Ear

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The focus of this study is to investigate the effects of intense sound stimulation on the function of the apical region of the inner ear. In order to do so we apply motion measurements, calcium imaging and electrophysiological measurements. The results give insights into the physiology and nanomechanics of the hearing organ and noise-induced hearing loss.
We used a high resolution laser interferometer with an integrated laser scanning confocal microscope to measure the motion of organ of Corti, during moderate sound stimulation before and after an intense sound stimulation. A calcium sensitive dye was used in order to track intracellular calcium fluctuations due to the intense sound exposure, and the fluorescence was monitored with the confocal microscope. The cochlear microphonic potential was measured via a microelectrode inserted into scala media to monitor the condition of the preparation. Our set-up allowed us to capture the sound evoked vibrations, cochlear potentials and image the calcium fluctuations subsequently in an isolated guinea pig temporal bone preparation over several hours.
Data shows that the amplitude of the motion decreases after intense sound and this decrease appears temporary. Calcium levels seem to be transiently elevated in the sensory cells after the intense sound exposure. The observed changes may be the result of the sensory cells adapting to the loud stimulus by changing their mode of action. We have also noted, in a number of experiments, that the Reissner's membrane bulge towards the organ of Corti immediately after the intense sound stimulation, to then return to its initial position.

122 Effects of Cholesterol Content on Viscoelastic Properties of Plasma Membrane

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Cochlear outer hair cells exhibit electrically induced movements known as electromotility, a membrane-based phenomenon which allows for the sensitivity and

frequency-resolving capability of mammalian hearing. It has recently been demonstrated that membrane cholesterol content modulates the membrane charge movement and electromotility in outer hair cells.
In this study, effects of cholesterol on viscoelastic properties of the plasma membrane are investigated. We use optical tweezers to extract nanotubes (tethers) from the plasma membrane of human embryonic kidney (HEK) cells as a model cell. We obtain time-resolved tether force measurements under cholesterol depleted and cholesterol enriched conditions. Using these data, elastic and viscous parameters of the plasma membrane are quantified and correlated to the changes in the membrane cholesterol level.
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123 On the Origins of the Compressive Nonlinearity of Hearing

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Much attention has been focussed recently on the so-called Hopf oscillator model for the compressive nonlinearity and amplification of mammalian hearing. Here we present an alternative model that is equally well able to capture the characteristic -2/3 power law type of amplification. The key feature of the model is bistability, which is generated robustly by a simple balance between linear terms and a cubic nonlinearity; it does not suffer from one of the main drawbacks of the Hopf model, where delicate tuning of two parameters is necessary to achieve the amplification effect. We also investigate a recent outer hair cell model proposed by Ó Maoiléidigh and Jülicher [personal communication] that accounts for physiological features such as hair bundle movement, adaptation and charge movement inside the cell. We find that it is not necessary to tune this model into the region where Hopf bifurcations occur. Instead, we show that there is a broad parameter regime in which bistability occurs, and where the charge of the hair cell shows -2/3 power law behaviour. Since the relation between the charge of the hair cell and its somatic motility is approximately linear, and since somatic motility can drive the organ of Corti, we suggest that the hair cell's bistable operation is fundamental to the compressively nonlinear amplification that is seen experimentally on the basilar membrane (e.g. see Rhode, J. Acoust. Soc. Am. 121, p2792-2804, 2007). Finally, the more physiological model shows that hair bundle movement is almost linearly proportional to the forcing. This suggests that it is the global motion of the organ of Corti and basilar membrane, rather than the localised motion of the hair bundles themselves, that underlies the generation of mechanical nonlinearity in the cochlea.

124 Consequences of Threshold Microstructure - A Modeling Approach

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Threshold in quiet of normal hearing listeners often show quasi-periodic fluctuations with frequency when measured with a high frequency resolution. These fluctuations are referred to as ”threshold finestructure“ or ”threshold microstructure”. This microstructure also affects psychoacoustical phenomena above threshold like e.g. modulation perception with low carrier levels. This effect has not yet been investigated with a model of the auditory periphery. The present study investigates this influence of threshold microstructure on the peripheral internal representation of sounds at the level of the cochlea. It was proposed that random fluctutations of the place-frequency map of the cochlea and an active process are neccessary conditions for threshold microstructure. Following this hypothesis, a one-dimensional nonlinear and active transmission line model of the cochlea is used. The model simulations show threshold microstructure and spontaneous otoacoustic emissions in microstructure minima, i.e. regions of high sensitivity. This model can also be used to predict data in finestructure in distortion product otoacoustic emissions. For sinusoidally amplitude modulated tones with low carrier levels, the cochleograms show differences in the representations of the modulation in microstructure minima and maxima. These differences are in line with the performance of human listeners in a modulation detection task [Heise et al. (2009), J. Acoust. Soc. Am. (125) EL33]. It is discussed if changes in the internal representation due to the underlying threshold microstructure can also account for other psychoacoustical data such as temporal integration of loudness growth functions in threshold minima and maxima.

125 Endocochlear Potential (EP)-Dependent K^+ Circulation Model Represents the Electrochemical Dynamics of the Cochlear Amplification System

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The cochlear duct is electrically isolated compartment filled by the endolymph, which contains high concentration of K+ and exhibits a potential of +80 mV, the endocochlear potential (EP). Two types of functional epithelial units line the cochlear duct. The first one is the secretory epithelium of the lateral cochlear wall that generates the EP as a biological battery, as well as secretes K^+ into the endolymph. The second one is the sensory epithelium,

which absorb the K^+ from endolymph upon depolarization of the hair cells. The K^+ transport through these two units results in the K+ circulation, which maintains the K^+ homeostasis, and EP is known to amplify the sharpness of hearing by enhancing the hair cell K^+ absorption. Although recent studies indicate that each ion channels and transporters is essential for EP and K^+ circulation, how the correlation of the functional units contribute to the electrochemical dynamics of the cochlear amplification system remains unknown. In order to elucidate the system, we developed the mathematical model using the theoretical property of currently known ion channels, transporters and structural data. The model is based on the assumption that the driving force of the K^+ circulation is EP-dependent and K^+ circulation current is equal throughout the K^+ transport pathway. Using this model, we simulated the ionic concentration together with the alteration of the membrane potentials, which are good agreement with the physiological data previously observed. In addition, we made some theoretical predictions under pathophysiological condition in hereditary deafness syndrome.

126 Bone Conduction Analyses in a Finite Element Model of the Human Middle Ear and Cochlea

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A three-dimensional finite element model of the human middle ear and cochlea, with a tapered box geometry, was developed to allow calculations of the basilar membrane (BM) velocity, as well as the round window (RW) to oval window (OW) volume displacement ratio (Urw/Uow). The model was used to explore the effects of bone conduction (BC) on cochlear function, by specifically simulating the following three cases: 1) Inserting a "third window" (TW) in the cochlear outer wall that might account for a reported imbalance in Urw/Uow under BC excitation (Stenfelt *et al*., 2004); 2) Applying BC excitation through translational acceleration (TA) of the ear, versus through compression of the bony capsule; and 3) Allowing the primary osseous spiral lamina (OSL) to be mobile versus immobile for both air conduction (AC) and BC.

When the TW was modeled as a simple compliant membrane, the model produced an imbalance of Urw/Uow that was consistent with experimental measurements, whereas when modeling the TW as a tube representing the cochlear or vestibular aqueducts, the results were inconsistent with experimental data. Terminating the tube with a compliant membrane produced better experimental agreement than with the tube alone, but poorer agreement than with the membrane alone.

For TA without TW, BM velocities, normalized by the OW velocity, were found to depend on the direction (*x*, *y*, or *z* - longitudinal, transverse, and normal to BM, respectively) of the acceleration at high frequencies, but not at low frequencies. By contrast, the normalized BM velocities generated by compression of the bony capsule were inconsistent with experimental measurements (Stenfelt *et al.*, 2003). A tentative conclusion is that, relative to compression, TA is the more dominant mechanism for BC, at least in the tapered box model geometry for the cochlea presently used.

For AC stimulation, adding a mobile OSL decreased the BM velocity (normalized by the OW velocity) at high frequencies, while at low frequencies a mobile OSL had a negligible effect. [Work supported by grant DC007910 from the NIDCD of NIH.]

127 Responses to Multi-Tone Stimuli in a Nonlinear Model of Human Cochlear Mechanics

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A nonlinear model of cochlear mechanics is described in which force-producing outer hair cells (OHC) are embedded in a passive cochlear partition. The OHC mechanoelectrical transduction current is non-linearly modulated by reticular-lamina (RL) motion, and the resulting change in OHC membrane voltage produces contraction between the RL and the basilar membrane (BM). Model parameters have been chosen to produce a tonotopic map typical of a human cochlea. Model RL displacement responses are similar to measurements of BM vibration in mammalian cochleae. Distortion-product otoacoustic emissions (DPOAE) in the model are plotted as iso-level contours against primary levels (L1, L2) for f2 = 1, 2, 4, and 8 kHz and for various frequency ratios f2/f1. The L1 at which DPOAE reaches its maximum increases as L2 increases and the slope of the "optimal" linear path generally decreases as f2/f1 increases. However, these relations are confounded by notches and valleys in the contour plots. DPOAE level is reduced by adding a third tone as a suppressor. The amount of suppression varies as a function of suppressor level L3 and suppressor frequency f3. In general, the amount of suppression increases as L3 increases and as f3 gets closer to f2, but there are counter examples. These results will be presented in comparison to existing data from normal-hearing human ears.

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128 Two-Tone Suppression in a Model of Specific Loudness

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Suppression is the decrease in cochlear response to one sound due to the presence of another sound. The difference between simultaneous masking (SM) and forward masking (FM) for the same masker may provide a psychophysical correlate of suppression. Specific loudness is the distribution of loudness across the dimensions of time and frequency. A model of specific loudness is described that exhibits suppression for the same stimulus waveforms used to elicit psychophysical tone-on-tone

masking. This dynamic loudness model (DLM) is defined in the time domain and takes any waveform as input. The DLM is calibrated to produce standard equal-loudness contours and to achieve expected intensity-discrimination performance for tones. Two-tone suppression in the DLM is similar to recent measurements of the difference between SM and FM (Rodriguez et al., 2009, ARO). The similarity between DLM predictions and empirical findings is noteworthy because the DLM provides no separate mechanism for suppression. Suppression in the DLM is only possible through the same mechanism that provides compression for single tones. Because of its computational efficiency, the DLM may be a useful tool for exploring the role of suppression in auditory signal processing. [This work was supported by grants R01-DC8318 and R01-DC2251 from NIH/NIDCD.]

129 Inter-Species Comparison of Cochlear Tuning with a Feed-Forward and Feed-Backward Organ of Corti Model

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The cochlear physiologies of four species, gerbil, chinchilla, cat, and human, are simulated with a three-dimensional hydro-dynamic cochlear model with "feed-forward and feed-backward" active mechanism for the organ of Corti and solution calculated using a "time-averaged Lagrangian". The basilar membrane velocity (V_{BM}) and scala tympani pressure (P_{ST}) simulation results for the gerbil and chinchilla cochlea show reasonable agreement with the physiological measurements (Ren and Nuttall, 2001; Ruggero et al., 1997; Olson 2001). Interspecies BM velocity (3.7-164 um/s depending on species) and displacement (0.1-3.3 nm) thresholds are estimated by equating the simulation results to neural thresholds at the best frequency (BF). The Q_{20} (BF/20dB-down-bandwidth) is qualitatively similar in velocity and displacement for all four species. In comparison to neurally measured Q_{20} values, the model Q_{20} for the cat and chinchilla have similar values (2.4 and 3.4 respectively). However, the model Q_{20} is less sharp than the neural measurements in the gerbil by about a factor about 1.5 (Schmiedt, 1989). The biggest discrepancy is for human where the model Q_{20} is lower by about a factor of 6 in comparison to the high Q_{ERB}≈13 estimated using forward masking methods (Oxenham and Shera, 2003). Parameters that control the tuning include the OHC gain factor and angle relative to the deiters cell and the reticular lamina and the phalangeal process angle. [Work supported by grant DC007910 from the NIDCD of NIH.]

130 The Real Part of the Axial Impedance of Isolated OHCs Is Governed by Shear Losses in the Basolateral Wall

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Electromechanical force produced by the soma of the outer hair cell (OHC) is essential for amplification of the travelling wave on the basilar membrane. This force depends on the mechanical impedances of the basolateral cell wall, composed of the motor complex, the cytoskeleton and the sub-surface cisternae, and on the intra- and extracellular fluids. As published by Eckrich et al. (2008, Assoc. Res. Otolaryngol. Abs. 179), with increasing frequency the axial impedance of the isolated OHC decreases in both its imaginary and real parts. The imaginary part is dominated by stiffness and the real part by damping. In this study, by using a three-dimensional finite element model, including the inner and outer fluid domains, we investigate different damping properties of the OHC system. The simulation suggests that damping derives predominantly from the basolateral wall and is characterized by pure shear losses. That is, only the application of an anisotropic damping formulation, in which the volume dilatation and normal strains are undamped, has the ability to match the experimental data satisfactorily. The dynamical viscosity was found to be 1e2 – 1e3 Pa*s, dependent on wall thickness (100 – 500 nm), and was independent of frequency. The finding that damping is due to shear loss should serve as a basis for understanding the dynamics of the basolateral wall.

131 Simulating Interactions Between Hair Bundle Tuning and Electrical Resonance in Turtle Auditory Hair Cells

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Hair bundle mechanics are implicated as part of the cochlear amplifier. Activation and adaptation of mechano-electric transduction currents are posited to provide a band pass filter, mechanically tuning hair cells. Electrical resonance is the major filtering and amplification mechanism in turtle auditory hair cells, thus the questions, What role does hair bundle filtering provide and how does it alter or impact electrical resonance? Can electrical resonance feed back to further tune the hair bundle? We have developed a model of electrical resonance that incorporates each component of electrical and mechanical filtering. This Hodgkin-Huxley type model of electrical resonance includes the time and voltage dependent

currents from voltage-dependent Ca^{2+} channels and Ca^{2+}-dependent BK-type K^+ channels. In addition, the model simulates activation and adaptation kinetics of mechano-electric transduction. The model was parameterized by fitting to voltage-clamped time-dependent tail currents through Ca^{2+}-dependent K^+ channels and to voltage-clamped and rapidly acquired time-dependent currents through mechano-electric transduction channels. The experimental currents through mechano-electric transduction channels were measured at various displacement distances of the hair bundle and at 2.8 mM and 50 μM Ca^{2+} in endolymph. The model allows us to vary kinetic components to simulate different characteristic frequencies and to investigate filtering of each component independently and then together. The model also allows us to probe the system with more complex paradigms than are experimentally feasible. Supported by NIH/NIDCD 5R01DC003896 to AR.

132 Linearization of a Physiological Nonlinear Model of Mammalian Hair Bundle Motility

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The hair bundle of the mammalian OHC has been observed *in vitro* to exhibit fast adaptation and to generate a force in a sub-millisecond time scale in response to mechanical stimulation by a flexible fiber. This result suggests that HB motility might play a role in cochlear amplification *in vivo*. The parameters of a nonlinear mathematical model are optimized to predict the measured *in vitro* response of the HB. In this model, fast adaptation of the transduction current is explained by the binding of calcium to a site located close to the transduction channel and slow adaptation by the action of the motor molecule myosin-1c. The nonlinear model is linearized for small harmonic motion around the operating point of the HB. The linear model predicts that the transduction channel acts as a poorly tuned band-pass filter, due to the combined effect of channel activation and adaptation. Near the peak of the filter, HB motility enhances the sensitivity of the mechano-electric-transducer function, which could have a significant effect on the cochlear macromechanics. In addition, the HB generates a frequency dependent complex force described by an active stiffness and an electromechanical coupling coefficient. Due to the presence of an imaginary part in the active stiffness, the linearized model predicts that the HB can add or dissipate mechanical energy depending on the values of the parameters of the nonlinear model. Simple analytic conditions for mechanical energy generation and for spontaneous oscillations are derived. We find that the parameters optimized for the *in vitro* experiment are consistent with mechanical energy dissipation. [Support from NIH-NIDCD RO1-04084.]

133 Vibration Response of the Organ of Corti at the Sensitive Location

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A 3D Finite element model of a section of the middle turn (4 kHz place) of the gerbil cochlea was designed to investigate the significance of the often neglected interstitial fluid in the organ of Corti (OC). The model is anatomically accurate and is based on measurements by Karavitaki [MIT Ph.D. Thesis, 2002] and includes most of the known components of the organ of Corti. Most importantly, radial movement of fluid between the pillar cells, the hair cells and the stereocilia is incorporated. The distribution of elastic properties within the OC was found via sensitivity analysis based on the experimental point stiffness profile obtained by Naidu and Mountain [Hearing Research, 124:124-131, 1996].

In this study we examined the vibration modes of the model in the presence or absence of fluid. It was found that the presence of the interstitial fluid greatly modifies the micromechanics of the OC, resulting in a more complex relative motion between the components of the OC than the case of the drained OC. Based on the fundamental mode of vibration in the presence of fluid, the model resonates an octave below the best frequency for the simulated location. The resonant frequency for the third mode was very close to the expected best frequency. The fundamental mode in the model reflected the classical bending mode, while it was found that the third mode of vibration in the model matched the pattern of vibration reported by Karavitaki and Mountain [Biophysical Journal, 92:3294-3316] when the outer hair cells were electrically stimulated. These results suggest that two vibration modes may be important in the normal cochlea: a low frequency mode which dominates the passive mechanics and a higher frequency mode which is driven by the outer hair cells and dominates active cochlear mechanics.

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134 Probing the Source of Stimulus-Frequency Otoacoustic Emissions Using Low-Frequency Biasing

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A low-frequency bias tone modulates the gain of the cochlear amplifier along the length of the cochlear partition. Here we use biasing experiments to understand where stimulus-frequency otoacoustic emissions (SFOAEs) are generated. We quantify biasing effects by measuring both the modulation of SFOAEs as well as suppression of compound action potential (CAP) amplitude during particular phases of the bias tone cycle. CAPs evoked from moderate level tone-pips are helpful for our purposes because they arise predominantly from traveling wave peak regions associated with particular tonotopic cochlear places. Preliminary results from guinea pig ears indicate that a bias tone of a given level suppresses CAPs evoked from high frequency tone-pips less than it

suppresses CAPs evoked from low-frequency tone-pips. Equivalently, a bias tone of higher level is needed to achieve criterion CAP suppression in the stiffer cochlear base than the more compliant cochlear apex. Turning to SFOAEs, we found that the bias level needed to achieve maximal SFOAEs modulation was correlated with the level needed to achieve criterion CAP suppression. These findings are consistent with models of SFOAE generation in which the dominant emission sources are located near the peak of the traveling wave.

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135 Exploring the Interrelationship Between Spontaneous and Low-Level Stimulus-Frequency Otoacoustic Emissions

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Otoacoustic emissions (OAEs) provide useful information about cochlear function, although the complexities associated with their generation have limited our ability to interpret the results of OAE testing. Some healthy cochleas produce spontaneous otoacoustic emissions (SOAEs), which can offer further insight into cochlear function. For example, those individuals who have SOAEs may exhibit more robust low-level stimulus-frequency otoacoustic emissions (SFOAEs). The purpose of this study is to explore the relationships between SOAEs and micro-structure of SFOAEs to gain further understanding of the underlying generation mechanisms. Shera (2003) proposed a theoretical model that described the interrelationship between SOAEs and SFOAEs, suggesting that OAEs be classified not on the basis of the stimuli used to evoke them, but on the mechanisms that produce them. The present study directly compares low-level SFOAEs and SOAEs within the context of model predictions, with the goal of improving interpretation of clinical OAE data. Magnitude and phase of SFOAEs were recorded in the right ears of normal-hearing young adults with prominent SOAEs. Low stimulus levels (10-40 dB SPL) were used with fine resolution for both level and frequency, particularly in regions about SOAE peaks. Where SOAEs were identified, corresponding SFOAE amplitude peaks were seen. This finding becomes distinctly apparent with decreasing stimulus levels. At the lowest levels tested, SFOAEs were indistinguishable from the noise floor, except at SOAE frequencies. Behavioral threshold fine structure was measured at frequencies around SOAEs and found to be lower about SOAE frequencies, consistent with previous studies. Elucidating the interaction between SOAEs and low-level SFOAEs could lead to the development of new strategies for adapting SFOAEs for novel clinical applications, such as rapid estimates of cochlear tuning.

136 Transiently Evoked Otoacoustic Emissions Decomposed Into Asymmetric Waveforms

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Transiently Evoked Otoacoustic Emissions (TEOAEs) are normally modeled as the sum of asymmetric waveforms. However, some previous studies of TEOAEs used time-frequency (TF) methods to decompose the signals into symmetric waveforms. This was justified mainly by a reduction in the complexity of the calculations. It is herein proposed to extend the set (or dictionary) of functions to incorporate asymmetric waveforms into the analysis. The calculations were carried out using an adaptive approximations algorithm that used a Matching Pursuit (MP) technique. The classic MP dictionary uses Gabor functions and consists of waveforms described by five parameters; namely, frequency, latency, time span, amplitude, and phase. A sixth parameter, the degree of asymmetry, was added to our enriched dictionary, in order to enhance the flexibility of this approach. The effects of this enlargement of the collection of available functions were evaluated by means of both simulation and their application to real otoacoustic emissions. The results were visualized by means of time-frequency-amplitude distributions and compared with decompositions using symmetric waveforms.

137 Temporal Adaptation of the Click-Evoked Otoacoustic Emission Level-Curve Reveals Dynamic Properties of Human Cochlear Processing

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The level of a click-evoked otoacoustic emission (CEOAE) depends on the level of the evoking click through the CEOAE level-curve. The CEOAE level grows linearly for clicks below 30–40 dB and saturates for higher input levels. This study shows that the CEOAE level-curve for a test click can be shifted when a suppressor click is presented less than 10 ms before the test click. This effect is referred to as temporal adaptation of the CEOAE level-curve, and its strength is determined by the levels and the temporal separation of the two clicks. Two cochlear mechanisms could underlie the observed temporal adaptation: (i) temporal overlap of the basilar-membrane (BM) excitation patterns of the two clicks and/or (ii) a temporal change in cochlear nonlinearities caused by the presentation of the suppressor click.

To investigate the origin of temporal adaptation, we developed a nonlinear adaptive transmission line model of the cochlea. Reverse travelling waves (OAEs) were produced by adding irregularities to the BM impedance along the cochlear partition. The implemented nonlinearity

modelled the compressive behaviour of BM impulse responses that preserves the intensity invariance of their temporal fine structure. These model features yielded realistic behaviour of the CEOAE level-curve and group delay of the simulated CEOAEs. Furthermore, model simulations successfully reproduced the characteristic features of temporal adaptation of the CEOAE level-curve. Because the model employed an instantaneous (i.e., time-invariant) nonlinearity, we conclude that temporal adaptation can be explained by the temporal overlap of the BM patterns of two closely spaced clicks. However, the model simulations do not rule out contributions to temporal adaptation from time-dependent processes, such as fast adaptation in the outer hair cells.

138 Characterizing Frequency Dependence of Human Cochlear Nonlinearity Using Distortion Product Otoacoustic Emission Input Output Function

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Introduction: A complete characterization of cochlear mechanics includes elucidation of the role of its active element, which contributes to its non-linearity. Distortion product otoacoustic emissions (DPOAEs) are a non-invasive measure of cochlear mechanics and studying their input output (I/O) function provides an assay of the nonlinearities of cochlear mechanics.

In the literature, there is disagreement as to whether there is difference in cochlear mechanics that are in play in the apex compared to the base of the cochlea. In a previous study by Gorga et al (2007), DPOAE I/O functions were measured in human subjects at 500 Hz and 4 kHz, representing the apex and base, respectively. In agreement with physiological data from animals, greater compression was observed at 4 kHz compared to 500 Hz. However, 4 kHz hardly represents the true base of human cochlea. Dreisbach et al (2001) investigated DPOAE I/O functions at much higher frequencies (up to 13 kHz) and observed a gradual increase in compression with increasing stimulus frequency. However, the limitation of their study was the use of a limited range of input levels thereby preventing the characterization of the entire I/O function at any given frequency. In the present study, DPOAE responses from a region closer to the base of human cochlea at 12.5 kHz was compared to those at 4 kHz over a wide dynamic range to see if there is any further disparity in compressive nonlinearity than what was seen between 500 Hz and 4 kHz. By using stimuli swept in frequency, we were able to investigate the role of DPOAE level fine structure (periodic fluctuation in DPOAE level) in determining DPOAE I/O characteristics.

Methods: DPOAEs were recorded around f2 frequencies of 4 and 12.5 kHz from 12 normal-hearing young adult human subjects using custom hardware and software. A stimulus frequency ratio of 1.2 was used while the two stimulus tones were swept in frequency over an 1/8th octave range centered around the test frequencies. Equal level stimulus tones between 25 and 100 dB SPL were used to record I/O functions.

Results: In agreement with previous work, I/O functions showed increased compression at higher frequencies. Data were in good agreement with Gorga et. al., (2007) at 4 kHz. Individual variability in I/O functions was observed and could be attributed to DPOAE level fine structure.

139 Towards Designing a Clinical Method for Estimating Basilar Response Input/output Response Characteristics in Listeners with Normal and Impaired Hearing

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It has been claimed that the input/output (I/O) response characteristics of the human basilar membrane (BM) may be inferred behaviorally and from distortion product otoacoustic emissions (DPOAEs). Behavioral methods are robust and reasonably well grounded but impractical in clinical contexts. DPOAEs could be highly useful in the clinic but it is controversial that they provide reasonable BM I/O estimates on a within-subject basis. The present study investigated the degree of correlation between behavioral and DPOAE I/O curves for listeners with sensorineural hearing loss. Test frequencies were between 1 and 6 kHz. Behavioral I/O curves were inferred from temporal (forward) masking curves. DPOAEs were measured for a fixed primary frequency ratio of f2/f1=1.2 and individual optimal primary levels. Reasonable within-subject correlation between behavioral and DPOAE I/O curves was observed for ~50% of the cases only. Data analysis was complicated by the wide range of response characteristics observed. Sometimes, for instance, high-level DPOAEs were observed even when corresponding behavioral I/O curves were almost linear. It is concluded that further research is necessary to design efficient clinical methods for estimating BM I/O curves. Work supported by the Junta de Castilla y León (GR221), the Spanish Ministry of Science and Innovation (BFU2006-07536) and the Oticon Foundation.

140 Interference Patterns in Distortion Otoacoustic Emissions at High Frequencies in Humans

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It is commonly accepted that distortion product otoacoustic emissions (DPOAEs) are generated initially in the cochlear region of overlap between the responses to the stimulus tones f_1 and f_2. While this region is generally assumed to be near the peak of the f_2 traveling wave, the actual magnitude and phase versus position of distortion sources within the generation region have not been characterized. Suppression experiments support the concept of a distributed generation region (Martin et al., Hear. Res. 136:105-23, 1999). We noticed in ongoing studies at Northwestern that, at high stimulus frequencies, the level of the $2f_1$-f_2 distortion product (DP) tends to decrease then

increase sharply to a peak with increasing f_2 before dropping into the noise floor. The frequencies of these features correlate well with the highest audible frequencies of each subject. We hypothesized that this pattern is due to interference between generators with place-dependent magnitude and phase within the interaction region. This interference pattern may result from an increasingly restricted generation region as the stimulus frequencies approach the upper limit of hearing.

We tested our hypothesis using a suppressor tone, varied in small frequency steps above f_2, to remove part of the population of DP generators. Vector analysis of the change in DP magnitude and phase resulting from small increments in the suppressor frequency yields an estimate of the spatial distribution of generators. With f_2 frequencies of 8 kHz and 10 kHz the phase rotates through several periods as the frequency of the suppressor is increased to approximately f_2 + 4 kHz. With f_2 frequencies of 12 kHz and 14 kHz the derived generator phase changes relatively little as the suppressor frequency is increased. The derived generator distributions are consistent with the hypothesis and appear to explain the trends in our data.

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141 Analysis of DPOAE (2f1-F2) Phase and Individual DPOAE Components in Human Newborns

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Apical DPOAEs are comprised of at least two components, as evidenced by the "interference" pattern of alternating maxima and minima known as fine structure. DPOAE fine structure is produced by the shifting phase relationship in the ear canal, between the generator (distortion) and characteristic frequency (reflection) components, each from a different cochlear region and according to theory, reflecting distinct generation mechanisms. The analysis of these dual DPOAE components and of DPOAE phase in newborns may provide a window into targeted aspects of cochlear physiology during development. $2f_1$-f_2 DPOAE fine structure was recorded from 15 young adults and 27 newborns over a 3-octave range using primary tones swept at 8 sec/octave with a fixed f2/f1 ratio of 1.22 and stimulus levels of 65-55 dB SPL. DPOAE group delay, as well as magnitude and phase of each DPOAE component were compared between age groups. Additionally, in conducting these experiments, fundamental differences in DPOAE fine structure recorded with two common probe microphones became apparent, prompting probe comparisons. Results show narrower DPOAE fine structure spacing, longer group delay (steeper phase gradient) in the low frequencies and a stronger relative contribution from the CF component in newborns. These age differences may reflect residual immaturities in the peripheral auditory system at term birth. Hypothesized sources include: 1) Immature basilar membrane motion in the apex of the cochlea, 2) The pristine condition of the newborn cochlea (compared to the aging adult cochlea that has been exposed to noise and ototoxins); 3) Immature middle ear transmission and/or 4) Immature medial efferent regulation of OHC function. In addition to age effects, clear differences were identified between DPOAE features recorded with the ER10B+ and ER10C probe microphone systems, including a marked DPOAE phase shift observed only when recording with the ER10C probe.

142 Stimulus Frequency Otoacoustic Emission Amplitude and Latency Estimates Using Time Domain Methods: Effects of Stimulus Level, Hearing Threshold and Aging

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We compared stimulus-frequency otoacoustic emission (SFOAE) amplitudes and latencies in 32 young subjects and 21 older subjects with hearing ranging from normal to mild hearing impairment. SFOAEs were recorded using a three-interval nonlinear residual technique in a two-tone suppression paradigm, following Keefe et al. (1998). Within this paradigm, a suppressor (s1) was presented first, followed by a probe (s2), and finally, both probe and suppressor were presented simultaneously (s12). The SFOAE is estimated as the nonlinear residual to the measured pressure in the ear in response to s1+s2-s12. Probe frequencies f2=794 or 3175 Hz were presented at fixed levels (L2) from 25 to 65 dB SPL. For each L2, the probe was paired with a 0.97*f2 suppressor (f1) and suppressor level (L1) was varied. Temporal envelopes of probe and SFOAE waveforms were obtained using a linear-least squares method for amplitude and phase extraction at the probe frequency. A time-domain simplex-based optimization methodology was used to fit the filtered probe and SFOAE waveforms to a rising exponential function to extract SFOAE amplitude, latency, and rise-time. We used multivariate random effects regression to jointly model SNR and latency over multiple L2 levels. There was a significant effect of both age and hearing on the bivariate outcome ($p<.001$). (Work supported by the grant NIH 1R21 DK079283-01A1, and VA RR&D Service, Advanced Research Career Development Award, C4447K).

143 Spontaneous Otoacoustic Emissions at Various Postures and Ear Canal Pressures

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The frequency and amplitude of spontaneous otoacoustic emissions (SOAEs) depend on ear canal pressure and on posture. This is presumably mediated by stiffness changes of the cochlear windows. Spectral peaks corresponding to an SOAE have a finite width due to the interactions of the

emission generators with intra-cochlear noise. If the change of ear canal pressure or posture does not influence the intra-cochlear generator signals, the corresponding spectral peak width is not expected to change. In contrast, if the intra-cochlear generator signals do depend on static pressure or posture, the spectral peak width will correspondingly change as well. We recorded SOAEs at various ear canal pressures (range: -200 to 200 daPa in steps of 50 daPa) in 8 normal hearing subjects; and at two postural positions (standing position and in supine position, 30 degrees below horizontal position) in 13 normal hearing subjects. The frequency, amplitude and width of 79 SOAE peaks (41 peaks in ear canal pressure change, and 38 peaks in postural change) were analyzed. On average, emission frequency increased and amplitude decreased by ear canal pressure change and postural change. For the majority of emission peaks, the width varied with ear canal pressure and posture and was inversely proportional to the emission amplitude for a large range of pressures and postures. This behaviour is consistent with that of a mathematical oscillator that interacts with noise: if the oscillator parameters vary such that the oscillation amplitude changes while the noise level is constant, the spectral peak width of the oscillation signal is inversely proportional to the oscillation amplitude. Therefore our results imply that changing the ear canal pressure or intracochlear pressure changed the intra-cochlear emission source signal amplitude.

144 Distortion Product Otoacoustic Emissions Suppressed by Bone-Conducted Ultrasound in Humans

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Humans are capable of hearing sounds with frequencies much higher than we can normally hear in the air when these acoustic waves are delivered via bone conduction, in some cases up to 120 kHz. Many studies have characterized the features of this phenomenon, but there has been little concrete evidence explaining the mechanism itself, in part because most have been limited to psychophysical assessments. One theory suggests that non-linear wave propagation of ultrasonic vibrations from the transducer to the cochlea result in subharmonic content in the normal audible frequency range. Alternatively, others have suggested that the ultrasonic stimulus frequency is preserved in its pathway to the cochlea, activating the base of the basilar membrane at regions dependent on stimulus intensity. The purpose of this study was to investigate these opposing theories using physiological responses in an attempt to further elucidate the mechanism of human ultrasonic hearing.

Distortion product otoacoustic emission (DPOAE) amplitudes were measured at f_2 frequencies from 1 to 18 kHz in normal hearing subjects in the absence and presence of a 30 kHz sinusoidal bone-conducted vibration set at five intensities (0, 3, 6, 9, and 12 dB SL). The presence of bone-conducted ultrasound (BCU) resulted in the suppression of high frequency DPOAEs (>10 kHz). Increasing BCU intensity resulted in a gradual decrease of DPOAE amplitude, as well as a downward spread of suppression to lower frequencies. Furthermore, no significant frequency content was observed outside of the 30 kHz suppressor frequency or the primary frequencies and their respective distortion products, suggesting that suppression was not caused by subharmonic vibrations created in the transmission pathway. These findings suggest that bone-conducted ultrasonic vibrations reach the base of the human basilar membrane and that these vibrations spread toward the apex with increasing BCU intensity.

145 Distortion Product Otoacoustic Emissions Evoked by Amplitude Modulated Tones in Humans

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The inner ear is a nonlinear transducer in which sounds are converted into electrical signals. When it is stimulated by two pure tones (f1 and f2, f1<f2), distortion product otoacoustic emissions (DPOAEs) can be recorded in the ear canal. One prominent DPOAE, the cubic difference tone (CDT, 2f1-f2), is commonly used to evaluate cochlear nonlinearity. The inner ear is also a dynamic system where sounds are processed in real time. Currently, most signals used in hearing tests are static tones. It would be necessary to use non-stationary signals, such as amplitude modulated (AM) tones, to measure the dynamic behaviors of the cochlea. In this study, one of the two primaries was an AM tone whose level and modulation depth (MD) were systemically varied to observe the changes of CDT amplitude. A major finding of this study was that multiple sidebands appeared around the CDT component in the frequency domain. The number and size of the sidebands increased with the level and MD of the AM tone. Amplitude modulated f1 produced fewer sidebands with the first one dominant in amplitude. More sidebands with similar amplitude appeared when f2 was the AM tone. Obtained from inverse fast Fourier transform (iFFT), the instantaneous amplitude of CDT followed the AM tone with a short delay when f1 was modulated. The delay decreased when the AM tone level or MD increased. When f2 served as the AM tone, the CDT showed a more complicated modulation pattern depending on the level of the AM tone. The extent of AM in CDT as measured by the total sideband energy relative to the CDT increased with the MD and with the reduction in AM tone level. Especially when f1 was modulated, the sideband energy exceeded the AM tone. The amplitude behaviors of CDT as a result of AM in the stimulus may reflect the dynamic signal processing in the cochlea. The results suggest that amplitude variation may be important for detecting non-stationary sounds at low levels.

146 Continuously Swept Tone Paradigm for DPOAE Measurements

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The continuously swept tone paradigm (e.g., Long et al. 2008) has been successfully used as a more time efficient method for obtaining broadband DPOAE measurements. In this method, the ratios of two primary tones being played in the ear canal are swept through a range of frequencies using either a linear or logarithmic swept paradigm, and then analyzed using a least squares fit filter. When filter parameters are carefully selected, the results obtained with this paradigm are equivalent to those obtained using a series of fixed tones while the measurement time for the swept tone can be substantially reduced. Other choices of the filter parameters, permits extraction of either the generator or the reflection site components. After a review of the experimental results of the continuous-tone versus swept-tone paradigms we will explore the underlying mathematical framework for these least-squares fit filters and the theoretical basis for the equivalence of the two paradigms in the measurement of DPOAEs in the human ear. Algorithms for selecting optimal parameter values for different outcomes (e.g., full DPOAE fine structure, generator site component or reflection site component) will be explored.

147 Comparison of Two Methods of Recording DPOAEs Over a Wide Frequency Range in a Large Population

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Tonal signals continuously swept in frequency were used in some of the earliest investigations of distortion product otoacoustic emissions (DPOAEs) (e.g., Brown and Kemp, Hear Res, 13:29-37, 1984). This technique has recently come into use again (e.g., Long, Talmadge, & Lee, JASA, 124:1613-1623, 2008) and provides the opportunity to rapidly record DPOAE data at very high frequency resolution. However, stimulus tones at discrete frequencies have been used in the vast majority of reports on DPOAEs to date. DPOAEs recorded using these two techniques need to be thoroughly and carefully compared. Long et. al., (2008) showed the equivalence between DPOAEs recorded using tones at discrete frequencies and tones swept in frequency at 8 or 16 s/octave in six human ears for f2 frequencies up to 4 kHz. In this report we compare DPOAEs recorded using swept- and discrete-frequency stimuli in a population of approximately 100 human ears. DPOAEs were recorded on the same day using two different hardware and software setups from each ear between f2 frequencies of 0.5 and 16 kHz using a stimulus frequency ratio of 1.22 and three stimulus level combinations (L1/L2 = 55/40, 65/55, 75/75). DPOAE levels recorded using the two different techniques will be compared statistically across the whole population as well as in specific age groups.

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148 Mirror, Mirror in the Ear, Scarcely a Reflection Here? Characterizing DPOAE Components in Four Species

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DPOAE level/phase maps were acquired from humans, chinchillas, rabbits, and rats for the $2f_1$-f_2 and $2f_2$-f_1 DPOAEs. To construct these maps, DPOAEs were measured in DPOAE-frequency steps of ~44 Hz from 0.5-6 kHz in response to primary-tone sweeps using constant f_2/f_1 ratio increments of 0.025 from 1.025-1.5. DPOAE level was directly plotted, while phase was corrected for primary-tone phase variation and unwrapped before plotting. Maps were collected with and without an interference tone (IT) placed either 44 Hz below the DPOAE frequency (f_{dp}) or at 1/3-octave (oct) above f_2. The IT was presented on alternate trials to minimize time-dependent changes, and vector differences were computed between the two conditions to obtain a residual. In all species, $2f_1$-f_2 DPOAEs showed horizontal phase bands (HPBs) characteristic of distortion emissions at standard f_2/f_1 ratios near 1.2, and varying degrees of vertical phase bands (VPBs) characteristic of reflection emissions for closely spaced f_2/f_1 ratios. When present, $2f_2$-f_1 DPOAEs exhibited VPBs. For humans, ITs at 65 dB SPL placed below f_{dp} (L_1,L_2=75 dB SPL) decreased fine structure and produced the expected residual with VPBs, but large areas with VPBs remained. ITs placed 1/3-oct above f_2 removed most VPBs in humans, and a DPOAE component with HPBs as well. In chinchillas, rabbits, and rats (L_1,L_2=65 dB SPL), ITs at 55 dB SPL placed below f_{dp} produced residuals to various degrees, but all were associated with HPBs. ITs at 75 dB SPL placed 1/3-oct above f_2 in these species typically eliminated VPBs and phase ambiguities in the maps. The resulting residuals were large and contained DPOAE components with both VPB and HPB characteristics. Together, the findings suggest that these nonhuman species do not exhibit DPOAE reflection components with steep phase gradients arising from f_{dp}. However, in all species tested, significant DPOAE components presumably generated basal to f_2 were evident. (NIH DC000613, VA/RR&D C6212L, C4494R, 4613, 6006).

149 DPOAE in Japanese Quail (Coturnix Coturnix Japonica): Defining Optimal Frequency Ratios and Characterizing Reflection and Distortion Source Responses

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Distortion product otoacoustic emissions (DPOAE) allow for observation of subtle changes in hair cell (HC) status and basilar membrane mechanics. Such subtle changes might be expected after HC regeneration in birds yet most studies show no significant change in DPOAE input/output functions or threshold following HC regeneration. Several

factors, including sub-optimal F2/F1 ratios, combining amplitude contributions from reflection and distortion sources, and anesthesia effects may contribute to this apparent lack of change in the face of substantially disorganized HC stereocilia on regenerated HCs. The current investigation examined the contributions of F2/F1 ratio and reflection and distortion sources on the normal DPOAE response in adult quail. Subjects were tested under either inhalant or injectable anesthesia, or both. Fifteen F2/F1 ratios were generated and tested using 5 fixed F2 (1.5-3.5 kHz) with varying F1 yielding F2/F1 ratios from 1.01 to 1.45. Reflection and distortion source components of the DPOAE were determined at a fixed ratio using an inverse FFT (Talmadge, et al 1999). Preliminary results show different F2/F1 ratios, 1.2 to 1.4, are needed for maximal DPOAE responses as F2 changes; amplitudes are unaffected by time or type of anesthesia within each session. Fine structure was evident throughout the range tested with reflection source phase dependent on frequency. Distortion source phase was independent of frequency and contributed more to the overall amplitude of emission. Our results in quail are similar to results in young chickens (Bergevin, et al 2008) confirming the presence of two distinct mechanisms for avian DPOAE generation analogous to the reflection- and distortion-source mechanisms in mammals. Future studies focusing on the two source contribution to the avian DPOAE may provide a more sensitive indicator of changes in HC status and basilar membrane properties following HC regeneration. Supported by NIDCD R01DC001372 & NIH P30 DC04664

150 Otoacoustic Emission Temperature Dependence Across the Lacertilia

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Given their robust emissions and diverse inner ear anatomy, lizards provide an excellent model for studying otoacoustic emission (OAE) generation mechanisms. We examined twelve different species of lizards with widely varying morphology, such as tectorial membrane (TM) structure and number of hair cells. Both spontaneous and evoked emissions were measured under two body-temperature conditions: ambient (~24-26° C) and warm (~32-34° C). Body temperatures were controlled via a regulated heating pad. For evoked emissions, low-level stimuli (10-45 dB SPL) were used. Spectral peaks and valleys in the magnitude of spontaneous and evoked emissions shifted upwards in frequency with increasing body temperature (by ~15% across the two conditions for most species), consistent with previous observations including neurophysiological studies. For evoked emissions, magnitudes extended out to significantly higher frequencies in the warm condition while phase-gradient delays were relatively insensitive to temperature. Differences in temperature-dependence were observed across species and appear to correlate with variations in TM structure. Specifically, the influence of temperature upon evoked OAEs appears less pronounced in species where the TM is either absent or discretized (sallets) as opposed to continuous over the majority of the papilla. For species lacking a continuous TM, our results are well described by a model (consisting of coupled oscillators with a small degree of irregularity) that assumes increasing body temperature produces an upward shift in the characteristic frequency of the underlying auditory filters. Our results suggest that body temperature has a strong effect upon the frequency range of auditory sensitivity in all lizards, but little influence upon sharpness of tuning (at least in species lacking a continuous TM).

151 Weak Inward Rectifier Channels in Root Cells Mediate Cochlear Potassium Recirculation

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A number of mechanisms regulate perilymphatic potassium levels, thus ensuring the sensitivity of auditory transduction and the longevity of sensory hair cells and neurons. Endolymphatic K^+ enters sound-stimulated hair cells via mechano-electrical transduction channels, exits via voltage-gated channels in their basolateral membrane, and is siphoned by underlying supporting cells. Hypothetically, K^+ is redistributed to the lateral wall by the epithelial cell gap junction network. To complete the recirculation, root cells in the outer sulcus are assumed to secrete K^+ from their basolateral processes, which are ensheathed by the processes of type II fibrocytes in the spiral ligament. Following its uptake by fibrocytes, K^+ is returned to stria vascularis via the connective tissue gap junction network, before its re-secretion into endolymph.

In order to determine the molecular basis of K^+ secretion from the epithelial gap junction network, we have carried out patch clamp recordings from root cells in a guinea pig lateral wall slice preparation. Root cells were coupled via gap junctions, as evidenced by extensive dye transfer. Gap junctional coupling could be abolished using 1-octanol. In uncoupled root cells, bath-applied Ba^{2+} blocked weakly inwardly rectifying currents. The *I-V* relationship of the Ba^{2+}-sensitive currents reversed around E_K. Kir4.1 immunofluorescence was detected in root cell bodies, and in their basolateral processes. Immunogold experiments localized Kir4.1 to the membrane of root cell processes.

In summary, our results suggest that the membrane properties of root cells are consistent with their hypothesized role in cochlear K^+ homeostasis. Root cells can carry out K^+ "spatial buffering" via extensive intercellular gap junctional coupling. The secretion of K^+ from the epithelial gap junction network to perilymph in the lateral wall is most likely mediated via Ba^{2+}-sensitive weak inward rectifier channels, comprising Kir4.1 subunits.

152 ClC-2 Chloride Channel in Reissner's Membrane

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Sensory transduction in the cochlea depends on regulated ion secretion and absorption. Flux studies have provided evidence for Cl^- transport by Reissner's membrane

(Konishi & Hamrick, 1978) and biochemical assays demonstrated a highly-active cAMP signal pathway (Thalmann & Thalmann, 1978). The present investigation utilized whole cell patch clamp, gene array and RT-PCR to determine the presence of Cl^- channels and transporters in mouse Reissner's membrane and to test for regulation by cAMP. Whole cell patch clamp recordings from epithelial cells under conditions where Cl^- was the only major permeable ion showed strong inward rectification. Channels expressed in the epithelial and/or mesothelial cells include ClC-2, Slc26a7 and ClC-Ka, but not ClC-1, ClCa1, ClCa2, ClCa3, ClCa4, Slc26a9, ClC-Kb, Best1, Best2, Best3 or the beta-subunit of ClC-K, barttin. ClC-2 is the only channel present that is a strong inward rectifier. The inward currents matched additional key characteristics of ClC-2 Cl^- channels, including activation by lowered external pH and inhibition by the divalent cations Zn^{2+} and Cd^{2+}. Further, inward currents were stimulated by membrane-permeant analogs of cAMP. Electroneutral Cl^- transporters found to be expressed in Reissner's membrane include K^+/Cl^--cotransporter isoforms Kcc1, Kcc3, Kcc4, anion exchanger isoforms Ae2 and Ae3 but not Kcc2, Ae1, Ae4, Slc26a3 or Slc26a6. This is the first direct evidence that Reissner's membrane epithelial cells contain a transport pathway for Cl^- under control of cAMP mediated by ClC-2. Supported by NIH grants R01-DC000212 and P20-RR017686

153 The Fibrocyte-Vascular Coupling in Control of Cochlear Blood Flow

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Increased hair cell activity must be accompanied by rapid, spatially localized delivery of elevated oxygen and glucose. The present study reports a local control mechanism for regulation of cochlear blood flow (CBF). Transmission electron microscope and fluorescence confocal images show fibrocytes are spatially distributed near pre-capillaries of the spiral ligament. Immunohistochemical techniques reveal that inter-connected fibrocytes are positive for Na+/K+ ATPase â-1 and S100. Connected fibrocytes display high intracellular signals of a fluorescent calcium indicator, fluo-4, compared to other cells in the cochlear lateral wall. Elevation of Ca2+ in fibrocytes, induced through photolysis of the caged divalent ion chelator NP-EGTA, results in Ca2+ signal propagation to neighboring vascular cells, including pericytes and endothelial cells, and induces contraction or dilation of the capillary. A cytochrome P450 epoxygenase (CYP), catalyzing production of 20-hydroxyeicosatetraenoic acid, and a cyclooxygenase-1, catalyzing production of PGE2, were found to be centrally involved in this fibrocyte-mediated control of capillaries. While increased sound stimulation to the ear increases CBF, acoustic-evoked vasoactivity is blocked by an inhibitor of COX1. Our findings demonstrate the key role of fibrocyte–to-vascular cell signaling in regulating cochlear blood flow, particularly in meeting metabolic needs during increased sound activity.

154 Lactate Dilates Pre-Capillaries of the Spiral Ligament Via Type V Fibrocyte-Pericyte Coupling

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Although lactate has been confirmed to play an important role in the regulation of local blood flow of many tissues, the underlying mechanism of lactate effects on the cochlear blood flow remain unclear. Our previous work showed that extracellular lactate induced the increase of intracellular Ca^{2+} in pericytes located at the pre- and post-capillaries of the spiral ligament. Accordingly, lactate caused the contraction of pericytes and constriction of these microvessels. Surprisingly, some pre-capillaries dilated when exposed to extracellular lactate. To investigate whether nitric oxide (NO), a key vasodilator, is involved in the lactate induced vessel dilation, time-lapse photography was used to visualize changes in the lumen diameter of the pre-capillaries of the spiral ligament. Fluo-4 and 4, 5-diaminofluorescein diacetate were used to measure Ca^{2+} and NO levels respectively. An immunofluorecent method was used to check the expression of nitric oxide synthase (NOS) in the cochlear lateral wall. During lactate exposure, NO levels in both pericytes and type V fibrocytes increased and vessel diameters dilated. This lactate induced dilation was totally blocked by pre-treatment of L-NAME, a non-specific inhibitor of NOS, suggesting the dilation was caused by NO. Immunohistochemistry showed that no Ca^{2+}-sensitive eNOS and nNOS expression were detected in pericytes. In contrast, type V fibrocytes had a robust expression of nNOS. We speculated from these results that type V fibrocytes were the source of NO production induced by lactate, which was further supported by photolysis of caged calcium in fibrocytes and pericytes. Photolysis caused increases both calcium and NO levels in fibrocytes, but it failed to up-regulate NO levels in pericytes in spite of the enhancement of intercellular calcium. These data reveal the role of lactate and NO in regulating cochlear microvessel tone and provide a mechanism for control of blood flow by fibrocyte-pericyte coupling.

155 Structure and Function of the Blood Labyrinth Barrier in the Developing Mouse Cochlea

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In this study, the structure of the blood labyrinth barrier (BLB) in the adult and postnatal days 1, 3, 7, 10, 14 mouse cochlea was investigated with immunohistochemistry

combined with confocal fluorescence and transmission electron microscopy (TEM) techniques. TEM examination of capillaries revealed that the BLB of the adult mouse was composed of endothelial cells, a high population of pericytes and perivascular macrophages. The endothelial cells contacted each other via tight junctions. Pericytes were tightly positioned adjacent to the endothelial cells and embedded within the basement membrane. Perivascular macrophages were distributed regularly along the microvessels. The serum protein IgG was observed to be restricted to the lumen of capillaries of the stria vascularis. Junction proteins such as ZO-1 and occludin were highly expressed in the endothelium. In contrast, in the pre-mature (postnatal day 1 to day 10) mice, TEM revealed that the endothelium was discontinuous, the basement membrane was incomplete, and IgG was observed to leak from the capillaries of the stria vascularis. The distribution of IgG outside of the capillary of the 1-, 3-, 7- and 10-day-old mice was significantly greater compared to the adult mice. The steady increase in the expression of occludin and ZO-1 was found from postnatal day five. Around postnatal day 14, endothelium was continuous and tight junctions between endothelial cells were uninterrupted. Perivascular cells such as pericytes and macrophages could be identified in the BLB areas. These observations indicate that the endothelial cell transport system is more robust in the developing cochlea and that the BLB becomes mature and intact by 14 days after birth in mice. The special features of the BLB at different developing stages create new opportunities for drug and gene deliveries to the ear in young mice with the consideration that potential increased ototoxicity due to lack of barrier integrity in young mice could occur.

156 Gap Junctional Intercellular Coupling in Lateral Wall Fibrocytes of the Developing Cochlea

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Intercellular communication via gap junctions is thought to be essential for K+ cycling and buffering in the mature cochlea. Previous dye transfer studies (Jagger, ARO, 2009) have shown extensive coupling between epithelial supporting cells and also between fibrocytes of the adult lateral wall, but these two networks are distinct and are not directly coupled to each other. It is thought that K+ ions buffered by the epithelial supporting cells are transferred to fibrocytes and returned via gap junctions to the stria vascularis and secreted back into the endolymph.

The role of gap junctional intercellular coupling (GJIC) during lateral wall development and K+ cycling has not been determined. In rats connexin expression is initially very weak in the lateral wall at post-natal day 0 (P0), but then rapidly increases in type I fibrocytes surrounding the stria vascularis at P2. From here connexin expression expands throughout the lateral wall fibrocytes before reaching adult expression levels (~P14).

To investigate the coupling properties of developing fibrocytes, dye transfer studies have been carried out in rat cochlear slices (P0-P6). Developing type I fibrocytes revealed extensive dye spread around the stria vascularis and correlated with connexin immuno-labelling. Cells injected just outside this region (type II fibrocytes) revealed neurobiotin spread between ~10-20 cells in a columnar direction that emanated from the outer sulcus/root cell region and spread to the edge of the type I zone. Intriguingly, neither connexin26 nor connexin30 (the two predominant subtypes) were detected in this region. A third fibrocyte type was also identified which had no coupling to other cells (no neurobiotin transfer).

Results here suggest the presence of three distinct zones according to the extent of GJIC during development of lateral wall fibrocytes. These may represent unique pathways for early K+ cycling in the cochlea.

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157 Characterization and Analysis of the Cochlear Strial Vasculature Proteome and the Role of Na^+, K^+-ATPase in the Blood Labyrinth Barrier

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The blood-labyrinth barrier (BLB) plays an important role in regulating the chemical composition of labyrinthine fluid and maintaining the functional integrity of the inner ear. In this study, to provide a broad view of the protein patterns of the BLB, we used a mass spectrometry-based, shotgun proteomics approach to identify the proteins from isolated and purified cochlear stria vascularis capillaries in CBA/CaJ mice. Mass spectrometry identified over 500 proteins, including protein function in energy metabolism, proteins involved in ion transport, Ca^{2+}regulation, signal transduction and proteins related to stress-response. In particular, the Na^+, K^+-ATPase alpha 1 subunit was the most abundant protein of the vascular tissue in the ear. Further immunohistochemistry studies confirmed Na^+, K^+-ATPase alpha 1 express predominantly in the abluminal BLB membrane. To investigate the involvement of Na^+, K^+-ATPase in maintaining the BLB integrity, the expression and activity of Na^+, K^+-ATPase associated with the changes of expression of tight junction proteins as well as barrier permeability were examined in normal and noise-exposed mice. We found that vascular permeability was significantly increased along with a decrease in tight junction membrane contact points and decrease in Na^+, K^+-ATPase activity immediately following noise exposure at 120 dB SPL broadband noise, 3 hours per day for 2 consecutive days. In vitro, inhibition of Na^+, K^+-ATPase activity by ouabain corroborated the in vivo studies, with swelling of capillary endothelial cells and impaired barrier function, supporting a functional link between Na^+, K^+-ATPase and the vascular barrier in the stria. These results implicate the participation of Na^+, K^+-ATPase in maintaining the functional integrity of the blood labyrinth barrier and shed light on the potential role of Na^+, K^+-ATPase in noise-induced hearing loss.

158 The Proteome of Human Perilymph

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Current diagnostic tools limit a clinician's ability to differentiate between many possible causes of sensorineural hearing loss at the level of the cochlea. This leads to the frequent diagnosis of the idiopathic condition, leaving patients without a clear prognosis and only general treatment options. Filling this diagnostic gap is important for the development of individualized treatment strategies ("personalized medicine") and critical for successful implementation of preservative and restorative therapies that may be developed in the future. As a first step toward developing new diagnostic tools, we analyzed the proteome of human perilymph using mass spectrometry-based proteomics techniques.

Four samples, containing pooled perilymph specimens obtained from 6 patients with vestibular schwannoma (VS) and 6 patients undergoing cochlear implantation (CI), were analyzed by LC-MS(/MS). Of the 245 proteins identified at high confidence within the four samples, 55 were found in every sample. This subset of identifications was used to conservatively define the proteome of normal human perilymph, generating a list twice as large as the known proteome (~30 proteins) and containing almost 90% of the previously identified proteins. Comparison with other bodily fluids (cerebrospinal fluid and blood plasma) showed significant similarity in protein content; however, a quantitative comparison of protein expression could not be made. Fifty-five percent of proteins identified in human perilymph were found to have direct homologs within the proteome of murine perilymph, indicating the potential applicability of mouse models in future studies. A list of 22 candidate biomarkers of VS was generated by comparing the VS and CI samples using uniqueness criterion and network analysis. This list will be used as the starting point for future proteomic investigations targeted at discriminating between VS tumors that induce hearing loss and those that do not.

159 Proteomic Analysis of Endolymphatic Sac Luminal Fluid in Patients with an Enlarged Vestibular Aqueduct (EVA) Associated with SLC26A4 (PDS) Mutations

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Endolymphatic sac (ES) is a highly specialized organ which is speculated to regulate the volume of endolymph. The ion composition and protein concentration in the ES luminal fluid are totally different from those of cochlea and vestibule. However, the protein profile of human ES luminal fluid has not been thoroughly reported. In the present study, we sought protein profile of ES luminal fluid which was obtained during cochlear implantation surgery (n=4) and compared it with that of plasma by proteomic analysis with 2-DE protein electrophoresis and MALDI-TOF method.

Protein concentration of ES luminal fluid is 500-1300mg/dL, which is lower than plasma (~35%). The protein profile is different from that of plasma as well. The major proteins of plasma such as albumin, immunoglobulin, ceruloplasmin, glycoprotein, VitD binding protein and ¥á1-antichymotrypsin were also detected in the ES luminal fluid; however, the concentration of each protein in the luminal fluid was different from that of plasma. Pre-albumin, several mitochondrial proteins, proteins involved in phagocytosis, ryanodine receptor 1 and several unnamed proteins were detected only in the ES luminal fluid, but not in the plasma.

ES luminal fluid has been reported to be originated from plasma in the studies using animals since it showed similar protein profile to plasma. In the present study, some major proteins in ES luminal fluid were also detected in the plasma, but their concentration was different from those of plasma. Furthermore, a lot of proteins detected in the endolymphatic sac luminal fluid did not appear in the plasma. Therefore, it is tempting to speculate that the ES luminal fluid is likely to be selectively filtered from plasma and partly originated from endolymphatic sac and inner ear.

160 Loss of Slc26a7 in Reissner's Membrane Leads to Hearing Loss in Mice

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Slc26a7 is a member of the Slc26 family that includes both pendrin (Slc26a4) and prestin (Slc26a5). Slc26a7 can function in two modes, as a Cl^- channel or as a Cl^-/HCO_3^- exchanger. Gene array analyses revealed high levels of Slc26a7 expression in Reissner's membrane, which prompted us to investigate whether Slc26a7 is functional in Reissner's membrane epithelial cells and whether Slc26a7 is essential for cochlear homeostasis, for hearing and, by extension, for balance. Cl^- currents were recorded in whole-cell patches of Reissner's membrane epithelial cells. Expression of Slc26a7 protein was localized by immunocytochemistry in developing and adult mice. Hearing and balance were evaluated by auditory brain stem recordings and RotaRod testing and cochlear morphology was assessed by immunocytochemistry in wild-type ($Slc26a7^{+/+}$) and in mice lacking Slc26a7 ($Slc26a7^{-/-}$). Reissner's membrane epithelial cells expressed Slc26a7 protein in the basolateral membrane and carried Cl^- currents that carried NO_3^- significantly better than Cl^- and that were characterized by a slight outward rectification when studied with symmetrical NMDG-Cl solutions in whole-cell patches. The onset of protein expression was postnatal. At 10 month of age, two

out of three Slc26a7$^{-/-}$ mice studied so far had a significant hearing loss at 16 and 32 kHz. No balance deficits were detected. Cochlear morphology was evaluated in one deaf Slc26a7$^{-/-}$ mouse. Reissner's membrane had a reduced number of nuclei and enlarged apical surface areas of the epithelial cells. Outer hair cell losses were found in the 16 and 32 kHz regions. In conclusion, the data demonstrate that Reissner's membrane epithelial cells express the Cl^- channel Slc26a7 in the basolateral membrane. Based on a very limited dataset it appears that lack of this channel leads to a degeneration of Reissner's membrane, to a loss of outer hair cells and to a loss of hearing.

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161 Determination of Molecular and Functional Properties of Voltage-Gated K^+ Channel, K_V1 in Spiral Ganglion Neurons

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K^+ currents are responsible for regulation of the resting membrane potential (V_{rest}), duration of repolarization phase of action potential (AP), frequency of adaptation and afterhyperpolarization (AHP) phase of AP in spiral ganglion neurons (SGNs).

To understand the underlying molecular and functional mechanisms of membrane excitability in SGNs, we are investigating the roles of K_V1 channels. Here, we focused on assembly of the subunits of K_V1 channels and their partners that confer native K^+ channels.

K^+ channel subunit profile was studied using immunostaining on primary culture of SGNs at different ages. To determine the assembly of K^+ channel, we used co-immunoprecipitation (Co-IP) method. Potassium currents were recorded using an extracellular solution (in mM, NaCl 125, KCl 6, $CaCl_2$ 0-8, D-glucose 10, $MgCl_2$ 1, HEPES 10), and intracellular solution (in mM, KCl 120, Na_2ATP 5, $MgCl_2$ 2, HEPES 10, EGTA 1-10, or BAPTA 1-10, D-glucose 10).

We will present data which demonstrate that multiple K_V1 channel subunits are expressed in SGNs. Also important, the K^+ current properties in SGNs are established by the promiscuous interaction between different subunits of K_V1 channels.

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162 What's with All the JNK in the Cochlea?

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c-Jun N-terminal kinase (JNK), a member of the mitogen activated protein kinase family is involved in responses to cellular stress and in apoptosis. JNK is activated both in vivo and in vitro in apoptotic spiral ganglion neurons (SGNs). While this suggests a possible use for JNK inhibition in maintaining neuronal survival, blockade of JNK inhibits neurite initiation and inhibits, by ~80%, growth of neurites already established. Imaging of the plasma membrane and microtubules showed that this reduction in SGN neurite growth was not accompanied by growth cone collapse, nor by gross morphological abnormalities in the growth cone or microtubules. Consistent with this, preliminary results using time-lapse microscopy suggest that, while a decrease in translocation across the substrate and neurite elongation is observed, motile structures of cells and growth cones, such as lamellipodia remain after JNK inhibition.

To facilitate biochemical studies of the mechanisms by which JNK inhibits neurite growth, we used the neuronal PC12 cell line, which extend neurites in response to NGF. PC12 cells, alone or co-cultured with SG Schwann cells, exhibited greatly reduced neurite growth when JNK was inhibited. Furthermore, this inhibition was associated with a significant decrease in cell-substrate adhesion. Therefore, we have begun to investigate the JNK-dependent mechanism controlling neuron-substrate adhesion. PC12 adhesion to collagen, like SGN adhesion to laminin depends on beta1 (CD29) integrins. Cell surface expression of beta1 integrin (CD29) and its associated proteins was assessed via immunoprecipitation of surface-biotinylated protein. There was no significant change in expression of CD29 or associated proteins after JNK inhibition. We are now assessing upstream proteins involved in adhesion, motility and cytoskeleton dynamics to determine the link between JNK signaling, de-adhesion, and neurite growth.

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163 Signalling Mechanisms Involved in Hearing Loss Associated to Insulin-Like Growth Factor I Deficit

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Insulin-like growth factor (IGF) I is fundamental for the regulation of cochlear growth, differentiation and metabolism, and its mutations are associated with hearing loss in mice and men (1,2). IGF-I and its high affinity receptor IGFR1 are expressed in specific spatiotemporal patterns in the cochlea during development. Peak expression of IGF-I occurs during the late embryonic and neonatal periods, being reduced in the adult, a trait that associates with age-related hearing loss. IGF-I/IGFR1 actions are mediated by intracellular signaling networks primarily activated by the phosphorylation of insulin

receptor substrates (IRS2) and down-regulated by the tyrosine phosphatase PTP1B, IRS2 activation leads to the sequential phosphorylation of lipid and protein kinases (c-RAF). IGF-I/IGFR1 output is connected to other signaling pathways activated by either lipid receptors (Lpa1) or G protein-mediated receptors (RasGRF1/2) (3,4).
To identify the main effectors of IGF-I in hearing, the analysis of auditory brainstem responses and cochlear morphology of mutant mice deficient in the above mentioned signaling molecules was carried out. In parallel, susceptibility to noise-induced hearing loss was studied in these mutant mice by using an acoustic reverberant chamber and a violet noise, both specially designed for noise exposure (5). By using these complementary approaches we have identified that both IRS2 and c-RAF are essential for cochlear development and hearing.
This work was supported in part by CIBERER (ISCiii) and MICINN (SAF2008-00470), in collaboration with the groups leaded by Drs. U. Rapp, AM Valverde, G Estivill-Torrús, A Fernández-Medarde and E Santos.

164 Cell Death Pathways in Acquired Hearing Loss

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Among the diverse causes of acquired hearing impairment, drug- and noise-induced as well as sensorineural age-related hearing loss have extensively been studies in animal models. A long-established anatomical hallmark is the preferential loss of outer hair cells and, in recent years, reports from several laboratories have also suggested intriguing commonalities between the molecular events associated with these pathologies. We have studied signaling pathways in outer hair cell death caused by aminoglycoside antibiotics, noise trauma and age in the mouse in vivo. Increased oxidative stress appears to be an early event, followed by changes in cell survival and cell death pathways. Classic apoptosis is the major form of cell death but we also observe necrosis in all three insults. Additional shared alterations in pathways include the translocation of endonuclease G, a mitochondrial protein, to the nuclei of outer hair cells. In contrast, activation of caspase 3 and p38MAPK/JUNK is involved in both noise-induced and age-related but apparently not in aminoglycoside-induced outer hair cell death. Several other signaling cascades potentially contributing to a diversity of cell death pathways, such as those linked to calpain or cathepsin, have not yet been investigated in all pathologies. The sum of our observations points to an up-regulation of cell death pathways (combined with a decreased survival signaling) as major molecular causes of hair cell death by the three different insults. Comparison of both shared and specific cellular responses might yield further insight into the development of auditory trauma and point to avenues of protection or attenuation.
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165 Neurotrophic and Apoptotic Signaling in Spiral Ganglion Neurons After Hair Cell Loss

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To model sensorineural hearing loss, we inject kanamycin (400 mg/kg) into rat pups daily from postnatal day 8 (P8) to P16, i.e., prior to hearing onset. Rats deafened in this manner never exhibit a detectable auditory brain response and the cochleae are devoid of hair cells by P23. Also, NT-3 mRNA levels have fallen to <10% of control (age-matched normal hearing rats) by P23. Nevertheless, at this time, spiral ganglion neuron (SGN) peripheral axons remain in contact with the organ of Corti, terminating immediately adjacent to the location at which the inner hair cells had been. Also, prosurvival signaling, detected as phosphorylated CREB, is still at control levels in SGNs and proapoptotic signaling, detected as phosphorylated Jun, is at control levels. One possible explanation for this apparent lack of degeneration over a week after hair cell loss is that organ of Corti supporting cells continue to provide trophic support to the SGNs, either residual NT-3 or another factor. However, if hair cell death is slow in this deafening paradigm, then it is possible that some inner hair cells persist after P16, In that event, SGNs at P23 will have been deprived of hair cell-derived neurotrophic factor for only a short time and have not yet begun to degenerate. To this end, we have started to investigate the loss of hair cells and of NT-3 in the third postnatal week in the kanamycin-injected rats. Preliminary data using anti-calretinin immunofluorescence indicates that some inner hair cells do persist until the end of the deafening period. Studies in progress will quantify NT-3 levels in this time interval.

166 Cre/lox Mediated *in Vivo* Mosaic Cell Ablation to Investigate Early Stages of Degenerative Disease: Generating a Model for the "Onset" of Gradual Hearing Impairment

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Most degenerative diseases begin with a gradual loss of specific cell types, and cell loss eventually reaches a threshold for symptomatic onset. Early stages of degenerative diseases are typically caused by partial loss of particular cell types, yet investigating the loss of cells has so far been difficult because creating reproducible models for limited ablation of a specific cell type was technically challenging.
Here, we generated a transgenic mouse model, Mos-iCsp3, in which an engineered dimerizable caspase-3 was expressed stochastically within a defined domain through a lox-mismatched Cre/lox expression cassette, to ablate a subset of specific cell types in a mosaic pattern. Target cell type is defined by the choice of Cre mouse; timing of the ablation is determined by the administration of a chemical

inducer, AP20187, that activates iCsp3 through dimerization. By crossing the mouse with a Pou4f3-Cre mouse, we created a stochastic mosaic-patterned hair cell ablation both *in vitro* and *in vivo*, along with two functionally reversible models in pancreatic β-cells and the skin with the appropriate Cre-mice. *In vitro* study showed that the hair cell loss was followed by coverage by surrounding supporting cells within 72 hrs of ablation by dimerizer. The percentage of hair cell loss in this model *in vivo* was 24.6 ± 1.8%, which led to irreversible hearing impairment with a moderate threshold shift (10.2 dB by ISO-DP). Immunostaining for Musashi-1 showed intact supporting cells. Spiral ganglion neurons were not changed by the dimerizer treatment. This rapid cell ablation model recapitulates an early stage of gradual deafness due to the loss of hair cells and will be useful for evaluating the "onset" of hearing impairment and potential regenerative therapies.

167 Inner Hair Cells Are Not Required for Survival of Spiral Ganglion Neurons in the Adult Cochlea

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Degeneration of spiral ganglion neurons (SGNs) is an important component of sensorineural hearing loss. The notion that inner hair cells (IHCs), and the trophic factors they produce, are necessary for SGN survival arises from the observation that IHC loss after acoustic trauma or ototoxic drugs is followed, over weeks to months, by SGN degeneration. However, recent studies suggest that the neurotrophin (NT) release from the IHC's supporting cells (SCs) may be more critical for SGN survival in the adult (Stankovic et al., J. Neurosci. 24:8651, 2004). To re-examine the role of IHCs in long-term SGN survival, we used a new model of selective IHC loss that does not involve noise or ototoxic drugs. Mice lacking the gene for the high-affinity thiamine transporter (Slc19a2) have normal cochlear structure and function when fed a regular (thiamine-rich) diet. However, dietary thiamine restriction causes widespread, rapid (within 10 days) and selective loss of IHCs (Liberman et al., JARO 7:211, 2006).

Groups of wild type and Slc19a2 -/- mice were fed a regular diet until 6 wks of age, then placed on thiamine restriction for 26 days, and finally returned to a regular diet for 12 wks. Additional control groups of both genotypes were maintained on a regular diet throughout. At the end of the observation period, ABR thresholds were severely elevated only in the Slc19a2-null group that had been exposed to the low thiamine diet. Serial cochlear sections from this group revealed selective and near-total IHC loss throughout the basal half of the cochlea; however, IHC supporting cells appeared normal and continued to immunostain strongly for the glutamate transporter (GLAST). SGN cell bodies appeared normal, and counts of their peripheral axons in the osseous spiral lamina were unchanged from control. In addition, immunostaining for vesicular acetylcholine transporter revealed a normal efferent innervation, even in the IHC area.

These results show that SGNs do not require IHCs for survival in the adult ear, for at least as long as 3 months. They also suggest that SGN death in regions of noise- or drug-induced IHC loss is due to direct effects on SGNs themselves and/or to alterations in SC function.

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168 Spiral Ganglion Neuron (SGN) to Organ of Corti (OC) Projections After Hair Cell (HC) Loss and Their Relationship to Apoptotic Signaling

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SGNs die slowly after the loss of hair cells (HCs). It is not yet known what provides trophic support for the SGNs during this period nor why it is ultimately insufficient in preventing SGN death. One possibility is that NT-3 derived from OC supporting cells (OCSCs) maintains SGN survival after loss of HCs. However, we have shown that NT-3 levels in the OC fall immediately to a very low level after HC loss and remain at this level throughout the >3 month period over which SGNs die. This implies that NT-3 from OCSCs is not what maintains SGNs after HC loss. Nevertheless, it is still possible that trophic support from the OC, other than NT-3, contributes to maintenance of SGN survival. This predicts that SGNs with peripheral axons still extending to the OC will not exhibit evidence of proapoptotic signaling (e.g., phosphoJun). In contrast, SGNs with peripheral axons that have already degenerated post-deafening will be likely to show proapoptotic signaling and unlikely to show prosurvival signaling (e.g., phosphoCREB). To determine this, we must trace individual axons from soma to the OC and detect phosphoCREB and phosphoJUN in the soma of each labeled cell. We are therefore fluorescently labeling small numbers of SGNs so that individual peripheral axons can be traced, and doing so in a manner that is compatible with immunofluorescence detection of apoptotic or intracellular signaling. Our current methods include use of transgenic mice expressing different fluorescent proteins in small random sets of neurons (Brainbow or Ngn1-CreER:Z/EG) and fluorescent lipophilic dye labeling methods. Although all methods have shown some promise, most effective is a modification of a fluorescent dye tracing technique in which the dye is applied to the cut end of the VIIIth nerve near the base of the cochlea. To ensure that only a small number of fibers are labeled, the dye is applied from 1 µm coated tungsten particles inserted into the nerve.

169 Re-Organisation of the Organ of Corti Following Hair Cell Loss

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Most forms of acquired sensorineural hearing loss, as a result of environmental agents or ageing, occur due to the

progressive loss of hair cells from the organ of Corti. Severe cochlear trauma, not only results in the loss of the majority of sensory cells but also in changes to the non-sensory cells within the organ of Corti to the extent that a non-specialised 'flat' epithelium replaces extensive regions of the organ of Corti.

Our study investigates the progression from an organised organ of Corti to a non-specialised epithelium using an *in vivo* mouse model. Immunohistochemistry was used to examine the characteristics of the cells comprising the epithelium following extensive damage, in particular whether remaining cells retain potassium channels and whether there is alteration in pattern expression of the connexins (cx26, cx 30). In addition, analysis of gross structural alterations of the cochlea, using both transmission and scanning electron microscopy, were carried out.

Following loss of outer hair cells, Deiters' cells remain differentiated for some time. Subsequently, cells from the Hensen's cell region and outer sulcus migrate towards the pillar cells. At later stages, in some regions, pillar cells collapse resulting in a loss of the tunnel of Corti and ultimately this degeneration leads to a flat epithelium, with squamous-like epithelial cells replacing the supporting cells.

Genetic background influences the rate and extent of reorganisation. CBA/Ca mice retain an organised organ of Corti even 6 months after trauma. The organ of Corti in C57Bl6 mice progress to a flat epithelium more rapidly within a 4-6 week period post-treatment. These 'flat' epithelial regions are not subject to a base to apex gradient, but seem to be located sporadically along the length of the organ of Corti. This correlates closely to the structural changes observed in human cochleae following extensive or prolonged trauma.

170 Role of IL-10 in Inner Ear Inflammation Secondary to Otitis Media

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Recently, we demonstrated that spiral ligament fibrocytes (SLFs) up-regulate MCP-1/CCL2 upon exposure to NTHI through TLR2-dependent NF-kB activation. Since the inner ear is an immune privileged organ, it is believed that there exists a protective mechanism inhibiting its excessive inflammation. We hypothesize that IL-10 plays a critical role in the protection of the inner ear from inflammation secondary to NTHI-induced OM. IL-10 is a potent anti-inflammatory cytokine repressing the expression of inflammatory cytokines. Interestingly, it is known that IL-10 differentially regulates MCP-1/CCL2 expression depending on the stimulatory state of monocytes, but how IL-10 affects NTHI-induced SLF-derived MCP-1/CCL2 expression remains unclear. In this study, we aim to determine a role of IL-10 in inner ear inflammation secondary to NTHI-induced OM. qRT-PCR data showed that recombinant IL-10 markedly inhibits SLF's NTHI-induced up-regulation of MCP-1/CCL2 expression. Accordingly, migration assays showed that recombinant IL-10 suppresses migration of THP-1 cells in response to the conditioned medium of NTHI-exposed SLFs. Furthermore, luciferase assays using a luciferase-expressing vector with an enhancer of MCP-1/CCL2 showed that silencing of IL-10 receptor A in SLFs suppresses the inhibitory effect of IL-10 on NTHI-induced MCP-1/CCL2 up-regulation. IL-10 deficiency of SLFs did not significantly affect NTHI-induced MCP-1/CCL2 expression, suggesting a critical contribution of exogenous IL-10 rather than endogenous IL-10. Our results provide the first demonstration of the involvement of IL-10 in inner ear inflammation secondary to NTHI-induced OM, which may protect the inner ear from immune-mediated damage due to excessive inflammation. Further studies are needed to elucidate an inner ear source of IL-10 as well as a signaling pathway associated. [Supported in part by NIH grants DC008696 and DC006276]

171 SLF-Derived MCP-1/CCL2 Is Involved in Inner Ear Inflammation Secondary to NTHI-Induced Otitis Media

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Although inner ear inflammation secondary to otitis media (OM) is not a common complication of OM, it is clinically important since the incidence of OM is extremely high in children and even mild hearing loss can affect language development. Excessive inflammation may lead to immune-mediate inner ear damage, but the understanding of its molecular pathogenesis is limited, particularly in the case of nontypeable *H. influenzae* (NTHI)-induced OM. In this study, we aim to elucidate a role of spiral ligament fibrocyte (SLF)-derived MCP-1/CCL2 in inner ear inflammation secondary to NTHI-induced OM. We first showed that THP-1 cells actively migrate and invade to the extracellular matrix in response to the conditioned medium of NTHI-exposed SLFs. This migratory activity was markedly inhibited by the viral chemokine inhibitor and by the deficiency of MCP-1/CCL2 in SLFs, which suggests that MCP-1/CCL2 is a main attractant of THP-1 cells among the SLF-derived molecules. We further demonstrated that CCR2 deficiency inhibits migration of monocyte-like cells among the splenocytes in response to NTHI-induced SLF-derived MCP-1/CCL2. The murine model showed that transtympanic inoculation of live NTHI develops inner ear inflammation in 10 out of 14 ears (71%), which is higher than the control group with inoculation of saline (25%) ($p<0.05$). Immunolabeling of the inner ears with OM-induced inflammation showed up-regulated expression of MCP-1/CCL2 in the cochlear lateral wall and limbus, compared to the control group. Unexpectedly, MCP-1/CCL2 deficiency did not significantly decrease the incidence of OM-induced inner ear inflammation (60%), which suggests a compensatory effect of other chemokines *in vivo*. Taken together, we suggest that NTHI-induced SLF-derived MCP-1/CCL2 contributes to OM-induced inner ear inflammation, but further studies are necessary to evaluate an involvement of other SLF-derived chemokines. [Supported in part by NIH grants DC008696 and DC006276]

172 Effect of Vestibular Labyrinth Destruction on Endocochlear Potential and Potassium Concentration of the Cochlea

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Background: Since the very earliest attempts at otologic surgery, it was generally believed that surgical violation of the bony labyrinth was incompatible with hearing preservation. Several recent lines of evidence, however, have challenged this conventional wisdom. Clinical and experimental reports support the fact that under certain circumstances partial labyrinthectomy can result in maintenance of hearing. We have previously reported that endocochlear potential (EP) was preserved during extensive destruction of the semicircular canals of the guinea pig but that vestibulotomy exterminated EP. The mechanism of the hearing impairment caused by labyrinthectomy is open to question. High positive EP (80-90 mV) and high concentration of potassium ($[K^+]$: ~150 mM) are essential conditions for excitation of hair cells. We supposed that the disruption of the membranous labyrinth caused the electrical leakage and electrolyte imbalance. The change of the $[K^+]$ during labyrinthectomy has never been reported. So the specific aim of this study was to examine the change of the cochlear function with recording of EP and $[K^+]$ caused by vestibular labyrinth destruction.

Method: Hartley guinea pigs were divided into 3 groups. 1) Lateral semicircular canal (LSCC) transection with suctioning of perilymph. 2) ampullectomy. 3) vestibulotomy. The EP and $[K^+]$ were monitored using double-barreled ion-selective microelectrodes in the second turn of cochlea.

Results: The results obtained in the present study demonstrate the following: 1) EP = 73.95±7.33 mV (n = 4), $[K^+]$ = 174.08±6.26 mM (n = 4), 2) EP = 80.48±8.17 mV (n = 4), $[K^+]$ = 169.38±6.89 mM (n = 4), 3) EP = 47.63±16.52 mV (n = 4), $[K^+]$ = 142.10±15.28 mM (n = 4).

Conclusion: The results of our experiments demonstrated that EP showed little to mild change in 1) and 2), however it declined drastically in 3). The EP did not recover but $[K^+]$ recovered considerably even in 3).

173 The Effect of the Insertion Speed of Cochlear Implant Electrodes on the Insertion Forces

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Background: Preservation of residual hearing gains more and more importance in cochlear implant surgery. Therefore reduction of the insertion trauma gets crucial. The forces being produced by the insertion of the cochlear implant electrode affect the insertion trauma and the preservation of residual hearing. The effect of the insertion speed on the insertion forces has been hypothesized but not proven yet.

Aim: Our aim was to examine the correlation between the insertion speed and the insertion forces and to study the clinical significance of these findings.

Method: Insertion force measurements were performed while inserting human electrodes in a Teflon scala tympani model with different speeds. For these measurements an Instron 5542 Force Measurement System with a 10 N measurement cell and Nucleus Contour Advance electrodes were used. Additionally, the insertion speed was measured through videos, which were taken during human implantations.

Results: Progressive increase in insertion speeds from 10 mm/min to 200 mm/min resulted in significant, proportional increase in the average insertion force from 0.09 N to 0.185 N and in the maximum force from 0.18 N to 0,42 N, respectively. The insertion speeds being used by the surgeons were found to range between 36 mm/min and 168 mm/min with an average of 86 mm/min.

Conclusions: There is a proportional correlation between insertion speed and insertion forces. High speeds at the stage of the cochlear implant electrode insertion cause significant increase of the forces and consequently most likely additional insertion trauma. Cochlear implant surgeons should apply low speeds during the insertion so as insertion trauma and loss of residual hearing could be reduced.

174 Systemic Dexamethasone as a Hearing-Protection Strategy in Experimental Cochlear Implantation

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Aim: The delivery of pharmaceuticals to the inner ear at the time of surgery is emerging as a promising adjunct to hearing-preservation cochlear implantation. The major challenge for clinical application is to ensure that adequate drug is delivered to the cochlea prior to surgery, given the practical constraints of the operating theatre. Local delivery of dexamethasone to the round window of the guinea pig can afford good protection when applied 1-2 hours prior to implantation, but this approach is likely lengthen clinical surgery unacceptably. Here we investigate whether systemic delivery of steroids may be a better alternative, given that the timing of administration is more flexible.

Methods: After baseline tone-pip auditory brainstem response (ABR) thresholds were estimated, normal-hearing guinea pigs received an intravenous injection of either 0.2 mg/kg or 2 mg/kg dexamethasone or saline (control) [n=6,5,6]. One hour after the injection, a dummy cochlear electrode was implanted 2.25 mm into the basal turn scala tympani, using a soft-surgery approach. ABR thresholds were estimated one month after surgery.

Results: In saline controls, ABR thresholds in the lower- (24 and 32 kHz) and upper- (8 and 16 kHz) basal turns were elevated by more than 22 dB (mean), but hearing in the second turn (2 kHz) was near normal, consistent with previous studies (Chang et al, Hear. Res. 255:67-72

2009). ABR threshold shifts were significantly lower in steroid-treated animals receiving 2 mg/ kg (ANOVA $p<0.01$); protection was observed across the cochlea with a mean threshold elevation of <10 dB. ABR thresholds did not differ from control after delivery of the 0.2 mg/kg dexamethasone dose.
Conclusions: High-dose systemic steroids, similar to those used clinically, were effective in protecting hearing during cochlear implantation. Systemic steroids offer greater flexibility than local methods, in that pre-operative treatment is possible and long intra-operative waiting times are avoided.

175 Cochlear Implant Electrode Array-Eluted Dexamethasone (DXMb) Conserves Hearing and Hair Cells in an Animal Model of Electrode InsertionTrauma Induced-Hearing and Hair Cell Losses: Mechanisms

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Objective: Determine if polymer-eluted dexamethasone base (DXMb) conserves hearing against trauma-induced loss and characterize cell signaling and gene expression in TNFalpha/DXMb treated cultures.
Methods: An animal model of electrode trauma-induced loss of auditory function/hair cells and an *in vitro* model of inflammatory cytokine-induced apoptosis of hair cells tested the otoprotective efficacy of biopolymer (SIBS)-eluted DXMb. Inhibitors and quantification of selected apoptosis-related genes examined the mechanisms of DXMb otoprotection. Significance=$p<0.05$.
Results: DXMb-eluted from SIBS-coated electrode arrays conserved ABR thresholds in an animal model of cochlear implantation and protected hair cells within TNFalpha challenged explants. *In vitro* studies using inhibitors demonstrated that activation of NFkB was required for the otoprotective action of DXMb against TNFalpha ototoxicity. Gene expression studies identified *Bax*, *Bcl-2*, *Bcl-xl* and *TNFR1* as targets of activated NFkB within DXMb-treated/TNFalpha challenged explants. TNFalpha exposure up regulates pro-apoptosis genes, e.g. *Bax*, and down regulates anti-apoptosis genes, e.g. *Bcl-xl*, while treatment of TNFalpha exposed explants with eluted-DXMb reverses inflammatory cytokine-induced changes in gene activity causing a down regulation of *Bax* expression and an up regulation of both *Bcl-2* and *Bcl-xl*. This results in a dramatic shift in the *Bax/Bcl-2* ratio favoring hair cell survival within the TNFalpha/DXMb treated explants. DXMb-treatment also reverses TNFalpha induced elevation of *TNFR1* expression.
Conclusions: Polymer-eluted DXMb retains its otoprotective efficacy activating NFkB signaling which down regulates pro-apoptosis and up regulates anti-apoptosis related genes. Development of a DXMb-eluting electrode array has the potential to conserve a patient's residual hearing allowing for improved electro acoustic based stimulation (EAS) of both auditory neurons/nerves and remaining functional hair cells.

176 Protection of Spiral Ganglion Cells in Vivo After Implantation of Model Electrodes Coated with BDNF-Producing Cells

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Long-term drug delivery to the inner ear may be achieved by functionalization of cochlear implant electrodes with cells providing neurotrophic factors to the spiral ganglion cells (SGC). In a previous study we demonstrated survival of NIH3T3/BDNF cells on model electrode surfaces and release of bioactive BDNF in vitro (Sasse et al., 2008). Aim of the present study was to investigate a potential in vivo effect of such modified model electrodes on deafened guinea pigs.
Lentivirally modified NIH3T3 cells producing green fluorescent protein and brain-derived neurotrophic factor (BDNF) were seeded on round silicone (silicone elastomer MED-4234; length: 1 cm; diameter: 4 mm) model electrodes (ME) at a density of $1{,}75x10^4$ cells/ME and allowed to adhere and proliferate for 7 days in 48-well plates. Cell-coated model electrodes were then implanted into the left ears of systemically deafened guinea pigs. The right deafened but untreated ears served as controls. Animals were sacrificed 30 days after implantation, electrodes were explanted and cochleae were histologically evaluated for SGC density. The density of SGC was 4.12 ± 1.07 (mean values ± standard error of mean) in the contralateral untreated side and significantly increased to 5.85 ± 1.07 in the implanted side ($p< 0.01$). Our data demonstrated a significantly increased protection of SGC after ototoxic deafening in the guinea pigs using cell-coated model electrodes for the delivery of BDNF to the inner ear.

177 Inhibition of JNK Pathway Protects Hair Cells and Prevent Inner Ear Trauma Induced Hearing Loss

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Background: Inhibition of JNK can prevent hearing loss in animals exposed to noise trauma and an aminoglycoside.
Material and Methods: In vitro study: Organ of Corti explants challenged with TNF alpha and treated with SP600125 10uM. Four groups: 1) control; 2) TNF alpha; 3) TNF alpha + SP600125; and 4) TNF alpha with 16 hr delay then SP600125. Explants were fixed and stained with FITC-phalloidin for HC counts.
In vivo study: One ear had an electrode inserted via the cochleostomy for 3 mm and then was gently withdrawn. In experimental groups we performed the same procedure, but instead of sealing the cochleostomy site, we inserted a catheter that delivered artificial perilymph (AP) with or without D-JNKI-1 peptide (AM-111) for 8 days. Hearing

thresholds were determined using ABRs (0.5, 1, 2 and 16 kHz) pre-surgery, days 0, 3, 7. Animals treated with DJNKI-1 were tested for 2 m post-EIT. The 4 groups were: EIT; EIT+ DJNKI-1; EIT + AP; contralateral control ears
Results: In vitro study: TNF alpha is ototoxic to explant HCs. SP600125 immediate treatment protects both HCs and stereocilliary bundles. SP600125 delayed treatment (i.e. after 16 hrs) protects HCs, but not stereocilliary bundles. TNF alpha significantly reduced HC counts ($p<0.001$) compared to control values. There was no significant difference ($p>0.05$) in HC counts between the control and both the immediate and delayed SP600125 treatment groups.
In vivo study: An immediate loss of auditory function was present in all EIT animals; this immediate loss was lessened by DJNKI-1 treatment but still occurred. There was a progressive loss of hearing function post-EIT in the EIT and EIT + AP groups between day 0 and day 7. This progressive loss of function was significantly reduced ($p<0.001$) in the EIT + DJNK-1 treated group.
Conclusion: Treatment with c-Jun N-Terminal Kinase Inhibitors (e.g. AM-111) prevents HC cell death in vitro and prevents hearing loss post-EIT in a guinea pig model of EIT-induced hearing loss.

178 Delayed Treatment of Tumor Necrosis Factor Alpha Challenged Organ of Corti Explants with Dexamethasone Base Prevents Apoptosis of the Auditory Hair Cells

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HYPOTHESIS: Delayed treatment of organ of Corti explants exposed to ototoxic levels of TNFα with dexamethasone base (DXMb) prevents auditory hair cells loss.
BACKROUND: Previous studies have shown that TNFα plays a significant role in multiple causes of sensorineural hearing loss, e.g. sound trauma. Immediate DXMb treatment of organ of Corti explants challenged with an ototoxic level of TNFα prevents apoptosis of the hair cells.
METHODS: P-3 rat organ of Corti explants were cultured for a total of 4 days *in vitro*. Two µg/ml of TNFα was added at 0 hr, and 70µg/ml of DXMb was added at one of 5 different time points. The experimental groups were: 1) untreated control; 2) TNFα; 3) TNFα + DXMb at 0 hr; 4) TNFα + DXMb at 6 hrs; 5) TNFα + DXMb at 12 hrs; 6) TNFα + DXMb at 18 hrs; and 7) TNFα + DXMb at 24 hrs. Explants were fixed and stained with FITC-phalloidin, and then inner (IHC) and outer hair cells (OHC) were counted per 415 µm of explant basilar membrane from the basal turns with total hair cell (HC) counts derived from this data.
RESULTS: Control group total HCs averaged 317.8 ±4.4/415µm while the TNFα group total HC count averaged 25.05 ±11.13. The TNFα + DXMb cultures at 0 hr and 6 hrs total HC counts averaged 319.1 ±6.3 and 320.8 ±2.86, respectively. The TNFα groups that were treated with DXMb after 12, 18 and 24 hrs of culture had a total HC counts that averaged 74.3 ± 27; 56.1 ±5.5 and 40.5 ±15.9, respectively. There were no statistical differences ($p>.05$) between control HC counts and the HC counts of both the 0 hr and 6 hrs DXMb treatment groups. The differences between the TNFα challenged cultures and both the control and the two DXMb at 0 hr and 6 hrs treatment groups were highly significant ($p<.001$). The differences in the HC counts between DXMb at 0 hr and 6 hrs treatment groups were not significant ($p>.05$). The differences between DXMb at 0 hr or 6 hrs treated cultures and the DXMb explants treated at 12, 18, and 24 hrs were highly significant ($p<.001$).
CONCLUSION: DXMb treatment prevents auditory hair cell loss for up to 6 hrs following exposure to an ototoxic level of TNFα. The present *in vitro* findings suggest that DXMb can be used as an otoprotective therapy against TNFα ototoxicity within a 6 hr therapeutic window.
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179 Improvement in Acoustic Thresholds Following Administration of a Phosphodiesterase (PDE) Inhibitor in Mice

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Both the guanylyl cyclases necessary for cyclic guanosine monophosphate (cGMP) production and the activators of those guanylyl cyclases (nitric oxide and natriuretic peptides) are present in the cochlea, suggesting that cGMP concentrations influence auditory transduction. Recent studies in our laboratory have established a correlation between decreased cochlear cGMP concentrations and age-related hearing loss. Furthermore, mice deficient in natriuretic peptide A receptors (which generate cGMP) are more likely to be deaf than wild-type mice, while natriuretic peptide C receptor mutants (which presumably have higher levels of natriuretic peptides) are protected against hearing loss. These results support the hypothesis that manipulation of cochlear cGMP concentrations alters cochlear sensitivity. cGMP is degraded by phosphodiesterases (PDEs); inhibition of PDE raises cGMP concentrations. In this study, two doses of zaprinast, a moderately selective PDE5 inhibitor, were administered and auditory brain stem response (ABR) thresholds.
Data were obtained from adult CBA/J and BALB/C mice that ranged from 50 to 460 days of age. 12 kHz thresholds were determined at 5 minute intervals, with three initial recordings (15 minutes) being used to establish a baseline. The diluent (DMSO) was then injected, and recordings made for an additional 15 minutes. 20 ng/kg zaprinast (a dose that does not modify blood pressure) was administered, and thresholds monitored for 1 hour or until they returned to control levels. A 40 ng/kg dose of zaprinast was then delivered, and thresholds measured for 1 hour or until the animal died.
Administration of a low dose of zaprinast resulted in a 10-15 dB decrease in thresholds, which was maximal approximately 30-45 minutes after administration. Thresholds returned to control levels within 110 minutes. A higher dose caused a larger initial improvement in

threshold, followed by an increase in thresholds as the systemic vasodilation compromised cochlear function (the higher dose was fatal). The improvement in thresholds induced by zaprinast was dependent upon the thresholds of the animal. No significant improvement was observed in animals with normal hearing or profound hearing losses. The largest changes in thresholds were observed in animals with mild to moderate hearing loss.
These results support the idea that modest improvements in cochlear cGMP concentrations improve hearing, while higher levels produce inner ear damage. These experiments provide additional evidence that cGMP, and the control systems that regulate its concentration, are important modulators of inner ear function.

180 Constitutively Active Forms of Mouse Hsf1: A Potential Model for Protecting the Cochlea

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Heat shock transcription factor 1 (HSF1) controls the protective heat shock or stress response. Many stressors can activate HSF1, leading to trimerization, DNA binding, and induction of genes for heat shock proteins (HSPs), including Hsp70.1, Hsp70.3, and Hsp 25. Several studies have demonstrated that activation of HSF1 by noise or heat protects the cochlea, that *Hsf1* KO mice are more sensitive to noise, and that transgenic mice expressing a constitutively active form of HSF1 are resistant to stress. The 503 amino acid HSF1 monomer is inactive because of intramolecular folding via the Negative Regulatory Region (NRR). Removal of NRR leads to a constitutively active form that trimerizes in the absence of heat. To develop a transgenic mouse model that expresses an epitope-tagged, constitutively active form of HSF1 in the cochlea, we first generated a series of mouse HSF1 constructs in pcDNA3.1 (CMV promoter) in which the NRR is deleted. Two additional aspects of HSF1 structure and function were considered in these studies. First, mammalian HSF1 exists in two isoforms. The longer mouse HSF1α isoform differs from the β isoform by an additional 22 amino acids following amino acid 407. The mRNAs for the two isoforms arise by alternative splicing. Second, there is little data on the effects of epitope tags on HSF1 transcriptional activity. We therefore compared N- and C-tagged versions of both isoforms by transient transfection experiments in a mouse cell culture model (NIH 3T3 cells). On Western blots, we detected all HSF1 constructs with anti-HSF1 antibodies, but only the FLAG®-tagged forms with anti-FLAG®antibodies. Both the un-tagged and the N-FLAG®-tagged Hsf1α deletion constructs activated HSP70.1 expression >10-fold in the absence of heat shock. A C-terminal FLAG® version of Hsf1α showed only 2-fold HSP activation. The series of Hsf1β deletion constructs showed fairly low levels of activation. Supported by NIH P01 AG025154.

181 Pharmacological Protection Against Endolymphatic Hydrops-Linked Hearing Loss in the Phex Mutant Mouse

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N-methyl D-aspartate (NMDA) receptor mediated glutamate excitotoxic injury has been implicated as a factor in type I spiral ganglion neuron loss observed with endolymphatic hydrops (ELH)-linked hearing loss. With excitotoxic damage, reactive oxygen species are produced resulting in cellular stress and, eventually, apoptosis. Here we test riluzole, a glutamate release inhibitor, and dimethylsulfoxide (DMSO), an anti-inflammatory and anti-oxidant solvent, to protect against hearing loss in a mouse model that spontaneously develops ELH and hearing loss post-natally. This mouse model carries a mutation in the Phex gene that causes bone remodeling defects and inner ear disease, which mirrors the ELH-linked hearing loss observed in the guinea pig model of Ménière's disease (MD). Starting at postnatal day 6 (P6), daily intra-peritoneal (IP) injections of riluzole+DMSO in phosphate buffered saline (PBS) vehicle or DMSO alone in PBS were administered to the mutant mice. At P21, 25, and 30, hearing function was assessed by recording auditory brainstem responses (ABRs). Because the hearing loss is asymmetrical and fluctuant in this mouse model, a cochlear function index (CFI) was developed to assess global cochlear function at each ABR time point. A statistically significant hearing protection for DMSO ($p < 0.05$) was observed. Riluzole+DMSO did not show greater protection than DMSO alone. The results demonstrate that DMSO has a protective effect in ELH-linked hearing loss.

182 Transplantation of Neural Differentiated Human Mesenchymal Stem Cells(HMSCs) Into the Cochlea of an Auditory-Neuropathy Guinea Pig Model

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Objective: Application of ouabain to the round window membrane of the guinea pig selectively induces the death of most spiral ganglion neurons and thus provides an excellent auditory neuropathy model for stem cell research. The purpose of this study was to investigate the effects of transplanted neural differentiated human MSCs (hMSCs) in the auditory neuropathy guinea pig model.
Methods:In this study, hMSCs were pretreated with a neural-induction protocol and transplanted into the scala tympani of the guinea pig cochlea 7 days after ouabain injury. Control model was made by transplantation of HBSS into the scala tympani of the guinea pig cochlea 7 days after ouabain injury. We established the auditory neuropathy guinea pig model using 1mM ouabain by application to the round window niche. After

transplantation of neural differentiated hMSCs, histologic analysis and functional analysis using ABR were performed.
Results:After application of ouabain to the round window niche, degeneration of most spiral ganglion neurons (SGNs) without hair cell losses within the organ of Corti and increasing of ABR threshold were found. After transplantation of neural differentiated hMSCs, SGNs were increased and which were stained by human nuclear antibody under a confocal laser scanning microscopy. ABR results showed mild hearing recovery after transplantation.
Conclusions:These findings suggest that it may be possible to replace degenerated SGNs by grafting stem cells into the scala tympani.

183 Geranylgeranylacetone Ameliorates Acute Cochlear Damage by 3-Nitropropionic Acid

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3-Nitropropionic acid (3-NP) induces hearing loss by impairing mitochondrial energy generation. Geranylgeranylacetone (GGA) is known to protect the cochlea from various injuries. The present study was designed to investigate the protective effect of GGA against acute 3-NP-induced cochlear damage. Female Hartley guinea pigs were divided into 4 groups. In group A, the 3-NP vehicle was injected, and in group C, only GGA was administered. 3-NP (500 mM, 4 µl) was administered with (group D) or without (group B) GGA pretreatment (800 mg/kg, 7 days). The auditory brainstem response (ABR) was recorded at click and at 8, 16, and 32 kHz before and after injection. After cochlear harvest, hematoxylin/eosin staining and immunohistochemistry for anti-HSP70 antibody were done.
3-NP exposure resulted in elevated ABR thresholds that exceeded the maximum recording limit, while GGA pretreatment before 3-NP exposure led to a significant decrease in hearing threshold shift. Histological analysis of above former group revealed loss of type II fibrocytes in the spiral ligament, hair cells in the organ of Corti, stellate fibrocytes in the spiral limbus, and spiral ganglion cells, while in above latter group, these cells were preserved. The injection of 3-NP vehicle revealed weak HSP70 expression in the nuclei of some supporting cells (pillar cells, Deiters' cells, and Hensen's cells) and interdental cells. Administration of only GGA showed strong HSP70 expression in the same area as in 3-NP vehicle group, while GGA pretreatment before 3-NP exposure demonstrated slightly decreased HSP70 expression in that area. These results suggest that GGA may protect 3-NP-induced acute cochlear injury by the up-regulation of HSP70.

184 The Effect of Intracochlear Electrical Stimulation on Intracellular Apoptosis Signaling in Spiral Ganglion Neurons After Deafening in Vivo

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Objective: Our goal is to establish the intracellular consequences of electrical stimulation (ES) to spiral ganglion neurons (SGNs) after deafferentation. SGNs die as a result of loss of hair cells, their sole afferent input. Some histologic animal studies of chronic electrical stimulation (ES) after deafening show that ES alone may improve SGN survival. This finding, however, is controversial. Here we use a rat model to determine the acute effect of both low and high pulse rate electrical stimulation on activation of the apoptotic pathway protein, Jun, in deafferented SGNs *in vivo*.
Methods: A single electrode was implanted through the round window of one month-old kanamycin-deafened rats for four hours of ES (monopolar, biphasic pulses, amplitude twice eABR threshold) at either 100 or 5000 Hz. c-Fos, a nuclear marker of neuronal activity, was used to localize the effect of ES within the cochlea. Jun phosphorylation (pJun), a proapoptotic signaling event known to be present in apoptotic SGNs after deafening, was assayed by immunofluorescence to quantitatively assess the effect of ES on proapoptotic signaling.
Results: No c-Fos immunofluorescence was detected in control deafened and hearing cochleae. cFos immunofluorescence was limited to SGNs in the basal region of electrically stimulated cochleae. Jun phosphorylation was reliably suppressed by 100 Hz ES in deafened cochleae. This effect was less robust after 5000 Hz ES.
Conclusions: Suppression of pJun occurs in deafferented SGNs after only four hours of ES. This finding supports the hypothesis that ES alone may provide sufficient trophic support to SGNs to decrease cell death after deafferentation. Furthermore these effects appear to be spatially limited to the SGNs most adjacent to the stimulating electrode. Stimulation frequency may be consequential: 100 Hz ES was significantly more effective than 5 kHz ES in suppressing pJun.

185 Enhanced Inner Ear Ion Homeostasis Gene Expression with Intratympanic Steroid Delivery

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Intratympanic delivery of glucocorticoids for hearing loss has been reported to improve the likelihood of hearing recovery compared to systemic delivery, presumably by increasing the amount of drug reaching the inner ear. Furthermore, although glucocorticoids are proposed to be acting by immunosuppression, they also have a significant

effect on the mineralocorticoid receptor and may be enhancing ion homeostasis in the cochlea as well to restore hearing. Clarifying these issues is critical if effective therapies are to be developed for the various forms of hearing loss. This study was conducted to better assess the role of steroids on inner ear ion homeostasis and compare the efficacy of systemic versus intratympanic delivery. BALB/c mice were given the glucocorticoid prednisolone (10 mg/kg) or the mineralocorticoid aldosterone (30 µg/kg). Each steroid was delivered by oral gavage (0.2 ml) or transtympanically (5.0 µl). Inner ear tissues were collected at 1, 6, or 24 hours and prepared for quantitative RT-PCR of several ion homeostasis genes (K^+ channels, Na^+,K^+-ATPase, gap junction connexin 26, tight junction claudin 3, epithelial sodium channel, and aquaporins 1 and 4. Analyses showed that both steroid delivery methods had an impact on gene expression, with the largest effect on Na^+,K^+-ATPase, connexin 26, and aquaporin 1. Furthermore, intratympanic delivery had a greater impact on gene expression than systemic delivery, particularly at 6 hours. Intratympanic prednisolone caused double the gene expression of aquaporin 1 at 6 hours compared to oral delivery, while intratympanic aldosterone induced nearly double the expression of Na^+,K^+-ATPase compared to oral. Thus, intratympanic delivery of steroids may indeed have a greater impact on cochlear genes than oral delivery. This study also establishes a valid gene assessment model system in which to compare the delivery and impact of steroids on the inner ear.
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186 Sjogren's Syndrome Histopathology in the Human Inner Ear

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Sjogren's syndrome (SS) is a chronic autoimmune disease characterized by chronic inflammation involving exocrine glands, primarily affecting the salivary and lachrymal glands. Histologically, SS is characterized by a lymphocytic infiltration of the affected gland. In one clinical study, approximately one-fourth of the SS patients presented with sensorineural hearing loss of cochlear origin (Ziavra et al. 2000). There are no temporal bone histopathology studies on subjects with a history of SS. We describe the histopathology of the inner ear of two patients diagnosed with SS. In patient 1 (55 yo female), there was a severe loss of intermediate cells of the stria vascularis bilaterally, and basement membrane (BM) thickening under the strial marginal cells, with relatively well-preserved fibrocytes in the spiral ligament (SL). There was collapse of Reissner's membrane and loss of hair cells. In the vestibule, the cristae epithelium was atrophied with BM thickening; the utricle is relatively well preserved. Patient 2 (65 yo female) showed a similar morphology as patient 1, however, there was pronounced loss of fibrocytes in the SL. The cristae and utricle in the vestibule were well preserved. Using immunohistochemistry, IgG deposition was detected in the strial BM blood vessels in the cochlea of both patients. This is consistent with a prior study demonstrating an association of SNHL of SS with anticardiolipin antibodies (Tumiati et al. 1997). The pathological changes in the inner ear of these two patients with SS parallels those found in the MRL/lpr (Ruckenstein et al. 1999) mouse models of autoimmunity: i.e. degeneration of strial intermediate cells, and IgG deposition on the BM of strial blood vessels, suggestive of a similar pathology. These results also highlight the importance of correlating the histopathology of available archival temporal bones with animal models to understand inner ear disease.
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187 The Vestibular Arch: Its Anatomy, Development, Physiology and Pathology as Pathways Towards an Understanding of Ménière's Disease

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We review here studies which may provide a basis for research into the origin of Ménière's disease.
The vestibular arch is a thin bony shell which is wrapped closely around most of the endolymphatic duct. The arch is made up of the inner layer of vestibular aqueduct and its vestibular extension. Within the vestibule it is bordered medially by the cochlear part of the otic capsule. The utricle and saccule lie on its lateral and anterior vestibular borders respectively .
It develops in membrane from the external perichondral layer of the primordial otic capsule to become by 8 weeks a prominent, very vascular, connective tissue sheet around the endolymphatic duct. It later ossifies to lamellar bone containing numerous Volkmann's canals. Commencing at about one year, an intricate network of microcanals grows from the latter and reaches close to the endolymphatic duct.
Large numbers of osteoblasts line the walls of the whole canal system in the mature arch. These cells show evidence, in moderate numbers, of apoptotic cell death. It seems possible that this process contributes to the normally high level of potassium ions in the endolymph, providing the correct electrolytic ambience necessary for the sensory processes of audition and balance.
The most striking pathologic change in the arch in Ménière's disease is a severe loss of osteoblasts. This is probably the result of massive apoptosis, since numerous apoptotic bodies can usually be seen in that disease in the canals of the arch. Denudation of osteoblasts from the canals in the arch produces a decayed appearance among them. It is possible that attacks of excessively high levels of potassium in the endolymph, resulting from such marked cell death in the arch, could cause the severe endolymphatic hydrops characteristic of Ménière's disease.

188 Deregulation of PDGFR Signaling in Vestibular Schwannomas

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Both sporadic and Neurofibromatosis 2 (NF2)-related vestibular schwannomas (VS) arise from mutations in the *NF2* gene, encoding merlin (schwannomin). While loss of functional merlin is presumed to initiate schwannoma development, the mechanisms underlying tumorigenesis remain to be elucidated. Upregulation of several receptor tyrosine kinases (RTKs) have been reported, including platelet-derived growth factor beta (PDGFRβ). Upregulation of PDGFRβ has also been reported in merlin-deficient mouse Schwann cells (SC), sporadic and NF2-related human VS specimens and an NF2-related schwannoma-derived cell line, HEI193. Research in both HEI193 and primary NF2-associated VS cultures has shown decreased cell proliferation in response to PDGFR small molecule inhibitors. Despite the abundant data establishing the upregulation of various growth factor receptors, evidence demonstrating which intracellular signaling pathways mitigate their tumorigenic effects is lacking. The mitogen-activated protein kinase (MAPK) and phosphoinositide-3-kinase (PI3K)/Akt intracellular signaling pathways have both been implicated.

The few studies evaluating PDGFRβ expression in VS have had small study numbers. We have collected a tumor bank of greater than twenty VS specimens, in which we have found downregulation of PDGFRβ at the mRNA level and upregulation of the PDGFRβ protein compared to normal human nerve specimens. Additionally, increased expression of activated (phosphorylated) downstream effectors in the MAPK and PI3K signaling pathways, Akt and Erk1/2, was evident. To further elucidate the early effects of merlin deficiency, we utilized our *in vitro* model of early VS tumorigenesis. After siRNA knockdown of merlin in cultured human SC, we found upregulation of PDGFRβ, phosphorylated and total Akt and Erk1/2. We propose that deregulation of PDGFRβ expression and resultant downstream mitogenic signaling pathways are the result of merlin deficiency, and contribute to VS tumorigenesis.

189 Cochlea Spiral Ganglion Cells Degeneration and Hearing Loss as a Consequence of Schwann Cells Death in the Saposin B KO Mice

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Saposin B derives from the multi-functional precursor, prosaposin, and functions as an activity enhancer for several glycosphingolipid hydrolases. To understand the in vivo functions of saposin B, mice were generated by introducing a point mutation into the saposin B domain of the prosaposin gene. This mutation disrupted a conserved disulfide bond that led to an unstable and undetectable saposin B protein, but preserved prosaposin, and saposin A, C and D processing and function (Sun et al 2008). Saposin B KO mice exhibit slowly progressive neuromotor deterioration and minor head tremor (Sun et al 2008).

The aim of this study is to investigate these KO mice in order to determine the biological function of saposin B in the cochlea and its effect on the hearing.

To gain better understanding of the effect of such a mutation on the hearing and the anatomy of the cochlea, the hearing in these KO mice was tested using standard ABR threshold analysis and the histology of the cochlea was analyzed at light and electron microscopy levels at different ages. Data on these mice at P21 and P60 shows that the KO has normal hearing and cochlea histology (normal organ of Corti, hair cell and spiral ganglion (SG) cell counts) when compared to wild-type (WT) littermates. However at 8 month the saposin B KO mice exhibited a statistically significant increase in ABR thresholds for each of the four sound stimuli (Click, 8, 16 and 32 KHz), when compared with the ABR threshold of the WT littermates. In contrast to what is typically seen, whereby hair cells death is followed by SG degeneration (eg Hurley et al 2007), in the Saposin B KO cochlea the organ of Corti appears normal with normal numbers of outer and inner hair cells but this mice show SG degeneration primarily. At the light and the electron microscopy levels there is degeneration of Schwann cells in association with SG neuronal degeneration. This degeneration is progressive with increased SG loss at 15 months. Interestingly at the EM level the SG myelin sheaths are reduced and sometimes absent in the KO when compared to wild type littermates.

These data suggest that in the auditory system, Saposin B is important for maintaining normal Schwann cells function through normal myelin production, while absence of saposin B leads to loss of hearing through degeneration of spiral ganglion neurons.

190 Parkin Deficiency Causes Progressive Hearing Loss in Mice

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Parkinson's disease (PD) is the second most common neurodegenerative disorder among elderly people. PD is characterized by muscle rigidity, tremor and a slowing of physical movement. PD is defined as one of ″synucleinopathy″, which is due to the accumulation of alpha synuclein (SNCA) and causes neuronal cell death. Most PD is sporadic, but 5-10% of PD cases are familial and presumably hereditary forms. In recent years, some specific genetic mutations causing PD have been discovered. One of these genes includes Parkin, encoding an ubiquitin-protein ligase that causes autosomal recessive juvenile parkinsonism in human. In Drosophila, *parkin* null mutants show decreased adult lifespan, apoptotic muscle degeneration and male sterility, but no neuronal phenotypes have been observed in Parkin deficient mice.Since sensorineuronal hearing loss can be

caused by neuronal cell death, we assumed that Parkin deficiency might cause hearing less. To confirm this, first, we performed auditory brainstem responses (ABRs) in *parkin* null mice (KO) and wild type mice (WT) at various ages, 4, 8 and 12-months-old. The results showed that the hearing loss progresses faster in KO. Next, we investigated the hearing of KO and their littermate by ABRs. KO showed significant hearing loss at their age of 2 and 3 months old, compared with WT and hetero mice. Their inner ears were collected in 3 months old and immunohistochemistry was performed in frozen sections. Immunohistochemistry showed that phosphorylated SNCA was expressed more intensely in KO than in WT and hetero mice at the spiral ganglion of apical turn. These findings indicate that the accumulation of alpha synuclein in spiral ganglion cells causes neuronal cell death and progresses hearing loss faster in *parkin* null mice. Progressive hearing loss is the first confirmed neuronal phenotype in Parkin null mouse.

191 Histogenesis of Otosclerosis and Its Relation to the Osteogenic Activity of the Fissula Ante Fenestram

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We have suggested that the *fissula ante fenestram* mediates the formation of new bone throughout life (Acta Oto-laryngol in press). The purpose of this study was to examine the histogenesis of otosclerosis and its possible relation to the osteogenic activity of the fissula.

We analyzed the mode of normal bone and canal formation in the fissula of 18 temporal bones from 14 patients aged between birth and 58 years. We also studied the histologic make-up of the lesions of clinical otosclerosis, in the light of otic capsule development, in 11 bones from 10 patients.

In the normal mature fissula, all three elements of the middle layer of the otic capsule – chondro-osseous canals, Volkmann's canals and lamellar bone – seem to arise continuously from mesenchymal cells, possibly by "appositional transformation" of each element at its fissular end.

The lesions of clinical otosclerosis are, like the external, periosteal layer of the otic capsule, formed of but two elements (although usually more active-appearing than the normal tissue): (a) primary, secondary or even smaller Volkmann's canals, all with marked osteoblastic activity in their walls, and (b) lamellar bone. In many cases the otosclerosis originates in the region of the fissula, and in some, appears to have obliterated it, but in no case have we yet seen direct origin, like that of the three-element middle layer otic capsule bone, from the fissula.

"Histologic otosclerosis" is a lesion of doubtful pathologic validity. In some cases there is a well-differentiated focus composed of normal Volkmann's canals and lamellar bone, anterior to, but not in contact with, the fissula. This may be normal tissue originating from periosteal mesenchyme on the external (middle ear) surface of the otic capsule.

Otosclerosis may thus be a focal, hyperplastic replication of normal otic capsule tissue (hamartoma). Relationship to fissular osteochondrogenic activity is still not defined.

192 The Skylab Mutation in Danio Rerio Affects Hair-Cell Function

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Zebrafish homozygous for the *skylab* mutation are insensitive to vibrational stimuli and unable to maintain balance. A closer look at the hair cells (HCs) in *skylab* mutants revealed that although they develop into morphologically normal HCs, some cell death was apparent at later larval stages. At 5 dpf, an average of 6 HCs remained in each neuromast compared to an average of 10 HCs in WT. DIC and EM images of mutant skylab HCs at 5 dpf revealed blebs that extrude from the apical surface adjacent to the ciliary bundles, which could be an early indication of apoptosis or that the cell surface has been disrupted. Visible spaces between the basal end of HCs and the support cells also developed as soon as 3dpf. At this early larval stage, calcium imaging demonstrated that stimulation of lateral line HCs in *skylab* mutants exhibited an 80% reduction in calcium flux compared to siblings. Additionally, no microphonics were detected in *skylab* mutants. We propose that the *skylab* mutation, while not important for early development, could be important for the maintenance of HC homeostasis. The *skylab* phenotype, however, could also be due to a defect in cell surface integrity. Investigations are underway to identify the mutation, and determine whether defective cellular homeostasis or cell surface integrity is the cause of the HC phenotype in *skylab* mutants.

193 Deficiency of the Ribosomal Protein L38 (RpL38) Causes Hearing Impairment in the Tail-Short (Ts) Mouse

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Hereditary and acquired hearing loss are among the most common forms of sensory impairment. Anatomy and physiology of the inner ear has been highly conserved during mammalian evolution making the mouse an ideal model to dissect molecular components and pathways relevant for normal and pathological function. An auditory-evoked brainstem response (ABR) screen of inbred mouse strains at The Jackson Laboratory identified a significant hearing impairment in Tail-short (TsJ/LeJ-Ts/+) mice. Homozygous (Ts/Ts) mutants are embryonic lethal at three days of gestation and heterozygotes undergo a transient fetal anemia followed by growth retardation, skeletal anomalies and occasional perinatal lethality.

To further examine the hearing impairment, we performed distortion-product otoacoustic emission (DPOAE) measurements. DPOAE amplitudes in Ts mutants were

significantly reduced at four weeks of age. Morphologically, the Ts cochlea presents with a bony overgrowth extending from the round window to the apex. In addition, deposition of mineral crystals that originate in the round window and extend to the middle ear coincides with abnormal ossification of the temporal bone. Both neo-ossification and crystal deposition are strongly associated with decreased DPOAEs.
To identify the underlying genetic defect, we positionally cloned the Ts locus. Genetic fine-mapping followed by molecular analyses demonstrated a 18 kb deletion comprising the entire ribosomal protein L38 gene (Rpl38). Immuno-localization of Rpl38 in inner ear tissues showed strong expression associated with the presence of red blood cells in the bony and membranous labyrinth. The data point towards a physiological link between Rpl38 function and mineralization in the inner ear.

194 Structure-Function Analysis of Grxcr1, the Gene Affected in the Mouse Deafness Mutant Pirouette

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The mouse mutant pirouette (pi) exhibits profound hearing loss and circling/head shaking behaviors inherited as recessive traits. Previous studies have demonstrated abnormally thin stereocilia during sensory cell maturation in the inner ears of pi/pi mice. We have identified pi mutations in the novel gene Grxcr1, which encodes a protein localized to stereocilia. GRXCR1 contains two recognizable domains: a central region of similarity to glutaredoxins, enzymes that reduce oxidized cysteines on cellular proteins; and a putative zinc finger in the C-terminus. These domains exhibit evolutionary conservation in likely Grxcr1 homologs in other vertebrates and in fly, worm, and plants. We have generated a variety of deletion and amino acid substitution mutations in GFP-tagged versions of the gene. Earlier studies indicated that the divergent N-terminus of GRXCR1 is necessary and sufficient for localization of the protein to actin filament-rich microvilli in transfected epithelial cells. We are currently evaluating the impact of these mutations on: the ability of the protein to localize to other actin filament-rich structures in cultured cells and in inner ear explant cultures; activities of the protein that impact actin filament-rich structures; and protein-protein interactions.
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195 BLEV-1: A Transgenic Mouse Model for BDNF Live Exon Viewing

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In various studies we showed an altered brain-derived neurotrophic factor (BDNF) expression in spiral ganglion neurons in aging animals (Rüttiger et al. Knipper, 2007), after acoustic trauma (Tan et al. Knipper, 2007) and after salicylate application (Panford-Walsh et al. Knipper, 2008; Singer et al. Knipper, 2008). BDNF plays a crucial role for activity-dependent plasticity, alteration of synaptic efficacy and the balance of inhibition and excitation. Deletion of the receptor of BDNF leads to progressive hearing loss (Schimmang et al. Knipper, 2003) after salicylate treatment.
The activity dependent usage of BDNF is due to its characteristic gene structure. The BDNF gene consists of a common protein-encoding exon (IX), which can be spliced to any of the eight non-coding upstream exons (I-VIII), resulting in different BDNF transcripts but only in one protein (Timmusk et al., 1993; Aid et al., 2007). Each of the eight upstream exons has a putative promoter on its 5'-flanking region, the activation of which is controlled by activity-dependent molecular components of calcium-dependent signaling cascades. The different exons are not, however, expressed ubiquitously. In the cochlea only BDNF exon IV, VI and IX are expressed.
To get a better insight into the mechanism of a trauma induced differential usage of BDNF exon IV and VI in the auditory system; we generated a transgenic mouse model, which was designed to label the activity-dependent differential usage of BDNF exon IV and VI in vivo by different fluorescence proteins.
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196 Auditory Function and Sensitivity in Chromosome 7 Disorders

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The goal of this research is to study the role of elastin (ELN) in peripheral and central auditory function. The *ELN* gene is located on chromosome 7q11.23. One of the better known *ELN*-disruption disorders is Williams syndrome (WS), which involves a deletion of approximately 28 genes including *ELN*. The *ELN* deletion causes insufficient production of elastin (*ELN* haploinsufficiency). Two other examples of *ELN*-disruption disorders are isolated supravalvar aortic stenosis (ELN^{+}/-), caused by a large spectrum of *ELN* mutations and leading to functional *ELN* haploinsufficiency, and cutis laxa (CL), in which a mutation of *ELN* leads to synthesis of a mutant elastin precursor, which disrupts the assembly of elastic fibers in a dominant fashion.
We obtained tympanometry, audiometric thresholds, and distortion product otoacoustic emissions (DPOAEs) in individuals with WS (n=81), ELN^{+}/- (n=10) and CL (n=3). Sixty-three percent of school-age children and 92% of adults with WS demonstrated mild to moderate-to-severe SHL. These results were also corroborated with the DPOAE conditions. In a group of children with WS with normal hearing (n=14), DPOAEs were significantly decreased, indicating that the auditory systems in this

subgroup were not normal. In addition, the DPOAE I/O function at 4000 Hz in this subgroup showed a loss of cochlear compression (in the absence of hearing loss). Individuals with point mutations of a single elastin gene, who do not have WS (ELN^+/-) have significantly depressed cochlear function (DPOAEs) but relatively preserved hearing sensitivity. Individuals with CL showed normal hearing sensitivity and DPOAEs. This data is an extension of those reported in Marler et al., (2005). Theoretically, WS ears may be an experimental model for "tender ears" (Maison & Liberman, 2000), or ears genetically predisposed to trauma from normal environmental noise. Further work needs to be conducted to identify the possible role of ELN in central auditory system function.

197 CLRN1 Mutant Mice Display Aberrant Cochlear Maturation

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Usher syndrome is a genetically heterogeneous disorder that affects one person in 25,000. The clinical symptoms are hearing loss and retinitis pigmentosa (RP). Usher syndrome is divided in three clinical subtypes: USH1, USH2 and USH3, dependent upon the severity of the disease. USH3 patients have a later initiation of deafness combined with variable RP and vestibular dysfunction. The USH3A gene encodes Clarin-1, a 28 kDa tetraspanin protein that is expressed from E18 to P6 in stereocilia and hair cell afferent terminals (Zallocchi et al, 2009). Recently a mouse model of USH3A was developed in which hair cell dysfunction and prolonged ABR peak latencies suggested that clarin-1 participates in neuronal as well as hair cell function (Geng et al, 2009). Here we examine the structure of the organ of Corti in the CLRN1 mouse during the period of neuronal reorganization and maturation.

Cochlea from mutant (KO) and wild-type (WT) mice at 6 and 9 DAB were isolated and prepared for histochemistry using tetramethylrhodamine-conjugated dextran (TMRD) to trace the type I afferent innervation. Alternatively, electron microscopy was employed to examine the synaptic fine structure of the inner (IHC) and outer (OHC) hair cell innervation. All observations were made from the upper basal turn.

TMRD dye uptake experiments at 6 DAB demonstrate a clear difference in the type I afferent innervation in the KO relative to the WT. The intensity of the dye is greater in Rosenthal's canal with a larger areas showing dye uptake under the OHCs and IHC. This suggests that the number &/or size of the type I afferents in the KO exceeds that of the WT. Ultrastructural investigation of the 9 DAB KO also showed more and larger afferent synaptic terminals at the OHC basal pole reminiscent of that seen in the WT mouse at an earlier age (6 DAB). Interestingly, while at 9 DAB the tunnel of Corti and spaces of Neul were near mature in size in the WT cochlea, the amount of tunnel of Corti opening in the 9 DAB KO mouse was equivalent to that observed in the 6 DAB WT. In sum, the data suggest that the CLRN1 mutant cochlea displays not only pathology in its innervation pattern but also in maturation.

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198 Mitochondrial Deafness Alleles Confer Misreading of the Genetic Code

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A number of diseases are associated with gene-specific defects in biogenesis, stability, processing, and translation of mRNA. A more general pathogenic mechanism presumably takes place in diseases associated with mutations in ribosomal nucleic acids. Despite the fact that important genetic diseases are linked to mutant mitochondrial ribosomes, the molecular mechanisms by which such ribosomes result in a clinical phenotype have remained largely unknown. Prominent examples are positions 1555 and 1494 in the mitochondrial genome which encode small subunit rRNA and map to the small subunit's decoding A-site. Mutation of these residues (A1555G, C1494U) is associated with maternally transmitted nonsyndromic deafness, with the phenotypic penetrance of the disease being variable. Here we report on the construction of bacterial hybrid ribosomes that carry various versions of the mitochondrial decoding region of small subunit ribosomal RNA, i.e. normal and deafness alleles. We show that the pathogenic rRNA mutations A1555G and C1494U decrease the accuracy of translation. Modelling the mitochondrial decoding site suggests that mt A1555G and C1494U reduce the contact between H44 and H27. Mechanistically, a relative movement between H44 and H27 is likely to be part of the induced conformational change required for decoding, and lowering the energetic penalty for such a change would allow near-cognate tRNAs to become accepted more easily. These findings suggest misreading of the genetic code as an important molecular mechanism in disease pathogenesis.

199 Screening Connexin Mutations in the Long-Term Storage of Human Temporal Bone Sections to Explore the Pathology of Connexin Mutation Induced Hearing Loss

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In the last decade, our understanding on the genetic causes of deafness has made a dramatic progress; many deafness-causing mutations have been identified and characterized. However, the pathological changes caused by these gene mutations in the human inner ear are less known, because the direct pathological study is almost impossible to be carried out in the cochlea of deaf individuals. Connexin mutations are a common genetic cause for hereditary deafness and induce a high incidence of nonsyndromic hearing loss. In this study, we have used the collected celloidin-embedded human temporal bone

sections from deaf individuals to screen connexin mutations in order to explore the mutation-induced pathological changes in the human inner ear. After de-celloidining of the samples, the genomic DNA was successfully extracted from those long-term storage human cochlear sections, amplified by PCR, and sequenced. We have screened Cx26 mutation in archival temporal bone sections from deaf individuals in two cases. We have found a point mutation (42G->C) in one case, which clinic and pathological diagnosis is ¡§Unexplained progressive hereditary hearing loss¡¨. The formalin-fixed, celloidin-embedded human cochlear sections were also processed by the antigen-retrieval treatment for immunofluorescent staining. Clearly labeling for Cx26 could be detected in the supporting cells in the organ of Corti and at the stria vascularis (SV) and spiral ligament (SPL) in the cochlear lateral wall. Our preliminary data show our established methods can successfully extract DNA and identify gene mutations from the long-term storage human cochlear sections, in which pathological changes have already been characterized or can be identified. Hence, this study can provide direct information about pathological changes in the human cochlea of deaf mutants.

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200 Unique and Essential Functions Played by Connexin26 in the Cochlea

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Mutations in genes coding for connexin26 (Cx26) and Cx30 are the most common causes of congenital deafness in humans. Molecular mechanisms of hearing impairments, however, are unclear. Since most of cochlear gap junctions (GJs) are co-assembled from Cx26 and Cx30, this molecular configuration suggests that homomeric GJs are still present in the cochlea of conditional Cx26 (cCx26) and Cx30 null mice. Both types of mutant mice are deaf, which may be caused by either insufficient number of GJs or an alteration to biophysical properties offered by homomeric GJs. Genetically over-expressing Cx26 in the Cx30 null mice completely rescued hearing of the mutant mice (Ahmad et al., 2007), indicating that the presence of Cx30 is not essential for normal hearing. To further test the essential role of Cx26 in hearing, we investigated whether the hearing in cCx26 null mice (Wang et al., 2009) could be rescued by over-expressing Cx30.

Multiple lines of mouse models were used, including five groups of control mice: (1) aged-matched WT control (n=24); (2) BAC^{Cx26} (mice carrying extra copies of Cx26 gene introduced by transgenic expression of a modified bacterial artificial chromosome (BAC)) (n=10) (3) BAC^{Cx30} (n=22); (4) $cCx26^{null}$ (Wang, et al., 2009) (n=24); (5) $Cx30^{null}$ (Teubner, 2003) (n=18), and four experimental groups: (6) BAC^{Cx26};$cCx26^{null}$ (cCx26 null mice that also carried a modified BAC that expressed Cx26) (n=40); (7) BAC^{Cx30};$Cx30^{null}$ (N=133); (8) BAC^{Cx26};$Cx30^{null}$ (Ahmad et al., 2007) (n=32); (9) BAC^{Cx30};$cCx26^{null}$ (n=34). Auditory brainstem response (ABR) measurements showed normal hearing in groups 1, 2, 3, 6, 7 and 8. In contrast, severe hearing loss was found in groups 4, 5, and 9. Morphology observations were consistent with the ABR data. Importantly, cochleae in groups 4 & 9 both showed abnormality in the postnatal development of the organ of Corti. The tunnel of Corti was never opened in these mice. Both immunolabeling and Western blot data indicated that the protein expression of Cx26 in the cochlea preceded that of Cx30 during the early postnatal period. Null expression of Cx26 may therefore result in a transient total elimination of GJs in some regions of the developing cochlea. Since the hearing in $cCx26^{null}$ mice was not rescued by over-expressing the Cx30 and the same BAC^{Cx30} construct was effective in rescuing the hearing of Cx30 null mice, we conclude that function of Cx26 can not be replaced by Cx30 in the cochlea.

201 Changes in Formation of Cochlear Gap-Junction Plaques in Dominant-Negative Connexin26 Transgenic Mice

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Hereditary deafness affects about 1 in 2000 children and mutations in the GJB2 gene are the major cause in various ethnic groups. GJB2 encodes connexin26 (Cx26), a channel component in cochlear gap junction. It has been hypothesized that gap junction in the cochlea, especially connexin26, provide an intercellular passage by which K+ are transported to maintain high levels of the endocochlear potential essential for sensory hair cell excitation. We have reported the generation and the phenotype of a mouse model carrying human connexin26 with R75W mutation (R75W Tg mice).

In this study, we analyzed the formation of gap-junction plaques in cochlear supporting cells of R75W Tg mice in different stages by confocal microscope. Gap junction composed of Cx26 in wild type mice showed horizontal linear gap-junction plaques along the cell-cell junction site with the adjacent cells and these formed pentagonal or hexagonal outlines of normal inner sulcus cells and border cells. The gap-junction plaques in R75W Tg mice did not show normal linear structure, but the round small spots were observed around the cell-cell junction site. We analyzed the size of the gap junction unit for wild type and R75W mutant, and the width of gap junction units of R75W Tg was significantly shorter than wild type. A number of these small spots were scattered around their cell-cell junction site. We also observed the formation of the gap junction plaques in the primary culture of the organ of corti including inner sulcus cell and border cell. The assembly of the connexons may decline due to R75W mutation in Cx26 and it may cause mis-localiazation of the connexons. Our results may suggest that dominant negative R75W mutation affects on the accumulation and localization of gap junction channels in the cell-cell junction site among cochlear supporting cells.

202 Correlating the Audiometric and Genotypic Profile of Connexin 26, Connexin 30 and A1555G Hearing Loss in Singapore

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To determine the audiometric & genotypic profile of connexin 26, connexin 30 & A1555G sensorineural hearing impairment (SNHI) in Singapore.

Significance: Connexin 26 mutation is the leading cause of idiopathic congenital SNHI worldwide. Cx30 and A1555G are other important genetic causes. They have not been evaluated in Singapore.

Methods: Prospective study of 94 SNHI and 98 hearing (Control) patients. Genetic testing for connexin 26, connexin 30 and A1555G, audiometry and clinical evaluation were performed.

Results: The prevalence of connexin 26 HI was 23% (22/94). The carrier rate of pathologic mutations amongst the HI group was 36.7% (69/ 188) and control 7.65% (15/ 196). The leading Cx26 mutation in Singapore is V37I, a missense mutation resulting in an amino acid change of valine to isoleucine at codon 37. The prevalence of V37I was 26.6% (50/188) in the HI group. All patients with V37I together with another pathologic allele for HI had HI, confirming V37I's pathogenicity. V37I/ V37I is associated with bilateral, down-sloping, mild to moderate SNHI. Four novel mutations I30V, 282(C-T), E120K, 558(G-A) were identified. No 35delG common to the Caucasians, and only four 235delC alleles common in Japan and China were found.

V27I/ E114G compound heterozygote prevalence was high, in controls16.8% (33/196) and in HI group 11.7% (22/188). Where V27I/ E114G occurred together with another pathogenic allele, all 4 patients had HI.

A1555G screen: no mutations found. Connexin 30 screen: 4 known polymorphisms of L132L/ WT, 4 novel mutations E101K/WT, 723G>A/WT, R32X/WT and I118K/WT.

Conclusions

There was a high prevalence of Cx26 deafness (23%) among patients with idiopathic congenital SNHL in Singapore. V37I is the leading mutation and its pathogenicity is confirmed by this study. Novel pathologic mutations I30V, 282(C-T), E120K, 558(G-A) were found. Interestingly, V27I/ E114G with a pathologic mutation was associated with SNHI and warrants further studies. There were no patients with A1555G or Cx30 related HI.

203 Is the Loss of Endocochlear Potential Solely Responsible for Causing Hearing Impairment in the Connexin30 Null Mice?

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Mutations in genes coding for connexin26 (Cx26) and Cx30 are a major cause of genetic hearing impairment in humans. Findings in Cx30 null mice demonstrated an unusually slow time course of degeneration in the cochlea (Sun et al., 2009). In contrast, endocochlear potential (EP) was never formed in these mice (Teubner et al., 2003). EP is generated by a number of membrane proteins (e.g., Kcnj10, Kcnq1, Kcne1, Na/K^+ ATPase and co-transporters) arranged in series in the stria vascularis. Among them, Kcnj10 is the only one localized in the intermediate cells across which EP is initially generated. Kcnj10 is known to be required for generating EP and its protein expression is significantly reduced in the Cx30 null mice. In this project, we tested whether over-expressing Kcnj10 in Cx30 null mice is sufficient to restore the EP and normal hearing in these mutant mice.

BAC^{Kcnj10} mice were generated by expressing extra copies of Kcnj10 gene from a bacterial artificial chromosome (BAC) introduced into mouse genome. Presence of extra copies of Kcnj10 gene was confirmed by PCR and Southern blot analyses. In our ongoing work, BAC^{Kcnj10} mice are bred with Cx30 null mice to obtain BAC^{Kcnj10};$Gjb6^{+/-}$ mice in first generation. The *Gjb6* heterozygote mice were interbred to obtain BAC^{Kcnj10};$Gjb6^{-/-}$ mice in the second generation. Measurements of the auditory brainstem responses (ABRs) of the BAC^{Kcnj10};$Gjb6^{-/-}$ mice showed that hearing thresholds across a frequency range of 4-32 kHz were comparable to the wild type mice in a subset of the transgenic mice (n=2). Measurement of EP, immunolabeling and examination of the cochlear morphology is on-going in the lab. Preliminary results suggest that the restoration of hearing in BAC^{Kcnj10};$Cx30^{-/-}$ mice is dependent on the copy numbers of the Kcnj10 gene incorporated in the mouse genome. Hearing was rescued only in those mice that incorporated larger numbers (>6) of exogenous Kcnj10 gene in their genome. These results suggested that hair cells in the cochlea of Cx30 null mice are fully functional before they are degenerated. Observed degeneration in the organ of Corti is secondary to the loss of EP. Loss of endocochlear potential was solely responsible for causing hearing impairment in the Cx30 null mice. A time window of opportunity may therefore be exploited for therapeutic interventions of deafness caused by Cx30 null mutation, with a focus on the regeneration of the EP.

204 mRNA Sample Size for Gene Expression Profiling of Mouse Inner Ears - How Low Can You Go?

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Gene expression profiling (GEP) using microarrays is a robust, affordable and now commonly used technique for comparative analysis of transcriptomes. RNA amplification methods allow for reliable gene expression profiling from as little as 0.5 ng of total RNA. The mouse auditory and vestibular sensory epithelia, however, are comprised of epithelial and non-epithelial components that are further divided into multiple cell types. Therefore, recommendations for mRNA starting amounts that pertain

to homogeneous cell lines may not be easily transferable to this complex tissue.
Total RNA was extracted from auditory and vestibular sensory epithelia of newborn mice, and a dilution series ranging from 100 ng to 1 ng of total RNA was made. RNA was amplified using two methods that are based on different chemistry: WT-Ovation™ Pico RNA Amplification system (NuGEN) and TotalPrep kit (Ambion). Amplified RNA was processed and hybridized to the mouse Illumina bead-arrays. The dataset was studied for differential gene expression in the auditory and vestibular epithelia, and for reliability in detecting mRNA signals of hair cell-specific genes.
After evaluating reproducibility between replicates and benchmarking against a large set of genes whose expression was recorded using real-time RT-PCR, we conclude that 10 ng of total RNA is sufficient to obtain reliable gene expression profiles from the inner ear sensory epithelia using the NuGEN system. This corresponds to less than the amount of RNA obtained from the auditory sensory epithelium of one mouse cochlea. We further discuss the differences between the amplification systems employed.

205 Identification of MicroRNA Targets in the Inner Ear Using an Integrative Approach

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MicroRNAs (miRNAs) are 17-24 nucleotide-long non-coding RNAs processed from transcripts of endogenous genes that function through the RNA interference (RNAi) pathway. miRNAs regulate gene expression by inducing degradation of mRNA of target genes and by inhibiting translation. In addition to down-regulating mRNA levels, miRNAs directly repress translation of genes. Their relevance to the inner ear has recently been emphasized by the discovery of miRNA mutations leading to deafness in humans and mice. In order to optimize the search for miRNA targets, we focused on enriched targets that were differentially expressed in protein datasets but were unchanged in mRNA datasets. To achieve this goal, we combined a comparative transcriptomic and proteomic analyses with a miRNA screen of early post-natal cochlear and vestibular sensory epithelia derived from mice.
Expression profiling was performed using Affymetrix microarrays. Proteomics analysis was conducted using a Q-TOF mass spectrometer with iTRAQ labeling. miRNAs were identified through the miRCURY LNA™ array system. We integrated the transcriptome, proteome and miRNA levels with sequence-based predictions to efficiently identify functional miRNAs and their targets. The integration of these data allowed us to significantly reduce the number of potential targets using bioinformatic methods, thus enabling experimental validation of these interactions. The predicted miRNA/target pairs were validated. The two miRNAs with the highest differential expression in our study were miR-124, with a 3.5 higher expression in the cochlea, and miR-135b, with a 2.5 higher expression in the vestibule. We identified eight targets that had reciprocal expression with the miRNAs in our proteomic dataset: Lamc1, Vat1, Ddx6, Snx6, Hadha, and Actn4 for miR-124 and Sub1 and Psip1 for miR-135b. The miRNA-target pairs are being evaluated further to determine their biological significance and function in the mammalian inner ear.

206 Cochlear Hair Cell Micro-Isolates Regulate the Soma Size of Spiral Ganglion Neurons

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Specific features of spiral ganglion neuron morphology, such as soma area and branching patterns, vary with cochlear location, yet little is known about how the size and shape of these neurons is established. Therefore, this study is designed to determine the factors that regulate the soma area of spiral ganglion neurons.
We initially determined that neurons isolated from the basal cochlear region have a larger soma area (303 ± 9 μm^2, n=12) than their apical counterparts (265 ± 6 μm^2, n=12) *in vitro*. This difference was enhanced when spiral ganglion neurons are co-cultured with hair cell micro-isolates. Basal neurons paired with basal hair cell micro-isolates were larger (351 ± 24 μm^2, n=5) while apical neurons co-cultured with apical hair cell micro-isolates were smaller (221 ± 5 μm^2; n=4) than the soma areas observed in neuronal cultures. By mixing and matching hair cell micro-isolates with spiral ganglion neurons isolated from different cochlear regions we noted that cochlear tissues could alter soma area. For example, when basal neurons were paired to apical micro-isolates neuron size was significantly reduced (206 ±7μm^2, n=5; $p<0.01$). Correspondingly, apical neurons paired with basal micro-isolates showed an enlarged soma area (361 ± 27 μm^2, n=5; $p<0.01$). In both cases anti-neurotrophin function blocking antibodies significantly reduced the effects of altering the cochlear location of the hair cell micro-isolates. Because cochlear hair cells can secrete both brain derived neurotrophic factor and neurotrophin-3 in differing amounts (Flores-Otereo et al., *JNeurosci*. 2007), we will next determine whether neurotrophins on their own or in combination with other factors can influence neuronal size. Our goal is to understand the regulatory mechanisms that control morphology and to determine how these features contribute to the initial stages of auditory processing.
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207 Influence of Central and Peripheral Glia on Spiral Ganglion Neurite Growth

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Spiral ganglion neurons (SGNs) are bipolar having processes that interact with peripheral glia (Schwann cells, SCs) and with central glia (oligodendrocytes, OLs and astrocytes, ACs). Here we investigated the ability of these

glial cells to support and guide SGN neurite growth. SG explants were plated on the border between cultured glial cell groups or on individual glial cell cultures in the presence or absence of cell permeant cpt-cAMP (1 mM) or forskolin (FSK, 5 □M).
In explants placed on OL-SC or AC-SC borders, the number of neurites per explant was significantly increased on SCs (84.04±23.12) compared with the number of neurites on OLs (17.70±6.18) ($p<0.01$), indicating that OLs inhibit SGN neurite growth. There was no significant difference between the number of neurites on ACs (88.24 ± 22.08) and SCs (119.29 ± 21.42) ($p=0.16$). Treatment with cpt-cAMP or FSK each significantly increased the number of neurites on OLs (133.54±25.59 and 292.25±83.57, respectively) compared with control condition (6.75±2.21) ($p<0.01$). cpt-cAMP and FSK each also increased the number of neurites on ACs (213.19± 36.06 and 208.64±59.25, respectively), however the difference was not significant compared with control (111.38±38.73) ($p=0.30$). Although elevating cAMP levels overcame the inhibitory effect of central glia, the neurites failed to grow radially in a well-fasciculated pattern as on SCs. In dissociated SG cultures grown in the presence of ACs, OLs and SCs, neurites tended to follow SCs and OLs and cross-over ACs. Remarkably, most neurites initially followed the type of glial cell on which the neuronal cell body was found (e.g. neurites from SGNs on OLs initially followed OLs rather than SCs.)
In summary, SCs support and guide SGN neurite growth better than central glia. Similar to observations in other neurons increasing cAMP levels overcomes the inhibitory effect of central glia. The type of glial cell in contact with the SGN cell body may influence neurite behavior.

208 Surface Protein Patterning for Schwann Cell Alignment and Cochlear Spiral Ganglion Axon Guidance

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Background**:** Cochlear implant is considered as the only effective therapy for profound hearing impairment and a mean to regain auditory perception for deaf people. The implanted electrical device elicits the hearing sensation by directly stimulating the auditory neural pathway. One critical parameter for the success of this strategy is survival of spiral ganglion neurons. Promoting survival and, even more so, regenerative outgrowth of these neurons in the vicinity of the implants will improve the efficacy of the device.
Objective**:** The overall goal of this study is to design a surface that stimulates migration and attachment of endogenous or transplanted neural cells.
Methods**:** Micro-scale patterned-surfaces were designed with different extra-cellular matrix proteins using the direct stamp technique. Auditory spiral ganglion and other peripheral ganglia (i.e. vestibular, trigeminal, sciatic, and facial) were grown on protein patterns.
Results**:** Spindle-shaped cells with bipolar processes similar to Schwann cells were able to recognize and align to the laminin stripes. Neurons plated simultaneously showed physical co-localization to Schwann cells, and the outgrowing neurites followed glial cell tracks. Cell identity was confirmed by morphological appearance and immunohistochemical neural and glial markers.
Conclusion**:** The relative importance of acellular and cellular cues for directed neurite outgrowth is discussed.

209 Purification and Transfection of Schwann Cells from Postnatal Mouse Cochlea

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Schwann cells line nerve fibers in the peripheral nervous system and synthesize myelin. In the sciatic nerve, immature Schwann cells support neuronal survival, neurite growth and regeneration. The possibility that cochlear Schwann cells may function in a similar manner has significance for experimental efforts to maintain survival of neurons, or stem cells, and to encourage the regeneration, pathfinding and myelination of their neurites. We have demonstrated that Schwann cells (P75, Sox10, connexin29 positive) and spiral ganglion neurites spontaneously associate in dissociated cultures of postnatal mouse spiral ganglia (Whitlon et al. (2009) Neuroscience 161:227-235.). The mechanisms and consequences of interactions between cochlear Schwann cells and spiral ganglion neurites have not been examined. The purpose of this study was to develop methods for purifying sufficient numbers of Schwann cells from postnatal mouse cochleas and for transfecting them with expression plasmids. Insufficient numbers of Schwann cells exist in acutely dissociated spiral ganglia. The dissociated spiral ganglia were therefore plated on poly-D-lysine/laminin in medium containing BDNF, NT3, LIF, N2 and serum and maintained for 5 days. Cells were harvested with trypsin and subjected to an immunomagnetic purification method using rabbit anti-P75 antibody (Chemicon) and goat anti-rabbit IgG coupled to superparamagnetic microbeads (Miltenyi). Purified cells were plated on poly-D-lysine/laminin coated T25 flasks in the above medium. After 24 hours, the cultures were >90% pure. AMAXA nucleofection of purified Schwann cells with pMax-GFP plasmid gave greater than 45% transfection efficiency. These methods will allow us to directly evaluate the biochemistry, function, and genetic mechanisms of neurite growth promotion by cochlear Schwann cells.
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210 Expression of Wnt Receptors in Adult Spiral Ganglion Neurons: Frizzled 9 Localization at Growth Cones of Regenerating Neurites

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Cochlear implant performance in patients with sensorineural hearing loss could be improved by reducing the distance between implanted electrodes and their targets, the spiral ganglion neurons. This could be achieved by activating resident signaling pathways to stimulate neurite growth towards the electrode array. To systematically identify neuronal receptors for regenerative and guidance cues in the adult cochlea, a genome-wide cDNA microarray screen was conducted with the modioli of normal-hearing and noise-exposed CBA/CaJ mice. A meta-analysis of our results and data from two other studies revealed the expression of neuronal transmembrane receptors in the mature cochlea for the four established axon guidance pathways: ephrin, netrin, semaphorin, and slit. Interestingly, comprehensive expression of the Wnt receptor family was also observed. These Frizzled (Fzd) transmembrane receptors and their extracellular Wnt ligands have recently been associated with axon guidance, synapse formation, and dendrite morphogenesis throughout the nervous system, but their presence in the mature inner ear has not been described. After qualitatively confirming the expression of all Fzd receptors with RT-PCR, in-situ hybridization was used to localize Fzd1, -4, -6, -9, and -10 in adult spiral ganglion neurons. Fzd1, -4, and -10 are expressed differentially along the cochlea's tonotopic map, and Fzd6 was found in the saccular macula and in Scarpa's ganglion. These results were further validated by using real-time RT-PCR. Finally, immunofluorescence microscopy was used to locate Fzd9 protein in the somata and growth cones of adult spiral ganglion neurons that were regenerating their neurites in culture. On the basis of these results, we propose Wnt signaling as a candidate pathway for guiding neurite outgrowth towards a cochlear implant after sensorineural hearing loss.

211 Expression of the Transcription Factor Sox2 in the Injured Cochlear Nerve of the Adult Mice

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Recent studies have shown that Sox2 protein, a Sex-determining Region Y gene (SRY)-related transcription factor, is required for the differentiation and maintenance of sensory cells in developmental and adult cochlea (Kiernan et al., 2005; Dabdoub et al., 2008; Oesterle et al., 2008; Mak et al., 2009). Sox2 is also a key neural stem cell marker involved in the proliferation and specification of neural progenitor cells during development of the central nervous system and adult neurogenesis. In our previous study, an animal model of cochlear nerve injury was established by application of ouabain to the adult mouse ear, which yields a selective loss of type I spiral ganglion neurons (SGNs) and induction of glial hyperplasia. Here we investigate whether Sox2 is involved in cell proliferation and glial hyperplasia in the auditory nerve after ouabain injury. Cell proliferation and expression of Sox2 in the auditory nerve of adult CBA mice were examined at 1, 3, 7, 14 days and 1 month after ouabain exposure. Real-time RT-PCR assays revealed that the expression of Sox2 mRNA in the cochlear nerve significantly increased at 1, 3 and 7 days after ouabain exposure compared to that in control ears. The increased expression of Sox2 mRNA was confirmed by a quantitative immunohistochemical analysis. Cell proliferation analysis using BrdU incorporation showed that the number of BrdU+ cells significantly increased in the cochlear nerve at 3, 7, and 14 days after ouabain exposure. Importantly, double-labeling studies with antibodies to BrdU and Sox2 demonstrated that the majority of dividing cells in the injured auditory nerves expressed Sox2. Together, these results suggest that 1) up-regulation of Sox2 is involved in cell turnover in the cochlear nerve after ouabain exposure; and 2) Sox2 may play an important role in preventing further neural damage at an early stage of ototoxic injury in the adult inner ear. Supported by NIHDC7506, NIHDC0422, NIHDC0713 and AAO-HNS CORE136165

212 Aggregation of Type I Unmyelinated Spiral Ganglion Neurons in Congenic Ly5.1 Mice

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Spiral ganglion neurons (SGNs) are the primary carriers of auditory signals from sensory hair cells to the brain. With the exception of humans, the cell bodies of type I SGNs in most mammalian species are myelinated. In a previous study, we used the Ly5.1 congenic mice strain to investigate the role of hematopoietic stem cells in the adult mouse inner ear (Lang et al., [2006a] J Comp Neurol 496:187-201). Surprisingly, morphological assessment revealed that the majority of SGNs in the apical and middle turns of Ly5.1 mice were unmyelinated. Here, we further examined the auditory phenotype of young adult Ly5.1 mice using light and electron microscopy, immunohistochemistry and electrophysiological recordings. Three different mouse strains (CBA/CaJ, C57/B6 and SJL/J) were used as controls. Our data revealed several interesting characteristics of the inner ear in the Ly5.1 mouse including: 1) large aggregates of unmyelinated SGNs in the apical and middle turns; 2) symmetric junction-like contacts on adjoining plasma membranes of the unmyelinated neural cell bodies; 3) abnormal expression patterns for CNPase, connexin 29 and connexin 43 in the aggregated neural clusters; 4) reduced SGN density in the basal turn of Ly5.1 mice relative to control strains without a corresponding loss of sensory hair cells; 5) significant delays in auditory brainstem wave I

latencies at low and middle frequencies as compared to those of C57/B6 mice with similar ABR thresholds; and 6) elevated ABR thresholds and a decline in wave I amplitudes at high frequencies. The Ly5.1 mouse strain is the only rodent model identified which has the "human feature" of unmyelinated type I SGNs in the cochlea. Thus, the Ly5.1 strain may be an animal model for studying neural development and neuronal degeneration associated with aging and other auditory insults. Supported by NIHDC7506, NIHDC0422 and NIHDC0713

213 Role of SDF-1 Expression and Hematopoietic Stem Cells in the Injured Auditory Nerve

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BACKGROUND: The degeneration of hair cells and spiral ganglion neurons (SGNs) is an important pathologic process in the development of sensorineural hearing loss. In a murine model, predictable and reproducible damage to SGNs occurs through the application of ouabain to the round window. Recent evidence has shown that the chemokine stromal cell-derived factor-1 (SDF-1) is a potent chemoattractant of hematopoietic stem cells (HSCs) and provides trophic support to injured tissues during development and maturation. Our hypothesis for the current study is that engrafted HSCs and expression of SDF-1 play an important role in protecting SGNs and preventing further degeneration in the setting of cochlear injury.

METHODS: Bone marrow (BM) cells obtained from transgenic mice expressing enhanced green fluorescent protein (GFP) were injected into tail veins of adult irradiated recipient mice for HSC transplantation. HSC engraftment patterns in the transplanted mice were analyzed three and seven days after ouabain application. Auditory brainstem response (ABR) and the expression of SDF-1 mRNA and protein were examined one, three, seven, fourteen, and thirty days after application of ouabain in adult mice without HSC transplantation.

RESULTS: Following ouabain application, HSC engraftment was significantly increased in the auditory nerve compared to control ears in BM-transplanted mice. Real-time RT-PCR for SDF demonstrates increased mRNA expression following ouabain injury in non-transplanted mice; a double peak of mRNA expression with peaks at one day and between seven to fourteen days post-injury was observed.

CONCLUSIONS: SDF-1 expression and HSC engraftment is increased in the auditory nerve following cochlear injury. Further knowledge about the cochlear microenvironment, including SDF-1, is critical to maximizing HSC engraftment in the injured cochlea and providing a therapeutic option for sensorineural hearing loss.

214 Can Cell Substitution Therapy Be Used for Regeneration of Sensory Cranial Nerves?

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Several organs and organ functions can be replaced today. Regarding our senses, however, their functions cannot be repaired. The hearing organ is one of the senses in which most progress in a replacement strategy has been made. Today more than 100 000 patients have been implanted with a cochlear implant (CI). Following implantation, patients with severe auditory dysfunction get a significantly improved hearing ability. The CI is functioning despite of many hair cells have been damaged, probably by stimulating directly on the spiral ganglion neurons (SGN). Consequently, the function of a CI depends on the integrity of the auditory neurons. Following longer time of severe hearing impairment the number and integrity of the SGN is reduced. A SGN loss cannot be counteracted. These patients may therefore not benefit from or disqualify for a CI. The present research project is focusing on replacement of SGN including their central connections in the cochlear nucleus.

The project consists of an in vitro part where progenitor cells or stem cells are co-cultured with brainstem slices containing the cochlear nucleus. In the following in vivo part the most successful cell candidates are implanted into the living animal.

In the future we believe that strategies to replace auditory neurons may also have significance for other injured sensory cranial nerves. Until then primary issues as type of implant and how/ where to implant have to be solved. In this context we hope the organotypic co-culture model can show to be valuable.

215 Intra-Auditory Nerve Trunk Delivery Approach Preserves Rat Auditory Function

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In order to rescue or even replace degenerated cells in the cochlea, different substrates such as drugs, non-viral/viral vectors, neuronal tissue and cells have been delivered into the cochlea via various surgical routes. Commonly performed surgical approaches to access the cochlea (e.g. cochleostomy) may disturb the intracochlear structure and jeopardize the residual hearing. Cell replacement therapies are currently under thorough investigation but only a few experimental studies have explored the possibility to access the central portion of the auditory nerve. In order to be able to inject cells and substrates directly to the auditory nerve without damaging the inner ear we have developed and tested a new surgical technique. This technique is based on an occipital approach which gives the surgeon access to the cerebellopontine angle, the internal meatus and the auditory nerve. The preservation

of auditory function and the inner ear has been controlled by measuring auditory brainstem response (ABR) pre- and post-surgery. Further we have monitored the possible distribution of injected substrates by Horse Radish Peroxidase (HRP) tracer injections.

216 Pyridoxine Preferentially Induces Auditory Neuropathy Through Mitochondrial Dysfunction

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Pyridoxine, a form of vitamin B6, is an essential dietary constituent. However, chronic abuse of oral pyridoxine supplements may contract progressive sensory neuropathy. Previously, we reported that pyridoxine-treated mice exhibited an increase in the hearing threshold shift and delayed latency of both ABR and AMLR in proportion to pyridoxine dosage with extensive loss of auditory nerve fibers. This study was designed to elucidate the signaling mechanisms of pyridoxine toxicity in auditory cells including HEI-OC1 auditory cells, VOT33 spiral ganglion cells, the rat cochlear explants of organ of Corti, and spiral ganglion cells. Treatment with pyridoxine significantly decreased the viability of HEI-OC1 cells and VOT33 cells, which was accompanied with apparent apoptotic features, including fragmentation of nuclei and increased fraction of subGo/G1 phase. Interestingly, spiral ganglion neuron cells were more vulnerable against pyridoxine cytotoxicity than sensory hair cells. Treatment of VOT33 cells with pyridoxine resulted in an increase in intracellular protein levels of heme oxygenase-1 (HO-1) and superoxide dismutase 1 (SOD1) and SOD2 whereas it decreased the protein expression levels of GCLM. These results eventually lead increased generation of H2O2, which further cause the oxidative stress in VOT33 cells. Taken together, these data suggested that the cytotoxic mechanism of pyridoxine was ascribed to ROS generation, which would result in the modulation of the intracellular redox cycle.

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217 Is the Projection from the Locus Coeruleus to the Cochlear Root Neurons Involved in the Mediation of the Acoustic Startle Reflex?

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The noradrenergic modulation of the acoustic startle reflex is well known, but there is a lack of information about their neuronal connection with cochlear root neurons (CRNs), a fundamental element of the acoustic startle pathway. To study the noradrenergic projection to the CRNs, we performed immunohistochemical visualization of tyrosine hydroxylase (TH), dopamine-beta-hydroxylase (BDH) and tract-tracing experiments injecting biotinylated dextran amine (BDA) into the locus coeruleus. To determine the effects of noradrenergic fibers on the startle reflex, seven rats of both sexes were injected intraperitoneally with 50mg/kg of DPS-4, a neurotoxin that selectively damage the noradrenergic neurons originating in the locus coeruleus. In control and lesioned animals, we tested the startle reflex and prepulse inhibition (PPI) at different interstimulus intervals prior to and 1-2 weeks after DSP-4 administration. After the last behavioral test, we studied the immunoreactivity to BDH and TH in the cochlear root.

After BDA injections into the locus coeruleus, we observed anterogradely labeled fibers with boutons in apposition to the CRN somata of both brain hemispheres with ipsilateral predominance; these BDA-positive boutons co-localized with dopamine-beta-hydroxylase immunoreactivity. Our behavioral tests showed a gradual decreasing of the startle reflex and an increase in PPI in the treated animals. Moreover, two weeks after DSP-4 administration, the treated animals loosed the BDH immunoreactivity in the cochlear nerve root. All together suggest that the noradrenergic system is directly involved in the modulation of the startle at the level of the CRNs, which are likely to play an important role in this paradigm.

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218 New Insights of the Ionotropic Glutamate Receptor Subunits Composition in Cochlear Nuclei. A SDS-Freeze Fracture Immunogold Labeling Study

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In the auditory pathway, the response of auditory neurons to acoustic stimulation must accommodate rapid transmission and maximize temporal fidelity through their synaptic networks. Consistent with these demands is the observation that targets of the auditory nerve (AN) often express fast kinetic receptors in the cochlear nuclei (CN) compared to other excitatory synapses (parallel fibers) that mainly play a modulatory role in auditory neurotransmission. The composition of AMPA and NMDA glutamate receptors at excitatory synapses in the CN has been well documented by postembedding immunogold labeling after freeze-substitution (Rubio and Wenthold, 1997, Neuron 18: 939; Wang et al., 1998, J Neurosci 18:1148; Whiting et al., 2009, Neuroscience PMID:19646510). This technique has several advantages such as the ability to identify the structures of interest and provides precise details of molecular localization in subcellular domains. Nevertheless, one of the major concerns with the technique is that reliable quantification of immunoreactivity is affected by low sensitivity and limited accessibility of antibodies to target molecules found deeper in the tissue. In this study, we have used SDS-digested freeze-fracture replica labeling (SDS-FRL) - a newly developed immunogold method that has high-spatial resolution and high-detectability (Masugi-Tokita et al., 2007; J Neurosci 27:2135) - to reinvestigate the subunit composition of specific AMPA and NMDA glutamate receptors at excitatory synapses of the ventral and dorsal CN. Data show a differential distribution of AMPA and NMDA receptor subunits between targets of the AN in the ventral and dorsal CN, and also between the targets of the parallel fibers in the molecular layer of the dorsal cochlear nucleus.

219 Excitatory Inputs to Bushy Cells

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The innervation by auditory nerve fibers of bushy cells in the anteroventral cochlear nucleus (aVCN) is at least in part through end bulbs of Held. Within the VCN, auditory nerve fibers bifurcate and terminate on cochlear nuclear neurons. Some small collateral branches of auditory nerve fibers terminate with one or a small group of synaptic boutons on bushy or stellate cell target neurons. At other branches the axon widens and ends in fingers and/or clusters of boutons that wrap around postsynaptic bushy cell bodies. There is considerable variation in the shape and complexity of those endings. We have recorded excitatory postsynaptic currents (EPSCs) in response to shocks to fiber bundles near bushy cells in slices from mice that were identified by their biophysical characteristics (Cao et al., 2007, J Neurophysiol 97:3961-3975). As the strength of shocks was gradually increased, the magnitude of the postsynaptic current increased in jumps. The amplitude of current jumps likely reflects the contribution to the synaptic current of a single auditory nerve fiber. On average, each excitatory input contributed 1.3 nA at -65 mV but individual inputs varied continuously in amplitude over a 50-fold range, between about 0.1 and 5 nA suggesting that there was not a distinct difference between end bulb inputs and smaller terminals that are not end bulbs. The distribution of numbers of inputs was bimodal with 60% of bushy cells having <4 inputs and 40% having >4 inputs. Bushy cells are known to differ in their projection patterns. Our findings are consistent with the interpretation that two populations of bushy cells can be distinguished on the basis of the number of excitatory inputs. This work was supported by a grant from the NIH DC00176.

220 The Endbulb of Held as a Model Synapse to Study Synaptic Transmission and Integration *in Vivo*

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Synaptic transmission is a crucial element of neuronal processing but due to the difficulty of intracellular recordings it is usually studied *in vitro* where physiological means of activation are missing. In the present study we provide a basis for *in vivo* studies of synaptic transmission and integration using the endbulb of Held/spherical bushy cell synapse (SBC) as a model system. Extracellular signals from this synapse have up to three wave components. Signals lacking the third component are frequently observed, but since the origin of each of the components is uncertain, interpretation of the failure has been controversial.

Here we have used *in vitro* methods to identify the electrophysiological basis of complex waveforms recorded at this synapse. We combined single- and multiunit methods and used pharmacological tools to block glutamate receptors. Simultaneous extra- and intracellular recordings from single SBCs demonstrated a presynaptic origin of the first component, consistent with data obtained by multielectrode array recordings of local field potentials. The later components originated from the EPSP and AP of the SBC, respectively.

These results will provide the basis for future investigation of convergence of excitatory and inhibitory inputs in modulating transmission at a fully functional neuronal system using physiological stimulation.

221 In Vivo Study of AVCN Principal Neurons: Input Convergence and Synaptic Plasticity

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Numerous in vitro studies have documented synaptic depression at the endbulb of Held and explored its impact on the bushy cells' (BC) most prominent physiological feature: the temporal sharpening of the phase-locked auditory nerve input. Synaptic depression together with inhibitory inputs seem to govern the response of BC to convergent inputs. However, few studies have addressed to which extent these observations hold true in the intact animal. It is not well understood what the characteristics of convergent AN inputs on BC are and how excitatory inputs and inhibition interact to influence the temporal precision of the BC response in vivo.

We tried to address these issues by analyzing the waveforms of complex spikes and failed events gathered from juxtacellular micropipette recordings in the anterior AVCN of the gerbil. Based on previous work we interpreted the complex spike waveforms as a three-component signal, consisting of the presynaptic action potential, the postsynaptic potential and the action potential in the postsynaptic neuron. We studied relative timing and amplitudes of these components and the effects of inter-event-intervals and features of the auditory stimuli. Our main preliminary conclusions are: 1) Evidence of short-term plasticity at the endbulb of Held can not be detected in vivo. 2) Non-monotonic frequency response areas appear to be shaped mainly by inhibition. 3) Existence of very short event intervals (shorter than the refractory period seen in the auditory nerve) underline the role for input summation in the principal function of BC. Furthermore, in certain stimulus situations (ie high levels) spikes show a higher synchrony to the stimulus than the totality of events comprising all inputs to the BC, again suggesting an important role for input summation in BC function in vivo.

222 A Model of Activity-Dependent Recovery from Synaptic Depression in the Avian Cochlear Nucleus Without Residual Calcium

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Short-term synaptic plasticity acts as a time- and firing rate-dependent filter that mediates the transmission of information across synapses. In the auditory brainstem, specific forms of plasticity are expressed at different terminals of the same auditory nerve fibers and contribute to the divergence of acoustic timing and intensity information into separate, parallel computation pathways. Using whole-cell patch clamp recordings of postsynaptic currents in the avian cochlear nucleus angularis and magnocellularis, we demonstrate that these synapses have a very rapid recovery from depression, explaining their ability to maintain responses during high rates of spontaneous and sensory driven activity. The rate of recovery showed a dependence on the presynaptic activity levels. In both the intensity and timing pathways, the time course of recovery from short-term depression was bi-exponential, with a fast time constant of ~40 ms, explaining ~80% of the recovery, and a slow time constant on the order of several seconds. In the intensity pathway, the fast recovery was not due to postsynaptic receptor desensitization, and was complicated by the presence of short-term facilitation that may partially contribute to the rapid time course of recovery. A cascade model of synaptic vesicle replenishment is described that accounts for the activity-dependence and time course of the recovery without requiring residual calcium feedback. The simple model of presynaptic short-term plasticity presented here provides an unexpected explanation for activity-dependent recovery and illustrates the dynamic nature of vesicle cycling.

223 The Effects of Delayed Release on Firing in the Cochlear Nucleus

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Synaptic transmission consists of both synchronous and delayed release components. The delayed component can become significant during long periods of high activity. At inhibitory synapses, delayed release contributes to tonic inhibition, but its role at excitatory synapses is unknown. This is a particular issue in the auditory system, as precise timing information about sounds could be disrupted by delayed release. To address this, we studied auditory nerve synapses onto bushy cells of the cochlear nucleus, using brain slices taken from P15–49 CBA/CaJ mice, at 34°C. Voltage-clamp recordings indicated that delayed release is prominent at frequencies ≥ 100 Hz with trains of 20 pulses at all ages studied. When single auditory nerve fibers were activated in current-clamp recordings, reducing delayed release with EGTA-AM or enhancing delayed release with strontium had little effect on spike probability or timing. To test the role of delayed release when more presynaptic fibers are active, we used dynamic clamp. We found a small increase in response probability with four active inputs, but little effect on spike timing. In immature synapses (P6–11), delayed release caused significant addition of misplaced spikes long after the end of the train. We found similar misplaced spikes in mature slices, when we applied α-dendrotoxin. Thus, it appears that the effects of delayed release are suppressed in mature bushy cells through the actions of $K_v1.x$ channels. This may indicate that delayed release is an unavoidable consequence of normal transmission.

224 Glycinergic Inhibitory Input Onto Bushy Cells in the Cochlear Nucleus

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Neurons within the cochlear nucleus, spherical bushy cells (SBCs) and globular bushy cells (GBCs), are important for sound localization as they initiate the pathways that

encode interaural time and level differences, respectively. As part of a plan to investigate the role of inhibition in sound localization pathways, we have quantified the inhibitory glycinergic input onto bushy cells. Tissue from young CBA/CaH mice was labeled using glycine immunohistochemistry and counterstained with cresyl violet so that the two types of bushy cells and their glycinergic inputs could be identified. Fluorescent confocal microscopy was used to investigate the three-dimensional distribution of glycinergic inputs onto SBC and GBC somata. Immunopositive glycine synaptic terminals were counted in the cochlear nucleus at the light microscope level using a 100x oil immersion lens. Three-dimensional reconstructions indicated that glycinergic inputs were distributed uniformly on the cell somata of both SBCs and GBCs. GBCs, however, showed a significantly greater number of glycine terminals (19 ± 0.8; n=15) than SBCs (13 ± 0.9; n=15; $p<0.01$). This difference was maintained when the number of terminals was standardized to cell circumference for GBCs (0.4 ± 0.02 terminals/µm) and SBCs (0.3 ± 0.02 terminals/µm; $p<0.01$). These results suggest important but different roles for glycine on the cells that initiate auditory pathways encoding interaural time and level differences. Future studies will combine these anatomical findings with electrophysiological recordings of inhibitory responses in these cells with quantification of the inhibitory inputs at the electron microscope level.

225 Dopaminergic Modulation of Action Potential Initiation in Auditory Brainstem Interneurons

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Dopamine is central to the neural mechanisms of reward, learning, and attention in many brain regions, yet its function in the auditory brainstem remains unclear. Dopaminergic signaling through G-protein coupled pathways can alter the activity of many ion channel types, including those present in the axon initial segment (AIS). The AIS is a specialized compartment with Na^+ and K^+ channels, and is the site of action potential initiation in many cells. Recently, we found that low voltage activated T- and R-type Ca^{2+} channels are also expressed in the initial segment (Bender & Trussell, 2009). These channels contribute to the subthreshold depolarization of the local membrane, and can influence the timing and number of action potentials generated by a given stimulus. Using a combination of electrophysiology and 2-photon Ca^{2+} imaging, we show that initial segment Ca^{2+} channel activity is regulated by dopamine. Slices were made from P16-21 mice, and experiments were performed in cartwheel interneurons of the dorsal cochlear nucleus, an auditory brainstem structure that aids in localizing sound in the vertical plane. In cartwheel cells, initial segment Ca^{2+} channels are functionally co-localized with Na^+ channels, and block of Ca^{2+} channels by local application of T- and R-type antagonists Ni^{2+} or mibefradil can delay or prevent action potentials triggered alone or in bursts. By monitoring action potential-evoked Ca^{2+} transients, we found that Ca^{2+} influx was reduced by dopamine type 2 family receptors (D2R) through protein kinase C (PKC). We then isolated T-type Ca^{2+} currents in voltage clamp and found that the D2-PKC pathway reduced Ca^{2+} channels activity in the AIS but not in the dendrites. We therefore hypothesize that the D2-PKC pathway has compartment-specific effects on T-type channels, and activation of this pathway could influence action potential generation through selective down-regulation of initial segment Ca^{2+} channels. To test this idea, we evoked action potential bursts (2-3 spikes per burst) with somatic current injection and bath applied PMA, a PKC activator. While no change was observed in intrinsic membrane properties, the number of action potentials evoked per burst was reduced by over 50%. To test if Na^+ channels were affected by this pathway, we monitored AP-evoked Na^+ transients in the initial segment and found that they were unaltered by the D2R agonist quinpirole, but were reduced by low concentrations of TTX (10 nM). Thus, the D2R-PKC pathway is specific for AIS Ca^{2+} channels, allowing for dopaminergic control of action potential output. This regulation could allow for attention-based modulation or reinforcement of proper sound localization in the dorsal cochlear nucleus.

226 P2 Receptors in the Cochlear Nuclues Neurons - In Vitro and in Vivo Study in Gerbil and Mice

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In mongolian gerbil, activation of P2 purinoreceptors was recently shown to change the firing pattern of developing spherical bushy cells (SBCs) from phasic to tonic (Milenkovic et al., J Neurophysiol., 102: 1821-33, 2009). While the involvement of ionotropic P2X receptors seems to be necessary for this effect, the role of metabotropic $P2Y_1$ receptors remained puzzling. To investigate the contribution of $P2Y_1$ receptors in purinergic signaling on SBCs, in vitro and in vivo experiments are conducted in gerbil, 129/SV mice and $P2Y_1$-knockout 129/SV mice. The abundance of large SBCs is different between gerbil and mouse cochlear nuclei due to diversity in hearing frequencies in these two species. Thus, we first compared the P2 receptor-mediated responses in acute brainstem slices of P5-14 gerbil and mice. Whole cell recordings from biocytin-filled neurons revealed responses in large SBCs but not in stellate cells. In gerbils, metabolically stable P2 receptor-agonist ATPγS evoked stronger depolarizations compared to mice. Moreover, activation of P2 receptors in combination with depolarizing current injection did not evoke multiple firing in mice but it was regularly observed in gerbil SBCs. This difference is probably due to higher input resistances measured in gerbil SBCs compared to neurons in mice of the same age. Activation of $P2Y_1$ receptors by ADPβS evoked transient Ca^{2+} signals and depolarization in gerbils and WT mice but no action potentials. In vivo extracellular recordings of pre-potential neurons in pre-hearing animals show significantly lower

frequency of spontaneous discharges in the $P2Y_1$-knockout mice with respect to WT mice and gerbils. This data indicate that $P2Y_1$ receptors possibly influence the excitability of SBCs before the onset of acoustically evoked signal processing.

227 Laser Photostimulation Mapping of Local Circuits in Rat Dorsal Cochlear Nucleus

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The local circuitry of the dorsal cochlear nucleus (DCN) provides a substrate for both auditory and cross-modal sensory processing. Anatomists have provided detailed descriptions of many different cell types and their axonal arborizations within the nucleus, but our knowledge of the functional synaptic connections within this nucleus is limited. In the present study, we used focal laser scanning photostimulation with caged glutamate to map excitatory and inhibitory connections in P11-P16 rat DCN brain slices. Slices cut both parallel to isofrequency sheets ("strial" slices) and orthogonal to isofrequency sheets ("transstrial" slices) were used. Recordings were made primarily, but not exclusively, from pyramidal cells. Local inhibitory connections to pyramidal cells were visible from sites in all layers as well as close to the cell body. Connections from layer 1 and the layer1-2 border sometimes were associated with bursts of IPSCs suggestive of cartwheel cell firing. Distant inhibitory connections crossing the isofrequency planes were also evident in layers 2 and 3 up to at least 300 microns from the recorded cell's soma. Weak excitatory inputs were seen from scattered sites in all layers. A consistent and unexpected excitatory input was seen from sites in the very deepest regions of the DCN. This input generated EPSCs with latencies of about 20 msec. Excitatory inputs also crossed the isofrequency axes. In summary, these experiments have revealed a novel functional excitatory connection within the DCN, and permit us to assay the spatial organization of sparse synaptic inputs to DCN pyramidal cells. Our results suggest that pyramidal cells integrate inputs from a larger number of sources than previously known.

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228 Comparison of Tone-Evoked Responses Measured Optically Using a Voltage-Sensitive Dye with Electrophysiological Responses Based on Multiunit Recordings

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We have been investigating the use of optical recordings based on voltage sensitive dye imaging as a means of measuring sound-evoked and spontaneous activity on the surface of the mammalian dorsal cochlear nucleus (DCN). In the hamster, the DCN straddles the dorsolateral surface of the brainstem with its tonotopic axis oriented more or less horizontally along the medial-lateral axis. Previous work has demonstrated vigorous sound-evoked electrophysiological and optical responses on the surface of the DCN. However, systematic studies comparing the responses obtained using electrophysiological and optical recording methods have not yet been carried out. We therefore compared responses of multiunit clusters in the DCN to tonal stimuli recorded by these two methods. The dye used for optical imaging was Di-2-ANEPEQ. We compared measures of temporal responses as well as responses to tones of increasing intensity. The results indicate similarities in some aspects of these responses and differences in others. Response latencies for electrophysiological responses tended to be in the range of 7-10 ms, compared to 15-30 ms in the optical responses. Thresholds were lower for electrophysiological responses, typically between 6 and 18 dB SPL compared to optical responses, which were in the range of 18-36 dB SPL. The rate intensity functions based on electrophysiological recordings were usually monotonic with clearly defined plateaus or non-monotonic with firing rates diminishing below the peak level in the mid-intensity range. Dynamic ranges were typically between 30-40 dB. In contrast, optically recorded response were monotonic but did not reach saturation levels and displayed dynamic ranges of at least 80 dB, or roughly twice the dynamic range of the electrophysiological response. These differences may indicate that the two types of response originate from different sources. (Supported by NIH grant R01DC006041).

229 Exploring Multisensory Integration Using a Three-Dimensional Silicon Microelectrode Array for Simultaneous Ventral and Dorsal Cochlear Nucleus Recording and Stimulation

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Somatosensory projections from the spinal trigeminal nucleus (Sp5) to the cochlear nucleus predominantly target the granule cell domains of the dorsal cochlear nucleus (DCN) and ventral cochlear nucleus (VCN). However numerous SP5 terminals are also found in the deep layers of DCN and magnocellular regions of VCN (Shore and Zhou, J. Neurosci Res., 2004; Haenggeli et al., J. Comp. Neurol., 2005), presumably contacting giant cells, multipolar cells and bushy cells. Sp5 stimulation can modify the responses of DCN pyramidal cells to acoustic stimulation but the influence of the Sp5 input on auditory coding in the VCN and deep layers of DCN has not yet been studied. An added complication in the interpretation of these experiments is the bidirectional connection

between DCN and VCN via tuberculoventral cells in deep DCN and stellate cells in VCN.
To address these questions we have developed a three-dimensional array composed of 4-shank/32 site silicon probes. One to two 32-site probes target the DCN allowing us to record in multiple cell-layers of the DCN across its mediolateral tonotopic axis. One to three 16-site probes target the VCN across its tonotopic axis, with the addition of 16 adjacent or concentric stimulation sites. This configuration allows for simultaneous recordings in VCN and DCN while stimulating Sp5, with the possibility of stimulating in VCN to explore the connections between DCN and VCN in multisensory processing.
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230 Sp5 Stimulation Leads to Prolonged Excitation in AVCN, and Inhibition in Deep DCN Neurons

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In addition to receiving inputs from the auditory nerve, the guinea pig cochlear nucleus (CN) also receives inputs from the somatosensory system (Shore et. al., J. Comp. Neurol., 2000; Zhou and Shore, J. Neurosci. Res., 2004). While most of these inputs terminate in the granule cell domain (GCD) of the CN, the magnocellular regions of antero-ventral cochlear nucleus (AVCN) and deep DCN also receive excitatory (glutamatergic) inputs from the spinal trigeminal nucleus, (Sp5; Zhou and Shore, J. Neurosci. Res., 2004; Zhou et. al., J. Comp. Neurol., 2007), a region associated with somatosensation from the vocal tract. Previous studies have investigated the short-term modulation of firing properties of CN neurons by electrical stimulation of the trigeminal system. However, in this study, we examine the long-term effects of Sp5 stimulation on the firing patterns of neurons in the AVCN and deep DCN.
Unit responses were recorded using multichannel electrodes placed into the DCN and VCN of ketamine-anesthetized guinea pigs. Rate-level functions (RLF's), obtained at best frequency, were used to determine changes in the sound-driven firing rates of units by Sp5 stimulation. RLFs were measured for several minutes before and after current stimulation (60 µA; 2 biphasic pulses, 100 µs/phase). Primary-like AVCN units showed a prolonged, significant increase in firing rate after Sp5 stimulation. In contrast, units in the deep DCN showed a long lasting decrease in firing rate. These results suggest that the Sp5 is exerting a direct excitation of AVCN units and an indirect inhibition of units in deep DCN through inhibitory interneurons.
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231 The Effect of Reverberation on the Representation of Single Vowels, Double Vowels and Consonant-Vowel Syllables by Single Units in the Ventral Cochlear Nucleus

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Reverberation is the persistence of acoustic energy in an enclosed space after the sound source has stopped; it is due to the multiple reflections from the surfaces within that space. Reverberation has a filtering effect, introducing distortion in both the spectral and temporal domains. For instance, spectral transitions are smeared in time and the slowly decaying 'tails', added at sounds offsets, effectively applies a low-pass filter to the temporal envelope. It is well known that reverberation can have a deleterious effect on the intelligibility of complex time-varying stimuli such as speech. For instance Culling et al. (1994) examined the effect of reverberation upon segregation mechanisms for double vowels. They found that the effect of differences in the F0 of the two vowels was robust in reverberation provided that the F0s were constant, however, when they were sinusoidally modulated (i.e. changing over time) this segregation effect was abolished. Little, however, is known about how reverberation affects the neural representation of these speech-like sounds. To address this question we have studied the effects of reverberation on the representation of single vowels, double vowels and consonant-vowel syllables from the responses of single units in the ventral cochlear nucleus of the anaesthetised guinea pig. Reverberation was added by time-domain convolution of CV's with impulse responses (IR) recorded in a corridor at source-to receiver distances of .32, 1.25, 5, 10m (courtesy of Tony Watkins) or with simulated IRs. In agreement with a previous study on the representation of the fundamental frequency (F0) of frequency swept harmonic complexes we show that the degradation of F0 is critically dependent on the neuron's best frequency, the listener's distance from the sound source, the F0 and, in this study, the F0 modulation.

232 Short Latency Forward Inhibition in the Ascending Auditory Pathways

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Acoustic information is relayed to the auditory cortex (AC) through a series of auditory structures. The complex synaptic connections cause time delays for neural signals to reach target structures, which depend on the diameters of axons and the property of synaptic transmissions. It has been reported that there are direct projections from the dorsal cochlear nucleus (DCN) to the medial geniculate body (MGB) and from the inferior colliculus (IC) to the AC. However, it is still unclear how these projections contribute to sound perception. In the present study, we investigated the time delays of sound-induced neural activity in the DCN, IC and AC of both anesthetized and unanesthetized

rats. Experiments were conducted in 12 adult Long-Evans rats in which the DCN, IC and/or AC were implanted with microwire electrode arrays (Clunbury Scientific). Acute recordings were performed 1-2 hours after implantation under anesthesia. Chronic recordings were performed 1-6 weeks after implantation while the animals were unanesthetized. Latencies of responses to both pure tones and broad band noise were characterized in the DCN, IC and AC. Our results demonstrated that 1) short latency inhibitions in the DCN, IC, and AC occurred prior to main excitatory responses, and 2) the inhibitions were often preceded with an excitatory response. These results suggest that there is a forward inhibition with a short latency before major neural activation occurs along the ascending pathways. The mechanisms may involve that the ascending fibers directly inhibit target neurons, activate local inhibitory circuitry, and/or bypass certain auditory centers. Unlike conventional forward inhibition that modulates frequency tuning curves, this forward inhibition might play an important role in sound perception by suppressing background activity to prepare the brain for major incoming acoustic stimuli.

233 Generation and Characterization of Mouse Lines Expressing a Tamoxifen-Inducible Cre-Recombinase Under the Central Auditory-Specific KCNK15 Promoter

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Temporally and spatially regulated gene silencing is essential for the analysis of genes and circuits within the auditory system. In contrast to the cochlea, this possibility does not yet exit for the central auditory system. Towards this end, we set out to generate a mouse with expression of a tamoxifen-inducible Cre-recombinase (Cre-ERT2) under the promoter of the KCNK15 gene. This gene encodes Task5, which is highly specific for the central auditory system (Karschin et al., 2001; Mol Cell Neurosci 18: 632-648). Task5 is present in the spiral ganglion neurons and the higher order auditory processing centers up to the inferior colliculus. Importantly, the protein is not found in the organ of corti. Using recombineering technologies, a bacterial artificial chromosome was generated, which expresses Cre-ERT2 under the KNCK15 promoter. After pronuclear injection of this construct, 9 founder lines with genomic integration of the DNA were identified. The progeny of these founders are currently characterized for expression of Cre-ERT2. These mice can then be crossed with the ever increasing number of mice containing floxed genes. Locally or temporally administrated tamoxifen to the progeny will enable time and region specific gene deletion within the central auditory pathway. This will provide new insight into the molecular requirements for development and function of the central auditory system and will contribute to an improved understanding of the mechanisms underlying central hearing impairment.

234 Organization of Neuronal Circuits in the Cochlear Nuclei of Mice That Lack Otoferlin

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The orderly innervation by auditory nerve fibers gives the cochlear nuclei a tonotopic organization. Connections between tuberculoventral cells in the deep layer of the dorsal cochlear nucleus (DCN) and their targets in both the dorsal and ventral cochlear nuclei (VCN), and connections between T stellate cells and their targets in the VCN and DCN follow that tonotopic organization. Even when mice never hear, the arrangement of both the auditory nerve fibers and the intrinsic connections is preserved (Cao et al., 2008, J Comp Neurol 510:297-308). Patterned spontaneous activity could play a role in forming or sharpening the tonotopic pattern (Tritsch et al., 2007, Nature 450:50-55). We therefore examined neuronal connections in mice that lack otoferlin (otof-/-) which support little or no synaptic transmission from hair cells to spiral ganglion cells (Roux et al., 2006, Cell 127:277-289; Beurg et al., 2008, J Neurosci 28:1798-1803), but do support synaptic transmission within the VCN and DCN. We found that although the cochlear nuclei are smaller in the otof-/- mice than in heterozygotes or wild type animals, the tonotopic relationship is largely preserved though some fibers made unexpected turns. End bulbs of Held were less common and wispier, and boutons were smaller in otof-/- than in otof+/-mice. The orderliness of the neuronal organization in the cochlear nuclei in mice that get little or no input from hair cells throughout life indicates that neither spontaneous nor sound-driven activity patterns play a major role in shaping the overall composition of the neuronal networks in the cochlear nuclei, but that it does affect the morphology of auditory nerve terminals. This work was supported by a grant from the NIH DC00176.

235 Is Pax6 Required for Normal Development of the Cochlear Nucleus?

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One approach to understanding how the cochlear nucleus (CN), the obligatory synaptic relay in the brain of inner ear afferents, becomes organized is to examine the role of gene function. A large body of data exists on the molecules that are expressed in juvenile and mature CN neurons, but studies are only beginning to examine the role of genes during development. We have previously reported on a role for the paired homeodomain transcription factor, Pax6, in CN development. In embryonic day 16.5 wild type mice, Pax6 mRNA is detectable in the ventral cochlear nucleus (VCN). As development proceeds, the expression domain expands to include the molecular/granule cell layer and portions of the dorsal CN (evident at least by postnatal day (P) 0). We have previously reported that the Math5-expressing region of the VCN is reduced in volume in Pax6 -/- mice.

Using an independent molecular marker for the CN, we have examined in more detail how Pax6 loss of function affects CN development. We have used to our advantage the fact that the receptor tyrosine kinase, erbB4, is

expressed in the developing CN. At P0, erbB4 mRNA is detected in wild-type (Pax6 +/+) mice in the molecular/granule cell layer and at a higher level in cells positioned around and within the VCN core. In Pax6 -/- mice at P0, high levels of erbB4 mRNA are expressed, indicating that erbB4 is not down-regulated nor a target of Pax6. However, there are only a few scattered cells near the edge of the VCN core and few, if any erbB4-positive cells distributed within the core region.
These findings suggest that the reduction in VCN volume that we have previously observed, may be due, in part, to the absence of erbB4-positive cells within the VCN. Pax6 is necessary for neuronal migration of other nuclei that are derived from the rhombic lip (Englekamp, et al., '99). Future studies will assess whether cell migration is also a function for Pax6 within the cochlear nucleus.
Supported by Deafness Research Foundation

236 Anatomical Plasticity in Brainstem Auditory Nuclei Following Unilateral Cochlear Ablation in Neonatal Rat

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Objectives: Hearing loss produced by cochlear damage during early development may result in persistent changes in the organization of the central auditory system in adults. The purpose of the present study was to investigate the neuro-anatomical changes produced in the auditory brainstem of rats with unilateral cochlear ablation conducted prior to the onset of hearing.
Methods: Following unilateral cochlear ablation during early development, we examined the anatomical plasticity of projections from the cochlear nucleus (CN) and the dorsal nucleus of lateral lemniscus (DNLL) to the inferior colliculus (IC) using retrograde tract tracing methods with fluoro-gold (FG). We compared the number of labeled neurons and patterns of brainstem projections to the ICs.
Results: Upon reaching adulthood, a marked decrease in volume of CN was observed in the ipsilateral to the cochlear ablation, particularly in the ventral nucleus of the CN (VCN). The number of retrograde labeled neurons in the ipsilateral VCN was also markedly decreased. Neurons labeled with FG were found in the DNLL on both sides. The number of FG-labeled neurons in the DNLL was almost the same as the normal control and the ratio labeled neurons in the bilateral DNLL was not significantly different across groups.
Conclusion: Unilateral congenital deafness with a volume reduction in CN did not induce changes in the number of retrograde labeled neurons in the DNLL when FG injections were made into the IC in rat. Because DNLL receives several inputs from lower brainstem auditory nuclei, the loss of neurons and a volume reduction in the unilateral CN did not influence upper brainstem auditory structure following unilateral congenital deafness.

237 Changes of Glutamate Concentration in Relation to Neuron Density in the Chinchilla Anteroventral Cochlear Nucleus Following Cochlear Ablation

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There is evidence for glutamate as a transmitter of auditory nerve fibers and cochlear nucleus projection neurons as well as a non-transmitter-related metabolite in neurons and glia. To obtain further insights into the relation of glutamate to auditory nerve fibers vs. cochlear nucleus neurons, we compared glutamate concentrations with neuron densities in the chinchilla anteroventral cochlear nucleus (AVCN). Among a group of chinchillas with ablation of the right cochlea, one with 1-month post-surgery survival was chosen for detailed mapping of amino acid concentrations in both left and right cochlear nuclei, by assay of samples microdissected from freeze-dried coronal sections. Gradients of glutamate concentration were found in both the left and right AVCN, and decreases of glutamate concentration in the right AVCN were proportionately larger in its more caudal parts. By superimposing the maps of the dissected sections using a drawing tube, at 50 times magnification, with adjacent Nissl-stained sections, the number of neurons corresponding to each microdissected sample was estimated. Also, the volume of each sample was measured so that its neuron density could be compared with its glutamate concentration. High and statistically significant ($P<0.01$) positive correlations between glutamate concentration and neuron density were found for both left ($r = 0.85$) and right ($r = 0.65$) AVCN. The proportional decreases of glutamate concentration in the samples of the right AVCN compared to corresponding ones on the left were larger for samples with lower neuron densities ($r = -0.44$, $P<0.01$), but the absolute concentration differences between the corresponding left and right samples showed no correlation with neuron density ($r = -0.09$). We conclude that glutamate is prominent in both auditory nerve fibers and AVCN neurons and that the decrease of glutamate 1 month after cochlear ablation predominantly reflects its loss from degenerating auditory nerve fibers.

238 Inhibitory Synaptic Transmission in Bushy Cells During Age-Related Hearing Loss

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Bushy cells in the anteroventral cochlear nucleus are innervated not only by the excitatory inputs from the auditory nerve, but also inhibitory inputs from various sources including the dorsal cochlear nucleus (DCN). While it has been shown that the excitatory transmission in bushy cells weakens during age-related hearing loss

(AHL), it is unclear whether inhibitory transmission also changes during the AHL process. In this study, we assessed inhibitory synaptic transmission in bushy cells using two different mouse strains, CBA mice with normal hearing and DBA mice with early onset AHL. Evoked IPSCs were recorded by electrically stimulating DCN deep layer while APV and CNQX were applied to block glutamatergic transmission. Spontaneous IPSCs were also measured under the same condition. We found an increased paired-pulse ratio of the evoked IPSCs at a 50ms interval in six-month old DBA mice (with AHL) in comparison with both age matched CBA mice (normal hearing) and young DBA mice at P21 (relatively normal hearing). This suggests that the release probability of the inhibitory transmission decreases during AHL. However, no significant difference was found in either the amplitude of the evoked and spontaneous IPSCs, or the spontaneous event frequencies. The results indicate that changes in inhibitory synaptic transmission can occur with AHL, and that these are distinct from changes in excitatory transmission. These changes in inhibition are expected to alter the physiological activity of bushy cells and thus may contribute to hearing deficits with AHL.

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239 Altered Expression of Synaptophysin and CD45 in the Cochlea and Cochlear Nucleus After Antioxidant Treatment in Acute Acoustic Trauma

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Acute acoustic trauma (AAT) can cause hearing loss and tinnitus. Excessive noise exposure promotes reactive oxygen species and reactive nitrogen species formation in the inner ear, which can induce cell death and hearing loss. However, up to date, there is very little information about the mechanisms of noise-induced tinnitus. It is believed that tinnitus is generated in the CNS but triggered by cochlear injury. We have previously demonstrated that an antioxidant treatment (4-hydroxy phenyl *N*-tert-butylnitrone, *N*-acetyl-L-cysteine, Acetyl-L-carnitine) could significantly reduce hearing threshold shift, as well as 4-HNE and NT formation in the cochlea. In the present study, we have used an indirect synaptic activity marker, synaptophysin, to examine effects of noise exposure and antioxidant treatment on synapses in the central and peripheral auditory systems 10 days after noise exposure (centered at 4 KHz, 105 dB SPL for 6 hours). Densities of positive stained fusiform cells in the dorsal cochlear nucleus (DCN) and of positive stained efferent nerve fibers at the basal turn of the cochleae were calculated and statistically analyzed. Compared with normal controls, significantly down-regulated synaptophysin expression was found in the fusiform cell layer of the DCN, and in the efferent nerve fibers in the cochleae of noise exposed chinchilla. There were no significant changes of total number of neurons in DCN (stained by anti-NeuN antibody) between groups. In the DCN, cartwheel cells deliver strong inhibition to fusiform cells through the synapses. Loss of inhibitory synapses in the DCN may be involved in noise-induced tinnitus. However, the significance of loss of efferent fibers in the cochlea is unclear. Antioxidant treatment significantly reduced the down-regulation of synaptophysin in those locations, suggesting the antioxidant treatment may not only treat the noise-induced hearing loss but also noise-induced tinnitus. We also used an inflammation marker, CD45, to study the role of inflammation in AAT. Increased numbers of $CD45^+$cells were found in the stria vascularis of noise exposed chinchilla. Antioxidant treatment reduced the number of $CD45^+$ cells, suggesting that antioxidant treatment can reduce inflammatory responses in the inner ear.

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240 Spontaneous Calcium Signals in the Dorsal Cochlear Nucleus After Noise Exposure in Mice

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A noise-induced increase in the spontaneous firing rate of neurons in the dorsal cochlear nucleus (DCN) has been associated with the development of tinnitus in animals. However, the underlying network activity is unknown. In this study, we used calcium imaging to map the spontaneous activity of populations of neurons from brain slices of the DCN. CBA/CAJ mice were exposed to an 8-16 kHz noise at 110 dB SPL for 2 hours in a paradigm established to produce tinnitus. Two weeks later, auditory brainstem response measurements confirmed a 20-40 dB shift in response thresholds to tone pips between 4 and 48kHz. Oregon-Green BAPTA-1 AM was injected into the DCN after preparing slices, and fluorescence measured at 30-100 frames/second. Spontaneous activity was observed throughout the DCN, and was greatest in the pyramidal cell and superficial deep layers. Spontaneous calcium events exhibit similar amplitudes in both the noise-exposed and control groups. This suggests that the generation of calcium signals in the noise-exposed and control groups is fundamentally similar. Conventional ACSF bathing medium contains a higher concentration of calcium than physiological ACSF, which has been shown to suppress spontaneous activity. When immersed in physiological ACSF (1mm $CaCl_2$), DCN neurons within the molecular and pyramidal cell layers displayed higher synchrony (measured as the analog cross-correlation of the calcium signals) in noise-exposed mice (R=0.3-0.4) when compared to age-matched controls (R=0.1-0.2). In conventional ACSF (2.5mM $CaCl_2$), the correlation amplitude was reduced by a factor of 2, but the difference in synchrony remained. Synchronous signals were consistently interspersed with intervals of asynchronous

activity in all conditions. These data suggest noise-exposure may enhance the inherent synchrony of spontaneous activity in the DCN, which may ultimately transmit an abnormal percept of sound to higher centers.
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241 Effects of "Benign" Noise Overexposures on AVCN Bushy Neurons in CBA Mice

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Noise exposures that only produce temporary hearing threshold shifts (TTS) are not inconsequential. Recent findings have demonstrated a sequence of events that lead to type I ganglion cell afferent terminal withdrawal from the inner hair cell and ultimate ganglion cell degeneration without the mechanosensory hair cell loss. Because normal auditory nerve fiber activities (both spontaneous and sound driven) require intact synaptic contacts between the spiral ganglion cells and the inner hair cells, we hypothesize that ganglion cell afferent terminal damages will affect central synaptic and neuronal properties of principal neurons in the anteroventral cochlear nucleus. We thus examined endbulb of Held synapse and bushy neuron properties in in-vitro slices from mature CBA mice (~P100 day) after TTS noise exposures. Compared to controls, we found that bushy neurons from TTS noise exposed animals 1) have higher membrane input resistance and action potential thresholds, 2) express less of a low-voltage-activated K current. Additionally, endbulb synaptic recovery from depression appears to be faster in noise exposed animals, indicating higher synaptic terminal Ca accumulation, perhaps, due to lower intracellular Ca buffering. On the surface, paradoxically, this may represent a short-term compensatory mechanism to maintain reliable endbulb synaptic transmission. However, loss of intracellular Ca buffering capability can render cells more prone to neuronal excitotoxicity, thus having detrimental longer-term effects. With incomplete recovery from acute injury, TTS noise exposures may not only have lingering peripheral effects but also consequences in the central auditory system.

242 A Model for Tinnitus Generation Based in the Ventral, Not Dorsal, Cochlear Nucleus

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Many neural representations have been proposed to underlie the tinnitus percept including elevated neural firing rate, abnormal temporal patterns of neural discharge, reorganized tonotopic maps, and increased correlation between neurons. By “increased correlation” we specifically mean that the spontaneous firing patterns of two or more neurons are correlated with one another to an abnormally high degree. Recently, Eggermont and co-workers reported enhanced inter-neuronal correlations within the auditory cortex of animals having a pattern of peripheral damage often associated with tinnitus. But cortex may not be special in showing such enhanced correlations. We suggest that cochlear nucleus neurons directly innervated by the cochlear nerve could show enhanced inter-neuronal correlation following peripheral deafferentation and that the spherical bushy cells of the ventral cochlear nucleus are particularly likely candidates for exhibiting this phenomenon. We will explain how several structural and functional aspects of cochlear-nerve fibers and spherical bushy cells may conspire to increase the degree to which spherical bushy cells are correlated with one another following cochlear hair cell or spiral ganglion loss. We will also discuss how these correlations might help account for various aspects of tinnitus phenomenology including, differences between people in the quality of the tinnitus percept, onset of tinnitus immediately following acoustic over-exposure, and the occurrence of tinnitus in some ears, but not others with seemingly identical damage.
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243 Effects of Inferior Colliculus Ablation on Noise-Induced Hyperactivity in the Dorsal Cochlear Nucleus

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Hyperactivity in the central auditory system, manifest as increases in spontaneous activity and/or increases in bursting activity, has been implicated as an important correlate of tinnitus induced by noise and other tinnitus-inducing agents. We have been investigating the influences of descending pathways as potential modulators of noise-induced hyperactivity in the dorsal cochlear nucleus (DCN) of hamsters. In this study we were interested in the potential modulatory influence of descending pathways to the DCN from the auditory midbrain. Hyperactivity was induced in the DCN of hamsters by exposure to intense sound (10 kHz, 115 dB SPL, 4 hrs). Recordings were conducted at tonotopic locations in the DCN where spontaneous activity was clearly elevated relative to control levels (i.e., where rates were above 40 events/s). Following this initial recording, input to the DCN from the contralateral inferior colliculus (IC) and higher levels was removed by ablation. Ablation was accomplished by electrocautery against the caudal wall of the inferior colliculus until the bulk of the IC was removed. Examples have been obtained in which removal of the contralateral IC resulted in an enhancement of the level of hyperactivity in the DCN. In other cases, IC removal produced a transient loss of DCN activity which recovered gradually to pre-ablation levels over a period of 15-20 minutes. These results are still preliminary, but they do suggest that DCN hyperactivity persists and may even be enhanced following IC ablation. This further supports the view that DCN hyperactivity does not follow a top-down explanation, although the descending input to the DCN from the IC or higher order structures may exert an overall inhibitory effect on DCN hyperactivity. (Supported by NIH grant R01 DC009097).

244 Longitudinal Changes in Gap Detection and Prepulse Inhibition Following Noise Exposure in Adult Mice

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Six-month-old mice on a mixed C57Bl6 x 129 background were anesthetized with isoflurane and exposed to unilateral noise (n=9), or sham exposure for controls (n=5), for one hour (16 kHz octave band signal, 116 dB SPL). Gap detection and prepulse inhibition were tested at 60 dB SPL (1,000 Hz bands centered at eight frequency bands) before and at post-exposure time points of 1, 3-4, 7, 14, 21, and 30 days, and monthly thereafter until 7 months post exposure. Auditory brainstem response thresholds were measured before and after the noise exposure, and 7 mo post exposure. Brains were then perfusion fixed, harvested, and frozen for later immunohistochemical analysis of vesicular glutamate transporter distributions that are altered after cochlear damage (Zeng et al., J. Neurosci., 2009). Noise exposed mice displayed changes in gap detection and prepulse inhibition behavior for 24 kHz stimuli consistent with the presence of tinnitus at that frequency. Several time points following noise exposure suggested evidence of hyperacusis (stronger responses to gap detection and prepulse inhibition) which was followed by the development of deficits in gap detection by 6 months post exposure. The temporal development of these changes following noise exposure are discussed in the context of the interactions between aging, noise exposure and the associated neurochemical changes that occur at early stages of auditory processing .

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245 Differential Effects of Prolonged Sound Exposure on the Enlargement of Auditory Neurons in the Brainstem

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In our previous study (Lu et al, 2009) we have showed that neurons at the auditory cortex enlarged their size markedly (~30%) following prolonged sound exposure at moderate levels, suggesting plastic changes as the result of over-activity. If so, similar changes should be observed in the subcortical auditory structures. To test this hypothesis, we measured cyto-histologically two obligatory brainstem auditory stations: inferior colliculus (IC) and cochlear nucleus (CN) the after exposing juvenile rats (4 weeks old, n=5) to a monotone (4 kHz, 65 dB SPL) for 7 days (10 hrs/day). Neuronal profiles (nuclei and perikarya) in the IC (central and external subdivisions) and CN (anterior ventral, dorsal and posterior ventral subdivisions) were digitized and measured on photomicrographs taken from 7 (m-thick histological sections stained with toluidine blue. To facilitate accurate measurement, we used image-analysis software (Image ProPlus) that contained a confocal-like image-merging function to sharpen edges of the nuclei and perikarya. Sound exposure expanded cell volumes (both nuclei and perikarya) at the IC markedly (~65%) compared with control ($p<0.0001$, Student's t-test). Such sound-induced changes were however not found in the CN. Neurons in other non-auditory structures (visual cortex and superior colliculus) were also unaffected by the same sound exposure. Results showed that the prolonged sound exposure had produced effects that cannot be explained by a simple mechanism linking over-activity with cell enlargement, but might involve a sensitive or critical period that determines whether or not over-activity can exert effects on cytomorphology. That sound has induced marked changes at the IC and the auditory cortex without affecting the CN also suggested the occurrence of tinnitus in these animals as likely induced by long-term sound exposure during their young age. Further behavioral experiments are necessary to confirm such conjecture.

Keywords: over-activity, neural plasticity, auditory brainstem, inferior colliculus, cochlear nucleus, tinnitus

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246 What Squid Hear: An Evoked Potential Study of the Longfin Squid (*Loligo Pealeii*)

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While many organisms hear and produce sound, little is known about marine invertebrate hearing. Squid are a key component of ocean biomass as an important predator and prey, however it is uncertain if they hear despite auditory implications. In particular, squid face substantial selective foraging pressures from echolocating toothed whales and anecdotal evidence suggests they respond to acoustic stimuli. Using auditory evoked potentials (AEPs) we measured squid (*Loligo pealeii*) hearing ranges and thresholds in response to pressure and particle motion components of a sound field ranging from 50 Hz to 10 kHz, and to acceleration stimuli from 20 to 1000 Hz. Responses were recorded from nerves in the vicinity of the statocyst. AEPs were observed for stimuli between 20 and 500 Hz, with lowest thresholds from 100-200 Hz. Good responses were obtained for pulsed sounds with significant low frequency components (peak frequency 100-150 Hz) but no AEPs were detected using simulated toothed whale echolocation clicks. Squid evoked potentials followed individual clicks up to 1 click/15 ms (66 Hz). Acceleration

alone produced AEP responses. The responses recorded here suggest squid have a hearing capability similar to "hearing-generalist" fishes and that the statocyst acts as an accelerometer by which squid detect the particle motion of the sound field. These data indicate that squid likely detect environmental low frequency sound signatures that may aid navigation and near-field acoustic stimuli from predators and prey.

247 Auditory Processing Changes Seasonally in Gambel's White-Crowned Sparrow

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Vocalization is the primary method of communication among songbirds. Dramatic seasonal differences in song behavior, and in the morphology and physiology of the neural circuitry underlying song production are well documented in variety of songbird species. Androgenic and estrogenic steroid sex hormones mediate these seasonal changes. While much work has focused on the hormonal mechanisms underlying seasonal plasticity in songbird vocal production, comparatively little work has investigated seasonal and hormonal effects on songbird auditory processing. To address this issue, Gambel's white-crowned sparrows (Zonotrichia leucophrys gambelii), a highly seasonal songbird with sharp, distinct breeding periods, were used in this study. Photoperiod and hormone levels were manipulated in the laboratory to simulate natural breeding and non-breeding conditions. Peripheral auditory function was assessed by measuring the auditory brainstem response (ABR) of males and females in both conditions. Birds exposed to breeding-like conditions demonstrated elevated thresholds and prolonged peak latencies compared with birds housed under non-breeding-like conditions. Females showed a greater seasonal threshold shift than males. These results suggest that seasons and hormones not only affect vocal production, but also substantially impact auditory processing in wild songbirds.

248 Serotonin Modulates Latency But Not Amplitude of Auditory Brainstem Response Waveforms

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Serotonin deficiencies have been linked to changes in the latencies and amplitudes of AEP waveforms, increases in absolute thresholds, and increased intensity dependence of waveform amplitudes. The purpose of the present study was to use the mouse model to investigate whether auditory responses were altered when endogenous serotonin was depleted. Young male CBA/J mice were implanted with chronic subcutaneous electrodes prior to the recording of baseline auditory brainstem responses (ABR). ABR stimuli consisted of pure tones presented across a range of subthreshold to suprathreshold levels. The chemical para-chlorophenylalanine (pCPA), an inhibitor of endogenous serotonin synthesis, was administered to subjects daily for a period of five days prior to reobtaining ABR measures. Using repeated measures ANOVA, serotonin-depleted subjects displayed significantly shorter latencies relative to baseline measures of approximately 0.1 ms ($F(6,1)=17.285$; $p=0.006$) at 16 kHz. However, no significant effect was found on absolute threshold, amplitudes, or the slope of amplitude-intensity functions. These results suggest that serotonergic effects on ABR slope found in cortex are not present in the brainstem of anesthetized mice. The roles of individual serotonin receptor subtypes in mediating such changes in latency are not well understood, and further investigation using specific serotonergic agonists will be required to determine which receptors are responsible for mediating such changes.

249 Acoustic Masking of Noised-Induced Tinnitus in Rats

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Tinnitus, a subjective perception of sound in the absence of acoustic stimulation, is a prevalent condition that causes somatic and psychological disturbances and affects quality of life at various degrees. Acoustic masking in some forms of background noise is a commonly used tool for tinnitus relief. However, the underlying mechanisms are still poorly understood. In this study, we evaluated and characterized the effect of acoustic masking on tinnitus by examining the changes in behavioral evidence and neural correlates of tinnitus. To induce tinnitus, Sprague-Dawley rats (n=16) were exposed to a 10 kHz tone at 120 dB SPL for 3 hours. GAP detection for tinnitus and prepulse inhibition (PPI) for hearing loss across 5 frequency bands were measured pre-/post-noise exposure as well as during acoustic masking with broadband and narrow band noises (10-18 kHz) at 55 dB SPL. Age-matched control rats (n=6) were also studied. In addition, electrophysiology is being conducted to investigate how acoustic masking affects neural correlates of tinnitus along the auditory pathway. Our preliminary results demonstrated that certain noise-exposed animals developed behavioral manifestations of tinnitus across different frequency bands with predominance at 12 kHz, as evidenced by significant gap detection deficits together with PPI. We also found that acoustic masking with broadband noise tended to mask tinnitus at 12 kHz band. However, when using 10-18 kHz band noise at the same level, we found that both GAP detection and PPI deficits at 12-16 kHz were enhanced. Our data in this animal model demonstrate that tinnitus can be masked with a low level broadband noise. Results from the 10-18 kHz masking may suggest that rats were more sensitive to band noise masking at frequencies that are similar to those of their tinnitus. Further investigation is needed to determine the masking effect of a lower intensity narrow band noise on tinnitus.

250 Sustained, High Dose Treatment with Sodium Salicylate Disrupts the Rat Auditory Brainstem Response

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High doses of salicylate (aspirin) are a well known cause of mild-to-moderate hearing loss and tinnitus mainly due to its ototoxic effect on outer hair cells. Hearing loss from high-dose salicylate treatment is considered temporary, but the effects of prolonged, high-dose treatment and potential neural effects are poorly understood. In our study we focused on the effects of a long-term, high-dose administration of salicylate on the central auditory pathway. A daily dose of 200 mg/kg of sodium salicylate was administrated to ten male Sprague-Dawley rats for 15 days. Auditory Brainstem Response (ABR) threshold and distortion product otoacoustic emission (DPOAE) $2f_1$-f_2 were measured for all animals before and after the end of the treatment (3 days, 2 and 4 weeks). The results showed in accordance with the literature a reversible decrease of DPOAEs with a full recovery of DPOAE. In contrast, the ABR showed abnormalities in the waveforms with a moderate to large reduction in amplitude of waves I thru IV. For frequencies below 20 kHz, the amplitude of wave II (i.e., P2-N2) measured at 100 dB SPL was significantly decreased. ABR thresholds were increased at all post-administration times. These findings demonstrate a reversible peripheral effect and a permanent central effect as reflected in ABR waveforms. Research supported in part by Tinnitus Research Initiative and NIH (R01DC009091; R01DC009219)

251 Somatosensory Modulation of Tinnitus, an FMRI Study

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Jaw protrusion may lead to a change of tinnitus. To identify the neural mechanisms that are responsible for this somatic modulation, we measured the fMRI response to jaw protrusion. Thirteen patients with tinnitus and 20 healthy controls were included in an fMRI experiment. All patients were able to modulate their tinnitus by performing jaw protrusion. Experiments were performed on a 3T Philips scanner, using sparse sampling (TR=10s). Experimental conditions consisted of (1) bilateral broadband noise, (2) jaw protrusion, (3) the combination of both and (4) a baseline condition. A region of interest analysis was performed to quantify responses to the experimental stimuli. A group analysis of the response to sound showed activity in the auditory pathway, consisting of the cochlear nucleus (CN), the inferior colliculus (IC), the medial geniculate body, and the primary and secondary auditory cortex. Jaw protrusion activated the following structures: the cerebellum, the ventrolateral nucleus of the thalamus, the putamen, the motor cortex and the somatosensory cortex. In addition, the auditory pathway showed significant responses to jaw protrusion. In contrast, the somatosensory cortex only showed a response to jaw protrusion and not to sound stimuli. A difference between subject groups was found in the CN and the IC, where patients showed a larger response to protrusion than controls. These data show that the brainstem auditory nuclei play a role in the somatic modulation of tinnitus.

252 Auditory Evoked Potentials in People with Tinnitus: A Relationship to Sound-Level Tolerance?

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A recent fMRI study of tinnitus and non-tinnitus subjects showed that (1) sound-evoked activation of auditory midbrain, thalamus, and primary cortex increases with decreasing sound-level tolerance (ST) gauged by loudness discomfort level and a questionnaire and (2) elevated activation in primary cortex is also associated with tinnitus. We measured auditory evoked potentials (AEPs) and assessed ST in threshold- age- and sex-matched tinnitus and non-tinnitus subjects to determine whether the synchronously active neurons producing AEPs contribute to the elevated activation seen with fMRI. AEPs were recorded between vertex, F3, F4 and either the left (binaural stim.) or the stimulated (monaural) ear in 6 men with tinnitus (age 43±2) and 6 without (age 43±1). Mean threshold for the tinnitus and non-tinnitus groups differed on average by 2 dB and by no more than 6 dB at any half-octave interval from 0.125 to 14 kHz. Mean amplitude of auditory brainstem response (ABR) wave I did not differ significantly between the tinnitus and non-tinnitus groups at any level and showed no correlation with ST (monaural 11/s clicks; 30, 50, 70 dB HL). However, mean amplitude for tinnitus subjects was smaller than for non-tinnitus subjects at all sound levels and the difference increased with level. This trend raises the possibility of diminished auditory nerve activity in the tinnitus subjects relative to the non-tinnitus subjects despite a close matching of thresholds between groups. Neither ABR wave V nor cortically-generated N100/P200 differed in amplitude between tinnitus and non-tinnitus subjects or correlated in amplitude with ST (binaural 3/s clicks; 50, 70 dB HL). These preliminary results suggest that neuronal populations generating the highly temporally synchronized activity underlying wave V and N100/P200 do not contribute to elevations in fMRI activation associated with abnormal sound-level tolerance and tinnitus.
American Tinnitus Association, Tinnitus Research Initiative

253 Neural Signatures of Speech-In-Noise Perception in Older Adults

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Hearing loss ranks third among chronic conditions in older adults, and the most common manifestation of this impairment is difficulty hearing in background noise. The

mechanisms contributing to poor speech-in-noise (SIN) perception are not well understood, but the roles of peripheral, central, and cognitive factors have been considered. Speech-in-noise perception is related to spectrotemporal encoding of speech in the auditory brainstem in young adults and children. We hypothesized that older adults with poor SIN perception demonstrate greater noise-induced deficits in the spectrotemporal representation of speech than older adults with good SIN perception. Participants included 24 older adults (ages 60 to 74) whose hearing levels ranged from normal hearing to moderate sensorineural hearing loss. SIN perception was evaluated using the Hearing in Noise Test (HINT). We recorded brainstem responses to a speech syllable presented in quiet and in speech babble. A frequency-shaping protocol was used to compensate for hearing loss during behavioral and electrophysiologic testing. We found that participants in both good and poor SIN performance groups had a wide range of hearing thresholds, consistent with previous findings that peripheral hearing loss cannot fully account for SIN difficulties. The older adults with poor SIN perception had reduced representation of the fundamental frequency (useful for 'tagging' a speaker's voice), greater peak timing delays, and smaller response magnitudes, particularly in the portion of the response reflecting the formant transition. Importantly, these differences were noted in the babble condition but not in quiet. These brainstem-level neural signatures can help guide future intervention efforts. Older adults with excessive neural degradation in noise are less likely to benefit from amplification-only strategies and will profit from the inclusion of auditory and/or cognitive training programs in their intervention plans.

254 Application of the Auditory Brainstem Response for Scaling Impulsive and Continuous Noise

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The aim of the work was to test whether it is possible to scale the impulsive noise and continuous noise by equivalent wave V latency or thresholds in the auditory brainstem responses (ABRs). A forward masking paradigm was used in which a 4-kHz tone pip was used to evoke the ABR. The tone pip was masked by the preceding 201-ms or 501-ms interval of click trains or band-pass noise. Effect of masking was measured for click/noise SPL varied in 10-dB steps. Masking click trains differed in number of clicks presented in a range from 50 clicks/s (dt = 20ms) to 10 clicks/s (dt = 100ms). Bands of continuous noise ranged in their center frequency from 250 Hz up to 4000 Hz. Results allowed for a comparison of masking effect of impulsive noise and that of continuous noise.

255 Auditory Brainstem Activity to Spectrally-Degraded Music

WITHDRAWN

256 Context-Dependent Encoding of Speech in the Human Auditory Brainstem as a Marker of Musical Aptitude

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Musical experience enhances subcortical encoding of speech and music, with musicians demonstrating more efficient auditory brainstem processing than non-musicians. Still, no data substantiate direct relationships between musical skill and subcortical neurophysiology. By establishing a direct relationship between musical skill and literacy-related aspects of auditory brainstem processing we could resolve a causal model for the co-dependency between musical and reading abilities and promote the claim that cognitive function modulates lower-level sensory processing via the corticofugal system.

The present study assessed auditory brainstem encoding, musical aptitude (Gordon, 1982) and reading abilities in school-aged children. Subcortical measures assessed context-dependent speech encoding by recording brainstem responses to predictable versus variable speech sounds. The auditory brainstem functionally adapts to fine-tune the online representation of predictable acoustic elements, which is important for extracting irregularities from auditory streams(1). This subcortical malleability provides an index of effective auditory processing, with a lack of dynamicity associated with reading deficits.

Outcomes reveal that higher musical aptitude relates with increased subcortical sharpening of predictable stimuli. This observation is consistent with the view that subcortical processing is modulated by cognitive function. Through structural equation modeling, data indicate that musical skill predicts over 40% of the variance in reading performance by means of auditory brainstem function. Outcomes provide a statistical model for causal relationships between musical aptitude, auditory brainstem function and literacy and could inform how remediation strategies might capitalize on music as a method for improving reading skills.

257 Brainstem Pitch Representation in Native Speakers of Mandarin Is Less Susceptible to Degradation of Stimulus Temporal Regularity

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It has been demonstrated that neural encoding of pitch in the auditory brainstem is shaped by long-term experience with language. To date, however, all stimuli have exhibited a high degree of pitch saliency. The experimental design herein permits us to determine whether experience-dependent pitch representation in the brainstem is less susceptible to progressive degradation of the temporal regularity of iterated rippled noise (IRN). Brainstem responses were recorded from Chinese and English participants in response to IRN homologues of Mandarin Tone 2 (T2IRN). Six different iterations steps were utilized to systematically vary the degree of temporal regularity in the fine structure of the IRN stimuli in order to produce a pitch salience continuum ranging from low to high. Pitch-tracking accuracy and pitch strength were computed from the brainstem responses using autocorrelation algorithms. Analysis of variance of brainstem responses to T2IRN revealed that pitch-tracking accuracy is higher in the native tone language group (Chinese) relative to the non-tone language group (English) except for the three lowest steps along the continuum, and moreover, that pitch strength is greater in the Chinese group even in severely degraded stimuli for two of the six 40-ms sections of T2IRN that exhibit rapid changes in pitch. For these same two sections, exponential time constants for the stimulus continuum revealed that pitch strength emerges 2-3 times faster in the tone language than in the non-tone language group as a function of increasing pitch salience. These findings altogether suggest that experience-dependent brainstem mechanisms for pitch are especially sensitive to those dimensions of tonal contours that provide cues of high perceptual saliency in degraded as well as normal listening conditions.

258 Identifying Relationships Between Neural Responses to Speech and Reading Using Structural Equation Modeling

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Structural equation modeling is an analytical technique that allows for the proposal of a model of the inter-relationships among variables and can determine, for example, the variance in reading ability in first grade accounted for by auditory perceptual skills in preschool in a predictive fashion (Boets et al., 2008). We applied similar analyses to a dataset from typically developing children and children with dyslexia which included measures of the neural representation of speech, reading ability, attention, memory, and phonological processing. We hypothesized that brainstem representation of speech would significantly predict variance in reading ability and that the brainstem's effects on reading ability might also be mediated by other cognitive factors such as phonological processing or attention. Brainstem representation of speech, specifically measures corresponding to the temporal and transient elements of the response, significantly predicted 32% of the variance in reading ability. When other cognitive measures were added to the model, brainstem measures significantly predicted phonological processing and attention. The influence of brainstem responses to speech on reading was mediated by phonological processing, which accounted for approximately 42% of the variance in reading. Short-term memory made a unique contribution to the model, also predicting approximately 42% of the variance in reading. This analytical approach supports our recent and long standing work suggesting that brainstem representation of speech is directly indicative of reading ability through the influence of other cognitive measures that may be considered the building blocks of reading ability.

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259 Language-Dependent Enhancement of Pitch Encoding in the Brainstem Transfers to Stimuli Beyond the Natural Voice Pitch Range

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Experience-dependent enhancements of the neural encoding of pitch in the auditory brainstem has been observed for only specific portions of native pitch contours exhibiting high rates of *pitch acceleration*, irrespective of speech or nonspeech contexts. The experimental design herein allows us to determine whether this language-dependent advantage transfers to acceleration rates that extend beyond the pitch range of natural speech. Brainstem frequency following responses (FFRs) were recorded from Chinese and English participants in response to four, 200-ms click-train stimuli with different rates of pitch acceleration. The maximum pitch acceleration rates ranged from low (0.3 Hz/ms; Mandarin Tone 2) to high (2.7 Hz/ms; 2 octaves). Pitch strength measurements were computed from the FFRs using autocorrelation algorithms with an analysis window centered at the point of maximum acceleration in each stimulus. Crosslanguage comparisons of pitch strength revealed that the Chinese group exhibits more robust pitch representation in the stimulus of greatest acceleration as well as in the linguistically-relevant stimulus (Tone 2). While both groups showed a decline in pitch strength with increasing acceleration rates, only the Chinese group maintains neural encoding at the highest acceleration level. These findings demonstrate that perceptually salient pitch cues associated with lexical tone not only influence brainstem pitch extraction in the language domain, but also extends to pitch extraction of stimuli that clearly fall outside the range of dynamic pitch in a listener's experience. Such findings are compatible with recent studies of brainstem

processing in music and language that reveal positive transfer effects across domains of experience.

260 Neural Representation of Pitch Salience in the Human Brainstem Revealed by Psychophysical and Electrophysiological Indices

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Pitch is one of the most important information-bearing parameters of species-specific signals. Acoustically, it can be related to the temporal regularity or periodicity of a sound. Behavioral studies have revealed that the salience of a pitch stimulus grows systematically with increasing periodicity. Results from functional imaging suggest that the neural correlates of pitch salience do not originate along the auditory pathway until after primary auditory cortex. Here, we show that subcortical structures are actively involved in processing pitch and that the neural manifestation of pitch salience emerges well before cortical involvement. Brainstem frequency following responses (FFRs) were recorded from participants in response to six linguistic tones which varied only in their degree of temporal periodicity (i.e., pitch salience). Neural pitch strength was computed from each FFR using autocorrelation algorithms. In addition, behavioral frequency difference limens (F0 DLs) were measured from each participant to obtain a perceptual estimate of pitch salience. Results showed that brainstem neural pitch strength increased systematically with increasing stimulus periodicity, thus indicating more robust encoding for salient pitch. Complementary results were found behaviorally. F0 DLs decreased with increasing stimulus periodicity revealing better pitch change detection for more salient stimuli. FFR neural pitch strength and behavioral F0 DLs were negatively correlated suggesting that subcortical processing can, in part, predict an individual's behavioral judgments related to pitch salience. These data imply that changes to the acoustic periodicity of a stimulus directly influence brainstem encoding and the corresponding behavioral responses to pitch. We infer that information related to pitch salience may emerge early along the auditory pathway and is likely rooted in pre-attentive, sensory-level processing.

261 Human Frequency Following Response: Differential Responses to Positive & Negative Gain of Iterated Rippled Noise (IRN) Stimuli

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Physiological and perceptual studies have shown differences in the representation of the pitch of iterated rippled noise (IRN) stimuli with positive and negative gain. These two conditions produce differences in waveform fine structure with minimal changes in waveform envelope. Perceptually, the pitch of IRN[+] corresponds to 1/d (d = delay in sec) whereas that of IRN[-] is an octave lower, 1/2d. Comparison of the neural autocorrelograms of responses obtained from the cochlear nucleus in response to IRN[+] and IRN[-] indicates that the temporal discharge patterns of primary-like units reflect the stimulus waveform fine structure, whereas those of chopper units reflect stimulus envelope. The aim here was to determine if these encoding differences are preserved in the scalp recorded human frequency following response (FFR), which reflects sustained phase-locked neural activity among a population of neurons in the rostral brainstem. FFRs were recorded from 12 subjects in response to IRN stimuli with positive and negative gains at delays (d) of 2 and 4 ms. Pitch related neural periodicity was computed from FFRs by examining response autocorrelation functions (ACFs). In addition, spectral magnitudes were measured to identify the dominant frequency in the responses and the mean frequency spacing between successive response harmonics. Results revealed harmonic spacing at 1/d Hz for IRN[+] and 1/2d Hz for IRN[-], consistent with the respective heard pitches of these stimuli. Dominant ACF peaks occurred a time-lag d for positive gain and 2d for negative gain conditions, respectively. These results suggest a different temporal pattern of phase-locked neural activity to IRN stimuli with positive and negative gain and may distinguish responses to stimulus fine structure and envelope. Our findings suggest that the temporal response pattern of the FFR reflects stimulus fine structure in at least the negative gain condition.

262 Afferent and Efferent Dopaminergic Projections of the Inferior Colliculus

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Dopamine can modulate release of GABA and glycine, two critical inhibitory neurotransmitters in auditory brainstem pathways as well as glutamate, the primary excitatory neurotransmitter of this system. Our previous studies provide evidence for deafness-related changes in dopamine levels in the inferior colliculus (IC). The targets of dopaminergic somata as well as the sources of dopaminergic terminals in the IC are currently unknown. In the present study we examine three dopaminergic projections that either originate or terminate within the IC. To determine targets of projections originating in the IC pressure injections of biotinylated dextran amine (BDA) were made either into the external cortex (IC_{ex}) or the central nucleus (IC_{cn}) of the IC. Sources of IC terminals were determined via pressure injections of the retrograde tract tracer fluorogold into the IC_{cn}. All studies were conducted in normal hearing adult Sprague Dawley rats (n = 3 – 5 per group). Consistent with previous tract tracing literature, labeled terminals resulting from injections of BDA into the IC_{ex} and IC_{cn} were observed throughout the superior olivary complex (SOC) and the medial geniculate body (MGB). Labeled cell bodies were observed in both the intermediate (INLL) and ventral (VNLL) nuclei of the lateral lemniscus following fluorogold injection into the IC_{cn}. In order to assess whether the pathways are dopaminergic

we have begun to use immunocytochemistry for tyrosine hydroxylase (TH). Dopaminergic terminals projecting from the IC to the SOC were primarily found rostrally within nuclei of the trapezoid body as well as throughout the lateral superior olive. In the INLL and VNLL approximately 40% of the cells projecting to IC_{cn} were dopaminergic. In the future we plan to examine MGB terminals, originating in the IC, that are dopaminergic. In addition, we plan to examine the SOC to determine the cell types upon which dopaminergic neurons originating in the IC terminate.

263 Origins of Glutamatergic Terminals in the Inferior Colliculus Identified by Retrograde Transport and Expression of VGLUT1 and VGLUT2 Genes

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The inferior colliculus (IC) receives both excitatory and inhibitory synaptic inputs from virtually all nuclei in the lower auditory brainstem as well as excitatory inputs from the auditory cortex. Recently, three types of glutamatergic synaptic terminals were found in the IC whose vesicular glutamate transporter (VGLUT) proteins differ: VGLUT1 only, VGLUT2 only, or both proteins. The VGLUT2-only synapses are of interest since only the largest neurons in the IC, the GABAergic tectothalamic neurons, have dense axosomatic VGLUT2-only endings. The glutamatergic synapses on other IC neurons terminate exclusively on dendrites. Here, we used a combination of the retrograde transport of Fluorogold (FG) injected into the IC and in situ hybridization for VGLUT mRNAs to determine the potential sources of the VGLUT2-only axosomatic synapses.

We found neurons projecting to the IC had one of three gene expression patterns: VGLUT1 alone, VGLUT2 alone, or VGLUT1 plus VGLUT2 (VGLUT1&2). After the IC injections, FG-positive (FG+) cells expressing only VGLUT1 were seen exclusively in the auditory cortex. FG+ neurons expressing only VGLUT2 were seen in the IC; in the ipsilateral intermediate nucleus of the lateral lemniscus; in the contralateral in the lateral superior olive, dorsal periolivary, and lateroventral periolivary nuclei; in the ipsilateral medial superior olive and ventromedial periolivary nucleus; and in the fusiform cells of the contralateral dorsal cochlear nucleus. In the ventral cochlear nucleus, almost all FG+ cells, presumably T-stellate cells, expressed VGLUT1&2. Smaller subpopulations of neurons in the medial and lateral superior olivary nuclei and other periolivary nuclei expressed both VGLUT1&2. In summary, the axosomatic VGLUT2-only terminals in the IC are most likely to arise from neurons in the IC, the intermediate nucleus of the lateral lemniscus, the main nuclei of the superior olive, or the dorsal cochlear nucleus.

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264 Organization of Glycinergic Inputs to the Inferior Colliculus

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The inferior colliculus (IC) receives a convergence of ascending inputs from both inhibitory and excitatory fibers. Neurons in the IC can be classified based on cell body size, expression of GAD67 or vesicular inhibitory amino acid transporter, expression of vesicular glutamate transporter 2 (VGLUT2), and VGLUT2 axosomatic inputs. However, the IC also receives glycinergic ascending projections, and the synaptic organization of these inhibitory inputs is still undefined. Using immunohistochemistry to label glycine transporter 2 (GLYT2), we have identified glycinergic synapses on IC neurons. We found GLYT2-positive axosomatic and axodendritic puncta present on nearly all neurons in the IC, including large and small GABAergic neurons as well as glutamatergic neurons. Using in situ hybridization for GLYT2, we have confirmed potential sources of these glycinergic afferents, including the ventral nucleus of the lateral lemniscus and the lateral superior olive. We further examined the axosomatic synapses of the IC by triple immunolabeling of GLYT2, GAD67, and VGLUT2 to determine the location of glycinergic, GABAergic, and glutamatergic terminals, respectively. We found that small GABAergic and GAD67-negative neurons have both GLYT2 and GAD67 positive axosomatic terminals, while large GABAergic neurons receive a combination of GLYT2, GAD67, and VGLUT2 axosomatic terminals.

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265 The Precision of Spike Timing Is Enhanced by Activation of Low-Threshold T-Type Calcium Channels in Auditory Midbrain Neurons

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The responses of IC neurons to temporal features of sounds reflect an interaction of synaptic inputs and neuronal biophysical properties. One striking biophysical property of IC neurons is the rebound depolarization that is produced following membrane hyperpolarization. To understand how the rebound is involved in precise spike timing, we made whole-cell patch clamp recordings from IC neurons in brain slices of young rats. Rebound neurons were encountered in about 70% of IC neurons at ages of 2-3 weeks. The proportion of the rebound neurons in IC gradually increased after the onset of hearing. With an injection of a sufficient depolarizing current after membrane hyperpolarization, the neuron produced a rebound on which 1-2 spikes were generated. The 1st spike generated on the rebound following hyperpolarization was more precise than that generated by depolarization alone. With repetitive depolarization (i.e., 0.5 Hz repeated 10 times) of a fixed level, the jitter of the 1st spikes generated on the rebound was 0.5±0.1 ms and that of the 1st spikes produced by depolarization alone at the same level was 2.5±0.5 ms, statistically significant

difference (P<0.001, n=20). The rebound and its associated spike were abolished altogether by the specific antagonist of the low-threshold T-type Ca^{2+} channel, mibefradil. The rebound depolarization was potentiated following 1-2 rebounds when the rebound was produced repetitively within a few hundred milliseconds. The spikes generated on the potentiated rebound were more precise than those on the non-potentiated rebound. When BAPTA, a fast Ca^{2+} chelator, was added to the internal solution of the recording electrode, there was no evidence of a rebound potentiation. The results suggest that IC neurons are able to produce very precise spikes at the onset of stimulation with rebound depolarization. The rebound depolarization is mediated by low-threshold T-type Ca^{2+} channels. The rebound potentiation may be attributed to an elevated level of intracellular Ca^{2+}.
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266 The Temporal Interaction of Excitatory and Inhibitory Synaptic Inputs Determines the Sound Response Properties of the Neurons of Inferior Colliculus

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Inferior colliculus (IC) is a midbrain auditory nucleus and contains neurons of various temporal response patterns to sound. Here, we performed single unit recordings and in vivo whole-cell patch recordings of IC neurons in mice to investigate underlying mechanisms to generate sound evoked responses. We classified IC neurons into five types on their temporal response properties to sound: transient (24.1%), sustained (43.2%), offset (3.9%), off (3.1%) and no spike response (24.1%) type. We examined firing responses to current injections and patterns of synaptic inputs to sound stimuli in order to understand the sound evoked spike responses. Under voltage clamp, we calculated excitatory and inhibitory conductance from synaptic currents measured at -80 mV and 0 mV, respectively. We found that the time courses of excitatory inputs were more diverged than those of inhibitory inputs and the balance of excitatory and inhibitory inputs principally contributes to the response patterns of IC neurons to sounds.

267 Inferior Colliculus Cells Rely Mainly on the Number of Input Spikes Rather Than Their Precise Timing to Discriminate Auditory Spectral/ Temporal Features

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The inferior colliculus (IC) receives afferents from virtually all the auditory brainstem nuclei before information ascends to the thalamus and cortex. Many brainstem nuclei encode auditory information in spike timing; indeed, some brainstem neurons are among the most temporally precise in the CNS, and such temporal precision is critical for detecting spectral/ temporal features of sound.
We tested the hypothesis that IC neurons discriminate spectral/ temporal features using the temporal patterns of their inputs. We used *in vivo* whole-cell recordings from awake bats to derive the synaptic conductances evoked by FM sweeps at various velocities and intensities. If neurons use mainly input timing to discriminate between sounds, then the temporal patterns of the synaptic conductances should be the primary determinant of output. Alternatively, if IC cells use mainly the number of pre-synaptic spikes rather than their precise timing, then the magnitudes of the conductances should be the primary determinant of output. Using modeling, we compared the magnitude components of the conductances to the timing components in terms of their ability to depolarize the post-synaptic cell. Preliminary analysis suggests that the best predictor of PSP peak was the conductance magnitude and not timing. We found that the single best predictor was the magnitude of the excitatory conductance, and this correlation was even stronger when combined with the magnitudes of the inhibitory conductance. In only a few cases were the temporal components strongly correlated with PSP peaks. These data suggest that IC neurons are more influenced by the information encoded in the number of afferent spikes rather than their precise timing. Supported by NIH grants DC007856 and1F 32DC009741.

268 Inferior Colliculus Cells Selective for the Direction of FM Sweeps Are Relatively Insensitive to the Timing of Inhibitory and Excitatory Inputs

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Here we used in vivo-whole cell recordings in awake bats to evaluate the role of the timing differences between excitation and inhibition for creating directional preferences for FM sweeps in the inferior colliculus (IC). In each cell, we first recorded the post-synaptic potentials (PSPs) and spikes evoked by an upward and downward FM sweep and derived the excitatory and inhibitory conductances evoked by the two signals. The excitatory conductances evoked by the preferred FMs were always larger than that evoked by the null FMs, and thus the preferred PSPs generated by excitatory conductances alone were also always larger than the null PSPs, although both excitatory PSPs were always suprathreshold. We next included the inhibitory conductances and time shifted the inhibitory conductance in 1.0 ms steps. Time shifts of 1.0 ms caused peak PSP changes of 1-2 mV, which had little or no effect on the directional preference of most cells. For the preferred FM, the excitation was so strong that time shifting the inhibitory conductance never reduced the PSP to below threshold, regardless of the amount of time shift. The effects for the null FM depended upon how close the PSP was to threshold. In most cells, the null PSPs were either very small or about half the amplitudes of the preferred. In those cells, shifts of 4-10 ms were required to bring PSPs to threshold level. Thus most cells were relatively insensitive to small changes in timing but rather the directional preferences were determined largely by the relative magnitudes of excitation and inhibition. In cells with null PSPs a few mV below threshold, shifts of only 1-3 ms brought the PSP to threshold or very close to it. The

directionalities of these cells were produced by a delicate balance between the timing of excitation and inhibition and their relative magnitudes. Supported by NIH grant DC7856.

269 Spectrotemporal Feature Selectivity for Conspecific Vocalizations in the Auditory Midbrain

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Previous studies have shown that response selectivity for conspecific communication signals can be observed as early as the inferior colliculus (IC) in the auditory midbrain. Receiving a convergence of excitatory and inhibitory inputs from the brainstem, it is not surprising that emergent response properties like feature selectivity can arise in the IC. While it has been previously shown that blocking inhibitory inputs greatly reduces response selectivity, it is still unclear which spectral and temporal features of conspecific vocalizations are encoded by an IC neuron and how excitation and inhibition interact with the intrinsic membrane properties of IC cells to produce a feature selective output.

In this study we used extra- and intracellular recordings as well as modeling to learn which stimulus feature produces an excitatory or inhibitory input to an IC neuron, and how these inputs are integrated nonlinearly to create a spiking response. By recording the extracellular response to a large repertoire of bat communication signals we were able to extract the relevant stimulus features (dimensions) that excite or inhibit the response of each neuron. We could then assess the nonlinear relationship between each feature and the output of the neuron. Additionally, we were able to compare this relationship, in a subset of cells, to the intrinsic membrane properties of each neuron. To assess the intrinsic properties of IC neurons, we used intracellular *in vivo* recordings and identified two main firing behaviors in response to current steps: sustained and onset-burst cells. While we didn't find a correspondence between intrinsic properties and the auditory features each neuron is selective for, we use conductance-based modeling to evaluate how intrinsic dynamics give rise to the nonlinear stimulus-response relationship and generate the spiking behavior in response to a specific feature of natural communication signals.

270 Pharmacological Dissection of Mechanisms Creating Selectivity for the Rate and Direction of FM Sweeps in the Pallid Bat Inferior Colliculus

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At least four mechanisms shape neuronal selectivity for downward FM sweep rate and direction in the pallid bat inferior colliculus. An early low-frequency inhibitory sideband prevents responses to upward sweeps, and thus shapes direction selectivity. An early on-best frequency inhibition makes a neuron shortpass selective for sound duration, and consequently made neurons bandpass or fastpass selective for sweep rate. A delayed high-frequency inhibitory sideband also shapes rate selectivity for downward sweeps. Finally, at least in neurons that respond exclusively to downward FM sweeps, two-tone facilitation shapes selectivity for both sweep rate and direction. These mechanisms can act alone, or in concert.

To determine whether these mechanisms that shape the complex spectrotemporal receptive fields of these neurons are discrete and created by different inputs, we measured the differential effects of blocking GABAa and glycine receptors. In neurons that responded to both tones and downward FM sweeps, blocking GABA receptors was twice as likely to eliminate direction selectivity than glycine receptor blockade, suggesting that GABAergic input was largely responsible for low-frequency inhibitory sidebands. Conversely, blocking glycine receptors was more likely to reduce or eliminate sweep rate selectivity by eliminating duration tuning or high-frequency inhibition. Of particular interest was the finding that, in neurons that responded only to downward FM sweeps, blocking glycine receptors had the seemingly paradoxical effect of abolishing a neuron's response altogether, suggesting that the underlying two-tone facilitation was dependent on a rebound from glycinergic inhibition. This is consistent with the fact that two-tone facilitation occurs at stimulus offset. These findings suggest that the multiple mechanisms that shape spectrotemporal selectivity for an important signal are discrete, and arise through the integration of different synaptic inputs.

271 Three-Dimensional Representation of Vocalizations in the IC

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Mice emit vocalizations with spectral content in the ultra-high frequency range (>45 kHz). However, there is limited representation of these frequencies in the central nucleus of the inferior colliculus (ICC). Previous studies of mouse IC showed that neurons with low frequency tuning curves responded to ultra-high frequency vocalizations (UHFVs), but it is unclear if these neurons are located in the ICC. In this study we determined whether neurons that respond to UHFVs are in the ICC. Extracellular responses to a suite of social vocalizations were recorded throughout the IC. The spatial location of each neuron was mapped by reconstructing electrode tracts from dye deposits made at the end of each electrode penetration. Each neuron's 3 dimensional position (caudal-rostral, medial-lateral, dorsal-ventral) was mapped onto one representative mouse IC. Many of the neurons that responded to UHFVs were located in the low frequency tonotopic regions of the ICC. Responses to vocalizations were not well predicted by each neuron's responses to pure tone stimuli. These results further illustrate that the way IC neurons respond to complex sounds cannot be predicted based on a neuron's tonotopic position and that responses to pure tones provide an inadequate understanding of the function of the IC.

272 Neuronal Responses to Tones in Quiet and in Noise in the Inferior Colliculus of the Awake Primate

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The inferior colliculus (IC) is an important subcortical auditory center that is the eventual target of all cochlear nucleus (CN) projections. Previous studies in the IC have shown that there are multiple response types in the central nucleus of the IC (ICC) as defined by the responses to tones as a function of frequency and sound pressure level (frequency response maps). These frequency response map types suggested functional segregation for ICC unit types, and one response type appeared specialized for representing signals in noise. Since cortex is not required for sound detection, CN or ICC or medial geniculate nucleus responses must match behaviorally observed changes in detection performance. We have previously shown that representation of tones in noise in the primate CN was inadequate to account for detection behavior. Our goal was to examine the responses to tones of single units in the ICC of awake macaques listening passively to sounds to characterize the ability to represent tones in quiet and in the presence of noise. Responses were obtained to tones of varying frequencies and sound levels, and to best frequency (BF, the frequency to which the unit responds at the lowest sound pressure level) tones as a function of sound level when BF tones were presented alone, and in continuous steady-state noise. Preliminary data indicate that frequency-response maps (a plot of firing rate as a function of tone frequency and sound level) of ICC units in our sample (mainly low BF units) showed mainly broad V-shaped excitatory responses, though some showed narrow I-shaped excitation. Many of our units, regardless of the frequency response map type, showed threshold shifts of 1 dB per dB of steady state noise, which matches the threshold shifts required for an ideal detector. Our results suggest that the responses of ICC units may be sufficient to explain detection behavior, which will be verified in future experiments.Supported by R03 DC 009338.

273 Functional Transformation Between Brainstem Inputs and Target Inferior Colliculus Neurons

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All ascending sound information to the auditory cortex is ultimately routed through the principal midbrain structure the inferior colliculus (IC). Despite its central position in the auditory pathway, how IC neurons process and transform brainstem input signals is poorly understood. Here we provide evidence for synaptic-like functional connectivity between presumed brainstem input neurons (PBIN) and IC neurons (ICN) and use this to identify how spectral and temporal sound information is transformed within the IC. Tetrode recordings in the IC were obtained in response to dynamic ripple sounds (Escabi and Schreiner 2002). The tetrode recording paradigm allowed us to simultaneously record single neuron activity as well as slow potentials that resemble S-potentials previously identified in the lateral geniculate nucleus (Hubel and Wiesel, 1961; Kaplan and Shapley,1984) and ascending auditory system (Kopp-Scheinpflug et al., 2002). The S-potential signals likely represent the spike train from single brainstem input neuron as evidenced by a clearly identified refractory period. Cross-correlograms between PBIN and ICN show a delayed (~ 2 msec) and sharp (~1 msec wide) synaptic-like correlation peak for ~10 % of identified PBIN and ICN pairs. Spectrotemporal receptive fields were then obtain for each neuron pair. BFs from recorded PBIN and ICN pairs were within 0.5 octaves and the strength of the correlation was stronger for pairs with more similar BFs. PBIN spectro-temporal receptive fields exhibited shorter latencies (3.4 msec v.s. 7.7 msec median) and shorter integration times (2.1 msec v.s. 5.0 msec median) consistent with a substantially faster processing for PBIN compared to ICN. Furthermore, PBINs exhibited substantially higher spike-timing precision (0.08 msec median) compared to ICN (0.6 msec median). These results indicate that temporal response properties undergo a dramatic transformation characterized by slower processing and reduced temporal precision within the CNIC. (Supported by NIDCD R01DC006397).

274 Modulation Tuning Characteristics Scale in the Inferior Colliculus: A Mechanism for Equalizing Natural Sounds

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Efficient coding principles propose that sensory systems evolved to accurately encode regularities found in natural sensory signals. To relate structural characteristics of natural sounds to neural coding strategies, we characterized the spectro-temporal modulations of several natural sound ensembles and compared these statistics with spectro-temporal tuning properties of cat inferior colliculus (CNIC) neurons. We demonstrate that modulation-tuning characteristics in the CNIC are particularly well suited for equalizing the modulation power of natural sounds. Specifically, natural sound modulations exhibit a 1/f modulation power spectrum (MPS) indicative of power-law scaling and self-similarity behavior that is not present for white noise. Neural tuning was highly overlapped with the MPS of natural sounds both of which exhibited a tradeoff between spectral and temporal modulations. Neural tuning approximated a proportional bandwidth filterbank in which modulation filter bandwidths scaled with the neuron's characteristic modulation frequency. A simulation that employs this modulation filterbank structure demonstrates that the neural scaling behavior opposes the 1/f scaling observed in natural sounds and enhances the signal representation by equalizing the MPS of natural sounds. The results provide evidence that inferior colliculus neurons represent natural sound cues by equalizing modulation power transfer in a

manner consistent with efficiency principles. (Supported by NIDCD R01DC006397).

275 Cortical Responses to a New Double-Shank Auditory Midbrain Implant in the Guinea Pig

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The auditory midbrain implant (AMI) is in clinical trials with a single shank array designed for stimulation along the tonotopic axis of the central nucleus of the inferior colliculus (ICC). Although initial results have been encouraging, the patients still do not achieve hearing levels comparable to cochlear implant patients. We believe this is, in part, due to the inability to effectively activate the 3-dimensional structure of the ICC. Therefore, we developed a double shank array with Cochlear Ltd. (Lane Cove, Australia) to assess how simultaneous stimulation of two regions along the isofrequency dimension of the ICC affects auditory cortical activity compared to stimulation of a single region.

We implanted the double shank array into the ICC and selected a site from each shank in a similar frequency region. We presented a single pulse (205 µs/phase) on each site with varying inter-stimulus delays and levels (multi-site stimulation, MSS). We also stimulated each site with two pulses with varying delays and levels (single site stimulation, SSS) for comparison. Local field potentials recorded from the primary auditory cortex (A1) were used for analysis.

For SSS, weak or no activity to the second pulse was observed for short inter-pulse delays (<1 ms). However, as the delay was increased (>2 ms), activity recovered to normal levels suggesting a refractory period. We also observed cases where the total activity to the first pulse was enhanced by the second pulse within a 2-6 ms window, suggesting a temporal integration mechanism from ICC to A1 consistent with psychophysics. Using MSS, we avoided the refractory effect and could enhance A1 activation. Both SSS and MSS were affected by stimulation location within the ICC.

Our findings demonstrate that stimulation of a single ICC location is not sufficient to effectively activate higher auditory centers, especially at rates above 500 pps. To accommodate finer temporal transmission algorithms and activation differences across ICC locations, development of a 3 dimensional array is necessary.

276 Binaural Processing in EI Neurons in the Inferior Colliculus Revealed with In-Vivo Whole Cell Recordings

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Neurons that are excited by one ear and inhibited by the other (EI cells) can code interaural intensity disparities (IIDs), the cues animals use to localize high frequency sound. EI cells in the inferior colliculus (IC) are characterized by firing to contralateral stimulation whereas ipsilateral stimulation evokes no response. With binaural stimulation the spike-counts evoked by contralateral stimulation are reduced or even completely suppressed. Previous iontophoretic studies have shown that in some IC cells, the ipsilateral inhibition occurs in a lower center (e.g., the LSO) whereas in other cells the ipsilateral inhibition actually occurs in the IC. In those cells in which blocking inhibition at the IC reduces or eliminates the ipsilaterally evoked inhibition, it was inferred that ipsilateral stimulation evoked IPSPs (inhibitory postsynaptic potentials), although the IPSPs could not be viewed directly from extracellular recordings. In order to measure PSPs directly, I made whole-cell patch-clamp recordings in 55 EI cells in the IC of Mexican free-tailed bats, and I also calculated excitatory and inhibitory conductances from the PSPs in some cells. In all cells contralateral stimulation evoked spikes and/or EPSPs, and the number of spikes or the EPSP magnitudes decreased with binaural stimulation. In 5/55 cells, ipsilateral stimulation evoked IPSPs, which suggests that the EI property was created locally in the IC. However, in a majority cells, the contralateral responses summed non-linearly with the ipsilateral responses. Sound presented to the ipsilateral ear evoked EPSPs, even though the ipsilateral stimulation suppressed spikes evoked by contralateral stimulation. Ipsilaterally evoked excitation in EI cells is surprising and shows that the circuitry activated by the ipsilateral ear is both different and more complex in EI neurons than was previously thought. Supported by NIH Grant DC007856.

277 An Improved Inferior Colliculus Cell Model for Interaural Time Difference Analysis

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The inferior colliculus (IC) is a key structure in the auditory brainstem. It is one of the main areas of convergence for auditory afferents. However, its role or roles in the auditory pathway is not clear. Part of the problem is that, although the sources of the afferents to the IC as a whole are fairly well-documented, the inputs to individual IC neurons are not. Although the pattern of responses of some IC neurons are similar to the responses of neurons in the nuclei that feed the IC, the responses of others are not clearly related to more peripheral responses. In order to look at the patterns of inputs that could produce the physiological responses to ITD inputs in IC neurons, a versatile, physiologically-based IC cell model was developed. Specifically, a neural processing model, including both cell membrane properties and a structural description of neural interconnections, was designed and implemented. The inputs to the IC model incorporate inputs from lower-level neurons that are sensitive to ITD, namely the medial superior olive (MSO) and the lateral superior olive (LSO), as well as monaural inputs. The model was used to try to understand the range of responses found in IC neurons by simultaneously feeding a single IC neuron model multiple inputs with different combinations of synaptic properties, frequency sensitivities, and ITD sensitivities of the inputs. Several specific strategies, alone or in combination, proved effective at reproducing a wide variety of response types.

For instance inhibitory inputs with narrowly-tuned ITD sensitivity combined with excitatory inputs with broader tuning can produce unusual rate-ITD curve shapes such as multiple peaks, dips, asymmetry, and plateaus. Similarly, combinations of inputs with different frequency tuning can produce rate-ITD curves with modulation in the mean and/or peak height.

278 Sensitivity to the Alignment of Sound Localization Cues in the Inferior Colliculus

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Previous studies have demonstrated that single neurons in the central nucleus of the inferior colliculus (ICC) are sensitive to multiple sound localization cues. In the present study we investigated whether single ICC neurons are specialized to encode multiple sound localization cues that are aligned in space (as would naturally occur from a single broadband sound source). Sound localization cues including interaural time differences (ITDs), interaural level differences (ILDs), and spectral shapes (SSs) were measured in a marmoset monkey. The results were used to impose aligned and misaligned combinations of cues on a set of broadband noise stimuli. The stimuli were presented to the same, unanesthetized marmoset monkey while recording single unit spike responses in the ICC. We computed mutual information between the set of spike rates and stimuli containing either aligned cues or cues with different degrees of misalignment. The results can be summarized as follows: 1) Neurons with best frequencies (BFs) less than ~13 kHz mostly encoded information about a single sound localization cue. Neurons with BFs below ~2 kHz mostly encoded information about ITD. Neurons with BFs ranging from ~4-13 kHz mostly encoded information about ILD. 2) Neurons with BFs above 13 kHz encoded information about multiple sound localization cues and were sensitive to their alignment. In some neurons mutual information between the set of stimuli and spike responses was greater for aligned cues while in others it was greater for misaligned cues. 3) In a control experiment the SS cues were artificially placed near the BF of the neuron under study using frequency scaling of the stimuli. In this case neurons at all BFs tested were sensitive to the alignment of multiple cues. 4) In general the results are consistent with the hypothesis that ICC neurons are sensitive to multiple localization cues if they are simultaneously present in the stimulus within the frequency response area of the neuron. The results suggest that there is not a qualitative specialization in the ICC for encoding aligned sound localization cues. (Supported by NIH grants DC00115, DC00023, and DC05211.)

279 Coding of Amplitude Envelope in Reverberation in the Inferior Colliculus of Awake Rabbit: Evidence for Mechanisms Compensating for the Acoustic Degradation

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Speech reception depends critically on temporal modulations in the amplitude envelope of the speech signal. These modulations are substantially attenuated by reverberation encountered in everyday environments. To assess the effect of reverberation on the neural coding of amplitude envelope, we recorded from single units in the inferior colliculus of awake rabbit using 100% sinusoidally amplitude modulated broadband noise stimuli presented in simulated anechoic and reverberant environments (direct-to-reverberant energy ratios of –3 and -7 dB). The modulation frequency was typically varied from 4 to 256 Hz.

Both the average firing rate and the modulation depth of the period histogram were studied as a function of modulation frequency to obtain rate and temporal modulation transfer functions (rMTF and tMTF), respectively. The maximum modulation gain of the tMTF as well as the range of firing rates of the rMTF were consistently lower in the reverberant condition than in the anechoic condition, indicating that reverberation degrades both temporal and rate coding. However, the maximum modulation gain of the reverberant tMTF was always better than predicted by a linear model, suggesting that nonlinear processing improves modulation coding in reverberation. Moreover, the strength of temporal coding was better for reverberant stimuli than for static anechoic stimuli whose modulation depth was adjusted to match the average demodulation induced by reverberation over the entire stimulus duration. As coding was more robust in the first 300 ms than in later portions of the 2-sec reverberant stimulus, consistent with the build up of reverberant energy over time, the coding advantage for realistic reverberant stimuli over static demodulated stimuli could be explained by simple mechanisms such as adaptation.

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280 A Comparison of Electrophysiological (Acoustic Change Complex) and Psychophysical Measures of Auditory Discrimination in Adults and Children

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The acoustic change complex (ACC) is an obligatory cortical response that is elicited by changes in an ongoing, long-duration sound. Although some evidence suggests that the ACC might serve as an objective index of auditory discrimination capacity, this function has not been systematically investigated. The aims of this study were: 1) to assess the dependence of the ACC on changes in stimulus intensity, frequency, and temporal envelope, and

2) to compare the ACC and psychophysical measure of auditory discrimination in normal hearing adults and children.
Just noticeable differences in intensity and frequency, as well as gap detection thresholds, were measured in children (7 to 15 y.o.).and adults (22 to 43 y.o.) using a two-alternative forced-choice procedure (2AFC) formatted as a video game. Stimuli were presented at a standard level of 70 dB SPL to the left ear. In each task, at least three threshold estimates were collected and averaged. The ACC elicited by changes in intensity, frequency and temporal envelope (gaps) were also obtained from the same group of listeners. During the ACC procedure, the observer sat in a reclining chair and watched a silent captioned movie of their choice. Only the response at electrode site Fz was measured.
Preliminary data analysis indicates that increasing magnitude of variation in all three acoustic dimensions results in greater ACC amplitudes in both child and adult subjects. Comparatively, ACC latency changes are less robust. Results will be discussed in terms of the association between psychophysical discrimination thresholds and ACC measures.

281 Modulation of Subplate Neuron Activity by 5HT

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The young mammalian cerebral cortex is different from the adult in that it contains neuronal circuits that are nonexistent in adults. These circuits are formed by subplate neurons, which relay thalamic information into the developing cortical plate (Kanold 2009, Luhmann 2009). Subplate neurons are crucial for the normal development of the functional organization and plasticity of the cerebral cortex. Serotonergic afferents are present in the subplate before they invade the cortical plate itself and altering 5HT levels in neonatal animals results in large scale cortical wiring deficits.
Because early 5HT projections are present in the subplate, they might form contacts with subplate neurons. Thus some of the developmental effects of altered serotonergic transmission might be mediated by subplate neurons. We characterized the responses of subplate neurons to 5HT and find that 5HT alters the excitability of subplate neurons. This effect of 5HT is strongest during the 1st postnatal week. Thus, hypo- or hyperfunction of the serotonergic system in development can not only influence thalamocortical transmission by acting on presynaptic fibers, but also do so by direct action on subplate neurons. This direct effect might contribute to some of the developmental abnormalities observed when 5HT levels are altered.

282 Null Mutations in EphB2 Decrease Sharpness of Frequency Tuning in Primary Auditory Cortex

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Primary auditory cortex (A1) exhibits a tonotopic representation of characteristic frequency (CF). The receptive field properties of A1 neurons emerge from a combination of thalamic inputs and intracortical connections. However, the mechanisms by which these inputs develop and form mature receptive field properties remain largely unknown. We previously showed that Eph family proteins help establish tonotopy in the auditory brainstem. Moreover, other studies have shown this family of proteins contributes to topography in visual and somatosensory cortices. Here, we examined the role that Eph proteins play in determining cortical response thresholds and sharpness of frequency tuning. To test mice with null mutations in *EphB2* and *EphB3*, we placed a 16-channel silicon multiprobe in A1 of urethane-anesthetized $EphB2^{-/-}/EphB3^{-/-}$ and $EphB2^{+/+}/EphB3^{-/-}$ mice, aged postnatal day 60-70. We recorded local field potentials and derived current-source density profiles based on responses to stimuli ranging in frequency from 1-40 kHz and intensities from below threshold to 70 dB SPL. Based on the shortest-latency current sink in the middle layers (presumed thalamocortical input), we generated frequency-intensity tuning curves for each recording site. Sharpness of tuning was quantified as the ratio of CF to the bandwidth at 20 dB above threshold (Q_{20}), and was determined using quarter-octave frequency steps. While both $EphB2^{-/-}/EphB3^{-/-}$ and $EphB2^{+/+}/EphB3^{-/-}$ mice had increasing CF values from posterior to anterior A1, we found that the double mutant $EphB2^{-/-}/EphB3^{-/-}$ had significantly lower Q_{20} values than $EphB2^{+/+}/EphB3^{-/-}$ mice, indicating that neurons lacking EphB2 were more broadly tuned. In addition, we found that the double mutants had significantly higher thresholds and longer onset latency for threshold intensities than mice with wild type *EphB2*. These results suggest that EphB2 influences cortical responses as a result of, or in addition to, its role in the auditory brainstem.

283 Organization and Experience-Dependent Modification of the Mouse Auditory Thalamocortical Circuit: Structure, Function and Behavior

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Experience plays an important role in the formation and dynamic maintenance of receptive field (RF) organization in the primary auditory cortex (AI) and auditory thalamus. Further study in transgenic mice will permit a deeper understanding of the molecular mechanisms that translate

auditory experience into long-lasting plasticity of these circuits. As a first step, we have characterized the tonal RF organization and plasticity in AI and the ventral division of the medial geniculate body (MGBv) in wild type C57BL6 mice using high-density mapping, neuroanatomical tracer injections, developmental sound exposure and prepulse inhibition (PPI) behavioral testing. The straightforward best frequency (BF) organization of AI was juxtaposed against a more complicated tonotopic organization in MGBv that was eventually resolved through reconstruction of electrophysiologically guided tracer injections and lesions. Critical period regulation of tonotopic map plasticity was assessed by exposing mice to pulsed 7 kHz tones either at the onset of hearing (P11-15) or from P16-20. A significant overrepresentation of BFs near the exposure frequency was observed in mice exposed from P11-15, but not P16-20. Interestingly, commensurate changes in BF distributions were not observed in MGBv or inferior colliculus. Experience-dependent modification of PPI behavior closely paralleled the developmental regulation of AI tonotopic map plasticity. However, tone-evoked PPI was reduced in P11-15 mice, rather than enhanced, and this effect generalized to frequencies other than 7 kHz. By describing the basic RF organization of the thalamocortical circuit, its developmental and hierarchical regulation and the impact of cortical reorganization on a simple auditory behavior, these data provide an effective backdrop for future studies in transgenic models.

284 Development of Intrinsic Cortical Circuitry in Mouse Primary Auditory Cortex

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Tuning and connectivity along the mature auditory pathway follow a general tonotopic arrangement. However, the underlying circuitry giving rise to this arrangement and its development are unknown. Given the high intrinsic interconnectivity in auditory cortex, we studied the organizational principles of local circuitry in primary auditory cortex (AI) during development using laser scanning photostimulation (LSPS) in mouse brain slices. We mapped the spatial and temporal locations of excitatory and inhibitory synaptic inputs to cells in the different cortical layers. We inferred synaptic distance based on the time-course of activation after photostimulation. By measuring synaptic maps at different ages (P2-P18), we are able to detect the changes in connectivity occurring during development. We find evidence for both intra- and extra-columnar inputs, occurring at different synaptic distances. In particular, we find strong extra-columnar inputs to layer 4 neurons originating from subplate neurons. Our methods and results offer a framework for quantitatively investigating the neural circuitry underlying cortical processing, and its development.

285 Developmental Changes in the Timing Properties of Auditory Cortical Synaptic Inputs

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Short and reliable spiking latency is essential for faithful information transmission between connected neurons within auditory cortex. Consequently, precise spike timing is critical for prompt and reliable representation of rapidly changing acoustic environment. Compared to adults, neural activity during development has different temporal properties, which may result in greater variability for cortical information processing (Oswald and Reyes, J. Neurophysiol. 2008; Dorrn et al., Soc. Neurosci. Abstr. 2008; Yuan et al., Soc. Neurosci Abstr. 2009). Previous studies have shown that the development of spike timing precision is correlated with maturation of fast synaptic transmission, but most of these studies were done *in vitro*. Here, instead we used *in vivo* whole-cell recording to investigate the developmental changes in the temporal properties of synaptic inputs (Froemke et al., Nature 2007). We found that the onset latency of excitation and inhibition decreased over development. Importantly, the lag between onset of excitation and onset of inhibition was initially significantly longer during the critical period (P12-P21: 5.5 msec) than afterwards (P22+: 3.1 msec). This decrease in inhibitory lag is expected to reduce spike variability. We will discuss the role of sensory experience in altering the timing of excitatory and inhibitory events.

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286 *In Vitro* and *In Vivo* Studies of $GABA_A$ Mediated Inhibition in Rat Medial Geniculate Body

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Age-related hearing loss is a complex disorder affecting 30% of the US population aged 65 to 74 years, and 50% of the population 75 years of age and older. The present *in vivo* and *in vitro* studies examined the nature of $GABA_A$ receptors ($GABA_A$Rs) at the level of the medial geniculate body (MGB). The MGB receives lemniscal and extralemniscal ascending inputs as well as reticular, limbic and descending inputs from auditory and nonauditory cortices. Subcortical temporal coding studies suggest that inhibitory GABA circuits gate input-output functions and are involved in coding temporal features of complex acoustic signals. Preliminary iontophoretic, receptor binding and measures of $GABA_A$ subunit protein suggest the presence of one specific $GABA_A$ receptor subtype is highly concentrated in MGB. These extrasynaptic $\alpha_4\delta$ containing $GABA_A$Rs mediate a tonic current and are thought to function as “spillover receptors” during periods

of high neuronal activity. A MGB slice preparation was used to establish the presence of this extrasynaptic subclass of $GABA_A$Rs. *In vitro* voltage-clamp studies revealed a tonic chloride current, induced by bath application of GABA (1-10 μM) or the $\alpha_4\delta$ $GABA_A$R subunit selective compound, gaboxadol (GBX)(0.1-5 μM). This current was revealed by focal application the $GABA_A$ antagonist, gabazine (50 μM). *In vivo* studies, in urethane anesthetized rat MGB, found that iontophoretic application of GBX rapidly shut down tone-evoked firing of most MGB neurons tested. GBX appeared more efficacious than GABA in inhibiting MGB tone-evoked responses. $GABA_A$ receptor blockade had limited effects on the shape of rate-level functions but significantly altered response to modulated stimuli. Collectively, these studies begin to characterize the functions of GABAergic projections onto MGB neurons and could provide a basis for development of selective agents which could potentially ameliorate certain kinds of tinnitus or age-related deficits. Supported by NIH DC00151.

287 Spatial Receptive Fields in the Auditory Thalamus of Awake Marmosets

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Determining the location of a sound in space is important for an organism's survival. In the auditory system, lesion studies have established that the auditory cortex is critically involved in sound localization behavior. Previous studies reported broadly tuned spatial receptive fields in the auditory cortex of anesthetized animals. In contrast, spatial tuning in the awake condition tends to be sharper and contains both onset and sustained phases of responses. How the thalamus contributes to these cortical responses in awake animals is unknown. Only a few studies have investigated the representation of auditory space in thalamus, mostly in anesthetized animals with a focus on azimuth locations. In the present study, we therefore sought to determine how neurons in the auditory thalamus respond to sound source locations in the awake condition.

We used a free-field speaker setup covering the upper hemisphere to study single-unit responses to noise bursts at different sound source locations in the auditory thalamus of the common marmoset (Callithrix jacchus). Most of the neurons encountered were tuned broadly to sound source location and the majority of sampled neurons preferred contralateral locations. However, we also found neurons that responded best to ipsilateral and other sound locations. Across the population of neurons tested, the preferred speaker locations varied across both azimuth and elevation. To investigate the neurons' input during spatial processing, we simultaneously recorded local field potentials with each single-unit. Local field potentials were mostly co-tuned to auditory space and often exhibited similar preferred speaker locations. These data suggest a possible transformation of the neural representation of auditory space from the auditory thalamus to cortex, resulting in sharper spatial receptive fields in auditory cortex.

288 Temporal Response Properties in Auditory Cortex Are Depth-Dependent

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In the visual and somatosensory cortices, many neuronal response properties vary systematically across layers within a cortical column. These laminar differences have provided the foundation for theories about how cortical columns transform visual and somatosensory information. In the auditory cortex, however, few clear laminar differences in neuronal response properties have been found, complicating attempts to understand intracolumnar cortical processing of auditory information. It has been suggested that temporal processing is a major function of the auditory cortex. We hypothesised that layer-dependence might therefore be most obvious for temporal response properties. To test this hypothesis, we recorded neuronal responses to short trains of noise bursts in the mouse primary auditory cortex (AI) and anterior auditory field (AAF). Responses of neuronal clusters were recorded simultaneously at different cortical depths using multielectrodes. Over 870 cluster recordings were obtained from a total of 118 penetrations in ten mice.

Following, quantified as the mean firing rate to second and later bursts normalised by mean rate to the first burst, was depth dependent. Following was strongest in more superficial recordings and weak in the deepest recordings, even at slow presentation rates. Moreover, at mid-to-superficial depths in AAF, responses to later pulses in 2-4 bursts/s trains were augmented. This augmenting response for slow pulse trains was less pronounced in AI, and was not observed in deeper layers in either area. We observed a different depth-dependence for neuronal excitability, which peaked at a depth presumed to correspond to layer V.

These results demonstrate that there are systematic depth-dependencies in the temporal response properties of neuronal clusters within auditory cortical columns.

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289 Expression of SMI-32 Neurofilament Protein in the Central Auditory System of the Rat

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SMI–32 is a neurofilament protein, expressed predominantly in the excitatory neurons. In the present study we evaluate the occurrence of SMI-32-immunoreactivity in the central auditory system of the rat.

In the inferior colliculus (IC), SMI-32-immunoreactive (SMI-32-ir) neurons were observed in all three subdivisions; however, their occurrence and morphology were significantly different between the dorsal and external cortices and the central nucleus of the IC. In the medial geniculate body (MGB), a rather uniform population of SMI-32-ir cells was present in the dorsal and ventral parts. Their numerical density, as well as their percentage of the total number of neurons, was unambiguously higher when compared with the IC and the auditory cortex (AC). This is in agreement with the fact that in rodents, inhibitory neurons are present in substantially lower numbers in the MGB compared to the IC and AC. At the cortical level, it was possible to delineate the AC from the non-auditory cortex and the central auditory core from the associative auditory areas on the basis of SMI-32 immunoreactivity. A striking demarcation line was found between the auditory cortex and the ventrally situated ectorhinal and perirhinal non-auditory areas. The demarcation borders towards the non-auditory parietal cortex were less pronounced and more gradual. Within the auditory cortex, the traditional Te1, Te3 and Te2 areas (Zilles, 1985) could be distinguished on the basis of the variable numerical density and volumes of SMI-32-ir neurons, emphasized especially in the large pyramidal cells of layer V. Since a relationship between the expression of SMI-32 protein and high myelin content in neurons has been suggested, the observed differences may reflect functional and wiring distinctions between the primary (Te1) and associative (Te3 + Te2) auditory areas as well as among subdivisions of the IC.

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290 Afferent and Intrinsic Input Systems Defining Spectral Frequency Integration in Primary Auditory Cortex

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Primary sensory cortex integrates sensory information by interaction of afferent feedforward thalamocortical microcircuits and convergent intracortical contributions. Here, we differentiate both by using a newly developed advanced analysis of the laminar current source density (CSD) distribution that allows quantifying relative contributions of horizontal intracortical input to a given cortical site. In combination with pharmacological cortical silencing we thus can evaluate the functional role of horizontal intracortical connections for such basic features as spectral frequency integration and for the construction of cortical receptive fields (CRF) on a laminar spatiotemporal level.

We found the temporal precise recruitment of convergent broadly tuned afferent thalamocortical and local recurrent excitatory and feedforward inhibitory intracortical inputs to be an important feature of suprathreshold cortical frequency tuning. Through separate global excitatory intracortical projections in upper layers II/III spectrally distant input is provided even over long cortical distances defining subthreshold spectral tuning width. Hence, the construction of CRF in primary auditory cortex depends on the temporal contribution of broad afferent thalamocortical inputs and different short- and long-distance horizontal intracortical networks. Ultimately, by describing refined models of thalamocortical and intracortical convergence we can developed a unified functional framework to bring different and partly controversial anatomical, as well as physiological models of spectral integration mechanisms into accordance with each other.

291 Evoked Potentials in the Macaque Auditory Cortex After Electrical Stimulation of the Midbrain

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Previous studies in the auditory cortex of rodents indicated that the dopaminergic system is involved in memory consolidation, motivational incentive for developing behavioral strategies and learning-induced plasticity. The group of Merzenich was able to show that repetitive pairing of a pure tone with electrical stimulation in the ventral tegmental area changes tonotopicity in the auditory cortex in rats (Bao et al., Nature 2001). As their data reflect only the acoustic responses, the direct electrical influence of the ventral tegmental area on the auditory cortex however has remained elusive.

Here we investigated the direct effects of electrical stimulation of the dopaminergic midbrain on the auditory cortex in the monkey. We recorded local slow wave field potentials and multiunit activity from the auditory cortex in *Macaca fascicularis* after electrical stimulation of different depths of the midbrain.

Our results revealed that it is possible to evoke responses in the auditory cortex by electrical stimulation of a large area in the midbrain. The electrically evoked field potentials had surprisingly stereotypic wave forms with three positive and three negative components. These field potentials resembled those which were evoked by acoustic click-stimulation, especially with regard to the latencies. The multiunit activity revealed three distinct response types with an early excitation (~40ms), followed by an inhibition response, an excitation-only response at ~75ms and an excitation-only response at ~110ms.

These data indicate that electrical stimulation of the midbrain can evoke firing in the auditory cortex. Our findings thus suggest that activation of the dopaminergic system can have immediate effects on the neuronal activity in the auditory cortex, in addition to the long-term effects observed in previous studies.

292 Differential Effects of Cortical Inactivation on the Ipsilateral and Contralateral Cochlear Potentials

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The profuse descending auditory system from the auditory cortex innervates nuclei all the way down to the brainstem and can have direct effect on the cochlea via the olivocochlear system. We have previously shown that when the cortex of the anaesthetised guinea pig is rapidly and reversibly inactivated by cooling, the cochlear potentials in the ear contralateral to the inactivated cortex are reduced in amplitude. We also demonstrated an apparent dissociation between the effects of cortical inactivation on the amplitude of the cochlear action potential (auditory nerve activity) and the cochlear microphonic (outer hair cell activity). The CAP was often little affected while the CM was simultaneously reduced. Both CM and CAP were reduced in a frequency dependent manner: the CM by 2-15 dB and the CAP by 0-7 dB. These effects are quite different from those due to stimulating the medial olivocochlear system.

Here we show that recording monaurally or from both ears simultaneously, a similar decrease in CM amplitude at both ears, whilst reductions in CAP at the ear ipsilateral to the inactivated cortex were larger than at the contralateral ear. In simultaneous recordings, at low frequencies, reciprocal effects on CAP amplitude in the two ears were occasionally seen with the CAP amplitude increased at the ipsilateral ear while it decreased at the contralateral ear. In preliminary data (N=2), lesioning the MOC fibers at the floor of the fourth ventricle did not abolish or reduce the effect of cortical inactivation on either the CAP or CM.

These results indicate that even in the anaesthetized animal the cortex exercises control over the function of the cochlea. Cortical inactivation does not mirror the classical MOC effects and at present it is unclear what pathway mediates the effect on cochlear function.

293 Comparison Between Task Related Plasticity in Primary and Secondary Auditory Cortex

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Rapid task-related receptive field plasticity alters the tuning properties of neurons in primary auditory cortex (A1) in a task-specific fashion that enhances their ability to encode the salient parameters of the current task (Fritz et al., 2003, 2005, 2007, Atiani et al., 2009). Such attention-driven sensory plasticity has been suggested to contribute to our ability to perform auditory scene analysis, navigate through a complex acoustic environment, attend to a selected sound stream and extract acoustic information of interest from the background. Previous studies have shown greater plasticity in secondary auditory areas induced by classical conditioning (Diamond and Weinberger1984) or increased frequency selectivity induced by Nucleus Basalis stimulation (Puckett et al. 2007). In this study we investigated task-related receptive field plasticity in two secondary auditory cortical areas in the ferret, in comparison with the known forms of plasticity in the primary auditory cortex. We trained ferrets on a conditioned avoidance acoustic task that required them to distinguish a set of band pass noise stimuli from tones. We recorded single unit activity from primary auditory cortex and two tonotopic posterior fields of the auditory cortex, which are anatomically and functionally linked to primary auditory cortex (Bizley 2005, 2006). We recorded in two behavioral states: while the animals were in a quiescent state of passive listening, and while they were in an active state during task performance. We observed robust task-related changes in firing rates and response dynamics in secondary areas. One difference was a large increase in task related responses to behavioral targets in secondary auditory areas compared to A1. This response may be pivotal for categorical encoding of target stimuli – attended auditory objects. Understanding the differential types and magnitude of task-related plasticity in secondary auditory cortex may clarify the functional contributions of these auditory areas to acoustic representation and auditory attention.

294 Sound Discrimination of the Guinea Pig Using a Non-Aversive Classical Conditioning Paradigm

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Guinea pig (GP) is one of the most common animal species that has been used in auditory research. In spite of relatively rich repertoires of its calls, neural mechanisms of discrimination of different natural sounds have not been studied. This seems to be mainly due to the difficulties in training this animal species with acoustic stimuli as well as non-acoustic stimuli. Thus, it is crucial to establish the association between specific acoustic signals and behavioral responses.

We trained this animal through 4 stages in a non-aversive classical conditioning paradigm. In the 1st stage, 2-3 GPs were kept in one home cage, and an animal keeper intentionally made stepping sounds whenever approaching the cage to feed them. It took around 7 days before animals came to gather around a pellet container as they heard stepping sounds. These animals were then moved to an arena in soundproof room. They were similarly fed by the same keeper but feeding frequency was much increased together with reduced quantity of pellet per feeding. This stage lasted 7 days. In the 3rd stage, the real stepping sound was replaced by a digitized one and the feeding was performed automatically. The digitized stepping sound was played back from a dynamic speaker within the soundproof room. Latency between the offset of the stepping sound and the feeding was 5 or 6 s. Following training for 2 to 3 days, one GP was chosen from the group and subjected to discrimination training for 3 days. In this final stage, 8 different natural sounds (7 novel

sounds added to conditioned one) were randomly played at an equal probability. Head movement above the pellet container was quantified during the period between the onsets of stimulus sound and the pellet supply. Results showed that significant changes in movement occurred only at the conditioned sound.

This discrimination behavior may be linked to electrophysiological recording to evaluate the neuronal basis of sound discrimination in the future.

295 Anatomical and Functional Connectivity from Prefrontal Cortex to the Auditory Cortex in the Mouse

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Attention-induced changes in auditory cortical response properties are likely to be the consequence of top-down signals originating in higher order cortical areas and, as previously demonstrated, mediated by neuromodulatory systems, such as those that use acetylcholine (i.e.. nucleus basalis). At this time, the precise identity and functional properties of the higher order regions that might orchestrate such changes in the auditory cortex, particularly in smaller mammals, is unclear. Here, using standard anatomical tract tracing methods, we identified regions in the mouse prefrontal cortex (PFC) that project directly to the auditory cortex (ACX) and characterized the projection patterns within ACX. In addition, we found that the PFC-ACX anatomical connection was indeed functional by applying electrical microstimulation in the PFC and simultaneously monitoring neural activity in ACX. Ongoing experiments are investigating the role of the PFC in mediating auditory cortical plasticity. We shall describe the effects of electrical activation of the PFC on activity of auditory cortical neurons as well as the effects on frequency tuning properties of ACX neurons. Collectively, these experiments may provide a more complete picture of the mechanisms underlying top-down control of auditory processing and may offer insight into the neural mechanisms underlying auditory cortical plasticity.

296 Early Auditory Novelty Processing in Humans: Auditory Brainstem and Middle-Latency Responses

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The detection of unexpected new events in the auditory environment is crucial for survival, as preparing the organism for rapidly changing surrounding conditions. Human auditory novelty detection has been associated to the mismatch negativity long-latency cortical evoked potential, peaking at 100-200 ms, but very few studies had been focused in earlier potentials such as auditory brainstem (ABR) within the first 10 ms from stimulus onset and middle latency (MLR) responses in the time range of 10 ms to 100 ms. Nevertheless, recent animal results showing novelty responses in individual neurons already at the level of the midbrain and thalamus suggest that novelty detection is a basic principle of the functional organization of the auditory system, expanding from lower levels in the brainstem along the auditory pathway up to higher-order areas of the cerebral cortex.

The present study aimed at testing whether the detection of frequency changes is already reflected at early stages in auditory processing. For that purpose, a typical frequency oddball paradigm and the respective control conditions were used, while the parameters of EEG acquisition were adjusted to record the ABRs and the MLRs in a sample of eighteen healthy human subjects. Stimuli consisted of 40 ms band-pass filtered broadband noises in the range 500 to 3000 Hz in steps of 500 Hz that were delivered monaurally to the right ear at an intensity of 80 dB SPL with a stimulus onset asynchrony of 96 ms. The left ear was masked with white noise at an intensity of 60 dB SPL.

The results showed that occasional changes in auditory frequency information are detected as early as 30 ms (Pa waveform of the MLR) after stimulus onset. The control block precluded these effects as resulting merely from refractoriness, altogether supporting the notion of “true” early auditory novelty detection in humans, matching the latency range of auditory novelty responses described in individual subcortical neurons of subhuman species.

297 Mismatch Negativity-Like Effect Observed in Epidural Evoked Potentials of Rat Auditory Cortex

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Previous research has shown that the auditory cortex plays some roles in detecting acoustical changes. The mismatch negativity (MMN)-like effect has been thought to be evidence for this. We examined the MMN-like effect in epidural-evoked potentials recorded from the auditory cortex of anesthetized rats. A standard oddball paradigm was used: We recorded responses to a sequence of tone bursts (100-ms duration; 500-ms inter-burst interval) involving two different frequencies in a randomly interleaved manner. One of these frequencies was infrequently presented as a deviant (5% probability) and the other as a standard (95% probability). As a control condition, these were presented with equal probabilities. A reverse condition was also included. In the results, we often observed the typical MMN-like effect: The difference between the responses for the deviant and the standard immediately preceding the deviant was large in comparison with the difference for the equal-probability condition. Nevertheless, the size of the MMN-like effect varied among recordings. A detailed examination of the response sequence revealed that the MMN-like effect was generally large when the responses for two sequential standard tones immediately preceding the deviant differed by a relatively small amount. This implies that the stability

of recorded responses to the standard, reflecting the recording condition and/or the cortical state, is a factor determining the observed size of the MMN-like effect.

298 Stimulus-Specific Adaptation to Frequency and Intensity in the Mouse Auditory Thalamus

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Mismatch negativity (MMN) is a cortical evoked potential associated with changes in sound parameters e.g. frequency, intensity or duration. Stimulus-specific adaptation (SSA), the phenomenon whereby a neuron responds more strongly to a rarely presented (deviant) stimulus than to the same stimulus when it is commonly presented (standard), has been proposed as a neuronal correlate of MMN. If SSA is related to MMN, it would be expected to occur for all of these sound parameters in some central auditory neurons. We tested neurons in the mouse auditory thalamus (medial geniculate body, MGB) for SSA to sound intensity as well as frequency, using presentations of standard and deviant pure tone stimuli or broadband noise. In the tone condition, the frequency difference between the two tones was no greater than 0.5 octaves; the intensity differed by no more than 20 dB. In the broadband noise condition, the intensity of the two stimuli differed by no more than 25 dB. Frequency-response curves and rate-level functions were used to choose stimuli which evoked similar firing rates. The probability of occurrence of the standard/deviant stimuli was 90/10, and stimuli were presented at 2 Hz. Extracellular responses were recorded from single and multiunit clusters distributed throughout the MGB. We confirmed our previous reports of SSA to frequency in the medial and ventral MGB; however we did not observe significant SSA to intensity when presenting pure tone stimuli (~1% of neurons at $p<0.01$ [randomisation test, Anderson et al, 2009, J Neurosci. 29, 7359-7363]). Conversely, when using broadband noise we saw significant SSA to intensity in a small population of MGB neurons (4%, $p<0.01$), even when controlling for the average power of the stimulus (10%, $p<0.01$). These neurons did not necessarily exhibit SSA to frequency. Thus, SSA in the mouse MGB may be elicited by intensity deviants as well as by frequency deviants, but differing populations of neurons could contribute.

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299 Frequency Response Maps in the Auditory Thalamus of Rat and Its Relation with Surprise Detection

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Neurons in the medial geniculate body (MGB) participate in auditory surprise detection (Antunes et al., 2009), but the relation between this property and the response type of neurons in this nucleus remains unknown. Here, we analysed frequency response areas (FRA) and their corresponding two-tone suppression FRAs of 93 single units throughout the MGB of anaesthetised rat to monaural pure-tones, and study its relation with the surprise detection profile of neurons. This profile was characterized based on the degree of stimulus-specific adaptation (SSA) of a neuron; surprise detector neurons were those that exhibited high values of SSA. Our results demonstrated that MGB neurons were broadly tuned as determined by the Q_{10} bandwidth measure. MGB neurons exhibited a wide variability of FRA shapes including V-shaped but mainly non-V-shaped. Non-V-shaped FRAs comprised diverse subtypes such as narrow, closed, multipeaked, U-shaped, mosaic and inhibitory. Our results show that none of the typical V-shaped FRAs exhibited high degrees of SSA, indicating that these neurons do not participate in surprise detection. By contrast, surprise detector neurons exhibited a high variety of FRA shapes. Neurons with high SSA values exhibited on average broader tuning widths than non-adapting neurons. Thus, we suggest that the best suited neurons to detect a surprising sound should be those receiving input over a wide range of frequencies. Moreover, our results based on two-tone suppression suggest that inhibitory mechanisms may underlie the modulation of SSA and surprise detection in the MGB.

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300 Assessing Tonotopy in Human Audition During a Change Detection Task – a Multi-Voxel Pattern Analysis Approach

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It is commonly agreed that regions in human auditory cortex are tonotopically organized. Although this tonotopy can be detected with fMRI (e.g. Bilecen et al., 1998; Engelien et al., 2001), the spatial scale causes the signal-to-noise ratio to be low when using standard imaging methods. Multi-voxel pattern analysis (MVPA, e.g. Cox & Savoy, 2003) is a new multivariate method that overcomes the spatial limitations of conventional fMRI and is additionally more robust to the substantial inter-individual anatomical differences that exist in auditory cortex (e.g. Feredoes et al., 2007). It, thus, provides a powerful new tool for examining the encoding of frequency information in auditory cortex.

It has, furthermore, been proposed that the primary sensory cortices do not only process incoming perceptual information but also play a role when it is necessary to hold information in short-term memory (e.g. Pasternak & Greenlee, 2005). By using MVPA and an event-related fMRI design with varying maintenance and inter-trial intervals, it was possible to separate the neural responses during encoding, maintenance and retrieval of the tones.

Participants listened to sequences of pure tones, maintained these tones in short-term memory for a variable time period and performed simple same/different

judgments after hearing a probe tone sequence. Distinct patterns of activity were observed during all three stages of the task with auditory cortex being strongly activated during encoding but deactivated during maintenance. Furthermore, the pattern of fMRI activation in auditory cortex was reliably shown to depend upon the frequency of the sounds, with those more similar in frequency evoking a more similar neural pattern. Interestingly, activity patterns during maintenance were negatively correlated with patterns evoked by the same frequencies during encoding suggesting that frequency specific suppression plays a role in maintaining information in auditory short-term memory.

301 Electrophysiological Study of Responses to Amplitude-Modulated Noise Within Human Lateral Superior Temporal Gyrus

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Periodic non-speech acoustic stimuli, such as sinusoidally amplitude modulated (SAM) noise, can elicit different percepts depending on modulation rate. Understanding cortical response properties to such stimuli can facilitate prediction of responses to complex natural sounds (*e.g.* speech). This study addressed representation of SAM noise within lateral superior temporal gyrus (STG) in humans where previous studies had described auditory response fields (Howard *et al*, *J Comp Neurol* 2000 416:79-92; Brugge *et al*, *J Neurophysiol* 2003 90:3750-63). Subjects were neurosurgical patients undergoing chronic invasive monitoring for refractory epilepsy. Stimuli were 1 s bursts of broadband noise, sinusoidally modulated at rates between 4 and 256 Hz, presented diotically via insert earphones. Recordings were made simultaneously using multi-contact depth electrodes implanted in Heschl's gyrus and high-density (5 mm spacing) subdural grids placed over the temporal lobe. Responses were analyzed as average evoked potentials (AEP) and event-related band power (ERBP).

Responses to SAM noise were localized to the lateral STG, adjacent to the junction between Heschl's sulcus and Sylvian fissure. Responses were characterized by onset and offset AEP complexes and increases in high frequency (70-150 Hz) ERBP. AEP envelope following was evident at low modulation rates (<16 Hz; *cf.* over 100 Hz in the core cortex within posteromedial Heschl's gyrus for SAM noise). ERBP rarely exhibited modulation by the stimulus envelope. No monotonic growth of the response with modulation rate was observed; however, higher modulation rates yielded responses with shorter latencies. No consistent "modulation tuning" pattern emerged across recording sites. Responses from the lateral STG were markedly different from those recorded from core auditory cortex and had a considerably lower limit of explicit temporal representation of envelope information.

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302 Effects of Stimulus Repetition on the Individual Generators of the N1 Peak of the Late Auditory Evoked Potential

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Speech and other complex sounds in the environment contain rich time-varying information. Understanding how the auditory system processes temporal cues of sounds in cochlear implant (CI) patients is critical for further improving their temporal processing abilities needed for sound perception. The temporal properties of neural responses following trains of successive acoustic stimulation can provide objective information regarding temporal processing of the auditory system. The goal of this study is to examine the temporal properties of the late auditory evoked potential (LAEP) in normal hearing (NH) listeners, which will serve as normative data to be compared with those in CI users. The electroencephalogram (EEG) was recorded from 14 NH listeners using a 40-channel Neuroscan system. Tone bursts (80 dB SPL) were presented in trains of ten with an inter-stimulus interval (ISI) of 0.7 and an inter-train interval of 15 s. Independent component analysis (ICA) was applied to concatenated single-trial EEG data and clustering was performed based on the common features of the event-related potential (ERP) components including the scalp topography, the component waveform, and the dipole location. Results showed that, at least 6 independent component clusters contributed to the N1. These clusters are located in the cingulate cortex, the frontal lobe, the reticular formation in the midbrain region, the left and the right superior temporal gyrus and the temporal-parietal junction, and the thalamus. The first 3 clusters showed obvious amplitude decrement following stimulus repetition, while the latter 3 clusters did not. The study confirms that the ICA may provide an important electrophysiological technique for determining generators of the ERP and examining their physiological features. This study also suggests that the N1 amplitude decrement in response to repeated stimuli reflects the adaptation and refractory features of different neural generators of the N1.

303 Does the Inter-Stimulus Interval Affect Hemispheric Asymmetry of the Late Auditory Evoked Potential?

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The functional neuroanatomy associated with hemispheric asymmetries in the auditory cortex has not been well addressed. Most studies have examined hemispheric asymmetry of the late auditory evoked potential (LAEP) using stimuli presented at short inter-stimulus intervals (ISIs: approximately 1 s). It is well known that the LAEP is composed of multiple components generated from different brain generators and some generators need much longer ISIs to recover from the preceding responses. The current study provided evidence that the ISI has effects on

hemispheric asymmetry of the LAEP. Multi-channel electroencephalographic (EEG) recordings were used to record the LAEP to monaural stimulation from normal hearing listeners. Stimuli were 1 kHz tone bursts presented in random orders at different ISIs ranging from 0.7 to 15 s. Independent component analysis (ICA) was applied to concatenated single EEG trials and clustering was performed based on the common features of the scalp map, the component waveform, and the dipole location across subjects. Hemispheric asymmetry was investigated for left and right ear stimulation. Results showed that there is a lack of hemispheric asymmetry in LAEP waveforms. However, the responses of the cluster from the temporal lobes showed hemispheric asymmetry. Results also showed that the contribution of other brain regions such as the cingulate cortex and the reticular formation in the midbrain region could be greater than that of the temporal lobes on both sides. The current study revealed that LAEP asymmetry can be reduced if the ISI is long enough to allow the recovery of other contributing structures than the temporal lobe. These findings have implications for future studies utilizing independent component clustering technique to examine LAEP asymmetry in normal and abnormal auditory systems.

304 MEG Measurement of Cortical Responses to Sound in Guinea Pig and Mouse

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Using a newly developed magnetoencephalograph (MEG) for small animals, we have recorded auditory-evoked cortical responses non-invasively in both guinea pig and mouse. The small-animal MEG system has 9 magnetometers placed in an 8x8 mm square array at 3 mm from the outer surface of the liquid helium-filled dewar. An additional set of 3 magnetometers and one accelerometer are used to measure and suppress environmental noise. Sound is delivered either free-field, or via short tubes from Etymotics transducers. Signal processing is crucial to extract the tiny brain responses from noise, and several new techniques have been developed for that purpose. Using these techniques, we can detect cortical responses evoked by sound onsets, transitions and binaural disparities in tone, noise and chirp stimuli, in both guinea pigs and mice. Up to 5 distinct spatio-temporal response components have been observed in these datasets. Additionally, we demonstrate that stimulus-specific adaptation (SSA), hypothesized to be a neural correlate of mismatch negativity (MMN), can be observed in MEG responses to deviant events within trains of standards, and we compare these responses to similar responses observed in humans. These results pave the way for joint MEG and electrophysiology in the same animals to elucidate the neural basis of the MEG response, bridging the gap between human brain imaging and invasive animal electrophysiology.

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305 Modification of Auditory Cortical Evoked Potentials by Irradiation of Near-Infrared Laser to Cortical Sub-Regions

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It is reported (Riquimaroux and Kataoka, ISBN: 0387209867, pp. 167-172, 2005) that the irradiation of a low-level laser at a near infrared wavelength (830 nm, 3-6 W / cm^2) reversibly inactivates neuronal activity with a short transition time (a few minutes). This preliminary study used this laser technique to investigate the contribution of sub-regions of the auditory cortex (AC) to auditory evoked potentials. Two small holes (1-2 mm diameter) aligned dorso-ventrally were drilled in the skull of an anesthetized rat, so that they were roughly above the primary and ventral auditory fields. A ball electrode was placed epidurally on the AC through one of the holes, while the laser irradiated the AC through the other hole. Before laser irradiation, the waveforms of the evoked potentials recorded through the ventral and dorsal holes had essentially the same shape. A typical waveform consisted of a rapid oscillation with positive and negative peaks (onset latency around 15 ms) followed by a slow oscillation (onset around 45 ms). During laser irradiation, we observed marked changes in the waveforms. Generally, the first negative peak decreased in amplitude. In addition to this general effect, the laser irradiation modulated the waveforms in a manner that depended on the recording / irradiation site. After laser irradiation, the laser effects disappeared. Next, we examined the responses to a tone sequence using two randomly alternating frequencies with unequal probabilities (i.e., the oddball paradigm). Generally, we observed a mismatch negativity (MMN)-like effect, namely a response enhancement for tones with the lower-probability frequency, indicating cortical sensitivity to rare events. In some instances, inactivating the cortical sub-region increased the MMN-like effect. This implies that the sub-region contributed negatively to the MMN-like effect.

306 Spatial Stream Segregation Tested with Rhythmic Masking Release

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We quantified spatial stream segregation of free-field sounds using an adaptation of Rhythmic Masking Release (Turgeon et al., JASA 111:1819-1831, 2002). Normal-hearing listeners heard a rhythmic sequence of brief noise bursts from a target loudspeaker at 0° or 40° azimuth and were asked to report which of two rhythms was heard. Interfering sequences of noise bursts having identical

spectra, interleaved in time, were presented from a nearby speaker. Performance was at chance levels for co-located target and interferer, but small displacements of the interfering speaker resulted in a perceptual segregation of target and interfering streams and a concomitant improvement in rhythm identification. Thresholds were given by the separation of target and interferer at which rhythmic patterns could be identified with 76% accuracy. For broadband sounds, threshold separations averaged 6.8° and 11.1° for targets located at 0° and 40°, respectively. For comparison, minimum audible angles for detection of the direction of a change in location of those targets were 2.6 and 4.8°. We evaluated the spatial cues responsible for stream segregation in this task by repeating the tests using low-frequency (400 to 1600 Hz) and high-frequency (4000 to 16000 Hz) bandpass sounds. Performance in the low-frequency condition was not significantly different from the broadband condition ($p = 0.5$), whereas threshold separations for the high-frequency condition averaged nearly double the broadband values ($p<.001$). Based on the degradation in performance in the high-frequency condition, we infer that stream segregation in the broad-band condition resulted primarily from use of interaural timing difference (ITD) cues. That result accords with the dominant role of ITD cues in broadband localization.

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307 Inharmonicity Affects the Detection of Signals in Maskers at Different Spatial Locations

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In our environment sounds are often masked by other sounds. The auditory system has evolved mechanisms to separate important signals from a noisy background, especially, if the signal is spatially separated from the distracting background noise. The auditory system can improve signal detection in acoustic scenes by analyzing interaural time and level differences. Spatial release from masking is the threshold difference between signal detection in maskers coming from the same direction and signal detection in maskers when signal and masker originate from different spatial locations. The amount of spatial unmasking depends on the type of signal and masker.

In the present study we examined if the harmonic relationship of a sinusoidal signal to a complex stimulus affects the ability to detect the signal in the masker originating from different spatial locations. In an operant Go/NoGo procedure humans had to detect a sinusoidal signal (1 and 8 kHz) in a masking background stimulus. The signal was either played from the same spatial location as the masker (M0S0 situation) or presented from a 90 degree angle to the right side of the subject (M0S90 situation). Five masker types were presented: a harmonic complex that either had a harmonic or an inharmonic relationship to the signal, an inharmonic complex that was either constant or in which the frequency components were continuously varied throughout the session, and a band-pass noise. For the 8-kHz signal the spatial separation of signal and masker decreased thresholds. For the 1-kHz signal thresholds were similar between the M0S0 and the M0S90 condition for the two harmonic maskers. Signal detection thresholds were always high in the noise masker condition. The results suggest an effect of the harmonic relation between signal and masker on the detection threshold.

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308 Head Saccade Precision and Latency to Sound Pairs Having Different Durations in the Barn Owl

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Barn owls make head saccades to simulated echoes more frequently, with greater precision, and with shorter latencies when the echo's delay is long. Recently, we demonstrated that this increase in the echo's localizability is due, not to the delay per se, but to the fact that the echo is present, alone, for a sufficient period of time after the offset the leading sound. To further test this idea, we presented two uncorrelated noise bursts simultaneously and increased the duration of one of the two sounds from 6 to 30 ms. The second uncorrelated sound was thus present, alone, for a variable period of time after the offset of the first sound (0 to 24 ms). As when a similar "lag-alone" segment was produced by the introduction of a variable delay between two correlated sounds, saccades were more frequently directed to the speaker that presented the longer sound (> 3 ms). The same saccades were also more precise and their latencies were shorter than when this "trailing" segment was absent or short (3 ms). The data suggest that in a precedence-effect paradigm, lag stimuli can become localizable regardless of correlation level, provided that the lag sound is present, alone, for a sufficient period of time following the leading sound's offset. [Supported by grants from the NIDCD F32-DC008267 and RO1-DC03925]

309 Dynamic Sound Localization in the Cat During Rapid Eye-Head Gaze Shifts

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Since sound localization, particularly in the horizontal plane, relies on interaural time and level cues which are implicit head-centered cues, one might expect localization during rapid head movements to be problematic. Studies have shown that humans are able to localize dynamic acoustic cues even during rapid eye-head gaze movements and the occulomotor system is capable of accurately issuing appropriate motor commands despite ensuing head and eye movements (Vliegen et al., 2004). We studied whether cats are able to process similar varying acoustic cues during rapid eye-head gaze shifts. We conducted visual-auditory two-step experiments in which we presented a 25 ms sound burst to cats as they made saccadic eye-head gaze shifts toward a visual target. Peak horizontal head velocity during the 25 ms noise burst was 89.0 ± 50.9 º/sec for one cat and 135.4 ±

90.1 º/sec for the other. The accuracy of localization performance was summarized by the slope (or gain) of the linear regression relating the localization responses of the cats to the target positions. Results show no consistent significant difference in accuracy or precision between performing this dynamic task and the static task (single saccade in which a 25 ms noise is presented when the head is stable) for targets localized in either horizontal or vertical directions. Cats, like humans, appear to be able to process dynamic auditory cues and execute complex motor adjustments to accurately localize auditory targets during rapid eye-head gaze shifts.

310 Free-Field Sound Localization During Passive Whole-Body Rotation

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Free-field sound localization behavior is accurate under open-loop conditions (i.e., in complete darkness, toward brief sounds). Even rapid active head movements during saccadic gaze shifts are fully incorporated in the localization response (Goossens & Van Opstal, Exp Brain Res, 1997; Vliegen et al., J Neurosci 2004). Here we investigate the role of the vestibular canals in dynamic sound-localization behavior. Head and body-restrained subjects localized broadband noise bursts (3, 10 and 100 ms duration) with saccadic eye movements, while being rotated sinusoidally (f = 1/9 Hz, V_peak = 111 deg/s) around the vertical body axis in total darkness with a vestibular chair. Since the loudspeakers were attached to the chair, the 100 ms sounds might be perceived, and subsequently localized, as rotating along with the chair, i.e. in a head-centered reference frame. During the 3 and 10 ms duration stimuli, however, the amount of chair rotation (less than 0.3 and 1.1 deg, respectively) remained well below the minimum audible movement angle (Chandler and Grantham, J. Acoust. Soc. Am. 1991), and therefore these short sounds would be considered as stationary in space. In other words, they would be localized in a world-centered reference frame. Multiple linear regression on the responses shows, however, that for all three stimulus durations subjects did not correct for the whole-body rotation that followed sound offset during the (approximately 200 ms) reaction time. In other words, all three stimuli were localized in a head-centered reference frame. These results suggest that the vestibular canal information is either not sufficient, or not used, to translate head-centered target location into a world-centered reference frame.

311 Sound Localization Acuity in the CBA/CaJ Mouse (Mus Musculus)

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Using a two-choice operant conditioning nose-poke task, sound localization thresholds were determined for five adult CBA/CaJ house mice (*Mus musculus*). Thresholds were measured for 2-s broadband noise signals, high-pass filtered noise at 18 and 8 kHz, and low-pass filtered noise at 18 and 8 kHz. The speakers were situated to the right and left of the midline and ranged from 20 to 160 degrees in total speaker separation distance. The mice were required to nose poke to a central hole to initiate a trial and variable waiting interval, and to remain in that hole until the signal was presented. Following that waiting interval, one of the above-mentioned stimuli was played randomly from the right or left with equal probability. If the sound was emitted from the left, the mouse was required to nose poke to a second nose poke hole to the left of the central hole. If the sound was emitted from the right, the mouse was required to nose poke to a third nose poke hole to the right. The responses had to be within 3 s of the beginning of the stimulus, and correct responses were reinforced with a small drop of Ensure. Incorrect responses were punished with a 10 s time-out period, where no new trials could be initiated by the mouse. Thresholds were calculated using a mean criterion of 75% correct response rate. Overall, the two-choice task yielded high localization thresholds, consistent to what would be expected in the house mouse. There were differences in localization thresholds amongst the five stimulus types; thresholds were lowest for the broadband noise (51 degrees) and highest for the 8 kHz low pass filtered noise (83 degrees). These results demonstrate that mice can, in fact, be trained on a two-choice operant conditioning task using positive reinforcement to produce results consistent to what would be expected from a small, non-predatory mammal.

312 Lateralization of Acoustic Stimuli by Budgerigars (*Melopsittacus Undulatus*)

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Sound localization allows humans and animals to determine the direction of objects to seek or to avoid and indicates the appropriate position to direct visual attention. Animals such as the barn owl and cat have “specialized” auditory systems that have been extensively studied, contributing a great deal to our understanding of the processes underlying sound localization. Virtually nothing is known about the mechanisms of sound localization in many other species with relatively “unspecialized” auditory systems, including the budgerigar. Although behavioral measures of sound localization from several species of birds in the free-field and physiological correlates to sound

localization from different stages in the auditory pathway of many species have been made, no study investigating the behavioral sensitivity to individual sound localization cues has been conducted in birds. The abilities of budgerigars to localize, detect, and discriminate a variety of sounds in quiet or noisy conditions are well known, but this is the first study to equip birds with headphones so that their sensitivity to interaural time difference (ITD) and interaural level difference (ILD) cues can be measured. Threshold ITD and ILDs were measured behaviorally in budgerigars in a two-alternative (left/right) identification task and the stimuli were pure tones (0.5-8 kHz) and broad-band noise. Budgerigars appear to be less sensitive than humans and cats as expected, and more similar to rabbits and monkeys in their abilities to lateralize signals having interaural intensive or interaural temporal disparities. These lateralization results are generally consistent with the free-field localization abilities of these birds, and add support to the idea that budgerigars may be able to enhance their cues to directional hearing (e.g., via connected interaural pathways) beyond what would be expected based on their head size (diameter 28 mm).

313 Acoustic Source Distance Discrimination in Rabbit

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The acoustical cues and physiological processing strategies underlying the perception of the distance of acoustic sources are not well understood. This behavioral study of distance discrimination in rabbit was undertaken in association with a physiological study of distance coding by midbrain neurons in rabbit (S. Kuwada and D. O. Kim, University of Connecticut). The goal of these experiments was to estimate the ability of the rabbit to discriminate between series of noise bursts presented from a near and a far speaker that were located directly in front of the rabbit. Wideband, low-pass (100-3000 Hz) and high-pass (3000-20,000 Hz) stimuli were used. The mean stimulus level was 60 dB SPL at the location of the rabbit's head during the task, and level was roved over a 12-dB range from trial to trial to avoid the use of level cues for distance. An operant one-interval two-alternative non-forced choice task was used, with a two-down-one-up tracking procedure. Food reinforcement was used, and bias was controlled by introducing a variable percentage of trials for which the amount of reinforcement was doubled. The tracking procedure varied the distance between the speakers based on the percent correct within 10-trial blocks. The near speaker was kept at a constant position, and the far speaker location was varied based on the tracking procedure. When the horizontal speaker position was changed, both speaker elevations were randomly varied over a 10 cm rove range to minimize the reliability of elevation cues that were unavoidable in the physical setup. Rabbits were able to consistently discriminate stimuli that were separated by approximately 50% of the distance to the near speaker. [Supported by NIDCD-R01-DC002178, PI: S. Kuwada]

314 Identification of Auditory Distance Cues by Zebra Finches (Taeniopygia Guttata) and Budgerigars (Melopsittacus Undulatus)

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The use of acoustic signals for long-range communication is well known in the animal kingdom. Birds, in particular, use these signals to defend territories, attract mates, and locate conspecifics. However, long-range acoustic signals progressively degrade during their transmission from the signaller to the receiver. This degradation makes perceiving these signals more difficult, but on the other hand, receivers can use this information to estimate the signaller's distance. The perception of auditory distance cues (overall amplitude, frequency-dependent attenuation, and reverberation) have typically been studied in birds through the use of playback experiments in the field. The present study examined auditory distance perception in a non-territorial songbird, the zebra finch (*Taeniopygia guttata*), and in a non-songbird, the budgerigar (*Melopsittacus undulatus*) in a controlled laboratory setting. Using operant conditioning procedures, three zebra finches and three budgerigars were trained to identify 1 m (undegraded) and 75 m (degraded) recordings of three budgerigar contact calls, one male zebra finch song, and one female zebra finch call. Once the birds were trained on these endpoint stimuli, other intermediate stimuli were introduced into the operant task. The stimuli differed from the trained 'near' and 'far' endpoints in five ways: overall amplitude, high-frequency attenuation, reverberation, all three cues, and natural recordings at intermediate distances. By examining each distance cue separately, we sought to determine which was the most salient for the birds. We found that both species could scale their perceptions on a continuum from undegraded to degraded and that amplitude was the most important cue for these birds in auditory distance perception, similar to humans and other animals.

315 Psychophysical Examination of the Accuracy and Precision of Sound Localization with Respect to Sound Source Diameter

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Sound radiated from a vibrating piston is typically assumed to be equivalent to that from a point source; however, a large speaker may extend to fill several degrees of a listeners frontal space. Humans can accurately discriminate sound source locations within a few degrees, thus one might expect localization precision to decrease as a function of sound source diameter, much as precision is lower for localizing the center of a wide, fuzzy light source. Acoustically, however, a large-diameter speaker will not provide any cues indicating the speaker width to a listener on-axis, in the far field. Nevertheless, it is possible that imperfections in the speaker design or other unexpected cues could educate the listener, and we may gain insight

into the source localization mechanism by testing localization performance with respect to speaker size. Human subjects were seated with their heads fixed by a bite bar in a darkened, echo-attenuating room facing a cylindrical, acoustically transparent screen at a distance of 2 meters. Auditory targets, consisting of repeating bursts (5 Hz) of low-pass noise (0.2 – 1 kHz, to emphasize inter-aural time differences), were presented from behind the screen by a 10" by 4" oval speaker oriented towards the center of the subject's head. Subjects were instructed to quickly and accurately guide a laser pointer mounted on a cylindrical joystick towards targets, presented randomly within a field ±40° in azimuth by ±10° in elevation but with oversampled points (ten repetitions) located every ten degrees along the primary meridians. Localization performance was evaluated with the speaker oriented both horizontally and vertically in order to reveal dependence on azimuthal size. Localization accuracy and precision (mean and variance in localization error at oversampled locations) were not significantly different between speaker orientations, and were comparable to baseline measurements recorded using a 3" circular speaker. We conclude that at low frequencies, sound source width does not affect localization performance, as predicted by the acoustics. Supported by NIH grant DC005409.

316 Validation of a Virtual Conductive Hearing Loss Technique in an Animal Model

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Unilateral conductive hearing loss (CHL) during development and in adulthood can result in physiological and anatomical changes in brainstem structures with resulting consequences to behavior. Experimental studies on the consequences of CHL and experience-dependent plasticity in binaural audition in mammals have measured neural spatial receptive fields (SRFs) or binaural sensitivity *after* the occlusion has been removed. Ideally, the SRFs and binaural sensitivity would be measured in the same animal both before and immediately after the CHL was removed. To overcome this limitation, a technique based on virtual acoustic stimulation methods was developed to study responses of auditory stimulation under conditions of experimentally-induced CHL. Acoustical directional transfer functions (DTFs) were measured via the cochlear microphonic (CM) signals in six animals with and without earplugs in the ear canal. From these DTFs, the spectral and temporal transformations due to the earplug were computed. Attenuation due to the experimental conductive hearing loss was measured in 11 ears via elevation in CM thresholds in pure tones. Mean attenuation of CM thresholds across the frequencies tested was ~25 ± 9 dB. Induction of CHL, the stimulus frequency, as well as interaction between the two were found to have a significant effect on the CM thresholds. Experimentally induced CHL produces an attenuation that was dependent on stimulus frequency. The attenuation estimated from the DTFs was not significantly different than that measured using pure-tone stimulation. DTFs generated by experimentally induced CHL can be used to implement a "virtual earplug" to study how unilateral CHL affects monaural and binaural response properties of neurons in the central auditory system without having a physical plug in place. Support: NIDCD R01-DC6865 (DJT) and 5T32HD041697-08(JLT).

317 Estrogen Effect on Age-Related Hearing Loss

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There are compelling evidences that innate differences between both sexes might affect the course of age-related hearing loss. Estrogen is major hormonal decrease of postmenopausal women. We tried to estimate the differences of hearing threshold in pre- and post-menopausal women and compared those to the men of the same age range. Subjects were the clients who visited at Health Promotion Center, Daegu Fatima Hospital, Daegu, Korea from January 2004 to September 2005. Pure-tone audiometric results from 3,470 subjects, who submitted hearing questionnaire voluntarily, were evaluated. Subjects who have any of positive history of otorrhea, usage of ototoxic drugs, head injury, job in noisy environment, and military service, and who have asymmetric hearing loss were excluded. Included subjects were 215 men and 903 women. To evaluate hormonal influence on hearing in aging human, subjects were divided groups by age of 50 and by sex into 4 groups; (1) young women for pre-menopausal, (2) old women for postmenopausal, (3) young men, and (4) old men. The slope of a linear regression was used to estimate the rate of changes in pure-tone thresholds at 0.25 to 8 kHz. And the rate of changes was compared by groups. Postmenopausal women showed significantly greater rate of hearing loss in all audiometric frequencies than pre-menopausal women did. However, in men, there was significant difference between age groups only at 8 kHz. Mid frequency range of 1 kHz showed greater rate of hearing loss in postmenopausal women after adjusting the effects of age and estrogen. These results suggest that there was significant estrogen effect on the rate of change in pure-tone threshold and estrogen is important in maintaining of hearing in elderly. This study was supported by a grant of the Korean Healthcare Technology R&D Project for Health, Welfare & Family Affairs, Republic of Korea (Project Number A084055).

318 Polymorphic Analysis of Mitochondrial Genome Sequence in Patients with Presbycusis

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Objective: Recently, single nucleotide polymorphisms (SNPs) and other mutations in mitochondrial DNA (mtDNA) have been found to be associated with sensoryneural hearing loss. Communication disorders among older people, and in particular age-related hearing loss (presbycusis), is a growing social problem. The aim of this study is to try to identify SNPs associated with presbycusis via investigation of the entire mitochondrial genomes of older people (over 65 years old) with hearing loss and those with normal hearing.

Methods: Whole blood from both a presbycusis group (n=76) and a normal hearing group of comparable ages (n=81) was collected and polymerase chain reaction (PCR) and direct sequencing were performed to analyze the nucleotide sequences.

Results: As a result of analyzing the mitochondrial genome sequence, we detected 398 SNPs. Nine of these (G676C, 961insC, A3434G, C3970T, C6455T, T6680C, G8784A, G11696A, A15874G) were more frequent in the presbycusis group. Of the nine SNPs, two (A3434G, G11696A) represent non-synonymous mutations. Haplotype analysis revealed that one haplogroup (A3434G, G5913A, G10320A and G13928C) may be related to presbycusis.

Conclusion: Some SNPs and haplotypes may influence mitochondrial activity and, moreover, have the potential to be predisposing factors that can lead to presbycusis.

319 Recovery from Forward Masking in Elderly Cochlear Implant Users

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As individuals age, changes often occur in the auditory system that are likely to impact auditory perception in patients with cochlear implants (CI). Studies have shown that auditory perceptual deficits with aging are primarily due to impaired temporal processing, both in the peripheral and central systems. Temporal processing has been shown to be slower in older, non-CI individuals. Other studies have indicated that in normal hearing individuals, peripheral hearing mechanisms are relatively less impacted than central ones as we age. The purpose of this study was to investigate the effect of aging on temporal processing in elderly CI users in both the peripheral and central auditory systems, in order to evaluate their respective contributions. We hypothesize that elderly CI users (>60 years of age) will exhibit abnormal recovery characteristics relative to younger CI users (<60) and that these differences are a central, not peripheral, phenomenon. To assess peripheral auditory processing, we measured physiologic recordings of 8th nerve ECAPs with a single-pulse forward masker (SPFM). We also measured psychophysical thresholds using a pulse-train forward masker, indicative of central processing. Recovery functions for both measures were plotted and individual/group recovery constants were extrapolated from an exponential decay and growth-to-max fit, respectively. Between groups, we found equal rates of recovery in ECAP functions, significantly slower psychophysical recovery in elderly CI users ($T=-4.45$, $p<.001$), with a significant effect of age ($R=-0.821$, $p<.0005$). We also found a significant relationship between psychophysical recovery and speech perception scores (CNC Word, HINT Quiet, HINT +8 SNR) but not with physiologic recovery. Our findings showed differences in recovery characteristics between older and younger CI users, and that central changes may be the primary cause.

320 Aging Alters the Neural Representation of Simple and Complex Sounds: Evidence from Human FFR Recordings

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Older adults, even with normal hearing sensitivity, have difficulty understanding speech. This difficulty may be related to age-related declines in the neural representation of steady-state and dynamic acoustic stimuli. The purpose of this study was to examine the neural representation of simple and complex stimuli in the same aging adults using the frequency-following brainstem response (FFR). Thirty four adults (ages 22 – 75) with clinically normal hearing sensitivity (equal to or better than 25 dB HL at octave frequencies 0.25 – 8.0 kHz) participated in this experiment. FFRs were elicited by a 1-kHz tone as well as a synthetic /da/ stimulus. The /da/ stimulus elicited responses to transient acoustic features of the CV such as onset and offset responses, as well as steady-state components related to the vowel which elicit the FFR. With increased age, older adults showed statistically significant latency delays in response to onset, steady-state, and offset portions of the CV syllable. Onset and offset amplitudes were also reduced with advanced age. These age effects were not specific to a time-varying complex sound such as speech; age effects were also observed for the steady-state tone. As age increased, phase coherence, reflecting phase-locked neural activity to the stimulus frequency, also declined. These results suggest that the auditory system, even in the absence of significant hearing loss, does not encode simple and complex tones as accurately as we age. Moreover, the people who had difficulty processing the simple stimulus (tone) were the same individuals who had difficulty processing the complex stimulus (/da/). Regardless of age, there was a significant correlation between the amplitude of the speech-evoked FFR and phase coherence of the tone-evoked FFR. These results indicate a decreased neural representation of simple sinusoids and spectro-temporally complex stimuli as chronological age increases. Work supported by NIH DC007705 (KT); T32-DC00033(CC).

321 The Influence of Aging on Human Sound Localization

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Sound localization relies upon central processing of auditory spatial cues. Below 2 kHz inter-aural time differences (ITDs) are the most salient cues to depict horizontal auditory space. Although past studies suggest auditory temporal processing, including ITD perception, declines with age, specific impairment of ITD-dependent sound localization under free-field conditions has not been reported in the elderly. Our goal was to investigate the influence of age on ITD-dependent sound localization in the free-field, and to correlate the results to ITD thresholds under headphones.

To test free-field sound localization, narrowband targets (150 ms noise bursts at 5 Hz) varied across the ITD-relevant range (250-2205 Hz in ~250 Hz intervals) were presented by a robot-controlled speaker hidden behind a cylindrical screen. Young (19-37 yrs.) and elderly (71-86 yrs.) subjects, with similar hearing thresholds in the range of frequencies tested, used a 2-axis cylindrical joystick to point a laser LED at perceived target locations. For low frequency narrow-band targets, localization proved similar for both age groups. However, while localization error in young subjects increased only for the highest frequency bands (≥1500 Hz), the elderly experienced the same phenomenon starting at even lower frequencies (≥1250Hz).

The same set of stimuli was presented under headphones to quantify ITD thresholds in these frequency bands. A two-interval, three-alternative (Left, Right, Center) forced-choice paradigm was used. For each trial, subjects heard a 'reference' burst (ITD = 0 μs) followed by a second burst of varying ITD led by the left or right ear. Subjects chose a Left, Right, or Center key-press to match the lateral position of the second relative to the initial burst. ITD thresholds for the elderly proved greater than those for young subjects, and deteriorated more dramatically with increasing frequency, beginning at surprisingly low frequencies (≥750Hz). These novel results reflect a robust age- and frequency-dependent deterioration of central auditory spatial processing even in a frequency range where peripheral sensitivity is otherwise similar with age.

322 Human Evoked Cortical Activity to Silent Gaps in Noise: Relation to Gap Detection and Processing Speed

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Auditory temporal processing declines are hypothesized to contribute to speech recognition difficulties of older adults. Recent evidence suggests that differences in auditory temporal processing are explained, in part, by differences in processing speed. Age-related declines in processing speed contribute to a broad spectrum of performance deficits in the cognitive abilities of older adults, including working memory and attention. These observations suggest that both auditory and higher order cognitive factors contribute to auditory temporal processing. The objective of this study was to measure cortical evoked responses to silent gaps in continuous noise in younger and older adults with normal hearing to identify the cortical levels at which neuronal activity underlies age-related differences in auditory temporal processing. We also examined the extent to which individual variability in cortical evoked responses was related to behavioral measures of gap detection and processing speed. Gap detection thresholds were obtained using a maximum-likelihood adaptive psychophysical procedure. Processing speed was measured using the Purdue Pegboard and Connections tests. Cortical evoked potentials were elicited by brief silent periods in an otherwise continuous broadband noise. Older adults exhibited significantly prolonged latencies and reduced amplitudes compared to younger adults. Smaller response amplitudes were generally associated with higher gap detection thresholds and individual differences in processing speed significantly predicted response latencies [Work supported by NIH/NIDCD].

323 Age-Related Changes in Auditory Perceptual Learning

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In young adulthood, performance on perceptual tasks can be significantly improved with training. Of interest here was whether the capacity for such perceptual learning changes with aging, given recent evidence that related cognitive and perceptual abilities can be poorer in older adults. We reasoned that any difference in the effects of the same training regimen between younger and older adults who have the same starting performance would indicate that at least one of the processes underlying perceptual learning changes with age. Therefore, we trained older adults (age > 65 years) on a basic perceptual task (auditory temporal-interval discrimination) over multiple days using a regimen known to yield learning in young adults (age 18-35 years). The older adults had normal hearing, passed a screening measure for dementia, and were selected because their performance before training was within the young adult range. The training was effective for all of the young adults (n=8), but for none of the older adults (n=5). Between pre- and post-training tests, only the young adults improved more than age-matched controls who had received no training. These results suggest that the processes that contribute to the effectiveness of perceptual training differ between younger and older adults, and change even before naïve performance is affected. Interestingly, a different group of older adults, who started worse than young adults (n=3), did improve with the same regimen, but they, too, ended with performance in the range of that of young adults prior to training. This result demonstrates that the present training regimen can yield improvements in older adults and raises the possibility that their performance may be limited by a higher noise floor as compared to the young adults. Taken together, it appears that there are greater constraints on the effectiveness of

perceptual training in this task in older than in younger adults. [Work supported by NIH/NIDCD.]

324 The Shape of Monaural Temporal Window in School-Aged Children and Adults

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Previous studies have indicated that monaural temporal processing is often poorer in children than adults. The underlying mechanism is unclear, but one possibility is that children have broader monaural temporal windows than adults. In the present study, a masking period pattern paradigm was used to assess the shape of monaural temporal window in 14 children (5.0 to 10.6 yo) and 10 adults (18.9 to 49.4 yo). The masker was a bandpass Gaussian noise (500-2000 Hz) that was square-wave amplitude modulated at 5 Hz, varying in level from 25 to 45 dB/Hz. Detection thresholds were measured for a 10-ms 1-kHz tone at one of six temporal positions relative to masker modulation. Thresholds were also measured in two fixed-level conditions, with the masker presented at 25 or 45 dB/Hz. Our results showed that children's masked thresholds were greater than those of adult in all conditions. This group difference was larger during modulation minima than maxima, particularly in temporal proximity to an increase in masker level. This finding is consistent with previous demonstrations of increased backward masking in children. Results are discussed in terms of the shape of the monaural temporal window as a function of age.

325 Spectral and Temporal Masking Release in the Low-Frequency Range for Normal-Hearing and Hearing-Impaired Listeners

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"Masking release" (MR), the improvement of speech intelligibility in fluctuating compared with stationary noise, is typically reduced for hearing-impaired (HI) listeners. MR is thought to rely on the ability to "glimpse" speech into the spectral and temporal dips of noise. Previous studies indicate that spectral MR is essentially constrained by cochlear frequency selectivity. Other studies suggest that temporal MR may be constrained by purely temporal mechanisms based on neural phase locking. Recent work suggests that these temporal mechanisms may be degraded in the low-frequency range for HI listeners showing normal absolute thresholds, and presumably normal frequency selectivity in this region. The goal of this research was to determine whether or not deficits in temporal MR can be demonstrated in the absence of deficits in frequency selectivity in such HI listeners.
Spectral and temporal MR were assessed for lowpass-filtered (at 1.5 kHz) speech and noise in young NH listeners and young and elderly HI listeners with normal hearing below 1.5 kHz and hearing loss at higher frequencies. Consonant identification was measured in the presence of a speech-shaped noise at signal-to-noise ratios of -6, -3 and 0 dB. The noise masker was either: (1) unmodulated; (2) rectangular-wave amplitude modulated with duty cycles of 25 and 50%; (3) spectrally modulated, by passing the noise through an auditory filterbank, and setting to zero the outputs of 1 filter out of 2, 2 out of 4, or 3 out of 4.
Overall, for all HI listeners, performance and phonetic-feature reception were normal in unmodulated noise but poorer-than-normal in modulated noise. A variety of deficits in spectral and temporal MR was found for all HI listeners. No clear dissociation between temporal and spectral MR was therefore observed, but a significant correlation was found between spectral and temporal MR data for HI listeners, suggesting that frequency selectivity constrains both spectral and temporal MR.

326 Contribution of Non-Simultaneous Masking in Masking Period Patterns of Ramped and Damped Noises

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Masking period patterns (MPP) for amplitude modulated noises [exponentially ramped, exponentially damped, and sinusoidal (SAM)] under simultaneous- and forward-masked conditions were measured. In the simultaneous conditions, the masked detection threshold of a 5-ms, 1-kHz tone pip was measured at five signal delays throughout one period (25 ms) of the masker envelope. The non-simultaneous conditions differed from the simultaneous conditions in that the masker was gated off during the signal presentation so that the masker did not overlap with the signal. The masker was either broadband (0-10 kHz) or narrowband (0.7-1.3 kHz) and had a spectrum level of 30 dB SPL. MPPs obtained under simultaneous and non-simultaneous conditions were similar, indicating a dominant role of non-simultaneous masking. MPPs in the broadband case were highly modulated whereas the narrowband MPPs were effectively flat for all types of amplitude modulation. Thresholds in the MPPs for the damped noise were much lower than thresholds for ramped and SAM noises for the broadband masker but not for the narrowband maskers. These results implicate the importance of across-frequency processes in the shape of the MPP and also suggest that a damped modulation pattern causes a larger release from masking than ramped or SAM noise.

327 The Time Course of the Temporal Effect and Its Relationship to an Efferent Mechanism

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Research in our laboratory has shown that the temporal effect (TE) is consistent with a decrease in gain. This decrease may be mediated by the medial olivocochlear reflex (MOCR). In simultaneous masking, the TE is defined as an improvement in signal detectability at masker onset when a precursor is added. We developed a forward

masking technique to measure the TE via growth of masking (GOM). This technique compares precursors well below the signal frequency with precursors at the signal frequency. On-frequency precursors are shown to decrease gain of estimated input-output functions (also called the TE). We assume that off-frequency precursors do not elicit an MOCR effect at the signal place.
Previously we have described a technique that measures the TE by comparing on and off-frequency precursor conditions at a single masker level on the lower leg of the GOM function. With this technique we examined the effects of precursor duration and precursor offset to signal onset delay on the TE. For short precursor durations we found that the TE increased then decreased as a function of delay in some participants. This pattern is consistent with the MOCR onset delay.
One physiological study has examined MOCR time course with short elicitors [James et al (2005). Dynamics of real time DPOAE contralateral suppression in chinchillas and humans. Int J Audiol 44:118 –129]. Those data suggest that MOCR strength persists for a minimum duration regardless of elicitor duration. It was unclear if strength continued to build for this minimum duration. In the current study we mapped the time course of the TE for short precursors with more precision. We also examined the effect of precursor level on time course. Results were modeled and interpreted relative to physiological MOCR time course and level effect data. These results may help determine possible influences of the MOCR in psychoacoustic tasks.
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328 The Role of the Temporal Effect in the Measurement of Temporal Masking Curves

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Temporal masking curves (TMCs) have been used to estimate the cochlear input-output (I/O) function. A signal is fixed at a low level and masker threshold is determined as a function of signal delay, for on- and off-frequency maskers. The TMC technique assumes that the decay of forward masking is the same, regardless of masker frequency. A recent study by Wojtczak and Oxenham [(2009). "Pitfalls in behavioral estimates of basilar membrane compression in humans," J. Acoust. Soc. Am. 125, 270-281] reported that forward masking decayed faster for an on-frequency masker than for an off-frequency masker at high masker levels. They assumed the decay of forward masking depended on a static I/O function when interpreting the results. Research in our laboratory suggests that the I/O function changes in response to sound. This change may be due to the medial olivocochlear reflex (MOCR). Based on this assumption, two types of forward masking may exist: excitatory masking, which dominates for signal delays of < 20 ms, and gain reduction, which dominates for longer signal delays. In the present study, the role of gain reduction masking in TMCs was examined by measuring forward masking decay for 20-ms and 100-ms maskers. Based on MOCR sluggishness, the short masker should not activate the MOCR for short signal delays. The long masker should activate the MOCR for all signal delays. Growth of masking (GOM) functions were measured to determine the change in gain produced by the long masker. The decay of forward masking was more rapid for the 20-ms masker than the 100-ms masker for certain masker levels. The rate of decay of forward masking was related to the GOM functions. For the short masker, the signal was compressed, while for the long masker; gain decreased, resulting in a steeper GOM function around that same signal level. Implications for the measurement of TMCs will be discussed.
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329 Constraining the Derivation of Auditory Filter Shape with Temporal Masking Curves

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Auditory filter shape was originally estimated from simultaneous notched-noise masking (NNM) data, and Irino and Patterson [J. Acoust. Soc. Am., 109 (5), 2008-2022, (2001)] have shown that the compressive gammachirp (cGC) filter could explain the compression observed in these data, Then, Patterson et al. [J. Acoust. Soc. Am., 114 (3), 1529-1542, (2003)] showed that only six parameters were required to explain a large body of NNM data distributed across a wide range of center frequencies. Nevertheless, the filter shapes derived from the data of individuals showed considerable variation, of a form which suggested that NNM data, on its own, does not properly constrain the compression parameters of the cGC filter model. Accordingly, we measured input-output (I/O) functions as well as NNM thresholds at 1 kHz for six normal hearing listeners, to see if the inclusion of compression data would reduce inter-listener variability in auditory filter shapes. The I/O functions were derived from temporal masking curves (TMC) [Nelson et al. J. Acoust. Soc. Am., 110, 2045-2064, (2001)]. The fitting process was used to test a "commonality assumption"; namely, that the parameter values of the passive stage of the cGC, which corresponds to the cochlear traveling wave, are common to all normal hearing listeners. The data of the individual listeners were fitted with (a) NNM data only, and no commonality assumption; (b) NNM data only, with the commonality assumption; (c) NNM and TMC data, with no commonality assumption; (d) NNM and TMC data, with the commonality assumption. Fitting NNM and TMC data simultaneously enables us to determine the relative stability of filter parameters across listeners. The results show that listener variability is concentrated in the level-dependent parameters associated with the later stage of the filtering process, as expected.

330 Auditory Filter Bandwidths in Behaving Ferrets Estimated with Notched-Noise

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Ferrets have been increasingly used in auditory research both in behavioral as well as neurophysiological experiments. However their ability to resolve frequency components of sound, in terms of peripheral filtering, is not known. This motivated the measurement of auditory filter bandwidths in behaving ferrets with a method standard in human psychoacoustics, the notched-noise method. Ferrets were trained to discriminate two types of trials: 1) presentation of a tone in a continuous masking noise ("sound trial") and 2) the absence of the tone (noise only; "silence trial"). The noise was either flat or had an asymetric notch centred at the tone frequency. The proportion of correct responses to sound and silence trials was measured for different tone levels using the method of constant stimuli. Thresholds were derived from the logistic fit to P(C)max, a criterion-free measure of performance, corresponding to the level at which P(C)max reached 71%. The shape and bandwidth of the auditory filter was then determined by fitting a roex(p,r) function to the thresholds estimated at increasing notch bandwidths. Preliminary results at 1 and 10-kHz show filter bandwidths about 30% of the notch centre frequency, suggesting that ferrets have poorer frequency resolution than humans.

331 Detection of Tones in Reproducible Noises: Combining Information Across Epochs and Across Cues

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Difficulty understanding speech in noise is a significant clinical problem. Despite decades of study, it is still not clear how listeners detect even pure tones in noise. This study focused on mechanisms for diotic and dichotic detection of a 500-Hz tone in wideband or narrowband reproducible noises. Previous analyses have focused on energy or temporal fine-structure cues over whole waveforms, yet listeners suggest that decisions are often based on short epochs. In this study, waveforms were separated into epochs to obtain temporally "local" cues, and decision variables (DVs) were computed by combining cues across epochs. For the dichotic case, DVs were the means of the standard deviations of interaural time difference (S_{ITD}) and interaural level difference (S_{ILD}) across epochs. For the diotic case, DVs were weighted sums across epochs, with weights based on reliability, evaluated from the distributions of cues for large sets of random noise-alone and tone-plus-noise waveforms using a likelihood ratio test. For most cues, these DVs yielded better correlations with the subjects' results than DVs computed for whole waveforms. Different epoch lengths were optimal for different cues. Epoch-based analysis also enabled an effective strategy for combining cues. Previous studies that fitted combined S_{ITD} and S_{ILD} cues to the data essentially selected the better of the two. In this study, cue weights for the dichotic case were derived based on the variability of S_{ITD} or S_{ILD} information across time. With this method, correlation results improved compared to those for a single cue. For the diotic case, information provided by a single cue was combined across epochs, and then each cue was weighted based on its reliability. For most subjects, the resulting predictions were superior to those based on any single cue, and predictions depended on multiple cues, similar to actual performance, as opposed to being dominated by a single best cue. [Supported by NIDCD-DC001641]

332 Backward Masking with Reproducible Noise Samples as a Function of Stimulus-Masker Interval

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We utilized a molecular psychophysical approach to evaluate the effect of stimulus-masker interval on performance in a backward masking task using reproducible noise samples. The task was to detect a 10-ms, 500-Hz sinusoid with 5-ms onset and offset ramps presented either 300ms, 100ms, 50ms, 25ms or 10ms before one of 25 reproducible broadband noise samples. The noise samples were presented alone or preceded by the signal. Data were collected at a fixed signal level to maintain a P(C)=70% for each time interval tested.

As expected, there was substantial influence of the individual noise samples on the detection of the signal as well as the signal level at short stimulus-masker intervals (<50ms). The hit and false alarm rates for the different noise samples were quite varied. As the interval between the stimulus and the masker increased, the experiment approached the expected behavior of a molar psychophysical task. The signal level approached the level of the signal when it was presented in isolation, and the variability of hit and false-alarm rates of the individual reproducible noise samples at long intervals decreased. In other words, the acoustic parameters of the individual noise samples do not significantly impact the detection rate of the signal when the signal is presented at long stimulus-masker intervals. However, unlike a molar task, the correlations between responses to the individual noise samples at different time intervals were higher than expected compared to a computer-generated random simulation.

Variations of a single-channel electrical analog model (EAM) [Jeffress, 1967, 1968; Gilkey & Robinson, 1986] were used to explain the variance in the subject responses at the varied stimulus-masker intervals. Implications of these findings will be discussed.

333 Discrimination of Frequency Ratios

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Our ability to distinguish among vowels and many natural sounds is widely believed to involve the perception of frequency ratios among partials. Few data, however, have measured the limits of our ability to discriminate frequency ratios [cf. Fantini and Viemeister (1987). In: Auditory Processing of Complex Sounds. (Hillsdale, N.J.: Lawrence)]. In an adaptive, two-interval, forced-choice

procedure with frequency rove, 11 highly-practiced listeners discriminated a change in the frequency ratio (R) of two equal-intensity tones. Threshold values of log(ΔR/R) corresponding to 71% correct performance were obtained for two center frequencies of the rove (559 and 4472 Hz), at two sound levels (67 and 90 dB SPL) and at standard frequency ratios of R = 1.25, 1.78, 2.00, 2.45 and 3.00. Thresholds varied widely across listeners (log(ΔR/R) = -1.0 to -2.5) with the lowest thresholds approaching those for standard frequency discrimination of a single fixed-frequency tone. Also, listener thresholds were found to be considerably lower for frequency ratios corresponding to the standard musical intervals, R=1.25 and 2.00. The data provide weak support for the notion that there are specific ratios for which listeners are most sensitive. [Work supported by NIDCD].

334 Do "F0 Difference Limens" Measure Residue-Pitch Discrimination?

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A commonly used approach in studies of pitch perception involves measuring difference limens for complex tones (DLCs) that differ in fundamental frequency (F0). It is assumed that these thresholds effectively reflect the discriminability of F0, or subjectively, residue pitch. However, when the complexes being compared contain corresponding resolved harmonics, discrimination may be based on other cues, such as differences in the frequencies (or pitches) of individual components, or differences in timbre (brightness). In view of the important role of DLC measurements in pitch-perception research, the evidence that DLCs effectively reflect residue-pitch discrimination is surprisingly weak. Here, DLCs were measured for harmonic and inharmonic tones in the same listeners under various conditions, including randomized or fixed lowest-harmonic number, trial-wise feedback or lack thereof, and background noise to mask distortion products. The inharmonic tones were produced by shifting the frequencies all harmonics upwards by a constant amount in Hz. Since such tones have a more ambiguous residue pitch than harmonic tones, if DLCs reflect residue-pitch discrimination, DLCs should be larger for inharmonic tones than for harmonic tones. In contrast, if DLCs reflect comparisons of component pitches, or timbre, they should not be systematically influenced by frequency shifting. The results of four experiments concurred to show larger DLCs for inharmonic tones than for harmonic tones, supporting the view that DLCs reflect residue-pitch discrimination. The results of a final experiment showed that the standard deviations of pitch matches were larger for frequency-shifted than for harmonic complexes, consistent with the effect of frequency-shifting on DLCs. [Work supported by NIH RO1 05216]

335 Insensitivity to Pitch-Change Direction with Fixed- And Roved-Frequency Tones

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Semal and Demany (J. Acoust. Soc. Am., 2006) demonstrated that some normally hearing listeners cannot correctly perceive the direction of small (but detectable) pitch differences between pure tones. However, the stimuli used in that study included frequency roving over a wide range (2000 Hz or 2.58 octaves), and it is unclear if this roving contributed to difficulties judging pitch-change direction. This study sought to clarify whether deficits in pitch-direction identification depend critically on wide roving, or if they can be observed under conditions with narrower or no roving. Difference limens for frequency (DLFs) were measured in "experts," with prior experience of psychophysical tasks involving pitch, and in "novices," selected using a pre-test that indicated they had a difficulty identifying pitch-change direction. DLFs were measured in a task that required identifying the direction of a pitch change (IDLFs), and in one that simply required detection of a change (DDLFs). The stimuli were pure tones presented in conditions where in a run of trials the standard frequency was either fixed or was roved (to different extents in different conditions) within and across trials. Wider roving increased DLFs monotonically. For experts, roving had a similar effect on DLFs in both tasks, and IDLFs were generally smaller than DDLFs. For novices, there was an interaction between task and roving range, such that IDLFs were elevated relative to DDLFs for stimuli roved widely, but less or not at all when the stimuli were roved narrowly or fixed in frequency. A trial-by-trial analysis of errors in conditions with the widest roving range suggested that poor pitch-direction identification was related to greater susceptibility to interference from irrelevant within-trial frequency changes. Implications of these results for psychophysical models of pitch perception are discussed. [SRM is supported by the UK BBSRC, CM is supported by NIH RO1 DC].

336 The Low Pitch of High-Frequency Complex Tones Relies on Temporal Fine Structure Information

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High-frequency complex tones containing only unresolved harmonic components with a frequency spacing Δf usually evoke a low pitch equal to Δf. However, for inharmonic components, the low pitch is often found to deviate slightly from Δf. Whether this pitch shift relies exclusively on temporal fine structure (TFS) cues has been a matter of debate. It is also controversial up to which frequency TFS information remains available, and to what extent envelope cues become dominant as frequency increases.

Using a pitch-matching paradigm, this study investigated whether the pitch of transposed tones with unresolved inharmonic components is determined by (A) the time intervals between the most prominent TFS peaks in their waveform (multimodal distribution of matches around subharmonics of the carrier frequency f_c), (B) the timing

between peaks in their envelope (unimodal distribution of matches around the envelope rate f_{env}), or whether (C) no salient pitch is evoked (random matches). Six musically-trained normal-hearing subjects matched the fundamental pitch of a broadband pulse train to that of transposed tones with carrier frequencies f_c = [3, 4, 5, 6, 7] kHz and envelope rates f_{env} = [f_c/11.5, f_c/14.5]. All stimuli were presented at 50 dB SPL in broadband pink-noise (13.5 dB/Hz at 1 kHz), and 40 matches per condition were obtained.

For f_{env} = f_c/11.5, the results favored hypothesis A for all values of f_c, indicating that TFS cues are available and used for pitch extraction, up to at least 7 kHz in most subjects. For f_{env} = f_c/14.5, hypothesis A was valid for values of f_c up to 5 kHz, and the distribution of matches showed a higher variance indicating a less salient pitch. In other conditions, hypothesis C was valid, suggesting that envelope cues do not take over as TFS cues become unavailable. These results strongly suggest that the monaural representation of TFS persists at high frequencies and that pitch does not rely on envelope coding as such.

337 Relative Effects of Increment and Pedestal Duration on the Detection of Intensity Increments

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The detection of a brief increment in the intensity of a longer duration pedestal tone is commonly used as a measure of intensity resolution. Increment detection is thought to improve with increasing duration of the increment and also with increasing duration of the pedestal, but the relative effects and interaction of these two parameters have not been explored in the same study to our knowledge. In past studies of effects of increment duration, pedestal duration was increased as increment duration increased. In the present study, increment and pedestal duration were manipulated independently. Increment-detection thresholds were determined for four subjects with normal hearing using a 500-Hz pedestal presented at 60 dB SPL. Increment durations were 10, 20, 40, 80, 160 and 320 ms. Pedestal durations were 20, 40, 80, 160 and 320 ms. Each increment duration was combined with all pedestal durations equal to or greater than the increment duration. Multiple regression analyses indicated that increment detection is determined by pedestal duration, with increment duration having little or no independent effect. This suggests that all reported effects of increment duration have been confounded by the practice of holding duration of non-incremented segments of the pedestal constant as increment duration was increased. Supported by R01 DC006648 and T32 DC000013.

338 An Order Effect in Monaural-To-Binaural Loudness Judgments

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It is well established that a sound presented binaurally is perceived as louder than when the same sound is presented monaurally, an effect that is termed binaural loudness summation. Considerable variation between studies exist as to the exact amount of summation, however. Reports have ranged from 4 dB to well over 10 dB increases in monaural level required to match equivalent binaural input for loudness.

This experiment demonstrates that the amount of binaural summation depends on the presentation order of monaural and binaural stimuli. Ten normally-hearing listeners participated in the experiment, where loudness matches were obtained via an adaptive, two-interval, two-alternative, forced-choice procedure. Pink noise stimuli of 1 s in duration were convolved with binaural room impulse responses, and played back over headphones to synthesize a sound source in front of the listener in different room acoustic conditions. On each trial of the experiment proper, the listeners were presented with a sound from the synthesized source monaurally and binaurally, and asked to judge which of the two presentations was louder. The presentation order was fixed within a block of trials, and the level of the monaural sound was adapted based on the listeners' responses.

When the monaural stimulus was presented first in a trial, the amount of binaural summation was 10 dB on average, while it was 7 dB when the binaural stimulus was first. Synthesized room acoustics did not have a marked effect on the results. In a follow-up experiment without binaural synthesis, and adapting both the monaural and the binaural stimulus, the order effect was of similar magnitude for a 1-kHz tone, but diminished for pink noise. The relation of the finding with sequential effects in loudness, as well as the role of interaural inhibition on monaural-to-binaural matches will be discussed. [Work supported by NIH DC008168, the Academy of Finland and Emil Aaltonen's Foundation]

339 An Efferent Hypothesis May Explain Why Long Duration Vowels Enhance Spectral Contrast in Vowel Masking Patterns

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The medial olivocochlear reflex (MOCR) may facilitate listening to speech in a noisy environment by reducing cochlear gain. When evoked by sound, this reflex exhibits a short onset latency (~20 ms) before gain begins to decrease. Our previous research describes a forward masking technique used to compare gain estimates for signals occurring during and after the MOCR's onset latency period. Using sinusoidal stimuli, we found that gain estimates were highest when the signal occurred during the MOCR's latency period. Conversely, gain estimates were reduced when the signal occurred at a time when MOCR strength was expected to be high.

The present study is our first attempt at investigating the dynamics of cochlear gain using a speech-related stimulus. We measured forward masking thresholds for several harmonics of a synthetic vowel to create vowel masking patterns (VMPs). To assess the possible contribution of the MOCR, we measured VMPs for two vowel durations and intensities. We selected the duration of the short vowel so that the signal occurred during the MOCR's onset latency period. Similarly, the long vowel's duration was such that the signal occurred when MOCR strength was expected to be high. Using a technique described by Schairer et al. [(2003). "Effects of peripheral nonlinearity on psychometric functions for forward-masked tones," J. Acoust. Soc. Am. 113, 1560-1573], we inferred cochlear compression at each harmonic frequency by comparing masking thresholds obtained with different tracking rules.

We hypothesized that spectral contrast would be enhanced in the VMPs from long-duration vowels. This hypothesis is based on the assumption that the MOCR affects vowel troughs more than vowel peaks at high vowel levels. Our preliminary data support this hypothesis. This enhancement in spectral contrast suggests the MOCR may aid in vowel identification, especially at high vowel levels.

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340 Amplitude-Modulation Detection Over Sonic and Ultrasonic

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Ultrasonic vibration generates a sensation of sound via bone-conduction. This phenomenon is called bone-conducted ultrasonic (BCU) hearing. Complex sounds also can be perceived by an amplitude-modulated BCU (AM-BCU). In order to clarify characteristics of perception of AM-BCUs in relation to that for AM sound in sonic or audio-frequency range, the influence of modulation frequency on the sensitivity for detecting amplitude modulation of sinusoidal carriers from 10, 20 and 30 kHz was examined. In addition, the detection sensitivity for lower or upper single sidebands for a 20-kHz "carrier" in relation to the detection for amplitude modulation was studied.

Measured temporal modulation transfer functions (TMTFs) obtained at each carrier frequency commonly had a flat portion at modulation frequencies from 10-100 Hz and an initially increasing potion at about 100-150 Hz. These low-pass characteristics in modulation frequency domain over the sonic range and the ultrasonic range are in line with common characteristics in temporal processing of AM air-born sound over the audio-frequency range. Results of single sideband detection curves with a 20-kHz carrier also showed low-pass characteristics, and the initial flat portion ran parallel to the TMTF with 20-kHz carrier with about 6 dB higher thresholds. These results support the idea of commonality in temporal processing of amplitude envelopes in audio-frequency range and ultrasonic range. The TMTF with a 30-kHz carrier did not show a decreasing portion at high modulation frequencies up to 6400 Hz, and the highest detection thresholds. These results seem to reflect that sideband components for a 30-kHz carrier could not be detected even if the carrier and sideband components are presented at almost same levels. These results can be reasonably interpreted on the bases of an assumption of "the end of the auditory channels", though it remains highly controversial.

341 Psychophysical Reverse Correlation with Multiple Stimulus-Response Alternatives

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Psychophysical reverse-correlation techniques have been used to uncover the decision strategies of participants in various auditory perception tasks, including tone-in-noise detection, profile analysis, sound-source discrimination, and speech identification. In the "correlational" technique, random perturbations are added to different stimulus components (e.g., frequency bands) on each stimulus presentation, and the relative importance (or "weight") of each component is estimated as the point-biserial correlation coefficient between the perturbations and the binary-coded responses (e.g., no = 0, yes = 1). Although this technique was originally designed with two-alternative experiments in mind, it has been extended to experiments involving more than two stimulus categories (e.g., several speech tokens). Such applications of the correlational technique are of particular interest, as they are more relevant to real-life listening experience than two-alternative experiments. However, their theoretical basis has so far remained largely elusive. Here we examined a theoretical framework under which the correlational approach can help uncover perceptual templates in tasks (recognition or identification) involving multiple stimulus-response alternatives. We used computer simulations to evaluate a variety of parameters and conditions, including different internal–to-external noise ratios, different degrees of correlation among the sensory observations, different statistical distributions of stimulus perturbations, and different ways of sorting and combining trials based on either stimulus or response types, to estimate the underlying perceptual templates. The results of this theoretical exploration clarify the conditions and assumptions under which the correlational technique can, and cannot, yield correct estimates of listeners' decision strategies in multiple-alternative tasks. [Work supported by University of Arizona and NIH DC05216]

342 Effects of Long-Term Deafness and Delayed Chronic Intracochlear Electrical Stimulation on the Primary Auditory Cortex

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Behaviorally relevant chronic intra-cochlear electrical stimulation (ES), when delivered from a young age, is

known to alter spectral (spatial) and temporal processing in the auditory system [1,2]. Whether these effects are limited to stimulation that is initiated during the early critical periods, or also occurs when stimulation is commenced after long-term deafness, is less clear. We neonatally deafened five cats via daily neomycin injections, and at two months of age implanted a multi-channel scala tympani electrode array. A modified clinical cochlear implant was used to deliver environmentally derived chronic ES from eight to fourteen months of age. We recorded from single- and multi-unit clusters (n = 300) in the primary auditory cortex (AI) using a combination of single tungsten and multi-channel silicon electrode arrays. We assessed spectral processing in AI by measuring the cochlea-to-cortex mapping, and the spread of cortical activation. We assessed temporal resolution in AI by measuring the jitter in response latency and the maximum rate at which clusters could be driven. Similar to chronic ES initiated early in life [1], delayed ES had little effect on the basic response properties of AI neurons, but did reverse the disruption of the cochlea-to-cortex mapping and reduction in maximum driven rate (Mann-Whitney; $p < 0.05$) seen with long-term deafness. The late initiation of ES did not, however, reverse the increase in spread of activation or increase in response latency jitter seen with long-term deafness. We hypothesize that the inability of electrical activation of the cochlea after the closure of the normal critical period to reverse the increased spread of activation and response latency jitter contributes to the poorer performance observed among congenitally deaf human patients implanted later in life.

343 Development of a Novel Rodent Model for Examining Central Auditory Plasticity with Cochlear Implant Use

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The plasticity of the auditory system is undoubtedly a significant contributor to the success of the cochlear implant. Work in our laboratory has been directed towards examining this issue in appropriate deaf animal models[1]. This paper describes the development of a novel rat model of cochlear implantation, which permits the delivery of behaviorally-relevant intra-cochlear electrical stimulation (ICES), together with ongoing behavioral and electrophysiological measurement of auditory acuity.

Our fully-implantable rodent stimulator is capable of delivering two channels of ICES. It is powered by an omni-directional inductive link. Stimulation is controlled over a 2.4 GHz radio connection, allowing dynamic adjustment in response to environmental cues. The implant is capable of generating up to 700 pps per channel, including amplitude modulation, at frequencies between 5 and 175 Hz. *In vitro* testing of the implant encapsulation has demonstrated viability for >6 months. We will present *in vivo* results of chronic stimulation, behavioral testing (using a conditioned avoidance paradigm), and electrophysiological recording in the rat. Our model allows us to examine the changes in temporal processing that occur following chronic ICES. Of particular interest is whether the behavioral relevance of the ICES, or exposure to an extended range of modulation frequencies during ICES, results in improved temporal processing.

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344 Perception of Pulse Trains in the Electrically Stimulated Cochlea: Effects of Preserving Acoustic Hearing

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Preserving acoustic hearing in ears with cochlear implants could have significant impact on perception of the electrical signals. In a previous study we found large differences in the slopes and levels of psychophysical electrical threshold vs pulse rate functions across guinea pigs that differed in the degree of cochlear pathology. In this study, we further explored these differences (1) using a greater range of pulse rates (5 pps to 5k pps); (2) using pairs of pulses with a range of interpulse intervals (IPIs: 0.2 to 200 ms); and (3) comparing psychophysical and cortical-extracellular-spike thresholds in the same animals. Guinea pigs were assigned to one of two treatment groups: (H) implantation in a normal-hearing ear and (D) implantation after neomycin deafening. Psychophysical and cortical-neural functions fell into two categories (A) functions in which thresholds decreased with increasing pulse rate over the full range of tested rates; and (B) cases in which thresholds showed little or no change as a function of pulse rate below 1k pps but decreased with increasing pulse rate above 1k pps. Functions for Group H animals were split between Categories A and B while functions for all of the Group D animals were in Category B. When tested with pulse pairs, none of the animals showed appreciable effects of pulse separation at IPIs > 1 ms but thresholds decreased with decreasing IPIs at IPIs < 1 ms. These results suggest that slopes and levels of the pulse-rate functions were primarily the result of multipulse integration over the duration of the pulse train with an additional contribution of interactions between adjacent pulses at high pulse rates. The multipulse integration seems to be dependent on the condition of the implanted cochlea as assessed by residual hearing, ensemble auditory nerve spontaneous activity, and counts of hair cells and auditory neurons, but the two-pulse interaction does not.

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345 Cochlear-Implant Modulation Detection Interference in the Auditory Cortex

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We have shown previously in an animal model that a sub-threshold pulse train presented through one cochlear-

implant electrode can influence the threshold of an interleaved pulse train presented through a second electrode. Such between-channel interference is sensitive to the carrier pulse rate and to details of inter-pulse timing. Here, we test the hypothesis that a supra-threshold pulse train through one electrode can mask detection of modulation of an interleaved pulse train on a second cochlear implant electrode. We recorded from cortical area A1 in anesthetized guinea pigs. Electrical pulse trains were presented through banded intra-scalar cochlear implants. Carrier pulse rates were 254, 1017, or 4069 pulses per second (pps). The pulse train on the most apical, "signal", electrode was modulated at 11 or 21 Hz at depths ranging from -40 dB (i.e., 1%) to -5 dB (56%). An unmodulated, "masking", pulse train was presented at varying levels on an electrode located 1.5 mm more basal. Temporal offsets between the two pulse trains were 82, 123, 491, or 1966 μs; the longest offsets were possible only at the lower carrier pulse rates. A signal detection procedure was used to detect cortical activity phase locked to the modulating waveform. The modulation threshold for each unit and each condition was the shallowest stimulus modulation depth at which phase-locked cortical activity could be detected.

Consistent with the hypothesis, cortical recordings exhibited modulation detection interference in the sense that increases in the current level of the unmodulated pulse train resulted in elevations in modulation thresholds, usually resulting in complete masking of modulation detection when the two competing pulse trains were approximately equal in current level. Contrary to our expectations, carrier rate and temporal offset parameters had little or no consistent effect on modulation detection interference.

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346 Central Component of Forward Masking Observed in Cortical Responses to Cochlear Implant Stimulation

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Study of forward masking in electric hearing allows direct measurement of temporal processing in the auditory system, independent of the nonlinearities introduced by the basilar membrane in acoustic stimulation. Previous studies have provided evidence for components of forward masking at the level of the auditory nerve, as well as more centrally in the nervous system. We measured the recovery from forward masking in the primary auditory cortex of anesthetized guinea pigs using a 200 ms electric pulse train masker at several electric pulse rates (250, 1000, and 4000 pps). Unexpectedly, the time course of recovery was shorter at higher pulse rates.

We fit a sum of two exponential decays to recovery-from-masking data for all pulse rates and current levels. The time constants obtained for these components did not differ across cortical units or stimulus conditions within the same animal. The rapid component had a time constant <10 ms, similar to auditory nerve adaptation. The longer component had a time constant between 100-500 ms, consistent with a more central mechanism. These time constants were similar to those observed in human cochlear implant listeners.

The magnitude of the slow adaptation was significantly greater for lower pulse rate, indicating stronger central masking in these conditions. It declined at higher pulse rates, presumably due to the rapid adaptation of the auditory nerve to high pulse rates that has been reported in physiological studies. In effect, higher areas of the auditory system receive less input at the end of a long, high-rate pulse train, leading to reduced masking.

The reduction in the magnitude of the slow component of central masking could account for the observed shorter recovery from masking by fast pulse rates.

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347 Effect of Short-Term Adaptation on Discriminability of Neural Responses to Psychophysical Stimuli

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Using a computational model of auditory nerve fibers, our laboratory has developed a procedure to estimate discriminability of stimuli based on the similarity of evoked neural responses. Such a procedure can potentially be used as an engineering tool to rapidly evaluate the fidelity of novel sound-encoding strategies prior to the commitment to a subject trial.

At the timescales typical to psychophysical stimuli (≈500 ms), short-term adaptation – a gradual decrease in the neural response rate – visibly affects the output of the auditory nerve. It is largely unknown, however, how short-term adaptation affects the perception of similar-sounding, psychophysical stimuli. In the following, we demonstrate the effect of adaptation on discriminability estimates of two stimuli: positive- and negative- Schroeder-phase (SP) harmonic complexes. We have chosen to use SP as our psychophysical stimuli because earlier studies have shown that discrimination of single-electrode SP complexes reflects the cochlear implant listener's sensitivity to temporal cues, which is an important component in recognition of tonal languages, melody perception, and sound source segregation.

To gauge the effect of adaptation, we compared neural output of CIS- and analog- encoded Schroeder-phase stimuli with short-term adaptation turned on and off within our model. Subsequently, we measured the similarity of the neural responses, thus estimating a discrimination performance of Schroeder-phase stimuli. In our presentation, we describe our estimation procedure and demonstrate how short-term adaptation affects temporal sensitivity of a modeled AN fiber population.

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348 Bilateral Effects of Unilateral Intra-Cochlear Electrical Stimulation on the Central Auditory Pathway

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Little is known about binaural interactions induced by simultaneous acoustic and electric stimulation of the auditory system in patients with a unilateral deafness. The present study therefore aims to investigate, at the cellular level, the microstructural and neurophysiological consequences of unilateral chronic intracochlear electrical stimulation within the ascending auditory pathway.

Normally hearing guinea pigs were single-sided deafened by the implantation of a standard HiRes 90kâ cochlear implant with a HiFocus1j electrode array. The first 4 or 5 electrode contacts could be used to stimulate the cochlear nerve fibers within the first turn of the cochlea. Four weeks after surgery, the speech processor (Auriaâ) was mounted on the back of the animals and programmed, based on the tNRI-values. The animals of the experimental group were stimulated for 3 months, both experimental and control groups experiencing a similar daily acoustic environment (16 hours).

Animals of the experimental group (stimulated) showed a significantly lower average spontaneous activity on the implanted as well as the non-implanted side of the cochlear nucleus (CN) and auditory cortex (AC) than the controls (implanted but not stimulated). In the inferior colliculus (IC) and medial geniculate body (MGB) only that side was affected which receives its direct afferent input from the non-implanted side.

The neuronal cell density of the CN was significantly higher on the stimulated side compared to the corresponding side of the implanted but not stimulated controls. However, the opposite holds true for the contralateral side of stimulation.

In the MGB, IC as well as in the AC, conservation of the neuronal structure was observed bilaterally upon electrical stimulation. The bilateral changes at the cellular level were accompanied by a slight hearing loss on the non-implanted side. The present findings indicate a neuronal plasticity to balance the input of simultaneous electrical and acoustical stimulation.

The results of the present study might be of particular importance when considering cochlear implantation for patients with single-sided deafness. They may also improve our understanding of the highly variable clinical course of auditory rehabilitation observed frequently after sequential, bilateral cochlear implantation.

349 Behaviorally Relevant Auditory Experience Improves Temporal Processing in Primary Auditory Cortex (AI) But Not in Inferior Colliculus (ICC) in Deaf Cats

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Our recent work (CIAP, 2009) indicates that behaviorally relevant intracochlear electric stimulation (ICES) significantly improves temporal processing in primary auditory cortex (AI) in neonatally deafened juvenile and long-deaf (>2.5 years) adult cats. We wondered if similar results might be found in the central nucleus of the inferior colliculus (ICC) in the same behaviorally trained animals.

Kittens were neonatally deafened by injection of ototoxic drugs. In three juvenile groups and in two groups of long-deaf cats, a feline prosthesis was implanted in the left scala tympani, and a regimen of continuous, passive ICES was initiated (~4 h/day, 5 day/wk). Two juvenile and one long-deaf group received additional behavioral training. A third long-deaf group and an acutely deafened adult control group received no ICES. For the passive stimulation and behavioral components of the study, stimuli were biphasic unmodulated (30 pps) or sinusoidally modulated (300 pps, 30 Hz, 100 % modulation depth) 0.2 ms/phase current pulses. Responses of single neurons in the right ICC to unmodulated pulse trains (~10 to 300 pps) were recorded with metal microelectrodes in anesthetized animals. The principal response parameters of interest were: Best repetition rate (BRR), the stimulus rate that produced the maximum number of phase-locked spikes; the cutoff rate at which the number of phase-locked spikes was just less than 50% of the number at BRR; and minimum neuronal response latencies.

Our preliminary results show: 1) Behaviorally relevant ICES did not affect temporal processing in the ICC of juvenile or long-deaf cats; 2) however, compared to long-deaf unstimulated cats, temporal processing was enhanced in long-deaf cats that received passive ICES. Our findings indicate that passive ICES can modulate temporal processing in the ICC, but in contrast to results obtained in AI, behaviorally relevant ICES has no effect on temporal processing in the ICC.

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350 Role of the Dorsal Cochlear Nucleus in Electrical Hearing

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Auditory brainstem implants (ABIs) restore hearing through electrical stimulation of the cochlear nucleus (CN) for patients who do not benefit from cochlear implants. To enhance ABIs, there is a need to better understand how each part of the CN contributes to electrical stimulation-

induced hearing. First, by using electrical pre-pulse inhibition (ePPI), a novel startle reflex behavior paradigm, we tested if electrical stimulation of the rat dorsal cochlear nucleus (DCN) induces hearing. In the ePPI experiment, electrical currents were delivered to the DCN as a substitute for acoustic pre-pulses used for hearing detection. Our results showed that electrical stimulation at certain levels induced suppression of startle responses that are similar to those induced by acoustic stimulation. This indicates that electrical stimulation of the DCN induced a hearing-like behavioral manifestation. Second, to understand the physiology of the induced hearing, we investigated whether electrical stimulation relays the tonotopic organization of the DCN to the inferior colliculus (IC) and produces frequency-specific activation. Frequency tuning curves in both the DCN and IC were obtained to determine the characteristic frequencies (CFs) of their stimulation and recording loci. Electrical spatial tuning curves (eSTCs) to randomized and non-randomized electrical stimulation were constructed to determine the best channels in the IC based on its lowest thresholds to DCN stimulation. Our results demonstrated a strong correlation between the CFs of the stimulation loci in the DCN and those of the best channels in the IC, and sharper eSTCs were produced by using randomized stimulation. In summary, our results demonstrate that electrical stimulation of the DCN induces hearing, which may involve relaying tonotopic organization from the DCN to the IC. Our findings also suggest that stimulation of the DCN should be well considered in the design of new generation of ABIs.

351 Amplitude Modulation Increases Tonotopic Spread of Inferior Colliculus Activation and Cochlear Implant Channel Interaction

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Psychophysical studies have shown that an amplitude-modulated masker is more effective than an unmodulated masker at inhibiting detection of modulation on a simultaneous probe. This phenomenon is known as modulation detection interference. To investigate a neural correlate of this phenomenon, we measured the distribution of activity along the tonotopic axis in the central nucleus of the inferior colliculus (ICC) evoked by acoustical stimulation, and also by intracochlear electrical stimulation. We report here our results using acoustic tone pairs and also using 1000-pps electrical pulse trains interleaved on cochlear implant channel pairs.

For an unmodulated stimulus (pure-tone or single-channel electrical pulse train), the distribution of ICC sustained responses was narrow. These responses were not greatly affected by the presence of a second distant stimulus. (For acoustic tones distance refers to frequency difference, and for electrical pulse trains distance refers to intracochlear electrode separation). As the distance between the stimuli decreased, interaction between them increased, as expected.

For an amplitude-modulated stimulus (tone or single-channel electrical pulse train), the distribution of ICC responses was broader than for the corresponding unmodulated stimulus. Interaction between two stimuli at a given distance was also greater for amplitude-modulated stimuli than for unmodulated stimuli. Interestingly, this was true even though the mean levels of the amplitude-modulated stimuli were always less than those of the unmodulated stimuli—consistent with the psychophysical phenomenon of modulation detection interference.

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352 Temporal Properties of Responses Measured in the Central Nucleus of the Inferior Colliculus (ICC) of Cats Chronically Stimulated with a High Pulse Rate

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Temporal Properties of Responses Measured in the Central Nucleus of the Inferior Colliculus (ICC) of Cats Chronically Stimulated with a High Pulse Rate.

It has been previously reported that neonatal deafening significantly decreases the temporal resolution of neurons in the ICC of cats (Vollmer et al., 1999, 2005). In those studies, chronic electrical stimulation improved ICC temporal resolution in animals that had been deafened at birth and studied in adulthood, as compared with similarly-deafened but unstimulated control animals. This effect was dependent on the frequency of the pulse train used for chronic stimulation – higher frequency (300 pulses per second – pps) pulse trains resulted in higher maximum frequency following and shorter response latencies in the ICC, whereas stimulation at 30 pps did not.

In the present study, we examined temporal properties of responses measured in the ICC of neonatally deafened cats that were chronically stimulated using a speech processor with a pulse rate of about 1000 pps. Six animals were deafened as neonates and implanted at the age of 4 weeks. Five of these were chronically stimulated for a period of several months. Four animals also received intracochlear infusion of the brain-derived neurotrophic factor (BDNF). Extracellular responses to unmodulated pulse trains (40 μs/phase) at two levels, 2dB and 6dB above minimum threshold, were recorded in the ICC using a 32-channel silicon recording probe. Responses were evaluated using vector strength analysis for pulse trains at rates from 20 to 400 pps.

In cats chronically stimulated with the higher 1000pps pulse rate, responses in the ICC were better able to follow higher frequency pulse trains, as compared to cats stimulated using a lower pulse rate or unstimulated control animals. The vector strength of the frequency following responses was correlated with stimulus level, such that stronger frequency following was seen at 6 dB above threshold than at 2 dB above threshold. Further, no

significant increase in average response rates was observed with higher stimulus pulse rates when responses were summed over all responsive ICC recording sites.

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353 Evaluation of Integration Effects on ECAP-Based Measures of Channel Interaction

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The electrically evoked compound action potential (ECAP) has been shown to be a useful method for measuring of channel interaction in multi-channel cochlear implant users (Abbas et al, 2004). Typically, a masker-probe paradigm is used in which the masker pulse is delivered to one electrode and the following probe pulse is delivered to another electrode. The spread of channel interaction is evaluated as changes to the probe-evoked ECAP as masker parameters (level, electrode) are varied. With a masker-probe interval (MPI) of about 0.5 ms, the effects seen in implant users are generally attributed to refractory properties (Cohen et al., 2003). At shorter MPIs and low masker levels, an enhancement of the probe's ECAP can occur, attributed to current integration effects (Stypulkowski & van den Hornet, 1984). Integration could be part of channel interaction protocols (Abbas et al, 2009).

Data collected from implant users are typically limited to symmetric biphasic pulses. Our data indicate that integrative effects are significantly reduced with that stimulus. We report on channel interaction measures in deafened cats, using monophasic and biphasic stimuli, as well as monopolar and bipolar presentation. ECAPs were assessed for different masker electrodes across an 8-band electrode array across a range of stimulus levels. MPI was fixed at 0.5 ms. Channel interaction ECAPs were analyzed using the well-established subtraction method (Brown and Abbas, 1990) and the "Miller" method (Miller et al, 2000), which is designed to avoid a complication of the Brown and Abbas method. ECAP amplitude versus masker level is generally nonmonotonic for monophasic stimuli. At low masker levels, there is a clear enhancement, suggesting masker-probe stimulus integration. At higher masker levels, refractory effects dominate and integration effects are less evident. The enhancement in probe responses was evident for both monopolar and bipolar delivery, but only for monophasic, cathodic, maskers. These results suggest that the choice of monophasic or biphasic stimuli can markedly affect the channel interaction measures and that monophasic or pseudo-monophasic stimuli may be advantageous for assessing refractory and integrative effects.

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354 Estimating ECAP Threshold from the Variability of the Response

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Electrical compound action potentials (ECAPs) of the cochlear nerve are used clinically for quick and efficient cochlear implant parameter setting. The ECAP is the aggregate response of nerve fibres at various distances from the recording electrode, and the magnitude of the ECAP is therefore related to the number of fibres excited by a particular stimulus. Current methods, such as the masker-probe or alternating polarity methods, use the ECAP magnitude at various stimulus levels to estimate the neural threshold, from which the parameters are calculated. However, the correlation between ECAP threshold and perceptual threshold is not always good, with ECAP threshold typically being much higher than perceptual threshold. The lower correlation is partly due to the very different pulse rates used for ECAPs (below 100 Hz) and clinical programs (hundreds of Hz up to several kHz). Here we introduce a new method of estimating ECAP threshold for cochlear implants based upon the variability of the response. At neural threshold, where some but not all fibers respond, there is a different response each trial. This inter-trial variability can be detected overlaying the constant variability of the system noise. The large stimulus artefact, which requires additional trials for artefact rejection in the standard ECAP magnitude methods, is not consequential, as it has little variability. The variability method therefore consists of simply presenting a pulse and recording the ECAP, and as such is quicker than other methods. It also has the potential to be run at high rates like clinical programs, potentially improving the correlation with behavioural threshold. Preliminary data is presented that shows a detectable variability increase shortly after probe offset, at probe levels much lower than those producing a detectable ECAP magnitude. Care must be taken, however, to avoid saturation of the recording amplifier saturation; in our experiments we found a gain of 300 to be optimal.

355 Audiovisual Integration in Cochlear Implants Users Measured by H_2O^{15}-PET

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Backgrounds: Cochlear implant (CI) users are equipped with better multisensory integration function than normal hearing population. With congruent audiovisual stimulation, associated cross-modal cortical activation examined in CI users correlates to the behavioral benefit. However, the enhanced audiovisual integration function might act as an obstacle attending to unimodal information in conflicting

auditory-visual environment. The aims of this study were to evaluate the role of the audio-visual integration function in the environment of agreeing/conflicting auditory and visual information and elucidate the cortical area that is crucial for processing audiovisual information.
Methods: Twelve cochlear implant users with good auditory performance and 12 matched healthy controls were included. Two speakers saying number 1 to 9 were videotaped. The video and audio tracts were separated and composed to produce three types of audiovisual stimulation; auditory only (A), audiovisual-congruent (AV congruent), and audiovisual-incongruent (AV incongruent) stimuli. During experiment, the subjects were instructed to recognize heard numbers, watching the monitor at the same time. Neural responses during the auditory, audiovisual, and silent baseline conditions were measured using H_2O^{15}-PET and compared between groups.
Results: The CI group showed lower rate of correct response and slower response time than controls in the AV incongruent condition. In the AV congruent condition, an increase of cerebral blood flow (CBF) was prominent in the visual cortices in the CI group. This was contrasted with the result of the control group that showed activation of both auditory and visual cortices for AV congruent stimuli. In the AV incongruent condition, CBF increase in the left prefrontal area was higher in CI group than control group and correlated with correct response rate in the CI group.
Conclusion: With agreeing audiovisual input, CI users depend mostly on visual information. In the AV incongruent environment, CI users activate left prefrontal cortex to cope with conflicting visual input which can impair auditory processing in CI users.

356 Cooperating Speech Processing Strategy and Pitch Mismatch in Bilateral Cochlear Implants: Predictions from an Acoustic Model

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The increasing rate of bilateral implantation necessitates the consideration of how the interaction between two cochlear implants can affect listeners' performance. This study explores two different types of interactions: the effect of a pitch mismatch between the devices and a cooperating bilateral speech processing strategy. In each case localization and speech recognition were tested on normal hearing subjects using an acoustic model.
Cochlear implant electrode array insertion depth varies from surgery to surgery due to factors such as physiology, implant design, and surgical conditions. This discrepancy in insertion depth can lead to differences in pitch between the two devices. In order to better understand the effects of this phenomenon, speech recognition and localization were tested for simulated insertion depth mismatches of 5 mm and 2.5 mm, along with a baseline condition with 0 mm mismatch, relative to the standard insertion depth of 25 mm. Statistically significant performance losses were observed between the 5 mm mismatch and standard conditions for localization and open-set word recognition, as well as for consonant recognition in noise. Localization responses tended to cluster toward the side associated with the shallower insertion depth, where the carrier frequencies were higher.
In the cooperating strategies implemented, the information from both implants was used concurrently. Two different variations of this strategy, each utilizing a modified N of M technique, were intended to provide enough redundant information to maintain localization abilities while presenting more detailed information to allow for improvements in speech recognition abilities. At least one of the cooperating strategies marginally outperformed the baseline strategy in every condition except consonant recognition in quiet. The results of this preliminary study indicate that cooperating bilateral implants may have the potential to improve speech recognition abilities.

357 Adding Adaptation to Cochlear Implant Processing

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Adaptation is a ubiquitous property of neural response. When a constant intensity stimulus is applied, firing rate is maximum at onset and then adapts to a steady state (SS) value. After stimulation, sensitivity is reduced and then gradually recovers. Adaptation may thus play a role in emphasizing changes in intensity and occurs at many levels of the auditory system including the auditory hair cell (HC). Cochlear implants (CIs) bypass the HC, and the present study was undertaken to introduce HC-like adaptation into CIs and observe whether this improved speech recognition.
CI processors were simulated with the Nucleus MATLAB Toolbox and Implant Communicator incorporating audiologist-produced maps. A simplified model of linear adaptation was produced by applying a 1st order high pass filter to the signal envelope in each electrode channel. Filters were defined by their time constants and onset-to-SS ratios in response to a constant-intensity input. Processing was adjusted to each channel's comfort and threshold levels so that the onset-to-SS ratio was equal on each channel. Time constants ranging from 2.5 to 50 msec simulated either rapid or short-term adaptation, and the ratio of onset-to-SS was 2-to-1 or 3-to-1.
Listeners were tested on vowel-consonant-vowel (VCV) and consonant-vowel (CV) discrimination tasks. Confusion matrices were used to compare results from tasks with adaptation to those without. Filters with varying parameters produced increased discrimination in some subsets of phonemes and deterioration in others. These phoneme changes will be analyzed with respect to spectrograms. The possibility that overall performance can

be enhanced by adding adaptation to selected frequency channels in some subjects will be discussed.

358 Effects of Spectral Shift on Speech Recognition in Background Noise

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Effects of spectral shift on speech recognition in quiet have been well investigated in acute studies and perceptual learning studies. Results of these previous studies showed that human listeners are able to somehow adapt to spectrally shifted speech in quiet. However, spectral shift may have stronger effects on speech recognition in background noise, since more fine information is required and more neural mechanisms are involved for speech recognition in background noise. The present study used normal-hearing (NH) listeners listening to acoustic CI simulations to examine the acute effects of spectral shift on speech recognition in noise. Speech recognition of seven NH subjects was measured with spectrally matched and shifted speech (vowels, consonants, and IEEE sentences), in quiet and in noise (5dB SNR speech-shaped static noise and 5dB SNR speech-babble noise). An 8-channel sine-wave vocoder was used to generate speech stimuli. One spectral match condition and three spectral shift conditions (2mm linear shift, 3mm shift with compression, and 4mm linear shift, in terms of cochlear distance) were investigated. Results of vowel and consonant recognition test showed that both spectral shift (larger than 2mm) and background sound have significant effect on speech recognition, and there is no significant interaction between spectral shift extent and background sound. For sentence recognition, performance did not significantly changed unless the shift extent was 4mm in quiet, but speech performance was significantly affected by the shift extent in background noise; there was significant interaction between the degree of spectral shift and background sound. These results suggest that spectral shifting has stronger effect on sentence recognition in background noise (especially in speech babble noise) than in quiet, at least in acute study. The clinical implication of these results is that spectral mismatch is one factor limiting performance of CI users in noise.

359 Effects of Duration and Fundamental Frequency Manipulation on Chinese Tone Recognition with Spectrally Degraded Speech

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Due to the limited pitch cues available from cochlear implants (CIs), tone recognition is challenging for CI users. Alternatively, CI users may use amplitude and duration cues to recognize tones. This study investigated the relative contributions of fundamental frequency (F0) and duration cues, as well as the effectiveness of enhancing these cues for Chinese tone recognition with spectrally degraded speech. Tone recognition was tested with 6 normal-hearing Chinese-speaking subjects listening to a 4-channel noise-band vocoder. In each experiment, tone durations, mean F0 values, and F0 variation ranges were manipulated one at a time without changing the others. The results showed that tone recognition significantly worsened as durations were normalized to 100 ms, but was not significantly different with normalized durations from 200 to 800 ms. Subjects' tone confusions were generally consistent with the duration distribution across original tones. One exception was the misidentification of Tone 4 (the originally shortest tone) as Tone 1 with the 100-ms duration, possibly due to subjects' reduced sensitivity to pitch variations. Tone 1 has significantly higher mean F0 values than Tone 3. However, when mean F0 values of different tones were shifted away from or close to the talker's average F0, tone recognition was not significantly affected. Tone recognition significantly improved by 11% when F0 variation ranges were increased from half to double original ranges. As a control, vowel recognition was not significantly affected by any of the F0 or duration manipulations. These results suggest that both tone durations and F0 variation ranges strongly contributed to tone recognition with spectrally degraded speech, while subjects did not use mean F0 cues to recognize tones. Time expansion may not benefit tone recognition. Exaggerated F0 variations observed in clear speech or resulted from speech pre-processing have the potential to improve tone recognition with CI.

360 Phonemic Restoration with Simulations of Cochlear Implants and Electric-Acoustic Stimulation

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Interrupted speech may be perceptually restored under certain conditions with help from bottom-up cues and top-down mechanisms in the auditory system. However, hearing impairment or front-end processing of hearing aids may negatively affect phonemic restoration, presumably due to the changes in the bottom-up cues (Baskent et al., 2007; 2009). The present study extends these findings to simulations of cochlear implants and combined electric-acoustic stimulation.

Sounds transmitted through cochlear implants become spectrally degraded and also lack salient pitch cues. The hypothesis of this study was that such degradations in the upstream signal, simulated with a noiseband vocoder, would reduce the phonemic restoration benefit. A second hypothesis was that the addition of low-frequency acoustic input, simulating a hearing aid, would bring back some of the pitch cues, counteracting the reduction in phonemic restoration.

These hypotheses were tested with normal-hearing listeners with speech interrupted at 1.5 Hz. The increase in intelligibility with the addition of noise bursts in the interruptions was the measure of restoration. In the cochlear implant simulation, stimuli were further processed with a vocoder with 4 to 32 noise bands. In the simulation of electric-acoustic stimulation, acoustic input low-pass

filtered at 500 Hz was added to the vocoded speech, while the noisebands with spectral content less than 500 Hz were excluded.
Preliminary averaged data supported both hypotheses. However, a more detailed analysis showed that the effects varied between stimuli with different talkers and between different noiseband conditions. These early data suggest another disadvantage of reduced spectral resolution of implant processing in complex listening environments, and that some of this disadvantage can be negated if the listener can make use of low-frequency residual hearing.
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361 Contribution of Low-Frequency Acoustic Cues to Talker Identification Training with Spectrally Degraded Speech

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Perceptual learning studies with normal hearing (NH) listeners demonstrate that training on indexical tasks can transfer to improvement in linguistic tasks, in spectrally degraded conditions. Here, we examined if talker identification (ID) training using additional low-frequency acoustic cues could improve talker ID in simulations of combined electric and acoustic hearing (i.e., EAS). Effects of talker ID training on sentence recognition were also assessed.
NH subjects participated in a 5-day talker ID training with visual feedback. A control group listened to an 8-channel cochlear implant (CI) simulation in their right ear, whereas an experimental group listened to the CI simulation in the right ear and 500-Hz low-pass filtered speech in the left ear (i.e., EAS simulation). Pre- and post-training measures included sentence recognition for both trained and novel talkers, in quiet and in speech babble noise. Talker ID was measured at the end of each training session; the experimental group was tested while 500-Hz low-pass filtered speech or a pure tone following speech fundamental frequency (F0) was presented in addition to the CI simulation.
Preliminary data showed that for both groups, sentence recognition for trained and novel talkers in quiet and in noise improved with training, although improvement varied across individual talkers. Talker ID also improved with training for both groups. Compared to the control group, the experimental group showed better sentence recognition before and after training, and better talker ID during training. F0 cues alone only partially accounted for the benefits to talker ID provided by the low-pass filtered speech noted in the experimental group. These results suggest that talker ID training may help both CI and EAS users improve talker ID as well as speech recognition.

362 Effects of Channel Interactions on Sensitivity to Interaural Timing Differences in Filtered Click Trains

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Most commonly used cochlear implants stimulate auditory nerve fibers with electrical pulse trains presented at multiple sites in the cochlea. Our lab has examined effects of interactions between cochlear implant channels on sensitivity to interaural timing differences (ITDs), which could have important implications for the development of speech processing strategies intended to give bilateral cochlear implant (BiCI) users access to binaural cues. However, one limitation of testing effects of interactions in BiCI users is that their auditory systems have pathologies about which only gross characteristics are known and which are not uniform across subjects. This can be overcome by testing sensitivity of normal hearing listeners to ITDs in filtered click trains.
In the current experiments ITD just-noticeable-difference thresholds (JNDs) were measured for a probe click train at 60 dB SPL that was temporally interleaved with an added click train and presented against a background of interaurally uncorrelated pink noise. In monaural experiments thresholds were measured for detection of a probe that was temporally interleaved with an added train. In all experiments thresholds corresponding to d' = 1 were determined using a modified version of a double staircase procedure. Results: 1) ITD JNDs were elevated in the presence of a diotic added train when the onset delay between trains was 5 ms but not when it was 60 μs, 2) results were unchanged when interaural phases of low-frequency harmonics of the click trains were randomized, and 3) the amount of the elevation in thresholds was not predicted by the separation between center frequencies of the two trains or by binaural sensitivity to single binaural click trains. These results generally support the findings of parallel experiments in CI users. Similarities and differences relative to stimuli and results in the "binaural interference" literature will also be discussed.
Work supported by NIH-NIDCD (R01 DC003083, F31 DC009361).

363 Auditory Training in Adult Cochlear Implant Listeners Using Spectrally-Rippled Noise Stimuli in an Adaptive, Single-Interval Paradigm

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Cochlear implant listeners commonly report difficulties in listening to music and understanding speech in the presence of background noise. While these deficits are likely a product of many factors, the ability to resolve frequency information is thought to be a contributor. Here we examine the use of auditory training exercises aimed at

improving the perception of spectral details. We used spectral ripple stimuli that contained "change" and "no-change" conditions. In the "change" condition, the second half of the ripple stimulus was an inverted version of the first half, while the "no-change" stimulus had no inversion. Thresholds were determined using an adaptive yes/no paradigm that tracked number of ripples per octave. Using a single-subject staggered baseline design, each subject completed baseline and training sessions with the onset of training being randomized across subjects. Each subject participated in 32 hours of testing/training over the course of three months. Three implanted participants (1 Advanced Bionics, 2 Cochlear) participated in the experiment. To date, training spectral ripple thresholds have improved on average 4.5 ripples per octave. Results involving outcome measures, including tests of speech in quiet, in noise, and music perception will be presented. [Supported by NIH grants F31DC010309, T32 DC005361, P30-DC04661.]

364 The Effect of the Number of Channels on Spectral-Ripple, Schroeder-Phase Discrimination, and Modulation Detection in Cochlear Implant Users

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The present study evaluated the effect of the number of channels on psychophysical performance in cochlear implant (CI) users. Previous studies have shown that speech recognition by CI listeners saturates around eight to ten channels. This study extends this finding of limitation in information transmission by CIs to more fundamental aspects of sound stimuli. Spectral-ripple discrimination, Schroeder-phase discrimination, temporal modulation detection, and CNC word recognition were measured with 1, 2, 4, 8, 12, and 16 channels. The three acoustic measures have been shown to correlate with speech perception in CI listeners. A 3-alternative forced choice (AFC), two-up and one-down adaptive procedure was used to determine the minimum ripple spacing that subjects could discriminate between standard and inverted ripple sounds. A 4-interval, 2-AFC paradigm at two fundamental frequencies (50 and 200 Hz) was used to measure the Schroeder-phase discrimination ability. The modulation detection thresholds for sinusoidally amplitude modulated noise were measured. Modulation frequency of 50 Hz was tested with a single-interval, 2-AFC, 2-down, 1-up adaptive procedure. Results showed: (1) spectral-ripple discrimination and word recognition improved as the number of channels increased; and (2) Schroeder-phase discrimination and temporal modulation detection did not improve with more than two to four channels. This observation reveals that the Schroeder-phase discrimination and temporal modulation detection are less dependent on the number of channels provided, than are spectral-ripple discrimination and word recognition. The results suggest that spectral-ripple discrimination ability benefits from having multi-channel information; whereas Schroeder-phase discrimination ability and modulation detection ability do not improve much with the additional channels. The latter measures may be useful to evaluate temporal processing ability in CI users. There was no evidence that broad-band spectral-ripple discrimination did not involve integrating information from multiple channels. [Supported by NIH grants R01-DC007525, P30-DC04661, F31-DC009755, and a graduate fellowship from Advanced Bionics.]

365 Customized Selection of Frequency Maps for Postlingually Deaf Cochlear Implant Users

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Despite the clinical success of cochlear implants (CIs), there remains substantial variability in speech perception outcomes. One factor that may limit speech perception by postlingually deaf CI usersis that, despite between-patient differences in cochlear size, electrode location, and neural survival, the vast majority of users of a given device receive essentially the same frequency-to-electrode table (or frequency table). There is evidence that the use of such "standard" frequency tables may limit speech perception for some patients. This may be due to a mismatch between the patients' long term memory for speech sounds, and the stimuli they actually receive from their device. The frequency mismatch may have its most negative effect on the speech perception performance of CI users shortly after implantation, and if a given CI user cannot adapt completely to a frequency mismatch, it may also affect their long term speech perception. To help ameliorate any detrimental effects of frequency mismatch on speech understanding, we have developed fitting tools that allow CI users to adjust the total bandwidth and average location of their frequency table in order to maximize speech intelligibility. When given the option to explore a number of different frequency tables, many CI users reliably selected a table that was different from the default standard table used in clinical practice. Moreover, some CI users had better speech perception scores with their self-selected frequency tables than with their clinical frequency tables, despite having used the self-selected frequency tables only for a few minutes in the laboratory.

366 Speech Quality and Intelligibility in Cochlear Implants with MP/BP Combined Mode MAPs

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In cochlear implants (CI), despite the notion that bipolar (BP) stimulation targets a more spatially focused neural population with a better channel specificity, monopolar (MP) stimulation is predominantly used for clinical applications, primarily due to a substantial reduction in power consumption in MP. As a result, the feature of BP stimulation available in most CI systems today is left unused, and any possible benefits of BP stimulation might

be overlooked. In the present study, the feasibility of BP stimulation for clinical mapping was examined in the Nucleus CI system. Postlingually deafened adult subjects (N=8) who had at least 6 months of experience with MP mode prior to the study were presented with speech sounds through MAPs with different combinations of MP and BP, and consonant/vowel identification and clarity/pleasantness ratings were measured. The combined-mode presentation was made by interleaving the pulses of MP and BP stimulation. All BP stimulation was in BP+1 mode and at the same stimulation rate as their regular setting, or at a half or lower when the hardware limit was reached. The following 6 conditions were tested: 1) full MP only (regular setting), 2) full BP only, 3) full MP + full BP, 4) full MP + full BP with MP/BP channels aligned based on pitch relations, 5) full MP + BP at mid-to-low frequencies (<3500 Hz), 6) staggered MP/BP (each mode using every other electrode). All testing was conducted at the laboratory or in their home for limited hours. Results to date indicate that, while all subjects showed poor speech recognition and quality ratings with a BP-only MAP, most subjects found at least one combined condition equally or more preferable than their regular setting and comparably intelligible, suggesting MP/BP combined-mode stimulation as a customizable tool to maximize benefits for individual subjects [Work supported by NIH/NIDCD R03DC009061].

367 Speech Enhancement Based on Partial Masking Effect

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To enhance the quality of the perceived speech on the present of the background noise, acoustic masking that is one of the most well-known property of human auditory have been used in many studies. In this study, we proposed an algorithm to enhance the perceived quality of speech signal masked partially by background noise. In the algorithm, standard equal loudness contour (ISO226) and loudness perception model proposed by Moore et al was used for modeling the human hearing properties. Absolute threshold from standard equal loudness contour and excitation level of noise and speech from absolute threshold and auditory filter proposed by Glasberg and Moore was calculated. Based on the modeling, specific loudness and partial specific loudness of the speech was also calculated from excitation level of speech and noise respectively. Then, comparing the specific loudness and partial specific loudness, gain for reducing partial masking effect was computed. We tried to make the gain optimal reducing the partial masking effect and distortion also. To confirm the effective of proposed algorithm, we compared the speech by proposed algorithm (PM) to the speech by conventional spectral subtraction (SS) and noisy speech (NS). In view of the results, SNR and PESQ score in the condition of PM enhanced more than that in the condition of SS or NS.

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368 SPARSE, an Enhanced Speech Processing Algorithm for Cochlear Implants

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Speech recognition in noisy environments is difficult for cochlear implant (CI) users. This paper proposes an enhanced speech processing algorithm by taking advantages of redundancy of speech signals.

The noisy signal can be represented sparsely, with components only necessary for correct speech recognition. The idea is to identify the key elements of speech and null the other components which are not important. This concept is similar to ideal binary masking, which only extracts areas with higher signal-to-noise-ratio (SNR). Identifying such areas without prior knowledge of speech and noise is a challenging task.

However, the proposed algorithm, named SPARSE, is based on information rather than SNR. It identifies the necessary components of speech by decomposition using Principal Component Analysis (PCA) and Independent Component Analysis (ICA) techniques. After ICA processing, independent components with higher amplitude represent important physical events. A threshold is then applied in the independent components of the spectrum of noisy speech signal. The reconstructed signals are used to create electrical pulse stimuli via the implant.

The SPARSE algorithm is compared with the standard Advanced Combination Encoder (ACE) algorithm in a speech recognition task. Both objective quality measurements of enhanced speech and subjective recognition experiments suggest that the algorithm will benefit CI users in babble noise conditions.

369 Influence of Source Talker F0 on Voice Conversion Algorithms for Cochlear Implant Users

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Given the limited spectral resolution provided by cochlear implants (CIs), CI users strongly attend to temporal cues (e.g., voice fundamental frequency, or F0) to discriminate voice gender. Additionally, CI users often report differences in voice quality and/or speech understanding across male or female talkers. Acoustic input signal optimization (e.g., voice conversion across gender and/or talkers) might improve performance for CI users who are sensitive to talker differences.

In this study, nine voice conversion algorithms were designed using Gaussian mixture models (GMM) with varying Mel-frequency cepstral coefficients (MFCCs) to

shift speech spectral components with or without variation of the source talker F0. MFCCs were obtained from source talker speech samples using adaptive interpolation of a weighted spectrogram or a fast Fourier transform. Six CI subjects were tested in sound field using their clinical speech processors and settings. Original speech from one male and female source talkers and the experimental algorithms were tested using two sets of IEEE sentences; sentence recognition, voice gender identification, and subjective quality ratings were obtained for each algorithm. Speech recognition was not significantly different among the conversion algorithms or the original talkers. When the source talker F0 was included in the converted speech, subjects easily identified the source talker gender, suggesting that temporal F0 cues dominated any spectral component conversions. There was a trend toward poorer performance and quality ratings for the male-to-female conversion algorithms with increasing MFCC vectors (from 25 to 75), suggesting that undesirable processing artifacts are associated with higher MFCCs. The results suggest that for GMM voice conversion algorithms, voice gender identification is strongly influenced by the source talker F0 information, despite variation in spectral envelope MFCC values.

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370 Improved Discrimination of Interaural Time Differences in Speech with an Asynchronous Cochlear Implant Sound Coding Strategy

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For normal-hearing listeners, the interaural time difference (ITD) is a major cue for sound localization and signal detection in noise. However, cochlear implant (CI) listeners with bilateral devices are typically not sensitive to ITD cues that are present in the acoustic signals picked up by the microphones in their sound processors, especially with real-world complex signals such as speech. We investigated an experimental sound coding strategy, called Fundamental Asynchronous Stimulus Timing (FAST), which has been designed to convey ITD cues in the timing of each electric pulse. Speech tokens were processed offline on a computer and streamed directly to the internal devices of each subject. Acute listening tests compared ITD discrimination of speech for a bilaterally synchronized version of the ACE coding strategy and the FAST coding strategy. Results show that most subjects were more sensitive to changes in ITD when listening to speech processed by FAST than by ACE. Furthermore, ITD discrimination thresholds were typically within the natural range of ITDs induced by the head using the FAST coding strategy. Future work will address whether or not the increased sensitivity to ITD cues seen here can form the basis for better sound localization and speech understanding in noise for bilateral CI recipients.

371 Regenerating Harmonics for Improved Intelligibility of Telephone Speech for Electric and Acoustic Stimulation and Cochlear Implants

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Several studies have shown that using telephone still presents a challenge for cochlear implant (CI) listeners, although no studies have been reported with CI listeners using electric and acoustic stimulation (EAS). The main reason for the reduced intelligibility of telephone speech for CI users is the narrow bandwidth introduced by the telephone network which has a passband between 300 and 3400 Hz. Compared with wideband speech, telephone speech does not convey information below 300 Hz and above 3400 Hz. This is problematic as information above 3400 Hz is used to identify certain high-frequency consonants (e.g., /s/). Several methods have been proposed to extend the bandwidth above 3400 Hz to partly recover the missing higher frequency information. In this study, we focus on bandwidth extension toward low frequencies rather than high frequencies.

In the present study, we propose to regenerate the harmonic structure below 600 Hz in the voiced segment of the telephone speech signal for EAS and CI processing. It has been proposed in several studies that improved speech recognition for EAS can be accounted for by a better F0 representation in the low frequencies. As the spectral content below 300 Hz is severely distorted in telephone speech, the F0 representation is most probably poorer in telephone speech compared with wideband speech; consequently EAS benefits will be considerably reduced with telephone speech. We hypothesize that by regenerating the harmonic structure in the voiced speech segments, the F0 representation can be improved, leading to improved intelligibility of telephone speech via EAS processing. For CI-alone processing, we hypothesize that harmonics regeneration can improve the F1 representation in the temporal envelopes of the vocoded telephone speech, leading to improved telephone speech intelligibility. Preliminary results indicated that harmonics regeneration can significantly improve telephone speech intelligibility for EAS and CI processing.

372 The Effect of Reducing Pulse Number Independent of Pulse Rate

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Multiple studies have indicated that the speech recognition performance of cochlear implant recipients can be significantly impacted by the stimulation rate used in their speech processing algorithm. Higher pulse rates have been hypothesized to be preferable due to the finer temporal resolution that they provide. This resolution better captures short-time transient speech components; however, subjects often do as well or better with lower pulse rates. This may be due to an inability to use the

additional information, similar to the limitations observed for increasing the number of spectral channels, or it may be an issue of the increased number of pulses leading to increased channel interactions.

This study considers a method to investigate these different hypotheses by reducing the number of pulses presented while retaining to some degree the representation of the transient speech components. A proportion of pulses are selected at random from a stimulation pattern generated using a high pulse rate and these pulses have their amplitudes set to zero. Thus, the number of pulses is reduced. However, while some of the pulses that represent the transient speech components will also be lost, others will remain thus potentially increasing the information available to subjects beyond that available using a lower pulse rate. Speech recognition using this method is compared to speech recognition using three standard rates: 250, 800, and 1600 pps. Speech recognition with pulse reduction is tested by first generating stimulation patterns using 1600 pps, and then reducing the number of pulses by random selection with the following retention rates: 75%, 50%, 25%, and 15%. Thus, at 50% and 15%, the reduced stimulation pattern has approximately the same number of pulses as the patterns using the standard 800 and 250 pps respectively. The results of this study may have implications for future speech processing algorithms that may intelligently select information for presentation.

373 Stream Segregation on a Single Electrode as a Function of Stimulation Rate in Cochlear Implant Listeners

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While cochlear implants usually provide a high level of speech recognition in quiet, speech recognition in noise and music appreciation remain challenging. In response to these issues, several studies have proposed increasing the number of channels of information, either through variable pulse rate strategies or current steering. In this study, stream segregation is proposed as a method to test whether different pulse rates on the same electrode can be perceived as independent channels of information. This approach differs from that of previous stream segregation studies which focused on stimulation of alternating electrodes, with the motivation of determining a relationship between electrode stream segregation and speech perception in challenging noisy environments. Additionally, the relationship between stream segregation results and rate discrimination is also explored.

This study considers stream segregation on a single electrode as a function of stimulation rate. Subjects were presented with two stimulus sequences following an A-B-A-B… structure via direct stimulation of a medial electrode. A and B were loudness-balanced pulse trains with different stimulation rates. One of the stimulus sequences had a regular rhythm throughout while the other had an increasing delay imposed on B, resulting in an irregular rhythm (Hong and Turner, 2006). Subjects were asked to identify the stimulus containing the irregular rhythm, which is a more difficult task if the streams are perceived as segregated. An adaptive procedure was used to find the minimum detectable delay for various pairs of stimulation rates. Additionally, subjects completed a rate discrimination task to determine the relationship between the stream segregation results and the discriminability of A and B. The results of this study indicate when fission or fusion for the selected subset of rates is perceived, and thus may aid in the selection of stimulation rates for multi-rate speech processing strategies.

374 Gap Detection for Pulsatile Electrical Stimulation: Effect of Carrier Rate and Stimulus Level

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In electric hearing, it has been shown that performance on measures of temporal acuity, including gap detection thresholds (GDTs), is optimal at high stimulation levels. However, distortion to the normal loudness growth can impact this ability. In a previous study, we showed that the mechanisms underlying GDTs are related to loudness growth. Yet, it is not known how these two variables are related to other parameters such as the carrier rate, which affects the temporal resolution of the electrical signal and the dynamic range (DR). In this study, GDTs were measured as a function of carrier rate and stimulus level to test the hypothesis that the shape of GDT vs. level function, the loudness-growth function, and carrier rate are interrelated.

T and C levels were measured in 6 postlingually deafened adults using a basal stimulation site. Stimuli consisted of 500 msec trains of symmetric-biphasic pulses, 40 μsec/phase, presented at a rate of 250, 1000, or 4000 pps using MP1+2 mode. GDTs were measured in random order for the three pulse rates at 10%, 30%, 50%, 70%, and 90% of the DR. Subjects were asked to discriminate a 500 msec stimulus with a gap created in the middle of the pulse train from a pulse train with no gap. Gaps were generated by omitting pulses starting at an initial value of 25 missing pulses and using an adaptive 2-interval forced-choice procedure to estimate the minimum gap duration that could be detected on 70.7% of trials. To test the relative growth of loudness as a function of carrier rate, two contrasting rates (250 and 4000 pps) were loudness matched at the same multiple levels of the DR.

As observed previously, DR increased as a function of pulse rate in all subjects and GDTs decreased as a function of stimulus level in %DR. In addition, GDTs in msec were either similar across carrier rates or smallest at the high carrier rate, depending on the subject. Loudness for the 250 pps carrier was similar or slightly louder than that for the 4 kpps carrier depending on the subject and level. However, across-subject differences in loudness matching results were not predictive of across-subject differences in effects of carrier rate on GDTs. Results suggest that the temporal processing of gaps as a function

of carrier rate is rather mediated by factors that are subject-dependent.
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375 Toward a Novel Method for Evaluation of Cochlear Implant Signal Processing Strategies

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Evaluation of user-independent efficacy of novel cochlear implant (CI) signal processing strategies is difficult. Current practice relies on customization based on user performance in the clinical setting. As a result, it is impractical to achieve theoretical optimality, and improvements to stimulation strategies are frequently achieved using trial-and-error. A computational framework that approximates the upper bound on CI performance is desirable to reduce the number of novel strategies that require evaluation in patients.

Our goal is a physiologically-based framework, using accepted models of the acoustically- and electrically-induced nerve activation patterns (NAPs), to predict discrimination and forced-choice identification among acoustic stimuli. An initial framework has been developed to predict behavioral performance of normal-hearing (NH) listeners, using NAPs generated from the Zilany-Bruce auditory-periphery model. The correlation-based metric presently predicts rank orders of errors in confusion matrices exhibited by NH listeners during psychophysical experiments, and extension to predicting absolute error rates is underway. Subsequently, we will shift to the application of predicting confusion matrices in CI users, replacing the physiological model with one of electrical stimulation. Here, accurate confusion matrix prediction will indicate that the framework meaningfully models interaction between the implant and auditory nerve, and may serve as a core element in optimizing novel CI electrical-stimulation.

376 A Model of Auditory Spiral Ganglion Neurons for Acoustic and Electrical Excitation

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For both normal hearing subjects and cochlear implant patients the most drastic step of sound coding for neuronal processing is when the analog signal is converted into discrete nerve-action potentials. As any information lost during this process is no longer available for neural processing, it is important to understand the underlying principles of sound coding in the intact auditory system and the limitations in the case of direct electrical stimulation of the auditory nerve.

Here we focus on a model of spiral ganglion type I neurons with Hodgkin-Huxley type ion channels, which are also found in cochlear nucleus neurons (K_A, K_{ht}, K_{lt}). Depending on the task, we model the neurons at different levels of detail. For acoustic stimulation, we model the postsynaptic bouton (1.5 x 1.7 µm) from high-spontaneous rate fibers and a synaptic excitation model fitted to results from Glowatzki and Fuchs (2002). To study the response to electrical stimulation in detail, we use a multi-compartment model and an approximation of the electrical field along the neuron. For automatic speech recognition and information theoretic calculations we use simplified single compartment versions.

We analyze the quality of coding with the framework of automatic speech recognition and the methods of information theory. Our results show that for acoustic stimuli, the model provides realistic refractoryness and generates more realistic spike trains compared to an artificial spike generator. Not surprisingly, speech discrimination in electrical hearing is lower than in acoustic hearing. This is probably due to the limited dynamic range of electric hearing and the wide current spread, which limits spectral resolution. On the other hand, the temporal precision of information coding seems to be very high because at levels well above threshold, action potentials are elicited quasi deterministic by the electrical stimuli. We argue that CIS strategies a) waste as much as 50% of this information and b) much of the information coded in the time domain can not be retrieved by the neurons in the cochlear nucleus.

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377 Are Slow K+ Channels Responsible for Sub-Threshold Masking?: A Computational Model Approach

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Our group has been developing a biophysical model of a feline ANF to investigate auditory-nerve-fiber (ANF) responses to electrical stimuli (Woo et al., IEEE T. Biomed. Eng., 2009). The model incorporates a K+ storage mechanism which alters the membrane voltage and produces simulations of spike rate adaptation. However, the model was limited in that it did not simulate sub-threshold stimulus effects that can reduce ANF¡¯s responsiveness to subsequent supra-threshold stimuli (Miller et al., ARO, 2009). We report here on simulation results obtained with a new model variant that includes both fast and slow K+ channels (Schwarz et al., J Physiol., 2006). The results support the hypothesis that sub-threshold pulse trains result in depolarization that can affect subsequent excitation and produce significant spike adaptation as assessed by the presentation of probe pulses after offset of the sub-threshold masker pulses.

We investigate both super-threshold and sub-threshold adaptation effects using a masker-probe paradigm similar to that used in our physiological experiments. Either a

high-rate (5000 pulse/s) or a low-rate (250 pulse/s) pulse train, each with 150 ms duration, was delivered as a forward masker, followed by a low-rate (100 pulse/s) probe train. Addition of the slow K+ channels results in sub-threshold masking when the modeled ANF is stimulated by high-rate, but not low-rate, pulse train masker, consistent with single fiber results (Miller et al ARO, 2009). We will also present parametric data showing how masker pulse rate, duration, and level influence the membrane and the probe response and compare those results, when possible, to recently acquired cat ANF responses.
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378 The Role of BK Channels in Vestibulo-Ocular Reflex Performance and Plasticity in Mice

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Large-conductance calcium-activated potassium (BK) channels are expressed widely throughout the brain and have been implicated in the regulation of neuronal excitability and plasticity. BK channels are abundant in the vestibular system, notably in cerebellar Purkinje cells and neurons of the vestibular nuclei, and thus may be involved in the control and adaptation of the vestibulo-ocular reflex (VOR).
We investigated the role of BK channels in VOR performance and plasticity in two transgenic mouse lines, one with a global deletion of the BK channel's pore-forming alpha subunit (BK nulls), and the other in which the absence of BK channels was restricted to Purkinje cells via the L7 promoter (L7 BK nulls). Wildtype littermates served as control. Adaptive changes in the VOR were induced either by subjecting mice to 30 min of gain-increasing or gain-decreasing visual-vestibular mismatch experience or by unilateral damage to the vestibular end-organ.
Both BK null strains displayed largely normal VOR performance and showed only minor decreases in optokinetic reflex (OKR) gain at low stimulus frequencies. VOR adaptation induced by visual-vestibular mismatch, however, was absent in both strains of BK null mice, in contrast to the robust gain decreases or increases induced by training in the respective wildtype littermates. Interestingly, recovery of VOR gain after unilateral vestibular damage was delayed but not reduced in BK null mice and the rapid increases in OKR gain that typically follow vestibular loss in wildtypes were completely absent in both strains of BK null mice.
These data indicate that in the vestibular system the contribution of BK channels to normal performance of the VOR is minor and that BK channels in Purkinje cells are critical for VOR and OKR adaptation and for the time-course of VOR compensation.

379 A Device for Quantifying Vestibular-Induced Eye Movements in Zebrafish Larvae

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To quantify vestibular function in larval fish, we created a device for measuring eye movements evoked by rotation about an earth horizontal axis. The device consists of a customized digital microscope system mounted on a rotating platform. For our experiments, larvae were placed near the microscopic lens and the platform was rotated sinusoidally at 0.25 Hz to stimulate the vestibular system. Immobilized specimens were illuminated with infrared light to avoid stimulating the optic kinetic response (OKR). An image processing method was designed to analyze the recorded videos, allowing quantification of eye movements in the rotational plane and the corresponding perpendicular plane. We observed that a robust response appeared as early as 72 hours post fertilization (hpf). The responses were absent in mutant *cdh23* larvae and larvae lacking anterior otoliths, demonstrating the correlation between the eye movements and the functionality of the anterior/utricular macula.

380 Effects of Stimulus Pulse Parameters on Eye Movement Responses to Stimulation Delivered by a Vestibular Prosthesis

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A multichannel vestibular prosthesis that measures 3D head rotation and encodes it via selective electrical stimulation of vestibular nerve branches could improve quality of life for individuals disabled by diseases of the inner ear that cause bilateral loss of vestibular sensation. Spread of current from a stimulating electrode to nerve branches other than its intended target manifest as a misalignment of the perceived and actual head rotation axes. The perceived head rotation axis and velocity can be assayed by monitoring the axis and velocity of eye movement caused by the angular vestibulo-ocular reflex (aVOR).
We investigated the effects of varying pulse duration (PD), interphase gap (IPG), and current amplitude of biphasic pulses on aVOR responses evoked by pulse rate modulated stimuli delivered to electrodes implanted in the three semicircular canals of 5 chinchillas rendered bilaterally vestibular deficient. We found that increasing the stimulation pulse rate resulted in a linear increase of the aVOR-mediated eye movement response velocity while maintaining a relatively constant axis of rotation. Increasing current amplitude increased eye velocity but also increased the misalignment between the intended and actual axes of rotation. Stimulation with shorter PD over the range of 28 – 340 μs required less charge per phase to elicit a given peak aVOR response velocity, and also evoked responses with less aVOR axis misalignment. Varying IPG from 25 – 175 μs had no significant effect on aVOR responses. We conclude that while pulse rate

modulation is the most appropriate basic code for prosthetic stimulation of the vestibular nerve, responses also depend on current and pulse duration, which can be optimized to better encode high velocity head rotations.
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381 Multichannel Vestibular Prosthesis Using Linear Coordinate Axis Transformation Stabilizes Eyes for Head Rotation About Any Axis

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There is no effective treatment available for individuals unable to compensate for bilateral profound loss of vestibular sensation. Development of a 3D gyroscope based vestibular prosthesis which electrically stimulates vestibular nerve branches to encode head movements has been limited by current spread resulting in distortion of the vestibular nerve activation pattern and consequent inability to accurately encode head movements. We investigated a precompensatory 3D remapping to accurately emulate semicircular canals for head rotations and velocities normally transduced by a healthy labyrinth.

We rendered four chinchillas bilaterally vestibular deficient via gentamicin treatment and/or canal plugging and implanted their left labyrinths with stimulating electrodes positioned in each of the semicircular canals. Velocity and axis of rotation r_i for angular vestibulo-ocular reflex (aVOR) eye movements, used to assay the perceived head rotation, were measured in response to sinusoidal pulse rate modulation for 65 different combinations of relative stimulation intensities s_i delivered simultaneously to the three stimulating electrodes. A 3x3 mapping matrix M was computed using least squares approximation to describe the electrical stimulus-to-aVOR response relationship, such that $r_i \approx Ms_i$. Then sets of 65 desired head rotations indicated by 3x1 vectors of gyroscope signals g_i, which spanned a space of possible 3D head rotations axes and velocities at a variety of frequencies, were each converted to the three individual electrode stimulus intensities $e_i = M^{-1} g_i$ needed to implement each desired rotation. The aVOR eye movement evoked in response to the combination of stimulus intensities e_i was compared to the desired rotation g_i. The aVOR responses spanned the 3D space of possible head rotations at the desired velocities and with 53±7% reduction in axis misalignments as compared to non-precompensated head rotations.

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382 3D Angular VOR Adaptation to Chronic Motion-Modulated Multi-Channel Prosthetic Stimulation of Semicircular Canal Ampullary Nerves

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Previous efforts to develop a 3D gyroscope-based multichannel vestibular prosthesis showed that current spread can cause distortion of the vestibular nerve activation pattern and consequent misalignment between the axis of perceived and actual head rotation. We hypothesized that over time, central compensatory mechanisms would adapt to the distorted signals provided by electrical stimulation, correcting for the distorted representation of head rotation presented to the brainstem by the prosthesis.

To investigate adaptation to chronically functioning vestibular prosthesis, we rendered 5 chinchillas bilaterally vestibular deficient via gentamicin treatment and canal plugging. Electrodes were implanted in each of the semicircular canals (SCCs) in the left ears and the prosthesis was connected and securely affixed to the top of the head of each chinchilla. We used the inverse of eye movements mediated by the angular vestibulo-ocular reflex (aVOR) and measured using 3D videooculography in darkness as an indication of perceived head motion. The aVOR responses to head rotations about each of the three axes corresponding to the orientation of each SCC were measured on the 1st, 3rd and 7th day of chronic, motion-modulated stimulation. The aVOR gain before the prosthesis was turned on was negligible at 0.05 ~ 0.1. On stimulation day 1, the aVOR gain was 0.4~0.6, similar to that of normal chinchillas, but the axis of observed eye movements aligned poorly (misalignment of 30~50°) with head rotation. Substantial improvement of axis misalignment (down to 20~30°) was observed after 3 and 7 days of motion modulated prosthetic stimulation while animals stayed in their usual well-lit cages. These findings suggest that the ability of central vestibulo-cerebellar pathways to compensate for errors of aVOR gain and alignment over time extends to the case of prosthetic stimulation with a multichannel vestibular prosthesis.

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383 Effects of Vestibular Electrode Implantation and Prosthetic Stimulation on Hearing in Rhesus Monkeys

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We measured auditory brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE) in two rhesus monkeys before and after unilateral implantation of vestibular prosthesis electrodes in each of 3 semicircular

canals (SCC). We repeated testing with the vestibular prosthesis both on and off, to investigate whether pulsatile current stimuli intended for the vestibular nerve negatively impacts auditory function. Each of the 3 left SCCs were implanted with bipolar pairs of electrodes via a transmastoid approach. The ear canal and ossicular chain were not disturbed. Right ears, which served as controls, were left intact. The hearing tests were conducted before implantation (BI), and then 4 weeks post-implantation both without electrical stimulation (NS) and with electrical stimulation (S). Stimuli were charge-balanced biphasic pulses rate modulated by measured head velocity around a baseline of 60 pulses per second on all three electrodes asynchronously. ABR hearing thresholds to free field clicks and tone pips at 1, 2, and 4 kHz increased by 5-10 dB from BI to NS and by a further increase of about 5 dB from NS to S in implanted ears. No change was seen in right ears.. DPOAE measurement showed a decrease of 2-14 dB from BI to NS in implanted ears. There was no observable difference of DPOAE between NS and S in implanted ears but the DPOAE/Noise ratio decreased, indicating the stimulation may cause some background noise.
There appears to be relatively small but measurable effect of vestibular prosthesis surgery and activation on hearing. Coupled with clinical experience, in which patients with cochlear implants only very rarely exhibit clear signs of spurious vestibular nerve stimulation, these results in a limited number of rhesus monkeys suggest that implantation and activation of vestibular prosthesis electrodes carries a risk of hearing loss, but that loss is not severe.
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384 A Micromachined Cupula: Toward a Low-Power Biomimetic Angular Velocity Sensor for a Vestibular Prosthesis

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The development of vestibular prostheses relies heavily upon inertial sensors—gyroscopes—for detecting angular head rotations. These movements are subsequently coded by electronic circuitry into current pulses serving to stimulate vestibular nerve fibers and replace lost vestibular function. Although such sensors are scaling in size, power consumption remains an issue. When considering a three-dimensional system, one sensor per dimension is required further increasing power demands thereby precluding the realization of a fully implantable system. As an alternative, we propose a biomimetic analogue to the natural human sensor, the semicircular canal (SCC) and ampullary organ. By employing micro-electromachanical systems (MEMS) fabrication technology, we translate the gelatinous, elastic nature of the cupula into a flexible membrane transversely spanning and totally occluding the flow of water in a hollow glass ring (SCC). In response to rotation about the axis normal to the plane of the ring, the water will apply pressure to, and deform the membrane, with the distance of deflection revealing angular velocity. In order to measure said deflection, a capacitive element will be placed within the flexible membrane, and a rigid reference electrode will be fabricated within the ring close enough to the membrane to sense deflection through capacitance changes. We chose polyimide as the membrane material due to its high degree of resistance to thermal and mechanical stresses. Our modeling work has shown that such a sensor requires an ultra-thin (~20 nm) membrane, presenting a significant fabrication challenge. A novel technique combining atomic force microscope lift-off lithography with metal sputtering will allow for the metallization and patterning of such a delicate element. Continued clean room testing and finite element analysis are being employed to determine an ideal fabrication protocol. This sensing methodology is both unique and efficient, allowing for power savings in excess of 66% over a gyroscopic scheme. This MEMS approach potentially presents a scalable, biocompatible, and low power sensor that enables the eventual realization of a fully implantable vestibular prosthesis.
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385 Blood Pressure and Heart Rate Changes from Labyrinthine Stimulation in the Anesthetized Rat

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Sinusoidal changes in head position re gravity during off-vertical axis rotation activate the otolith organs to produce sinusoidal changes in blood pressure (BP) at the frequency of rotation (Kaufmann et al. 2002). Here we examined the impact of sinusoidal galvanic vestibular stimulation (GS) on autonomic activity. Binaural, sinusoidal GS was given at 1, 2 and 4 mA from 0.01 to 0.5 Hz via electrodes inserted behind the external auditory meati in isoflurane-anesthetized Long-Evans rats. BP was measured intra-arterially from the carotid and femoral arteries with a BP transducer and Grass amplifier. BP and stimulation parameters were acquired at 250 Hz. Heart rate (HR) was identified from the BP peaks, stored as pulses and converted to beats/sec. FFT's indicated a response in BP and HR at both the frequency of stimulation and at double the frequency. Changes in BP and HR were fitted with sinusoids, using a least mean square algorithm at the frequency of stimulation and at double the frequency. BP and HR modulation amplitudes were normalized to the stimulus current. One mA stimuli were ineffective at any frequency. Stimulation at 2 and 4 mA were most effective at low frequencies, and responses were inconsistent or absent at frequencies ≥ 0.2 Hz. The onset of stimulation was associated with a 20 mm Hg drop in BP and a decrease in HR from 4 to 3 beats/sec ($p<0.001$). Both parameters recovered within 2-3 min, even with continuous stimulation. As BP and HR dropped at stimulation onset, both parameters oscillated sinusoidally at both the stimulus frequency and at twice the stimulus frequency. The rapid drop and slow recovery of BP indicate that the labyrinths were stimulated directly, producing the oscillations in BP and HR. It is likely that

these autonomic responses were mediated by activity arising in the otolith organs. The finding that there was oscillation at a double frequency suggests that the GS was activating the labyrinths on both sides.
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386 Low Level Blast Overpressure Exposures Initiate Brain Microvascular Remodeling and Repair

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Blast-induced, traumatic brain injury (BI-TBI) is a common battlefield injury in Iraq and Afghanistan. Symptoms of mild BI-TBI include tinnitus, hearing loss, emergent and delayed post-traumatic balance disorders, and migrainous disorders in the absence of overt histological or radiological evidence of damage. This study examines the effects of low intensity (4-18 psi) blast overpressure (BOP) in Sprague-Dawley rats, delivered as a single Friedlander wave with a pneumatic shock tube Brain tissues were analyzed with quantitative PCR arrays and histological markers of injury at 2, 24 and 72 h after BOP exposure. Behavioral measurements were performed on other animals out to 21 days post-BOP exposure. A battery of rat vascular remodeling mRNAs and interleukins showed BOP related up- or down-regulation (≥1.8-fold) of greater magnitude and/or duration with increasing BOP intensity (ANOVA, $p<0.01$). This group included mRNAs for angiopoietin 1, inhibitor of DNA binding 1, endothelial differentiation sphingolipid G-protein-coupled receptor 1, endothelial PAS domain protein 1, α-laminin, endothelial cell growth factor 1 (platelet-derived), matrix metalloproteinase 2, Fibronectin 1 and Platelet/endothelial cell adhesion molecule. Other mRNAs were affected at higher threshold (~15 PSI peak), including mRNAs for vascular endothelial growth factors A and B, tissue inhibitors of metallopeptidase 2 and 3, thrombospondin 4, mitochondrial superoxide dismutase 2 and Bcl2-associated X protein. A late up-regulation (72 hours after blast wave exposure) was also observed for a number of stress genes (e.g., Bax, Nos1, Sod1, Sod2, Hsp family genes, Hmox1 and Hmox2) at the higher exposures, suggesting more severe direct and secondary brain injury at the higher peak intensity exposures. Histological findings from decalcified whole heads were consistent with a primary vascular (venous) mechanism of injury. Brain parenchyma appeared normal. There were microthrombi in small veins and limited areas of protein extravasation and hemorrhage near veins within the subdural and subarachnoid spaces, particularly in the velum interpositum. The extravasated protein often contained cellular infiltrate, presumably from the adjacent veins. Within the subarachnoid space, the superficial cortical veins appear to have more injury than veins in the choroidal fissure. Immunohistochemical findings included upregulated angiopoietin 1 staining of superficial vessels and upregulated C-X-C receptor 3 staining throughout telecephalic white matter. These findings are consistent with a tissue injury response to microvascular wounds that increased with peak shock overpressure intensity in mild BI-TBI.

387 A Compensatory Mechanism Mitigates the Detrimental Effects of Otoconin-90 Deletion on Otoconia Formation

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Otoconins and $CaCO_3$ are the major components of otoconia, which couple mechanic force to sensory hair cells in the vestibule for motion detection and bodily balance. As the endolymph has an extremely low Ca^{2+} concentration, otoconins have been hypothesized to be essential for $CaCO_3$ crystal formation. Yet, deletion of the predominant protein, otoconin-90 (Oc90), does not cause absence of crystals, but instead leads to formation of largely inorganic, giant crystals albeit with an overall 50% reduction of $CaCO_3$ incorporation. We therefore hypothesized that compensatory deposition of another protein(s) must have taken place to alleviate the detrimental effects of Oc90 deletion, and analyzed a number of candidates, including proteoglycans, tectorins, calbindin and bone/dentin matrix proteins, to see if they are present in otoconia crystals in wildtype (wt) and Oc90 null mice. Here we have found a great increase in the deposition of Sparc-like 1 (aka SC1 or hevin) in Oc90 null crystals. In the macular epithelial cells, SC1 protein level is 1.5-fold and the transcript level 1.9-fold in Oc90 null mice over that in wt tissues at P0. The expression and otoconial deposition of Sparc itself is also significantly increased in the neonatal Oc90 null vestibule compared to wt mice, but to a less degree than SC1. Other candidates show no significant difference in their otoconial deposition, or absence of it, in Oc90 wt and null mice. Together, the data show a Sparc family-specific adaptive response to Oc90 loss, and suggest a possible alternative pathway to ensure that otoconia morphogenesis is least impacted by an adverse condition. Based on the structural similarities between Oc90 and SC1, we posit these features as critical structural requirement for the otoconial matrix backbone protein. Such information will serve as the foundation for future regenerative purposes.

388 Cooption of Secretory Phospholipase (SPLA2) for Different Aspects of Gravity Receptor-Associated Mineralization in Vertebrate Phylogeny

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Otoconin22 (OC22) and Otoconin90 (OC90), the principal matrix proteins of aragonitic amphibian and calcitic avian and mammalian otoconia are homologs of secretory phospholipase A2 (sPLA2). OC22 represents the simplest type of cooption of the enzyme for CaCO3 modulation, since it consists of a single, strategically modified molecule (modified enzymatic site; increased anionic residues, with

moderate clustering). According to our molecular modeling experiments the rigid scaffold of the sPLA2 (disulfide bonds and alpha helical structure) is conserved, and adaptation to mineral modulation is effected by strategic folding of surface loops. Thereby the moderate anionic clustering of the primary sequence is amplified maximally, resulting in confluent anionic stretches - presumed active sites. OC22 is generally considered the prototypical CaCO3 modulator: 1. consisting of a single molecule, 2. representing an earlier evolutionary stage, and 3. expressing the unstable aragonitic polymorph.
OC90 can be considered an improvement over the basic design of OC22 because: 1. of duplication of sPLA2-like domains; 2. birds and mammals represent the highest evolutionary stage; and 3. calcite is the most stable polymorph. This concept of an evolutionary progression toward OC90 was shaken by the discovery of Otoc1, an ortholog of OC90 in the zebrafish, shown to be essential in early otolith nucleation [Petko et al., 2007]. Although Otoc1 fulfills the criteria as OC90 ortholog, the linker and terminal extensions are far longer than in OC90 and contain unprecedented levels of glutamate with polyglutamate stretches. These regions are also predicted to be highly phosphorylated. Consequently these regions are highly acidic and could act as principal nucleators in analogy to sialoprotein, an established nucleator of hydoxyapatite [Hunter et al., 2005]. By contrast, the sPLA2-like domains exhibit a near neutral electrostatic surface potential and may be poorly functional.
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389 Significance of Tertiary Structure in CaCO3 Modulators

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Evans et al. (2003) and others have proposed that unordered structure is an essential characteristic of CaCO3-modulating proteins, and that, in fact, folding is undesirable. This concept was formulated in invertebrate model systems, such as mollusk shell, and tested by advanced biophysical technique in biomimetic synthetic peptides. However, these concepts are not universally applicable to vertebrate CaCO3 systems, including vertebrate otoconia. The prototypical vertebrate otoconial matrix protein, otoconin22 is based on adaptation of a single secretory phospholipase A2 (sPLA2) molecule, a globular protein, whereas the corresponding mammalian otoconin90 (OC90) consists of two globular domains, but in addition contains large segments of unordered structure. Our biophysical solution state studies on recombinant otoconins indicate that the globular structure of sPLA2 is conserved in both otoconins. The only changes are present in some of the surface loops that contain most of the anionic residues. For instance in sPLA2-like domain 1 of OC90, folding results in amplification of the moderate anionic clustering of the primary sequence and two long contiguous anionic stretches at the solvent-exposed surface. The anionic stretches are surrounded by clusters of hydrogen-bonding residues, resulting in two-dimensional putative active regions. Clearly, linear synthetic peptides are inadequate to characterize these regions. Rather systematic substitution mutagenesis would be the strategy to define the mineral-modulating characteristics of these regions. Analogous anionic clusters are present in other vertebrate CaCO3 modulators, such as pancreatic lithostatin and the egg shell protein ansocalcin.
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390 Quantiative Analysis of Nystagmus by Image Analysis Technique

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It is essential for investigating vestibular disturbances to use an infrared CCD camera for recording eye. We devised an original eye movement image analysis technique using an infrared CCD camera, a personal computer and public domain software. The analysis was performed using the publish domain soft ware ImageJ program (developed by the U.S. National Institutes of Health). The video image from an infrared CCD camera was captured at 30 frames per second in 320*240. For analysis of the horizontal and vertical components, the X-Y center of the pupil was automatically calculated using the original macro. For analysis of torsional components, the whole iris pattern, which was rotated each 0.1 degrees, was overlaid with the same area of the next iris pattern, and the angle at which both iris patterns showed the greatest match was calculated. For quantitative analysis, slow phase velocity of each nystagmus, average of slow phase velocity, the visual suppression value, were analyzed automatically.
Using this technique, it is possible to inexpensively perform nystagmus analysis, including in quantitative analysis, from video images recorded by many types of infrared CCD cameras.

391 Posterior Semicircular Canal Dehiscence and Bony Findings of Posterior Semicircular Canal in Human Temporal Bone

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Objective: To evaluate the incidence of dehiscence of the posterior semicircular canal and the development of the distance between the posterior semicircular canal and posterior cranial fossa.
Background: Posterior semicircular canal dehiscence might have the potential to cause clinical symptoms, however, no histopathologic study of abnormally thin bone overlying the posterior semicircular canal has been reported.

Methods: The shortest distance between the posterior semicircular canal and posterior cranial fossa was measured in 1051 adult human temporal bones (557 cases) and temporal bones with a distance under 0.1 mm were evaluated. We also measured the shortest distance of 4 fetal human temporal bones (2 cases) and 110 temporal bones of children (55 cases).
Results: Of 1,051 temporal bones, 23 temporal bones (2.19%) had a distance under 0.1 mm. Two temporal bones (0.19%) had dehiscence and 2 temporal bones had micro-fractures in the thin bone, however, related clinical symptoms were not confirmed. In children, linear regression revealed a relationship between thickness and age ($r = 0.70$).
Conclusion: The histopathological incidence of dehiscence of the posterior semicircular canal was low. The etiology of dehiscence of the posterior semicircular canal may be a developmental anomaly, similar to dehiscence of the superior semicircular canal. In our study, the cases with a distance under 0.1mm and the cases with dehiscence of the posterior semicircular canal did not show apparent clinical symptoms related to canal dehiscence syndrome. Other factors, in addition to thinning of the bone, might be required to cause clinical manifestations.

392 Multi-Slice CT Overestimates Superior Canal Dehiscence Size

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The gold standard for diagnosis of superior canal dehiscence (SCD) has been multi-slice CT. However, partial volume averaging and filtering may confound the ability to detect thin bone next to low-radiodensity brain and inner ear fluids. We correlated radiographic and surgical findings in SCD to determine if multi-slice CT overestimated the size of SCD and if a threshold radiodensity could be defined, below which actual dehiscence could be predicted. Participants were 34 humans with a clinical diagnosis of SCD syndrome who underwent middle fossa approach for SC plugging. High resolution temporal bone CT scans were acquired axially with a Toshiba Aquilion scanner (step-mode, 120 kV, 300 mA, 1 s rotation, 0.5 mm collimation). Reconstructions (512 X 512 pixels) from a field of view ≤24 cm were made in 0.2 mm increments in planes parallel, vertically orthogonal, and radially orthogonal to the SC. Dehiscence length and width measured from these reconstructions were compared to measurements made at microsurgery. Differences between radiographic and actual length and width were both >0 ($p < 0.001$, one-sample t-test), indicating that CT tends to overestimate the size of SCD. Receiver operating characteristic analysis found that a threshold of -375 Hounsfield units predicted actual dehiscence.

393 Comparison of Cone-Beam and Multislice CT for Temporal Bone Evaluation

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Cone Beam Volumetric Tomography (CBVT) is gaining popularity because its smaller radiation dose (e.g., 5mA and 120kV) compared to conventional multislice CT (MSCT, 250mA and 135kV) allows the technology to be deployed directly to otolaryngology and dental offices. A disadvantage of CBVT is that the use of low energy photons results in less image contrast. However, there is potentially better spatial resolution compared to MSCT when inherent tissue contrast is high. This should be the case with superior canal dehiscence (SCD), where thin bone must be detected between low-radiodensity brain and perilymph, and with delineation of the stapes and cochlear implant arrays. Data from cadaveric temporal bones scanned with CBVT and MSCT are compared. Results demonstrate better spatial resolution of CBVT in these applications.

394 Lessons from Follow-Up Examinations in Patients with Vestibular Neuritis: How to Interpret VFT Findings at a Compensated Stage

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Objectives: Most patients complaining of dizziness seek medical services in the interictal period, which is thought to be a compensated stage. Thus, we wanted to investigate the results of vestibular function tests (VFTs) at a compensated stage in patients with vestibular neuritis to determine the presence and the sides of vestibular hypofunction.
Methods: We analyze the results of VFT including spontaneous nystagmus (SN), caloric, vibration-induced nystagmus (VIN), head-shaking nystagmus (HSN), and subjective visual vertical (SVV) tests in 38 patients (M/F = 23:15; age range, 15Y85 yr) with vestibular neuritis observed at around 2 months after the onset of vertigo.
Results: Thirty-seven (97%) of 39 patients showed pathologic results in at least 1 test. Pathologic results, based on caloric, SN, VIN, HSN, and SVV tests, were observed in 29 (76%), 20 (53%), 24 (63%), 33 (87%), and 15 patients (39%). Twenty-nine showed pathologic canal paresis (CP) on the affected side and 9 patients (24%) showed normal CP. There was no patient with pathologic CP on the intact side. In 29 patients with pathologic CP, pathologic results, based on SN, VIN, HSN, and SVV tests, were observed in 16 (55%), 20 (69%), 26 (90%), and 13 patients (45%). Three (10%) of 29 patients showed pathologic VIN or HSN, indicating that the intact side is pathologic. In 9 patients with normal CP, pathologic results, based on SN, VIN, HSN, and SVV tests, were observed in 4 (44%), 4, 7 (78%), and 2 patients (22%). Five (56%) of 9 patients showed pathologic results on the

intact side at least in 1 test, and the pathologic sides by each test were not the same.
Conclusion: Our findings suggest that we can detect vestibular imbalance in patients with unilateral vestibular hypofunction through a set of VFTs even when CP is normal at a compensated stage. The CP side indicated by caloric test was the real affected side when CP was pathologic, even if the results of other tests were normal or rarely indicated that the intact side was pathologic. If CP was within reference range, other tests can show the previous presence of vestibular imbalance; however, they could not predict the side of the vestibular hypofunction. These data provide strong support for enrolling a set of VFT when evaluating a dizzy patient.

395 Sinusoidal Off-Vertical Axis Rotation Test as a Clinical Otolith Function Test

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There is no practical test of the otolith-ocular reflex (OOR) in clinical use. The development of an evaluation tool for OOR is potentially important, because disorder of the otolith organs may be responsible for the complaints of some undiagnosed vertigo patients. It is well known that positional nystagmus and positional vertigo are usually successfully cured by canalith repositioning procedures in benign paroxysmal positional vertigo (BPPV), but some patients still complain of dizziness after treatment. The purposes of this study are to investigate the contributions of the semicircular canal versus otolith organ signals to the vestibule-ocular reflex (VOR) by providing canal-only (earth vertical axis rotation: EVAR) and canal plus otolith 30-degrees nose-up and nose-down conditions (off-vertical axis rotation: OVAR) and to investigate whether there were otolith dysfunctions or not in the patients with BPPV. We used 23 healthy adults and we also used 18 patients with BPPV. The rotational stimuli were delivered using a conventional rotation chair, except that the floor, the chair, and the walls surrounding the chair could be tilted up to 30 degrees to provide OVAR stimuli. In the OVAR session, we tilted the chair to 30 degrees both in nose-up and nose-down conditions. Stimuli were carried out sinusoidally at frequencies of 0.4 Hz and 0.8 Hz and a maximum angular velocity of 60 deg/sec both in the EVAR and OVAR. Horizontal eye movement was recorded and processed using infrared video oculography (SMI, GmbH, Berlin, Germany). Chair position signal was fed into the VOG and used to calculate the gain of the VOR.
There was no difference in the VOR gain between EVAR, and both nose-down and nose-up OVAR at 0.4 Hz and 0.8 Hz. In patients with BPPV, there was no difference in the VOR gain between EVAR, and both nose-down and nose-up OVAR at 0.4 Hz. However, gain during OVAR at 0.8Hz in the nose-up position in BPPV patients was significantly less than that during EVAR. The BPPV patients who had not only a history of episodes of positional vertigo, but also a dizziness or floating sensation all the time may have otolith dysfunctions.

396 Translational Vestibulo-Ocular Reflexes During Off-Vertical Axis Rotation

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The translational vestibulo-ocular reflex (tVOR) is an otolith-mediated response that stabilizes near vision during linear acceleration at higher frequencies where visually mediated reflexes are not adequate. The modulation of horizontal and vergence eye movements during Off-Vertical Axis Rotation (OVAR) are presumed to reflect the tVOR in response to the continuously varying linear acceleration in the interaural and naso-occipital axes, respectively. The purpose of this study was to examine the effect of frequency and fixation distance on the modulation of slow phase eye velocity (SPV) as further evidence that the tVOR is elicited during OVAR. Eighteen subjects were rotated about their longitudinal axis tilted by 30 deg off-vertical. Rotational velocities varied between 18 and 288 deg/sec corresponding to a frequency range of 0.05 to 0.8 Hz. Fixation distance was altered by asking subjects to imagine stationary targets that were briefly presented at 0.5, 1 and 2 m during some rotation cycles. The target flash was 40 msec in the nose-up position at eye level. Oculomotor responses were recorded in the dark using infrared binocular videography. Sinusoidal curve fits were used to derive amplitude, phase and bias velocity of the eye movements across multiple rotation cycles. Consistent with previous studies, the modulation of both horizontal and vergence SPV increased with stimulus frequency. The effect of fixation distance was negligible at lower frequencies. The modulation of horizontal and vergence SPV was; however, proportional to fixation distance during OVAR at 0.8 Hz. This increasing sensitivity and dependence on fixation distance of horizontal and vergence SPV during OVAR is consistent with tVOR characteristics measured during other types of linear motion. We conclude that the modulation of horizontal and vergence SPV will be diagnostically more useful at higher stimulus frequencies where the tVOR is more robust.

397 VEMPs Can Be Measured Even with Moderate and Varying-Intensity Contractions

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The Vestibular Evoked Myogenic Potential (VEMP) is a modulation of the electromyogram (EMG) from a contracted neck muscle, produced by the activation of the saccule by brief acoustic stimuli. VEMP responses are typically measured by stimulus-locked averages of the EMG from the sternocleidomastoid (SCM), and are being used to test peripheral vestibular function in the clinic.
VEMP amplitude is strongly influenced by the intensity of the muscle contraction. VEMP amplitude increases roughly linearly with root-mean-square (rms) EMG level. As a consequence, current methods of measuring VEMP require the subject to maintain a steady, sustained and intense voluntary contraction of the SCM. To compensate

for differences in contraction, some studies compute a "normalized" VEMP in which the VEMP is scaled by an estimate of the contraction effort, usually computed from EMG measured just prior to the acoustic stimulus. The test is physically demanding, and the ability to maintain the required contraction effort varies widely across subjects and test sessions, particularly for patients in poor health.
We examined whether the requirement of sustained, maximal effort is necessary in order to acquire a robust VEMP response. From a small population of normal subjects, we recorded the EMG in response to identical stimuli but with various contraction efforts. We found that even moderate contraction efforts yielded VEMP waveforms that are as informative as those recorded with maximum effort. By normalizing the response to each stimulus (each trace) by the contraction effort estimated from the rms EMG level, we compared responses recorded at different effort levels. We found differences in the efficacy of different normalization methods, but normalization by EMG level computed continuously is superior to scaling by a single pre-stimulus level. We conclude that the requirement of sustained, intense effort may be relaxed, provided trace-by-trace normalization is used.

398 Outcome Analysis of Vestibular Evoked Myogenic Potentials in Children with Enlarged Vestibular Aqueduct: Clinical Value and Implication

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Introduction: Enlarged vestibular aqueduct (EVA) is the most common inner ear anomaly in children with permanent hearing loss. This pathologic condition is not only responsible for hearing impairment but also accountable to vestibular symptoms in some patients. While the association of hearing loss and EVA has been well investigated in the past, the vestibular function is often over-looked, especially in young children. Vestibular evoked myogenic potential (VEMP), elicited by acoustic stimuli, provides a unique prospect to explore the inner ear dynamics in both auditory and vestibular apparatus.
Methods: Retrospective study of children with EVA seen in a pediatric tertiary care facility over the last three years was taken. A total of 25 cases (37 ears) of EVA were identified with complete records, including otologic evaluation, imaging studies and audiologic assessment. The diagnosis of EVA was confirmed by CT scan or/and MRI of the temporal bone. Pure-tone audiogram, tympanometry and VEMP testing were also performed. All results underwent correlation analysis.
Results: Hearing loss was found in 97% (36/37) of the ears with EVA. Air-bone gaps (conductive components) were found in all hearing losses with normal middle ear pressure and mobility. Abnormally low threshold VEMP responses were found in 92% (34/37) of the ears with EVA. VEMP were absent unilaterally in three EVA patients who had vestibular complaints. No clear correlation was found between the size of EVA and the audiologic findings.
Conclusions: The presence of air-bone gaps was found unrelated to any middle ear pathology. The abnormally low threshold VEMP responses suggested a "third" window effect in the pathologic condition of EVA. Unilateral absence of VEMP may implicate vestibular/balance impairment. These findings are valuable in clinical evaluation of young children who usually give limited and ambiguous input regarding their hearing and balance problems.

399 An Implementation of the Subjective Visual Horizontal Static Bias Vestibular Test for IPhone OS-Based Mobile Devices

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The Subjective Visual Horizontal Test (SVH) is a clinical measure of otolith function commonly used within a battery of oto-neurological tests assessing patients with inner-ear dizziness and balance problems. Existing systems typically utilize an LED array, or laser projection, to display a line which the patient must rotate to the horizontal using button press input. Prior to adaptation, vestibulopathic patients may present a bias of up to 20° towards the affected side. Our implementation of the SVH test uses the iPhone or iPod Touch device, which may prove more accessible and portable than existing systems, as well as being a more sensitive measure of vestibulopathy. Our system presents a bar, at a random orientation, on the screen. The subject rotates the device until the bar is perceived as horizontal, and taps the screen to indicate their response. Using inbuilt accelerometers, our software compares the dynamic device orientation with the actual bar angle to estimate any response bias. This paper will compare our implementation of the dynamic SVH test with an existing static LED clinical system, under various conditions, both in normal subjects, and in those presenting with a suspected otolith disorder. We aim to examine whether the bias measured using these two approaches is comparable, and if additional clinical data may be extracted through continuous recording of the device angle prior to the user response.

400 Accelerometry as a Measure of Vestibulospinal Function: Test-Retest Reliabilty and Relationship to Computerized Dynamic Posturgraphy

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There is a need for a clinically feasibly, inexpensive, quantifiable technology to measure vestibulospinal functioning. Accelerometry (ACC) technology shows promise for development of an easily implemented clinical measure of static postural control. The purpose of the study was to estimate test-retest reliability and determine if there was a relationship between ACC measures at the waist and center of pressure (COP) data from a force plate during the Sensory Organization Test (SOT) of computerized dynamic posturography. Subjects: Fifty-six subjects were recruited from the community with no known

orthopedic or vestibular deficit (33 females; mean age 50; SD 22; age range-19-84). Persons were screened over the phone and on site. Methods: Subjects were asked to complete 3 trials for each of the 6 SOT conditions while concurrently wearing the accelerometer. Data Analysis: The test-retest reliability of SOT COP (three trials) and ACC (RMS) was estimated for each test condition using the intraclass correlation coefficient (ICC) based on a two-way random effects model analysis of variance. The association between pelvic acceleration and center of pressure movement for each test condition was estimated using simple linear regression with COP as the dependent variable and measured pelvic acceleration as the predictor. Analysis for the relationship between ACC and COP for each condition was done separately for the first trial value and for the average value of each obtained from repeated measures. The coefficient of determination (r-squared) was used to compare the models using single and average measures. Results: Test retest reliability of ACC across 3 trials of all SOT test conditions ranged from ICC = .22 (condition 1) to ICC = .67 (condition 6). SOT COP ICC values ranged from ICC = .48 (condition 2) to ICC = .78 (condition 6). Pelvic acceleration was a significant predictor of SOT COP under every condition using both single and average measures. Using single measures, coefficient of determination (r-sq.) ranged from 0.10 (condition 1) to 0.60 (condition 6). Using average of three SOT trials, r-sq ranged from 0.17 (condition 1) to 0.62 (condition 6). Discussion/Conclusion: Accelerometry data displays similar test-retest reliability to the established measure of the SOT. Accelerometry best predicted SOT COP movement in Condition 6. The degree of association between ACC and SOT COP was equivalent when using the first or 3-trial average measures of performance. The use of an accelerometer may have value in estimating vestibulospinal function and one trial may be sufficient, thus minimizing clinical evaluation time.

401 Portable Quantitative Assessment of the Horizontal Vestibulo-Ocular Reflex

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An improved method for evaluating vestibular function using video-oculography can provide a quantitative bedside measurement of horizontal vestibulo-ocular reflex (VOR) function in response to the head impulse test. Currently available quantitative methods for peripheral vestibular testing are not suitable for bedside or routine clinical use. A quantitative bedside method will enable appropriate diagnosis of vestibular disorders.

We evaluated 10 healthy volunteers and one with profound bilateral vestibular loss with hand-delivered passive head impulses. Head and eye movements were measured with a head-set incorporating a head velocity ratemeter and high speed video-oculography. VOR gain was calculated by dividing the slope of the eye velocity tracing (eye acceleration) by the slope of the head velocity tracing (head acceleration) over a 40 ms window centered on peak head acceleration. We measured VOR gain during earth-horizontal head impulses from the midline to lateral and from lateral to midline positions.

The gain of normal subjects to head impulses was 1.00±0.13 (mean±SD). The gain of the vestibular deficient subject was 0.03±0.08. Gain in head impulses from midline to lateral and lateral to midline were not significantly different ($p>.05$). Gains were constant across peak head accelerations of 1500 to 3000 deg/sec^2.

Quantitative VOR measurements from our device are comparable with those obtained from other techniques, while retaining portability and ease of use suitable for busy clinical environments or the bedside. Potential applications include serial testing of patients receiving vestibulotoxic medications.

402 Frequency and Velocity Dependence of Vestibular Psychometric Thresholds

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Testing of vestibular function has relied almost exclusively on the responses of the vestibulo-ocular reflex. These techniques are unable to test higher balance-related circuits. Psychometric testing offers the potential to quantify function in these circuits. This technique has been used in many sensory systems but only in a very limited way in the vestibular system. We determined the discrimination thresholds to earth-horizontal rotations of normal and vestibular-deficient subjects using a two-alternative forced-choice paradigm. We used a stimulus trajectory minimizing the effect of the velocity-storage mechanism, which would otherwise have distorted the results of this testing paradigm. We found that the 79% psychometric thresholds were higher at a frequency of 0.3 Hz than 0.5 Hz, consistent with previous reports of frequency-dependent detection thresholds. We found that thresholds at 0.5 Hz rose from 2 deg/sec at at the detection threshold to 6 deg/sec at higher velocities, with poorer thresholds at 0.3 Hz. The bilaterally deficient subject had thresholds of at least 30 deg/sec. The sinusoidal paradigm considered here is successful at identifying patients with peripheral vestibular loss and is a promising tool for balance testing.

403 Path Integration and Vestibular Impairments at Different Speeds

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Path integration is the ability to keep track of one's location with reference to the starting point. This study tested the hypotheses that gait speed influences path integration performance and that patients with vestibular impairments will be impaired on the task. Normal adults, patients with benign paroxysmal positional vertigo, and patients with unilateral vestibular weakness were tested on a simple path integration task: walking 7.62 m straight ahead with eyes opened or closed at three frequencies timed by a metronome: 60 beats/min, 120 beats/min, and 176 beats/min. Preliminary analyses showed that at the slow speed normals walked significantly further than unilateral

weakness subjects before veering to one side, at the medium speed both patient groups veered more than normals, and at the fast speed patients drifted sooner than normals and or drifted at a greater angle than normals. These findings confirm the previous finding that vestibular impairments affect path integration skill and extend that finding to include the role of movement speed.
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404 Motor and Perceptual Inhibition in Patients with Vestibular Disorders

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Purpose. The purpose of this study was to examine motor and perceptual inhibition in patients with vestibular dysfunction. Subjects. Forty seven patients (mean age = 50 y, SD = 15 y) were tested using a Motor and Perceptual Inhibition Test (MAPIT) designed by Nassauer and Halperin (2003). Patients' performance was compared to that of a healthy group (n = 50, mean age = 53, SD = 19 y). Materials and methods.The MAPIT test measures subjects' manual reaction times (RT) when responding to different stimuli presented on a computer screen. During the perceptual inhibition (PI) task, subjects press a button that corresponds to the direction of a right- or left-pointing arrow that appears on the right or left side of the screen; in incongruent trials, the participant needs to inhibit a response toward a spatial stimulus (right arrow location) that is incongruent with the arrow's direction (e.g. left arrow). In congruent trials, the spatial and directional cues are the same. The PI time is the difference between the median RT during incongruent trials and congruent trials. The motor inhibition (MI) task consists of 2 blocks of centrally presented arrows. During the first block, subjects press a button toward the same side the arrow is pointing, and during the second block, toward the opposite side (i.e. inhibiting a motor response). The MI time is the difference between the median RT for the opposite block and the median RT for the same block.
Results. After adjusting for the effect of age, our results indicate that patients with vestibular disorders did not show any significant differences from healthy subjects on any of the measures ($p > 0.07$).
Conclusion. Recent evidence has shown that patients with vestibular disorders exhibit cognitive impairments in addition to the well documented balance difficulties. The results of this study demonstrate that vestibular dysfunction does not affect the patients' neural inhibition abilities during a manual button press task.
Key words. Motor inhibition, perceptual inhibition, vestibular dysfunction

405 Ocular Pursuit and Visual Suppression of the VOR Interfere with Auditory Information Processing Task Performance in Older Persons

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This study extends our research regarding interference between visual-vestibulo-ocular reflexes and cognitive task performance in older persons. Subjects performed dual-task paradigms that included either ocular pursuit or fixation suppression of the vestibular ocular reflex (VOR-fix) coupled with one of two auditory choice reaction time (RT) tasks. Subjects were healthy young (n=20;10F;24+/-1.8yrs), old (n=18;10F;69+/-3.3), and old-old (n=19;9F;79+/-2.4) adults. Ocular pursuit conditions consisted of 3 sinusoidal horizontal frequencies (0.1, 0.2, and 0.4 Hz) and a sum-of-sines stimulus. VOR-fix was conducted at 0.2 Hz and with a sum-of-sines stimulus during yaw rotation. The RT task was either a frequency discrimination task or a lateralization task. A no-movement baseline in darkness was obtained. Results indicated that RT during ocular pursuit and during VOR-fix was significantly slower than baseline ($p<.001$). This effect was significantly larger for ocular pursuit than for VOR-fix ($p<.001$). Stimulus predictability (i.e., a single frequency vs. sum-of-sines) had no effect. There was no age group effect, no task effect or any significant interactions with age. This study provides further evidence that ocular pursuit and VOR-fix employ somewhat different neural mechanisms. VOR-fix appears to be more "automatic," thereby leading to less interference than "voluntary" ocular pursuit. Despite the influence of stimulus predictability on ocular motor performance, stimulus predictability does not appear to influence interference with cognitive tasks. Also, this study corroborates our previous finding that the type of cognitive task (i.e., spatial vs. non-spatial) does not appear to influence interference and that age does not influence the pattern of interference.
This study was supported by NIH Grants AG 10009 and DC 05205.

406 Simple Inverted Pendulum Feedback Control Model for Posture

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The purpose of this research was to implement a feedback control model pertaining to quiet-stance posture. Experimental, quiet-stance center-of-pressure (COP) traces were used in conjunction with a computer model. A simple model of human postural control was implemented in MATLAB and Simulink. In this model, the human was treated as a single inverted pendulum of which the appropriate dynamics were determined. Stabilization of the human modeled as an inverted pendulum has been shown to require a component of corrective torque proportional to angular deviation (Kp), a component

proportional to the time derivative to angular deviation (Kd), as well as a third component proportional to the integral of the deviation signal (Ki). (Johansson et al., 1988). Thus, the neural control consists proportional, integrative and derivative (PID) control. Model parameters, such as Kp, Kd, Ki, noise gain (Kn), time-delay (τd), and sensory gain, could be determined by utilizing a multidimensional optimization procedure which minimized the error between the experimental COP and model-simulated data sets (as in Maurer and Peterka, 2005).

We have used the model for characterizing differences between human normal and vestibular loss patients under different test conditions (i.e. eye-open and eyes-closed conditions). However, the model could be used in conjunction with characterizing spontaneous sway of vestibular loss patients assisted by noninvasive sensory substitution, or even modified for characterization in animals assisted by an invasive device, such as the vestibular implant. For example, posture of normal rhesus monkeys, monkeys with bilateral vestibular hypofunction (BVH), and BVH-implanted monkeys could be compared using this model. We are currently working on these modifications.

407 Comorbidity of Vestibular and Neuropsychiatric Disorders: The Search for Causality

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Neuroanatomical and physiological studies have demonstrated extensive interaction of the vestibular system with cognitive and limbic brain sites. These observations are frequently cited to account for the clinical cases of comorbidity between vestibular and neuropsychiatric deficits. However, the causality governing the comorbidity is yet to be demonstrated. To this end we studied Headbanger (*Hdb*), a dominant ENU-induced mouse mutant with a mutation in the myosin VII gene that is a progressive model of vestibular deficit. We showed that from about two months of age, *Hdb* mice present a significant reduction in balance skills on the rotarod test, reduced vestibulo-autonomic reflex in the form of reduced rectal temperature response to hypergravitation, deficient exploration pattern in the open-field, and enhanced anxiety in the elevated-plus-maze and open-field tests. Rehabilitation of the balance skills, by raising the mice in cages with 'acrobatic' devices, ameliorated the symptoms of anxiety. These findings support the suggestion that vestibular system is causally related to disorders of anxiety and cognition. The clinical implication is that some psychiatric disorders should be treated by neurological means.

408 Preliminary Results Using Sensory Substitution for Balance Disorders

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Objective: Balance disorders are common sequela for our wounded service members with traumatic brain injury. These balance disorders result in substantial functional disability which may prevent return to duty. Depending on the specific etiology of the disorder, approaches for treatment may include medication, physical therapy, and/or surgery. Despite these modalities, used singly or in combination, some patients are not able to recover. Some promising research has been completed in utilizing sensory substitution devices for patients with peripheral sensory losses. This project seeks to determine whether tactile sensory substitution using the BrainPort® balance device is an effective treatment for patients with balance disorders who have failed to improve with traditional physical therapy.

Study Design: Human, pilot study.

Methods: Patients meeting inclusion criteria are offered enrollment. Participants take part in 20 training sessions in the clinic setting over the course two weeks, returning weekly for follow-up. They are then instructed to self-administer the device twice a day until symptom resolution or up to one year. The Dynamic Gait Index (DGI) and the Neurocom Computerized Dynamic Posturography (CDP) system using the Sensory Organization Test (SOT) are performed as objective outcome measures of functional status before and at the conclusion of treatment.

Results: Ten of 11 patients showed improvement in composite SOT score and/or DGI. Four patients normalized their composite SOT scores. Only one individual demonstrated a decrease in *both* SOT and DGI.

Conclusion: This pilot investigation provides preliminary evidence that BrainPort® may be a useful application in patients as most patients did derive benefit when all other treatment options had been exhausted. Additional study is warranted to better define the effect as well as possible applications of this device in wounded service members. This pilot data can be used to develop new protocols for use of this technology.

409 Analysis of Mechanism of Malignancy Enhanced by Epithelial-Mesenchimal Transition in Squamous Cell Carcinoma of the Temporal Bone

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Objective: We investigated the prognostic factors for SCC of the temporal bone, and show that extensive bone erosion correlates with a worse prognosis of the squamous cell carcinoma (SCC) of the temporal bone . Moreover we recentry investigated the roles of Epiterial-Mesenchymal Transition(EMT) in SCC of the temporal bone . Patients and methods: Clinical symptoms of the patients with primary

SCC of the external auditory canal (EAC) or middle ear (ME) were reviewed based on medical records. Correlation of clinical symptoms and cancer severity staging using the modified Pittsburgh classification was analyzed, along with disease-specific survival (DSS). Expressions of E-cadherin, vimentin, in 16 patients were also examined to investigate the roles of EMT in SCC of the temporal bone by immunohistochemical analysis. Results: Sixteen patients with primary SCC of the EAC (n=13) or ME (n=3) were included in the study population. DSS was not influenced by whether a hearing disturbance or otalgia was noted at the first medical examination. Extended bone involvement identified with imaging studies significantly correlated with worse prognosis ($p<0.05$). Prognoses of patients without extensive bone erosion were good, and extensive (>/=0.5 cm) soft tissue involvement did not correlate with prognosis in this study. In this study, we also show the roles of Epiterial-Mesenchymal Transition(EMT) in SCC of the temporal bone Conclusion: Extensive bone erosion correlated with a worse prognosis of the squamous cell carcinoma (SCC) of the temporal bone but extensive soft tissue involvement did not correlate with prognosis in this study.

410 Incidence Trends for HPV-Related and Unrelated Squamous Cell Carcinoma of the Head and Neck in New Mexico, 1981-2006

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Objectives: We are utilizing New Mexico cancer surveillance data to:
1) analyze and describe temporal trends in the incidence of human papillomavirus (HPV)-related squamous cell carcinoma of the head and neck (SCCHN) and HPV-unrelated SCCHN, among the populations of New Mexico from 1981-2006.
2) evaluate the relationship between incidence trends for HPV-related SCCHN and cervical carcinoma, in parallel with HPV-unrelated SCCHN and lung cancer.
3) compare the incidence of these malignancies in New Mexico with their national incidence trends.
Methods: This descriptive epidemiological study is being conducted with the use of data that have been collected through the New Mexico Tumor Registry (NMTR), a member of the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) Program. We are querying existing records contained within the NMTR to identify all cases of HPV-related SCCHN, HPV-unrelated SCCHN, lung malignancy, cervical carcinoma in situ, and invasive cervical carcinoma that were diagnosed in New Mexico from 1981-2006. Data is being analyzed using SEER*Stat and the investigators will calculate age-adjusted cancer incidence rates by the direct method. Rate ratios and associated 95% confidence intervals will be used to compare rates by sex and race/ethnic groups. Trends in age-adjusted incidence rates will be assessed with the Joinpoint regression analysis tool developed by the National Cancer Institute.
Results: Head and neck cancer outcomes are significantly and independently affected by race and poverty level, and nationally, 25% of all SCCHN is HPV-related. Existing research indicates that the incidence of in situ and invasive cervical carcinoma, attributed to HPV 16 and 18 in New Mexico is heavily influenced by racial and socio-economic factors. Prior studies have demonstrated a significant reduction in the incidence of invasive cervical cancer following secondary prevention efforts targeting these groups.
Conclusions: We expect that the incidence of head and neck carcinoma in New Mexico is significantly affected by oncologic HPV infection with disparate frequency in the various populations of New Mexico. Based on our results, we hope to institute a program of primary prevention in high-risk groups that may impact the incidence of HPV-related SCCHN in New Mexico

411 Imaging Mass Spectrometry for the Analysis of Head and Neck Tumor

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Mass spectrometer enables us to identify thousands of known and/or unknown molecules in a sample and it has been one of the main tools for the proteome analysis in the post-genomics era. However, it was impossible to locate the molecules on a tissue section. Recently, imaging mass spectrometry (IMS) has been developed in several research groups including Hamamatsu University group. IMS is a technique used in mass spectrometry to visualize the spatial distribution of molecules, such as phospholipids, proteins, and glycolipids. In IMS, the tissue sections is directly raster-scanned by matrix-assisted laser desorption/ionization (MALDI) and the ions are separated by time-of-flight (TOF). The distribution of a biomolecule is two-dimensionally visualized as the relative signal intensities among the measurement points of the tissue section. Although IMS is spreading explosively in many fields including biology and pathology, there has been no report in otolaryngology. This study is the first to analysis the head and neck tumor by IMS. Two cases of adenomatous goiter and three cases of thyroid papillary carcinoma were involved in the study. Sample tissue was crio-sectioned and the sections were used for the analysis. Thousands of molecular peaks were obtained by IMS and the distributions of known lipid molecules were analyzed. Sphingomyelin was found to be highly expressed in tumor portions. Some phosphatidylcholine molecules were strongly expressed in cancer area than in normal thyroid tissue, whereas the expression was lower than control in adenomatous goiter cases. IMS is a powerful tool to perform in situ proteomics and will lead to novel findings in the mechanisms of carcinogenesis, diagnosis, and treatment of head and neck cancer.

412 Ototoxicity and Bacteriostatic Activity of Methylrosaniline Chloride (Gentian Violet) in the Guinea Pig

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Introduction and Purpose: Empirical treatment of chronic otitis externa with Gentian Violet (GV) has shown excellent therapeutic usefulness. When a GV solution is used in an ear with perforated ear drums, the drug may come into contact with the round window membrane, and thus finds it way to the inner ear. We have previously examined the ototoxic effects of 0.5% GV in the guinea pig cochlea, using eighth nerve compound action potentials (CAP), (ARO meeting in February 2008). In this report, we examine the ototoxic effects of more dilute 0.13% GV and evaluate bacteriostatic activity of GV.

Materials and Methods: Ototoxicity was evaluated in guinea pigs by measureing CAP. The stimulus consisted of click sounds and tone bursts of 4 and 8k Hz. The middle ear cavities of the animals were filled with 0.13 % solution of GV and 5 minutes later the cavity was washed with saline. CAP was measured after 24hours. The bacteriostatic activity of the solution against two strains of *MRSA, P.aeruginosa, S.pneumonia, M.catarrahalis* and *H.influenzae* isolated from ears of patients in our clinic was also studied.

Results: We previously reported no ototoxicity was detected at 30 minutes when using a 0.5% solution, but that same concentration caused complete abolishment of CAP by 24 hours. In this report, the middle ear cavity was filled with an 0.13% GV for 5 minutes, and the cavity was then thoroughly washed with saline solution. The CAP measured at 24 hours showed severe reduction. GV was found to be effective against all bacterial species tested except for *P.aeruginosa*

Conclusions: Although GV has excellent antibacterial and antifungal activity, the use of GV should be limited to the external ear canal. The use of this drug in the middle ear cavity in not recommended even with concentrations as low as 0.13%. CV was not effective against *P.aeruginosa*.

413 Effect of Burow Solution on the Basement Membrane Anionic Sites in the Stria Vascularis

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Burow solution comprises aluminum sulfate and acetic acid; it is an otic preparation that is directly applied to the tympanic cavity. Recently, we have shown that the application of Burow solution induces a loss of the outer hair cells in the basal turn of the cochlea. Application of acetic acid to the round window membrane (RWM) causes a decrease in the pH of the perilymph and endolymph, which leads to a decrease in the endocochlear potential. Therefore, application of Burow solution may induce a decrease in the pH in the inner ear tissue, and thereby cause a loss of hearing. A decrease in the number of anionic sites in the basement membrane is attributed to the decrease in pH in the inner ear tissue. In this study, we investigated time-dependent changes in the auditory brainstem response (ABR) and basement membrane anionic sites in the strial vessels. Burow solution was applied to the tympanic cavity of normal guinea pigs and retained on the RWM for 2 h. In the control pigs, distilled water was used instead of Burow solution. In each animal, ABRs were recorded at 4, 8, and 20 kHz immediately before the surgery and immediately or on the 2 days after the surgery. Decapitation was performed immediately or on the 2 days after the surgery. The bony labyrinth of the animal was first immersed in a 0.5% cationic polyethyleneimine (PEI) solution for 15 min, after which it was immersed for 2 h in a solution of 1% phosphotungstic acid and 2.5% glutaraldehyde; finally, it was immersed in a 10% EDTA solution. After decalcification, ultrathin sections of the basal and third turns of the cochlea were examined using a transmission electron microscope. The number and size of the PEI particles in the capillary basement membrane of the stria vascularis were measured. Immediately after the surgery, ABR threshold shifts at 4, 8, and 20 kHz significantly increased in the test animals as compared to those in the control animals. On the 2 days after the surgery, ABR threshold shifts at 4 and 8 kHz recovered to the control level. As compared to the control animals, the distribution of the PEI particles in the basal and third turn of the cochlea significantly decreased immediately after the surgery. However, on the 2 days after the surgery, PEI particle distribution recovered to the control level. These findings suggest that intratympanic application of Burow solution induces a decrease in pH in the stria vascularis, and thereby causes a temporary ABR threshold shift; further, that a decrease in pH in the stria vascularis may not be the only cause of the permanent ABR threshold shift at 20 kHz.

414 A Pre and Postoperative Bacteriological Study of Chronic Suppurative Otitis Media; a Multicenter Study

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Objectives: Although many bacteriological studies on preoperative otorrhea in patients with chronic suppurative otitis media (CSOM) have been performed, there are few studies on postoperative otorrhea. In this study we analyzed the pathogenic microorganisms, changes in the bacterial species before and after surgery, and the antibiotic sensitivity on preoperative and postoperative cultures.

Subjects and Methods: This was a retrospective study of 87 patients with chronic suppurative otitis media; preoperative and postoperative otorrhea samples were obtained from January 2002 to April 2009.

Results: Four species of organisms, methicillin sensitive Staphylococcus aureus (MSSA), methicillin-resistant Staphylococcus aureus (MRSA), Pseudomonas, and coagulase-negative Staphylococcus showed higher

prevalence than others on both the preoperative and postoperative cultures. Among 67 patients with positive preoperative cultures, the same bacteria was cultured in 15 patients (22.4%), another bacteria and fungi in 34 (50.8%) and 2 (3.0%) each, and no growth was confirmed in 16 patients (23.8%) on postoperative bacteriological testing. Of the 20 patients with no growth on preoperative cultures, bacteria were cultured in 9 patients (45.0%), fungi in 3 patients (15.0%) and there was still no growth confirmed in 8 patients (40.0%). MSSA and MRSA were rarely recultured in the immediate postoperative bacteriological testing due to good control with antibiotics and surgery, but usually detected in late onset postoperative otorrhea. However, this was not true for Pseudomonas.
Conclusions: Ciprofloxacin resistant Pseudomonas aeroginosa occasionally causes early onset postoperative otorrhea. There are few highly sensitive antibiotics for this species. To reduce the frequency of postoperative otorrhea, and cure CSOM, otolaryngologists should perform bacteriological studies early during the course of the infection and monitor for nosocomial infections.

415 Evaluation of a Prototype Robot for the Microsurgery of the Middle Ear in Human Temporal Bone Specimens

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Introduction: Previously, we designed a tele-operated assistance robot with 2 arms for the microsurgery of the middle ear. In order to assess the performances and the ergonomics of this robot, a prototype with only one arm was manufactured. The micromanipulator carried by the robotic arm has the particularity of yielding a mimimal view obstruction, and being able to achieve complexe mouvements without a dextrous tool or intracorporal mobility. The aim of this work was to assess the accessibility to different target points in the middle ear cleft and the visual obstruction with the robot in human temporal bones under operative microscope.
Materials and Methods: The prototype was composed of a command unit with a pen-like interface (Phantom Omni, Sensable ©, Woburn, MA), a robotic arm carrying a high precision, tool-carrier micromanipulator. The micromanipulator was connected to a microhook. The robot base was placed in front of the surgeon and the commande interface under his right hand. An operating microscope with a 400 mm focal was used for these experiments. Assessment was carried out with and without ear speculum (diameter = 7 mm) on 3 human temporal bones. Accessibilty of the tympanic membrane was tested in 4 cardinal points with the microhook. Then, an endaural approach was performed without the robot, and the tympanic membrane was displaced anteriorly. Accessibility of oval and round windows, and the hypotympanic region was evaluated.
Results: All target regions of the tympanic membrane and the middle ear cleft could be reached with the microhook with minimal visual obstruction and without damage to the ossicular chain in the 3 temporal bones. Moreover, instrument declination backward in the sinus tympani, and upward in the attic region were possible with moderate visual obstruction. Introduction of the microhook into the incudostapedial joint and rotation of the instrument allowed ossicular separation without any translation. Command delay between the interface and the robot appeared to be crucial in the ergonomics of the robot.
Conclusion: Evaluation of this prototype in human temporal bones demonstrated the validity of the workspace evaluation and the choice of the kinematics. The visual obstruction was moderate to minimal in all target points and can allow procedures in the oval window or on the ossicular chain

416 Assessment of Mental Representation of Mastoidectomy by a Computer-Based Drawing Tool

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Objective : The aim of this study was to elaborate a simple computer-based drawing tool to assess the mental representation of mastoidectomy.
Materials and methods : Twelve trainees in otology (5 beginners and 7 mid-level) and 4 otology experts were included in this prospective study. The image of a left mastoid was displayed on a 17" screen in a full-screen mode. All subjects reproduced the movements of mastoidectomy with a pen on a graphic tablet (Cadboy Ultratablet, NGS, Errenteria, Espagne). Virtual drill movements appeared as grey or black lines on the image. Surgeons were evaluated before and after a 2-day dissection course. Total surface of mastoidectomy (% of reference), surface in each anatomic zone of mastoid, perimeter, circularity (4π(surface/perimeter2)) and the angle between the traces and the edges of the cavity were measured by Image J software (NIH, Washington DC).
Results : Total surface of mastoidectomy was higher in experts than in mid-level and beginner trainees (respectively 99 ± 6,5 %, versus 57 ± 1,5 %, et 22 ± 5,6 %, $p<0,01$ for experts versus beginners and $p<0,05$ for experts versus mid-level, ANOVA and Bonferroni). Circularity was also higher in experts than in trainees. After training, total surface and circularity increased in trainees. Angle between traces and cavity edges was lower in experts than in trainees and was reduced after training.
Conclusions : This simple computer-based drawing tool provides valid and precious information on mental representation of mastoidectomy at its initial phase.

417 Canalostomy as a Surgical Approach for Cochlear Gene Therapy in the Rat

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Mice and guinea pigs are established in vivo models for cochlear gene therapy. The anatomy of the rat inner ear, with a relatively thick bone is a challenging system for gene delivery. Vector delivery via cochleostomy or injection through the round window causes concomitant sensorineural hearing loss and is therefore not suitable for studies where the change in hearing is being followed. Compared to the mouse, the rat does not demonstrate easily recognizable landmarks that allow for use of the semicircular canals as an approach to the inner ear.

We analyzed sagittal and coronal temporal bone sections of Long Evans rats and identified the bony entrance of the facial nerve as a crucial landmark for canalostomy. ABR and DPOAE measurements revealed minimal differences in the hearing threshold after adenovirus vector application when large volumes of vector were infused to the inner ear. Canalostomy and infusion of adenoviral vectors also resulted in temporary balance disturbance in the rat. Immunohistochemical assessment after delivery of a green fluorescent protein (GFP) expressing vector showed significant GFP expression in the cochlea compared to controls.

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418 The Ultrasonic Approach to Cochleostomy: Inner Ear Effects of Otic Capsule Drilling in the Rat

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Hypothesis: Drilling on the otic capsule for cochleostomy should be less traumatic to the cochlea using the Piezosurgery® Medical device (PZ) rather than a standard diamond drill (DD). Background: Soft cochleostomy is used for preservation of residual hearing in cochlear implant patients. PZ drilling can be used for accurate cochleostomy placement with minimal soft tissue damage and may be superior for atraumatic drilling on the cochlea, compared to a DD. This study compared inner ear effects after drilling the rat otic capsule using the PZ versus the DD.

Study Design: Prospective animal study utilizing rats.

Methods: Otic capsule drilling, with irrigation, was performed on the left ear using the DD (n=5) or the PZ (n=5), monitoring otic capsule temperature. Contralateral ears served as controls. The animals were sacrificed after one week. Organ of Corti damage was morphologically evaluated and compared between groups.

Results: Basal turn hair cell loss was observed in all ears in the PZ group, regardless of drilling depth. However, no cochlear damage was found in any ears in the DD group. Overheating of tissue was not a factor, as average otic capsule temperature decreased for both groups during surgery.

Conclusion: Otic capsule drilling with the PZ results in greater trauma to the rat inner ear than conventional drilling methods.

This study has been submitted to Otology and Neurotology for publication.

419 Effect of DC Current on Biofilm Formation on Platinum/Iridium Electrodes

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Formation of bacterial biofilm on cochlear implants causes infections refractory to antibiotic treatment. These infections necessitate the removal and replacement of a cochlear implant, with the potential loss of hearing function. Therefore, devising an effective and easily accessible antimicrobial treatment, eliminating the need for removal, would be most beneficial. Taking into consideration that charged surfaces interfere with adhesion of bacteria, we tested the effect of DC electrical stimulation on development of biofilm and viability of bacteria. Using constant DC currents at levels tolerated by the human cochlea, we grew *S.aureus* biofilms on platinum-iridium ball electrodes; we examined changes in biofilm formation using SEM, live/dead stain, and confocal microscopy. *S.aureus* was still able to form biofilm under all conditions tested after 12 hours incubation. The ultrastructure of the biofilm and bacterial survival were current-dependent. The greatest reduction in biofilm was observed on both the positively and negatively charged electrodes in response to 100μA current. Significant reduction in biofilm coverage and decrease in cell viability was also seen on positively charged electrodes at 0.1μA. More biofilm extracellular matrix was seen on the positively versus negatively charged electrodes. We conclude that biofilm formation on platinum/iridium electrodes is affected by DC current at levels of physiologic interest. Additional data on other bacteria of interest will be combined with these results and reported in this presentation.

420 A Case of Intracochlear Schwannoma with Progressive Hearing Loss

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Intralabyrinthine schwannomas are rare tumors. It is difficult to diagnose them, and their hearing disturbance has not been sufficiently elucidated. Recently, however, the development of the imaging technology enables the diagnosis of intracochlear schwannoma before operation. We experienced a case of intracochlear schwannoma diagnosed with mild hearing loss.

In our rare case, pure tone audiometry (PTA) showed hearing loss at mid-frequency in the right ear, with a mean threshold of 33.3 dB. Alternate binaural loudness balance test revealed complete recruitment, and distortion product

otoacoustic emission levels at 1 kHz were in the region of uncertainty in the right ear. Auditory brain stem response was normal at the beginning of follow-up. However, magnetic resonance imaging (MRI) revealed an abnormal lesion in the cochlea. For approximately 2 years after the diagnosis, we followed up with serial audiometric test, which indicated progressive hearing loss. Finally, PTA showed profound deafness and MRI revealed invasion of the schwannoma into the fundus of the internal auditory canal.
Intracochlear schwannoma may be difficult to diagnose even with gadolinium-enhanced MRI. However, the development of the imaging technology help the diagnosis of intracochlear schwannoma. In our case, a constructive interference in the steady-state sequence image was useful to the evaluation of intracochlear schwannoma. In order not to overlook it, it is necessary to pay attention to abnormal lesions within the cochlea or vestibule, and a suspicious lesion should be confirmed by a repeat scan.

421 Repetitive Transcranial Magnetic Stimulation for Tinnitus Treatment: Identification of Clinical Predictors for Treatment Response

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Background: There is increasing evidence that repetitive transcranial magnetic stimulation (rTMS) can reduce chronic tinnitus. However treatment results are characterized by high interindividual variability. Therefore the identification of clinical predictors for treatment response is of utmost importance for the correct patient selection.
Methods: Clinical data of 194 tinnitus patients were evaluated. All patients were treated with a standardized rTMS procedure (1 Hz, 10 days, 2000 stimuli/day, over the left temporal cortex). It was investigated, whether the following demographic and clinical parameters had a significant effect on the outcome as assessed with a standardized tinnitus questionnaire (TQ): age, gender, depression scores in Beck Depression Inventory (BDI) and tinnitus severity (TQ) before rTMS ,lateralization, frequency and duration of tinnitus, extent of hearing loss and measures of cortical excitability .
Results: An effect of tinnitus laterality was observed. In patients with left-sided or bilateral tinnitus rTMS results in a statistically significant reduction of TQ scores whereas patients with right-sided tinnitus did not show a significant improvement after rTMS treatment. In correlation analyses we found that in the subgroup of treatment responders tinnitus duration influenced rTMS outcome in a statistically significant way. A short history of complaints was associated with better treatment outcome. In addition, a multiple regression analysis identified the TQ score at baseline as a significant predictor for treatment outcome: the higher the TQ score was at baseline, the greater was the reduction of the TQ score after rTMS therapy. For all other investigated parameters no statistically significant effect was found.
Conclusion: Tinnitus laterality, tinnitus duration and baseline TQ score appeared to be clinical factors with significant impact on treatment outcome after rTMS therapy.

422 Neuroimaging of Deaf Children Following Cochlear Implantation: Speech-Evoked Activity in the Auditory Cortex

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Cochlear implants (CI) can provide auditory stimulation with the potential to allow normal speech and language development in a deaf child. However, CI programming is a challenging, individualized, and iterative process with variable success. There is currently a gap our clinical ability to assess CI function at the level of cortical activity in response to speech. Near-infrared spectroscopy (NIRS) offers a viable alternative to functional magnetic resonance imaging (fMRI), electroencephalography, and positron emission tomography for regular imaging of speech-evoked cortical activity in awake, deaf children using CIs. Using fMRI and NIRS in normal hearing adults listening to the same stimuli, we have detected similar speech-evoked auditory cortex activity. In subjects able to complete testing, we have demonstrated speech-evoked auditory cortex activity using NIRS in 73% normal hearing adults (8 of 11), 82% of normal hearing children (9 of 11), 60% of deaf children using CIs (22 of 37), and 83% of deaf children at the time of CI activation (5 of 6). We were able to successfully complete NIRS testing in at least 90% of the subjects from the first three cohorts, and 60% of children at their CI activation. NIRS shows promise as a powerful adjunct to current CI assessment tools, especially for children whose behavioral responses can be variable or difficult to measure.

423 In Vivo Determination of Olfactory Mucus Cation Concentrations in Normal and Inflammatory States

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Background: Olfaction is impaired in allergic rhinitis. We hypothesize that chronic rhinitis will induce changes in cation concentrations, which may affect the “chemo-electrical” transduction.
Objective: This animal study is designed to determine changes in cation concentration in the olfactory mucus of mice with allergic rhinitis.

Methods: Inflammation was induced by sensitization and chronic exposure of 16 C57BL/6 mice to *Aspergillus fumigatus*. The control group included nine untreated mice. Ion-selective microelectrodes were used to measure free cation concentrations.
Results: Olfactory mucus of chronically inflamed mice had lower [Na^+] than controls, 84.8 ± 4.45 mM versus 93.73 ± 3.06 mM, $p<0.05$, and higher [K^+] 7.2 ± 0.65 mM versus 5.7 ± 0.20 mM, $p<0.05$. No significant difference existed in the [Ca^{2+}], 0.50 ± 0.12 mM versus 0.54 ± 0.06 mM $p = 0.39$.
Conclusion: Chronic inflammation elevates the potassium and lowers the sodium ion concentration in the olfactory mucus, possibly affecting the microenvironment of olfactory transduction.

424 Cortical Electrophysiology of Infant Hearing for Tones and Speech

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Audiologists are asked to fit hearing aids and program cochlear implants for infants less than a year of age. There are behavioral and electrophysiologic methods that can be used to estimate hearing thresholds needed to fit these devices, yet the evaluation of hearing abilities beyond sensitivity remain elusive in this age group. The current research is aimed at developing methods for determining if a hearing device provides access to the acoustic cues needed to discriminate speech features. The first step was to evaluate the correspondence between psychophysical measures of pure tone and speech sound detection and cortical auditory evoked potentials (CAEP). The participants in this experiment were infants between 5-12 months of age (mean= 8.5 mo) and young adults (mean = 22 yr). All participants had normal hearing. Perceptual thresholds were obtained for 50 ms tonebursts at 500, 1000, 2000 and 4000 Hz and for 50 ms tokens of the following speech sounds: /a, i, u, o, m, s, ʃ/. Observer-based psychophysical methods were employed for threshold tests. CAEPs were obtained in response to the same tone and speech tokens presented at -20 to 40 dB re: perceptual threshold. All participants were awake and alert during CAEP tests. Stimuli for perceptual and CAEP tests were presented in sound-field. Perceptual thresholds in infants were elevated, on average, by 24 dB for tones, and 28 dB for speech tokens in comparison to adults. Initial analyses of the CAEP data indicate latency and amplitude differences in the P1-N1-P2 complex obtained at supra-threshold levels that are attributable to spectral characteristics of the speech sound tokens. CAEP thresholds for adults are elevated by 10-20 dB with respect to perceptual threshold, while in infants, CAEP thresholds are highly variable with respect to perceptual threshold. Amplitude input-output functions for infants are steeper than those of adults suggesting differences in loudness perception.

425 Ten-Year Follow-Up of a Universal Newborn Hearing Screening Programme Based on Multiple Transient-Evoked Otoacoustic Emissions and Clinical Brainstem Response Audiometry

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Follow-ups were performed 6.5 and 10 years after the start of a six-year universal newborn hearing-screening programme based on multiple transient-evoked otoacoustic emissions (TEOAEs) and clinical click-evoked brainstem response audiometry (BRA), to study programme efficacy, prevalence and effects of sex and left/right ear on permanent hearing loss (n=31,000). The proportion of screened newborns was high, 98%. Multiple TEOAE recordings reduced the need for clinical BRA, only 0.8% of the screened newborns underwent BRA. Maximum TEOAE pass ratio occurred at postnatal day 5 (generally higher pass ratio in right ears). Bilateral permanent hearing loss (exceeding about 30 dB HL) was found in 0.18% of the screened newborns (n=57, 32 males and 25 females). They showed a median ABR threshold of 60 dB nHL (median age=2.5 months). A lower proportion revealed unilateral hearing loss (overweight of males and left ears). The proportion of congenital hearing loss was higher in males and in left ears than in females and right ears, thus corroborating the previously reported effects of sex and ear in TEOAE levels, at birth.

426 The History of Screening for Hearing Ability - Part 1- Pediatrics

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The need for hearing screening of school children (HSSC) was noted in 1876 but its implementation awaited the establishment of a valid and reliable test and documentation of effectiveness of early intervention(s).The first school age screening was carried out after the development of the electronic audiometer in 1922. Newborn Hearing Screening (NHS) was conceived as need in the late 1950's. L. Fisch in 1956 appears to be the first to recommend the use of a high risk registry. A prospective trial of testing new born, 4 and 12 moth olds was carried from1959 to 1962. It was then concluded that the newborn testing was unreliable and this report again suggested the use of a high risk registry. The newborn nursery was identified in 1964 as the optimal setting to carry our NHS. The high risk registry became the standard tool for NHS until the application of transient evoked otoacoustic emissions for NHS in the early 1990's. Universal NHS was adopted in the end of the 20th century and recently genetic NHS programs have been initiated. The criteria for passing NHS accounts only for sever to profound and for HSSC it is based on an empirical average that does not take into account the variable needs of children. There is now, in much of the world, NHS and HSSC. A major unaddressed concern of childhood hearing

screening is what is considered as a pass and how this differs in children with diverse needs.

427 The Dichotomy in Hearing of Cystic Fibrosis (CF) Children Following High Exposure to Aminoglycosides: A Study Using DPOAEs and Extended High-Frequency Audiometry

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The toxic effect of aminoglycosides (AG) on hearing has been known since their discovery in the late 1940s yet controversy over incidence still exists with a reported incidence of ototoxicity ranging from 0-41%.
In this study, auditory testing was performed for CF children at Great Ormond Street Hospital, UK, as they are exposed to aminoglycosides to treat respiratory infections. 45 children were tested using conventional and extended high frequency audiometry and DPOAEs. They were divided into 3 groups according to AG exposure history into; non-exposure (n=6), low (n=13) and high (n=26) exposure groups. Eight (31%) of the high exposure group showed ototoxicity. This was evidenced through significant increases in audiometric thresholds at frequencies 8-20kHz and through decreased amplitudes of 2f1-f2 DPOAEs at f2 frequencies 3.2-6.3kHz. These results did not overlap with those of the other children who showed completely normal, even exceptionally good, hearing.
Conclusion: There appears to be an unknown factor influencing whether the high AG exposure does or does not result in HF hearing loss. This could be an exposure intensity factor, other environmental factors or genetic susceptibility. Further investigation taking into account better dose documentation, genetic analysis, frequent audiological testing using a HF audiometry rather than conventional audiometry may help explain this dichotomy.

428 Influence of Age Upon Hearing Function in Patients with Fabry Disease

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Fabry disease is an X-linked genetic disorder of glycophingolipid metabolism caused by alpha-galactosidase A (a-Gal A) deficiency. Glycosphingolipids, mainly globotriaosylceramide (GL-3), accumulate in various types of cells and organs, resulting in clinical manifestations in skin, heart, cerebrovascular system, and kidneys. As patients with Fabry disease suffer from many life-threatening disorders, otological problems have been relatively neglected until recently. However, many patients experience otological symptoms such as tinnitus, hearing loss, and dizziness. Although they are not life-threatening, they may severely affect quality of life.
Our aim of this study is to investigate hearing function in patients with Fabry disease. Twenty patients (hemizygote/male ; nine, heterozygote/female; eleven), who are treated or observed in our hospital, were tested by auditory examinations such as auditory brain stem response (ABR), pure-tone audiometry (PTA), distortion product otoacoustic emission (DP OAE), and tympanometry. Most patients frequently have suffered from otological symptoms such as tinnitus (66.7% in male, 54.5% in female), hearing loss (33.3% in male, 36.4% in female), and vertigo (11.1% in male, 18.2% in female). The data of audiogram indicated that hearing impairment (threshold shift; more than 30 dB in PTA) started from 30 years of age in male and sensorineural hearing loss were also detected in female patients over 60 years of age. Moreover, our result suggested that unusual response of DPOAE was observed earlier than that the threshold sift in PTA.

429 Measuring Reliability of a Subjective Rating of Listening Effort

WITHDRAWN

430 Development and Evaluation of a Method for Deriving Initial Fittings of Extended-Bandwidth Hearing Aids with Multi-Channel Compression: CAMEQ2-HF

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Moore et al. (1999) described a procedure, CAMEQ, for the initial fitting of multi-channel compression hearing aids, derived using a model of loudness perception for impaired hearing. We describe here the development and evaluation of a new fitting method, CAMEQ2-HF, which differs from CAMEQ in the following ways: (1) CAMEQ2-HF gives recommended gains for centre frequencies up to 10 kHz, compared to 6 kHz for CAMEQ; (2) CAMEQ is based on the assumption that the hearing aid user faces the person they wish to hear and uses a free-field-to-eardrum transfer function for frontal incidence. CAMEQ2-HF is based on the assumption that the user may wish to hear sounds from many directions, and uses a diffuse-field-to-eardrum transfer function; (3) CAMEQ2-HF is based on an improved loudness model for impaired hearing; (4) CAMEQ2-HF is based on recent wideband measurements of the average spectrum of speech. Preliminary evaluation of the method used linear filtering to implement the CAMEQ2-HF-prescribed gains for speech with an input level of 65 dB SPL. The results obtained from four normal-hearing (NH) and 15 hearing-impaired (HI) listeners showed: (1) The gains were sufficient to make components above 5 kHz audible when those components were presented alone, and when they were presented together with the lower-frequency components; (2) NH listeners preferred a wider bandwidth (10 or 7.5

kHz versus 5 kHz) for both pleasantness and speech clarity, while HI listeners usually preferred a narrower bandwidth for pleasantness but a wider bandwidth for clarity; (3) HI listeners performed better on the "S-Test" (detection of word-final /s/ or /z/) with a wider than with a narrower bandwidth (7.5 versus 5 kHz); (4) Identification of vowel-consonant-vowel nonsense syllables improved with increasing bandwidth from 5 to 7.5 kHz for the NH but not for the HI listeners.

431 Closed-Set Intelligibility Tests for a Multilingual Society: A New Spanish Digit Triplet and Sentence Speech Test

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The development and validation of reliable, efficient and comparable speech intelligibility tests across six languages were part of the Hearcom project [1]. A closed-set format enables examiners to test subjects in their first language without being able to speak it. To achieve a high efficiency of the tests for speech reception threshold (SRT) estimation, a steep slope of the discrimination function was obtained by maximizing the homogeneity in intelligibility across the recorded word materials.

The current contribution presents the extension of these speech intelligibility tests by Spanish: a digit triplets test, using spoken numbers in a noise background (screening test of speech recognition for telephone and internet use) and a sentence intelligibility test ("Spanish Matrix test" motivated by [2] and [3]). The latter consists of ten names, verbs, numerals, adjectives and nouns which can be composed to a large number of syntactically equal, but semantically unpredictable sentences permitting repeated measurements.

The results of the optimization procedure will be described where the word-specific speech discrimination functions were measured with native Spanish-speaking subjects. This led to high similarity in intelligibility across words, sentences and test lists. To demonstrate the compatibility with the languages covered so far with appropriate closed-set test methods, the resulting overall discrimination functions and between-list variability will be compared to the functions obtained for the respective other languages.

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432 Improvement of Speech Intelligibility in Noise of the Hearing Impaired with Cochlear Representation Algorithm

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There is a persistent complaint among the hearing impaired (HI) of difficulties in understanding speech in background noise. Although most of the hearing assistive devices (hearing aids as well as cochlear implants) include some sort of advanced digital speech enhancement algorithm, the outcome so far is not satisfactory.

The purpose of the present study was to evaluate the effectiveness of a new noise reduction algorithm based on our time-domain cochlear model (Cohen and Furst, JASA 115, 2185-2192, 2004).

The algorithm's input is noisy speech and its output is a reconstructed signal. The stimulus is analyzed by the cochlear model which derives the basilar membrane velocity as a function of time along the cochlear partition. The output reconstructed stimulus is obtained as a weighted sum of all the cochlear partition outputs. Those parts of the cochlea that receive only noise yield relatively low weights.

The algorithm was evaluated in HI and normal hearing (NH)) listeners. Stimuli consisted of monosyllabic Hebrew words (AB words) which were embedded in Gaussian noise at several signal to noise ratios. The results demonstrate that the use of the present algorithm significantly improved speech intelligibility in noise of the hearing impaired. No benefit, however, was demonstrated when using this algorithm in the NH. The cochlear implant users demonstrated the most significant improvement. For example subjects with initial recognition rate below 35% reached recognition rate of about 55% with the algorithm. We believe that such an algorithm can significantly improve the ability of HI to understand speech in a background noise.

433 Pure Tone Auditory Thresholds Can Change According to Tone Duration in Functional Hearing Loss Patients

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Backgrounds: The results of pure tone audiometry in functional hearing loss (FHL) patients are different from actual hearing threshold. Test-retest reliability is sometimes poor in FHL patients, and duration of the stimulus tone may affect the results. Materials and Methods: Twelve FHL patients (21 ears) were enrolled in this study. At first, their hearing thresholds were measured by intermittent tones with duty cycle 50% lasting for 2 seconds (2 seconds' thresholds), as performed in the usual pure tone audiometry. Then, the other hearing thresholds were obtained by intermittent tones lasting for 5 seconds (5 seconds' thresholds), which are 3 second longer than those used in the usual method. The average gained thresholds (5 seconds' thresholds minus 2 seconds' thresholds) at all frequencies were compared to those of 15 controls with normal hearing (25 ears); 15 patients with cochlear hearing loss (23 ears); and 4 patients with retrocochlear lesion (4 ears). Results: The average gained thresholds of FHL patients except profound FHL patients at all frequencies were 20.5dB, which was significantly larger than those of other groups: 0.3dB (profound FHL patients); 3.8dB (controls with normal hearing); 3.0dB (patients with cochlear hearing loss); and 3.2dB (patients with retrocochlear lesion). FHL patients presenting with profound hearing loss (4 patients, 6 ears) hardly responded any sound, regardless of the tone duration. Discussions: Hearing thresholds in FHL patients

typically show 'saucer-shaped audiogram', which resembles an equal-loudness contour. This fact is explained as the result that FHL patients track their loudness memory. Attention to the sound may improve their thresholds in FHL patients by suggestion audiometry (Hosoi, et al, Int. J. Pediatr. Otorhinolaryngol, 1999). The longer stimulation may affect their attention and short-term memory of FHL patients, and lead to the improvement of hearing thresholds.

434 A User-Operated Two-Alternative Forced Choice Audiometry System for Use in Monitoring Hearing in Professional Musicians

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Professional musicians are at risk of developing hearing loss after long time exposure to loud sounds. Hearing loss may develop with time and a regular check-up of musicians hearing thresholds can identify early signs of hearing loss. However, the testing of a large sample of musicians in a symphony orchestra is work-intensive, especially since it is desirable to have several measurement stations working in parallel, for example to measure temporary threshold shifts as an early indicator of loud sound exposure.

We have developed a portable system, based on a Tucker-Davis RM2 DSP that can be used to measure auditory thresholds. The system is operated by the test subject and uses the Two-Alternative Forced Choice (2AFC) paradigm to estimate thresholds. The program uses maximum-likelihood fitting of the most probable psychometric function to minimize the number of tests and it automatically controls for subject related false alarms. To evaluate the method, we also compared the 2AFC results to the results obtained by conventional audiometry conducted by a trained technician.

The audiometry based on 2AFC is reliable and comparable to traditional audiometry. Testing time with 2AFC audiometry is typically around 2 minutes per frequency tested. 2AFC audiometry gives thresholds of 1-2 dB lower than those obtained by traditional audiometry. Results from test-retest studies of 2AFC audiometry give test-retest standard deviations below 3 dB. This is comparable to the most optimal test-retest variation seen under standard clinical settings.

The system is used for reliable testing of musicians under non-optimal testing conditions and is used successfully to detect hearing loss in classical musicians. The method will be effective for hearing tests in tests on other occupational groups as well as in clinical use.

435 Auditory Thresholds in Quiet and Background Noise During a Visuo-Spatial Task

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In some occupational settings workers are required to attend to important auditory signals while performing manual tasks in relatively noisy backgrounds. In this investigation, auditory thresholds of 20 young adults with normal auditory sensitivity and middle ear function were determined using the 5Up/5Down procedure in four randomly ordered conditions: 1. Quiet with attention, 2. Quiet with distraction, 3. Noise with attention, and 4. Noise with distraction. In the 'attention' conditions the participants pressed a button each time they heard the signal. In the 'distraction' conditions participants were asked to respond to tones by turning their heads towards the loudspeaker while solving a cardboard jigsaw puzzle as quickly as possible. In the noise conditions the background noise was speech noise presented at 60 dB SPL. The stimuli were warbled tones presented in the sound field through a loudspeaker in the following order: 0.5, 1, 2, & 4 kHz. Control trials were interspersed among the test trials to get an estimate of the false alarm rate. Three participants were excluded from the analyses due to a relatively high rate of false alarms. As expected noise worsened the thresholds and the effect of noise varied across frequency due to the speech spectrum noise resulting in a noise-frequency interaction. The effect of noise was similar across 'attention' and 'distraction' conditions. In addition, the thresholds were worse during the performance of the visuo-spatial task in the 'distraction' conditions than in the 'attention' conditions at the beginning of the threshold test procedure. However, thresholds during the 'distraction' task were better than those in the 'attention' task at the end of the test procedure suggesting the possibility of a combination of learning and intersensory facilitation effects. It appears that training and presentation levels above the background noise can allow workers to attend to important auditory signals.

436 Contribution of Temporal Envelop and Fine Structure in Mandarin Lexical Tone Perception for Sensorineural Hearing-Impaired Listeners

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This study was aimed to investigate how hearing-impaired native Mandarin speakers achieved lexical tone recognition using the temporal fine structure and envelope cues. The hypothesis was that people with sensorineural hearing loss (SNHL) may rely on more temporal cues rather than temporal fine structure, because it has been suggested that people with SNHL still has intact ability to use temporal envelope cues as normal-hearing people, but

their ability to perceive temporal fine structure is dramatically reduced.
Four groups of subjects, including 20 normal-hearing, 8 moderate, 13 moderate to severe and 10 severe sensorineural hearing-impaired listeners, participated in the study.. Monosyllabic words were processed through "auditory chimera" in which the temporal envelope from a monosyllabic word of one tone was paired with the temporal fine structure from the same monosyllable of other tones. A total of 1,536 tokens were created under the conditions of 4, 8, and 16 frequency bands and were presented to the listeners for identification.
Hearing-impaired subjects showed significantly reduced ability to use temporal fine structure cues to perceive tones in comparison with the normal-hearing group. As the hearing loss became more severe, patients relied more on the temporal envelope cues to perceive tones rather than the fine structure. On average, 69.6%, 57.4% and 39.4% of the tone responses were consistent with temporal fine structure for moderate, moderate to severe, and the severe hearing-impaired groups. On the other hand, 22.6%, 31.2% and 43.7% of the tone responses were consistent with temporal envelope cues for these hearing-impaired groups, respectively. Therefore, consistent with the hypotheses, our results indicate that patients with SNHL progressively loss their capability of using temporal fine structure as hearing loss becomes more and more deteriorated. The implications of these results in tone perception and cochlear implants will be discussed.

437 Relative Fundamental Frequency in Patients with Vocal Hyperfunction

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Vocal hyperfunction, which has been defined as "conditions of abuse and/or misuse of the vocal mechanism due to excessive and/or imbalanced muscular forces" (Hillman et al., 1989), is associated with a high percentage of voice disorders (Boone, 1983). Current diagnosis of vocal hyperfunction is dependent upon subjective interpretation of patient history and physical assessments, as no current objective measure for detection of hyperfunction has been identified. However, our previous work has shown relative fundamental frequency (RFF) to be a candidate for an objective measurement of hyperfunction, with lowered RFF values in individuals with hyperfunction relative to controls. The goal of this work was to continue to explore the utility of RFF as an objective measure of hyperfunction, and more specifically, to determine if RFF of patients with hyperfunctionally related voice disorders would normalize after a successful course of voice therapy.
RFF is an analysis of fundamental frequency on a phonemic level, considering only the ten cycles prior to and after a voiceless consonant. Pre- and post-therapy measurements of RFF were analyzed and compared in 16 subjects undergoing voice therapy for voice disorders associated with vocal hyperfunction. A two-way ANOVA found a statistically significant ($p < 0.05$) effect of both cycle and therapy phase (pre vs. post). A post-hoc t-test (one-sided) showed that post-therapy RFF measurements were significantly higher than pre-therapy, migrating toward normal patterns.
The migration of RFF in patients with hyperfunctionally related voice disorders towards normal patterns after a full course of successful voice therapy further demonstrates its potential as an indirect measure of vocal hyperfunction. Future work will be designed to determine the specificity and sensitivity of RFF as a clinical measure of vocal hyperfunction, as well as to better define its relationship to underlying phonatory physiology.
This work was supported by NIDCD Grant Number T32 DC00038.

438 Semantic Ambiguity as a Factor Influencing Speech Comprehension in Noise

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Linguistic as well as acoustic factors can influence reception of speech in noise. For example, words in sentences are more intelligible than words in isolation, presumably due to syntactic and semantic constraints on word identification. Here, we manipulate semantic constraints by comparing word report for 58 naturalistic sentences containing ambiguous words (high-ambiguity, HA, sentences: e.g., "There were dates and pears in the fruit bowl") with that for 58 sentences matched on a number of psycholinguistic variables (including word frequency) but without ambiguous words (low-ambiguity, LA, sentences: e.g., "There was beer and cider on the kitchen shelf"). Half of the sentences of each type were presented in quiet, and half were presented in signal-correlated speech-spectrum noise at a signal-to-noise ratio of -2 dB (2x2 factorial). We tested 20 normally hearing participants aged 19-65 and observed a significant interaction between sentence type and noise level: the difference in mean word report scores between HA and LA sentences was larger in noise than in quiet (82.5% vs 88.5% respectively in noise; 98.4% vs 99.1% in quiet). The interaction persisted when we compared word report for the ambiguous keywords and their LA homologues, (e.g., dates, pears vs beer, cider), and when we compared non-keyword words. An fMRI study of the same materials in a separate group of normally hearing participants (n=28, aged 18-32) revealed that the brain networks recruited for the two main effects (HA vs LA and noise vs clear) overlapped in frontal regions including the SMA and left inferior frontal gyrus (LIFG), and an interaction effect was also observed in LIFG. Behavioral and imaging results suggest that the processes recruited to cope with

background noise and with ambiguous words in sentences are not independent. This may reflect the common demand that both manipulations make on processes that use semantic context to help identify ambiguous speech segments.

439 Temporal Aspects of Sine-Wave Speech

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Sine waves that track the first three formants of speech have been shown to be intelligible to linguistically-primed listeners. Previous studies on sine-wave speech have emphasized the spectral aspects, particularly the vocal tract resonance frequencies, of speech that the three sine waves represent. The purpose of the present study was to investigate the temporal aspects of sine-wave speech using the methods of noise-excited vocoder and time reversal. Sine-wave speech stimuli were generated based on the frequency and amplitude values derived every 10 msec at the center frequencies of the first three formants of the CUNY sentences. Experiment 1 examined the intelligibility of sine-wave speech that was further processed through a noise-excited vocoder, in which the temporal envelopes of the sine-wave speech were extracted and then used to modulate bandpassed noises. Eight normal-hearing, native English-speaking adult listeners participated in the perceptual experiments. Recognition performance of the vocoded sine-wave speech as a function of number channels (from 1 to 8) was only slightly poorer compared to that of the vocoded natural speech. The results indicate that the temporal envelopes of the three spectral peaks adequately represent the temporal envelope information of speech that is crucial for speech recognition. Experiment 2 examined the effects of time reversal on the recognition of sine-wave speech. Sine-wave speech was time reversed at segments of 8 different durations (i.e., 20, 40, 60, 80, 100, 120, and140 msec). Fifteen normal-hearing listeners participated in this experiment. Recognition performance of reversed sine-wave speech was significantly lower than that of reversed natural speech at segment duration of 60 ms or longer. This result suggests that the already degraded signals as sine-wave speech is more susceptible to the effects of temporal distortion than the natural speech.

440 Breakdown of Speech Intelligibility Enhancement in Reverberant Rooms

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Results from a recent study have demonstrated that prior listening exposure to the acoustics of a reverberant listening environment can enhance speech intelligibility in the same environment by as much as 20%. Although the precise mechanisms underlying this effect are not yet known, it appears to be related to certain dynamic aspects of echo suppression – specifically precedence effect buildup – that have been widely confirmed using echo detection or echo discrimination paradigms. Here we demonstrate an additional similarity to dynamic precedence effects, namely that speech intelligibility enhancement from prior listening in reverberant environments can breakdown when a rapid change is made to the acoustics of the listening environment. Speech intelligibility testing was conducted for 11 listeners using the CRM speech corpus. Each listener was tested in three conditions: a congruent carrier (CC) condition in which sentence carrier phrases from the CRM were simulated in the same reverberant room as the CRM color/number targets, an incongruent carrier (IC) condition in which sentence carrier phrases were simulated in a different reverberant room from the color/number targets, and a condition with no carrier sentences (NC). Three different reverberant room environments, with broadband reverberation times (T60) ranging from 0.3 to 1 s, were simulated for use in the experiment via virtual auditory space techniques. In all conditions the speech signals were presented at a location directly in front of the listener (1.4 m source distance) and a competing masker (broadband noise) was presented opposite the listener's right ear. Consistent with previous results, speech reception thresholds were found to be lower in the CC condition relative to the NC condition. This enhanced intelligibility was generally reduced in the IC condition relative to NC, although the amount of reduction, or breakdown, was room-dependent. Overall, these results appear to be functionally consistent with buildup and breakdown of echo suppression. [Work supported by NIH DC008168].

441 Pitch Level and Pitch Shape as Determinants of Hemisphere Dominance in Automatic Auditory Processing of Chinese Lexical Tones

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Mandarin Chinese is a tonal language that uses lexical tones to signal word meaning. The voice fundamental frequency (F0) contour provides the dominant cue for lexical tone recognition. The F0 contour of a Chinese lexical tone can be decomposed as pitch level and pitch shape. In Mandarin Chinese, a change in pitch level is considered by some researchers to facilitate the pronunciation and make the speech melodious and rhythmical while the pitch shape is considered to contribute to defining the word meaning. In the present study, we studied the role of pitch level and pitch shape in determination of hemisphere dominance for automatic auditory processing of Chinese lexical tones. We used Chinese vowels as stimuli and odd-ball paradigms to create contrasts resulting in changes in pitch level and pitch shape for recording the mismatch negativity, an index of automatic auditory processing. Our data show that automatic auditory processing of the pitch level component is lateralized to the right hemisphere whereas the pitch shape component is lateralized to the left. Our study provides the first demonstration of the opposite hemisphere laterality for the two components of Chinese lexical tones. This work was supported by the National

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442 Level Difference Cues for the Segregation of Interleaved Speech Signals

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In two-talker listening tasks, there is some evidence that listeners are able to take advantage of level difference cues to help focus attention on either the louder or quieter talker. However, relatively little is known about the role that level cues might play in the segregation of more than two voices. In this experiment, listeners were asked to use to identify a five-word target sentence that was interleaved on a word-by-word basis with two other five-word sentences. Performance in this task was found to improve systematically when the target sentence was reduced below the level of the two masking sentences, with a plateau in performance when the level difference increased to 9 dB. However, no performance benefit was found when the level of the target was adjusted to fall between one louder masker and one quieter masker--- in fact, when the target was 9 dB louder than the first masker and 9 dB quieter than the second masker, performance was actually substantially worse than in the condition where all three talkers were presented at the same level. These results show that level cues can serve as an effective speech segregation cue when the target talker is either the loudest or quietest talker in the stimulus, but that listeners are generally unable to use level cues to focus attention on a talker who is speaking at the middle level in a three talker stimulus.

443 Discrimination of Degraded Speech Sounds by Rats: Behavior and Physiology

Kamalini Ranasinghe[1], Will Vrana[1], Chanel Matney[1], Gabriel Mettalach[1], Tara Rosenthal[1], Erika Renfroe[1], Tara Jasti[1], Michael Kilgard[1]
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We examined the ability of rats to discriminate consonant and vowel sounds that were degraded spectrally and temporally and identified the neural basis for impaired discrimination at the highest levels of degradation. The degraded speech sounds were created using a cochlear implant simulation processed via a noise vocoder. Five levels of spectral degradations were defined by the number of channels used in the vocoder (1, 2, 4, 8, or 16), and four levels of temporal degradations were used determined by the low pass cutoff frequency of the envelop extractor. The rats were trained on three consonant discrimination tasks and three vowel discrimination tasks in an operant training paradigm. Rats performed with 80% accuracy when 8 or more channels were used. Performance dropped to chance when only one channel was used. Temporal degradation significantly impaired both consonant and vowel discrimination tasks. However, performance was well above chance even at the highest level of temporal degradation (4 Hz low pass). These observations are consistent with observations in humans. Discrimination behavior was compared with neural discrimination based on activity recorded from rat primary auditory cortex (A1) and inferior colliculus (IC). The rat performance on consonant discrimination tasks was significantly correlated with neural discrimination when precise spike timing (1 ms) information is used, where as the behavior performance of vowel discrimination tasks was significantly associated with neural discrimination when average spike count information was used. Our results reveal that A1 neural activity better predicts the consonant discrimination while IC neural activity better predicts the vowel discrimination suggesting that consonants and vowels are represented by neural activity on two different time scales.

444 Effect of Background White Noise and Speech Shaped Noise on Speech Discrimination Performance in Primary Auditory Cortex of Rats

Jai Shetake[1], Jordan Wolf[1], Ryan Cheung[1], Kinsey Ram[1], Will Vrana[1], Tara Rosenthal[1], Michael Kilgard[1]
[1]*University of Texas at Dallas*

Rat speech sound discrimination ability is highly correlated with discrimination by primary auditory cortex (A1) neurons. Different consonants generate distinct patterns and are represented with temporal precision in the order of 1-10ms over the onset response period. Speech sound discrimination is harder in noisy situations. Addition of background noise degrades the neural onset response in the primary auditory cortex. We were interested in testing if consonant sounds are encoded using temporally precise onset response bins even when the neural onset response in A1 is degraded.

Speech shaped noise masks speech sounds equally compared to white noise, which masks the high frequency spectrum more. Therefore, we hypothesized that white noise will mask more high frequency speech sound discriminations and high frequency neurons as compared to speech shaped noise. Also, since high frequency neurons are more sensitive to white noise than low frequency neurons, we hypothesized that their performance at any given noise level would be worse than low frequency neurons. If confirmed, this hypothesis might explain why place of articulation is more sensitive to high frequency noise than voicing and manner of articulation.

To test the above hypotheses, we played the 20 English consonant sounds (60 dB) in four different levels of continuous white noise and speech shaped noise(0, 48, 60, and 72 dB) and measured neural responses in the A1 of 10 rats. Preliminary results show that neurons are differentially affected by noise depending on their characteristic frequency. Ongoing studies will evaluate behavioral correlates of our new neurophysiologic observations.

445 NIDCD Workshops: Trainees and Early Stage/New Investigators

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NIDCD will offer two concurrent mini-workshops targeted to specific audiences. Session 1 is specifically targeted to individuals interested in Training and Career Development; Session 2 is targeted to Early Stage/New Investigators. The Training and Career Development session will include an overview of research training and career development opportunities appropriate for graduate students, medical students, postdoctoral fellows/medical residents, and new and budding clinician investigators. Discussion topics will include the submission and review of individual NRSA fellowship awards (F30, F31 and F32) as well as the mentored career development and transition awards (K08, K23 & K99/R00). The NIH Loan Repayment Program will also be presented. Drs. Janet Cyr, Dan Sklare, and Melissa Stick will lead the presentation.

The Early Stage/New Investigator session will provide practical information on how the NIH/NIDCD works (Institute and study section assignments, Advisory Council activities, pay lines and the roles of program, review, and grants management staff). Specific information will be provided regarding funding opportunities for early stage investigators including the NIDCD Small Grant Award (R03) with a goal of providing funding information for a successful transition from trainee to independent investigator. Drs. Christine Livingston and Christopher Platt will lead the presentation.

446 Mouse Models of Deafness; the Human Otopathology Perspective

M. Charles Liberman[1,2], Saumil N. Merchant[1,2]
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There are more than 350 syndromic and non-syndromic types of hereditary hearing loss in the human. Although the mutations responsible for genetic deafness have been characterized in many cases, little is known about the pathology and pathophysiology of the great majority of genetic hearing impairments.

The first part of this talk will focus on the use of mouse models in the study of human genetic deafness. Issues to be discussed include 1) the nature of the genomic alteration used to match the human condition, 2) effects of the background mouse strain, and 3) use of appropriate metrics to study the histopathology and pathophysiology.

The second part of this talk will focus on temporal bone histopathology in patients with molecular confirmation of the underlying mutation. The available data, which is relatively sparse, has shown heterogeneity in the inner ear phenotypes, with selective or mixed losses of sensory and neural structures. Reasons why examination of inner ear tissues in human genetic deafness has lagged behind the rapid progress in clinical and mouse genetics will be discussed.

Mouse and human data are complementary; both are needed to make progress in understanding deafness. An integrative approach has great potential to improve diagnosis and treatment of genetic deafness.

Supported by NIDCD

447 Supporting Cells in Organ of Corti Regeneration and Otopathology

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In mammals, loss of sensory hair cells in the organ of Corti leads to permanent deafness due to the failure of these cells to regenerate. In contrast, regeneration occurs normally in non-mammalian vertebrates through a combination of supporting cell proliferation and re-differentiation, as well as through a process of direct transdifferentiation of supporting cells into hair cells. The discovery of these processes in non-mammalian vertebrates, suggested the possibility that a better understanding of the basic biology of supporting cells may lead to the means of therapeutically manipulating mammalian supporting cells to take part in hair cell regeneration in the damaged organ of Corti.

The first part of this talk will focus on advances in our understanding of organ of Corti development and regeneration in the mouse, with specific emphasis on the regulation of proliferation, and the maintenance of cell fate in postnatal supporting cell populations. In the second part, we will briefly discuss new data analyzing and categorizing the survival of supporting cell populations in archival human temporal bones, from the temporal bone laboratories of the House Ear Institute and the Massachusetts Eye and Ear Infirmary, from deaf individuals. These supporting cell populations remain largely uncharacterized, but will, of necessity, form a part of any therapeutic strategy for hair cell regeneration and hearing restoration.

448 Using Human Temporal Bones, Combined with Histological and Proteomic Methods to Study the Underlying Molecular Changes Associated with DFNA9

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Formalin-fixed, celloidin-embedded (FFCE) or paraffin-embedded (FFPE) tissues are a largely unexplored archive in MS-based proteomics, mainly due to the fact that it is extremely difficult to identify the unpredictable nature of the chemical modifications that take place as a result of fixation. Current methods such as immunohistochemistry (IHC) suffer from a lack of sensitivity and scalability. Exploring capabilities to conduct large-scale analyses of tissues using proteomic approaches becomes of paramount importance and could have far reaching implications on the morphological, histopathological as well as molecular characterization of temporal bones associated with hearing loss or balance disturbance.

DFNA9 is a form of sensorineural hearing loss, clinically characterized by onset in the fourth or fifth decade of life

and initially involves the high frequencies. Deafness is progressive and usually complete by the sixth decade. In addition to cochlear involvement, DFNA9 patients also exhibit a spectrum of vestibular dysfunctions. Affected individuals have mucopolysaccharide depositions in the inner ear (spiral ligament, limbus and the dendrites under the limbus, and supporting tissues of the vestibular system), in the middle ear (incudomalleolar joint) and cartilage formation in the eardrum. This is the first time in DFNA-9, where a gene defect has been reported to affect both the inner and middle ear structures.

We will show how we have successfully leveraged the application of powerful mass spectrometry-based proteomic approaches towards the molecular investigation of archival temporal bone specimens associated with the non-syndromic sensorineural deafness autosomal dominant type 9 (DFNA9), the ideal test case whose underlying molecular defect has already been linked at the genetic level to three different base mutations in the Cochlin gene.

449 Molecular and Cellular Approaches to the Human Temporal Bone II

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The purpose of the present study is to present the advances in applying immunocytochemical localization in the human temporal bone using auditory and vestibular endorgans microdissected from temporal bones acquired post-mortem. With this technique there is minimal or no decalcification steps and immunohistochemical staining can be conducted and evaluated within one week. Microdissected vestibular endorgans from temporal bones were sectioned with a cryostat, or used as whole mount surface preparations. Specimens were analyzed using light and fluorescent microscope. The resulting specimens demonstrated excellent morphology with minimum tissue alteration and good immunoreactive signal-to-noise ratio. Immunohistochemical staining has been successfully performed in auditory and vestibular endorgans using antibodies against aquaporins, basement membrane (BM) proteins and connexins 26 and 30 (Lopez et al. 2007, Ishiyama et al. 2009; McCall et al. 2008). Using this methodology, we have provided the first comprehensive immunolocalization of aquaporins and BM proteins in the human inner ear. This method can be used to increase the resolution of unbiased stereology on nerve fiber counts in the human vestibular endorgans using immunocytochemistry (Lopez et al. 2005). Using microdissection and immunohistochemistry of human temporal bone, we have recently detected the von Willebrand A domain-related protein (WARP) an extracellular matrix molecule with restricted expression in a subset of BMs in the vasculature (Trac et al. ARO Abst 2010). WARP-immunoreactivity was located in the internal (luminal) portion of blood vessels and in calyx like structures that surround type I hair cells. The combination of microdissection and immunohistochemistry can potentially open up a new field for future human temporal bone research. Pathological changes in the sensory epithelia can be documented with the use of these techniques.

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450 Temporal Bone Surgical Simulator I: A Resource for Clinicians and Trainees – from Cardboard Models to the Visible Ear Simulator and Beyond

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From anatomical cardboard models over simple pc-based wire frames and surface models sophisticated volumetric 3-D representations with haptic user interaction have evolved. Most existing virtual simulators for middle ear surgery are based on CT or MRI data in which image quality is limited by the lack of natural colour, texture and detail (max. 50 voxels/mm^3) of the source material. Interaction requires the purchase of software, a customized computer, and expensive peripherals dedicated exclusively to this purpose.

This has limited the benefit and dissemination of virtual simulators for ear surgery.

The VES Visible Ear Simulator is based on the "Visible Ear" freeware library of digital images from a fresh frozen human temporal bone, which was segmented and volume rendered into a 3-D model of high-fidelity, true color and great anatomical realism and detail (125voxels/mm^3) of the surgically relevant structures. A real-time rendering was achieved by combining advanced GPU raycasting and standard rasterization. A haptic drilling model was calculated by using volumetric techniques.

Program solutions were constantly selected to provide the highest quality of images and haptics possible in an application designed for real time interaction on any standard pc platform with GPU acceleration.

Realistic visualization in 2-D or optional anaglyph stereoscopic 3-D was achieved on a Core 2 Duo® pc with a GeForce 8800 GTX® graphics card (or better), and real-time surgical interaction was provided through a Phantom Omni® haptic 3-D pointing device. A range of controls allows the user to adjust the virtual environment, to save, restore and even "undo" surgical work, to collect screenshots for external use and calibrate visual and haptic performance to match the default pc equipment.

At: http://temporalboneconsortium.org/educational-resources/simulator/ the VES is offered as freeware to meet a world-wide oto-surgical demand.

Future developments may include tutor/censor functions and haptic tutorials. Moreover, the source material may support increased resolution up to 8000 voxels/mm^3 and interaction with deformable soft tissue components such as skin, tympanic membrane, dura and cholesteatomas developed at random by the program. New technology may provide enhanced stereoscopic and haptic quality even with low budget commercial equipment.

451 Temporal Bone Surgical Simulator II: The Ohio State Experience, Towards Validation

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The potential impact of interactive, multimodal simulation technology to teach technical skill in surgical endeavors is undeniable. Simulation technology has become a mainstay of training in the airline and other industries and recently has been applied to technical skills training in many types of surgery. Otologic surgery presents a unique challenge of this technology in that it requires an integration of the spatial comprehension of the exacting anatomy with the application of fine motor skills to develop expertise. In order to provide an advantage to Otologic surgical training, systems must be developed following an iterative design. Development must be followed by assessment followed by modification and reassessment. The process must be repeated to achieve validity in training and skill assessment. Systems must have core features including sufficient structural and visual realism to engage the user, the ability to provide an opportunity for structured repetition of skill, and the provision for active feedback to the user supplying continuous and quantified assessment. These components must be integrated within the midst of a metrics driven curriculum. This presentation will outline our approach with a focused overview of objectives, followed by progress emphasizing data acquisition, integration, dissemination, and validation within the resident curriculum. Additionally, we will present our future plans and recommendations to those involved in temporal bone research to provide data that can be integrated into more complex simulations. Supported by NIH/NIDCD RO1 DC006458-01

452 Technical and Educational Resources Developed by the Temporal Bone Consortium

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The inner ear is inaccessible for examination during life by biopsy or other techniques. Post-mortem examination of the inner ear is challenging because the delicate membranous labyrinth is encased within the dense petrous bone. There currently exist only a handful of laboratories in the world that conduct systematic investigations of human inner ear specimens.

The Human Temporal Bone Consortium for Research Resource Enhancement was recently established as a cooperative agreement using the U24 funding mechanism between NIDCD and three member laboratories: the Massachusetts Eye the Ear Infirmary, the House Ear Institute, and the University of California at Los Angeles. The goals of the Consortium are to improve and enhance methodologies for studying human temporal bones, promote sharing of tissues and technologies, and promote the recruitment and training of new investigators.

The Consortium has assembled a variety of resources of value to the research and clinical communities to facilitate research and teaching, as well as the diagnosis and treatment of otologic disorders. These resources are available as freeware, using interactive, web-based formats (www.temporalboneconsortium.org).

These resources, which include the following, will be demonstrated : 1. Techniques of removal of the human temporal bone. 2. Methods of study, including microscopy, immuno-, genomic- and proteomic- assays. 3. An interactive image library of normal 2-D morphology, allowing the user to perform virtual remote microscopy at magnifications ranging from 10x to 400x. 4. Interactive image libraries correlating radiology and histology of the temporal bone. 5. Interactive, downloadable 3-D models of the inner ear and temporal bone. 6. A library of searchable and downloadable images of morphology and pathology (1,500+ images). 7. A database of archived specimens, fully searchable by text, similar to PubMed. 8. Webcast recordings of seminars in otopathology.

Supported by NIDCD

453 Comprehensive Analysis of Gene Expression in the Otocyst

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The chicken otic vesicle contains all necessary information to generate the major inner ear cell types. At embryonic day 3 (E3), it consists of otic progenitor cells that have already sub-compartmentalized into non-sensory regions and into regions that will give rise to prosensory domains. Many of the active genes/pathways behind sub-compartmentalization are still unknown. Our goal is to identify a comprehensive set of genes that are expressed in otic progenitors. Toward this, we conducted a Serial Analysis of Gene Expression (SAGE) of transcripts present at HH stage 18 (E3). SAGE provides an unbiased, comprehensive and quantitative readout of gene expression, which is unequaled by any gene array currently available. We sequenced 39,326 17-mer tags comprising 16,008 unique sequences. Initial analysis of these tags with three reference libraries (Ensembl 52, RefSeq, and Unigene) resulted in matches for 4,153 genes that were eligible for Ingenuity Pathway Analysis software. As expected, the highest expression levels were detected for common housekeeping genes, COX-1 being the most abundant (tag count: 840). Our analysis identified 355 transcriptional regulators of which 303 are previously unknown in the context of the inner ear. In addition, 8 out of 14 growth factors detected were previously not linked to the inner ear. On the other hand, we identified 41 transcription factors or growth factors with a previously described expression in the otic vesicle, such as PAX-2 and TGFB2. To validate our findings, we are currently performing a medium-throughput *in situ* hybridization screen on chicken otic vesicle sections. About 90 % of the identified genes tested thus far have been confirmed by *in situ* hybridization. Some genes show a regionally restricted

expression pattern while others occur more widespread. Our goals are to use the outcome of this investigation to select individual genes for in-depth functional studies, and to obtain a comprehensive set of transcriptional regulators and cell surface markers expressed in otic progenitor cells. These genes will be important tools for future studies aimed to generate pure otic progenitor cell populations from stem cells.

454 Discovery and Characterization of FGF-Regulated Genes Involved in Otic Placode Induction

Suzanne L. Mansour[1], Lisa D. Urness[1], Christian N. Paxton[1], Xiaofen Wang[1], Gary C. Schoenwolf[1]
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The inner ear, with its intricate array of sensory, non-sensory and neuronal cell types, is derived from a small patch of ectodermal cells known as the otic placode. Once induced, the placode invaginates to form a vesicle, which subsequently undergoes complex morphogenesis and cell-type specification leading to the generation of the elaborate cochlear and vestibular structures. Mice lacking both *Fgf3* and *Fgf10* fail to initiate inner ear development, and the role of FGF signaling in this case is to specify the appropriate patterns of gene expression within the prospective otic placode (Wright and Mansour, 2003). To understand the transcriptional "blueprint" for inner ear development, we sought to identify otic placode genes that are up- or down-regulated in response to inductive FGF3/10 signals. We isolated RNA from the otic placode of 4-8 somite-stage *Fgf3*$^{-/-}$/*Fgf10*$^{-/-}$ and control embryos and performed microarray analysis. Several putative FGF effectors have been identified, including *Sox9*, *Hmx2*, *Hmx3*, *Foxg1* and *Has2*, and differential expression for each has been validated in the double mutant. FGF signals are not only required for placode induction, but also for subsequent otic vesicle morphogenesis and differentiation; thus, expression studies with our target genes at a variety of developmental ages are underway.

We also assayed candidate genes suggested by other studies of otic induction. *Foxi3,* a placode marker, is often down-regulated in *Fgf3*$^{-/-}$/*Fgf10*$^{-/-}$ embryos, whereas *Foxi2*, a marker of cranial epidermis, is expanded in our mutants, similar to its behavior when WNT responses are blocked in the otic placode. Assays of hindbrain-expressed *Wnt*s show that only *Wnt8a* is down-regulated in FGF-deficient embryos, and chick explant assays show that FGFs are sufficient to induce *Wnt8a*, suggesting that it may provide the link between formation of the *Pax2*+ preotic field and restriction of the otic territory to hindbrain proximal ectoderm.

455 Sprouty1 and Sprouty2 Limit the Size of the Otic Placode and Restrict Dual Fgf and Wnt Inductive Domains

Katherine Shim[1], Amanda Mahoney Rogers[1]
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Multiple signaling molecules, including Fibroblast growth factor (FGF) and Wnt, induce two patches of ectoderm on either side of the hindbrain to form the progenitor cell population for the inner ear, or otic placode. Sprouty (Spry) genes encode antagonists of receptor tyrosine kinase signaling, including FGF signaling. Here, we find that in mouse embryos with loss-of-function mutations in both the Spry1 and Spry2 genes (Spry1-/-; Spry2-/- embryos), the pre-otic field, or region of ectoderm that gives rise to the otic placode, is initally correctly formed. However, the definitive otic placode is expanded in Spry1-/-; Spry2-/- embryos. Consistent with a role in otic placode induction, we find that both Spry1 and Spry2 are normally co-expressed in the pre-otic ectoderm and underlying mesenchyme. The enlargement of the otic placode observed in Spry1-/-; Spry2-/- embryos is preceded by an expansion of a Wnt8a expression domain in the adjacent hindbrain. Interestingly, the enlargement of the otic placode can be rescued in Spry1-/-; Spry2-/- embryos by reducing the gene dosage of Fgf10. Our data define a FGF-responsive window during which cells can be recruited into the otic domain and uncover Spry regulation of the size of a putative Wnt inductive center.

456 Role of Wnt and Hedgehog Signaling in Axial Patterning of the Developing Inner Ear

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The inner ear develops from a simple otic placode into a complex structure that is asymmetrical along all three axes. How these axes are set up is poorly understood. The frog, *Xenopus*, provides an excellent model system to study inner ear development because of the ease with which embryological and molecular manipulations can be done. We have shown that dorsal (D) or ventral (V) half ablations result in the loss of corresponding structures, unlike the mirror duplications seen after anterior or posterior half ablations. Here we explore the role of Wnt signaling on axial patterning in *Xenopus* with gain and loss of function experiments. Gain of function experiments were done by placing beads containing Wnt3a protein on the remaining otocyst after D or V half ablation. These were capable of rescuing the loss of tissues seen after dorsal but not ventral half ablation, suggesting that Wnt3a protein alone is sufficient to rescue the severe loss in inner ear structures resulting from dorsal half ablations. The *Wnt3a* gene is expressed dorsally during early inner ear development. Blocking of canonical Wnt signaling by injecting morpholinos to β-*catenin* results in a broad range of inner ear defects depending on how specifically the inner ear is targeted. When our knock down of Wnt signaling was restricted to the inner ear we found a severe reduction in the number of sensory organs with some resembling mirror posterior duplications. If instead Wnt signaling was knocked down more broadly, in both the inner ear and surrounding tissues, we found that mirror anterior duplications or medial instead of lateral formation of cristae were the two most common results. The broader blocking of Wnt signaling may also be affecting hedgehog (Hh) signaling as we have found that blocking Hh signaling also results in mirror anterior duplications. Hh signaling is

necessary for posterior half identity in zebrafish and *Xenopus*. (Supported by NIDCD)

457 SoxE Genes in Mouse Inner Ear Development

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SoxE genes are a subgroup of the large family of Sox HMG domain transcription factors, found throughout the animal kingdom. In mammals the SoxE subgroup contains three genes, Sox8, Sox9 and Sox10 . All three genes play crucial roles during vertebrate development. Among others they are involved in vertebrate sex determination, cartilage development and the development of neural crest derived structures. Expression of SoxE genes has also been detected during mouse inner ear development and we have shown previously that Sox9 is required in mouse for otic placode invagination. We have performed a detailed analysis of the otic expression patterns of all SoxE family members. We could detect all three proteins in very distinct, partially overlapping patterns during mouse inner ear development. Expression of all three is prominent from otic vesicle formation on and can be traced until birth and beyond. To further address the function and potential redundancies of SoxE genes we are currently analysing inner ear phenotypes found in single and compound SoxE knockout mice. Our data suggest that SoxE genes are required for inner ear morphogenesis and epithelial integrity in a partially redundant manner.

458 Characterization of Jab1, a Major Downstream Effector of the Cytokine Mif, in Zebrafish Inner Ear Development

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Otocyst-derived factor (ODF) is secreted from a specific region of the otocyst called the otic crest for a limited time in early development and serves as both directional neurite outgrowth factor(s) and survival factor(s) for early statoacoustic ganglion (SAG) neurons. Two of the cytokines that make up ODF are macrophage migration inhibitory factor (MIF) and monocyte chemoattractant protein 1 (MCP1). As the epithelium matures, supporting cells (SCs) produce MIF and the hair cells (HCs) produce MCP1. The receptors for both cytokines (CD74 and CCR2, respectively) are expressed on the neuroblasts and neurons of the SAG and on adult spiral ganglion neuron (SGN). Studies in the Barald lab have shown that MIF is capable of inducing neurite outgrowth from explanted SAG and supporting the survival of dissociated SAG neurons: MCP1 alone does not. MIF also affects cell fate specification. In MIF knock-out mice, large numbers of inner and outer HCs are missing in the basal cochlea. We hypothesize that many, if not all of these effects depend on MIF's binding to a highly conserved effector protein, Jun activation domain-binding protein 1 (Jab1), which was found in ODF.

Molecules downstream of Jab1 are critical for inner ear development. Jab1's interactions with p27kip1 could be critical in the role p27kip1 plays in HC specification. Jab1 interacts with Id3, an up-stream regulator of Atoh1, a specific and critical gene for HC specification. Potential downstream targets of Jab1 activation include: Hand2, critical for the formation of many neuronal lineages and Pou3f2, critical for the specification of many inner ear cell lineages. Hand2 is essential for the neuronal development of neural crest derived inner ear cell lineages. We are investigating if Mif activation of Jab1 signaling plays a major role in otic development, specifically in neurite outgrowth and survival of the developing SAG neurons as well as HC/SC cell fate specification in the zebrafish inner ear.

459 Inner Ear Neural Stem Cell Development and Morphogenesis Are Positively Regulated by the Chromatin Remodeling Protein CHD7 Via Effects on Proneural and Otocyst Gene Expression

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CHD7 encodes a chromatin remodeling protein commonly mutated in human CHARGE Syndrome, a multiple anomaly condition that includes delayed growth and puberty, deafness, and vestibular dysfunction. We previously generated Chd7 gene-trap mice ($Chd7^{Gt/+}$) which have lateral and posterior semicircular canal malformations and defects in posterior sensory innervation. Prior studies also demonstrated that $Chd7^{Gt/+}$ mice are anosmic with malformed olfactory bulbs and reduced proliferation of olfactory neural stem cells. While CHD7 is predicted to regulate transcription, target genes and enhancer regions are likely tissue and developmental stage specific. $Chd7^{\mathbf{Gt/Gt}}$ mice are embryonic lethal after E10.5, prohibiting examination of Chd7-null tissues beyond the otocyst stage. We show here that Chd7 is highly expressed in neuroblasts and early born neurons in the vestibulocochlear ganglion. $Chd7^{\mathbf{Gt/Gt}}$ mice have significantly smaller ganglia, suggesting a role for Chd7 during inner ear neurogenesis. We also report the first analysis of inner ear specific Chd7 conditional null mice. Foxg1-Cre;$Chd7^{Gt/flox}$ mice survive to birth with complete absence of the semicircular canals and cristae and cochlear hypoplasia. Interestingly, Foxg1-Cre;$Chd7^{Gt/flox}$ mice also have small vestibulocochlear ganglia, fewer proliferating neuroblasts, and reduced expression of the proneural transcription factors NeuroD and Isl1. While delamination of early born neuroblasts appears normal, expression of genes responsible for establishment of the neurogenic (Otx2) and prosensory (Bmp4, Fgf10) domains is absent from the E10.5 FoxG1-Cre;$Chd7^{Gt/flox}$ otocyst. These data indicate that epigenetic regulation of transcription must be tightly coordinated by chromatin remodeling for proper inner ear morphogenesis and neural

stem cell development, and suggest that therapeutic strategies for treating deafness and vestibular dysfunction may target early neural progenitors. Supported by NIH grant R01DC009410.

460 Bmp2 Is Essential for the Formation of Semicircular Canals in the Mouse Inner Ear

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The sensory cristae and their associated semicircular canals comprise the vestibular apparatus of the inner ear that is responsible for detecting angular head movements. During development, a series of complicated morphogenetic events gives rise to the semicircular canals. First, an epithelial outpocketing of the otocyst forms a canal pouch, which then flattens to form a disc-like shape that is thin in the center and thicker around the edge. The epithelia in the center region of the flatten disc known as the fusion plate disappear through mechanisms that are not entirely clear, and leave behind the rim of the disc forming a canal. Throughout these morphogenetic events, the developing canal pouch/canal continues to grow and expand.

In *Bmp2* conditional knockout (*Fogx1*$^{cre/+}$*;Bmp2*$^{lox/-}$) inner ears, the canal pouches are present, though smaller than wildtype. However, by the stage that the canals should be formed, *Bmp2* conditional knockout inner ears are missing all three canals, even though the cristae are present. Our results indicate that the absence of canals is due to a failure of the rim of the canal pouch to maintain its identity and growth as well as an increase in the resorption process, which may co-opt canal epithelial cells for resorption. Markers for the canal rim such as *Dlx5*, *Lmo4,* and phosphorylated Smad1/5/8 immunoreactivities are down-regulated, followed by reduction in cell proliferation. In addition, *Netrin1,* which is known to be required for proper canal resorption, shows an expanded expression domain in the canal pouches of *Bmp2* mutants. Removal of one copy of *Netrin1* from *Bmp2* conditional knockout mutants partially rescues some of the canal phenotypes suggesting that Bmp2 normally antagonizes Netrin1's activities in mediating resorption of the fusion plate. Together, these results indicate that Bmp2 functions to maintain canal growth and at the same time restricts the process of canal resorption.

461 Opening of Scala Media During Embryonic Development Depends on Local Cochlear Ion Transport

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Scala media and the endolymphatic duct and sac develop from the otic vesicle as epithelial protrusions that subsequently open to enclose a fluid-filled lumen (Sher 1971). Luminal fluid spaces develop an enlargement in a mouse model of *SLC26A4*–related deafness, which is a key event in the etiology of deafness. The goal of the present study was to determine whether opening of the lumen in scala media depends on the endolymphatic duct and sac or on local cochlear ion transport. Embryonic otocysts were cultured for 2 days. Paired experiments were set up by culturing one otocyst originating from one embryo with the endolymphatic sac and duct and the other otocyst without the endolymphatic sac and duct. Procedures were adapted from methods developed by Dr. Thomas van de Water (1973). Freshly isolated and cultured otocysts were evaluated by confocal immunocytochemistry. The lumen of scala media was found to be closed prior to E13.5 and to open between E13.5 and E14.5. Expression of Na^+/K^+ ATPase was present in all epithelial cells lining scala media at the time the lumen opened. No expression of Kcnq1 and Slc12a2, both known to mediate K^+ secretion in postnatal stria vascularis, was found at the time scala media opened. Cultured otocysts were used to observe the development of the lumen as a function of the presence or absence of the endolymphatic sac and duct. Without endolymphatic sac and duct scala media developed an enlargement in otocysts that were obtained from E14.5 mice. No difference was observed in cultured otocysts obtained from older embryos (E15.5 and E17.5). In conclusion, these data suggest that the endolymphatic sac and duct engage in fluid reabsorption during embryonic development and that local cochlea ion transport contributes to the opening of scala media.

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462 Fgf9 Subfamily Is Essential for Organ of Corti Development

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Deafness caused by inner ear defects is one of the most common genetic diseases, affecting more than 1 in 1000 births. Understanding the molecular and cellular mechanisms that regulate inner ear development is paramount to identifying genetic etiologies for deafness and for understanding degenerative and regenerative processes in the inner ear. During organogenesis, Fibroblast Growth Factors (FGFs) regulate cell proliferation, differentiation and survival. Hypomorphic or conditional deletion of *Fgfr1* in the developing ear caused decrease hair cells and gaps throughout the cochlea (Pirvola, 2002). Here we have generated mice null for *Fgf20* by insertion of a β-galactosidase gene. β-galactosidase activity identified sites of *Fgf20* expression in the developing inner ear as early as E10.5 and extending at least through P7. *Fgf20* expression partially overlaps with p27, which marks the sensory patch at E13.5. At P0, *Fgf20* expression is restricted to supporting cells, Hensen's cells, and inner phalangeal cells and excluded from hair cells. Auditory Brainstem Response tests showed that *Fgf20*$^{-/-}$ mice have complete hearing loss. Analysis of the inner ear using histology and marker studies, including phalloidin, myosin 6, prox1, and p75, indicates that hair cells and supporting cells can differentiate normally, however, there was a selective decreased number of outer hair cells compared to inner

hair cells. *Fgf20*$^{-/-}$ mice also formed gaps in the cochlear sensory epithelium that lacked all components of sensory region (hair cells and supporting cells). Additionally, *Fgf9*$^{-/-}$; *Fgf20*$^{-/-}$ compound embryos showed a similar cochlear morphology but a significantly shorter cochlea than *Fgf20*$^{-/-}$ embryos. *Fgf9*$^{-/-}$ embryos showed no cochlear defects in the absence of disruption of *Fgf20*. These data indicate that FGF9 and FGF20 are essential regulators of organ of Corti development and are likely to be the endogenous functional ligands for FGFR1 in the developing cochlea.

463 Organ of Corti Defects in Mouse Model for DFNB8/10 *TMPRSS3* Deafness

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DFNB8/10 is an autosomal recessive, non-syndromic deafness that is due to mutation of the *TMPRSS3* gene, a member of the Type II transmembrane serine protease family. Proteolytic cleavage of this protein is required for function of the epithelial sodium channel (ENaC), thus its defect leads to abnormal Na^+ transport. Because this channel is prominent in the inner ear, its defects lead to sensorineural deafness as reported in human studies. To better understand the role of this gene defect in hearing, mice heterozygous for the defective *Tmprss3* gene were obtained from Genentech, Inc. (San Francisco, CA). Offspring from heterozygote matings were genotyped and tested for cochlear function by auditory brainstem response audiometry. Wildtype mice and heterozygotes had normal hearing thresholds out to 5 months of age. On the other hand, *Tmprss3* null mice had no brainstem response at 4 weeks, the earliest age tested. Histology showed absence of the organ of Corti in the null mice, with no detectable hair cells or supporting cells. Vestibular epithelia and stria vascularis appeared qualitatively normal. These preliminary findings suggest organ of Corti degeneration, or failure to develop, is the genetic mechanism in this form of deafness. Ongoing studies of embryos are determining if the hair cells ever develop. The development of this animal model will permit further studies of the underlying molecular genetic basis of this form of deafness.

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464 Forced Activation of Canonical Wnt Signaling Blocks Cartilage Differentiation in the Otic Capsule of Chicken Embryos

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Regulation of otic capsule morphogenesis involves epithelial-mesenchymal interactions to promote chondrogenesis; some of these events are likely to be mediated by secreted signaling molecules. The chicken embryo offers an *in vivo* experimental model to explore the role of Wnt signaling in this process. Our approach was to manipulate the intracellular mediator of canonical Wnt signaling, β-catenin, and thus to override the influence of endogenous Wnt inhibitors.

On E2-E3, the otic anlage was infected with replication-competent avian retrovirus encoding an activated form of β-catenin. Embryos were fixed on E5-E10, sectioned, and processed by immunohistochemistry with antibodies to detect viral infection, cell proliferation, apoptosis or collagens II and III. Counterstaining selected sections with Alcian blue marked cartilage formation. In virus-injected embryos, the otic capsule was infiltrated with foci of infected cells notably lacking an aura of Alcian-blue staining. There was no obvious increase in either cell proliferation or cell death within these foci as compared to the nearby, uninfected cartilage. Virus-infected cells were surrounded by collagens that normally encase the otic capsule and distribute throughout the soft tissue mesenchyme between the otic capsule and the otic epithelium.

We provisionally interpret the β-catenin-transfected cells of the otic capsule to have progressed from multipotent progenitors into the soft connective tissue lineage, which are unable to further differentiate into chondrocytes. This interpretation is consistent with that described for Wnt regulation of mesenchymal-to-chondrocyte progression in the chicken limb bud (ten Berge *et al., Development,* 2008).

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465 Branchial Arch Skeletal Elements Develop at Disparate Rates During the First Postnatal Week in Mice

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Congenital middle ear pathology is often correlated with other skeletal defects, especially in the orofacial region. These correlated abnormalities reflect underlying developmental linkages and may predict correlated treatment effects. To better understand the nature of these linkages, I analyzed growth and development of middle ear ossicles and other skeletal derivatives of the branchial arches in mice, beginning with the first week of postnatal development. Clearing and staining revealed that only the dentary, tympanic ring, and hyoid bar (transverse body) are ossified at P1, but by P7, only the horns of the hyoid and laryngeal cartilages remain unossified. Quantitative analysis using locations of discrete anatomical points, found dramatic changes in both size and shape of the dentary between P1 and P7, but little change of either in the malleus. The other elements have various intermediate amounts of change during this interval. Thus there appears to be little coordination of development among branchial arch elements of the mouse during the first postnatal week. There are differences between dermal (membrane) and endochondral bones, between branchial arches, and between elements of the same arch. The best evidence for developmental correlation lies in the early onset of ossification in bones involved in feeding (dentary and hyoid), and early completion of development by bones involved in hearing (malleus, incus and stapes). These results also indicate that the first postnatal week is a

crucial period for ossification of the middle ear bones, but earlier time is more important for development of their shape. Therefore, correlations between the shapes of the ossicles and the shapes of other branchial arch derivatives must have been established prior to this interval. This suggests that therapies targeting abnormal development of the middle ear in humans may be most effective if directed at a comparably early interval of human development.

466 The Mentorship of US Underrepresented Minorities and Women in Research-Education Integrated Environment

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A glance at science, technology, engineering and mathematics (STEM) fields show that roughly speaking we are still in awe of individuals who are successful mentors of underrepresented US minorities (URMs) or women. This perspective suggests that most researchers, members of the majority population, see the mentoring-to-success process of diverse individuals as a rare and inherently difficult enterprise.

In 1996, I established the Mathematical and Theoretical Biology Institute (MTBI) at Cornell University in order to generate a sustainable pool of URMs and women, primarily from non-selective institutions in order to show that existing efforts were ignoring large segments of our undergraduate populations showing in the process that large scale efforts were feasible.

MTBI has fulfilled this objective via the participation of 25-36 undergraduates (on the average) per summer in sequential summer research experiences, in environments that resemble the typical science lab models, where undergraduates, graduates, postdoctoral students and visitors carry out joint research. The MTBI experience lasts 8 weeks per summer and we are in our 14th year. Our efforts have served nearly 400 undergraduate students. The first earned her Ph.D. from Cornell in 2003 and from 2005-2009, MTBI alumni have been awarded 55 Ph.D.s (mostly in the mathematical sciences) with 41 being URMs (primarily US Latinos). Since 1996 MTBI students have produced over 120 technical reports (available through our website - http://mtbi.asu.edu/Research.html), several have published in refereed journals. MTBI participants have presented their work at national professional conferences and received numerous awards.

MTBI students participate not only in one or two or three sequential summer experiences, but also in life-long professional development activities. MTBI provides its alumni continued academic encouragement and support throughout their graduate studies and afterwards. Since the program's inception at least 153 US alumni have pursued advanced degrees, and 110 (87 URMs) have or are pursuing Ph.D.s; 79 (68 URMs) have received advanced degrees and 55 (42 URMs) have received Ph.D.s. Several of our nearly 400 alumni have become MTBI mentors often helping members of community for six summers or more. In 2005, we expanded our efforts by connecting our high school Mathematics Science Honors Program (http://www.asu.edu/mshp/) and year round undergraduate and graduate mentorship efforts through (http://mcmsc.asu.edu/)_at Arizona State University in order to engage an even larger pool of talent. MTBI's multi-layer mentoring model elements, some of the model results, and the use of this mentorship model in establishing and expanding the pool of potential quantitative scientists are highlighted in this presentation.

467 That None Shall Perish

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Despite efforts to increase the number of women faculty in the STEM disciplines, the underrepresentation of women in higher academic ranks remains disproportionately low. As a means of addressing this issue, the National Science Foundation (NSF) ADVANCE Program has as its mission to increase the participation and advancement of women in academic science and engineering careers. As such, the Program utilizes advances in social science research, as well as both demonstrated and novel strategies rooted in organizational change theory as a means of targeting gender diversity issues in the science, technology, engineering, and mathematics (STEM) disciplines.

This presentation will provide an overview of the current status of women faculty, as well as the ADVANCE Program and the mechanisms by which it has supported institutions of higher education. Additionally, vital best practices and the concomitant incorporation of them into the institutional infrastructure will be discussed. These include, but are not limited to: strategic training on implicit bias, programmatic focus on departmental leadership, use of professional development grants, institutionalization of mentoring, incorporation of transparency in policies and procedures, demonstration of sensitivities toward work-life balance issues and women of color.

It is envisaged that with the development of a broad awareness of the accomplishments of the ADVANCE Program and its role in addressing issues of gender diversity, our nation will be well poised and positioned to create more equitable environments for women faculty, a changed STEM departmental culture, reduced faculty attrition, increased institutional competitiveness for recruiting highly qualified STEM faculty, increased faculty job satisfaction and an enhanced academic culture beyond the STEM disciplines.

468 Segregating and Selecting Auditory Objects

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In order to analyze the spectral and temporal content of a behaviorally important sound, a listener, be it a scientist arguing a point at an ARO poster session or a songbird listening for the call of a potential mate in the forest, must perceptually segregate the sound source of interest from other sources. In addition, the listener must be able to focus attention on a desired source as needed in order to make sense of its content.

Many human studies have explored the features of natural sound that enable us to segregate sources from one

another in everyday settings, forming the sound mixture into appropriate perceptual objects. These studies show that we exploit the inherent structure of natural sound to determine what sound elements belong to the same sound source, including common amplitude modulation, common harmonic structure, continuity in time-frequency, and rhythmic regularity.
The ability to selectively attend to a pertinent auditory object (i.e., to select out which sound is behaviorally relevant in a complex sound mixture) is an equally critical skill in everyday settings. This process of selection, although intricately linked to how we perceptually segregate sound sources from one another, is nonetheless distinct. Selection of what auditory object to process at a given moment is guided by knowledge of acoustic features of the desired source that distinguish it from other sources (such as its location, its pitch, its timbre, or even the time at which it begins), but is also influenced by the relative salience (loudness, distinctness) of all the sounds in a given mixture.
This talk will review recent human studies from our laboratory that explore the relationships between perception, sound segregation, and selection in complex sound mixtures, highlighting both the differences between as well as the interconnectedness of object formation (segregation) and attention (selection).
This work was supported by NIDCD grant R01 DC009477 and ONR grant N000140410131].

469 A Songbird as a Model for Understanding Auditory Streaming

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Most cues that provide for salient perceptual differences can lead to auditory streaming. Behavioral studies indicate that the European starling (Sturnus vulgaris) experiences auditory streaming of sequential tones in a similar way as humans do (MacDougall-Shackleton et al. 1998, Klump & Cordes, unpublished data). Sounds with similar features (e.g., tones close in frequency) are perceived as one stream, whereas sounds with very different features (e.g., tones differing by an octave) are perceived as two streams. Neural responses recorded in starling primary auditory cortical fields when listening to tone series typically eliciting streaming (e.g., ABA- sequences, A and B being tones of two different frequencies) show patterns that are compatible with the results from the behavioral studies (Bee & Klump 2004, 2005). Suppression of the response to the B signals comprising one stream by the response to the A signals comprising the other stream occurs that may result in a representation of the streams by separate neuron populations. Forward masking supports the segragation. Suppression builds up over time in a way that parallels the build-up in psychophysical streaming studies in humans (Micheyl et al. 2008). Neural response patterns observed in response to sinusoidally amplitude modulated (SAM) tones suggest that the mechanism of streaming by amplitude modulation in ABA- sequences are similar to those observed for streaming of pure tones (Itatani & Klump 2009). A dissociation of the neural responses in the ABA- sequences can also be found for harmonic-complex stimuli with a constant amplitude spectrum in which the components of the different signals have different phase relations resulting in distinct temporal patterns. Starling forebrain neurons exhibit a segregation of the responses towards these stimuli resembling stream segregation by such temporal patterns that is observed in humans (Roberts et al. 2002, Itatani & Klump 2010).
Study funded by the DFG (SFB/TRR31).

470 The Cocktail Party Problem: Insights from Electromagnetic Recordings

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A complex listening situation, commonly illustrated by the cocktail party example, requires listeners to decipher a composite acoustic wave into its constituent parts. To solve this problem, listeners can capitalize on spectral and/or location differences among the various sound objects. In this presentation, I will review prior work on concurrent sound segregation and present new findings from a concurrent vowel study in which we examined the interaction between spectral and location cues. The role of attention and learning in concurrent sound segregation and identification will also be discussed.

471 Independent Neural Populations Embody Perceptually Discrete Auditory Streams

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In a complex auditory scene, normal-hearing listeners readily segregate sequences of sounds originating from discrete sources. We are evaluating the contribution of spatial separation of sources to stream segregation in psychophysical experiments in humans. Parallel physiological experiments in cats are exploring the underlying cortical mechanisms. A rhythmic masking release procedure was used to quantify spatial stream segregation in normal human listeners. Listeners discriminated between two target rhythmic patterns consisting of sequences of brief noise bursts presented from a free-field loudspeaker. An interferer consisting of interleaved sounds presented from a second loudspeaker confounded rhythm discrimination when target and interferer were co-located, but spatial separation of the sources by ~5-10 ° permitted criterion levels of discrimination. In anesthetized cats, we recorded the synchrony of cortical neurons to interleaved sequences of noise bursts presented from pair of loudspeakers, similar to the stimulus conditions for the humans. When the sources were co-located, neurons synchronized to the aggregate pulse pattern. Spatial separation of the sources by ~5-10 ° resulted in a bias in the proportion of spikes synchronized to one or the other of the two sound sequences. Further separation often resulted in complete

capture of the neural response by one of the sequences. Comparison of the human and animal results leads to the hypothesis that discrete dynamically recruited neural populations underlie the perception of perceptually segregated auditory streams.
Support from NIH RO1 DC00420

472 Foreground and Background at the Cocktail Party—A Neural and Behavioral Study of Top-Down and Bottom-Up Auditory Attention

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The mechanisms by which a complex auditory scene is parsed into coherent objects depend on poorly-understood interactions between task-driven and stimulus-driven attentional processes. We use a simultaneous psychophysical-neurophysiological (MEG) experimental paradigm to manipulate human listeners' attention to different features of auditory scenes. In a series of experiments, our findings reveal a role of attention in enhancing the sustained neural representation of the foreground. This enhancement, in both power and phase coherence, originates in auditory cortex, occurs exclusively at the frequency of the target rhythm, and is only revealed when contrasting two attentional states that direct subjects' focus to different features of the acoustic scene. These results have substantial implications for models of foreground/background organization and mechanisms mediating auditory object formation.

473 Inner Hair Cell Functional Maturation Is Impaired in Myosin VIIa Mutant Mice

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Mutations in the gene that encodes the unconventional, non-muscle myosin, Myosin VIIa, are associated with Usher syndrome Ib (1), a rare genetic disorder that leads to deaf-blindness in humans, as well as non-syndromic deafness (2). In *Shaker-1* mice, the orthologous recessive gene also causes deafness (3) with impaired cochlear function and progressively disorganized stereocilia of the auditory hair cells (4). Mechanotransduction in hair cells of mutants deficient in Myosin VIIa is impaired requiring force on the bundle beyond the physiological range (5).
We demonstrate, for the first time, that adult $Myo7a^{6J}/Myo7a^{6J}$ (P20-P30) inner hair cells retain immature ionic properties. The fast, outward potassium current $I_{K,f}$, normally expressed by around P12, is absent: measured at 0 mV it was -210±33 pA (n=6) in $Myo7a^{6J}/Myo7a^{6J}$ and 5200±1400 pA (n=5) in $+/Myo7a^{6J}$ controls; $p<0.01$. The cells display immature-like stimulated spiking behaviour (n=8) unlike the typically mature graded receptor potential of age-matched $+/Myo7a^{6J}$ controls (n=4).
Neonatal (P2-P4) $Myo7a^{6J}/Myo7a^{6J}$ mutants (n=10) show spontaneous and evoked spiking behaviour like $+/Myo7a^{6J}$ controls (n=8). Also the outward potassium currents of neonatal (P2-P4) $Myo7a^{6J}/Myo7a^{6J}$ and $+/Myo7a^{6J}$ hair cells appear similar: 2470±290 pA (n=9) and 2470±370 pA (n=8) respectively measured at –25 mV.
These data explicitly show that lack of Myosin VIIa not only impairs mechanoelectrical transduction but also causes dysfunctional development of inner hair cell basolateral currents.
Supported by the Medical Research Council and EuroHear.

474 Usher Syndrome IIIA Gene Clarin-1 Is Essential for Hair Cell Function and Associated Neural Activation

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Usher syndrome IIIA (USH3A) is an autosomal recessive disorder characterized by progressive loss of hearing and vision due to mutation in the clarin-1 (*CLRN1*) gene. One of the most common genetic alterations in USH3A patients is the Y176X mutation in *CLRN1*, a presumptive null mutation. Lack of an animal model has hindered our ability to understand the function of the protein (CLRN1) and the pathophysiology associated with USH3A. Here we report on a the phenotypic characterization of *Clrn1* knockout ($Clrn1^{-/-}$) mutation in the mouse, and our results show that the inner ear phenotype in the knockout animals is similar to the clinical presentation seen in patients harboring the Y176X mutation. The $Clrn1^{-/-}$ mouse should, therefore, be a good model for ear disease in USH3A patients. *Clrn1* mRNA is expressed as early as embryonic day 16.5 in the auditory and vestibular hair cells and associated ganglionic neurons. At 2-3 weeks postnatal (P14-21), $Clrn1^{-/-}$ mice showed elevated auditory brainstem response (ABR) thresholds and prolonged peak and interpeak latencies. By P21 ~70% of $Clrn1^{-/-}$ mice had no detectable ABR, and by P30, almost all $Clrn1^{-/-}$ mice were deaf. Distortion product otoacoustic emissions were not recordable from $Clrn1^{-/-}$ mice. Vestibular function in $Clrn1^{-/-}$ mice mirrored the cochlear phenotype, although it deteriorated more gradually than cochlear function. Disorganization of OHC stereocilia was seen as early as P2, and by P21, OHC loss was observed. Also, previous studies have shown that USH3A patients with a single mutant allele of USH1B (the myosin 7A gene) exhibit the USH1 phenotype, suggesting a genetic interaction between *CLRN1* and *MYO7A.* In sum, we hypothesize that *Clrn1* is necessary for hair cell function and associated neural activation, and that CLRN1 interacts with MYO7A to mediate its function. Experiments to test this hypothesis are underway and results from these studies will be presented at the meeting.

475 TRIOBP Bundling of Actin Filaments Is Indispensable for Hearing

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Mutations of TRIOBP have been identified as the cause of human hereditary deafness DFNB28, and TRIOBP KO mice do not develop stereocilia rootlets thereby causing deafness. In this study, we performed biochemical analyses to determine how TRIOBP forms stereocilia rootlets. FLAG-tagged TRIOBP protein was expressed in Sf9 cells, and purified using anti-FLAG resin and gel filtration. Purified TRIOBP protein co-sedimented with actin filaments in vitro, indicating that TRIOBP binds directly to actin. However, time-lapsed TIRF imaging of fluorescently labeled actin showed that TRIOBP does not change the rate of actin polymerization. Transmission electron microscopy images showed that actin filaments formed very tight bundles in the presence of TRIOBP. These TRIOBP-bundled actin filaments are much more densely packed than those bundled in vitro by purified espin, a protein that is localized in stereocilia cores and not in rootlets. In combination with the phenotype of our TRIOBP KO mouse, we conclude that TRIOBP is a novel actin bundling protein necessary for the formation of stereocilia rootlets.

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476 Stereocilia and Espin Haploinsufficiency: Novel Developmental Stereociliary Defects Observed in Heterozygous Jerker Mice by Scanning Electron Microscopy

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We have been examining espin-deficient jerker mutant mice to ascertain the roles of the espin actin-bundling proteins in stereocilium morphogenesis. To minimize the impact of age-related hearing loss, we are examining a congenic jerker mouse line prepared in the CBA/CaJ background strain. Previously, we have determined that a lack of espin in CBA/CaJ jerker homozygotes reduces the length and width of stereocilia and ultimately results in loss of stereocilia. In contrast, the cochleas of adult CBA/CaJ jerker heterozygotes showed relatively few abnormalities, even at up to 1 year, and ABR measurements on 13-month-old jerker heterozygotes was similar to wild-type controls. Thus, the hair cell pathology and hearing loss noted previously by others in aged heterozygous jerker mice of the original background strain are likely the result of age-related hearing loss. A careful examination of the CBA/CaJ jerker heterozygotes, however, revealed a group of novel defects early during stereocilia development that are presumably the result of espin haploinsufficiency. At P0, cochlear stereocilia in the jerker heterozygotes were shorter and thinner than those in wild-type CBA/CaJ mice, but by P5 there was no difference from wild-type. We also saw tapering of the longest stereocilia on inner hair cells in the apical region of the cochlea in CBA/CaJ jerker heterozygotes. In the vestibular system, especially the cristae, the tallest stereocilia showed an uneven width in CBA/CaJ jerker heterozygotes. At P0, stereociliary width along a 2-3 micrometer basal segment was similar to wild-type, but then suddenly narrowed to about half this width above that level. A milder, gradual tapering was observed in other vestibular stereociliary bundles before P10. We conclude that espins are so crucial for the maturation and maintenance of stereocilia that defects in stereocilium morphogenesis are evident even when espins are present at half-normal levels. (NIH DC004314, JRB)

477 Fascin 2 Is a Major Actin Crosslinker in the Hair Bundle

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Recent advances in mass spectrometry based protein identification has made it possible to probe entire proteomes with unprecedented sensitivity. We previously described a method to purify and identify proteins in the sensory hair bundle using mass spectrometry (Shin et al., Neuron 53, 371-86; 2007). We have further developed the method to gain quantitative information about proteins identified in our screen. We find that fascin 2 (FSCN2) is the most abundant actin-crosslinking protein in chicken hair bundle. Immunolabeling experiments confirm that FSCN2 is specifically localized to the bundle. FSCN2 distribution in the hair bundle is not uniform, however, but is concentrated in longer stereocilia and towards stereocilia tips. In the mouse cochlea, FSCN2 expression is absent at birth, but appears in the bundle at around P10, progressing from inner to outer hair cells. Interestingly, FSCN2 maps to a locus for early onset, progressive hearing loss phenotype in the DBA/2J inbred mouse, a strain extensively used in hearing research. The FSCN2 gene in DBA/2J mice has a mutation that changes a highly conserved arginine to histidine, a mutation that is absent in all other non-affected mouse strains examined. Immunolabeling experiments demonstrate that the mutation does not disrupt expression and localization of FSCN2 in the hair bundle. We hypothesize that FSCN2 is important for postnatal maturation and maintenance of the sensory hair bundle, and that the early-onset progressive hearing loss in DBA/2J mice is caused by a gradual decline of hair bundle integrity and function, likely caused by a suboptimal function of the mutant FSCN2 protein.

478 Otoferlin Function in Ca^{2+}-Dependent Exocytosis at Cochlear and Vestibular Hair Cell Ribbon Synapses

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Otoferlin, a large Ca^{2+} binding protein with six C2-domains, has been proposed as an essential Ca^{2+} sensor for transmitter release at auditory hair cell ribbon synapses (Yasunaga et al., 1999; Roux et al., 2006; Beurg et al., 2008). Indeed, otoferlin knock-out (Otof $^{-/-}$) mice are deaf because inner hair cells lack Ca^{2+}-evoked exocytosis. Intriguingly, while otoferlin is also highly expressed in type I and type II hair cells of vestibular organs, Otof $^{-/-}$ mice do not display apparent vestibular disorders suggesting that otoferlin might not be essential for exocytosis in all types of hair cells.

Here, we show that the vestibular nerve compound action potentials evoked during linear acceleration pulses in Otof $^{-/-}$ mice display higher threshold, lower amplitude and increased latency compared to wild-type mice, suggesting a partial synaptic deficit in vestibular hair cells. Using patch clamp capacitance measurement in intact utricles, we show that wild-type type I and type II hair cells display a remarkable linear transfer function between Ca^{2+} entry, flowing through voltage-activated Ca^{2+} channels, and exocytosis. In Otof $^{-/-}$ vestibular hair cells, exocytosis displays slower kinetics, reduced Ca^{2+} sensitivity and non-linear Ca^{2+} dependence, despite morphologically normal synapses and normal Ca^{2+} currents.

Overall all, our data show that vestibular hair cells in contrast to cochlear hair cells still show residual exocytosis, however with changes in the Ca^{2+} dependence of release and kinetics. These subtle changes give additional insights into the role of otoferlin that likely operates in conjunction with other Ca^{2+} sensors. We conclude that otoferlin is essential for a high affinity Ca^{2+} sensor function that allows efficient and linear encoding of low intensity stimuli at the hair cell synapse. The residual nonlinear and poorly Ca^{2+}-sensitive exocytosis in Otof $^{-/-}$ vestibular hair cells is likely driven by other low affinity Ca^{2+} sensors that remain to be uncovered.

479 Using Cameleon to Measure Zebrafish Hair-Cell Function *in Vivo*

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The zebrafish inner ear is well characterized both developmentally and anatomically and is required for balance and the acoustic startle reflex. Many zebrafish genes associated with inner ear defects have been shown to be orthologues of human deafness genes, demonstrating the relevance of zebrafish as a hearing model.

In order to understand the cellular basis of hearing and balance and probe the molecular mechanisms of mechanosensation in zebrafish, we have created transgenic fish expressing the genetically encoded calcium sensor (GECI) cameleon in hair cells. In these fish, robust calcium responses can be measured in response to a variety of stimulations.

Using these transgenic fish we have begun to characterize several zebrafish vestibular mutants. To start, we examined *gemini* mutants, defective in the L-type calcium channel Cav1.3. These mutants displayed calcium responses that were reduced by 50% compared to wild-type. This reduction was phenocopied by 10uM isradipine. To determine if the residual response in *cav1.3* mutants was due solely to the MET channel, we applied a series of drugs including amiloride (MET channel blocker), suramin (P2 channel blocker), ryanodine (RyR blocker at high concentrations), and CPA (SERCA blocker). The MET channel blocker amiloride completely blocked the remaining calcium response in *cav1.3* zebrafish mutants, and abolished all response in wild-type hair cells. Of the other drugs, only CPA had a specific effect, significantly reducing the calcium response in wild-type hair cells, while having little effect on *cav1.3* mutant hair cells. This indicates that the 50% reduction seen in *cav1.3* mutants may include both calcium influx directly from Cav1.3 and also secondarily from release of calcium from the ER. The potential for other calcium sources is currently being explored.

This preliminary data indicates that our transgenic fish provide a useful tool for exploring hair-cell function. By testing additional zebrafish auditory and vestibular mutants, we hope to acquire more detailed information about the complex molecular process of hearing and a greater general understanding of the process of mechanosensation. An update of these studies will be presented.

480 Hair Cell Damage in the Neonatal Mouse Cochlea Using Forced Expression of Diphtheria Toxin

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Hearing loss caused by cochlear hair cell (HC) damage is permanent in mammals; however, non-mammalian vertebrates can regenerate HCs. Much of the work involving HC damage in mammals has been conducted in adult guinea pigs and chinchilla. As the technology of transgenic and knockout mouse models advances, there is a great need to model HC damage in mice, especially in neonates when the organ of Corti appears to be more plastic and thus more likely to regenerate. Unfortunately, there has been little success in damaging HCs of neonatal mice using ototoxic drugs. Noise damage is also not effective in neonates as hearing is not fully mature until postnatal day 21 (P21). Recently our laboratory has used a HC-specific, inducible Cre mouse line (Atoh1-CreER) to specifically manipulate genes in neonatal HCs (Weber et al., *Proc Natl Acad Sci*, 2008). Here, we have crossed the Atoh1-CreER line with ROSA26-eGFP-DTA mice to induce the expression of diphtheria toxin, fragment A (DTA) in neonatal HCs. ROSA26-eGFP-DTA mice express eGFP ubiquitously with DTA expression prevented by the

presence of a strong stop codon. Upon Cre-mediated excision of the floxed eGFP, DTA is translated, resulting in specific ablation of Cre-expressing cells (Ivanova et al., *Genesis*, 2005). Using this novel technique of Atoh1-CreER; ROSA26-eGFP-DTA mice induced with tamoxifen at P0-P1, ~60% HC loss occurred at P7 and complete HC loss occurred by P15. Both inner and outer HCs were killed throughout the length of the cochlea, resulting in deafness. We are currently investigating the effect of DTA-induced HC damage on supporting cells. This model likely induces HC death by reactive oxygen species-induced apoptosis, which is similar to the cell death pathway implied in drug-induced HC death. Moreover, it is possible that the neonatal organ of Corti is able to respond to HC damage, perhaps showing signs of regeneration.

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481 What Is the Role of Release Mechanism in the Cochlear Amplifier?

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Reverse transduction in hair cells, which includes electromotility and adaptation that brings about partial closure of the MET channel, has been a focus of hearing research due to its role in enhancing sensitivity and frequency selectivity. While their relative significance has been an issue for mammalian outer hair cells, adaptation is the only known active process capable of functioning as an amplifier in non-mammalian hair cells that lack electromotility.

Of the two components of adaptation, slow adaptation is considered to regulate the operating point and fast adaptation is thought to have a role of an amplifier. In this regard, the observation that fast adaptation is based on a reduction of tension at the tip link (Stauffer et al., 2005; LeMasurier and Gillespie, 2005) is puzzling because such a delayed relaxation is a characteristic of a damper, not of an amplifier.

To address this issue, we examine a simple release model. For small displacement of the hair bundle, we show that release mechanism together with negative stiffness, a result of gating compliance involving the MET channel, indeed works as an amplifier. We further suggest that this feature is not specific to this particular model but it is generic to any relaxation mechanism combined with negative stiffness, including the model proposed by Tinevez et al. (2008).

482 Mechanical Motility in Hair Bundles Coupled to Artificial Membranes

Dolores Bozovic[1], Joshua Tokuda[1], Van Mai[1], Keita Onoue[1]
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To study the role of inter-cell coupling in shaping the response of the overall system, biologically functional in vitro preparations of the internal ear were interfaced with artificial membranes and electronic feedback circuits. Experiments were performed on epithelia of the bullfrog sacculus, with the natural overlying membranes removed. Artificial coupling elements of various sizes were fabricated from polylactide material, and conjugated so as to improve connection to the tissue. Transepthelial electrical stimulation was applied to induce and modulate movements of the underlying hair bundles. Responses induced in the hybrid system will be discussed and compared to hair bundle movements evoked under natural coupling conditions.

483 Coupling a Sensory Hair-Cell Bundle to Cyber Clones Enhances Nonlinear Amplification

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The vertebrate ear benefits from nonlinear amplification of mechanical vibrations by sensory hair cells to operate over a vast range of sound intensities. Hair cells are each endowed with a hair bundle which can oscillate spontaneously and function as a frequency-selective, nonlinear amplifier. Intrinsic fluctuations, however, jostle the response of a single hair bundle to weak stimuli and seriously limit amplification. We report that a hair bundle can effectively reduce noise and enhance amplification by teaming-up with other hair bundles. We implemented a dynamic force-clamp procedure to couple a hair bundle from the bullfrog's saccule to two cyber clones that emulated flanking neighbours. We argue that the auditory amplifier relies on cooperation of a small group of hair bundles to overcome intrinsic noise limitations and achieve high sensitivity and frequency selectivity.

484 Regeneration of Stereocilia of Cochlear Hair Cells by Math1 Gene Therapy

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Cochlear hair cells transduce mechanical stimuli into electrical activity. The site of mechanoelectrical transduction is the stereociliary bundle in the apical surface of hair cells. The delicate hair bundle is susceptible to acoustic trauma and ototoxic drugs. Hair cells in lower vertebrates and mammalian vestibular organs can spontaneously regenerate the stereocilia once lost. Mammalian cochlear hair cells, however, no longer retain that capability. The inability to self-repair the damaged stereocilia subsequently leads to hair cell death and hearing loss. We explored the possibility of regenerating stereocilia in the noise-deafened guinea pig cochleas by over-expression of *math1*, a gene encoding a basic helix-loop-helix transcription factor and a key regulator of hair cell differentiation. After impulsive noise exposure, a 60-80 dB hearing loss was seen in the middle to high frequency regions. SEM examinations performed at 7, 10, and 14 days after noise exposure revealed extensive stereociliary damage and loss in both inner and outer hair cells in the first, second and third turns. However, majority of hair cells were able to survive the

bundle damage or loss for up to 7 to 10 days, although sporadic hair cell loss was also seen in some areas. M*ath1* inoculated within the first week after noise exposure was able to induce stereociliary regeneration. The newly regenerated stereocilia were functional, as ABR and CM measured 1 and 2 months after *math1* inoculation showed significant hearing threshold improvement in the frequency range that was mostly affected. Our results suggest that *math1* over-expression promotes regeneration of the stereocilia. *Math1*-based gene therapy, therefore, has the potential to restore hearing after noise-induced hair cell damage. (Supported National Natural Science Foundation of China grants No. 30871398 and 30730040 to SY and by NIH grant R21 009908 to DH)

485 Adaptive Functional Switch: A New Stratagem for Repopulation of Non-Renewable Inner Ear Sensory Cells

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Loss of sensory hair cells (HC) is the major cause of hearing impairment, often leading to spiral ganglia neuron (SGN) degeneration. The HC and SGN loss is irreversible in mammals, because they have a limited capacity to regenerate. In pursuit of effective cell replacement therapy, an attractive solution to solve both the loss of non-reproducible cells and immune rejection is using an autologous source of target cells.

Here, we report that in the adult brain of both rodents and humans, the ependymal layer of the lateral ventricle contains cells that retain the ability to proliferate, and share morphological and functional characteristics with HCs. In addition, putative neural stem cells (NSCs) from the subventricular zone of the lateral ventricle can differentiate into functional SGNs. Also important, the NSCs can incorporate into the sensory epithelia, demonstrating their therapeutic potential. We assert that ependymal cells and NSCs can undergo an adaptive functional switch to assume essential characteristics of HCs and SGNs. This study also revealed that functional adaptation should be recognized as an effective component of cell replacement therapy.

486 Tinnitus Research and Treatment: The Next Frontier

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Tinnitus is a prevalent hearing disease, affecting 15% of the population, particularly hearing impaired, veterans and even young people. The mechanisms underlying tinnitus remain controversial. There are few available treatment options and many of the most severe cases of tinnitus remain intractable. The goals of the present workshop are threefold. First, the workshop will bring together prominent tinnitus researchers and clinicians to give the ARO audience an overview of the problem and its potential solutions. Second, as the premier avenue for otolaryngology research, ARO provides an excellent platform to exchange information, stimulate interest, and attract both students and seasoned researchers to the tinnitus field. Last but not the least, by engaging tinnitus patients, the workshop will promote communication and understanding between researchers and patient advocates to achieve common goals.

487 Tinnitus: A Patient Perspective

Mari Quigley-Miller[1]
[1]Speaker

I have had hearing loss for more than 20 years as a result of a progressive loss in one ear and an acoustic tumor in the other. Tinnitus started with hearing loss on a come-and-go basis since the late 1980's and has been on going on a steady basis since 1995. It's been frustrating to cope with both hearing loss and tinnitus, but I've been trying to stay positive by relying on a positive attitude and helping other tinnitus sufferers first as a telephone support volunteer for the American Tinnitus Association (ATA), then progressing into a tinnitus self help group coordinator in Orange County, CA. On May 16, 2009, I organized the first ever National Walk to Cure Tinnitus and raised $60,000 for ATA. I have seen members get off medications for sleep by switching to sound therapies, counseling, and alternatives such following a healthy diet, herbs, and exercise. I would like to see the scientific community pay more attention to tinnitus and to look outside of the box including, but not limited to, the interactions of tinnitus with the jaw, neck, food and allergies along with continued brain research.

488 Neural Mechanisms of Tinnitus

Jos J. Eggermont[1]
[1]University of Calgary

Tinnitus resulting from different etiologies has different ignition mechanism, albeit that the way it is represented in cortex is likely the same. Because of the strong neural feedback loops from the cortex to thalamus, midbrain and lower brainstem, the neural mechanisms of long-standing tinnitus may involve the entire auditory system network. In addition the amygdala may be involved and be at the basis for the strong emotional content of certain forms of tinnitus. Animal research and non-invasive studies in humans have suggested that tinnitus is often accompanied by increased spontaneous firing rates and increased neural synchrony. In case noise-induced hearing loss is the etiology there are indications that changes in the auditory cortical frequency place (tonotopic) map may also be present. The underlying mechanisms are likely a (temporary) imbalance in the level of excitation and inhibition cause by the reduction of afferent input or by an increase thereof (potentially in the case of somatic tinnitus).

489 Central Tinnitus as Prime Example of Thalamocortical Dysrhythmia

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Tinnitus, and auditory dysfunction of central or peripheral origin, is characterized by the perception of auditory noise, most commonly a whistling or a roaring sound, in the absence of an objective physical sound source. While it is

now universally accepted that central tinnitus is a disconnection syndrome the underlying neuronal mechanism is presently unresolved. Indeed, central tinnitus provides a unique opportunity to define the intrinsic neuronal and ionic mechanisms capable of supporting such stable auditory hallucination. A very likely mechanisms relate to recurrent thalamo-cortical resonance known as thalamocortical dysrhythmia. This presentation will address such possibility.

490 Sound Therapy and Counseling for Tinnitus

Rich Tyler[1]
[1]*U of Iowa*

A variety of counseling and sound therapies have been developed over the past two decades. The counseling approaches follow a broad range, depending on patients needs. Theoretical approaches vary, focusing on habituation, cognition, behavior, fear, control or acceptance. There is now emerging evidence that some of these counseling treatments provide relief for the primary handicaps of tinnitus; changes in 1) thoughts and emotions, 2) hearing, 3) sleep and 4) concentration. Many sound therapy approaches have also been proposed, including the use of partial and total noise masking, music and modulation tonal complexes. There is less agreement on the benefit of devices, but my personal clinical experience is that they help some individuals.

491 How Do We Find a Cure for Tinnitus? Approaches and Challenges for Medical Intervention Research

William Martin[1]
[1]*Oregon Health & Science University*

Although tinnitus management strategies are often successful at reducing the emotional and psychological impact of tinnitus, patients have one common desire – to turn the tinnitus off. The more we understand of the brain's role in tinnitus, the higher the likelihood of accomplishing this goal. Brain-based strategies come in two main forms: One attempts to change the firing pattern of the brain through variations of electrical stimulation. The other attempts the same goal through modifying the chemistry of the brain. The hope is that abnormal firing patterns, perceived as tinnitus, can be eliminated.

Clinical research evolves in cycles. Clinical observation, exploration, trial and error help build hypotheses. The hypotheses then must be tested in extremely controlled, rigorous experiments designed to demonstrate whether or not an intervention really works, and if so, for whom. This presentation will discuss several brain-based interventions to disrupt tinnitus and also some of the great challenges facing tinnitus researchers conducting clinical trials.

492 Reconsidering the Conventional Model for Transduction

David P. Corey[1]
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Several years ago, there was a fairly comprehensive view of transduction and adaptation by hair cells. We knew how the adaptation motor, myosin-1c, regulates tension on transduction channels and also how Ca2+ entry controls myosin-1c. We had good evidence from Ca2+ imaging that transduction channels are at both ends of the tip link, which in turn suggested that the tip link was a symmetric structure. For lack of a better candidate, the tip link was often drawn as—and sometimes assumed to be—the elastic gating-spring element of biophysical models for transduction. We even had a good candidate for the transduction channel itself.

New results in the past few years have upended this understanding. The identification of the tip link proteins as cadherin-23 and protocadherin-15 has forced recognition that this structure is asymmetric. Higher-resolution and higher-speed Ca2+ imaging suggests that transduction channels are only present at the lower end of the tip link, far from the myosin motors they are supposed to regulate with Ca2+ influx. Thus models in which myosin mediates both fast and slow adaptation must be rethought. Evidence from both crystal structures and molecular dynamics simulations of cadherins suggest that the tip link is not very elastic and that we must incorporate an additional elastic element into structural models of transduction. And a variety of candidates for the transduction channel have risen and then fallen when tested with knockout animal models.

Key questions remain: What is the structure of the tip link, and how are the transduction channels attached to it? What is the transduction channel protein? How does Ca2+ control "slow" and "fast" adaptation? Do the same processes mediate adaptation in mammals and lower vertebrates? Is active motility of stereocilia the basis for frequency tuning in the cochlea, and what drives it? Defining the issues is the first step in creating a post-modern view of transduction.

493 Calcium Imaging Localizes Mechanotransducer Channels to Tops of Stereocilia

Anthony Ricci[1]
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High speed swept field confocal calcium imaging was used to localize mechanotransducer channels to the uppermost region of stereocilia. Rat inner and outer hair cells were investigated. Calcium signals in shorter stereocilia (rows 2 and 3) were several fold larger and faster in rise time than those in the tallest, first row. Imaging at three heights along the stereocilia showed larger signals for the second and third row near to their top while the first row showed larger signals near the base. Together these data suggest mechanotransducer channels are localized near the tops of stereocilia and not along the sides of stereocilia. Also examples where third row stereocilia were active while

second row were not further supported the argument that the top and not the side connection of the tip-link insertion was the site of mechanotransduction. Diffusion times measured were slow in comparison with measured adaptation rates. Thus channel localization calls into question existing hypotheses regarding adaptation mechanisms. Previous work has suggested a climbing, slipping model that requires myosin (likely myosin Ic) to regulate tip-link tension in a calcium-dependent manner. Although an adaptation mechanism occurring at a distance from the channel is plausible, the kinetics of the adaptation responses, at least in the mammalian auditory system, are too fast for a process that will be limited by diffusion, let alone coupled with the known kinetics of myosin Ic. Thus, it seems unlikely that the myosin motor mechanism can be directly responsible for either fast or slow adaptation. However, it is possible that tip-link tension is maintained independently of adaptation, in which case the myosin hypothesis may play a role.

494 Revealing the Structure of the Stereocilia Transduction Complex Using Imaging

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The hair cell mechanoelectrical transduction (MET) apparatus is presumed to be organized around a structural tip link between pairs of adjacent stereocilia actin protrusion that form a staircase shaped bundle on the apical surface of the hair cells. The dynamic properties and molecular composition of these tip links has been the subject of intense debate over the past two decades. Two members of the cadherin family of adhesion proteins, cadherin 23 (CDH23) and protocadherin 15 (PCDH15), were recently identified as tip link constituents. The asymmetry formed by heterophilic interaction of these molecules together with the recent localization of calcium entry at the lower end of tip links provides new opportunities for understanding MET at the molecular level. Despite these and other advances, the current understanding of the MET molecular machinery is still very rudimentary. Electron micrographs of the presumed site of MET shows characteristic electron dense structures - the tip link insertion plaques - whose size and density suggests the presence of at least hundreds of proteins. For example, the well-studied cell-matrix adhesion plaques, which have a comparable size to stereocilia insertion plaques, contain dozens of copies of hundreds of different proteins. It is noteworthy that the stereocilia tips are also the site of complex actin polymerization processes and dynamic stereocilia length and shape regulation machinery. Only very few of the molecules that make up the tip density have been identified. While putative relationships between the MET channel properties and stereocilia myosins and scaffolding proteins have been inferred from functional and molecular assays, the molecular identity of the channel itself and intricacies of the MET machinery remain unsolved. The presence of stereocilia top-connectors and other ever-present stereocilia radial links in proximity to the tip links keep open the possibility that the tip links may not be the sole mediators of mechanical input to the MET channel gating mechanism when bundles are deflected. In this presentation we will review the current state of the art ultrastructural investigations (including electron tomography) of the transduction apparatus, focusing on localization and mobility of proteins relevant to MET function. The limits of protein localization and imaging methods will be discussed in context of essential hair-bundle proteins. We will also present and discuss structural correlates of MET properties during developmental changes as well as in hair bundles from mice deficient in essential stereocilia proteins. Finally, we will discuss the potential reciprocal influence between the MET and actin regulation machineries in helping dynamically maintain the mechanosensory bundle at an optimal operating range.

495 Molecular Constituents of the Tip-Link Complex of Hair Cells

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Tip links are thought to gate mechanotransduction channels in hair cells. Tip links consist of a cadherin 23 (CDH23) homodimer that interacts in trans with protocadherin 15 (PCDH15) homodimer to form the upper and lower part of tip links, respectively. The distribution of CDH23 and PCDH15 at tip links suggests that the mechanotransduction machinery of hair cells is inherently asymmetric. In support of this model, recent studies suggest that the mechanotransduction channels in cochlear hair cells are localized to the lower end of tip links. To identify molecules that might be important for tip link function, we have searched for proteins that interact with the cytoplasmic domains of CDH23 and PCDH15 and are localized in proximity to tip links. We show here that the PDZ-domain protein harmonin is a component of the upper tip-link density (UTLD) where CDH23 inserts into the stereociliary membrane. Using forward and reverse genetics approaches, we have generated mouse lines with mutations in tip-link cadherins and harmonin. Some of the mutations are similar to those that are associated with deafness in humans and affect mechanotransduction and tip-link maintenance. Collectively, our finding define essential components of the tip-link complex in hair cells and provide strong evidence that some forms of human deafness are caused by defects in tip-link function.

496 Structural Determinants of Cadherin-23 Function in Hearing and Deafness

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The tip link is an essential component of the hair-cell transduction apparatus and has been proposed to be part of a biophysically defined "gating spring". Cadherin-23 and protocadherin-15 likely form the tip link; both proteins belong to the cadherin superfamily of calcium mediated cell-cell adhesion proteins and are involved in hereditary

deafness, yet their molecular structures and elasticity are unknown. Here we present crystal structures for cadherin-23 repeats 1 and 2 (EC1+2). Overall, the structures show a typical cadherin fold for both repeats, but reveal a novel calcium binding site and an elongated N-terminus that impairs classical cadherin-cadherin binding interactions. The linker region between the repeats closely resembles a classical cadherin calcium-binding motif and contains the site of the D124G mutation causing non-syndromic deafness (DFNB12). The crystal structure of EC1+2 carrying this mutation displays a different angle between the repeats than the wild type protein. Molecular dynamics simulations of wild type and mutant structures suggest that deafness mutations and removal of calcium ions control cadherin inter-repeat motion and unfolding strength of hair-cell tip links. The new structures along with simulations indicate that cadherins forming the tip link are too stiff to be the gating spring. In addition, the new structures define a previously uncharacterized family of cadherin proteins and begin to suggest mechanisms underlying disease as well as ways in which cadherin-23 may bind end-to-end with itself and also with protocadherin-15 to form the tip link.

497 Molecular Structure of the Hair Bundle

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We are using multiple approaches to identify proteins of avian and mammalian hair bundles, including components of the mechanotransduction apparatus. In one approach, we purify hair bundles from the chicken utricle with the twist-off procedure, yielding >95% pure bundles, then subject them to shotgun mass-spectrometry analysis. Using this approach, we identified ~300 major hair-bundle proteins, including some (e.g., cadherin-23, protocadherin-15, myosin-1c) known to be involved in transduction. By quantifying proteins in both bundles and whole utricular epithelium, we found that ~50 proteins were enriched >5-fold in hair bundles and another ~125 were at comparable levels in both samples. These 175 proteins are the major proteins of the hair bundle. In a second approach, we used oligonucleotide microarrays to identify transcripts expressed at higher levels in the mouse organ of Corti or vestibular system than in the stria vascularis. A total of 1338 genes (of 45,100 on the array) had transcripts with significantly altered expression sensory epithelia. Strikingly, we found nearly 100 genes on both lists: their proteins were found in hair bundles and their transcripts were elevated in hair cells. This overlap validates the power of each approach, and together, the two approaches illuminate the array of molecules used to build a stereocilium and its transduction apparatus.

498 Tip Link Orientation and Mechano-Electrical Transduction

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Mechano-electrical transduction in hair cells depends upon the integrity of tip links, the extracellular filaments that run from the tips of the shorter stereocilia to the lateral surface of the adjacent taller stereocilia. Oblique orientation of these links is thought to be crucial for proper gating of the transduction channels at the tips of shorter stereocilia. We have studied mechano-electrical transduction in the inner hair cells of young postnatal shaker 2 mice that do not have tip links but their equally short stereocilia are interconnected only with “top-to-top” links. These abnormal “top-to-top” links are still able to mediate mechanotransduction responses with the normal amplitude and speed, suggesting that the transduction channels can function even if they are dislocated from the stereocilia tips. However, fast adaptation is disrupted in shaker 2 inner hair cells and the transduction current is insensitive to extracellular Ca^{2+}. In the same shaker 2 mutant mice, outer hair cells still have obliquely oriented tip links that mediate mechanotransduction responses with apparently normal fast adaptation and sensitivity to extracellular Ca^{2+}. Thus, Ca^{2+} sensitivity of the transduction machinery is not an intrinsic property of the transduction channel but a phenomenon that seems to depend on the orientation and/or positioning of a tip link. These observations favor a “tension release” model of fast adaptation over a “channel re-closure” model.

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499 Amplification by Active Hair-Bundle Motility

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In addition to responding to mechanical stimuli, the hair cell's transduction apparatus mediates active hair-bundle motility, one mechanism underlying the active process that increases responsiveness to sound, sharpens frequency selectivity, and compresses the dynamic range of hearing. An exuberant active process even causes spontaneous otoacoustic emissions. In non-mammalian tetrapods—and perhaps in mammals as well—mechanical amplification is accomplished by active hair-bundle motility, which results from the interaction of two phenomena. First, the hair bundle displays negative stiffness owing to the concerted gating of transduction channels. And second, the adaptation process implemented by the motor protein myosin-1c supplies energy by continually restoring the bundle to its range of negative stiffness.

The operation of the active process near a dynamical instability, the Hopf bifurcation, explains many of the characteristics of hearing. In particular, the dependence of response amplitude on stimulus force for such a critical oscillator is expected to follow a power law with an exponent of one-third, as is measured experimentally. Again as observed in a variety of species, threshold stimuli entrain a critical oscillator without affecting the amplitude of its movement; only substantially stronger stimulation enhances the response magnitude. A system operating near a Hopf bifurcation additionally produces distortion products with the level dependence observed for human hearing. Finally, a critical oscillator can become unstable,

providing a natural explanation for spontaneous otoacoustic emissions.

500 Syllable-Evoked Activity Simultaneously Recorded from Heschl's Gyrus and the Lateral Superior Temporal Gyrus

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Previous studies from our laboratory have identified 3 distinct functional fields in human auditory cortex based on averaged evoked potentials (AEPs) (Brugge et al., 2008, Hear Res 238: 12-24) and event-related band power (ERBP) in the electrocorticogram (ECoG) (Brugge et al., 2009, J Neurophysiol doi:10.1152/jn.91346.2008). These fields include the posteromedial (PM) portion of Heschl's gyrus (HG), the anterolateral (AL) portion of HG, and the immediately adjacent posterior superior temporal gyrus (pSTG). Definition of these fields was obtained through characteristic response patterns elicited by simple stimuli such as brief 100 Hz click trains (5 pulses) or more prolonged click trains with varying repetition rates. PM HG responses were characterized by short-latency, high amplitude AEPs with superimposed phase-locked responses to high click rates and robust changes in the ERBP. In contrast, AEPs recorded from AL HG were of longer latency, lower amplitude, failed to exhibit phase-locked responses to even low click rates, and had an ERBP response only to stimuli with higher click rates. Responses from pSTG were intermediate between these two extremes. It is unclear, however, whether this functional categorization is applicable for the processing of more complex sounds such as speech.

In this study, we examine AEPs and ERBP in the ECoG elicited by syllables varying in their voice onset time (VOT) and consonant place of articulation (i.e., /ba/, /ga/, /da/, /pa/, /ka/, and /ta/). Recordings were simultaneously acquired from AL and PM HG, and pSTG. On-going analysis supports, in general, the previously described classification scheme. AEPs and ERBP in PM HG are of short-latency, high amplitude, and reflect with high fidelity the temporal features of VOT and the syllable fundamental frequency (f0). Recordings from AL HG are of long-latency, lower amplitude, and respond with generally poor fidelity to the temporal features of the syllables. Once again, AEPs and ERBP recorded from pSTG are intermediate in activity patterns, with high-amplitude responses having slightly longer latency than PM HG. VOT is represented in the temporal AEP morphology and the amplitude of the ERBP, while phase-locked activity to the f0 is not observed.

We conclude that this basic categorization (i.e., PM HG, AL HG, and the immediately adjacent pSTG) offers a reasonable starting point for the development of more complex parcellation schemes of auditory cortex.

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501 Spectro-Temporal Encoding of Speech by ECoG Signals in Human Auditory and Motor Cortices

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Research in non-human mammals has described the spectro-temporal representation of speech and other natural sounds in cortex, but little is known about how these findings generalize to humans. To study auditory representation in humans, we recorded electrocorticographic signals (ECoG) from epileptic patients during a 24 hour period. Subdural recordings were made using a grid of electrodes (spacing 10 mm) placed over temporal and frontal lobes. Patients were presented with a sequence of speech sounds (isolated words and sentences) during passive listening.

To characterize auditory tuning, spectro-temporal receptive fields (STRFs) were estimated using the time-varying high gamma power (100-300 Hz, 10 ms time bins) in the ECoG signal at each recording site. STRFs were estimated by normalized reverse correlation, a procedure that compensates for correlations present in speech and other natural sounds that can bias STRFs estimated using other methods.

Sites along the lateral surface of the superior temporal gyrus showed clear tuning to sound features, and the corresponding STRFs were able to predict high gamma activity in a validation data set with correlations up to r=0.4. STRFs measured from power at lower ECoG frequencies and the raw signal showed weaker tuning; high-gamma STRFs performed consistently better. Preliminary topographic data revealed systematic variability in bandwidth across sites. For a small number of sites in primary motor cortex, STRFs also had significant predictive power, suggesting that these areas participate in processing the basic features of speech, even during passive listening. A large number of sites in temporal cortex that could not be characterized with STRFs did show phase-locked responses to auditory stimuli. Characterization of tuning at these sites may be possible with nonlinear spectro-temporal models or models that incorporate high-level abstract sound features.

502 Predicting the Selective Encoding of Phonemes in the Primary Auditory Cortex

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There is evidence that humans and animals can robustly perceive speech despite considerable variability across speakers and context, and in the presence of acoustic distortions arising from background noise and sound reverberation. The neurophysiological basis of these perceptual abilities remains unknown. It has been shown that the population of A1 neurons encode the perceptually important features of phonemes along various dimensions

(Mesgarani et. al., 2008). Here we show that this rich multidimensional representation is explained by linear Spectrotemporal Receptive Field models of the neurons. The predicted responses of the cortical neurons to speech show a similar selectivity to different speech elements. This shows that neurons are most responsive to phonemes with spectrotemporal patterns that match their STRFs. The dimensions of tuning considered include frequency, temporal and spectral modulation tuning, and the directionality of the STRFs. This multidimensional decomposition provides insights into the encoding scheme of complex sounds such as speech by the auditory cortical neurons and enables the incorporation of this knowledge into biologically inspired speech applications.

503 Cortical First Spikes Can Encode the Peripheral Transformation of Natural Sounds

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There is a general interest in trying to understand how the auditory cortex in awake animals encodes natural sounds. This encoding question has usually been approached by assuming a black box between the sound and the cortical neurons, and simply relating its spiking response to acoustic features. Here, we applied an alternative paradigm where we tested a specific encoding of sounds at the auditory periphery, and tried to predict the timing of first spikes evoked in cortical neurons by natural communication calls. The peripheral encoding was modeled by the Leaky Integrator, Event Formation, Temporal Summation (LIEFTS) process, which has previously been shown to accurately account for first spikes evoked by pure tones at an auditory nerve fiber's best frequency (BF). We made an assumption that this peripheral representation is transformed only by the excitatory convergence of different frequency channels, along with systematic latency dispersion due to central auditory processing. If this simplified view were inappropriate to explain cortical spiking, first spike latency prediction errors would likely be large and random from neuron to neuron. Surprisingly though, we were able to predict first spikes with an accuracy of <5ms for a physiologically distinct subset of cortical neurons (~20%) in the awake mouse. Importantly, neurons that did and did not fit this model were systematically different in their sensitivity to sound amplitude and frequency, their spiking precision, and the time course of their full response to natural calls. Hence, the fidelity of the peripheral first spike representation for communication calls actually separated out neurons that responded in an acoustically faithful manner from those that reflected higher nonlinearities. These results therefore suggest that the auditory cortex contains parallel encodings of natural calls. Support provided by an NSF IGERT fellowship (FL), NIH R01 DC008343 and the NSF Center for Behavioral Neuroscience (RL).

504 Responses of Marmoset Primary Auditory Cortex Neurons to Noisy Vocalizations

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Sounds of interest such as vocalizations are often experienced in acoustic environments with competing sounds or background noise. Despite substantial potential interference from extraneous sounds, target sounds can often be perceived quite robustly. In the case of interfering wideband noise, for example, speech can be fairly intelligible in normal hearing listeners even at extremely unfavorable signal-to-noise ratios (SNRs). Some neurons in the primary auditory cortex (A1) of marmoset monkeys appear to be sensitive to particular spectral contrasts and could serve as a substrate for robust detection of vocalizations in noise. When marmoset A1 neurons were tested with species-specific vocalizations and simultaneous white noise at a range of SNRs, a common neuronal response mirrored psychophysical observations in humans. In the psychophysical case, noisy speech intelligibility degrades monotonically with added noise. In this neuronal population, mutual information between the vocalization and elicited spikes decreased monotonically with added noise. About the same number of neurons, however, exhibited their peak mutual information at some intermediate SNR. Neurons tuned to spectral contrast were more likely to fall into the latter category. A number of neurons exhibited mutual information measures in response to pure noise on par with the same measure for noise-free vocalizations. Both noise-free vocalizations and pure noise elicited consistent phase-locking in these neurons, and they were often sensitive to the lowest spectral contrasts. Altogether, these results indicate that robust vocalization encoding in wideband noise may be aided by auditory neurons most responsive to intermediate SNR values, and in marmoset A1 such neurons often are tuned to spectral contrast.
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505 Neural Processing of Competing Sounds in Auditory Cortex

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In a naturalistic acoustic environment, irrelevant acoustic events impede target detection. The similarity in neural response patterns evoked by target and masker sounds has been proposed as one of the factors attributed to the perceptual masking (Watson 2005). However, few studies have directly tested the neural processing of competing sounds in auditory cortex pertaining to masking and its release. In the present study we examined the effects of spatially close and far-apart masker on neural responses to a target sound in the primary auditory cortex (A1) and adjacent caudal-medial field (CM) of awake marmosets. Fifteen loudspeakers were positioned in the semicircular frontal field (-90^o to 90^o along the horizontal axis and at 0^o,

45°, 90° elevations). The target (CF tone) and masker (flat-envelope broadband noise) were delivered simultaneously from either the same or different spatial locations and resulted in spatial-temporal interactions in neural firing. We observed that the target responses can be suppressed by the noise masker from a broad range of spatial locations, which often exceed the excitatory spatial receptive field of a neuron. In addition to the suppressive effects on firing rate, the masker presented from an inhibitory spatial location sharpens the temporal firing patterns of the target sound. In contrast, the masker presented from an excitatory spatial location often smears the target firing patterns. These findings demonstrate spatial location-based effects of noise maskers on the timing of neural firing in the awake auditory cortex. We propose that spatial inhibition in auditory cortex facilitates unmasking by enhancing the saliency of the target representation and reducing the interferences between neural response patterns to multiple competing sounds. [Supported by NIH grant DC03180 (X.W.).]

506 Moderate Hearing Loss Decreases Spike-Timing Precision But Not Averaged Firing Rate of Auditory Cortex Neurons in Response to Conspecific Vocalizations

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Over the last 10 years, studies have evaluated the consequences of acoustic trauma on the functional properties of auditory cortex neurons. Changes in spontaneous and evoked activity, shifts of characteristic frequency (CF), and map reorganizations have been described in anesthetized animals (Norena & Eggermont 2003, 2005). However, so far, the consequences of acoustic trauma on the processing of communication sounds have not been investigated. In normal hearing subjects, recent results indicate that cortical responses to conspecific vocalizations show temporal patterns of activity allowing more robust stimulus discriminations than the overall firing rate (Schnupp et al 2006; Huetz et al 2009). Here, we examined how the firing rate and the spike-timing precision of cortical cells are modified after partial hearing loss.

Using chronically implanted microelectrodes, single units were recorded in awake, restrained guinea pigs from a few days before up to 15 days after acoustic trauma (induced by a 5kHz tone at 120dB for 2h). When tested with pure tones, cells showed a 15-25dB increase in threshold at the CF and a transient increase (~ 5ms) in response latency. However, the responses to four conspecific vocalizations (presented at 70dB SPL) did not elicit weaker responses (in terms of average spike count) after trauma compared to before trauma. Assessing spike-timing reliability by a between-trial correlation index reveals that the spike-timing precision was decreased for cells with CF in the 5-10kHz frequency range. Histograms generated by pooling responses of all recorded cells indicated that the temporal patterns of responses were disorganized for all the vocalizations after hearing loss. These preliminary findings suggest that quantification of temporal patterns of neuronal responses is more appropriate than studying the averaged firing rate to understand how the perception of socially meaningful communication sounds are impaired after partial hearing loss.

507 Is IRN Really a Well-Controlled Pitch-Evoking Stimulus?

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In order to investigate the neural processing of pitch in humans, many researchers have used one specific type of pitch-evoking stimulus: iterated ripple noise (IRN). IRN is created by taking a sample of Gaussian noise, delaying it, then adding it to or subtracting it from the original. IRN is easy to create, and the pitch salience can be manipulated simply by increasing or decreasing the number of iterations. The spectrum of IRN contains a number of harmonic peaks, and also broad spectral features that vary slowly in time, in a manner determined by the number of iterations. High-pass filtering IRN removes the harmonic peaks that are peripherally resolvable; this has often been assumed to leave only the temporal regularity that gives rise to the pitch sensation. However, resolvable broad spectral features remain after filtering. The current study used a novel type of stimulus, IRNo (no-pitch IRN), which is created by processing IRN in a way that removes the fine temporal structure responsible for the sensation of pitch, whilst leaving the broad spectro-temporal features intact. Behavioral measures showed that these features are perceptible: IRNo is clearly discriminable from Gaussian noise even though the pitch is absent. Furthermore, discrimination performance improves with increasing number of iterations, as is also the case for IRN. Preliminary functional magnetic resonance imaging (fMRI) data suggest that brain activation patterns for IRNo show the same pattern as for IRN, with a peak of activity in lateral Heschl's gyrus and an increase in activation with number of iterations. If confirmed, these findings have serious implications for the use of IRN as a controlled pitch stimulus: IRN contains features not associated with pitch that may confound results. Previous neuroimaging studies of pitch that used IRN should be interpreted with caution.

508 Enhancement of Sustained Fields for Pitch and Vowels Map to Similar Sites of Human Auditory Cortex

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Several studies have shown enhancement of sustained fields in the auditory cortex for periodic over non-periodic sounds and for vowels over non-vowels. Source analysis indicated enhancement related to periodicity in lateral Heschl's gyrus, and sustained field sources for non-periodic sounds at more posterior sites (Gutschalk et al 2002). A similar source decomposition was suggested for vowel sounds (Hewson-Stoate et al 2006).

Here, we directly compared the sustained fields evoked by periodicity and vowels using synthesized speech with a "damped" amplitude modulation. These stimuli were

parametrically varied to yield four classes of matched stimuli (Uppenkamp et al 2006): (1) periodic vowels (2) non-periodic vowels, (3) periodic non-vowels, and (4) non-periodic non-vowels. Sequence 1 comprised vowels A, E, I, O, and U; sequence 2 comprised only the vowel A. 12 listeners were studied with combined MEG and EEG.
Sustained fields were reliably enhanced for vowels and periodicity. One set of bilateral dipoles was fit to each of the following three contrasts: (I) non-periodic, non-vowel vs. silence [4]. (II) periodic vs. non-periodic [1+3 – 2+4]. (III) vowel vs. non-vowel [1+2 – 3+4]. The source analysis revealed that the location for vowel (III) and periodicity (II) mapped to similar sites at antero-lateral Heschl's gyrus. In contrast, the non-periodic, non-vowel condition (I) mapped to a more medial and posterior site. The vowel enhancement was significantly more prominent for sequence 1, where the vowel identity was varied, indicating selective adaptation of the response.
These results render it unlikely that there are spatially distinct fields for vowel and pitch processing in the auditory cortex. Moreover, they raise the question whether there is early specificity for speech-sounds in the auditory cortex, or if the sustained field enhancement reflects a more general mechanism, e.g. related to the behavioral importance of sound.
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509 Neural Coding of Simultaneous Fast and Slow Temporal Modulations in the Human Auditory Cortex

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Natural sounds such as speech contain multiple levels and multiple types of temporal modulations. Because of nonlinearities of the auditory system, however, the neural response to multiple, simultaneous temporal modulations cannot be predicted from the neural responses to single modulations. Perhaps even more importantly, the observed neural representations of multiple, simultaneous temporal modulations can disambiguate among competing candidate neural mechanisms, which would otherwise be left undetermined if only the neural representations of single modulations were examined. Here we demonstrate the cortical neural representation of an auditory stimulus simultaneously frequency modulated (FM) at a high rate, $f_{FM} \approx 40$ Hz, and amplitude modulated (AM) at one of several slow rates, $f_{AM} < 15$ Hz. Magnetoencephalography recordings demonstrate fast FM and slow AM stimulus features evoke two separate but not independent auditory steady state responses (aSSR) at f_{FM} and f_{AM} respectively. The power, rather than phase locking, of the aSSR of both decreases with increasing stimulus f_{AM}. The aSSR at f_{FM} is itself simultaneously amplitude modulated and phase modulated with fundamental frequency f_{AM}, demonstrating that the slow stimulus AM is not only encoded in the neural response at f_{AM} but also encoded in the instantaneous amplitude and phase of the neural response at f_{FM}. Both the amplitude modulation and phase modulation of the aSSR at f_{FM} are most salient for low stimulus f_{AM} but remain observable at the highest tested f_{AM}, 13.8 Hz. The instantaneous amplitude of the aSSR at f_{FM} is successfully predicted by a model containing temporal integration on two time scales, ~25 ms and ~200 ms, followed by a static compression nonlinearity.
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510 Invariant Feature Encoding Across Five Auditory Cortical Fields

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To interpret vocal calls, listeners must be able to generalize particular features of sounds despite changes in the acoustic signature. For instance, in human speech, they must recognize an /a/ sound and distinguish it from an /i/ sound despite ongoing changes in the pitch or spatial location of a speaker's voice. While this phenomenon has previously been studied using psychophysical tasks, little is known about how cortical representations of vocalizations might support this type of feature invariance. In a recent report, we showed that neurons throughout five fields of ferret core and belt auditory cortex have responses that are modulated by multiple features of artificial vowel sounds (Bizley et al., 2009). Here, we use an information theoretic approach to investigate how neurons in each field encode the pitch, timbre and azimuth of these sounds in a manner that is invariant to changes in nuisance variables (i.e. the other two features). Across fields, we find significant differences in the number of neurons that encode information about each feature. Although neurons representing the pitch, timbre and azimuth of complex sounds are distributed throughout auditory cortex, the way in which they typically represent these features, (for example, by modulations of spike rates within specific response windows or by first-spike latencies), differs according to the stimulus feature in question and across fields. Thus, single neurons may represent multiple features of sounds unambiguously via independent modulation of different aspects of their spiking response.

511 Formation of Associations in Monkey Auditory Cortex

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We will describe types of neuronal activities in auditory cortex that are typically observed only when subjects are engaged in behavioral tasks that include auditory stimuli. During task performance, but not outside, tonic firing emerges that slowly changes over periods of up to several seconds. Both increases and decreases of firing can be observed. The changes in tonic firing typically start after some behaviorally significant event of the behavioral procedure and end with some other event. The events can be either auditory or visual stimuli, motor acts, or (appetitive or aversive) reinforcers. These types of slow firing changes have been observed by us and others (Gottlieb et al., Exp. Brain Res. 74: 139-148, 1989; Shinba et al., 1995, Brain Res. Bull. 37:199-204, 1995; Quirk et

al., Neuron 19: 613-624, 1997; Armony et al., J. Neurosci. 18:2592-2601, 1998) during the performance of auditory detection, discrimination, working memory, and categorization tasks and during classical conditioning. We speculate that the slow changes in tonic firing level could provide a neuronal correlate for the formation of associations between events that are related to auditory stimuli and that are of behavioral relevance. These types of firing likely emerge from connections of auditory cortex with brain structures generally not considered parts of the auditory system, like basal ganglia, prefrontal cortex or neuromodulatory systems. Thus these findings suggest a stronger involvement of auditory cortex in cognitive and executive functions than previously assumed.

512 Learning Strategy Determines Cortical Plasticity: Implications for Behavioral Treatments of Auditory Disorders

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The auditory cortex is now known to be a substrate for auditory learning, in addition to its role in the analysis of sounds. Most extensively studied is the primary auditory cortex (A1): highly-specific physiological plasticity develops when both animal and human subjects learn the meaning of sounds. First discovered in studies of associative learning, frequency receptive fields (RFs) can shift to the frequency of a signal tone (Bakin & Weinberger, *Brain Res.*, 1990), producing a specific increase in its representational area as a direct function of the level of its acquired behavioral importance (Rutkowski & Weinberger, *PNAS*, 2005). Such learning-induced plasticity provides the neurobiological basis for clinical treatments of disorders such as speech comprehension and tinnitus. Although it is currently assumed that auditory learning is invariably accompanied by cortical plasticity, we have found that *how* learning occurs is critical to the formation of plasticity. Specifically, auditory tasks can be solved using different learning strategies and plasticity in A1 of animals depends on their use of a particular strategy. We present findings from groups of animals (rats) trained in a variety of tasks. The findings reveal that cortical plasticity in the form of signal-specific increased sensitivity (decreased threshold), increased selectivity (decreased bandwidth) and increased area of representation develop when the learning strategy of subjects depends on attending to acoustic onset transients while ignoring tone offsets. Thus, remedial auditory training regimens that aim to induce learning-related plasticity need to incorporate the use of appropriate specific auditory learning strategies.

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513 Arc Expression and Neuroplasticity in Primary Auditory Cortex During Initial Learning Are Inversely Related to Neural Activity

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Prevailing models of learning-based sensory cortex re-organization in adults assumes that ascending sensory activity is crucial to the genesis of plasticity. We directly tested this by using optical intrinsic signal imaging to identify high-frequency and low-frequency regions of A1 in rats performing an auditory detection task for a low frequency tone. Rats were given one detection session or were allowed to rehearse the detection task for 14 days. Using in-vivo microelectrode recordings to reconstruct A1 tuning, we found that action potential responses in the one session group were significantly changed in a manner non-selective for the target tone across both frequency regions. Following 2 weeks of detection rehearsal, responsiveness in low-frequency regions of A1 continued to be enhanced without spectral selectivity, but the high-frequency region became increasingly tuned to the target tone. Expression of the plasticity-related immediate-early gene Arc matched the action potential response plasticity in the single session animals, being greatly increased in both A1 frequency regions. Arc mRNA remained slightly elevated at 14 days, but only in the low-frequency region. These findings identify Arc as a potential mediator of early A1 plasticity in instrumental learning. They also show that direct activation of A1 by target stimulus is not necessary to induce neuroplasticity. This lack of correspondence between Arc mRNA and action potential plasticity suggests that early neuroplasticity in A1 is substantially directed by higher levels in the auditory hierarchy.

514 Behavioral Uncertainty in a Tone-In-Noise Detection Task Reflected in Decreased Amplitude and Greater Latency of Neuronal Responses in Ferret Frontal Cortex

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The detection of faint sounds in the presence of background noise is a ubiquitous acoustic challenge in active hearing for humans and animals. In a previous study (Atiani et al., 2009) ferrets were trained to detect a faint tone embedded in white noise. The level of difficulty of the tone-in-noise (TIN) task was parametrically varied by changing the signal-to-noise ratio (SNR) of the pure tone relative to background noise. Ferrets took longer to recognize the presence of the tone as SNR decreased, reflecting the rising difficulty of extracting signals from increasingly noisy surroundings and the uncertainty in very low SNR as to whether the tone was present. We also observed rapid task-related plasticity in responses and spectrotemporal receptive field (STRF) properties in A1 that may enable better TIN detection. To study the mechanisms and time course of neuronal recognition of

the tone in cortical areas beyond A1, and the neural basis for categorizing the two types of stimuli (TIN and white noise) we recorded from frontal cortex of two ferrets engaged in the TIN task. We recorded over 80 single units in quiescent conditions and in two behavioral task conditions of pure tone detection, and TIN, with variable SNR. Frontal neurons during behavior showed: (1) similar selective responses to the tonal target, even when embedded in noise, (2) greater latency of target response in the TIN task compared to target latency in pure tone detection reflecting greater processing time to detect the TIN, (3) diminished amplitude of target responses in the TIN task compared to target responses in pure tone detection, reflecting a greater degree of uncertainty about the presence of the target tonal stimulus. The intriguing parallel between task performance and neural responses in frontal cortex and the adaptive A1 receptive field responses suggests that both areas participate in a broader auditory network during pure tone detection and TIN tasks.

515 Broadcast Media Training or Why Most Scientists Would Rather Go to the Dentist Than Talk to a TV Reporter

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This interactive session is customized for scientists and covers the benefits and risks of working with the news media, including strategies for accommodating the differing methods of print and broadcast journalists. P articipants will learn what to do when a reporter calls, as well as their rights to obtain information from the news media before agreeing to an interview. A series of exercises will teach participants how to prepare for a successful interview and how to develop strategic media messages and talking points for public presentations. The course will also cover the topic of public versus protected information, and offer tips for handling crisis communications. Techniques are demonstrated through a series of exercises including critiqued on-camera interviews. Topics covered through on-camera exercises will include message development, speaking to a group and aggressive interviews.

516 Modulation of Notch Signaling by Fringe Activity: Making Boundaries in the Developing Cochlea

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Notch receptors are some of the most highly post-translationally modified proteins in the cell, with hundreds of sugar moieties being added to their 36 extracellular EGF repeats. Addition of fucose groups to Notch by the O-fucosyltransferase Pofut1 is an important modification for Notch signaling, although there may be different requirements for O-fucosylation between invertebrates and vertebrates. Fringe proteins are glycosyltransferases that add GlcNAC moieties to O-fucose residues on both the Notch receptors and their ligands. This GlcNAC modification differentially affects the activity of Notch receptors, making them more sensitive to Delta class ligands, and less sensitive to Jagged class ligands. Expression of Fringe proteins are frequently seen at biological boundaries, where they modulate Notch signaling (high on one side, low on the other) to sharply define cell identity on either side of the boundary. We and others have shown that Lunatic Fringe is expressed in Kölliker's organ, immediately adjacent to the developing prosensory domain of the cochlea. As inner hair cells differentiate, Manic Fringe is also expressed at the Kölliker's organ/prosensory domain boundary. To examine the role of Fringe proteins in defining this boundary, we have examined mutants of Pofut1 (which should lack all Fringe function), LFng and MFng. Pofut1 knock out mice display a similar phenotype to RBPJ and Notch1 knock outs, dying around E9.5. However, inner ear specific Pofut1 conditional knock outs display a phenotype that is different from Notch1 or RBPJ conditional knock outs, consisting of only supernumerary inner hair cells. Our preliminary data suggests that the same phenotype occurs in Lunatic/Manic Fringe double mutants. Taken together, these results suggest a role for Fringe proteins in modulating Notch signaling at the border between Kölliker's organ and the developing organ of Corti.

517 BMPs Act as Morphogens to Pattern the Developing Mammalian Cochlea

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The mammalian inner ear detects sound with the organ of Corti, an intricately patterned region of the cochlea in which one row of inner hair cells and three rows of outer hair cells are surrounded by specialized supporting cells. Very little is known about the mechanisms that generate this highly organized cellular array from prosensory progenitors. We present evidence that a gradient of bone morphogenetic protein (BMP) signaling patterns the cochlea in its abneural-neural axis, whereby high levels of BMP signaling induces the future outer sulcus, moderate levels of BMP signaling induce the prosensory domain destined to form the organ of Corti, and BMP signaling suppresses the development of Kolliker's organ. Ear-specific disruption of the Alk3 type I BMP receptor by Pax2-Cre mice leads to a loss of the future outer sulcus, a mirror-image duplication of the organ of Corti and Kölliker's organ and massive over-production of hair cells with reversed polarity. Further reduction of BMP signaling in Alk3/6 compound mutants eliminates both the future outer sulcus and the organ of Corti, with all cells expressing markers of Kölliker's organ. Conversely, organ cultures of outgrowing Math1-GFP mouse cochlea at the presence of intermediate concentration of exogenous BMP4 result in an over-production of Math1+ cells and Prox1+ supporting cells, while high BMP4 concentration suppresses the organ of Corti cells and up-regulates markers of outer

sulcus. Our results are consistent with a model in which the cochlea is patterned by a morphogen gradient of BMP activity.

518 Phenotypic Analysis of *vangl2* Knockout Mice and Morphological Comparison to *looptail*

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Hair cell Planar Cell Polarity (PCP) is the polarization of the stereocilia bundle and coordinated orientation of bundles between adjacent cells. PCP requires the activity of the polarity protein Van Gogh-like2 (Vangl2), and in *looptail* mutant mice a single point mutation in the *vangl2* gene is sufficient to misorient auditory and vestibular hair cells (HCs). To further evaluate Vangl2 function we generated *vangl2* knockout (KO) mice that lack DNA encoding the Vangl2 transmembrane domains. *Vangl2* KOs have neural tube deficits similar to *looptail*. Despite this most *vangl2* heterozygotes (>95%) lack the looped tail that is characteristic of *looptail* hets. This difference in the heterozygous phenotype suggests that there may also be differences in HC PCP between *vangl2* KO and *looptail* mutant mice. Consistent with this we find a vestibular HC phenotype in *vangl2* KOs that is restricted to the striola. These KO HCs are misoriented and are no longer patterned about a line of polarity reversal (LPR). We also find changes in the distribution of PCP proteins including Pk2 which is normally localized to one edge of wild type HCs. In KOs Pk2 is present throughout the circumference of misoriented HCs. HCs outside of the striola are largely unaffected with normal PCP in the lateral region adjacent to the cristae and only mild changes to HCs in the medial region of the maculae. Thus the general organization of vestibular HCs into two groups of opposite bundle polarity persists in *vangl2* KOs although the striola lacks a defined LPR. This is in contrast to a description of the *looptail* mutant utricle where all aspects of PCP patterning are absent. By comparison, the auditory HC phenotype is similar in organ of Corti from *looptail* mutants and *vangl2* KOs. Together these observations suggest that the *looptail* mutation has mildly dominant effects that may only be revealed in the patterning of large fields of polarized cells such as the neural tube and vestibular maculae.

519 Cell Adhesion Underlies the Role for the Vertebrate Planar Cell Polarity Signaling Pathway in Convergent Extension of the Hearing Organ

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The vertebrate planar cell polarity (PCP) signaling pathway regulates coordinated cellular polarization that drives a type of cellular rearrangement known as convergent extension (CE) and provides directional information to orient uniformly hair bundles in all the hair cells of the mammalian hearing organ, the organ of Corti. Defects in the PCP pathway cause apparent CE phenotypes and randomization of hair bundle orientation in the mouse cochlea. However, it is not known whether the PCP pathway acts differentially on downstream targets for CE and hair bundle orientation.

Here, we show that cell-cell contacts and cell geometry change drastically in the hearing organ during CE. An essential PCP protein, Vangl2, that is asymmetrically partitioned and required for both CE and hair bundle orientation is localized to the adheren junction. Disruption of p120, a component of adheren junctions in the inner ear diminished the levels of E-cadherin and resulted in characteristic CE defects of a shortened and widened hearing organ. The CE defects in p120-/- mice are enhanced by an additional loss-of-function allele of Vangl2, but are not accompanied by loss of asymmetric partition of Vangl2 protein or misorientation of hair bundles. We further found that E-cadherin levels are also drastically reduced in Vangl2 PCP mutants. Together, these results indicated a requirement for proper remodeling of cell-cell contacts during CE and a role for the PCP pathway in CE that is mediated by cell adhesion molecules and independent of the role for PCP pathway in hair bundle orientation.

520 Function of HES and HEY Transcriptional Repressors in the Developing Mammalian Organ of Corti

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The mammalian organ of Corti (OC) is characterized by a highly complex sensory epithelium with an intricately patterned assortment of specialized cell types and a rigidly stereotyped histoarchitecture. The molecular mechanisms underlying the developmental timeline of the OC, including the factors regulating differentiation and fate specification of its multiple specialized cell types, remain poorly understood and difficult to elucidate. HES and HEY transcriptional repressors are known to play important roles in the development of several different organs and tissues including the OC. We have previously demonstrated that HES/HEY factors divide the OC into distinct compartments based upon their differential combinatorial expression patterns in the various supporting cell types.

Here we continue to characterize the roles of HES/HEY factors in cochlear cell fate specification and maintenance by focusing on Hes5, Hey1, and HeyL, which are the three factors expressed in Deiters cells of the OC. We generated Hes5, Hey1, and HeyL mutant mice, including the potential double and triple knockouts, in order to analyze any phenotypic variation in OC development imparted by the various mutations. We present evidence that Hes5, Hey1, and HeyL function not only in limiting the number of outer hair cells that develop within the auditory

sensory epithelium but in the developmental patterning of Deiters cells as well. We further anticipate that our analysis of the differential HES/HEY gene expression patterns in the various knockout mice will shed light on possible mechanisms of redundancy and signaling compensation between them, clarifying the necessity for expression of multiple HES/HEY factor subtypes within individual supporting cells.

521 Specific Ablation of Neonatal Cochlear Supporting Cells in Vivo by Cre-Mediated Expression of Diphteria Toxin

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Different studies show that cochlear hair cells (HC) require the integrity of the surrounding cellular and acellular components of the organ of Corti to effectively perform their roles as sensory receptors. Pillar and Deiters cells are cochlear supporting cells (SC) with unique architecture lying in close contact with inner and outer HCs. In this study we aim to specifically ablate pillar and Deiters cells postnatally in mice and determine the effects in the morphological and functional development of the organ of Corti. We used a mouse line in which Cre expression is driven by the endogenous Prox1 promoter and its activity is induced by tamoxifen (Prox1CreERT2). Characterization of this mouse line with different reporter mice has shown that Cre is expressed in the organ of Corti specifically in pillar and Deiters cells when tamoxifen is injected at P0 and P1, with higher expression in the apical region of the cochlea. We crossed the Prox1CreERT2 mice with Rosa26-eGFP-DTA mice in which Diphteria Toxin fragment A is expressed after Cre-mediated deletion of a stop codon, resulting in specific ablation of cells expressing Cre. Prox1Cre/+;DTAloxp/+ and control mice were injected with tamoxifen and their cochleae harvested at different ages. Hearing was tested by auditory brainstem responses (ABR). Immunostaining of cochlea whole mounts showed SC loss and disruption in the HC layer at P8 in the apical region of the organ of Corti in the mutant mice, but not in control mice. This phenotype was more severe at P15 where some HCs were missing, although many SCs remained. ABR thresholds were increased in the mutant mice relative to their control littermates at P25. This mutant mouse line will offer valuable information about the developmental roles of pillar and Deiters cells in the postnatal organ of Corti and is a novel model to achieve SC death prior to HC damage.

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522 Gelsolin Plays a Role in the Actin Polymerization Complex of Hair Cell Stereocilia

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A complex of proteins scaffolded by the PDZ protein, whirlin, reside at the stereocilia tip and are critical for stereocilia development and elongation. We have shown that in outer hair cells (OHCs) whirlin is part of a larger complex involving the MAGUK protein, p55, and protein 4.1R (Mburu et al. 2006 PNAS). Whirlin interacts with p55 which is expressed exclusively in outer hair cells (OHC) in both the long stereocilia that make up the stereocilia bundle proper as well as surrounding shorter stereocilia structures. In erythrocytes, p55 forms a tripartite complex with protein 4.1R and glycophorin C promoting the assembly of actin filaments and the interaction of whirlin with p55 indicates that it plays a similar role in OHC stereocilia. However, the components directly involved in actin filament regulation in stereocilia are unknown. We have investigated additional components of the whirlin interactome by identifying interacting partners to p55. We show that the actin capping and severing protein, gelsolin, is a part of the whirlin complex. Gelsolin is expressed in OHC stereocilia and the pattern of localisation at the apical hair cell surface is strikingly similar to p55. Like p55, gelsolin is ablated in the *whirler* and *shaker2* mutants. Moreover, in a gelsolin mutant, stereocilia in the apical turns of the cochlea become long and straggly indicating defects in the regulation of stereocilia elongation. The identification of gelsolin provides for the first time a link between the whirlin scaffolding protein complex involved in stereocilia elongation and a known actin regulatory molecule.

523 Ribeye Is Required for Correct Afferent Synapse Innervation of and CaV1.3a Channel Clustering in Zebrafish Hair Cells

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The exquisite temporal and spatial sensitivity of hair cell neurotransmission is due, in part, to the specialized structure of ribbon synapses. Ribbon synapses contain a unique protein component, Ribeye, which is important for their physical integrity and function. Previous studies (Dick et al, 2003; Wan et al, 2005; Regus-Leidig et al, 2009) have indicated that Ribeye plays an important role in synaptic formation and maturation. Therefore, we used morpholino knockdown and transgenic over-expression of Ribeye in zebrafish hair cells to investigate its role in ribbon synapse development.

There are two *ribeye* genes in zebrafish, *ribeye a* and *ribeye b*, and both are expressed in hair cells. Knockdown of either isoform of Ribeye induces hearing and balance defects in 3-5 day old larvae. Subsequent

immunohistochemical labeling in either Ribeye a or b morphants reveals defective afferent innervation, but no disruption of post-synaptic MAGUK staining juxtaposing pre-synapses. However, knockdown of both Ribeye isoforms shows both reduced afferent innervation and disruption of MAGUK morphology and localization. This disruption of pre- and post-synaptic co-localization is striking because it mimics what we observe in zebrafish with mutations in the L-type voltage-gated Ca^{2+} channel $Ca_V1.3a$. Finally, we observe that knockdown of Ribeye b leads to an absence of $Ca_V1.3a$ immunohistochemical label and $Ca_V1.3a$ clusters co-localize with ectopic aggregates of overexpressed Ribeye b-GFP.
These findings suggest Ribeye is required for afferent neuronal innervation and pre-synaptic clustering of $Ca_V1.3a$. Moreover, $Ca_V1.3a$ plays a crucial role in correctly localizing ribbon pre-synapses in zebrafish hair cells with afferent postsynaptic densities.

524 GATA3 Regulates the Timing of Neurite Extension and Targeting of Spiral Ganglion Neurons

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Auditory circuits must be precisely organized for sound information to be processed by the brain. Spiral ganglion neurons play a key role in this process, receiving input from hair cells in the cochlea and communicating all aspects of complex sound stimuli to neurons in the cochlear nucleus. At the beginning of auditory circuit assembly, spiral ganglion neurons arise together with vestibular ganglion neurons within a common neurogenic domain. An early sign of the segregation of these two neuronal populations is the restriction of the expression of the transcription factor GATA3 to spiral ganglion neurons. GATA3 is mutated in the human deafness syndrome hypoparathyroidism, sensorineural deafness, and renal anomalies (HDR), but the origin of deafness is unclear. In other systems, GATA factors are master regulators of cell fate and cooperate with other transcription factors to regulate cell-type specific aspects of differentiation. We hypothesized that GATA3 guides the integration of spiral ganglion neurons into functional auditory circuits.
Our investigations of GATA3 mutant mice indicate that GATA3 regulates the timing of spiral ganglion neuron differentiation. We generated mice where GATA3 was selectively removed from neurons using a BhlhB5-Cre driver. In GATA3 conditional knock-outs, spiral ganglion neurons prematurely extend their processes into the cochlear duct. To understand how GATA3 regulates the timing of neurite extension and target selection, we investigated the changes in gene expression at the onset of premature neurite extension and correlated this to the genes directly regulated by GATA3. By elucidating the role of GATA3 in spiral ganglion neurons, we can better understand the mechanisms of circuit formation in the developing cochlea and gain insights into the etiology of deafness in HDR patients.

525 Permanently Impaired Axonal Projection and Synapse Formation in Hypothyroid Mice

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Mice are born deaf and start to hear toward the end of the second postnatal week when the organ of Corti is fully developed (Mikaelian and Ruben, 1965; Ehret, 1985). The maturation of the organ of Corti involves axonal growth and co-ordination of a massive rearrangement of afferent and efferent fibers and synapses. These processes take place during the thyroid hormone critical period. Here we report the characterization of a mouse model of severe, secondary hypothyroidism (*Pit1*^{dw}) with profound, congenital deafness. The advantages of this model over other hypothyroid mutants are the severity of hearing impairment, long-term viability of the animals, and ease of thyroid hormone replacement. Although the lack of thyroid hormone causes developmental delay in cochlear development, some processes mature eventually. We demonstrate that the function and innervation of both inner and outer hair cells are permanently affected. Both outer and inner hair cells have abnormalities in the pattern of synapses, ribbon formation, and presynaptic and postsynaptic marker expression. There is a reduction in both otoferlin, a protein required for synaptic release, and the large-conductance Ca2 -activated K (BK) currents. Comparison of the cochlear transcriptome of adult *Pit1*^{dw} mutant and wild-type littermates using microarray analysis of gene expression reveals differential expression of genes that are involved in axonal guidance and synapse function. We are using cochlear explant cultures to assess the function of these candidate genes. We anticipate that these studies will enhance our understanding of neuronal development in the cochlea as well as the mechanism of action of thyroid hormone on cochlear maturation.
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526 Profiling of Auditory-Specific Gene Expression Throughout Auditory Circuit Assembly Identifies Npr2 as a Regulator of Spiral Ganglion Axon Bifurcation

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Spiral ganglion (SG) neurons transmit sound from hair cells in the cochlea to the brain, but little is known about how their circuitry is established. To uncover the molecular programs controlling auditory circuit formation and function, we generated a database of genes expressed at key points (E12.5, E13.5, E16.5, P0, P6, and P15) during SG development. In parallel, we profiled gene expression in the vestibular ganglion (VG), since the VG arises from the same precursor pool and shares many features in common with the SG, but develops slightly earlier and innervates different targets. Using this microarray data, we identified cohorts of genes that may be involved in the development or function of SG neurons.

As expected, the SG and VG share many genes in common. However, among the differentially expressed genes are a small number of transcription factors, which may play a role in cell fate or regulation of SG- or VG-specific target gene expression. Different members of the same transmembrane protein families are often expressed, perhaps allowing the SG and VG to respond differentially to environmental cues but to use common downstream machinery to elicit similar behaviors. Notably, many ion channels and synaptic proteins are expressed well before the onset of functional hearing. Moreover, many MHC class I and complement genes are specific to the SG at late stages, suggesting a possible role for these genes in auditory function. Many genes we identified are linked to human deafness or auditory dysfunction, indicating that our database may be a useful tool for identifying candidate disease genes.

Our database permits us to explore the molecular mechanisms of specific steps in auditory circuit formation. For example, SG axons bifurcate upon entering the hindbrain and send branches to the dorsal and ventral cochlear nucleus, but nothing is known about the molecular mechanism or functional significance of this branching. One gene we identified by our microarray screen is Natriuretic peptide receptor 2 (Npr2). In Npr2 mutant embryos, SG axons turn instead of bifurcating upon reaching the hindbrain. Since Npr2 mutant mice are viable and are able to hear, they will permit us to investigate the role of SG axon branching in complex auditory functions, such as gap detection and sound localization.

527 A Spiral Ganglion Neuron-Specific Cre Mouse Line

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Sonic Hedgehog (Shh) has been reported to be highly expressed in the embryonic spiral ganglion region at E14 and expressed at a lower level at E16. To further understand the Shh expression pattern at later ages, we analyzed ShhCreEGFP/+ knock-in mice where the fusion protein, of recombinase Cre and EGFP, is driven by the endogenous Shh promoter. Cre/EGFP expression was not detectable in the cochlea at P0, indicating that Shh is inactive at P0. Additionally, by cell lineage labelling approach, we analyzed ShhCreEGFP/+; Rosa26-eYFPloxp/+ mice at P0 and P6 in which the offspring of Shh expressing embryonic progenitor cells were labeled with eYFP after Cre mediated recombination. We found that the cell bodies of all eYFP+ cells were distributed in the spiral ganglion region at P0 and P6. Double staining of eYFP and TUJ1 (a widely used neuronal marker) confirmed that these eYFP+ cells were spiral ganglion neurons and not glia cells. There was no distinguishable difference of eYFP expression between P0 and P6, suggesting that there were no additional cells expressing Shh between P0 and P6. In summary, our data supports that 1) Shh becomes undectable at postnatal ages in the cochlea; 2) Shh is exclusively expressed in spiral ganglion neurons during the embryonic development; 3) ShhCreEGFP/+ is a valuable genetic tool for analyzing gene functions in spiral ganglion neurons.

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528 Introduction to the Symposium on Signal Processing in First- And Second-Order Vestibular Neurons

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The vestibular system shows a remarkable capacity to maintain posture and balance, despite perturbations in the external environment and after peripheral vestibular deafferentation. Recent research has been targeted toward defining the synaptic transmission and ionic conductances involved in signal processing from the peripheral receptors to the first brain centers located in the vestibular nuclei. The vestibular system is characterized by a high degree of plasticity, not only between first- and second-order vestibular neurons, but also from non-labyrinthine inputs, in particular, from the cerebellum. Experiments are performed on whole animals, as well as isolated vestibular circuits in culture, and brain slice preparations. The symposium is directed toward 1) clinicians, who want to bridge the gap between the fundamental sciences and treating patients for vestibular disorders; 2) vestibular neuroscientists using both structural and functional approaches to understand the labyrinth and its central pathways at the system, cellular, and molecular levels; and 3) auditory neuroscientists intrigued by the similarity and differences in signal processing in the two systems.

529 The Organization of Signals in Mammalian Otolith Organs

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Afferent nerve fibers from the central (striolar) and peripheral (extrastriolar) zones of mammalian otolith organs have strikingly different spontaneous and evoked discharge properties. Striolar neurons fire irregularly and have a number of specializations that favor high-speed transmission from the hair cell to target neurons in the brain. Extrastriolar neurons fire regularly and appear designed for the sensitive representation of low-frequency head motions. This talk will discuss advances in our understanding of the origins of these zonal differences, considering such factors as hair bundle morphology and the expression of voltage-gated ion channels by hair cells and afferent neurons.

530 Membrane Proteins Define Micro-domains in the Vestibular Afferent Calyx

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The vestibular calyx ending performs at least two very different functions, receiving input on its inner surface from

ribbon synapses, and generating an action potential at the heminode that is transmitted to the brain. Until recently, this afferent terminal was considered to be rather homogenous in membrane composition. We have found that the calyx ending consists of different membrane domains, each marked by a set of cell adhesion, scaffolding, and extracellular matrix proteins, and voltage-gated ion channels. The domains consist of: 1) a synaptic zone, 2) an apical zone, and 3) an initial segment zone leading up to, 4) a heminode. Furthermore, calyx endings in dimorphic and calyx afferents can be distinguished immunohistochemically by subsets of voltage-gated channels, by differences in the distribution of these channels within the calyx and within the sensory epithelium, and by the locations and sizes of their heminodes. This talk will discuss the evidence for membrane ionic channel composition in the calyx ending contributing to these diverse functions in vestibular afferents.

531 Neurotransmission Between the Vestibular Type I Hair Cell and Its Calyx Ending

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The type I hair cell and its calyx ending are recent phylogenetic acquisitions, being found exclusively in amniotes (reptiles, birds and mammals). Because of their peculiar properties, it is unclear how these structures function. Yet, calyces are found in >90% of afferents in the monkey crista and provide the sole innervation in 40% of these fibers. In this talk, we will consider three problems. 1) The type I hair cell has a peculiar voltage-gated, outwardly rectifying current whose hyperpolarized activation range and large magnitude might prevent the hair cell from depolarizing sufficiently to trigger neurotransmitter release. 2) As the calyx ending covers the basolateral surface of the hair cell, supporting cells cannot function to transport glutamate and K^+ ions out of the synaptic cleft. 3) The large surface of the calyx may lower its input impedance, thereby attenuating synaptic potentials. Intracellular recordings, including recent whole-cell recordings from calyx endings, offer suggestions as to how these problems are solved.

532 Neurotransmitters and Modulators of Vestibulo-Sympathetic Pathways

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Otolith afferents provide a major input to the relatively short latency vestibulo-sympathetic reflex (VSR), which allows humans to stand up without losing consciousness. The VSR works in concert with the longer-latency baroreflex, which maintains sympathetic tone. VSR signals from the vestibular nuclei are conveyed to multiple brainstem regions including the rostral and caudal ventrolateral medulla (RVLM and CVLM, respectively). However, convergence of baroreflex and VSR signals is most likely to occur in the RVLM. Of the numerous neurotransmitters and modulators localized in the region, glutamate is considered the key neurotransmitter of RVLM projections mediating excitatory cardiovascular responses, whereas CVLM exerts GABAergic sympatho-inhibition on the RVLM. Catecholaminergic neurons provide important neuromodulation in both regions.

The caudal vestibular nuclei were injected with anterograde tracer and vestibular processes were visualized in RVLM and CVLM in order to identify the transmitter phenotypes of vestibulo-autonomic neurons and vestibulo-recipient cells in rat R/CVLM. We found that the perikarya and dendrites of glutamatergic neurons in RVLM receive vestibular input. Most of these glutamatergic cells produce nitric oxide and co-express imidazoleacetic acid-ribotide (IAA-RP), a neuromodulator that participates in the regulation of systemic blood pressure. Vestibular projections do not target the somata of catecholaminergic cells but occasionally terminate on their distal dendrites. In contrast, vestibular axons form axo-somatic as well as axo-dendritic synapses with catecholaminergic neurons in CVLM. Our results demonstrate that vestibular input to RVLM is positioned to rapidly influence premotor sympathetic circuitry, whereas vestibular input to CVLM is sparser and is directed toward local interneuronal processing. We suggest that the axo-somatic vestibular projections to glutamatergic RVLM cells mediate the short-latency VSR. Supported by NIH R01 DC008846.

533 Expression of Glutamate Receptors and Potassium Channels in Second-Order Chicken Vestibular Neurons During Development of Signal Processing

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Central vestibular signal processing depends in part on the gradual acquisition of specific subsets of glutamate receptors and potassium channels by second-order vestibular neurons for rapid and precise synaptic transmission. Here, we present data from a select group of vestibular reflex projection neurons, the principal cells of the chick tangential nucleus, a major avian vestibular nucleus. In the late-term chick embryo (E16), principal cell excitability is low, as demonstrated by their lack of spontaneous spike activity and generation of single spikes on depolarization. However, about one week after hatching (H5-H9), vestibular reflex activity is robust, and most principal cells display spontaneous spike firing and repetitive firing on depolarization.

To determine the receptors and channels underlying the maturation of principal cell excitability, immunolabeling and confocal imaging of AMPA receptor subunits (GluR1-4) and dendrotoxin-sensitive, potassium channels (Kv1) were performed during the perinatal period. From E16 to H9, AMPA receptor subunits GluR1 and GluR2 are characterized by low levels of expression. In contrast, GluR3 and GluR4 are expressed at high levels at E16 and continue to increase after hatching. These findings are consistent with recording faster kinetics of excitatory

spontaneous postsynaptic events in the hatchling principal cells.
The expression of Kv1.1 and Kv1.2 subunits is high in E16 principal cells bodies, but decreases by H1, consistent with recording decreased dendrotoxin-sensitive potassium conductances after hatching. Moreover, these specific potassium subunits undergo transformation in their subcellular compartmentalization. Specifically, Kv1.1 concentrates in the cell body and at the axon initial segment, whereas Kv.1.2 decreases in the cell body and appears within presynaptic terminals surrounding the principal cell bodies. Altogether, these developmental changes contribute to define the mature spike firing pattern and provide a developmental model to identify the protein targets which are involved in changes in signal processing in vestibular nuclei neurons after vestibular deafferentation.

534 Differential Dynamic Signal Processing in Frog Second-Order Vestibular Neurons

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During locomotion, vestibular signals have to be transformed into appropriate motor commands for gaze stabilization. Given the wide range of motion dynamics - from tonic head deviations to fast head accelerations - it is indispensable that the transformation into extraocular motor commands for compensatory eye movements occurs in parallel, frequency-tuned channels. Second-order vestibular neurons (2°VN) are the central element of this circuitry and thus play a key role for the sensory-motor transformation. In various vertebrates, these 2°VN have been shown to form differently tuned functional subgroups. In frog, 2°VN distinguish into two separate subpopulations (tonic - phasic 2°VN) with distinctly different intrinsic membrane properties, discharge dynamics and synaptic response characteristics. Tonic 2°VN exhibit low-pass filter characteristics and membrane properties that cause amplification of synaptic inputs, whereas phasic 2°VN are band-pass filters that allow frequency-dependent shunting of repetitive inputs. The differential, yet complementary membrane properties render tonic 2°VN particularly suitable for synaptic integration and phasic 2°VN for signal detection. Differential insertion into local inhibitory and excitatory circuits reinforces the functional consequences of the membrane properties of the two cell types, respectively. A feed-forward inhibition through local vestibular interneurons in phasic 2°VN concurs with the highly phasic membrane properties of these neurons. Thus, differences in synaptic response dynamics and preferred frequency-bandwidths are generated by co-adapted intrinsic membrane properties and emerging properties of the network in which the two types are embedded. Accordingly, the synergy of cellular and network properties creates sets of neuronal elements with particular filter characteristics that form flexible, frequency-tuned components for optimal transformation of all dynamic aspects of vestibular sensory signals.

535 Signaling and Plasticity of Vestibular Nerve Synapses Onto Functionally Distinct Vestibular Nucleus Neurons

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Information about head movements in the horizontal plane is processed in the medial vestibular nucleus (MVN), which comprises multiple evolutionarily conserved cell types that differ in their connectivity and functions during behavior and spatial cognition. Despite decades of elegant signal processing analyses of vestibular nucleus neurons in vivo, little is known about the cellular and synaptic mechanisms that underlie the remarkable performance and adaptive capabilities of the vestibular sytem. To bridge this cellular-systems-behavior gap, we perform slice physiology and vestibulo-ocular reflex plasticity experiments in mice, who are excellent at using vestibular mechanisms to stabilize gaze. By targeting intracellular recordings to fluorescent subsets of MVN neurons in brain slices from transgenic mouse lines, we have found that neurons differentially connected with the cerebellar flocculus, ocular motoneurons, the thalamus, and the reticular formation have intrinsic cellular physiological properties that are distinct both from each other and from local and commissural inhibitory neurons. Vestibular nerve synaptic transmission onto both projection and inhibitory neurons is rate-invariant over short time scales but plastic over longer time scales. Bidirectional non-Hebbian synaptic plasticity of the vestibular afferent synapse can be evoked in most neurons by pairing high frequency nerve stimulation with postsynaptic depolarization or hyperpolarization. Interestingly, the relative balance of synaptic potentiation and depression induced by different protocols differs across projection and local inhibitory neurons, as does the balance of postsynaptic glutamate receptor subtypes and the propensity for postinhibitory rebound firing. Identifying key mechanisms responsible for differences in signaling and plasticity in functionally distinct cell types will enable experimental analyses of the molecular influences on vestibular behaviors.

536 JAG1-Mediated Notch Signaling Specifies Sensory Organ Development in the Mammalian Inner Ear

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The mammalian inner ear is composed of six separate sensory organs that function in hearing and balance. Each sensory organ is composed of two basic cell types, the hair cell and the supporting cell, that have been shown to arise from a common progenitor. Our previous studies have shown that inactivation of JAG1 in the inner ear leads to missing or smaller sensory regions. A reduction in both hair cells and supporting cells in the mutant ears indicated that JAG1 is important for the development of the sensory progenitors. However, it is not clear how JAG1-mediated Notch signaling was acting in these progenitors. One

possibility is that JAG1 keeps these progenitors proliferating, or is required for their survival. Another possibility is that JAG1 specifies the progenitors. To investigate these possible mechanisms, we analyzed early sensory markers in *Jag1* conditional knockouts (*Jag1cko*). These studies revealed that the sensory markers were altered or absent at E10.5. We also investigated whether loss of JAG1 function lead to a decrease in proliferation or increase in cell death. Results of these studies showed that proliferation or cell survival were not affected at early time points in the *Jag1cko* mutant ears. To investigate these possible mechanisms further, we expressed an activated form of the Notch receptor (NICD) in non-sensory regions of the early otocyst using a combination Cre-tetracycline inducible system. Results of these experiments showed that we could induce ectopic sensory regions in the non-sensory areas of the cochlea and saccule. Taken together, these data support a role for JAG1-mediated Notch signaling in sensory specification in the mammalian inner ear. [Supported by NIDCD 1R01 DC009250]

537 Cooperative Functions of Hes/Hey Genes in Auditory Hair Cell and Supporting Cell Development

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Notch signaling pathway has been reported to regulate hair cell and supporting cell development. The basic helix-loop-helix transcriptional repressors Hes1, Hes5 and Hey1 are known to act as Notch effectors. They are expressed in the developing cochlea, but deletion in either of them causes mild or no abnormality. This raised questions about their possible specificity or redundancy. To explore the functions of Hes/Hey genes in auditory hair cell and supporting cell development, cochleae of various genotypes that lost single or double alleles of Hes1, Hes5 and Hey1 were examined. We found that graded increase in hair cell formation was accompanied by a reduction in Hes/Hey gene dosage. When one or two genes of Hes1, Hes5 and Hey1 were knocked out, hair cells significantly increased in number, as reported previously. However, this increase was accompanied by excessive formation of supporting cells adjacent to the excessive hair cells, although it had been thought that the increase of hair cells was due to the fate conversion of supporting cells into hair cells. Our data indicate that if at least one allele of Hes1, Hes5 and Hey1 was intact, both hair cells and supporting cells increased in number, suggesting that there was little fate conversion. The increase of hair cell and supporting cell numbers seemed to occur after the specification of prosensory domain because the size of prosensory domain and the timing of cell cycle exit appeared to be unchanged in the mutants. By contrast, when all of Hes1, Hes5 and Hey1 were inactivated, the number of hair cells increased at the expense of supporting cells. Moreover, the array of cells was irregular, and the quiescent state was disturbed. These results suggest that Hes1, Hes5 and Hey1 cooperatively inhibit hair cell differentiation, and at least one of Hes1, Hes5 or Hey1 is sufficient for operation of lateral inhibition and production of supporting cell property.

538 The Role of Bone Morphogenetic Proteins in Cochlear Hair Cell Formation: Analyses of Noggin and Bmp2 Mutant Mice

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The mammalian organ of Corti is a structurally complex sensory organ that converts sound waves in the form of vibrations to biochemical signals, which are then relayed to the brain via the auditory neurons. The cells comprising the organ of Corti are originated from the prosensory domain of the developing cochlea. Noggin, a secreted glycopeptide, antagonizes the activities of bone morphogenetic proteins (Bmp) by binding to Bmps and prevents their interactions with Bmp receptors. We show that inner ears of *Noggin-/-* mutants *(Nog-/-)* display increased rows of inner and outer hair cells in the organ of Corti, suggesting that too much Bmp signaling affects hair cell formation. We report that multiple Bmps are expressed in the developing cochlear duct including, Bmp4 and Bmp7 in the abneural region adjacent to the prosensory domain and Bmp2 in the nascent auditory hair cells. To investigate the role of *Bmp2* in auditory hair cell formation, we deleted *Bmp2* in the developing cochlea using *Foxg1*$^{cre/+}$and *Gfi1*$^{cre/+}$mice. The *Foxg1*$^{cre/+}$;*Bmp2*$^{lox/-}$ mice show no change in hair cell patterning within the organ of Corti but these mice die at birth. The *Gfi1*$^{cre/+}$;*Bmp2*$^{lox/-}$ mice are viable and they show normal hearing and auditory hair cell arrangement. Taken together, these results indicate that *Bmp2* is dispensable for hair cell formation and function. The phenotype of *Nog*$^{-/-}$ cochleae is likely due to gain-of-function of other Bmps rather than Bmp2 in the developing inner ear.

539 Analysis of the Maturation of Genotypically Atoh1-Null Hair Cells in the Cochlea of Chimeric Mice

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Previously, we demonstrated that genotypically Atoh1-null hair cells were able to be generated and were differentiating normally when given interactions with genotypically wild-type cells within the chimeric environment (Du et al., Dev. Biol. 305, 2007). However, the analysis was conducted in neonatal mice before the animal was able to hear, and thus, the Atoh1-null hair cells (HCs) may simply be surviving longer rather than truly forming functional hair cells. To distinguish between these two possibilities, we are examining Atoh1-null HCs in

chimeric mice up to postnatal day 20. To assist in the identification of the Atoh1-null HCs, GFP was bred onto the Atoh1 background and, therefore, the genotypically Atoh1 cells were identified by the presence of GFP. Distinguishing which chimeric combinations contained Atoh1-null cells, rather than Atoh1 heterozygous cells, was done using abnormal cerebellar morphology as previously described and by using PCR genotyping of the microdissected cells from the Atoh1 component of the chimeras. The maturation of the genotypically mutant HCs was examined using immunofluorescent labeling of several markers including myosin VI, acetylated alpha tubulin, Brn3c and phalloidin. At all of the ages examined, the genotypically mutant hair cells appeared similar to controls in the labeling pattern of all of the markers examined to date. Several different wild-type components were used in making the different chimeras, and all gave comparable results. This data demonstrates that genotypically Atoh1-null hair cells exhibit normal expression of various HC-specific proteins. Further, this data supports the hypothesis that these cells are differentiating normally and, likely becoming fully functional hair cells. This data also supports the idea that, given the right environmental cues, genotypically Atoh1-null HCs can bypass the Atoh1 pathway and still form hair cells. Supported by grant number DC009462 from NIDCD.

540 Morphological Development of the Reticular Lamina

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The reticular lamina, apical surface of the organ of Corti, is composed of the cuticular plates in the hair cells and the supporting cells, that are properly arranged in a plane and tightly connected each other via apical cell junction. The maturation of the cuticular plate is required for establishing a barrier between endolymph and perilymph as well as for maintaining the structural integrity. In order to elucidate the mechanism underlying the establishment of the reticular lamina, we observed the temporal change in the morphology of the cuticular plate and the property of the cell junctions during postnatal development. Using confocal laser microscopy, remarkable change was observed in the shape of OHC in mouse cochlea from postnatal days (P) 4 to P7 when the cuticular plates altered from circular to oblong and finally to matured heart shape with a notch formation in the medial portion. This morphological change occurred longitudinally from the base to the apex, which is indicated by the formation of the ″medial notch″ recognized at P5 in the basal turn and at P7 in the apical turn. Throughout this postnatal period, OHC-Deiter′s cell (DC) junction contains E-cadherin which is localized to the DC-DC junction but not to the OHC-DC junction in mature cochlea. Myosin II accumulates beneath the membrane at the apical cell junction and is activated by phosphorylation of its light chain at 19-Ser. These data suggest that the remodeling of the adherence junction and the contraction force mediated by it regulate the structural maturation of the reticular lamina.

541 TAK1 Expression in the Murine Cochlea: A Novel Marker for Supporting Cells

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TGF-b activated kinase-1(TAK1) is a member of the MAPKKK family and regulates diverse biological functions across species. Acting downstream of TGF-β and BMP signaling, TAK1 mediates the activation of the NF-κβ and c-Jun N-terminal Kinase (JNK) signaling pathways. TAK1 also serves as the target of a number of pro-inflammatory cytokines, and has been shown to play a role in Wnt/Fz signaling. Expression of TAK1 during the development of the cochlea has not been explored. Here we examine the expression levels of TAK1 in E16, P0, P4, P16, and adult cochleas, via DAB chromogenic immunohistochemistry and TSA-amplified fluorescent immunohistochemistry. The results indicate that at E16 TAK1 is broadly expressed throughout the developing cochlear sensory epithelium and is co-localized with the hair cell markers Myosin 7a, calbindin, and Atoh1. From P1 to P4, TAK1 expression is limited to cells of the stria vascularis, hair cells, supporting cells, and cells of the greater epithelial ridge. By P16, TAK1 expression is limited to Deiters cells, inner phalangeal cells, and inner border cells. TAK1 expression remains specific to these cells into adulthood, with little to no expression present in any other cell types throughout the mature cochlea. While the role of TAK1 in the cells is unclear, we propose that TAK1 expression may be used as a novel marker for specific populations of supporting cells.

542 Expression of the IGF Signaling Pathway During Mouse Cochlear Development

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Organogenesis of the inner ear is a highly ordered process that includes cell proliferation, coordinated cell cycle exit, differentiation and cell specification. This process is presumed to be regulated by a variety of growth factors and transcription factors, but only a limited number have been identified and fully studied. One such group is the insulin-like growth factor (IGF) signaling family. Mice deficient in IGF-I exhibit severe hearing loss as well as loss of spiral ganglion neurons (Cediel et al., 2006), and Woods et al. (1997) described a 15-year-old boy with a homozygous mutation in the IGF-I gene, that was associated with growth failure, mental retardation, and severe sensorineural hearing loss. These observations demonstrate an important role for the IGF-signaling pathway plays in development of the inner ear. However, the specific role of the IGF pathway, and in particular its role in the development of the cochlea, remains to be elucidated.

The IGF signaling pathway consists of two ligands (IGF-I and IGF-II), two receptors (type 1 IGF receptor: IGF1R and type 2 IGF receptor: IGF2R) and six soluble binding

proteins (IGFBP 1-6). IGFBPs play an essential part in the binding of IGF ligands to IGF receptors and in the control of IGF bioavailability. To begin to examine the role of this pathway in cochlear development, we used RT-PCR and in situ hybridization to determine the temporal and spatial patterns of expression for all the members within the developing cochlear duct. *IGFBP5* and *IGF1R* are already expressed in the epithelium of the otocyst at E10.5. *IGF-I* and *-II*, and *IGFBP-3* and *-4* begin to be expressed within the cochlear duct around E13. Moreover, at P0, *IGF-I* and *-II*, *IGF1R*, and *IGFBPs 2-5* are all expressed in unique domains within the cochlear duct. These results support the hypothesis that the IGF pathway is involved in one or more aspects of cochlear development such as cell growth and/or proliferation, differentiation, or cell specification.

543 Differential Expression of CTL2/SLC44A2 Isoforms During Development of Mouse Inner Ear

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CTL2/SLC44A2 is a membrane glycoprotein belonging to the solute carrier family of osmolyte transporters. In the ear, it was discovered as a supporting cell antigen that appears to be the target of antibody induced inner ear damage. Since antibody binding to CTL2 in vivo leads to loss of outer hair cells and hearing deficits, it is likely that CTL2 has an essential role in inner ear homeostasis. The purpose of this study was to examine CTL2 isoform expression in the developing inner ear. We used reverse transcription PCR techniques with full length primers designed to amplify individually the P1 and P2 isoforms of CTL2 in murine inner ear tissues obtained at e14, e16, e17, e18, p0, p1, p7, p14, p21, and 6 weeks of age. CTL2 expression was present at all stages from e14 to 6 weeks. Both isoforms were expressed at the earliest developmental stage examined, e14. The expression of the P1 isoform increased until about p7 but then it diminished in intensity relative to the P2 isoform. In contrast the P2 isoform became the predominant isoform and was highly expressed in the young mature inner ear (6weeks). This finding supports the concept that CTL2 has an important role in the developing ear and indicates that there is an isoform change that occurs with development. Future directions will assess the impact of knocking out either or both of the major CTL2 isoforms on inner ear development. Because of the early expression in the developing ear and the abundance of the protein we observed previously in both sensory and non-sensory cells, we postulate that like antibody induced damage, CTL2 abnormalities could have detrimental effects on development and function of the inner ear. CTL2 maps in the DFNB68 marker area suggesting that mutation of CTL2 could be a cause of congenital hearing loss.

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544 MicroRNA-Associated Argonaute Protein Expression in the Mouse Inner Ear

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The development and maintenance of sensory hair cells in the mammalian inner ear require the function of microRNAs as posttranscriptional regulators of gene expression. MicroRNAs function through association with Argonaute proteins in the microRNA-induced silencing complex (miRISC), which represses translation and effects degradation of complementary messenger RNA targets. Mammals possess four Argonaute proteins (Ago1-4), each of which is poorly understood with regard to differential expression and function. Preliminary immunohistochemical analysis of Argonaute expression in the mouse inner ear shows that each Argonaute has a distinct expression pattern among supporting and hair cells within the organ of Corti. The data suggest that differential expression of microRNAs and Argonautes might combinatorial effect histogenesis within the developing organ of Corti. Moreover, the cell-specific expression of Argonuate proteins could facilitate the biochemical isolation and identification of microRNA-regulated target genes that are relevant to hair cell development and maintenance.

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545 Transient Expression of PTEN in the Hair Cell and Neuronal Lineages During Mammalian Inner Ear Development

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The PI3K/Akt pathway is known to play a pivotal role in the survival of many tissues including the inner ear (Nagy et al., 2005; Jiang et al., 2006). PTEN (phosphatase and tension homologue of deleted on chromosome 10) is a tumor suppressor gene, and normally acts to inhibit the PI3K/Akt pathway by dephosphorylating PIP3. In the zebrafish, ptena, one of the two PTEN homologues, was revealed to be expressed in the developing inner ear, and ptena-deficiency caused the irregularities in the inner ear (Croushore et al., 2005). However, very little is known about the substantial function of PI3K/Akt pathway during the development of the mammalian inner ear. We reported in 2009 ARO MWM that PTEN-IR (Immuno-Reactivity) was detected in the prosensory region in the developing cochlear epithelium of the mouse at E14.5, which gradually became localized in the hair cell progenitors, and disappeared at P7. This time, we investigated the expression pattern of PTEN in the neuronal lineage during the mouse cochlear development. We observed that the expression of PTEN was also transient in the neuronal lineage, and the cellular localization of PTEN was temporally changed. The results imply that the PTEN might have some specific roles in the regulation of the

proliferation and the differentiation of the hair cells and neurons during the development of mammalian inner ear.
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546 Expression Pattern of Olig Gene Family in the Developing Inner Ears

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Olig gene family consists of Olig1 and Olig2 that were originally cloned as oligodendrocyte lineage-specific transcription factors and Olig3 that was found by homology search. Olig family genes are basic helix loop helix (bHLH) transcription factors that were important for cell fate specification in various organs of mammals. Other than oligodendrocyte differentiation, Olig1 regulates repair of demyelination in adult mammals and Olig2 regulates astrocyte and motoneuron development at the embryonic stage and glial scar formation after brain injury.
Since many kinds of bHLH transcription factors, such as Hes1, Hes5, Hey2, Atoh1 and Neurogenin1, regulates inner ear development in various stages, we hypothesized that Olig family might have some important roles in inner ear development. To confirm this hypothesis, we checked expression pattern of Olig1, 2 and 3 in E10.5, E13.5 and E15.5 inner ears by in situ hybridization.
All Olig genes were expressed in E10.5 otocyst epithelia and cochleo-vestibular ganglion (CVG) cells. Although Olig1 and Olig2 were expressed only in ventral portion of otocysts, Olig3 was expressed in all over the otocyst epithelia. Olig3 was strongly expressed both in inner ear epithelia and CVG cells even in E13.5 and E15.5 suggesting Olig3 might be important in the development of all inner ear epithelia. In contrast Olig2 was expressed in CVG cells and weakly in inner ear epithelia of E13.5 inner ears but not expressed in E15.5 inner ears. These results suggested that Olig2 might be involved in early stage of inner ear development. On E13.5 Olig1 was expressed only in CVG cells and not in inner ear epithelia but on E15.5 Olig1 became expressed in inner ear epithelia again, suggesting Olig1 affects inner ear development differently at early and later stages.
In conclusion each Olig family gene has different roles in inner ear development.

547 Excitatory and Inhibitory Synaptogenesis in the Cochlear Nucleus

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Interplay between excitation and inhibition is essential for signal processing in the cochlear nucleus. An imbalance between excitation and inhibition may be involved in disorders such as sensorineural hearing loss or tinnitus and such an imbalance may be even more critical when produced during development. In this regard, developmental studies are essential to understand pathological responses involving excitatory and inhibitory neurotransmission. Vesicular neurotransmitter transporters fill synaptic vesicles with specific neurotransmitters, thus contributing to neuronotransmitter phenotype. There are three known vesicular glutamate transporters (VGluT1, 2 and 3). VGluT1 is involved in excitatory neurotransmission in the adult rat cochlear nucleus. The vesicular inhibitory amino acid transporter (VIAAT) carries both GABA and glycine into synaptic vesicles. Because of the specificity of these two markers for labelling inhibitory and excitatory terminals, respectively, they may be used to study synaptic circuit development. Using specific antibodies, we are investigating the developmental distribution of VIAAT and VGLUT1 in the rat cochlear nucleus by immunohistochemistry.
VIAAT and VGLUT1 immunoreactivity are differentially distributed across different subdivisions of the cochlear nucleus during postnatal development. Immunoreactivity patterns undergo quantitative and qualitative changes during development, notably before (P7) and after (P15), after hearing onset. Results indicate that VIAAT immunoreactivity precedes VGLUT1. This may reflect neurotrophic effects mediated by GABA and/or glycine, a delayed auditory nerve arrival compared to interneuron maturation, or both. The correlation between hearing onset and immunoreactivity changes may reflect possible causal effects to be further studied. Supported by: MEC (BFU2006-13974) and JCCM (GCS2006C-15, SAN06-009, PEII0901526233, MOV-2007_IE/05).

548 A Clarin-1/integrin α 8 Complex May Function in the Developing Hair Cell Synapses

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Usher syndrome is a genetically heterogeneous disorder. The clinical symptoms are hearing loss and *retinitis pigmentosa*. Depending of the severity it can be divided in USH1, 2 and 3. The USH3A gene encodes Clarin-1, a protein that belongs to a hyperfamily of small proteins with four transmembrane domains. A subset of these proteins associates with the alpha subunit of integrins increasing the affinity of the heterodimer for its ligand. A closely related tetraspanin, stargazin, is known to regulate the targeting of AMPA receptors at the synapse. Based on this it has been proposed that Clarin-1 may function at the synapse.
We have localized Clarin-1 to the stereocilia and at the type I afferent synapses of developing cochlear hair cells. At the synapse, Clarin-1 is present both pre- and post-synaptically, correlating with the maturational switch of type I afferent to efferent synapses. TMRD staining showed abnormal type I afferent synapses in Clarin-1 KO mice.
OC-1 cells showed a filamentous pattern of expression for Clarin-1. Treatment with vesicle transport inhibitors disrupts this pattern suggesting an association with

vesicles. Sucrose density gradients and subcellular fractionation confirmed the presence of Clarin-1 in vesicles. Itga8 knockdown resulted in reduction of Clarin-1 protein, but not mRNA suggesting the Clarin-1/itga8 interaction is required for Clarin-1 stability.
Developmental studies showed colocalization of Clarin-1 and itga8 (which functions in synaptogenesis in the brain) at the hair cell synapses. We co-immunoprecipitate Clarin-1 with itga8 antibodies from organ of Corti and OC-1, demonstrating an interaction between both proteins. The Clarin-1/integrin complex is present in microsomal/vesicular fractions in OC-1 cells shown by subcellular fractionation and sucrose gradient.
These findings suggest that Clarin-1/itga8 complex is involved in vesicle trafficking at the type I afferent synapses where it may play a critical role in synaptogenesis.

549 Neurod1 Regulates Neuronal Differentiation and Controls Hair Cell Differentiation by Negatively Regulating Precise Spatial and Temporal Expression of Atoh1 in the Ear

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bHLH protein family members play a diverse role in proliferation and differentiation in many developing systems by precisely regulating the coordinated transition among various cell fate states. For example, in the ear, the first bHLH gene to be expressed, Neurog1, is required for proliferation and fate determination of neurosensory precursors. Neurosensory precursors then differentiate into all neurons and some hair cells through upregulation of Neurod1 and Atoh1, respectively. We recently showed that the absence of Neurod1 leads to enhanced expression of Atoh1, aberrant proliferation and death of granule cells in the cerebellum. This implies that Neurod1 regulates the continued expression of Atoh1 through a negative feedback loop. Using a Pax2-cre line to knock out the floxed Neurod1, we show that a similar negative feedback loop between Neurod1 and Atoh1 exists in the ear: absence of Neurod1 causes neuronal precursors to express Atoh1 and differentiate as ectopic hair cells near the displaced spiral and vestibular ganglia. In addition, absence of Neurod1 results in the premature upregulation of Atoh1, Pou4f3 and Nhlh1 in the apex instead of the base of the cochlea. This premature apical upregulation of Atoh1 results in a severely disorganized apex with multiple rows of inner and outer hair cells. Our data confirm and expand the previous observations on Neurod1 knock-out mice and show that Neurod1 is essential for proper migration and segregation of spiral and vestibular neurons. Furthermore, Neurod1 regulates the central projection of cochlear and vestibular afferents, which enter as a single root into the cochlear nucleus in Neurod1 mutants. In conclusion, our data suggest that Neurod1 is not only essential for neuronal viability and differentiation (including central projection) but also suppresses a hair cell phenotype in the delaminating neurons by controlling a group of genes that regulate the spatiotemporally accurate onset of hair cell differentiation.

550 Spiral Ganglion Neurite Outgrowth in NCAM Null Mutant Mice

WITHDRAWN

551 Spatiotemporal Differences in Cre-Mediated Recombination May Relate to Dose-Dependent Differences of Cre Needed to Recombine Different Floxed Genes

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The Cre/loxP system is extensively used for tissue-specific deletion of target genes in mice. A Cre recombinase expressed under the control of a promoter can excise genomic DNA flanked by loxP sites in a tissue-specific manner. Cre-mediated recombinations are generally monitored by breeding the cre line with reporter mouse lines, which express a reporter gene after Cre-mediated recombination is activated. However, previous studies have suggested that different reporter lines have different sensibilities to the Cre recombinase. Here we directly compared reporter gene expression of two reporter lines, R26R (which expresses LacZ after activation) and Z/EG (which express LacZ prior to and eGFP after activation), in three cre transgenic lines, Atoh1-cre, Foxg1-cre and Pax2-cre. Our data show noticeable differences in the expression patterns and levels between these two reporter lines. For example, using Pax2-cre we show apparent recombination with the R26R reporter line in the posterior canal (PC) crista whereas the Z/EG line does not show any recombination in the PC crista. The lack of defects obtained with floxed genes, such as reported for the floxed Dicer1 gene (Soukup et al., 2009), may reflect the inability of Pax2-cre to recombine certain genes in the PC crista. We also analyzed the expression of Cre recombinase by immunohistochemistry (IHC) and the results reveal that the expression of LacZ gene in R26R line requires less Cre protein than what is detected by IHC. Furthermore, we examined the residual expression of a floxed Neurod1 gene in these three cre lines by in situ hybridization. Our results suggest that the Cre expression shown by IHC is a more reliable approach for monitoring the recombination event for Neurod1 deletion. In conclusion, our studies demonstrate that different floxed genes require different thresholds of effective Cre-mediated excision and it is necessary to specifically analyze the deletion efficiency of each individual floxed gene.

552 Transplantation of Xenopus Laevis Ears Reveals Ubiquitous Rerouting of Motor Neurons to Become Efferents

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Comparative and developmental studies have suggested that cholinergic inner ear efferents are redirected facial branchial motor neurons, historically destined to innervate striated muscle fibers. We want to understand whether cranial motor neurons have the unique property to innervate the ear or whether any motor neuron can be rerouted to innervate the ear if the ear is placed in its trajectory. Transplantation of *Xenopus laevis* ears into the path of spinal motor neurons could reveal the potential to become efferent innervation by these neurons. In addition, the ability of the ear to develop in a novel location, complete with afferent connection with the spinal cord could be assessed. Otic placodes from stage 24-26 embryos were transplanted to the trunk and allowed to mature until stage 46. Of the 109 transplanted ears, 73 developed with otoconia. The presence of hair cells was confirmed by detection of *miR-183* and myo VI, suggesting that transplanted ears could develop histologically normal in a novel location. Injections of dyes ventral to the spinal cord revealed motor innervation of transplanted ears. This was confirmed by detection of vesicular acetylcholine transporter in synaptic terminals on hair cells, thus supporting the hypothesis that spinal motor neurons can reroute to innervate the ear as efferents if placed in its trajectory. Injections of dyes into the spinal cord labeled ganglion cells with axons fasciculating with existing spinal nerve fibers to project back to the spinal cord. These results demonstrate that the ear can develop normally, with efferent and afferent connections, in a novel location. In conclusion, evolutionary rerouting of facial branchial motor neurons relates to the topographical development of the ear which happened to be within the trajectory of the facial branchial motor neuron fibers, thus causing their rerouting. Future tests will assess whether this reinnervation of the ear is also possible by other cranial motor neurons.

553 TNF Mediates Antibacterial Clearance Via CCL3-Dependent Activation in Otitis Media

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Rationale: The middle ear (ME) mucosa provides immune defense by recognizing microbes and triggering inflammation via recruitment of phagocytes during otitis media (OM). Previously we found that in the absence of TNF, a key effector of innate immunity, OM induced by *NTHi* becomes persistent, accompanied by greatly impaired clearance of bacteria. Since bacterial clearance and killing are often mediated by macrophages, we evaluated the function of these cells in WT and mutant mice.

Methods: Phagocytic and intracellular killing capacity of TNF-/- and WT macrophages was assessed, with and without rTNF and rCCL3. Cytokine expression in macrophages was evaluated by qPCR and ELISA, and production of highly reactive oxygen species (hROS) was detected by vital staining. In vivo, NTHi-clearance and OM were assessed in rCCL3- and rTNF-treated or unstimulated MEs of TNF-/- and WT mice.

Results: In the absence of TNF, mice showed ME defects in inflammatory cell recruitment and OM recovery. Moreover, mutant macrophages showed reduced hROS production, phagocytosis and intracellular killing of NTHi, and failed to upregulate TLRs and downstream chemokine and cytokine genes, including CCL3. The impaired capacity of TNF-/- macrophages was partially corrected by rTNF, but was fully rescued by rCCL3. Furthermore, *in vivo* treatment with rCCL3 restored normal ME bacterial clearance and OM recovery in TNF-/- mice.

Conclusions: CCL3 is a potent stimulus of middle ear innate immunity in vitro and in vivo. Therapeutic manipulation of CCL3 and/or TNF might provide an effective approach in the treatment of OM induced by innate immune deficiency.

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554 Ion Homeostasis Channels in Middle Ear Epithelium

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Ion homeostasis has been proposed as a mechanism for regulating fluid balances within the middle ear. Various ion channels and transporters have been identified as key regulators of ion homeostasis within the inner ear, tightly controlling auditory and vestibular functions. These same ion channels may be involved in middle ear ion homeostasis and their disruption during infection may lead to the inability to clear effusions. To begin assessing these potential fluid control mechanisms, we conducted an immunohistochemistry investigation of normal mouse middle ears to evaluate protein expression of nine ion homeostasis channels or transporters commonly found in the inner ear. These included Na^+,K^+-ATPase α1, epithelial sodium channel (ENaC), gap junction protein beta 2 (connexin 26), aquaporins 1, 4, and 5, tight junction claudin 3, potassium voltage-gated channel (KCNQ1), and potassium inwardly-rectifying channel (KCNJ10). Paraffin-embedded normal mouse ears were evaluated with antibodies against these structures, using the inner ear as a positive control. The most prominent staining within the middle ear epithelium is seen for aquaporins 1, 4 and 5, claudin 3, ENaC and Na^+,K^+-ATPase. Other channels have not stained significantly, implying they are not present or occur at very low levels. These findings imply significant ion and fluid movement mechanisms may exist within the middle ear. Further elucidating these transport

processes in the middle ear may allow for improved medical treatment of effusions present after acute otitis media, as well as chronic effusions leading to protracted ear disease.
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555 Inner Ear Inflammatory Cytokines During Acute Otitis Media in the Mouse

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Although the inner ear has long been reported to be susceptible to middle ear disease, little is known of the inflammatory mechanisms that might cause permanent sensorineural hearing loss. Recent studies from this laboratory have suggested that inner ear tissues are capable of expressing inflammatory cytokines during otitis media. However, little quantitative information is available concerning cytokine gene expression in the inner ear and the gene products that result. Therefore, this study was conducted of mouse inner ear during acute otitis media to measure the relationship between inflammatory cytokine genes and their products with quantitative RT-PCR and ELISA, respectively. Balb/c mice were inoculated transtympanically with heat-killed H flu and inner ear tissues collected at 6 hours and 24 hours. Tissue homogenates were prepared for either quantitative RT-PCR microarrays (SABiosciences) or ELISA multiplex arrays (Quansys and Searchlight). Our quantitative RT-PCR and ELISA assays used cytokine profiles designed by our laboratory to evaluate those most relevant to middle ear disease. Expressed RNA for several cytokine genes was significantly increased in the inner ear at 6 hours. These included MIP-2 (273 fold), IL-6 (70 fold), IL-1β (4.6 fold), IL-10 (16 fold), TNFα (2 fold), and IL-1α (1.3 fold). The 24 hour samples showed a similar pattern of gene expression, although generally at lower levels. By comparison, the 24 hour ELISA showed the related cytokines were present in the inner ear at concentrations higher by 8, 45, 3, 2, 1.6, and 6 fold, respectively. These findings demonstrate considerable inflammatory gene expression and gene products in the inner ear following acute otitis media. These cytokine levels suggest one mechanism for the permanent hearing loss seen in some cases of acute and chronic otitis media.
[Research supported by NIH-NIDCD R01 DC009455]

556 Measurement of Mouse Middle Ear Inflammatory Cytokines with Multiplex ELISA Assays

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A recent advancement in ELISA technology is the multiplex array that quantitatively measures multiple proteins simultaneously within a single sample. This allows reduction in sample volume, time, labor, and material costs, while increasing sensitivity over single ELISA. Current multiplex platforms include planar-based systems using microplates or slides, or bead-based suspension assay with microspheres. To determine the applicability of this technology for otitis media, we used 4 different multiplex ELISA-based immunoassays to measure cytokine levels in mouse middle ear tissue lysate extracts following transtympanic H flu inoculation. Middle ear tissue lysates were analyzed using testing services from Quansys BioSciences, Aushon Biosystems SearchLight (both microplate-based), Milliplex Map Sample (bead-based), and RayBiotech, Inc. (slide-based). Samples were assayed in duplicate or triplicate. Results were compared to determine their relative sensitivity and reliability for measures of middle ear inflammation. The cytokine pg/ml amounts varied among the multiplex assays, so we compared the mean fold increase in cytokines from untreated controls at 24 hours. Several cytokines and chemokines were elevated, the extent dependent upon the assay sensitivity. The most significant were IL-1α, IL-1β, IL-6, TNFα, VEGF, MIP-1α, MIP-2, ICAM-1, KC, G-CSF, RANTES, and LIF. Where we harvested samples at 18, 24, 36 hr and 48 hr after inoculation, the peak elevations appeared between 18 -24 hours. Overall, the Quansys and Searchlight arrays showed the greatest sensitivity, both employing the same multiplex methodology of spotted arrays within each well of a microplate with chemiluminescent detection. Thus, the multiplex ELISA procedures appear suitable and reliable for study of middle ear inflammatory cytokines, providing accurate, quantitative, reproducible results with considerable improvement in economy.
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557 Impact of Middle Ear Inflammation on Ion Homeostasis Gene Expression

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Ion homeostasis genes are responsible for movement of ions and water in the various spaces in the inner ear and epithelium of the middle ear. To what extent ion homeostasis is a factor in the fluid accumulation seen in the middle ear with otitis media is not well known. Balb/c mice were transtympanically injected with heat-killed *Hemophilus influenza* bacteria to create an inflammatory condition in the middle ear. Untreated mice were used as controls. Mice were euthanized at 6 and 24 hours after injection, the bullae harvested, and total RNA isolated from the middle ear tissues. A total of 23 ion homeostasis genes were analyzed for up-regulation or down-regulation with quantitative RT-PCR from the following gene families: Na^+,K^+-ATPase, claudins, K^+ transport channels, epithelial Na^+ channels, gap junctions, and aquaporins. Genes in every category were shown to experience down-regulation ($p<0.05$) at 6 hours and increasing down-regulation at 24 hours. Down-regulation was most prominent in the K^+ transport channel genes at both time points and in the Na^+, K^+-ATPase genes at 24 hours. Inflammatory genes were also analyzed: MIP-2, IL-6, IL-1β, IL-10, TNF, IL-1α, VEGF, and Mapk8. All of the inflammatory genes were significantly up-regulated, more at 6 hours than at 24

hours, with the exception of VEGF and Mapk8. The up-regulation of the inflammatory genes indicates that an inflammatory condition was created in the middle ear of the experimental mice. Down-regulation of the ion homeostasis genes, however, may indicate that in the early stages of inflammation, the ion homeostasis genes are shut off which leads to the fluid accumulation within the middle ear seen with otitis media, both acute and chronic. Evaluation of PCR results at time points beyond 24 hours are underway. [Research supported by NIH-NIDCD R01 DC009455]

558 Effect of Glucocorticoids on Tumor Necrosis Factor Alpha Concentration in Middle Ear Effusion from Lipopolysaccharide Induced Otitis Media with Effusion in Chinchilla

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Otitis media with effusion is an inflammatory condition of the middle ear cleft typically brought on by bacterial infection or Eustachian tube dysfunction. This inflammation leads to production of middle ear effusion (MEE), which contains inflammatory mediators. Tumor necrosis factor alpha (TNF-á) is considered to be one of the most important inflammatory mediator in the production of MEE because it strongly induces a number of pro-inflammatory cytokines such as IL-1,-2, -6, -8 and endothelial adhesion molecules. Topical application of glucocorticoids has been shown to be effective for the treatment of otitis media with effusion in the presence of tympanostomy tube or tympanic membrane perforation. However, the mechanisms of glucocorticoid treatment have not been evaluated. This study was designed to determine if glucocorticoids reduce the concentration of TNF- á in MEE. Forty chinchillas were divided into four treatment groups: vehicle, 0.1% dexamethasone, 0.05% fluticasone propionate, and 1% rimexolone. Otitis media with effusion was induced in animals with injection of 0.3ml (1mg/ml) of lipopolysaccharide through the superior bullae. Chinchillas were treated with test substances at -2, 24, 48, and 72 hours relative to LPS inoculation. At 120 hours after inoculation, animals were euthanized. Samples of MEE were collected, solids were separated out by centrifugation, and supernatants were stored at -80°C. TNF- á concentrations were determined by western blot analysis with a standard curve. Concentrations were normalized with an albumin internal control. All three glucocorticoids significantly reduced the concentration of TNF- á in the MEE. The reduction of TNF- á may contribute to the amelioration of otitis media with effusion after glucocorticoid treatment. Further study is needed to determine if glucocorticoids have a direct or secondary effect on TNF- á and if this reduction affects the downstream inflammatory mediators.

559 S100 Protein Expression in the Middle Ear and Response to Streptococcus Pneumoniae

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Middle ear infection or otitis media (OM) is one of the most common diseases of childhood and the most frequent reason for pediatric surgery causing an estimated 5 million annual episodes at a cost of about $6 billion in the United States. Inflammation and biofilm formation are hallmarks of OM which may lead to conductive hearing loss due to secretory fluid accumulation in the middle ear cavity or defects of tympanic membrane and, more rarely, sensorineural hearing loss due to the inner ear damage. S100 protein A8, A9 and A12, members of the calcium binding protein family, have multiple functions including antimicrobial properties and inflammation regulation in a variety of diseases including cancer and infections. Data from our laboratory suggests that S100A8, S100A9 and S100A12 may play important roles in OM, however, the mechanisms regarding the regulation and function of S100 proteins in OM still remain undefined.

Utilizing an *in vivo* human middle ear epithelial tissue S100A8, S100A9 and S100A12 expression in the middle ear was characterized. Utilizing an *in vitro*, human middle ear epithelium cell culture (HMEEC) model S100A8, S100A9 and S100A12 expression following co-infection with *Streptococcus pneumoniae* was evaluated. Significant increased expression was demonstrated for each of these S100 proteins under these conditions.

This study is the first to characterize S100 protein expression in *in vivo* middle ear tissue and in response to *S. pneumoniae*. Similar to our previous work with *Haemophilus influenzae*, these results indicate that the S100 proteins are significantly up-regulated in the middle ear during periods of bacterial infection. This work would suggest that a more thorough understanding of the mechanisms in which the S100 family of proteins interacts with middle ear epithelium and bacterial pathogens in the pathogenesis of OM is warranted.

560 Extending the Chinchilla Middle Ear Epithelial Model for Mucin Gene Investigation

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Otitis media is the most common diagnosis in pediatric patients who visit physicians for illness in the United States. Mucin production in response to otitis media causes significant sequelae including hearing loss and the need for surgical intervention. Because mucins play an integral role in the mechanisms of otitis media, investigating the expression of mucin genes in this tissue is vital to the understanding of the pathophysiology of otitis media. The chinchilla (*Chinchilla lanigera*) model has been well defined for both *in vivo* and *ex vivo* studies of otitis media and has historically been the most widely used animal model in studying the pathophysiology of otitis.

Our laboratory has previously characterized the expression of mucin genes in the chinchilla including Muc1, Muc2, Muc4 and Muc5AC and Muc19. In this study we investigated the expression of additional mucin genes which have been expressed in epithelial tissues and which we have previously been characterized in the human and mouse. An *in vivo* chinchilla model was utilized and the expression of Muc6, Muc17 and Muc18 genes was assessed. In addition, sequencing of the cDNA of each of these genes was performed. The expression of these three genes was identical to that which we have previously identified in the human and mouse.

This study further characterizes mucin gene expression in the chinchilla middle ear and provides additional sequence data for chinchilla middle ear genes. This data will be helpful in future investigations examining middle ear pathophysiology and allows for the design of future reagents needed in utilizing the chinchilla for these and other investigations. The concordance of this gene expression data to that of both the human and mouse models further demonstrates the utility of this animal model in otitis media investigations.

561 Polymicrobial Biofilms as Reservoir for Chronic Otitis Media with Effusion (COME)

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Previous investigations have demonstrated the presence of biofilms containing middle ear pathogens (MEPs) in both the adenoids and middle ear effusions (MEEs) of children with acute otitis media (AOM) and recurrent AOM. Biofilm phenotypes have been identified in 65% of human infections and provide insight into chronic and recalcitrant infections. The pathogenesis of Chronic Otitis Media remains an elusive entity. The role of persistent middle ear infections in children with OME has been suggested.

Biofilms are an assemblage of microbial cells enclosed in an exo-polysaccharide (EPS) matrix that exist attached to inert abiotic or biotic surfaces. These microorganisms have shown increasing resistance to antimicrobial compounds. The EPS matrix allows bacteria to evade normal host immune response. These unique properties and planktonic shedding by biofilms provide a model for persistence of chronic and recurrent infections.

There is a growing body of data that underscores the role of nasopharyngeal polymicrobial biofilms in patients with OME. The effectiveness of adenoidectomies may provide a link between nasopharyngeal biofilms and their near ubiquitous presence in COME by serving as a bacterial reservoir. A similar link was recently investigated by our lab by analyzing the MEEs and nasopharyngeal tissue specimens of COME patients. Molecular analysis revealed the presence of DNA from MEPs in MEEs. Scanning Electron Microscopy (SEM) imaging analysis showed distinct biofilm ultrastructure on matched adenoid specimens.

Specimens were obtained from patients diagnosed with COME undergoing bilateral myringotomy tubes and adenoidectomy. Obstructive sleep apnea patients were used as controls. SEM imaging analysis revealed distinct biofilm architecture and significant coverage on COME specimens versus negligible biofilm density in controls. Polymerase chain reaction was performed on COME adenoid specimens and matched effusions to detect the presence of DNA from *S. pneumoniae, M. catarrhalis and H. influenzae*. All effusions tested positive for one or more of the aforementioned pathogens. Also, any pathogen identified in the effusion was also found to be present in the matched adenoid specimen. These data suggest that nasopharyngeal biofilms play a role in harboring bacteria which seed the middle ear space.

562 Clonal Spread of Beta-Lactamase-Producing, Amoxicillin-Clavulanate-Resistant Strains of Non-Typeable Haemophilus Influenzae (BLPACR) Among Young Children Attending a Day Care Center in Japan

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Background: Resistant strains of non-typeable Haemophilus influenzae (NTHi) is one of the principal causes of recurrent acute otitis media, rhinosinusitis, and pneumonia in young children. beta-lactamase-nonproducing ampicillin-resistant (BLNAR) strains are particularly commonin Japan, and β-lactamase-producing amoxicillin-clavulanate resistant (BLPACR) strains are now emerging. We investigated the nasopharyngeal carriage status of these resistant strains among children attending same day care center during the ten years period.

Methods: From 1999 to 2008, we obtained nasopharyngeal swab specimens from young children attending a same day care center in central Japan and examined the incidence of resistant strains of NTHi. Antimicrobial resistances of NTHi were identified based on polymerase chain reaction analysis of mutation of the penicillin binding protein (PBP) genes. Pulsed-field gel electrophoresis (PFGE) was performed to examine the clonal relationship of each resistant strain.

Results: The prevalence of genetically BLNAR (gBLNAR) among the children attending day care has been significantly increased during the past ten years. In addition to the gBLNAR, genetically BLPACR (gBLPACR) strains have been dramatically increased since 2007. Most of the day cared children had resistant strains with PBP gene mutations in their nasopharynx recently. PFGE analysis demonstrated that all gBLPACR strains were clonally identical. This represents the first report of the apparent clonal dissemination of gBLPACR strain of NTHi occurred in a certain condition such as day care.

Conclusions: Rapidly increasing prevalence in resistant strains, especially gBLPACR in a day care may predict the high incidence of these resistant bacteria from clinical isolates in the near future and the serious medical problems worldwide.

563 Establishment of an Experimental Otitis Media Model with Bioluminescent Pneumococcus in Chinchillas

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Objectives: Otitis media remains the leading pediatric diagnosis. Animal models provide much of what is known about the disease. Bioluminescent imaging has emerged as a powerful new tool to enhance animal models by offering non-invasive infection monitoring. Using this technology we hypothesized we could qualitatively and quantitatively monitor progression of acute otitis media in chinchillas. We proposed that early detection and monitoring of otitis media were possible using bioluminescent pneumococcus in the chinchilla model.

Methods: Thirty-six chinchillas cleared pre-morbid inspection by otomicroscopy and tympanometry. Bioluminescent pneumococcus was injected into the chinchillas' epitympanic bullae (50 CFU each). Animals were randomized into a control group and a group receiving Amoxicillin. Otomicroscopy, tympanometry, and bioluminescent imaging monitored infection progression.

Results: Animals developed otitis media by 24 to 48 hours. Controls showed robust middle ear infections with detectable bioluminescent activity on day 1 ($2.3x10^6$ photons/s/cm^2) and peak intensity on day 3 ($5.1x10^6$ photons/s/cm^2, difference p=0.005). Animals receiving Amoxicillin were imaged on day 3 after receiving 4 doses and photon counts approached baseline levels. Antibiotic-treated animals showed significant reduction in photon counts by day 3 compared to controls (average difference $4.6x10^6$, p=0.001). Many untreated controls developed labyrinthitis and meningitis. Infection in surviving controls ceased by day 14; middle ear effusion remained for six weeks. Antibiotic-treated animals cleared infections by day 2 of treatment and effusions between days 6 and 12.

Conclusion: Chinchilla-based otitis media models remain a standard investigational tool. Bioluminescent microbes promise to reinvent this model to study otitis media in finer detail. Bioluminescent technology offers a deeper understanding of otitis media pathogenesis and treatment.

564 The Genetic Basis of the "Otitis-Prone" Condition: The Association of Chronic or Recurrent Otitis Media with the Loci *Fbxo11* and *Evi1*

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Introduction. Studies have shown that susceptibility to both chronic otitis media with effusion (COME) and recurrent acute otitis media (RAOM) has a genetic component of 60-70%. Genetic associations are likely to be at several loci. Published studies suggest an association of RAOM with polymorphisms of HLA-A molecules, CD14, TNFα, IL1, IL10, IL6, mucin-5, surfactant-protein-5, plasminogen-activator-inhibitor-1, and TLR4. However, the strength of these associations has been weak and studies have lacked statistical power. The mutagenesis program at MRC Harwell has developed two novel mouse models of chronic otitis media. The *Jeff* mouse has a point mutation in the *Fbxo11* locus and the *Junbo* mouse has a mutation in the *Evi1* locus. These loci are known to be involved in TGF-β/SMAD signaling pathways, which are activated in human COME. These loci represent good candidate sites for association with human susceptibility to otitis media.

Methods. We are in the process of collecting DNA from 1,000 predominantly Caucasian children undergoing insertion of ventilation tubes to treat RAOM or COME. We are also acquiring DNA from their parents and their siblings. We are genotyping recognized single-nucleotide-polymorphisms at the loci *Fbxo11* and *Evi1,* using the Pyrosequencing® platform. Statistical comparison will be made of observed allele frequency to expected allele frequency based upon parental genotype. In addition, we plan a genome-wide association study, including the loci previously suggested to be associated with RAOM.

Results. We will report our progress on the establishment and analysis of the DNA collection. Any associated polymorphisms will be re-sequenced to determine the causative mutation, which may allow assessment of functional consequence.

Conclusions. These studies should identify single-nucleotide-polymorphisms having a moderate association (RR>1.8) with susceptibility to RAOM or COME in the Caucasian population, with a statistical power of 80%. This genetic database for otitis media will be one of the largest yet created and allow us to dissect further, alongside our use of mouse models, the genes and genetic pathways involved with chronic OM.

565 Histopathological Incidence of Facial Canal Dehiscence in Chronic Otitis Media

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Background: In clinical studies, the incidence of the facial canal dehiscence in chronic otitis media is lower than that in histopathological studies using normal human temporal bones.

Objective: To disclose the histopathologic findings of facial canal dehiscence in chronic otitis media.

Methods: We divided the human temporal bones into two groups (Group 1, 4 years old and over; Group 2, 3 years old and under) because facial canal development has been reported to be completed by the age of 4. The group of 4 years old and over consisted of 132 human temporal bones. 17 temporal bones were examined for the group of 3 years old and under. Age-matched normal temporal bones were also examined as the controls. We evaluated the incidence and the area of the facial canal dehiscence in chronic otitis media under light microscopy.

Result: In the group of 4 years old and over, 68.9% of temporal bones with chronic otitis media and 71.9% of controls had the facial canal dehiscence. There was no significant difference between the two groups (P = 0.71). The area of the dehiscence in temporal bones with chronic

otitis media was not statistically different from controls (P = 0.53). In the group of 3 years old and under, 88.2% of temporal bones with chronic otitis media and 76.5% of controls had dehiscence. No significant difference was found between the two groups (P = 0.66). The area of the dehiscence in temporal bones with chronic otitis media was not statistically different from controls (P = 0.43).
Conclusion: In temporal bones with chronic otitis media, the incidence of facial canal dehiscence was high, but was not different from controls. These findings indicates that chronic otitis media does not affect facial canal dehiscence. Surgeon should keep in mind that the facial canal dehiscence is more frequent than that reported in clinical literature.

566 Effect of Intracellular Chloride on Pre-Pulse Sensitivity of Outer Hair Cell Electro-Motility and NLC

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Mammalian outer hair cell (OHC) electro-motility is believed to result from voltage-dependent conformational changes of the membrane protein prestin. The interaction of prestin with intracellular chloride (Cl) modulates the cell's nonlinear capacitance (NLC), the electrical signature of electromotility. A number of physiological factors shift prestin's voltage dependence, including changes in intracellular Cl and membrane holding potential (pre-pulse effect).
We recently observed that the OHC's magnitude of pre-pulse effect is dependent on intracellular Cl level (Song and Santos-Sacchi 2008, ARO). Hyperpolarizing membrane potentials shift the NLC curve toward depolarizing potentials only when intracellular Cl level is normal (10 mM) or higher, but not when intracellular Cl is low (1 mM). Depolarizing membrane potentials do the opposite, shifting NLC to hyperpolarizing potentials when intracellular Cl concentration is normal or low, but not when intracellular Cl concentration is a at saturating 140 mM. To understand the consequence of combined Cl and pre-pulse manipulations on the mechanical output of OHCs, i.e. electromotility, we combined measurement of NLC with video imaging. This effort is to determine whether mechanical (motility) and electrical (NLC) activity can uncouple under extreme conditions, such as when Cl and pre-pulse potentials are far from 'in vivo' conditions. We find that OHCs patched at 140 mM intracellular Cl show robust depolarizing shift of NLC with prepulse potentials of -80 mV, with mechanical measurements from video images displaying a similar shift. Investigations of prepulse effect on NLC and motility at other intracellular Cl concentrations are underway.
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567 Assessing Chloride Flux Across Cell Membranes of Stable Prestin Cell Lines

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The mechanical feedback loop in mammalian cochlear amplification relies on prestin, the motor protein in the basolateral membrane of the outer hair cell (OHC). Recently, we developed a HEK cell-line expressing prestin induced by tetracycline (2008 ARO Abstract **1096)**, which can be used as an alternative platform to explore prestin's characteristic features, such as the dependence of non-linear capacitance (NLC) on anions. The benefit of this cell line is the greater expression rate of prestin, hence better signal to noise ratios during manipulations that alter NLC.
Prestin's Cl dose-response relationship in the HEK cell-line is similar to that in the OHC, except for a 40~60 mV left-shift of Vh to negative potentials. With local perfusion, 140 mM extracellular pentane sulfonate can hardly affect the HEK cell-line, while the same concentration of this anion can penetrate into outer hair cells, markedly decreasing and left-shifting peak NLC. Also, extracellular Cl^- (Cl^-_o) has little affect on the intracellular Cl^- (Cl^-_i) concentration in the HEK cell-line, since neither V_h nor amplitude of peak NLC has any change. In prestin-transfected CHO cells, similar results were found.
There are two possible interpretations. First, extracellular anions cannot enter HEK cells since they lack a corresponding pathway like G_{metL} in OHCs, indicating that prestin is independent of G_{metL}. Second, the washout by pipette solution, which dilutes the anions' impact inside the cells, is so fast that entering anions cannot affect NLC. We are currently working to circumvent washout problems to rule out this issue.
(Supported by NIDCD DC 000273 to JSS)

568 Only One Isoform of Prestin Out of Several Expressed on the Surface Is Important for NLC

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Prestin (SLC26A5) is a member of the SLC26 family of anion transporters responsible for electromotility in mammalian outer hair cells (OHC). Prestin is a membrane protein of 744 amino acids with 10 or 12 transmembrane regions, depending on the prediction paradigm
used. Cysteine residues, because of their ability to form disulfide bonds, can play a number of important roles in a protein's function. Prestin contains 9 cysteine residues, of which six (C192, C196, C260, C381, C395, and C415) lie in the transmembrane regions. Of the remaining three, two (C52 and C679) are located in the intracellular N and C termini respectively, while the remaining cysteine (C124) residue lies in a loop connecting two potential transmembrane regions. Previous studies showed that mutating individual cysteines in prestin didn't alter the protein function. We generated multiple cysteine mutants of prestin and tested them for functionality by measuring non-linear capacitance(NLC) and for membrane targeting

by performing surface labeling experiments followed by detection of prestin by western blot. These experiments revealed that both wt-prestin and cysteine mutants were delivered to the membrane in two forms; one a slightly larger form and a second slightly smaller form. Both were in the molecular weight range expected of the prestin monomer. We observed a linear relationship between a measure of prestin function in the membrane (Qsp) and the ratio of the upper band to total prestin expressed. Such a relationship was not observed of the lower band. Since both these bands could be detected with antibodies to the N and C terminus of prestin it is likely that the lower band is misfolded. These data suggest a delinking between proper folding and surface delivery. Additionally, they suggest that surface measurement by fluorescence cannot be used as a reliable measure of how much functional protein is delivered to the surface of the cell. Supported by NIDCD DC 007894, DC 000273 and DC 008130.

569 Prestin Upregulation in TECTAC1509G Mice Increases Electromotility and Membrane Permeability to Propidium Iodide

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Prestin-mediated outer hair cell (OHC) electromotility improves auditory sensitivity by amplifying the traveling wave. Previously, our laboratory created a mouse with a point mutation in TECTA, the gene encoding α-tectorin. Humans with one copy of this mutation are born with a partial hearing loss that progressively worsens with time. We found that $TECTA^{C1509G/+}$ mice exhibit partial hearing loss because their tectorial membrane only contacts the first OHC row. Surprisingly, $TECTA^{C1509G/+}$ and $TECTA^{C1509G/C1509G}$ mice also expressed ~25% more prestin in their OHCs. We sought to determine whether increased prestin levels increased electromotility and whether this might affect OHC membrane permeability. OHC electromotility was measured in freshly-excised mouse cochleae stimulated with a sine wave electric field. Electrically-evoked displacements of the reticular lamina were measured by laser doppler vibrometry. Over the frequency range of 1-40 kHz, larger displacements were found in $TECTA^{C1509G/+}$ and $TECTA^{C1509G/C1509G}$ mice compared to $TECTA^{+/+}$mice (4.58±0.24 dB and 4.95±0.15 dB; mean±SEM, n=33-34, $p<0.05$). No movements were detected in control prestin-null mice (n=12). Cell toxicity after electrical stimulation was assessed by identifying membrane-compromised OHCs with propidium iodide. The proportion of labeled OHCs was greater in $TECTA^{C1509G/+}$ and $TECTA^{C1509G/C1509G}$ cochleae (42.3±5.5% and 26.5±3.8%, n=11) compared to $TECTA^{+/+}$or prestin-null cochleae (10.6±2.7%, n=12; 6.4±3.9%, n=7, $p<0.5$). As well, there were no genotypic differences in propidium iodide labeling in controls where no stimulus was applied (1.8-2.5%, n=12-13, $p=0.41$). Our data demonstrate that increased prestin in $TECTA^{C1509G}$ OHCs produces greater electromotility and that this is associated with an increased risk of membrane permeability after electrical stimulation.

570 Electromechanical Forces in Lipid Bilayers

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Electromechanical coupling in cellular membranes is important for cell physiology. The outer hair cell (OHC) plasma membrane is an important part of this cell's motor complex necessary to mammalian active hearing. The membrane contributes to generation of the active force and affects outer hair cell nonlinear capacitance. An experimental technique combining optical tweezers with cell voltage clamp has been recently used to probe the cell's electromechanical properties. In this experiment, membrane tethers were pulled from OHC and HEK cells, and the corresponding pulling (holding) force was measured as a function of voltage. Here we propose a physical mechanism explaining the voltage dependence of the membrane force. This mechanism is associated with the electric field-dependent distribution of local charges, including the membrane surface charges and adsorbed counter ions. We also describe a redistribution of the system of local charges in response to changes in the membrane curvature. In our analysis, we solve a Poisson-Boltzman equation for the system of local charges and find the corresponding potential field. Then, we derive the free energy of the tether system and obtain the tether force as a function of voltage. The membrane characteristics, such as polarization and free energy, are also obtained as functions of the membrane curvature. We find that the computational tether force increases with hyperpolarization and decreases with depolarization of the cell which is consistent with the experiment (Qian et al., 2004). The local electric field associated with the system of membrane charges is of significant interest itself because it affects the prestin-related charge transfer and membrane nonlinear capacitance (Sun et al., 2009). The developed analysis will help deeper understand the physical picture of the active force production in the cochlea. Supported by research grants DC 000354 and DC 002775 from NIDCD.

571 Evidence That the Subsurface Cisternae Influences the Electrical Properties of the Outer Hair Cell

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The plasma membrane (PM) of the lateral wall is separated from the subsurface cisterna (SSC) membrane by the cortical lattice and partitions the outer hair cell (OHC) into two compartments; the extra cisternal space (ECS) and the axial core (AC). The role of the SSC is unknown but it may be electrically coupled to the PM. We measure the linear capacitance (LC) of OHCs across the cochlea with admittance techniques by whole-cell patch clamping at the synaptic pole of the cell. The apparent surface area (ASA) of the OHC was calculated from the

length and the diameter obtained from images of the cell, where the length was measured from the cuticular plate to the basal pole. We find that the LC does not increase monotonically with length or ASA. The relationship fits poorly to a linear function (LC=mx+c, m=0.005pF/μm^2, c=12.8pF and R^2=0.38) because the LC of cells from the apical region exhibits two values; it either saturates or increases linearly with length or ASA. The data requires two linear functions ($LC_1=m_1x+c_1$ and $LC_2= m_2x+c_2$) for a better fit either as function of length ($m_{1length}$=0.29pF/μm, $c_{1length}$=6.77pF, $R^2_{1length}$=0.88 and $m_{2length}$=0.10pF/μm $c_{2length}$=15.0pF, $R^2_{2length}$=0.61) and of ASA (m_{1ASA}=0.0103pF/μm^2, c_{1ASA}=5.69pF, R^2_{1ASA}=0.80 and m_{2ASA}=0.0046pF/μm^2, c_{2ASA}=11.8pF, R^2_{2ASA}=0.65). There are two possibilities: (1) some cells originating from the apical region have a larger membrane reservoir; or (2) the (AC) and the (ECS) have different resistivity and the measured LC is lower because both membranes act like capacitors in series. Because (1) cannot explain the saturation-like function we suggest (2) is more plausible. Where the higher slope (1.03μF/cm^2) reflects the apparent specific capacitance of the electrically uncoupled PM and the lower slope (0.46μF/cm^2) reflects the apparent specific capacitance of the coupled membranes from which we derive a value of 0.83μF/$cm^2$for the apparent specific capacitance of the SSC.

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572 Modulation of Prestin-Associated Charge Movement by an Omega-3 Fatty Acid

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Docosahexaneoic acid (DHA), a polyunsaturated omega-3 fatty acid, has been shown to affect membrane protein function. This study investigates the effects of DHA concentration on the voltage dependence and magnitude of prestin-associated charge movement in HEK 293 cells. Prestin-transfected cells were incubated with increasing concentrations of DHA (30, 45, 50, 60 µM), morphology was observed and prestin-associated charge density and linear capacitance were measured by admittance by use of the patch clamp technique. At higher concentrations of DHA (≥45 µM) the voltage shifted the function of the charge movement to hyperpolarizing values and increased the amount of charge moved. Cells became rounded and their membranes more fragile at higher concentrations. We also observed an effect of prestin transfection efficiency. In cell populations in which control cells had higher baseline charge density, DHA treatment did little to influence prestin-associated charge movement. However, when baseline charge density is low, DHA treatment becomes significant and increases prestin-associated charge movement. Two mechanisms of action for poly unsaturated fatty acid have been proposed: 1) they alter the mechanical and/or electrical properties of the membrane; and 2) they remove the protein from cholesterol rich rafts. We will discuss how each of the mechanisms may be involved in modulating prestin-associated charge movement.

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573 Voltage-Induced Molecular Movement in Prestin

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The electromechanical activity of cochlear outer hair cells is essential for mammalian hearing. Electromotility is driven by the transmembrane motor protein prestin, which acts as a voltage sensor and presumably undergoes conformational changes in response to altered transmembrane potential. However, direct experimental measures of voltage-induced motion on a molecular scale are lacking. We investigated whether changes in the transmembrane electric field induce molecular motions by measuring fluorescence resonance energy transfer (FRET) between C-terminally tagged prestin proteins. Our data demonstrate that prestin-prestin FRET decreases upon membrane depolarization and increases upon hyperpolarization over an operating range of voltages relevant to electromotility. These changes in the FRET signal occur over a similar voltage range as prestin-associated charge movement (nonlinear capacitance) and are sensitive to salicyalte, suggesting that both measures are tightly coupled by a voltage-dependent conformational change in prestin. These results are direct evidence of voltage-induced motion in prestin and demonstrate a fluorescence signature associated with prestin-dependent motility. [Supported by NIDCD R01 R01DC009622]

574 Characterization of Prestin Oligomerization and Diffusion at the Single Molecule Level

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Prestin is the motor protein that drives outer hair cell electromotility. Several groups have shown evidence of prestin-prestin interactions and prestin oligomerization using biochemical techniques and optical imaging. Furthermore, studies have demonstrated that alterations of cholesterol in the cell membrane, which modify prestin function, affect membrane fluidity and prestin mobility. However, the role prestin-prestin interactions or oligomerization play in electromotility and the molecular motifs that mediate these interactions are unknown. It is also unknown whether the effects of cholesterol modifications on prestin function are due to changes in prestin mobility or changes in prestin interactions. Ensemble optical measurements of prestin self-association cannot provide molecular level details of prestin-prestin interactions, nor can they distinguish the oligomeric states these interactions produce. Likewise, fluorescence recovery after photobleaching cannot elucidate non-Brownian modes of diffusion which could indicate interactions with the cytoskeleton. We have thus developed our ability to detect individual prestin-citrine

molecules using single molecule fluorescence (SMF) microscopy. We have applied SMF to measure the distribution of intensities emitted by individual prestin clusters in the HEK cell membrane. The distribution displays peaks spaced at multiples of the unitary intensity which one would expect for a distribution of non-interacting fluorescent emitters. We have resolved the stoichiometries up to tetramers, however the data makes clear that higher populations also exist. We have also explored the effect of membrane cholesterol depletion on the oligomerization of prestin using SMF microscopy to asses whether this treatment dissociates prestin oligomers or simply removes a bulk population of intact oligomers from microdomains. We will further utilize total internal reflection fluorescence microscopy and site-directed labeling to measure prestin diffusion at the single molecule level and explore the effects of cholesterol depletion and cytoskeletal inhibitors. [Supported by NIDCD R01DC009622]

575 Dissecting the Mechanoelectric Coupling of Outer Hair Cell Electromotility

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Electromotility is a voltage-induced rapid force-generating cell length change of mammalian cochlear outer hair cells (OHCs) that is essential for normal hearing of mammals. Charge movement in the lateral membrane of OHCs accompanies OHC electromotility. This charge movement is manifested in the bell-shaped voltage-dependent cell membrane capacitance, which is referred to as nonlinear capacitance (NLC). Prestin has been identified as the membrane protein that is responsible for generating electromotility and NLC. It is believed that electromotility and NLC are fully coupled, and thus, NLC measurement is generally accepted as a proper substitute for evaluating the electromotile function of prestin and its mutants. However, a rigorous quantitative proof for the validity of this substitution has not been provided. Especially, knowledge regarding the relationship between the degree of cell displacement and the degree of charge movement has not been attained. Recent evidence that zebra-fish ortholog of mammalian prestin lacks electromotility while retaining NLC implies that NLC does not necessarily represent electromotility. In the present study, we simultaneously measured electromotility and NLC for dissecting the mechanoelectric coupling of OHC electromotility. We found solid quantitative evidence that NLC indeed represents electromotility in OHCs. In other words, the same parameters of voltage dependence can be derived from either measure. By gradually reducing the charge movement in OHCs by salicylate, we also found that there is a linear relationship between OHC displacement and the charge movement, and that the slope varies from one cell to another. The result strongly suggests that the mechanoelectric coupling of prestin is modulated by cellular cues, which is probably important for optimizing cochlear amplification under various conditions. [Supported by NIH Grant DC00089 and the Hugh Knowles Center].

576 Investigating the Relationship Between Two OHC-Specific Proteins: Prestin and Oncomodulin

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Outer hair cells (OHCs) of the cochlea constitute the cochlear amplifier, essential for providing high sensitivity and frequency selectivity of mammalian hearing. Two unique proteins, prestin and oncomodulin, are abundantly expressed in these cells. Prestin is a voltage–dependent motor protein responsible for somatic electromotility of OHCs (Zheng et al., 2000). These membrane proteins are densely packaged, exclusively in the basolateral membrane of OHCs. Lack of, or dysfunctional prestin can cause OHCs death (Dallos et al., 2009). Oncomodulin is an uncommon calcium-binding protein that was original identified from tumor cells. In OHCs, an exceptionally high level of oncomodulin proteins is found in the soma, which is surrounded by the prestin-embedded basolateral membrane (Hackney et al., 2005). Expression of prestin and oncomodulin are both developmentally regulated. In addition, prestin and oncomodulin mRNAs were are regulated by the same microRNA (Lewis et al., 2009). However, the relationship between these two OHC-specific proteins remains unknown.

In order to explore the relationship between prestin and oncomodulin, we collected cochlear RNA from different hearing developmental stages range from P0 to adult. Quantities of prestin- and oncomodulin-mRNA were measured by real-time PCR. Amounts of oncomodulin-mRNA isolated from different developmental stages of wild type mouse cochleae were also compared to those collected from prestin-KO mice. The possible physiological connection between prestin and oncomodulin is discussed. (Work supported by NIH Grants DC00089 and DC006412).

577 The Size of R Group Rather Than the Charge Alone Plays a Role in Voltage Sensing of Prestin

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Nonlinear capacitance (NLC) and motility are two unique features of mammalian prestin (SLC26A5). The regions or amino acids essential for voltage sensing or motility are still unclear. Intracellular anions are thought to trigger the conformational change of prestin. Therefore, charged amino acids were most likely to be the candidate of voltage sensor of prestin, because of their potential capability to serve as the anion binding sites. Factors critical for this function may include the charge and size of the R group, as well as the existence of the R group itself, while previous studies focused mostly on the charges of the R groups and their individual effects on NLC. In our study, the roles of three positively charged amino acids (R197, K227 and K449) in voltage sensing are examined based on the comparison of paralogs of the mammalian SLC26A family. All of these three residues are in the predicted transmembrane domains. Two of them, K227 and K449

are conserved in all the paralogs, while R197 is unique to only the mammalian SLC26A5 orthologs. Mutual positive (R to K and K to R), negative (R and K to E) and neutral (R and K to A) replacements were constructed, to test the influences of the size, charge and existence of the R groups, respectively. Furthermore, the combinations of these three sites (double and triple mutations) were also made and tested considering the possibility of multi anion binding sites in the molecule. The results showed that: 1. Mutations on all three sites showed significant changes on NLC and increased slope factors of NLC were found on two mutual positive mutations. This result indicates that all three sites are important for voltage sensing and the size of R group is essential in addition to the charge; 2. More deteriorated NLC were detected on double and triple mutations, revealing the participation of multiple sites in the voltage sensing; 3. A negative correlation between slope factor and peak voltage of NLC were observed, while the maximum charge movement didn't increase with the slope factor. This suggests other electrical features such as dielectrical properties rather than the number of charges or their traveling distance were changed in these mutations.

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578 Sound Transduction in the Mammalian Outer Hair Cells: Prestin Activity Is Required for Proper Deflection of the Stereocilia Bundle

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The outer hair cell (OHC) body is capable of prestin-driven electromotility leading to force generation that increases the vibration of the hearing organ critical for auditory sensitivity. At the cell's apex, the stereocilia bundle deflects as a unit during sound stimulation (Fridberger et al, 2006). Such a deflection converts nanometric displacements into electrical signals transmitted to the auditory nerve.

Very little is however known about how sound stimuli cause the bundle to deflect, especially, the possible contribution of prestin-induced cell body vibrations to this deflection has never been investigated.

Here we investigated the influence of the membrane protein prestin activity on the bundle deflection, in an intact ear preparation from the Guinea pig. Prestin was previously shown to be specifically inactivated by salicylate and tributyltin. Using an approach combining rapid confocal imaging and optical flow-based computation, the bundle deflection was studied under simultaneous sound stimulus administered at 50-350HZ, a frequency band typical of OHCs vibrations in the apex of the cochlea.

To our surprise and irrespective of the prestin inhibitor used, sound-induced bundle deflection drastically increases, specifically, near the best frequency whose position was altered. Likewise, the vibration of the bundle tip intensified. Moreover, the shape of the bundle deflection's pattern was affected.

Our data challenge the general assumption that prestin inactivation decreases the vibrations of the cochlear´s structures. Because no consistent change was observed for vibrations of the reticular lamina, the increase in the bundle deflection may be caused by a robust vibration seen for the top. The data suggest that prestin motor's activity regulates the tuning of the bundle vibrations and may explain how the stereociliary and saumatic amplifiers interact during sound transduction in the mammalian ear.

579 Prestin Possesses Two Voltage-Dependent Gates for Electromotility

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Prestin or outer hair cell (OHC) electromotility is voltage-dependent. Chloride ions have been found to act as an external voltage sensor to trigger prestin protein conformational changes. However, its voltage gating mechanism still remains unclear. Despite the relatively systematic prestin mutagenesis has been performed, the gating and gating structure in the prestin protein have still not been identified based on the traditional one-gate model analysis. Prestin is a member of transporter (SLC26A5) belonging to a sulfate-anion transporter family. Distinct from general ionic channels (such as K^+, Na^+, and Ca^{++} channels), transmembrane transporters usually possess two gates located near the cytoplasmic and extracellular sides to block or permit movement of ions to carry out transporter function. In this study, we used two-gate model to analyze the prestin voltage-dependent gating. In the direct recording of gating charge movement, two time-constants were visible corresponding to two-gate activity. Also, two-gate model shows a perfect fit to nonlinear capacitance (NLC). F-test demonstrates the improvement did not result from the increase in the fitting freedoms (parameters). In comparison with the traditional one-gate Boltzmann model, the two-gate model also shows a perfect fit to NLC at the reduction of intracellular Cl^- concentration. As the concentration of intracellular Cl^- decreased, prestin two-gating responses became one-gating activity. Salicylate can inhibit OHC electromotility by competitively binding to the prestin anion binding side. Two-gate model also shows a goodness of fit to NLC at the application of salicylate, which usually can not be perfectly fitted by the traditional one-gate model, and demonstrated inhibition of one gate activity. Prestin mutants D154N and D342Q have been reported shifted the NLC peak voltage to extremely negative and positive voltage ranges, respectively. The gating analysis shows that only one-gate activity was detectable in these mutants. Mutation impaired one voltage-dependent gate activity, resulting in their voltage-dependence shifted to negative and positive voltage ranges. Computer structure modeling also revealed that prestin has two voltage-dependent gating clusters. Thus, consistent with other transporters, these data indicate that prestin possesses two voltage-dependent gates rather than the traditionally assumed one voltage-dependent gate for electromotility.

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580 High Resolution Imaging by Atomic Force Microscopy of Prestin Purified and Reconstituted Into an Artificial Lipid Bilayer

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The electromotility of cochlear outer hair cells, which realizes cochlear amplification, is considered to be driven by the motor protein prestin. To date, several attempts to clarify the structure of prestin have been made. Recently, electron microscopy (EM) of purified prestin in a soluble state and atomic force microscopy (AFM), employed with Qdot labeling, of the isolated plasma membrane of prestin-expressing CHO cells have revealed the shape of prestin. Although those studies are significant, there are some problems. One problem with the above-mentioned EM research is that the structure of prestin in a soluble state is not necessarily identical to that of prestin embedded in the lipid bilayer. Thus, the native structure of prestin should be observed when it is so embedded in such bilayer. As for the study with AFM, proteins in the plasma membrane other than prestin hindered high-resolution imaging of prestin. To resolve those problems, prestin should be purified and reconstituted into the lipid bilayer, followed by its observation. Thus, in the present study, an attempt was made to reconstitute prestin which was purified from CHO cells stably expressing prestin into an artificial lipid bilayer and to observe it by AFM. Such reconstitution was performed after the method of Mihiet et al. in 2006. The lipid bilayer was formed on a freshly cleaved mica disk by the deposition of small unilamellar lipid vesicles. Such bilayer was destabilized by incubation with a detergent. Afterward, prestin purified from CHO cells stably expressing prestin was added to the destabilized lipid bilayer. Extensive rinsing then removed the excess prestin. Finally, a high resolution image of the prestin-reconstituted lipid bilayer was acquired by AFM. As a result, densely embedded prestin was visualized. From the obtained image, prestin was found to form a ring-like structure with a diameter of about 11 nm.

581 Molecular Determinants of Electromotility and Anion Transport Identified by Domain Swapping Between Mammalian and Non-Mammalian Prestin

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We have recently shown that non-mammalian prestin (SLC26A5) orthologs are electrogenic anion transporters that stoichiometrically exchange chloride for divalent anions. In contrast to mammalian prestin, however, they lack electromotility-associated fast charge movement and are not capable of generating voltage-driven membrane motility.

Because electromotility by prestin depends on anions, we hypothesized that the molecular events underlying generation of motility by prestin derived from the anion exchange mechanism of SLC26A5 transporters present in pre-mammalian ancestors. In other words, we suggest that electromotility and anion transport are evolutionary homologous processes and are thus mechanistically related.

Although functionally divergent, mammalian and non-mammalian isoforms feature a reasonably high degree of amino acid sequence conservation. This allowed us to generate chimeric SLC26A5 proteins with various sequence stretches transplanted between orthologs in order to identify the motifs that confer electromotile and transport capabilities to prestin.

Transfer of the cytoplasmic N- and C-termini from rat prestin to zebrafish prestin or vice versa did not alter functional properties of the recipient protein. Thus, functionality originates from the transmembrane core region of prestin, as suggested previously.

Extensive exchange of parts of the core region led to the identification of two narrow sequence stretches that, when transplanted from rat prestin into the zebrafish backbone, conferred fast charge movement (non-linear capacitance) to the non-mammalian SLC26A5. Moreover, this 'gain-of-function' chimera generated electromotility, as measured in outer hair cells from prestin knock-out mice expressing the chimera by viral transduction. Surprisingly, this chimeric prestin was still a functional anion transporter, indicating that abolishment of electrogenic anion exchange is not a prerequisite for the emergence of electromotility. Equivalently, transferring circumscribed parts of zebrafish SLC26A5 onto the mammalian ortholog enabled electrogenic divalent anion transport.

Thus, minor changes in amino acid sequence can convert an SLC26A5 anion exchanger into a voltage-driven membrane motor and vice versa.

582 Effects of MβCD Are Mediated Via G Protein Related Pathway

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[Introduction] Cholesterol is an essential composition of cell membranes, and determines their rigidity and fluidity. Our preliminary studies showed that MβCD changed stiffness, capacitance and motility of the outer hair cells (OHCs). These results suggest that the reconstruction of the cytoskeleton may be induced by MβCD. In this study, intracellular signaling pathways involving G proteins were studied to determine whether they modulate the effects induced by MβCD.

[Method] OHCs were isolated from cochleae of guinea pigs. They were patch clamped by whole-cell mode. One group of OHCs were treated with 100uM Guanosine 5′-O-(3-thiotriphosphate) tetralithium salt (GTPγS); the GTP analog, into the cell via patch pipettes and the other group had internal perfusion buffer only as a control. External perfusion buffer was delivered around the cells continuously (1 ml/min). After 7 minutes, 1 mM MβCD was delivered externally and electrical stimulation was administered (from -150 to 150 mV/0.2 msec) every 1 minute.

[Results] Application of 1 mM MβCD shifted non-linear capacitance curves of the control group with reducing the peak capacitance (C_{max}). After 10 minutes application, shifts of voltage at C_{max} (V_{pkcm}) and C_{max} were 74.95±13.37 mV and -9.09±2.10 pF respectively (n=4). On the other hand, in the GTPγS treated group, the shift of V_{pkcm} and reduction of C_{max} were attenuated. The shift of V_{pkcm} and reduction of C_{max} after 10 minutes were 11.17±12.97 mV and -3.08±1.91 pF respectively (n=7). MβCD decreased the cell length 16.53±4.27% in control and 6.45±6.22% in GTPγS, respectively.
[Discussion] This study demonstrated that internally perfused G protein reduced the MβCD effects on capacitance and cell length, suggesting that G proteins may be involved in the MβCD mediated pathway. More study is required to clarify the detailed role of G proteins in the relation between the cholesterol and the OHC cytoskeleton.

583 Oligomerization in the Slc26 Family

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Prestin-driven somatic motility is fundamental to cochlear amplification. Prestin, part of the solute carrier 26 (Slc26) family, acts as a motor protein in the outer hair cell basolateral membrane. The other members of the Slc26 family, however, are anion transporters rather than motor proteins. Recent reports suggest that prestin monomers form a homo-oligomer, but it is not known whether this property is exclusive to prestin or shared by other Slc26 anion transporters. Our bioinformatic analysis indicates that mammalian prestin's most distant Slc26 relative is Slc26a11. Using a Foerster resonance energy transfer (FRET) technique, HEK cells transfected with either prestin/prestin or Slc26a11/Slc26a11 fluorescent protein-conjugated donor-acceptor FRET pairs showed a significant increase in FRET efficiency over donor alone. When cells were transfected with a prestin/Slc26a11 FRET pair, however, no significant increase in FRET efficiency was detected. This evidence indicates that homo-oligomerization is conserved in the Slc26 family and is not unique to prestin, but the structures underlying oligomerization are sufficiently different that hetero-oligomerization cannot occur.

584 Anthracene-9-Carboxylic Acid, a Chloride-Channel Blocker, Reversibly Reduces the Non-Linear Capacitance of Outer Hair Cells

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Anthracene-9-carboxylic acid (9-AC) is a voltage-dependent chloride-channel blocker (Kawasaki 1999), which reduces the displacement amplitude of the organ of Corti evoked by electrical stimulation of outer hair cells (OHCs) (Nowotny and Gummer 2006; Scherer and Gummer 2004) and also reduces the axial stiffness of the isolated OHC (Eckrich et al., 2008, Abstract 517). To examine whether this substance might be acting on the motor protein prestin, we measured the non-linear capacitance of isolated OHCs.
Measurements were made using the patch-clamp technique in the whole-cell configuration. The intracellular and extracellular chloride concentrations were set to 140 mM. The absolute electrical admittance parameters of the OHC were determined using the Lindau-Neher algorithm with the *LockIn* extension of the PULSE software and EPC9 patch-clamp amplifier system. The voltage-dependent membrane capacitance data were fitted with the sum of a linear component and a non-linear component, which was given as the sum of the first derivative of the two-state Boltzmann function and a sigmoidal function (Santos-Sacchi and Navarrete 2002). Application of 500 µM 9-AC from a fluid-jet capillary positioned at a distance of 100 µm from the basolateral wall significantly reduced the peak non-linear capacitance by 22 ± 6 %, without significantly changing the voltage at half amplitude and without changing the effective valence; the peak charge reduction was 26 ± 6 %. Peak amplitude and charge transfer returned to normal after washout, 92 ± 3 % and 98 ± 3 %, respectively. The presence of 500 µM 9-AC in the patch pipette solution did not significantly influence the blocking effect of the extracellularly applied drug.
These data suggest that 9-AC blocks the motor complex of OHCs.

585 Prestin Undergoes Thickness Changes

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Prestin, the membrane protein responsible for electromotility of outer hair cells, has a larger membrane area at a negative membrane potential than a more positive membrane potential. If we assume that the volume of prestin is conserved during conformational transitions, the conformation with larger membrane area (extended state) must have a thinner profile in the membrane.
Phosphatidylcholines (PCs), including PC12:0, PC13:0,PC16:1, and PC24:1, were incorporated into the plasma membrane of HEK cells which is transfected with plasmid encoding prestin.The membrane capacitance was monitored under the whole-cell recording configuration while PCs were incorporated to the plasma membrane using 5mM carboxyethyl-gamma-cyclodextrin as vehicle.PCs with short acyl chains increased the linear membrane capacitance by up to 20% and positively shifted the voltage dependence of prestin's nonlinear capacitance by as much as 120 mV for PC12:0. PCs with long acyl chains decreased the linear membrane capacitance by up to 10% and negatively shifted prestin's voltage dependence up to 20mV for PC24:1. Since the linear capacitance is proportional to S/d, where S is the membrane area and d is the thickness, we can interpret that PCs with short acyl chains decrease the bilayer thickness d and PCs with long chains increase it.
These observations therefore indicate that a reduction in bilayer thickness prefers the extended state of prestin whereas an increase in bilayer thickness prefers the compact state. Since the energy of hydrophobic mismatch

between a membrane protein and the lipid bilayer must be significant, these observations further indicate that the state with larger membrane area has thinner hydrophobic profile in the membrane.

586 Gentamicin-Induced Selective Inner Hair Cell Loss in Mouse Explant Cultures

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Mouse inner ear explant cultures have been widely used for in vitro ototoxicity studies. We have observed a novel phenomenon, in which selective inner hair cell death was induced in mouse inner ear explant cultures after gentamicin (GM) administration. Very few methods for induction of selective inner hair cell death have been reported, as compared to induction of outer hair cell death. In this study, we further investigated the effect of age and hair cell death in inner ear explant cultures.

Inner ear explants (cochlear duct, utricle, saccule, cristae ampullaris) were prepared from C57BL/6J mice at postnatal day 1 (P1) and P5. The explants were incubated either with or without GM, and harvested at 24 and 48 hours. The cells were labeled with Phalloidin and Hoechst 33342 and visualized under fluorescent microscopy.

In the cochlea, selective loss of inner hair cells without damage to the outer hair cells was observed in the P5 explants, with greater loss at 48 hours compared to 24 hours of GM incubation. In P1 explants, loss of both the inner and outer hair cells was observed, with inner hair cells being most sensitive and the outermost outer hair cell being the most resistant to GM toxicity.

In both P1 and P5 explants, the greatest hair cell loss was observed in the middle turn, whereas the basilar turn was least affected and the apical turn had intermediate cell loss.

In P1 explants, hair cell loss in the vestibular organs occurred in the ampullae and saccule at 48 hours. In P5 explants, the saccule and utricle showed hair cell loss at 48 hours, while the ampullae were unaffected.

Our results indicate that selective inner hair cell death induced by GM is dependent on age, and a time window for this novel phenomenon is around P5. The differences occurring in hair cell death between the different ages of mouse explant cultures can be used to identify molecules involved in GM ototoxicity and search for methods to prevent hair cell death in the inner ear.

587 Cochlear Hair Cells Exhibit Rapid, Mitochondria-Mediated Responses to Gentamicin Treatment

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We recently performed the first direct investigation of gentamicin (GM)-induced alterations in mouse cochlear hair cell metabolism using two-photon fluorescence excitation of the metabolic intermediate, reduced nicotinamide adenine dinucleotide (NADH). Basal turn inner hair cells (IHCs) and outer hair cells (OHCs) exhibited significant decreases in NADH fluorescence within 30 mins of GM treatment. This GM-induced decrease in NADH fluorescence was less in the middle turn and essentially absent in the apical turn. Consistent with the known ototoxicity of GM, the greatest change in NADH fluorescence occurred in basal turn OHCs. To elucidate the relevance of these observations to GM-induced hair cell loss, we first used an antibody against the mitochondrial redox chain protein COX IV, and quantitative fluorescence, to determine mitochondrial density (measured as fluorescence intensity divided by volume). OHCs at 60% and 80% of total cochlear length (measured from the apex) contained a significantly greater density of mitochondria than apical (20%) OHCs. Mitochondrial densities were also significantly greater in OHCs than IHCs at the more basal cochlear locations. We next examined GM-induced redistribution of the normally mitochondria-sequestered, pro-apoptotic protein apoptosis-inducing factor (AIF). AIF released into the cytoplasm after GM-induced mitochondrial stress was detected by antibody labeling and quantitative fluorescence. An apex to base increase in GM-induced anti-AIF concentration was observed in both OHCs and IHCs after 30 mins of GM exposure. This increase was significantly greater in OHCs than IHCs, consistent with their different eventual fates.

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588 Locating Outer Hair Cell Damage Using the Cochlear Microphonic

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The long-term goal of this research is to develop diagnostic techniques that locate the anatomical sites of lesion within the cochlea and auditory nerve that result in hearing loss. The cochlear microphonic (CM) is a physiologic signal that comes from the receptor currents of outer hair cells (OHCs) and may be useful in examining OHC integrity. However, when recorded from the round window, the CM is dominated by OHCs in the base of the cochlea. Additionally, high frequency stimuli create opposing OHC currents that result in vector summation at the recording electrode. These factors limit the ability to examine OHC integrity in apical regions of the cochlea and confound the overall interpretation of the CM. The purpose of this study was to evaluate a low-frequency stimulus and a high-pass masking technique to assess OHC function along the length of the cochlea.

CM recordings were obtained in Mongolian gerbils with a 733 Hz tone-burst presented at 80 dB SPL in the presence of high-pass noise (cutoffs between 0.4 and 45 kHz). Repeatability was assessed by acquiring recordings twice with a 20-minute break between sessions, followed by 20 minutes of exposure to either a 1 or 8 kHz pure tone presented at 100 dB SPL. After exposure, a final set of recordings was obtained.

Masker cutoff frequencies were converted into distances along the cochlea and the CM masked amplitude relative to the unmasked amplitude was plotted as a function of length to create a cumulative distribution function (CDF).

Pre-exposure CDF curves were sigmoidal in shape and saturated approximately 2 mm from the base. Post-exposure curves were also sigmoidal, but plateaued at regions farther from the base. This shift in the location of the plateau after damage indicates that a different population of OHCs contributed to the CM and that the low-frequency stimulus and masking technique may assess OHC function along the length of the cochlea.

589 Inhibition of JNK by SP600125 Protects Against TNFα-Induced Auditory Hair Cell Death by Regulating the Expression of *Bax* But Not *Bcl-2*

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Activation of c-Jun by c-Jun N-terminal kinase (JNK) has been associated with the initiation of apoptosis of auditory hair cells. SP600125 (a JNK inhibitor) can prevent phosphorylation of c-Jun by binding to and inhibiting activation of JNK signaling. SP600125 may play a role in preventing TNFα-induced auditory hair cell loss through interrupting pJNK/c-Jun interaction.

One hundred and twenty organ of Corti explants (OC) were dissected from three-day old rats and cultured in one of three conditions: 1) no treatment (control); 2) TNFα (2 µg/ml); or 3) TNFα (2 µg/ml) + SP600125 (10µM). Thirty six OC were cultured in the above conditions (n=12 OC/condition) for 4 DIV and subsequently fixed and stained with FITC-phalloidin for hair cell counting. The remaining 84 OC were cultured in one of the above three conditions for 0, 24, and 48 hrs (n=12 OC/condition) for RNA isolation and real time PCR using primers for *B-actin*, *Bax,* and *Bcl-2* genes.

Treatment of the TNFα exposed OC with SP600125 protected the auditory hair cells against TNFα's ototoxicity. The TNFα exposed OC demonstrated an up regulation of pro-apoptotic *Bax* expression that was inhibited by treatment with SP600125. There was a small increase in anti-apoptotic *Bcl-2* expression in TNFα treated OC, which was not significantly affected with the treatment with SP600125. The *Bax/Bcl-2* ratio was increased in TNFα damaged explants; however, there was a significant decline in this ratio following treatment with TNFα + SP600125 because of the reduction in *Bax* expression.

SP600125 can inhibit JNK and ultimately prevent downstream up regulation of pro-apoptotic *Bax* expression that is characteristic of TNFα damage to auditory hair cells *in vitro*. These results support the use of SP600125 as a treatment to prevent TNFα induced auditory hair cell loss following a trauma to the inner ear.

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590 Hair Cell-Specific MicroRNA Depletion Affects Hair Cell Maintenance and Causes Progressive Hearing Loss

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MicroRNAs (miRNAs) are small regulatory RNAs that function post-transcriptionally to regulate target gene expression. We have recently demonstrated the functional significance of small RNAs in development of the mouse inner ear using *Dicer* conditional knockout (CKO) to block miRNA production and function from E8.5 in the otic placode using *Pax2-Cre*. Sensory epithelia of CKO mice exhibit severe defects in morphohistogenesis, neurogenesis and innervation. Moreover, the extent of hair cell (HC) development correlates with residual expression of miR-183 family members (miR-183, miR-96, and miR-182), which are normally expressed in HCs and spiral ganglion cells and shows basal-apical expression gradients in the mature inner ear. To further examine HC miRNA expression and function, we generated HC-specific *Dicer* CKO from E14.5 using *Atoh1-Cre*. In this model, HC miRNA depletion is apparent by P18, and neither substantial HC loss or hearing deficits were observed at this age. However, Affymetrix microarray analysis of RNA isolated from apical versus basal organ of Corti demonstrates that expression profiles are longitudinally more disparate in the absence of HC miRNAs. These data suggest that HC miRNAs function to subdue rather than establish longitudinal expression gradients in the organ of Corti. By P28, the model shows significant basal HC loss and some aberrant stereocilia of HCs located in the apical turn of the cochlea. Moreover, CKO mice exhibit progressive hearing loss and diminished DPOAE responses by P28. Interestingly, the specificity of this *Dicer* CKO model results in less severe morphohistological defects and auditory deficits than other sensory epithelial-specific *Dicer* CKO models. Strikingly, the model also exhibits less severe consequences than germline mutation of one HC miRNA (miR-96) in the *Diminuendo* mouse. These data demonstrate that miRNAs are not only critical in the development of HCs, but are necessary for HC maintenance and survival.

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591 Expression of Phosphodiesterases in Hair Cells Modulating CAMP and CGMP Signaling

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A role for phosphodiesterases in regulating cAMP signaling in hair cells was originally suggested by the finding that rolipram, a specific inhibitor of cAMP-phosphodiesterase PDE4 which converts cAMP to AMP, was highly effective in elevating cAMP in a teleost model

hair cell preparation (Drescher et al., Soc. Neurosci. Abstr. 20: 969, 1994). Evidence has now been obtained that mRNA for phosphodiesterases PDE4D, as well as PDE6C, is expressed in these saccular hair cells. Degenerate primers in PCR yielded 45% of full-length amino acid sequence of PDE4D with 96% identity to zebrafish PDE4D (Accession No. XP_695374). Likewise, 36% of full-length sequence of PDE6C was obtained for teleost saccular hair cell cDNA with 72% aa identity to Danio rerio phosphodiesterase 6C (GenBank Accession No. NP_957165). Primers for PDE5 did not elicit amplification in PCR. PDE4D acts primarily on cAMP (K_m = 1.2-5.9 μM) whereas PDE6C is more sensitive to cGMP (K_m = 17 μM). PDE4D is predicted to regulate intracellular levels of cAMP at microdomain sites of adenylyl cyclase activity coupling to G-protein receptors. For saccular hair cells, such sites would exist at foci of putative efferent dopaminergic input. The components of the corresponding adenylyl cyclase pathway, i.e., AC5/6, Gαs/olf and dopamine D1A4 and D2 receptor, have been identified (Abu-Hamdan et al., Assoc. Res. Otolaryng. Abstr. 31: 215, 2008). PDE4D5 is reported to bind to RACK1, and RACK1 is a binding partner for HCN1 in saccular hair cells (Ramakrishnan et al., ARO Abstr. 30: 31, 2007). HCN1 (in addition to its stereociliary localization) is found on the hair cell basolateral membrane, consistent with sites of efferent input. In contrast, PDE6C is primarily known for its role in hydrolyzing cGMP in cone photoreceptor sensory transduction, regulating levels of cGMP available to CNGA3 channels. We hypothesize that PDE6C may also be coupled to CNGA3 in saccular hair cells.

592 Type XVII Collagen/the 180-KDa Bullous Pemphigoid Antigen Have a Role in Mechanosensory Hair Cell Development in Zebrafish

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The COL17A1 gene encodes type XVII collagen (known as the 180-kDa bullous pemphigoid antigen), an integral component of hemidesmosomes, attachment complexes providing integrity to the dermal-epidermal junction. We have identified two COL17A1 orthologues in the zebrafish genome, col17a1a and col17a1b, which are expressed in the skin and in the sensori-neural system, respectively. Zebrafish hair cells have ability to regenerate from damage whereas mammalian hair cells have not. If a peculiar gene of zebrafish hair cell differs from gene of mammalian hair cell, it can be one of candidate genes for making the difference of regeneration.

Zebrafish col17a1b was discovered by analysis of the online gene database (Ensemble Database). To characterize the expression of col17a1b, we performed in situ hybridization (inclusinding two color in situ hybridization) with a probe recognizing col17a1b sequence.

Subsequent injection of a morpholino corresponding to 5¡¯UTR of col17a1 was performed. Morpholino injected embryos were breed for 3 days. Those embryos were dyed YO-PRO-1 fluorescent dye and hair cells were analyzed. We performed immune-staining of neuromasts with acetylated ¥á-tubulin, a marker for hair cells and analyzed with confocal microscope. Neuromasts were also analyzed with scanning electron microscope (SEM).

In two color in situ hybridization with Atoh, Notch, and Gicerin, col17a1b revealed it as a hair cell gene and not the supporting cell gene.

Morpholino injected embryos did not reveal any change in gross morphology when examined at 72hpf and followed up to 5dpf. However, they demonstrated destructed neuromasts in YO-PRO-1 fluorescent examination. Conforcal microscope and SEM showed destructed hair cells and intact supporting cells in neuromasts.

COL17A1 in zebrafish appears to be required for mechanosensory hair cell development and it differs from mammalian auditory hair cell. Col17a1b can be a candidate gene which make hair cell regenerate and must be analyzed further.

593 A Study of Mitochondrial Populations in Vestibular Hair Cells and Implications for the Function of the Striated Organelle

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We used electron microscope tomography to reconstruct the apical region of type I hair cells. In type I cells, a group of large mitochondria appears to be restricted by the striated organelle (SO) to the subcuticular portion of the hair cell, suggesting that a highly energetic process occurs in this portion of the cell. The overall architecture of the apical region in type I hair cells – a striated structure (the SO) segregating a group of large mitochondria – resembles the arrangement of a fast contracting muscle (Franzini-Armstrong, 2007). This suggests that, in type I hair cells, the subcuticular mitochondria are part of a functional complex that includes the hair bundle, cuticular plate and SO. In type II hair cells, mitochondria are associated with the SO but they are not unusually large and they are not restricted to a particular portion of the cell. We measured the volumes and surface areas of the subcuticular mitochondria in hair cells and found that they vary with location of the hair cell (central vs. peripheral) within the sensory epithelium and with hair cell type. Overall, they are very large compared with mitochondria in type II hair cells. We will present a structural data analysis for several mitochondrial populations in type I and type II hair cells from different locations and discuss possible implications for SO and hair cell function.

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594 Creating a Hair Cell-Specific Flp Transgenic Mouse

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Atoh1 is a transcription factor that is required for differentiation of hair cells in the inner ear and granule cells

in the cerebellum. This gene is expressed in both tissues during embryonic development and is down-regulated by postnatal day 6-7 (P6-7). We aimed to create transgenic mice that express Flp, a site-specific recombinase derived from yeast, specifically in cochlear hair cells and cerebellar granule cells of neonatal mice. Similar to the Cre-loxP system, Flp is able to recognize two FRT sites resulting in genetic recombination for the gene of interest. Here we used a construct that contains the well characterized Atoh1 enhancer followed by Flp-IRES-hPLAP-SV40 Intron-poly A. With the internal ribosome entry site (IRES)-human placental alkaline phosphatase (hPLAP) gene in the construct, the Flp transgene expression can be visualized through histochemical analysis (Chow et al., Dev. Dyn. 2006). We have obtained ten transgenic founder mice, all of which resulted in germline transmission. We are characterizing these transgenic mice using the hPLAP staining and further crossing them into Flp reporter mice to examine Flp activity in inner ear hair cells and cerebellar granule cells after birth. These Flp mice will serve as valuable resource and can be used in combination with other existing hair cell specific Cre or CreER mice for genetic manipulation of genes in neonatal inner ear hair cells.

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595 Generation of Mice That Specifically Express Inducible Cre Recombinase in Developing and Adult Outer Hair Cells

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The inducible Cre-loxP system has been successful in manipulating gene expression specifically in neonatal mouse cochlear hair cells. We have shown that Atoh1-CreER (Cre recombinase fused with estrogen receptor or ER) transgenic mice can specifically express inducible Cre activity in both cochlear inner and outer hair cells when induced with tamoxifen at postnatal day 0-1 (P0-1). However, Atoh1-CreER is down-regulated after P7 in cochlear hair cells. Here we aim to generate mice that specifically express inducible Cre recombinase in outer hair cells (OHCs) at postnatal and adult ages. Prestin is a specific OHC marker that is first turned on after birth and maintained at a high level in adults. We generated a prestin-CreER knockin mouse where an internal ribosome entry site (IRES)-CreER cassette is inserted into the prestin locus after the stop codon. We have obtained germline transmission from 3 independent recombinant ES clones. We are currently characterizing the inducible Cre activity and distribution by crossing with Rosa26-eYFP reporter mice. These prestin-CreER mice will be valuable for examining gene functions in OHCs, particularly in cochlear amplification.

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596 Dexamethasone Inhibits IL-1beta - Induced MMP-9 Expression in a Cochlear Cell Model

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Objectives;Several cytokines, especially IL-1beta, have been found in a high percentage of otitis media with effusion. To investigate the effect of IL-1beta on MMP-9 expression in cochlea and the molecular and signaling mechanisms involved. Materials and Methods: HEI-OC1 (House Ear Institute-Organ of Corti 1) cells were used and exposed to IL-1beta with/without dexamethasone (Dexa). Glucocorticoid receptor (GR) antagonist, RU486, was used to see the role of dexamethasone. Also PD98059 (an ERKs inhibitor), SB203580 (a p38 MAPK inhibitor), SP600125 (a JNK inhibitor) were used to see the role of JNKs, ERKs, and/or p38 MAPK signaling pathway(s) in MMP-9 expression in response to IL-1beta in HEI-OC1 cells. RT-PCR and gelatin zymography were used to measure mRNA expression level of MMP-9 and activity of MMP-9, respectively.

Results: Treatment with IL-1beta resulted in concentration-dependent increase of MMP-9 protein. IL-1beta(5ng/ml)-induced MMP-9 expression was inhibited by Dexa. Interestingly, p38 MAPK inhibitor, SB203580, significantly inhibits IL-1b-induced MMP-9 mRNA and MMP-9 activity. However, inhibition of JNKs and ERKs by SP29004 and PD98059, respectively, had no effect on the IL-1beta-induced MMP-9 expression.

Conclusion: These results suggest that the pro-inflammatory cytokine IL-1beta strongly induces MMP-9 expression in HEI-OC1 cells and the induction seems to be inhibited by Dexa and activation of p38 MAPK signaling pathway

597 Outer and Inner Hair Cells Are Metabolically Dissimilar

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Outer hair cells (OHCs) are more vulnerable than inner hair cells (IHCs) to a variety of injury mechanisms, including loud sound, aminoglycoside antibiotics, the anti-proliferative agent cisplatin, and aging. This observation suggests that OHCs are metabolically different from IHCs in some fundamental way, but no direct evidence addresses this question. To test for putative metabolic differences between the two cells types, we used fluorescence lifetime imaging (FLIM) of the metabolic intermediate reduced nicotinamide adenine dinucleotide (NADH) in an explanted but intact mouse cochlea. The significance of fluorophore lifetime, in contrast to fluorescence intensity, is that increased lifetime may indicate a transition of the fluorophore from free to bound pools. NADH fluorescence lifetimes in hair cells were found to be organized into populations of from 400 ps to more than 5000 ps, some of which were common to the two cell types. IHCs and OHCs also showed lifetime unique lifetime populations and fractions of NADH in each

lifetime population were distinctly different. When the preparation was challenged by the metabolic poisons cyanide and FCCP, the distributions of NADH lifetimes by population in IHCs and OHCs were observed to change in different ways. Thus, our evidence suggests, not only that there are innate differences in the metabolism of IHCs and OHCs, but also that there are important dissimilarities between their responses to metabolic challenges that may relate to their differential vulnerabilities.

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598 Characterization and Morphological Analysis of Targeted Deletion of Oncomodulin in the Inner Ear

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The tight regulation of Ca^{2+} is essential for cochlear function, and yet the role of Ca^{2+} binding proteins remains elusive. While most Ca^{2+} binding proteins are found extensively throughout the nervous system, oncomodulin (Ocm), a member of the parvalbumin family, has a restricted expression pattern. Recent studies find differences in the subcellular localization and distribution of Ocm in outer hair cells and striolar hair cells. In both outer hair cells and striolar hair cells, however, Ocm is also found in the hair bundle.

We constructed a targeting vector to knockout the Ocm gene (exons 2 - 4) conditionally and generated three Ocm conditional knockout mouse models to determine the effect of deleting the Ocm gene at different developmental periods. In a companion study, we show preliminary results that embryonic but not postnatal targeted deletion of Ocm results in significantly elevated DPOAEs and ABRs. A preliminary analysis of the *β-actin-Cre;Ocm-/-*, *Pax2-Cre;Ocm-/-*, and *Prestin-Cre;Ocm-/-* mice reveal no obvious histopathologies in young adult mutants. They have normal looking inner ears, sensory epithelia, and no obvious hair cell loss in homozygous mutants compared with controls. Both cochlear outer hair cells and saccular striolar hair cells demonstrated an absence of Ocm immunoreactivity in *β-actin-Cre;Ocm-/-*, *Pax2-Cre;Ocm-/-*, and *Prestin-Cre;Ocm-/-* ears compared to wild type littermates. Immunolabeling for Ca^{2+} binding proteins such as calretinin appeared normal across all mutant ears. RT-PCR analysis is consistent with the immunocytochemistry.

Although targeted deletion of Ocm can result in profound deafness, it does not lead to any obvious histopathology or significant changes in the levels of Ca^{2+}- binding proteins in the young adult mouse ear.

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599 Oncomodulin Is Necessary for Normal Cochlear Development But Not Postnatal Function

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Oncomodulin (Ocm), a member of the parvalbumin family of calcium binding proteins, is present in outer hiar cells in the basolateral wall and the hair bundle, suggesting a role in either mechanoelectric transduction or electromotility. To study the role of oncomodulin in the cochlea, we generated conditional Cre-lox knockout lines with Cre-recombinase driven by three different promoters expressed in outer hair cells at different developmental periods: actin (E1), Pax2 (E12) or prestin (P6). In all conditional lines, the absence of Ocm expression was confirmed with immunocytochemistry.

Cochlear function was tested in mutant and wild-type littermates from each line at 5 - 6 weeks. Actin-Cre;Ocmflox mutants showed no distortion product otoacoustic emissions (DPOAEs) and auditory brainstem response (ABR) threshold shifts of 70 - 80 dB. Pax2-Cre;Ocmflox mutants showed slightly more robust cochlear responses: small DPOAEs were measurable at low frequencies and ABR threshold shifts ranged from 30 dB at low frequencies to 70 dB at high frequencies. In contrast, the Prestin-Cre;Ocmflox mice showed normal DPOAEs and ABRs at all frequencies.

These results suggest that embryonic expression of Ocm is essential to cochlear function, whereas, deleting Ocm after the onset of prestin expression, after P6, has no measurable effect on cochlear responses.

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600 Two-way Effects of Fractalkine in the Damage or Survival of Hair Cells in the Corti¡¯s Organ

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Fractalkine, as an exclusive ligand for CX3CR1 expressed on microglia, has recently been reported to be released out by neurons, and induce microglial activation as a neuron-to-glia signal in the rodent brain. However, the exact role of this chemkine was not determined in the auditory nervous system. Our preliminary data reported that microglia-like cells were presented in the Corti¡¯s organ. Then, we demonstrated that the microglia-like cells were OX-42 immunoreactive and they were activated in the inner ear following neomycin administration. In our recent in vivo studies, the increased level of CX3CR1 but not fractalkine in the inner ear of mice was observed by real-time PCR. On the other hand, CX3CR1 expression were increased and co-localized with OX-42 positive cells (microglia-like cells) in the Corti¡¯s organ compared with control group after injury. During in vitro studies, the basilar membranes cultured with neomycin were examined by

immunohistochemistry. The number of hair cells was decreased. However, fractalkine completely co-localized with myosinVIIA, a hair cell marker, and its immunoreactivity dramatically increased in the survival hair cells, while there was few OX-42 or CX3CR1 positive cells on the cultured organ. Furthermore, when the basilar membrane cultured with different concentrations of exogenous fractalkine, the lower dose promote the survival of hair cells, however, the higher induced cells death. These results suggest two-way effects of fractalkine in the damage or survival of hair cells and fractalkine-to-CX3CR1 signal may be involved in neomycin induced hearing loss.

601 Depolymerization of F-Actin Produces a Pulling Force at the Plasma Membrane in Vivo

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We report that depolymerization of F-actin filaments produces a pulling force on the plasma membrane as predicted by calculations based upon energetics. We do this by monitoring the axial membrane force produced upon forming a long (> 15 μm) membrane tube filled with an actin bundle formed from a mammalian cell. The filopodium is formed with an optical trap which is also used to measure the force. We observe a dynamic sawtooth force riding atop the equilibrium force which increases slowly (10 s of seconds), stalls and decays rapidly back (ms) to equilibrium. Examination of the magnitude and time course of the force shows that the rise and decay of the axial membrane force is due to depolymerization and polymerization of F-actin at the barbed end of the bundle. From the magnitude of the force we determine the number of filaments (< 20) within the bundle, and establish that the on and off rate decays exponentially with the axial membrane load exhibiting a length constant of ≈3 nm. We determine the on and off rates of G-actin at the barbed end and calculate that a filament produces a pushing and pulling force of 4 to 5 pN upon polymerization and de-polymerization. Cooperativity within the filaments of the bundle is observed; the load is borne by > 1 filament. The results are discussed in relation to early development of stereocilia which protrude at the plasma membrane of the hair cell by actin polymerization similar to that observed in filopodia.

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602 Detection System for Transplanted Bone Marrow Stem Cells in Inner Ear by SPIO

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Damage to sensory hair cells in the mammalian cochlea results in hearing loss and sets in place a number of irreversible changes, which eventually result in the progressive degeneration of auditory neurons. Although human application of regeneration therapy has been subsequently developed in other tissues, it has not been established the method to monitor the biological dynamics of transplanted cells non-invasively in vivo. Hence, superparamagnetic iron oxide (SPIO) nanoparticles have demonstrated their utility as an important tool for enhancing magnetic resonance contrast, allowing researchers to monitor not only anatomical changes, but physiological and molecular changes as well. We therefore examined both the effectiveness of cell labeling in vitro and the expression in the cochlea after transplanting bone marrow stem cells labeled by SPIO in vivo.

603 The Examination of Monitoring the Engraftment of Transplanted SPIO Labeling Stem Cells in the Inner Ear on 1.5T-MRI

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Growing evidence from preclinical and clinical studies suggests that stem cell therapy may provide a viable therapeutic alternative to restore the target organ. Stem cells have the capacity to give rise to any cell type in the body, and the fate of their progeny is determined by the microenvironment in which the stem cells reside. Thus, it has been proposed that if intrinsic stem cells in the inner ear could be activated or if extrinsic stem cells could be transplanted into the ear, they could produce new hair cells or neurons to replace those that have been lost. Recently, magnetic resonance imaging (MRI) using superparamagnetic iron oxide (SPIO) cell labeling techniques is emerging as the main diagnostic modality to track the transplanted stem cells. Therefore, we will make a report after conducting an examination of monitoring the engraftment of transplanted SPIO labeling stem cells in the inner ear on 1.5T MRI, and we can assess the localization, quantification, and viability of transplanted human mensenchymal bone marrow stem cells in vivo. Stem cells were magnetically labeled with SPIO, and then we transplanted SPIO labeling cells by directly injection to the scala tympani of the guinea pigs and performed at clinical 1.5T-MRI device (General Electric Medical Systems) to track the cells at 3 hours, 2 weeks, and 4 weeks after transplantation. Scanning was performed using a balanced steady-state free precession (SSFP) sequence (3D-FIESTA). MRI demonstrated a clear low signal area in the basal turn of cochlea. Similarly, morphological study showed that the Berlin blue positive cells located on basal turn at 4 weeks after transplantation. In conclusion, both the low signals of 3D-FIESTA MRI and enfragments of transplanted stem cells were observed until 4 weeks after transplantation. It may be feasible to magnetically label and visualize SPIO labeling stem cells. MR visualization of magnetically labeled may be a valuable tools for in vivo tracking of stem cells.

604 Supporting Cell Survival in Temporal Bones of Patients with Known Hearing Loss

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Multiple investigators are attempting to examine the potential role of mammalian inner ear supporting cells as targets for regenerative therapies. However, no studies to date have examined whether supporting cells survive in humans after insults to the inner ear that result in hair cell loss and subsequent hearing loss. Thus, the objective of this study was to examine supporting cell survival in temporal bones obtained from patients with documented severe-to-profound sensorineural hearing loss. Histopathological review of archival temporal bone specimens obtained from the House Ear Institute and the Massachusetts Eye and Ear Infirmary was performed. These archival temporal bone specimens included patients who were noted to have hearing loss in the following categories: aminoglycoside-induced ototoxicity and sudden sensorineural hearing loss (SSNHL). The analysis specifically targeted the identification of regions along the cochlea where supporting cells existed in the absence hair cells. Analysis included a position-based quantification of supporting cells and hair cells along the entire length of the cochlea. 63% of all patients examined exhibited regions of supporting cell survival in the absence of hair cells. All otoxocity-induced hearing loss patients had evidence of supporting cell survival in regions of hair cell loss. Furthermore, 12 (52%) of our SSNHL patients also exhibited regions of supporting cell survival. However, it is important to note that 9 patients (75%) of this group was also noted to have other pathological lesions that could account for their hearing deficit. Histopathological analysis of supporting cell presence was confirmed with proteomic confirmation of supporting cell markers from microdissected histopathologically verified supporting cells. These data show that supporting cells survive in regions of hair cell loss after a variety of insults in humans. This suggests that, in certain patients, supporting cells are a potential target for hair cell regeneration.

605 Proliferative Activity of IPS Cells After Transplantation Into the Cochlea Varies Among Cell Lines

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Several iPS cells derived from various sources were generated using different combinations of reprogramming factors and different methods of gene transfer. Recently, large variations in the risk of tumorigenesis among iPS cell lines have been demonstrated (Miura et al., Nat Biotechnol 2009). We have reported that iPS cells can be a source of transplants for regeneration of auditory spiral ganglion neurons (Nishimura et al., Neuroreport 2009). In the present study, we examined differences in proliferative activity among iPS cell lines following transplantation into the cochlea. Mouse iPS and ES cells were neurally induced by the stromal cell-derived inducing activity (SDIA) method (Kawasaki et al., Neuron 2000), and then were transplanted into the cochlea of neonatal mice. For iPS cells, three different cell lines were used. We evaluated the survival, proliferative activity of transplanted cells 4 weeks after transplantation. The survival of transplanted iPS or ES cell-derived cells was identified. No significant difference was found in the localization of transplants among cell lines, while proliferative activity varied among cell lines. What mattered in proliferative activity was not the difference in gene transfer methods or transfected reprogramming factors, but the difference in a source of iPS cells. Teratoma formation was identified in one cochlea in which iPS cells that were derived from adult tail-tip fibroblasts were transplanted. In conclusion, selection of appropriate iPS cell lines is critical for avoiding tumorigenesis.

606 Transplantation of Bone Marrow Mesenchymal Stem Cells Into Neonatal, Adult and Aged Mouse Cochlea

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Recently, a number of clinical studies for cell therapy have been reported and clinically used for several intractable diseases. Inner ear cell therapy for sensorineural hearing loss also has been studied using some laboratory animals, although the successful reports for the hearing recovery are still few. We previously reported that mesenchymal stem cell (MSC) transplantation accelerates hearing recovery through the repair of injured cochlear fibrocytes (Kamiya et al.Am.J. Pathol. 2007).

MSCs are multipotent cells that can be isolated from adult bone marrow and can be induced to differentiate into a variety of tissues in vitro and in vivo. It has been expected that the new cell therapy using MSC transplantation is established also for sensorineural hearing loss.

In the present study, we transplanted MSCs stably expressing EGFP into the posterior semicircular canal of neonatal (10 days), adult (8-10 weeks) and aged (over 5 months) by using perilymphatic perfusion..Auditory functions were monitored by thresholds of auditory brain stem responses (ABR), that was recorded before surgery, 4 and 7 days after surgery.

The cochlea morphology and transplanted MSC were observed by the immunolabeling with the antibody for GFP and Myo7a.

A number of these stem cells were detected in cochlear and vestibular tissue. The elevation of ABR threshold after the operation was less than 20 dB.

607 Intracochlear Injection of Adeno-Associate Virus Vector to a Mouse Model Created by a Conditional Knockout of Gjb2 Gene

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Hereditary deafness affects about 1 in 2,000 children and mutations in the GJB2 gene are the major cause in various ethnic groups. In order to establish the fundamental therapy of congenital deafness, we generated targeted disruption of Gjb2 using Cre recombinase controlled by P0. Using this animal model, we examined the potential of gene therapy in the inner ear, using the homozygous mutant mice and the heterozygous mutant mice.

Adeno-associated virus vectors carrying the gap junction beta 2 protein (Gjb2) gene were injected into the scala tympani through the round window of the cochlea of the homozygous mutant mice. The expression of Cx26 was not seen in the supporting cells and did not improve the hearing ability.

We succeed in gene introduction to the supporting cells of neonatal mice without hearing loss using adeno-associated virus vectors (2008, Iizuka T, et al.). We are going to introduce this virus into the Gjb2 knockout mouse in future to cure hereditary deafness.

608 Differential Regulation of Connexin Expression in the Regenerating Avian Inner Ear

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Supporting cells in the vertebrate inner ear are extensively coupled via gap junctions. Gap junctions play an important role in the maintenance of tissue homeostasis and their constituent proteins, connexins, have been implicated in the coordination of cell proliferation, migration and differentiation in both a gap-junction dependent and independent manner. These cellular processes are often associated with changes in the pattern and level of connexin expression and in their post-translational modification. Here, we examined the regulation of chicken connexin 30 (cCx30) and cCx43 in the sensory epithelia of hatchling chicks in response to ototoxic damage. Using a combination of immunohistochemistry and real-time RT-PCR, we show that cCx30 and cCx43 are differentially regulated in response to gentamicin-induced hair-cell loss in vitro and that the modulation of their expression accompanies key stages during repair and regeneration of the sensory epithelium in the avian inner ear. Coinciding with the extrusion of hair cells and the repair of the lesions, cCx43- protein levels rapidly decreased in supporting cells. However, no significant changes in its mRNA levels were observed. This suggests that cCx43 expression is regulated predominantly on a post-translational level. In contrast, cCx30-mRNA levels significantly increased, particularly around the time of maximum supporting cell proliferation. Although the inhibition of gap-junctional communication by pharmaceutical agents reduced the number of EdU-positive supporting cells in the damaged chick inner ear, a positive correlation between cCx30 and supporting cell proliferation remains to be determined.

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609 Gene Expression Profiling of Supporting Cells in the Chick Inner Ear

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Inner ear disturbances caused by ototoxic drugs, aging and several diseases originate mainly from loss of hair cells. In mammals, hair cell loss leads to a permanent deficit of hearing and balance. In contrast, birds and other vertebrates can spontaneously regenerate lost hair cells through differentiation of supporting cells and restore auditory and vestibular function. Hair cell progenitors are thus thought to reside in a population of supporting cells.

In the present study, we sought to identify novel genes related to inner ear regeneration by gene expression profiling of supporting cells in chick utiricle. Gene expression of supporting and hair cells prepared by Laser capture microdissection (LCM) was analyzed by using Affymetrix GeneChip Chicken Genome Array and Gene spring GX. The condition tree of each sample data after quality control demonstrated a validation for our method. The volcano plot (fold change 2, p=0.05, MTC: benjamini and hochberg) revealed 408 genes, and 175 genes were well annotated. Among these genes, we focused particularly on Musashi1 (Msi1), a neural stem cell marker gene and its related genes. Immunohistchemistry analyses demonstrated that Msi1 mainly locates at the basal side of supporting cells of 14 day undamaged chick utricle. In aminoglycoside sulfate damaged models of chick utricle, gene expression of Msi1 and HES5 was up-regulated during the regenerating time. Msi1 may facilitate the proliferation of adult stem cells by repression of translation of Numb and p21CIP1.

These findings suggest that Msi1 plays, at least in part, a critical role in hair cell regeneration in both stable and damaged conditions of inner ear.

610 Identification of Genetic Regulators of Transdifferentiation in Cochlear Sensory Epithelia for Hearing Restoration

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It is estimated that 28 million Americans suffer from hearing loss. The most common cause of hearing loss is damage of the mechanosensory receptor hair cells within the cochlear sensory epithelium. Hair cells can be damaged by ototoxic drugs, acoustic trauma, disease, and

the effects of aging. In mammals, damage to the hair cells is irreversible. Non-mammalian vertebrates however, posses glia-like supporting cells, that in response to hair cell damage, are capable of either proliferating to generate new hair cells or directly changing fate into hair cells in a process called transdifferentiation.

Regeneration in lower vertebrates, through this combination of proliferation and transdifferentiation, is sufficient to restore function to the sensory epithelium and thus restore hearing. Although mammals retain similar types of supporting cells, they do not proliferate and generate hair cells in response to hair cell damage. Recent studies have shown, however, that cochlear supporting cells in the mouse do posses the ability to transdifferentiate into hair cells during the early postnatal period, under the right conditions. However, the plasticity of mammalian supporting cells is limited to developmental periods, as these cells abruptly lose the capacity to transdifferentiate into hair cells as they age beyond postnatal day 3 (P3). In this study, we attempt to elucidate the genetic regulators that allow mammalian supporting cells to transdifferentiate prior to P3 but limit the plasticity of these cells at later ages. We used Hes5-GFP transgenic mice and fluorescence-activated cell sorting to identify and collect a purified population of supporting cells from cochlear epithelia at P0 and P5. From these two purified supporting cell populations we extracted RNA and performed a microarray expression analysis to compare the gene expression profiles at these two postnatal periods between which the supporting cells lose the capacity to transdifferentiate into hair cells. Results from the microarray analysis will be presented. Further characterization of the identified genetic regulators will provide targets for therapeutic manipulation which may result in conferring support cells the ability to transdifferentiate into hair cells and restore audition to hearing impaired patients.

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611 Combinatorial Transcription Factor Coding to Enhance Transdifferentiation Into Hair Cells in the Organ of Corti

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Current evidence suggests that cellular specificity of gene expression and function can result from combinatorial coding by multiple transcription factors (TFs). Because Atoh1 can induce a hair cell (HC) fate in nonsensory inner ear cells, but specifies alternative fates in many other cell populations, we determined whether the presence of additional TFs might alter the effects of induced Atoh1 expression on nonsensory inner ear cells. We chose TFs for which binding sites are present on an enhancer in the *pou4f3* gene that directs gene expression to HCs and that contains binding sites for Atoh1. Electroporation was used to transfect cells of the greater epithelial ridge (GER) of P1.5 organ of Corti (oC) explants, from transgenic mice in which expression of GFP is driven by *pou4f3* upstream DNA that contains the enhancer. Plasmids encoding a constitutive promoter driving human Atoh1 (hAtoh1), hE47, hSP1 or hGATA3 were used singly or in combination at 0.5 µg/µl. The expression of GFP and myosin VIIA were monitored in the GER 5 days after transfection. When the entire length of the oC was considered, co-transfection of hAtoh1 with either hE47 or hGATA3 induced 2-3X more GFP+ cells than hAtoh1 alone ($p < .01$). Co-transfection of hAtoh1 plus hE47 plus hGATA3 did not induce more GFP+ cells than the double TF combinations, and hSP1 did not enhance the effects of hAtoh1. Similarly, co-transfection of hAtoh1 with either hE47 or hGATA3 induced 2-3 fold more cells positive for myosin VIIA than hAtoh1 alone, with a stronger response in the cochlear apex. However, in the middle turn only the combination of hAtoh1 plus hE47 plus hGATA3 induced more myosin VIIA+ cells than hAtoh1. The results suggest that Atoh1 can act in a combinatorial fashion with E47 or GATA3 to induce *pou4f3* expression and a HC phenotype in nonsensory cells of the GER.

612 Guiding Neuronal Subtypes: BDNF Enhances Expression of Auditory Nerve-Associated Channels in Differentiating Neurog1-Induced Mouse ES Cells

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A mouse embryonic stem (ES) cell line containing an inducible Neurogenin-1 (Neurog1) transgene differentiated into glutamatergic neurons with auditory nerve-like neurotrophic factor (NTF) receptor expression. Application of brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) significantly enhanced survival of ES cell-derived neurons and further-promoted their differentiation into the target phenotype. This pluripotent cell line provides an interesting in vitro framework to study the effects of exogenous factors on sensory gangliogenesis downstream of Neurog1. Electrophysiological characterization of induced cells found Neurog1 rapidly captured a functional neuronal phenotype, with cells exhibiting fast adaptation to current-clamp stimuli. This feature is similarly found in basal SGN, which process high frequency acoustics. Neurog1-induced cells also highly upregulated the expression of voltage-gated potassium and sodium channels known play important roles in the specialized firing patterns of SGN. In this study we examined whether application of BDNF + GDNF, BDNF, or NT-3 during Neurog1 induction in differentiating mouse ES cells could further promote the SGN subtype by influencing different ensembles of ion channels. Quantitative real-time PCR revealed at 2 DIV BDNF alone and in combination with GDNF, but not NT-3, brought about earlier expression of the voltage-gated sodium channel Nav1.6 and the SGN-associated potassium channels Kv3.1 and Kv4.2. At 2 and 3 days in vitro, BDNF also elevated expression of the DRG-associated channel Kv1.4. These results demonstrate that SGN-associated channels can be selectively modulated by NTFs, allowing

us to shape aspects of the neuronal phenotype in differentiating ES cells. The potential use of NTFs in customizing the composition of ion channels downstream of Neurog1 more broadly impacts the field of sensory neuron replacement.

613 Inhibition of Notch Activity Promotes Hair Cell Regeneration in the Chicken Utricle

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In birds, vestibular epithelia undergo slow hair cell turnover throughout life. This is accompanied by continual low rates of progenitor cell division and new hair cell differentiation. These processes are increased after aminoglycoside-triggered hair cell loss. Signaling through the Notch receptor regulates progenitor cell behavior and cellular differentiation during regeneration in post-hatch chicken basilar papillae. In this study, we explored the role of Notch signaling in hair cell regeneration in control, undamaged vestibular epithelia and in vestibular epithelia after drug damage. Notch1 and its ligands Serrate1 and Delta1 are expressed in the control utricle and expression is upregulated after damage. Using qRTPCR and ISH, we found that the Notch effector, Hes5, and the Notch modulator, Lnfg, are expressed in control utricles and are upregulated in the utricular striola, the predominant region of hair cell loss, after damage. Treatment of organotypic cultures of control utricles with either DAPT or TAPI-1 (at 10-50 µM) blocks Notch activation via two separate mechanisms. Compared to negative controls (DMSO-treated cultures), both DAPT and TAPI-1 triggered a dramatic increase in supporting cell entry into the cell cycle and in hair cell numbers, as identified by Atoh1 and MyosinVI immunolabeling. Similar results were seen in isolated utricular supporting cells. We are currently conducting experiments to determine the specificity of this effect for the Notch signaling pathway by overexpressing Notch intracellular domain to induce constitutive Notch activation. We are also exploring other signaling pathways that may be modulated by DAPT and TAPI-1 treatment in utricular epithelium.

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614 The Role of Growth Hormone in Zebrafish (*Danio Rerio*) Auditory Hair Cell Regeneration

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In order to develop treatment for, or prevention of, deafness, a thorough understanding of the process of auditory hair cell regeneration, which is possible in fish and birds but not in mammals, must be established. Our previous microarray and quantitative real-time PCR analysis of noise-exposed zebrafish ears, showed that growth hormone (GH) was significantly upregulated during zebrafish auditory tissue cell proliferation and hair cell regeneration. In a subsequent preliminary study, salmon GH (10 µg/g BW) was injected intraperitoneally into zebrafish, resulting in a significant increase in cell proliferation in the zebrafish ear, particularly in the utricle. To investigate the potential role of growth hormone in recovery from hearing loss, we will utilize the zebrafish model, of which a time line of acoustic trauma-induced auditory hair cell death and regeneration has been established. We will induce auditory hair cell damage by exposing zebrafish to a 100 Hz pure tone at 179 dB for 36 hours. Immediately afterwards, the fish will be injected intraperitoneally with GH and placed in a recovery tank. At 2, 7, and 14 days following acoustic trauma, auditory evoked potentials will be recorded to test functional recovery and hair cell densities, quantified by counting phalloidin-labeled stereocilia bundles, will be recorded to examine the effects of GH on hair cell regeneration. At the same time, cell proliferation and apoptosis will be measured by BrdU- and TUNEL-labeling, respectively, to identify possible cellular mechanisms associated with auditory hair cell regeneration in zebrafish.

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615 Synaptic Ribbons in *Atoh1*-Induced Ectopic Hair Cells

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The transcription factor, Atoh1, initiates hair cell differentiation in the developing inner ear. Delivery of the *Atoh1* gene to the organ of Corti has shown promise as a means of triggering hair cell regeneration by inducing the transdifferentiation of supporting cells to a hair cell-like phenotype. We previously found that in cultured mouse organ of Corti, forced *Atoh1* expression induced the appearance of ectopic myosin VIIa-positive cells with apical hair bundles. For acoustic waveforms to be transduced into action potentials in the auditory nerve, synaptic transmission between hair cells and spiral ganglion neurons must take place. This involves exocytosis of neurotransmitter via ribbon synapses. Strategies to recreate the normal function of hair cells must, therefore, give rise to synaptic ribbons in addition to stereocilia. Here, we use immunohistochemistry for the synaptic ribbon protein, RIBEYE, via a CtBP2 antibody to probe for the presence of synaptic ribbons in ectopic myosin VIIa-positive cells. In cultured postnatal mouse organ of Corti explants treated with Ad.*Atoh1-GFP*, ectopic hair cells were found lateral and medial to normal hair cell positions. Cultured explants treated with vector containing reporter in the absence of *Atoh1* lacked ectopic hair cells. In the cultured explants, nuclear label typical of CtBP2 staining was absent in the organ of Corti, while ribbon label was detected in hair cells. Some of the ectopic cells in both lateral and medial locations exhibited punctate RIBEYE/CtBP2 labeling that was similar to that of synaptic ribbons in the inner hair cells. The finding of ribbon-like structures in ectopic hair cells suggests that such cells have the potential to make ribbon synapses with spiral ganglion nerve fibers, possibly to restore hearing if they were to receive acoustic stimulation in deaf ears.

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616 Atoh1 Mediated Recovery of Balance Function After Exposure to IDPN

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Loss of balance is often due to loss of vestibular hair cells. In mammals regeneration of functional hair cells in the mature sensory epithelium is not possible therefore loss of sensory cells can lead to debilitating balance problems. Delivery of the transcription factor atoh1 after aminoglycoside ototoxicity can induce the transdifferentiation of supporting cells into new hair cells and restore function. In order to more closely mirror the clinical balance dysfunction we have developed an animal model using systemic application of 3,3'-iminodipropionitrile (IDPN), a vestibulotoxic nitrile compound known to cause vestibular hair cell loss and have tested delivery of atoh1 using a new adenovirus vector based on Ad28. The Ad28 adenovector is based on a rare human serotype with a low prevalence of neutralizing antibodies that appears to specifically target vestibular supporting cells. In order to further provide cell type selective gene delivery we express atoh1 from the supporting cell specific glial fibrillary acid protein promoter. Delivery of this vector to IDPN damaged vestibular organs resulted in recovery of vestibular hair cells and restoration of balance. *Supported by NIDCD R01DC008424*

617 Injection of Virus Vector Targeting Vestibul in Mice

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It is known that a lot of children with a congenital deafness have a disorder in vestibular function. We examined the gene transfer to the inner ear using glass tube. In the mouse, three main routes of gene delivery are possible, namely scala media approaches (via a cochleostomy), semicircular canal approaches (via a canalostomy) and round window membrane approaches. In this study, adenovirus (AdV) carrying the green fluorescent protein (GFP) gene were injected in mouse inner ear through the round window and a canalostomy for the purpose of the gene transfer to vestibule. To evaluate the influence of the operation on auditory function and balance function, ABR and the balance test were assessed pre- and post-operatively. Thereafter, transgene expression was observed in the mouse cochlear and vestibular organ. In injection of AdV through the round window, GFP-positive cells were present at the perilymphatic spaces and ampulla. In canalostomy approach, GFP-positive cells were present at perilymphatic space in most samples, and at endolymphatic space in several samples. The signs of vestibular dysfunction were not observed. ABR thresholds did not show any significant changes between before and after the operation of cochleostomy.

618 Identification of 11 Novel Mutations in USH2A Among Japanese Patients with Usher Syndrome Type 2

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Objective: Usher syndrome (USH) is an autosomal recessive disorder characterized by retinitis pigmentosa (RP) and hearing loss (HL). USH type 2 (USH2) is the most common type of USH and is frequently caused by mutations in *USH2A*, which accounts for 74–90% of USH2 cases. To date, several mutation analyses of *USH2A* were performed in European Caucasian patients, but not in Japanese. By screening *USH2A* in Japanese patients, we evaluated the mutation spectrum and clinical findings. Methods: Ten unrelated Japanese patients from various regions throughout Japan participated in the study and met the criteria of USH2. The clinical evaluation of the affected patients consisted of elicitation of medical history, and ophthalmological and audiovestibular examinations. Mutation analysis of *USH2A* was performed by direct sequencing technique using genomic DNA extracted from peripheral lymphocytes. Results: Mutation analysis of *USH2A* in 10 unrelated Japanese patients revealed 14 different probable pathogenic mutations in 8 patients. Of these, 11 mutations were novel. Splicing mutation c.8559-2A>G was identified in 4 of 10 patients and accounted for 4 of 15 mutated alleles (26.7%); the other mutations were seen in 1 patient each. Thus, c.8559-2A>G occurs frequently in Japanese patients. Mutations were widely distributed throughout almost the entire *USH2A*, without any apparent hot spot. All 8 *USH2A* patients showed RP, moderate-to-severe HL, and normal vestibular function. However, clinical findings were varied in them. In particular a patient showed rapid progressive HL, which is atypical for USH2, and the severely constricted visual field for his age. Conclusion: The mutation spectrum that we identified differed from that for Caucasians, but the incidence of mutations in *USH2A* was 80% for all patients tested, which is consistent with previous report. Further mutation screening for c.8559-2A>G may prove very effective for early diagnosis of *USH2A* in Japanese patients.

619 The Genetic Etiology of Congenital Hearing Loss in Deaf Probands from Newfoundland's Founder Population

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The province of Newfoundland and Labrador, Canada, has a founder population consisting of deaf probands with

large extended pedigrees. This unique population has already played a role in the discovery of novel deafness genes such as WFS1, TMPRSS3, and PCDH15. The purpose of this study is to identify the cause of hearing loss in unsolved families with an autosomal recessive (AR) hearing loss, and to possibly discover new genes.
Mutations in connexin genes, specifically GJB2, are the most common cause of recessive hearing loss in most world populations, though the frequency in the Newfoundland population is not known. A second connexin gene, GJB6, also plays a role in hearing loss in homozygous individuals or in a compound heterozygous form with a GJB2 mutation. Sixty-two congenitally deaf probands with a family history consistent with AR hearing loss were sequenced for both exons of GJB2 and the common deletion (del13S1830) in GJB6. Nine out of 62 were found to have mutations in GJB2, GJB6, or both. Two probands were heterozygous for 35delG, two homozygous for 35delG, and one was a compound heterozygote for the 35delG mutation and M34T (in GJB2). As well, four deaf probands were compound heterozygotes for 35delG and del13S1830 in GJB6. This leaves 53 of the 62 probands unresolved, meaning only 15% of hearing loss in Newfoundland and Labrador's deaf probands is due to mutations in GJB2 and GJB6, compared to 33.1% reported in a recent study of the UK population. A surprisingly low result for Newfoundland since it was colonized by Irish and English (Northern European) settlers in the 1700s. This may indicate that one or more genes, possibly novel, are causing deafness in these probands. In order to look for other "usual suspects", sequencing of candidate genes such as SLC26A4, OTOF, CDH23, and TMC1 is underway. The detection of an underlying cause of congenital deafness will improve the clinical diagnosis and management of these patients.

620 Discovery of a Novel 3bp Deletion Within the P-Loop Domain of KCNQ4 Causing Late-Onset Deafness in a Large Newfoundland Family

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Newfoundland is one of the few founder populations in the world, typically has large extended families, similar lifestyle (low confounding factors), deep genealogies, and a willingness to participate. The discovery of a large Newfoundland family harboring a deafness causing mutation in a novel gene, WFS1, is just one example of a gene discovery using the founder population. Candidate genes COCH, TECTA, and KCNQ4 are being screened in 28 probands with late onset autosomal dominant hearing loss, because they have a higher frequency of recurrent mutations in autosomal dominant families. Mutations in GJB2, GJB6, and WFS1, previously identified in Newfoundland, were excluded in all 28 probands. After screening KCNQ4, a novel 3bp heterozygous deletion in exon 5 was found in the proband of a 6 generation family. This was subsequently shown to cosegregate with deafness in 13 affected relatives; and of 90 ethnically matched population controls none harbored the putative mutation. This deletion predicts an in frame removal of a serine residue at amino acid position 269 within the P-loop domain of the KCNQ4 protein. Interestingly, the P-loop domain is a mutational hotspot where missense mutations causing late onset hearing loss have been described. While this deletion is not seen in four affected family members, these four individuals present a distinctly different audiological profile compared to deletion carriers. Audiology reports of affected relatives with the novel deletion in KCNQ4 show a high frequency late-onset hearing loss, supporting the current genotype-phenotype correlation that KCNQ4 deletions associate with a late-onset and milder hearing impairment (high-frequency loss) than the missense mutations. The entire KCNQ4 gene is currently being sequenced to confirm the deletion is the causative mutation, and not just a closely linked marker. This is the third deafness causing deletion found in KCNQ4 (DFNA2) in the past 15 years, and the first outside of exon 1.

621 DFNA34 Maps to Chromosome 1q44 and May Be Allelic with Hearing Loss-Autoinflammation Syndromes Caused by Mutations in *NLRP3*

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We ascertained LMG113, a North American family segregating autosomal dominant sensorineural hearing loss (SNHL) that appears to be nonsyndromic. The hearing loss initially affects middle and high frequencies in the 2nd to 4th decade of life and typically progresses to mild-to-moderate severity. A genome-wide scan with 440 short tandem repeat (STR) markers revealed probable linkage (maximum two-point LOD=3.15 at θ=0 for D1S2836) on chromosome 1q44. There were no other DFNA loci mapped to this region that was thus defined as DFNA34. We also completed a genome-wide linkage analysis of 6,090 single nucleotide polymorphisms (SNPs) on the Illumina HumanLinkage-12 Genotyping BeadChip to confirm the result of our STR analysis. Five SNPs on chromosome 1q43-44 showed two-point LOD scores >2.3 (maximum two-point LOD=2.94 at θ=0 for rs974893), which overlaps the linkage interval defined by STR markers. Combined SNP-STR haplotype analyses of all other SNPs with LOD scores >1.5 ruled out linkage to other regions of the genome. We used novel STR markers to narrow DFNA34 to a 3.9-Mb interval harboring 26 annotated genes. One candidate gene is *NLRP3*, in which dominant mutations cause SNHL and autoinflammation in patients with Muckle-Wells syndrome (MWS) and neonatal-onset multisystem inflammatory disorder (NOMID). We detected a heterozygous missense substitution, c.G2753A (p.R918Q), in the leucine-rich repeat domain of NLRP3. p.R918 is conserved in mammals. Although p.R918Q co-segregated with DFNA34, it was detected in one of 186 normal control samples from Coriell Cell Repositories. Ages and audiologic phenotypes of these controls were not available

to confirm normal hearing. Rheumatologic evaluations of affected members of LMG113 revealed only nonspecific signs and symptoms that might reflect autoinflammation but did not meet diagnostic criteria for MWS or NOMID. We conclude that DFNA34 may be allelic with MWS and NOMID or may be caused by a mutation elsewhere on chromosome 1q44.

622 Application of Mass Spectrometric Methods to Identify and Validate the A119T Mutation in the Cochlin (COCH) Protein, Using Temporal Bone Samples from an Individual Characterized with DFNA-9

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Objectives: In this study, we implement a novel sample preparation method to extract, and identify the proteins in human temporal bone sections using LC-MS/MS. In addition, we show that, in cases where genomic DNA is either not available or of poor quality to perform basic genetic analyses, proteomic techniques can be successfully utilized to identify the underlying genetic mutations associated with hearing loss.

Study Design**:** Extract and identify proteins isolated from formalin-fixed human temporal bone samples and compare the amount coverage of COCH protein between a known DFNA9 HTB sample and a pure COCH protein sample acquired commercially. Proteins are extracted using a heat-induced antigen retrieval method in the presence of 1% citraconic anhydride. LC-MS/MS used to identify and characterize proteins and targeted MRM analysis to identify and validate specific COCH mutations.

Methods: Samples were placed in a 1% citraconic anhydride homogenization buffer and treated in 99ºC for 20 minutes and 80ºC for 2 hours. After centrifugation, the supernatant was subjected to Chymotryspin (Promega) digestion and the resultant peptides were purified using a C_{18} column (Pierce). Peptides were separated by liquid chromatography (Eksigent, Inc.) and identified and sequenced using a 4000 Q-Trap mass spectrometer (ABI). Spectrum analysis was conducted using Analyst 4.1 software and bioinformatics performed using MASCOT/SwissProt database and Scaffold2 proteome software.

Results: purified COCH protein exhibited 14 unique peptide sequences, with 28% coverage over the sequence. The HTB sample had 1 unique peptide comprising 4% coverage of the protein. Furthermore, the HTB sample generated a list of 875 probable proteins with 593 identified peptide sequences at the lowest level of stringency (20% probability and 1 peptide).

Conclusions: 1) We used heat-induced antigen retrieval method in the presence of 1% Citraconic Anhydride to extract ~250µg of purified protein using two section (~50µm) of a human temporal bone associated with DFNA9, 2) We identified 46 proteins from this sample with >80% probability with 1 unique peptide match, 3) Confirmation of the A119T in this temporal bone sample validates the use of proteomic methods as a powerful, yet complimentary tool in temporal bone research.

623 Pre-Implantation Genetic Diagnosis (Embryo Screening) for Enlarged Vestibular Aqueduct Due to SLC26A4 Mutation

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Pre-implantation genetic diagnosis (PGD) is used to analyze embryos genetically before their transfer into the uterus. For families with genetic diseases, PGD offers a chance to have an unaffected child, without facing termination of pregnancy. Although PGD has been performed for many monogenic disorders, such as cystic fibrosis and beta-thalassemia, the application of PGD to hereditary hearing impairment has not been explored. In the present study, we reported the development and application of PGD protocols to address enlarged vestibular aqueduct (EVA), which is a common type of hereditary hearing impairment associated with mutations in the SLC26A4 gene. The family requesting PGD had a history of EVA, segregating the SLC26A4 c.919-2A>G mutation. In short, the PGD process was composed of two steps: the development of a single-cell testing protocol and clinical PGD cycles (i.e., selection and implantation of unaffected embryos using the single-cell testing protocol). First, protocols for genetic testing in a single cell were established for the c.919-2A>G mutation using GenomiPhi technology and primer extension mini-sequencing. These protocols were validated on single lymphocytes collected from both parents and their affected child. Two clinical PGD cycles were then performed for the parents, with the second cycle successfully leading to a singleton pregnancy. The baby was homozygous for the wild type SLC26A4 allele and revealed a normal audiological phenotype after birth. To our knowledge, there has not ever been reported in the literature describing successful PGD in families with genetic hearing impairment. In our opinion, the application of PGD in the field of hereditary hearing impairment involves fewer ethical controversies than other novel applications of PGD and traditional indications for PGD for other monogenic diseases. Therefore, the approach demonstrated in the present study can also be used in a large number of families with other types of hereditary hearing impairment.

624 Identification of Novel Mutation of TECTA Gene in the Korean Nonsyndromic Hearing Patients

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TECTA gene encodes alpha-tectorin (TECTA), a major noncollagenous component of the tectorial membrane. Mutations in the TECTA locus cause dominant (DFNA8/A12) or recessive (DFNB21) form of nonsyndromic hearing loss in humans. Eighteen different mutations in the TECTA locus have been identified from various ethnic groups. Thus far, there was no TECTA mutation reported in Korean population. In this study, we screened the entire coding region of TECTA gene by directly sequencing of the genomic PCR amplicons from 43 autosomal dominant and 19 autosomal recessive (sporadic) nonsyndromic hearing loss probands showing various severities. From an autosomal dominant patient, we identified a point mutation at nucleotide 5597, cytosine to thymine. This nucleotide change converts a threonine to a methione at the amino acid position 1866. This variation was not present in the proband's father, and the mother and the affected sisters were heterozygotes. This variation was not found in 46 normal hearing individuals, suggesting that this nucleotide change may be the cause of hearing loss in this family. In addition, five different polymorphisms were identified; 2256C>T, 2795T>C, 2805T>C, IVS17+40C>G, and 5634C>T. This is the first report for mutation in the TECTA locus identified in Korean population. Our finding emphasizes the importance of genetic screening of the TECTA gene for the patients with autosomal dominant nonsyndromic hearing loss in Korean population.

625 Pathogenetic Role of the Deafness-Related P.V37I Variant of GJB2: Evidences from a Large Clinical Cohort, Cell-Line Studies and the Knock-In Mouse Model

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p.V37I, a GJB2 allele with debatable pathogenicity frequently identified in East Asians, was reported to contribute to mild-to-moderate sensorineural hearing impairment (SNHI). The purpose of the study is to elucidate the pathogenicity of p.V37I through investigations in a clinical cohort, cell-lines and the knock-in mouse model. A total of 732 unrelated Han Chinese patients with idiopathic non-syndromic SNHI and 1005 Han Chinese controls were enrolled. According to the GENDEAF criteria, 356 were classified as severe-to-profound SNHI, and 376 were classified as mild-to-moderate SNHI. Allele frequencies of p.V37I in these two groups and the controls were 12.8%, 30.3% and 9.2%, respectively (chi-square test, $p < 0.001$). Both patients with severe-to-profound SNHI and those with mild-to-moderate SNHI revealed a deviated genotype distribution from estimation (chi-square goodness-of-fit test, both $p < 0.001$). Homozygosity for p.V37I was identified in 77 (20.5%) and 9 (2.5%) patients with mild-to-moderate and severe-to-profound SNHI, respectively, and co-segregated with the phenotypes of SNHI in 56 kindreds. Further mutation screening in the GJB2 promoter and GJB6 coding regions did not detect sequence variants in the 86 p.V37I homozygotes, excluding the possibility of a near-by mutation in linkage disequilibrium with p.V37I. The pathogenetic mechanisms of p.V37I were then investigated in cell-lines by transfecting HCx26wt and the p.V37I variant into HeLa cells. HeLa cells transfected with p.V37I expressed protein levels comparable to those transfected with HCx26wt, but were less able to form clear junctional plaques and demonstrated a less permeability for intercellular dye transfer. A knock-in mouse model homozygous for p.V37I, Gjb2tm1Dontuh/tm1Dontuh, was also established. Preliminary characterization of the audiological phenotype revealed near-normal hearing levels at 4 weeks. These lines of evidence indicated that p.V37I might mildly impact the function of connexin26.

626 Characterization of Two Missense Mutations in the GJB2 Gene Associated with Non-Syndromic Hearing Loss

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Mutations in the GJB2 gene, which encodes the gap junction (GJ) protein connexin26 (Cx26), are the most common cause of inherited non-syndromic hearing loss (NSHL). We identified two missense mutations, p.D46E (c.138T>G) and p.T86R (c.257C>G), of GJB2 in the Korean HL families. The novel p.D46E mutation exhibited autosomal dominant inheritance, while the p.T86R mutation, which is exclusively found in Asians, segregated with an autosomal recessive pattern. Thus, we sought to elucidate the pathogenic nature of such different inherited patterns of HL. We studied protein localization and gap junction functions in cells transfected with wild-type or mutant Cx26 tagged with fluorescent proteins, which allowed visual confirmation of homozygous or

heterozygous mutant GJs. The Cx26-D46E mutant was targeted to the plasma membrane, but this mutant protein failed to transfer Ca(2+) or propidium iodide intercellularly, suggesting disruption of both ionic and biochemical coupling. Heterozygous GJs also showed dysfunctional intercellular couplings and hemichannel opening, confirming the dominant-negative nature of the p.D46E mutation. The Cx26-T86R mutant protein did not form GJs, since the mutated protein was confined in the cytoplasm and not transported to the cell membrane. When Cx26-T86R was co-expressed with Cx26-WT, ionic and biochemical coupling was normal, consistent with the recessive nature of the mutation. These studies revealed distinct pathogenic mechanisms of two GJB2 mutations identified in Korean families

627 The GJB2 and GJB6 Mutations Associated with Nonsyndromic Hearing Loss in the Korean Population

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Aims: The *GJB2* are the most common genetic cause of nonsyndromic sensorineural hearing loss worldwide. However, contribution to hearing loss and mutation spectrum of the *GJB2* varies widely among population. Recently, the *GJB6* has been emerged as a common, second mutation in patients with single heterozygous *GJB2* mutation. In this study, we fully analyzed the *GJB2* and *GJB6* gene of sixty unrelated patients to investigate genetic cause of nonsyndromic sensorineural hearing loss in Koreans. Results: Only three cases (5%) were molecularly diagnosed with compound heterozygous mutation of c.[235delC]+[427C>T]. Other five cases were single heterozygote for c.38T>G, c.109G>A, c.143A>G, c.235delC, and c.427C>T, respectively. We have identified two novel missense mutations, c.38T>G (p.V13G) and c.143A>G (p.Q48R), and these mutations were likely to be pathogenic through in-silico predictions. Neither the large deletion including the *GJB6* nor mutation in non-coding region of the *GJB2* were identified in this study. Conclusions: The *GJB2* gene might be an important cause of nonsyndromic sensorineural hearing loss in Koreans. However, relatively low contribution to hearing loss and different mutation spectrum of the *GJB2*, and no presence of the *GJB6* deletion need a careful consideration for establishing screening strategies and further investigation for mutational etiology in the Korean population.

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Keyword: Hearing loss, GJB2, GJB6

628 Preliminary Gene-Therapy Studies for Rescue Hearing of Conditional Connexin26 Null Mice

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Non-sensory cells in the cochlea are connected extensively by gap junctions (GJs) that facilitate intercellular ionic and biochemical coupling. Mutations in the gene coding for connexin26 (Cx26) is the most common cause of human nonsyndromic hereditary deafness that affect millions of patients. No mechanism-based therapy is available for treatment. To investigate the pathological process in the cochlea and study treatment options, we have generated three independent lines of conditional Cx26 (cCx26) null mice (Wang et al., 2009). All mutant mice displayed severe hearing impairment. Results show that the cell differentiation and gross cochlear morphology at birth were normal in these mutant mice. Hair bundles of both inner and outer hair cells were clearly visible and normally arranged. However, development of the organ of Corti was arrested in the early postnatal period. The opening of the tunnel of Corti and the Nuel's space, which are hallmarks of normal development of the organ of Corti, was not observed in all three lines of cCx26 null mice. Cell degeneration was first observed in the Claudius cells and outer hair cells around the onset of hearing in mice (~P12) at middle turn when inner hair cells were still intact. Massive cell death occurred in the middle turn thereafter and gradually spread to the basal turn, resulting in secondary degeneration of spiral ganglion neurons in the corresponding cochlear locations. These results demonstrated that Cx26 plays essential roles in postnatal maturation and homoeostasis of the organ of Corti before the onset of hearing.

One implication of these observations made from cCx26 mice is that a gene therapy approach to treat deafness in cCx26 mice at the adult stage is unlikely to be successful. We therefore performed gene-therapy studies using early postnatal cCx26 mice. Preliminary studies injecting a control lentiviral vector at P0 into the scale media resulted in the expression of GFP signal in most of the marginal cells in the stria vascularis. However, only scattered supporting cells in the sensory epithelium was transfected with GFP. One promising results was that injections made by glass micropipttes at P0 into the scale media only resulted in slight damage to the hearing of mice. We plan to continue the study by testing other types of control virus vectors first, and eventually examining whether hearing of cCx26 mice could be restored by viral-mediated expression of Cx26 in early developmental stages of the cochlea.

629 Auditory Characteristics in the Mucopolysaccaridosis II Mice and Therapeutic Effects of Enzyme Replacement

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Mucopolysaccaridosis (MPS) II (Hunter syndrome) is an X-linked metabolic disorder caused by dysfunction of iduronate-2-sulfatase (IDS). The defect results in the progressive accumulation of incompletely degraded glycosaminoglycans (GAGs) in lysosome of various tissues and organs. Until recently, there have been few reports about the auditory characteristics in MPS II mouse model. The purpose of this study was to evaluate the auditory characteristics in IDS knock-out (k/o) mice of the MPS II mouse model. Furthermore, we estimated the effect of enzyme replacement therapy in the otologic field. We have examined the thresholds of auditory brainstem response (ABR) from 7 to 12 weeks of age and the ear histopathology in male IDS wild type and k/o mice. IDS k/o mice showed normal histologic findings in the cochlea with few lysosomal storage and retained good hearing at 7 weeks of age. However, at 12 weeks of age, hearing thresholds of IDS k/o mice at click and tone burst (8, 16, 32kHz) sounds were elevated, compared to those of wild type of IDS mice. Idursulfase for enzyme replacement therapy was administered weekly to IDS k/o mice in the form of oral injection at dose of 0.5mg/kg from 7 to 12 weeks of age for 5 weeks. Hearing thresholds of IDS k/o mice which were treated with Idursulfase, were approximately similar to those of the IDS wild type mice at 12 weeks of age. These data indicate that hearing deficits in MPS II mouse model can be prevented if enzyme replacement therapy with Idursulfase is started before hearing impairment.

630 Mcph1 and Spns2: Two Novel Genes Implicated in Hearing Impairment, Identified Via a High-Throughput Targetted Mutagenesis Phenotyping Screen

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Measurements of the auditory brainstem response (ABR) were used to evaluate hearing sensitivity across a range of frequencies in mutant mice produced by a high-throughput targeted mutagenesis programme. Individual mice aged 14 weeks were anaesthetised with Ketamine/Xylazine before being placed on a heating pad, 20cm from a loudspeaker. Needle electrodes, inserted in the skin overlying each bulla and the vertex, with associated hardware and software, are used to record brainstem responses to free-field acoustic stimuli. Clicks (10µs duration) and tones pips (5ms duration, at 6, 12, 18, 24 and 30kHz) were presented across a range of levels from 0–95dB SPL to assess ABR threshold. For each mutant line, at least 4 mice are tested and compared with results from wildtype mice of the same genetic background. Several of the 170+ lines tested to date have demonstrated varying degrees of hearing loss and other anomalies of the ABR waveforms.

Mcph1-deficient mice demonstrated a moderate hearing impairment of 22-38dB. This impairment (recorded at 14 weeks) appears to be progressive, because homozygous mutants show no hearing loss at P21 or P42. Further investigations are ongoing to characterise the time-course of the emergence of the impairment and its underlying mechanisms.

Spns2-deficient mice showed a severe hearing impairment, with ABR threshold elevations of 59-77dB at frequencies up to 18kHz and no responses up to 85dB SPL at 24 & 30kHz. This impairment was present also in young mice (P21). Anatomical observations suggested that the gross morphology of the cochlea and middle ear ossicles, along with the fine structure of the organ of Corti are normal in young homozygotes. An endocochlear potential of 20-40mV suggests that the deficit may result from strial dysfunction.

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631 Genetic Variation Produces Multiple Hearing Phenotypes in NIH Swiss Mice

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NIH Swiss mice exhibit wide distributions of hearing threshold across all frequencies. This variation produces several distinct auditory phenotypes, including a previously unrecorded frequency-specific hearing impairment. Since the genetic architecture of hearing impairment is of considerable interest, we began to investigate the basis of these different phenotypes. The basis of the frequency-specific loss of hearing is of particular interest since it might elucidate the mechanism responsible for the tonotopic arrangement of the cochlea along a frequency gradient. The variation present in the NIH Swiss population allowed us to select for specific phenotypes and create two new lines by brother x sister matings between mice exhibiting increased thresholds at all frequencies (all frequency hearing loss line, AFHL) or between mice with increased 32 kHz thresholds only (high frequency hearing loss line, HFHL). Currently at F14 generation, the phenotypes segregate with complete penetrance in the two lines. While AFHL mice exhibit stereociliary hair bundle irregularities and develop the typical cochlea-wide degeneration common in sensori-neural hearing loss, the HFHL mice exhibit no discernable hair cell abnormalities and cochlear degeneration is late-onset and limited to the basal region. The HFHL line also has a unique DPOAE phenotype that suggests an outer hair cell dysfunction restricted to the basal region of the cochlea. A preliminary genome-wide scan of an (NIHSwiss x C3HeB/FeJ) x C3HeB/FeJ backcross revealed 3 QTL influencing ABR thresholds, one affecting hearing thresholds at all frequencies tested (click, 8-, 16-, 32 kHz) and two affecting only the 32 kHz ABR threshold. Genetic complementation tests and morphological analyses suggest that the QTL responsible for AFHL is the *ahl5* allele. Additional crosses

have been produced from the HFHL line to segregate the two putative QTL from one another and further investigate the HFHL phenotype.

632 Mitochondrial Haplotypes May Modulate the Phenotypic Manifestation of the Deafness-Associated 12S RRNA 1555A>G Mutation

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Mitochondrial 12S rRNA 1555A>G mutation is one of the important causes of aminoglycoside-induced and nonsyndromic deafness. Our previous investigations showed that the A1555G mutation was a primary factor underlying the development of deafness but was insufficient to produce deafness phenotype. However, it has been proposed that mitochondrial haplotypes modulate the phenotypic manifestation of the 1555A>G mutation. Here, we performed systematic and extended mutational screening of 12S rRNA gene in a cohort of 1742 hearing-impaired Han Chinese pediatric subjects from Zhejiang Province, China. Among these, 69 subjects with aminoglycoside-induced and nonsyndromic deafness harbored the homoplasmic 1555A>G mutation. These translated to a frequency of ~3.96% for the 1555A>G mutation in this hearing–impaired population. Clinical and genetic characterizations of 69 Chinese families carrying the 1555A>G mutation exhibited a wide range of penetrance and expressivity of hearing impairment. The average penetrances of deafness were 29.5% and 17.6%, respectively, when aminoglycoside-induced hearing loss was included or excluded. Furthermore, the average age-of-onset for deafness without aminoglycoside exposure ranged from 5 and 30 years old, with the average of 14.5 years. Their mitochondrial genomes exhibited distinct sets of polymorphisms belonging to ten Eastern Asian haplogroups A, B, C, D, F, G, M, N, R and Y, respectively. These indicated that the 1555A>G mutation occurred through recurrent origins and founder events. The haplogroup D accounted for 40.6% of the patient's mtDNA samples but only 25.8% of the Chinese control mtDNA samples. Strikingly, these Chinese families carrying mitochondrial haplogroup B exhibited higher penetrance and expressivity of hearing loss. In addition, the mitochondrial haplogroup specific variants: 15927G>A of haplogroup B5b, 12338T>C of haplogroup F2, 7444G>A of haplogroup B4, 5802T>C, 10454T>C, 12224C>T and 11696G>A of D4 haplogroup, 5821G>A of haplogroup C, 14693A>G of haplogroups Y2 and F, and 15908T>C of Y2 may enhance the penetrace of hearing loss in these Chinese families. Moreover, the absence of mutation in nuclear modifier gene TRMU suggested that TRMU may not be a modifier for the phenotypic expression of the 1555A>G mutation in these Chinese families. These observations suggested that mitochondrial haplotypes modulate the variable penetrance and expressivity of deafness among these Chinese families.

633 Control of ATP on K^+-Recycling in the Cochlear Supporting Cells

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Gap junction-mediated K^+-recycling in the cochlear supporting cell has been proposed to play a critical role in hearing. However, how potassium ions enter into the supporting cells for recycling remains undetermined. In this study, the effect of ATP on K^+-sinking in the cochlear supporting cells for K^+-recycling in the guinea pig was investigated. Application of micromolar and submicromolar physiological levels of ATP evoked a significant inward current in the cochlear supporting cells. The evoked inward current had a K^+-dependence, proportionally increased with extracellular K^+. At the resting membrane potential of -80 mV, the amplitude of the inward current was a linear function of the extracellular concentration of K^+. The inward current increased as the concentration of ATP was increased. However, without ATP, there was no apparent inward current evoked for extracellular K^+ challenge. Antagonists of ionotropic purinergic (P2x) receptors eliminated the ATP-evoked inward current in the cochlear supporting cells. Application pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS, 50 µM) or pre-incubation of irreversible P2x7 antagonist oxidized ATP (oATP, 0.1 mM) completely abolished the inward current evoked by ATP at the negative membrane potentials. ATP also evoked an inward current at the positive membrane potential, which could be eliminated by inactivation of positive holding potential or the replacement of intracellular K^+ by Cs^+, suggesting different mechanisms underlying ATP-evoked inward currents at the positive and negative membrane potentials. These data indicate that ATP can activate P2x receptors to induce K^+-sinking at the resting membrane potential in the cochlear supporting cells for K^+-recycling and plays an important role in the cochlear ionic homeostasis.

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634 Identification of Lateral Wall-Specific KCNQ1 Channels and KCNE Subunits in Inner Ear

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Functional KCNQ1 channel comprises 4 pore-forming alpha-subunits and can be modulated in part by binding with beta-subunits of the KCNE family. The KCNE family consists of 5 members (KCNE1-KCNE5), each of which is potentially capable of associating with KCNQ1. Although it has been demonstrated that KCNQ1 and KCNE1 are expressed in marginal cells to maintain K+ homeostasis, the stria vascularis-specific channel has not been identified. We cloned lateral wall-specific KCNQ1 and KCNE subunits using RT-PCR techniques, performed with mRNA purified from lateral wall of mouse cochlea. We demonstrated that KCNQ1 and KCNE1-4 channels are expressed in the mouse stria vascularis via RT-PCR methods and confirmed by using immunofluorescence microscopy. In a parallel series of experiments, the cloned KCNQ1 and KCNEs were expressed singly and in

combination in CHO cells and whole-cell and single channel recordings were performed. Co-expression of KCNQ1 and KCNE1 produced a conductance ~30-fold smaller than the combination of KCNQ1 and KCNE3. We will demonstrate that a combination of KCNQ1/KCNE1/KCNE3 may associate to produce the native current phenotype in marginal cells of the stria vascularis.

635 Identification of Aquaporin-4 in Rana Pipiens AP Hair Cells

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Recent physiologic experiments in isolated anuran AP hair cells have suggested that they change volume in response to variation in the osmolarity of their surrounding medium in a mercury-independent manner. This demonstrated increase in permeability over that of a lipid bilayer may depend on the presence of water channels in the AP hair cell membrane. To investigate this hypothesis, immunofluorescence and confocal microscopy were used to identify aquaporin-4 (AQP4) in anuran AP hair cells. AQP4 is one of the more than 10 known water channels of the aquaporin family and is known to be mercury-insensitive.

Rabbit anti-AQP-4 antibody (sc-20812) was purchased from Santa Cruz, USA. Amphibian papillae of *Rana pipiens pipiens* were microdissected and fixed in paraformaldehyde. Both whole mounts and sections were used in double-immunohistochemical staining experiments along with AP hair cell and hair bundle markers. AQP4 was identified in the basal and apical aspects of the AP hair cell, and appears to be prominent in the cell membrane. AQP4 was also identified in supporting cells of the amphibian papilla as well as in saccular hair cells. Control experiments included mouse brain, in which AQP4 was identified lining the ventricles, and mouse cochlea, in which it was found in spiral ganglion cells and supporting cells, but not in inner or outer hair cells (OHCs). These controls agree with previous reports. Whether water channels exist in mammalian OHCs remains controversial; although a polyclonal antibody raised against the conserved amino acid sequence of aquaporins has been found to label the OHC membrane in rats, no specific water channel has been identified. The presence of a water channel in the anuran AP hair cell either constitutes a major physiologic difference between amphibian and mammalian hair cell structure, or the mammalian counterpart has yet to be identified.

636 Immunocytochemical Distribution of WARP (Von Willebrand a Domain-Related Protein) in the Human Inner Ear

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The basic components of the epithelial, perineural and perivascular basement membranes in the inner are well-documented in several animal models and are recently being elucidated in the human inner ear. The von Willebrand A domain-related protein (WARP) is a recently identified extracellular matrix molecule with restricted expression in cartilage and a subset of basement membranes in peripheral nerves, muscle and central nervous system vasculature. We determined the distribution of WARP by immunocytochemistry in the human inner ear using antibodies against WARP. For this purpose we used vestibular and auditory endorgans microdissected from human temporal bones obtained at autopsy. All subjects (n=5, ages 55-87) had documented normal auditory and vestibular function. WARP-immunoreactivity was consistently found in the vasculature throughout the stroma of the cristae ampullaris, the maculae utricle and saccule. Specifically, WARP-immunoreactivity was located in the internal (luminal) portion of blood vessels. WARP-immunoreactivity was also found in calyx-like structures that surround type I hair cells. In the cochlea, WARP-immunoreactivity was well-localized to the blood vessels of the stria vascularis and spiral ligament, as well as sub-basilar blood vessels allowing clear delineation of the vasculature. The distinct localization of WARP in the human inner ear vasculature suggests an important role in maintenance of vessel integrity. In addition, WARP allows specific and clear delineation of microvessels in the inner ear permitting future study into the possible role of microvascular disturbances in the development of otopathology.

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637 Immunohistochemical Localization of P2Y4 Receptor in the Inner Ear

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Puringergic receptors are widely distributed in the mammalian inner ear. Substantial in vitro and in vivo data support a role for extracellular nucleotide and associated P2 receptors in the inner ear function. The purpose of this study is to investigate the expression of P2Y4 receptor in the inner ear of gerbil using immunohistochemistry. In the cochlea, immunoreactive staining for P2Y4 was observed in the organ of Corti, Reissner¡¯s membrane, spiral ligament and apical membrane of stria marginal cells where P2Y4 is known to inhibit K+ secretion via KCNQ1/KCNE1 K+ channel. In the organ of Corti, most of cellular types showed immunoreactivity. The most intense

immunolabeling was detected in the Deiters¡¯ cells. In the outer hair cells, immunohistochemical distribution of P2Y4 receptors was heterogeneous; intense staining was observed at the apical part, close to the cuticular plate which is facing endolymphatic space than at the lateral wall of cell body. The inner hair cells were moderately stained in the lateral wall of cell body. Intense staining was seen in the upper middle portion and head portion of outer and inner pillar cells. In the utricle and saccule, sensory hair cells were strongly stained. In the crista ampullaris, sensory hair cells were also strongly stained, among them type I cells showed the most intense staining, by contrast apical membrane of vestibular dark cell showed moderate staining. P2Y4 receptors are expressed in many cell types of the inner ear. In the cochlea supporting cells such as Deiter¡¯s cells and head portion of inner and outer pillar cells showed the most intense staining, whereas in the vestibular end organ sensory hair cells were strongly stained.

638 P2X Receptor of Endothelial Cells and Pericytes of Spiral Ligament Capillaries

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The cochlear lateral wall generates endocochlear potential (EP), which is essential for normal hearing. EP is highly dependent on oxygen, which is provided by the blood flow of the cochlear lateral wall. Modulation of microcirculation at the cochlear lateral wall plays an important role in normal and pathophysiological hearing. In the current study, using whole cell recording, we show that ATP elicits the characteristic current through the P2X channel at both endothelial cells and pericytes of spiral ligament capillaries and consequently produces a substantial increase of intracellular Ca^{2+} concentration. Two kinds of tissue preparations from guinea pig were made; 1) isolated capillaries with endothelial cells only and 2) capillaries with endothelial cells and pericytes. Patch recording at endothelial cells (without pericytes connected) shows that ATP (1 mM) induced the characteristic inward current (~97 pA) with desensitization at a holding voltage (Vh) of -70 mV. At 0 mV of Vh, the ATP sensitive current is around zero (~1.0 pA), suggesting a non-selective cation channel (NSCC). Recording at pericytes (associated with endothelial cells) shows that ATP (1 mM) induced a similar inward current (~115.5 pA) with desensitization at a Vh of -70 mV. At 0 mV of Vh, the ATP sensitive current is around zero (~0.3 pA). The gap junction blocker, 18β-glycyrrhetinic acid (30 mM), blocked the ATP sensitive current by 73.5%. Consistent with the patch clamp data, time-lapse imaging of Ca^{2+} fluorescence signal shows that ATP (1 mM) elicited a significant increase at both endothelial cells (by 77%) and pericytes (by 63%). We hypothesize that ATP regulates microcirculation of the cochlear lateral wall in a manner of negative feedback, i.e. an increase of ATP level activates P2X channel and the subsequent transient Ca^{2+} entry in both endothelial cells and pericytes elicits a transient constriction of capillaries and reduction in blood flow *in vivo*.

639 RNA Expression of Claudins in the Rat Endolymphatic Sac

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Claudins are a family of tight junction membrane proteins regulating permeabilities of paracellular transport in epithelia and consist of more than 20 members.

The endolymphatic sac (ES) is a part of the membranous labyrinth. The ES epithelia are considered to absorb the endolymphatic fluid produced by marginal cells of the stria vascularis and the dark cells in the vestibular organ. The ES dysfunction could result in endolymphatic hydrops. Electron microscopical observation of tight junctions in the ES epithelia suggests that ES epithelia may conduct paracellular ion transport. In this study, we examined claudin mRNA expressions in the ES to know the properties of tight junction in the ES epithelia. This study is the first report revealing that claudin RNA was expressed in the rat ES by RT-PCR and *in situ* hybridization.

Total RNA was isolated from the whole ES epithelia of rat by the laser capture microdissection, which is able to dissect ES epithelium without neighboring tissues as shown previously (Akiyama et al., BBRC 376: 611-614, 2008). RT-PCR evidenced expressions for *cldn2, cldn4, cldn6, cldn7, cldn9, cldn11, cldn12,* and *cldn14*.The individual expressions of these claudins in the epithelial cells of ES were confirmed by *in situ* hybridization.

This study suggests that claudins may have important roles in paracellular ion transport in the ES epithelia.

640 Tumor Necrosis Factor Alpha Promotes Vasoconstriction in Cochlear Arteries – A Novel Sphingosine-1-Phosphate Dependent Mechanism for Sudden Hearing Loss

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Growing clinical evidence suggests that antagonizing tumor necrosis factor alpha (TNFα) significantly improves auditory function in patients suffering sudden hearing loss (SHL). The present study indicates a causal link between inflammation and inner ear vascular dysfunction. Recovery profiles of auditory function in a small SHL patient group treated with TNFα inhibitor etanercept were consistent with a vascular origin. Using an animal model of the gerbil

spiral modiolar artery (SMA), the functional end artery feeding the inner ear, we demonstrate that TNFα induces a pro-constrictive state via activation of sphingosine-1-phosphate (S1P) signalling. Detailed analysis of the molecular signalling pathway identified the phosphorylation of sphingosine kinase 1 (the S1P-generating enzyme activated by TNFα) as a potential new therapeutic target for SHL. We conclude that any pathology linked to the release of TNFα has the potential to reduce cochlear blood flow and cause SHL. The present study integrates SHL into the family of cardiovascular pathologies, with immediate implications related to risk stratification, diagnosis and treatment.

641 Diarrhea-Dehydration and Auditory Thresholds in Children

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Poor perfusion of the cochlea is an implied contributor to sensorineural hearing loss. In human infants, rarely can poor perfusion be isolated from other contributors (hypoxia, elevated bilirubin, prolonged hyperventilation, and aminoglycosides and diuretics).

Data of childen followed prospectively from birth in a population based study of otitis media, afforded a natural observational experiment. Of 35 children, in whom contributors to elevated auditory thresholds had been excluded, nine had suffered diarrhea-dehydration such that they were hospitalized (at age 1-14 months, mean 6.5 months).

At ages 4-8 years (median 77 months for the nine hospitalized for diarrhea-dehydration, median 71.5 months for the 26 comparison children), all 35 children had normal voluntary thresholds for pure tone stimuli, and normal thresholds for contralateral middle ear muscle reflexes. However, at 4 kHz, the diarrhea-dehydration children had slightly worse thresholds (P = .05). And, those children had slightly lower thresholds for contralateral elicitation of middle ear muscle reflexes.

Transient poor perfusion may have lasting effects on cochlear performance.

642 Targeted Genetic Delivery of MiRNA Against Vasopressin Receptor 2 in Mouse Inner Ear

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To test the hypothesis that hearing and balance depend on vasopressin-mediated regulation of fluid homeostasis in the inner ear we are attempting to suppress the expression of vasopressin receptor type 2 (Avpr2) in mouse inner ear. We have constructed plasmid and lentiviral vectors expressing miRNAs against Avpr2 as well as negative control miRNA. The lentiviral vectors are delivered into murine inner ear by transuterine microinjection of embryonic otocyst. The plasmid vectors are delivered by transuterine microinjection of embryonic otocyst followed by in vivo electroporation. Both plasmid and lentiviral vectors have been incorporated into parts of the inner ear including the endolymphatic duct and sac where Avpr2 and its downstream effector aquaporin 2 (Aqp2) are expressed. We aim to achieve long-term inhibition of Avpr2 and Aqp2 in murine inner ear so that hearing and balance can be measured in adult mice. Our targeted genetic delivery method can facilitate the study of genes in the inner ear that are not amenable to study in transgenic mouse model because of lethality.

643 Determining the Volume of the Elliptical Cone Simulating the Vestibular Aqueduct in Ménière's Disease Using Multiplanar Reconstruction Images: A Novel Index for the Vestibular Aqueduct Evaluation

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Using the vertical multi-planar reconstruction (MPR) image besides the axial image of computed tomography (CT), vertical and axial sizes of the vestibular aqueduct (VA) was evaluated in both ears with unilateral Meniere's disease and in control ears. The sizes of the VA in axial and vertical plains could be measured in all subjects. The vertical sizes of the VA were not correlated with the axial sizes. The volume of the elliptical cone simulating the VA , which reflects the VA sizes of axial and vertical plains, showed a significantly smaller value in both ears of patients with unilateral Meniere's disease than that in control ears, whereas two dimentional analysis in each plain failed to reveal a significant difference between ears with Meniere's disease and control ears. The present results were in good accordance with those of previous reports on the temporal bone histology and radiology in Meniere's disease. The present results suggest that the volume of the elliptical cone simulating the VA may be useful for evaluating the VA size in Meniere's disease. This is the first report evaluating the VA quantitatively in three dimensions in living Meniere's disease patients.

644 Pharmacokinetic and Toxicity Profile of OTO-104 : A Sustained Release Dexamethasone Hydrogel for Inner Ear Delivery

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In recent years, intratympanic drug delivery has been investigated as a route of administration to treat a variety of inner ear disorders, such as Meniere's disease and Sudden Sensorineural Hearing Loss. While constituting a significant improvement in safety and efficacy over the traditional systemic dosing approach (oral, intravenous), several issues still remain to be addressed: large differences in dosing schedules and regimen, as well as high variability in clinical outcomes and patient acceptance. These disparities are primarily the result of the nature of the current formulations, namely drug

solutions with short residence time and rapid elimination from the middle and inner ear.
OTO-104, a poloxamer-based hydrogel containing micronized dexamethasone (DEX) was developed. Poloxamers are tri-block co-polymers (PEO-PPO-PEO) with mucoadhesive and thermoreversible properties that behave as sustained release drug delivery vehicles. OTO-104 was administered to guinea pigs via intratympanic injection and its pharmacokinetic and toxicity profile was examined.
Following a single intratympanic injection, significant and prolonged exposure to dexamethasone in the inner ear (as measured in perilymph) was observed. Increasing the concentration of dexamethasone resulted in higher dexamethasone levels in the perilymph as well as a more prolonged duration of exposure. At the maximally deliverable drug concentration of 20% DEX, therapeutic levels of dexamethasone could be sustained over a 3-month period.
Evaluation of potential adverse effects included an assessment of auditory function (Auditory Brainstem Response) and histological analyses (cochlear paraffin sections, cytocochleograms). A small and transient shift in hearing threshold was observed, most probably of conductive nature. No significant histological changes in either the middle or the inner ear tissues were noted.
In conclusion, OTO-104 appears to provide a well-tolerated and controllable delivery system to achieve prolonged sustained release of dexamethasone at multiple concentrations within the inner ear.

645 Towards Predicting Human Inner Ear Pharmacokinetics: Allometric Scaling Using Guinea Pigs and Sheep

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Deriving predictable drug levels in humans from pharmacokinetic studies performed in animals is a critical component of drug development. In particular, this exercise lends knowledge to the efficacy and safety profiles of the drug candidate under consideration. The evaluation includes multiple species, typically small and large mammals, in a process known as allometric scaling.
To date, there are no published studies on allometric scaling as applied to inner ear pharmacokinetics. A study was designed to explore the relationships between guinea pigs and sheep following intratympanic injection of either a dexamethasone sodium phosphate solution (DSP) or a dexamethasone-loaded poloxamer hydrogel (OTO-104).
Detailed pharmacokinetic profiles of dexamethasone in the inner ear compartment (perilymph) as well as in the plasma and cerebrospinal fluid will be presented. The robustness of the allometric scaling analysis will be demonstrated by comparing the data derived from the guinea pig and sheep studies to that reported in human studies using intratympanic steroid administration.

646 Sequential Sampling of Perilymph from the Lateral Semi-Circular Canal

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Studies of perilymph pharmacokinetics have been complicated by the technical difficulties of obtaining perilymph samples from animals. We have shown that small samples collected sequentially from the cochlear apex allowed gradients of drugs along scala tympani (ST) to be quantified. In the present study a similar technique was used in which perilymph was collected from the lateral semi-circular canal (SCC) after it was perforated, to establish whether drug distribution throughout the entire perilymphatic space could be quantified.
The lateral SCC of guinea pigs was exposed and an injection pipette was sealed into the perilymphatic space of the canal and surrounded by silicone glue. Solution containing 2 mM trimethylphenylammonium (TMPA), a marker ion, was injected at a rate of 1 uL/min for 30 min. Ion-selective electrode measurements from perilymph in vivo show that during such injections, the outlet for flow is the cochlear aqueduct so that the entire perilymphatic space becomes loaded with marker. This allows the elimination of marker (to blood) to be assessed while minimizing the influence of marker distribution in the fluid spaces. Perilymph was sampled by removing the injection pipette and collecting fluid emerging at the injection site. Sixteen separate 1 uL samples, were collected, each taking approximately 30 sec to collect. Pairs of samples were pooled to provide eight, 2 uL samples for analysis. Samples were diluted in 25 uL of buffer and TMPA was measured with an ion selective electrode. In different experiments, perilymph was sampled at 30, 60, 120, 180, or 240 min after injection. The decline of TMPA concentration with time was not uniform throughout the perilymphatic compartment. The most rapid decline occurred in samples originating from ST, with slower declines in samples originating from scala vestibuli and the vestibule. Computer simulations of the data were consistent with a far higher rate of elimination from ST than from other locations.
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647 Perilymph Sampling from the Apex and the Basal Turn of the Cochlea Shows the Pharmacokinetic Profile of Cisplatin in Vivo

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Introduction: Cisplatin is a potent anticancer drug and hearing loss is a dose limiting side effect. We wanted to evaluate the pharmacokinetic profile of cisplatin in the cochlea after an intravenous administration of the drug. We have used two different techniques for perilymph sampling i.e. from the basal turn and from the cochlear apex (Salt et al., J Neurosci Methods. 2006 May 15;153(1):121-9). With capillary tubes sequential samples

of perilymph were taken from the apex. It is thus possible to determine the concentration of cisplatin in the cochlear base and apex at the same time point. Materials and methods: Thirty female albino guinea pigs were used in the experiment. All animals received a single intravenous dose of cisplatin (1 mg/ml, 8 mg/kg, given 1 ml/minute). The animals were divided into 2 groups. Group 1; a dorsolateral approach was used to collect scala tympani perilymph from the basal turn of the cochlea and one sample of 1 µL were taken from each cochlea. Group 2; a ventrolateral approach was used to collect sequential scala tympani perilymph samples from the cochlear apex. Altogether 10 samples of 1 µL were taken from the right ear. One blood sample, one sample of CSF through a suboccipital puncture and the samples of scala tympani perilymph were drawn at the same target time; 10, 20 and 30 minutes after intravenous administration of cisplatin. Liquid chromatography with postcolumn derivatization was used for quantitative determination of the parent drug. Results: In all animals the concentration of cisplatin was lower in CSF than in scala tympani perilymph. Thereby the risk of CSF contamination of the perilymph samples can be excluded to cause "false high" concentrations of cisplatin in the perilymph. Up to 20 minutes after administration of the drug the concentration of cisplatin was considerably lower in the apex compared to the basal turn. At 30 minutes the concentrations were similar.

648 Apical-Basal Concentration Gradients of Gentamicin in Perilymph of Scala Tympani Following Systemic Applications

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A robust baso-apical concentration gradient of drugs e. g. gentamicin in the perilymph of scala tymphani (ST) showing maximum concentrations in the basal turn of ST could be observed following experimental round window membrane (RWM) application (Salt et al., 2006; Plontke et al., 2007). We were interested whether a baso-apical gradient of gentamicin was present in the perilymph after systemic applications.

We analyzed the pharmacokinetics of a gentamicin sulfate drug (Refobacine, Merck, Darmstadt) in clinical use for the treatment of bacterial gram negative infections. Gentamicin was applied systemically with concentrations of 100, 300 and 600 mg/kg/body weight. Three and 5 hours post start of application perilymph of ST was aspirated from the apex of the right and left cochlea, respectively. Ten times 1 µl perilymph fluid from each cochlea was analyzed quantitatively using a fluorescent polarization immunoassay (Abbott Diagnostics, Wiesbaden).

In contrast to local RWM applications systemic applications of gentamicin showed an apical-basal concentration gradient in the perilymph of ST. Peak concentrations were measured in the apical turn and decreased towards the basal turn. The concentrations along the cochlea increased with time and dose applied.

A finite element model of the guinea pig cochlea (http://oto.wustl.edu/cochlea/) was modified to interpret the results. Calculated concentrations were consistent with concentrations determined in the experiments showing concentrations that were higher in the apical part of the cochlea and decreased towards the basal turn.

We conclude that apical-basal gradient might be caused by different perilymph blood exchange properties for the drug in the basal and apical turn and involvement of the endolymphatic space in drug entry into ST.

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649 Quantitative Evaluation of Magnetic Assisted Transport of PLGA Nanoparticles Through a Human Round Window Membrane Model

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Treatment of auditory and vestibular dysfunction has become increasingly dependent on inner ear drug delivery. Recent advances in molecular therapy and nanotechnology have stimulated the development of a variety of delivery methodologies involving both transtympanic and direct intracochlear infusions. Hearing loss is a major public health problem, and its treatment with traditional therapy strategies is often unsuccessful due to limited drug access to the cochlea deep in the temporal bone. Multifunctional nanoparticles may help to resolve this problem since they have the potential for targetting specific cell populations, biodegradability and traceability in vivo as well as allowing controlled drug release.

Coumarin-6 labeled superparamagnetic nanoparticles (CMNPs) 250nm in diameter were formulated by an oil-in-water emulsion/solvent evaporation method and characterized by transmission electron microscopy and dynamic light scattering. A human round window membrane (RWM) model was established by seeding epithelial cells on both sides of a small intestinal submucosal (SIS) matrix (100 µm in thickness) with fibroblasts seeded in between the epithelial layers. The magnetic assisted transport of CMNPs was carried out by application of external magnetic forces and quantified by analysis of the fluorescence intensity of the coumarin-6 extracted from the transmembrane deliveries.

This study employed the tri-layer RWM model to evaluate the effectiveness of magnetic assisted transport of nanoparticles. Factors such as time course of magnetic exposure, composition and concentration of hyaluronic acid hydrogel, concentration of magnetite nanoparticles inside PLGA polymer nanoparticles, and physical characteristics of magnetic fields were studied. The quantification of CMNPs tranversing the membrane indicated that magnetic assisted transport could increase the delivery of particles two fold in 1 hour compared to the

controls without influences of external magnetic forces. The results suggest that there are optimal conditions for each factor under study. Balancing these variables may be of critical importance to optimize drug delivery across a biologic membrane.

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650 Tissue Distribution of PLGA-Magnetite Nanoparticles Targeted to the Guinea Pig Cochlea

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Nanoparticles are increasingly being explored as drug delivery vehicles due to the ability to tailor particle size, surface charge and chemical properties. Tissue targeting presumably minimizes non-specific tissue delivery and uptake. We are studying the effect of magnetic targeting of magnetite (Fe_3O_4) encapsulated PLGA (polylactic-co-glycolic acid) nanoparticles (MP) to the cochlea through the round window membrane (RWM). MP tagged with ^{99m}Tc were used to measure tissue distributions as part of their pharmacokinetics.

MP were synthesized and characterized, then tagged with ^{99m}Tc. Radio-labeling efficiency of ^{99m}Tc on the nanoparticles was 84 ± 9 %. The average particle size and zeta potential of these nanoparticles was 310 nm (hydrodynamic size) and -30.2 mV respectively. Anesthetized guinea pigs were administered 0.4 µl of MP-^{99m}Tc in the RWM niche. In the experimental group nanoparticles were pulled across RWM for 45 minutes under the influence of a magnet. Control animals experienced diffusion only. Samples were collected from blood, skin, kidney, spleen, liver, lung, urine, brainstem, eye and muscle and analyzed with a NaI gamma counter to quantify ^{99m}Tc content. Preliminary data from the control animals suggested MP-^{99m}Tc in blood, muscle, liver and kidney and smaller amounts in urine, lung, brainstem and eye. The experimental animals showed significantly greater nanoparticle delivery into the cochlea by non-parametric test (Mann-Whitney Test) and indicate a trend of lower amount of MP-^{99m}Tc in blood, muscle, liver, and kidney tissue samples as compared to the control group. These results demonstrate that magnetically targeted nanoparticles can be more effectively targeted to cochleae, with less non specific delivery to other tissues/organs.

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651 Characterization of Reciprocating Flow Parameters for Inner Ear Drug Delivery

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To address the need for precise, well-controlled drug delivery directly to the inner ear, we are developing a fully-implantable, long-term microelectromechanical system (MEMS)-based delivery device. This reciprocating delivery device operates through a single implanted cannula, dispelling a fixed volume over a few seconds into the cochlea and slowly withdrawing the same amount over a few minutes. We have characterized the effects of varying flow parameters on the efficiency and safety of drug delivery throughout the length of the cochlea. Tonotopically generated auditory brain stem responses (ABRs) served as bioassays at discrete locations along the length of the cochlea for the presence of administered DNQX, a hair cell neurotransmitter antagonist that attenuates the ABR. Increases in flow rates reduced the onset time for drug effects throughout the cochlea. A lumped-element model, accomplished using circuit simulation software, was constructed to simulate drug distribution. Based on curve fits of the experimental data, we hypothesize that reciprocating delivery distributes the drug into a volume in the base of the cochlea, and that the primary determinant of distribution throughout the rest of the cochlea is diffusion. Increases in flow rate distributed the drug into a larger volume that extended more apically. Over short time courses (less than 2h), the apical extension, though small, significantly enhanced apical delivery of drug. Over longer time courses (>5h) or greater distances (>3mm), maintenance of drug concentration in the basal scala tympani may provide more advantage for apical delivery than increases in flow rate.

652 Effects of Molecular Weights and Osmotic Pressure of Various Acids on Cochlear Function in the Guinea Pig

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Purpose

This study examines the ototoxic effects of acidic solutions with different molecular weights (MW) and different osmotic pressures.

Materials and Methods

The three different acids that we studied were: (1) Formic acid (HCOOH), pH 4 (MW 46), 120 mOsm; 300 mOsm;.430 mOsm (2) Acetic acid (CH3COOH)pH 4 (MW 60), 300 mOsm. (3) Propionic acid (CH3CH2COOH) pH 4 (MW 74), 300 mOsm. All solutions studied were at pH 4. Ototoxicity was measured in guinea pigs by measuring CAP. The stimulus consisted of click sounds, and tone bursts of 4 and 8kHz. Baseline CAP measurements were first made, thenthe middle ears of the animals were filled

with an acid solution. CAP compared to baseline was measured at 30 minutes or at 24 hours.
Results 1: Effects of molecular weights
30 minutes after the acid solution was applied in the middle ear cavity, no significant change was observed in CAP. At 24 hours, all 3 solutions showed significant ($p<0.01$) changes in CAP, and the magnitude of change was similar.
Results 2: Effects of Osmotic pressure
At 24 hours, formic acid solution with lowest osmotic pressure (120mOsm) showed no adverse effects in CAP, but the solutions with 300mOsm and 430mOsm showed the same degree of ototoxic effects.
Conclusions
Ototoxicity of three different acidic solutions, formic acid (MW 46), acetic acid (MW 60), and propionic acid (MW 74), were studied. Those solutions had the same pH 4, and the same osmotic pressure, 300mOsm. All three solutions showed the same degree of ototoxicity at 24 hours. We postulate that the difference in the molecular weights studied here was insufficient to reveal if round window membrane permeability shows subtle selectivity for compounds of different masses.
Using the formic acid solution at pH 4, the effect of three different osmotic pressures, 120, 300 and 430mOsm, was also studied. The formic acid solution at 120mOsm did not show any ototoxicity whereas other two solutions showed ototoxicity.

653 Temporal and Spatial Gentamicin Distribution in the Peripheral Vestibular Organ After Intratympanic Injection

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Intratympanic gentamicin injection has been considered an effective approach to manage patients with incurable vertigo due to Meniere¡¯s disease. Low dose of purified Gentamicin conjugated to Texas Red (GTTR) can be used as a direct tracer to track gentamicin uptake and accumulation by cells in the guinea pig vestibular end organs after a single dose intratympanic application.
Thirty adult ablation guinea pig were administered with 50ul of gentamicin conjugated to Texas Red (GTTR0.1mg/ml) survived for 1d, 3d, 7d, 14d and 28d (five animals in each group). An additional nine animals separated in three groups (3 animals in each group) were administered with different concentration (0.1mg/ml, 0.4mg/ml or 0.8mg/m) of GTTR sacrificed at 7d after local GTTR¡¡injection. Nine animals served as control which were divided into three groups (three animals in each group), those animals were administrated with 50ul of 0.065mg/ml, 0.205mg/ml and 0.51mg/ml Texas Red solution. Confocal fluorescence microscopy was used to determine the localization of gentamicin in the semicircular canal cristae and macular.
We observed the peak intensity of gentamicin was during 3 to 7 days after local injection which has a time delay compared cochlear basilar membrane. Gentamicin has a concentration gradient accumulation from the central zone type I hair cell to the peripheral type I hair cell and then type II hair cell in the ampullar sensory epithelium and from striola to extra-striola hair cell in macular sensory epithelium. Subsequently in hair cell gentamicin first localized the infra-cuticular regions of hair cells, with weak, diffuse cytoplasmic labeling in the cell body. With the dose increased more and more gentamicin diffused into cell body. The transitional cells also display gentamicin labeling in supra-nucelar regions shortly after intra-tympanic injection, and retained this distribution for up to 4 weeks. Gentamicin was seldom observed in the supporting cells (of the sensory epithelium) or in the dark cells. The saccule accumulated more gentamicin than the utricle and the ampula.Among three semicircular canals, the hair cells in the posterior semicircular canal showed strong GTTR labeling.
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654 Adenoviral-Mediated Gene Transfer Into the Mouse Cochlea in Vivo

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Gene therapy may provide a way to restore cochlear function to deaf patients. A variety of viral and nonviral gene transfer vectors have been developed for implementation of gene therapy and the delivery of the therapeutic gene. An ideal route of gene delivery to the adult inner ear would be minimally invasive and non-destructive to hearing. The major sensory and functional components of the inner ear, such as the organ of Corti and the stria vascularis are located within the endolymphatic fluid compartment, the scala media. Based on published studies, the most promising surgical approaches to deliver chemicals, viruses or cells atraumatically to the inner ear and preserve hearing are via a semicircular canalostomy, basal turn cochleostomy and round window membrane. In preliminary experiments, we injected adenovirus into the mouse cochlea using these access routes and found that the majority of infected cells were located adjacent to the perilymphatic space in the scala tympani and scala vestibuli. We present a new method to reliably deliver adenoviral vectors to the scala media of the mouse cochlea and target the organ of Corti in vivo with some preservation of hearing.

655 Intratympanic Delivery of Antivirals and the Effects on SNHL

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Congenital Cytomegalovirus (CMV) is the leading cause of infectious-related sensioneural hearing loss (SNHL) worldwide. Approximately 90% of newborns infected with CMV are asymptomatic at birth, of these 20% exhibit SNHL. Clinicians are developing novel ways to treat SNHL caused by CMV infection. The lab is exploring the Intratympanic route (IT) for delivery of established antivirals to treat CMV related SNHL with promising preliminary results.

Local administration of antivirals by IT injection provides an advantage over systemic delivery and prevents serious side effects. In this model, surgical inoculation of the cochlea via the round window with guinea pig CMV (GPCMV) shows that there is replication of the virus. Titers in blood and cochlear fluids have shown viral replication and auditory brain stem response (ABR) data reveals an associated hearing loss. At the same time, we are working on Cidofovir (CDV) injections given IT. After infecting guinea pigs with GPCMV, IT injections of CDV are administered at Days 1, 3 and 7. ABR, *real-time PCR,* and histological data confirm that CDV given IT inhibits viral replication, and improves hearing without manifesting any side effects.

A unique application of drug-delivery is using a temperature sensitive copolymer injected via the tympanic membrane, as a transporter. In vitro data has shown that CDV put into a temperature sensitive copolymer is viable for a longer period than just the drug itself. The concentration of the drug is successfully released over time. In vivo studies are ongoing, they have shown that the gel can be injected IT, and cover the round window. Hearing and kinetic data will determine if the polymer gel can be used as a transporter for controlled drug release into the inner ear.

656 Adeno-Associated Virus Transduction of the Adult Mouse Cochlea in Vivo

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BACKGROUND: Hearing loss associated with hair cell damage is well-described. Non-mammalian vertebrates replace lost hair cells via transdifferentiation of supporting cells; inducing this process in humans represents a target for gene therapy. Adeno-associated virus (AAV) has limited immunogenicity and toxicity as a vector and can transduce hair cells and supporting cells. Studies have described AAV inoculation in the perinatal mouse cochlea *in vitro* or in the guinea pig *in vivo*. In this study, we aim to investigate the efficiency and specificity of AAV transduction in the adult mouse cochlea.

METHODS: Normal and deafened adult mice underwent inoculation with AAV2-GFP (green fluorescent protein) serotypes 1, 2, 5, 6, and 8 via scala media cochleostomy. Viral stocks, with an internal CMV promoter, were provided by Harvard Gene Therapy Initiative. Auditory brainstem response (ABR) was used prior to and three and seven days post-treatment. After whole mount preparation, apical and basal portions of cochleae were analyzed under fluorescent microscope.

RESULTS: Rates of transduction with AAV-GFP were 50% (4/8), 71.4% (5/7), 55.6% (5/9), 100% (6/6), and 100% (7/7) for serotypes 1, 2, 5, 6, and 8, respectively. Mean total number of positive inner hair cells (IHCs) in the apical and basal portions, respectively, was 36.7 and 50.3 (serotype 1), 44.4 and 65.2 (serotype 2), 7.4 and 9.8 (serotype 5), 6.8 and 79.8 (serotype 6), and 34.7 and 73.3 (serotype 8). Supporting cells were transduced by serotypes 1, 2, and 8, though a small number of transduced cells were noted. Serotypes 2, 5, 6, and 8 transduced outer hair cells (OHCs) of control mice.

CONCLUSION: For all five serotypes, IHCs were most effectively transduced; IHC transduction is best achieved with serotypes 2 and 8. Further investigation into the proper promoter, optimal time window for inoculation after hair cell loss, and appropriate inoculation dose are necessary to increase the efficiency of targeting supporting cells.

657 Nanoparticles as Carriers for Rolipram to Increased the Neuroprotective Effect on Spiral Ganglion Cells

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Objective: Nanoencapsulation is a powerful technique to allow the protection and controlled delivery of a wide range of pharmacological substances. Nanoparticles can be used as non-viral, biodegradable and cell-specific vectors to increase the efficacy of already existing application methods. Brain-derived neurotrophic factor has been demonstrated in numerous studies to be an important survival factor for spiral ganglion cells (SGC). Composed of 119 aminoacids with a molecular mass of 14 kDa BDNF is to massive to be incorporated in nanoparticles. Therefore, it may be reasonable to identify smaller molecules with neurotrophic properties for the incorporation in nanoparticles. Rolipram ($C_{16}H_{21}NO_3$), a phospodiesterase inhibitor, appears due to his small molecular size as suitable. However, the effect on the survival of SGC remains to be elucidated yet. We therefore hypothesize, that rolipram acts neuroprotective on SGC.

Methods: Cultivation of SGC after extraction from neonatal Sprague-Dawley rats and cultivation for 48 h in a serum-free medium and the addition of rolipram. The neuroprotective properties of rolipram were compared with those of BDNF (50 ng/ml) and negative controls (medium without the addition of any growth factors).

Results: Similar survival rates were achieved under treatment with BDNF or rolipram. Both factors significantly

increase the survival of SGC when compared to negative controls. Conclusions: Rolipram acts as neuroprotective on SGC derived from neonatal rats as BDNF. First cell culture experiments with in nanoparticles incorporated rolipram are promising but further research is necessary. Provided that rolipram is as effective in *in vivo* experiments as demonstrated *in vitro*, it may be an ideal candidate for the application to the inner ear via nanoparticles.
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658 Use of the Biodegradable Polymer Chitosan as a Vehicle for Applying Drugs to the Inner Ear

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Development of efficient local delivery systems for the auditory organ has an important role in clinical practice for the management of inner ear disorders using pharmacological means. Chitosan, a biodegradable polymer, is a good drug carrier with bioadhesive properties. The aim of this study was to investigate the feasibility of using chitosan to deliver drugs to the inner ear across the round window membrane (RWM).
Three structurally different chitosans loaded with a tracer drug, neomycin, were injected into the middle ear cavity of albino guinea pigs (n=35). After 7 days the effect of chitosans and neomycin was compared among the treatment groups. The hearing organ was analysed for hair cell loss and the RWM evaluated in term of thickness.
All tested chitosan formulations successfully released the loaded neomycin which then diffused across the RWM, and exerted ototoxic effect on the cochlear hair cells in a degree depending on the concentrations used. Chitosans per se had no noxious effect on the cochlear hair cells. It is concluded that the chitosans, and especially glycosylated derivative, are safe and effective carriers for inner ear therapy.

659 Hyaluronic Acid Enhanced Cochlear Gene Delivery Via the Round Window Membrane

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Delivery of reagents into the cochlea often requires an invasive surgical procedure which can damage the membranous labyrinth, alter the auditory function, cause loss or mixing of fluids or lead to post-surgical infection. Development of non-invasive means of transducing sensory and/or non-sensory cells in the cochlea could potentially increase the feasibility of viral vector mediated inner ear treatments in the clinic. Hyaluronic acid (HA) is a non-toxic, viscous, high-molecular-weight polysaccharide commonly used in ocular surgeries. HA has also been reported to enhance the diffusion of reagents (e.g. dexamethasone) through the round window membrane in humans. Our goal was to determine whether placing HA on the round window membrane can facilitate diffusion of viral vectors into the inner ear. We used a postauricular incision to approach the middle ear and filled the round window niche with 10-14% HA. Ten minutes later, we removed the HA and subsequently applied 5µl of adenovirus with a *GFP* gene insert (Ad.*GFP*) to the round window membrane. Ad.*GFP* without HA or HA alone was applied to control ears. Four days later, we assessed whole-mounts of the auditory epithelium, labeled for F-actin to localize the GFP-positive cells. Ears treated with HA plus Ad.*GFP* had robust GFP expression in cells lining the perilymphatic and endolymphatic spaces whereas little or no GFP expression was seen in ears treated with Ad.*GFP* alone. Delivery of transgenes to the inner ear by diffusion across the round window membrane is important because it can be performed without violating the cochlea. This method is more feasible for clinical applications. Our data demonstrate that HA can be used for enhancing viral vector delivery into the inner ear through the round window membrane.
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660 Rodent Intracochlear Infusion Systems: Technology Advances for Implantable Micropumps

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Advanced deafness therapies that restore normal auditory function will require carefully timed and dosed, site-directed delivery of multiple therapeutic compounds over a period of time. Syringe and osmotic pumps have proven effective for baseline investigations in animal models, but lack the flexibility required for more sophisticated therapy development. An implantable micropump with controllable flow rate and dosing profile is being developed using MEMs technologies. The pump initially targets murine intracochlear drug delivery for deafness therapy research.
Key integration technologies have been created to minimize both size and power for this implanted device. A method for coupling fine capillary tubing to microfluidic channels via in-plane interconnects is presented. Capillary tubing inserted into micro-channels etched in the surface of a silicon wafer is sealed in place via room temperature polymer deposition providing a low volume, biocompatible interconnect. These microfluidic connections withstand pressures as high as 827 kPa (120 psi) with average pull test strength of 2.9 N. The interconnects consume less than 20 nl of volume and exit in-plane with the pump, facilitating implantation. Resistive micro-bridges integrated into the middle of the fluid channel provide an optimal means of flow rate measurement via hot-wire anemometry.

Multiphysics modeling is used to determine the sensor geometry that provides the most favorable trade-off between sensitivity and power consumption. Analog circuit solutions are explored for in-situ fluid temperature compensation without digital computation. A process for creation of deformable membranes over pump chambers with simultaneous coating of the microfluidic channels has been developed allowing integration of a biocompatible fluid flow path. These integration technologies represent critical steps towards micropump development suitable for implantation in mice.

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661 Flow Rate as a Parameter for Reducing Concentration Gradients in Murine Intracochlear Infusions

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Compound delivery to the cochlea generally results in strong basal-to-apical concentration gradients, limiting the efficacy of therapeutic approaches for apical structures, and constraining therapeutic dose ranges. These gradients depend on the location of cochlear entry, the resulting fluid flow path, and diffusion / clearance mechanisms, offering few options for control and reduction. In the present study, infusion flow rate was examined as a parameter for reducing concentration gradients within the cochlea for basal turn scala tympani cochleostomy infusions with and without a posterior semicircular canal canalostomy. Fixed volumes of artificial perilymph (AP) and sodium salicylate (10 mM) were infused. Cochlear function was measured via closed-system DPOAE threshold measurements from 8-44 kHz, with threshold shifts compared for both surgical approaches using two flow rates; 16 and 32 nl/min. Neither flow rate had an acute impact on auditory function as demonstrated by stable thresholds during the initial AP infusion and subsequent threshold recovery during the washout phase. The cochleostomy-only approach exhibited statistically significant enhancements in DPOAE threshold shifts with the higher flow rate at 12 and 16 kHz, suggesting higher concentrations of salicylate at these more apical locations. Flow rate did not impact DPOAE thresholds for the cochleostomy-plus-canalostomy approach. Simulations were used to investigate the impact of higher flow rates on salicylate concentration along the length of the cochlear spiral, with clearance rates examined as a potential influencing factor.

Infusion flow rate may be an important variable in optimization of intracochlear drug delivery paradigms. Elucidating the details of threshold shifts will provide information critical for development of more advanced models of cochlear infusate flow patterns and optimization of inner ear infusion protocols.

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662 Agarose Encapsulated Lentivirally Modified Fibroblasts as Model for Hydrogel Based Drug Delivery to the Inner Ear

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Biofunctionalisation of cochlea implant-electrodes with cells providing neurotrophic factors to the spiral ganglion neurons (SGN) may be an approach to realize drug delivery preventing SGN degeneration. Attention has to be drawn to the mode of electrode coating: Beside the immobilisation of cells by their adherence on different kind of surfaces encapsulation of cells in hydrogel compositions may be an option for controlled drug delivery to SGN. In the presented study we first established an in vitro model for the cellular delivery of BDNF from NIH3T3 cells lentivirally modified to synthesize the green fluorescent protein (GFP) and BDNF (NIH3T3/BDNF) following occlusion in low melting agarose (LMA). Different cell numbers were seeded in 2% LMA and cultivated up to 7 days. Additionally, a suspension of about 10.000 NIH3T3/BDNF cells/µl were mixed with 6% LMA and the tips of electrodes were dipped into the cell suspension and cultivated up to 14 days. The BDNF release of both assays was determined by ELISA. We found an increase of BDNF in the culture medium up to 11,15 ng/ml ±1,095 (350.000 cells, 3 days) in correlation to cell number (0,436 ng/ml ±0,062, 10.000 cells and 4,13 ng/ml ±0,06, 100.000 cells). However, enhanced BDNF expression in correlation to the cultivation period was not demonstrated. In contrast, a decrease of BDNF release was found in the culture assay with the highest cell seed following 7 days. Coating of electrode tips with 6% LMA containing NIH3T3/BDNF cells revealed lower BDNF release of about 0,231 ng/ml ±0,031 following 10 days of cultivation due to the overall reduced hydrogel surface and subsequent smaller cell number. Our study showed in vitro the feasibility of the encapsulation of genetically modified cells as potential drug delivery-system. Other hydrogels rather than agarose may enable drug release and, thus, investigations on hydrogel compositions for clinical applications will be in the focus of our research.

663 Development of a Micropump for Dispensing Nanoliter-Scale Volumes of Concentrated Drug for Intracochlear Delivery

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We are developing a fully-implantable, micro-electromechanical system (MEMS)-based device for long-term precision-controlled drug delivery directly to the inner ear. The device operates through a single implanted cannula, dispensing a fixed volume over a few seconds into the cochlea and slowly withdrawing the same amount over a few minutes. The fluid delivery system consists of a microliter-scale displacement chamber for dispensing and

withdrawing fluid, and a micropump and valve system for dispensing smaller volumes of concentrated drug into the injection line flow stream. Because of the need to limit drug storage volume in an implantable device, drugs must be highly concentrated and therefore dispense rates must be controlled at the microliter/minute level. In order to enable a wearable system for long-term animal experiments and ultimately a fully implantable device, we are developing a miniature, low-power delivery system with the requisite precision flow control. Our prototype micropump is a positive displacement pump composed of an electromechanical actuator, a membrane-based displacement chamber, and two inline check valves, all integrated on a substrate composed of machined and laminated polymer sheets. The volume of the fluidic components of the pump is less than 30 mm^3. We have demonstrated controlled dispense volumes over the range 5 to 125 nanoliters and flow control from 0 to 25 microliters/min by varying the duty cycle of the input square wave voltage to our electromechanical actuator.

664 Decreased Variability of High-Frequency DPOAE Measures in Patients Using an Alternate Calibration Method Compared to Non-Patients Using a Traditional Calibration Method

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High-frequency (> 8 kHz) distortion-product otoacoustic emissions (DPOAEs) have been shown to be repeatable in normal-hearing adults (Dreisbach et al., 2006). To use high-frequency (HF) DPOAEs as an effective monitoring tool, the repeatability of these measures needs to be established in a patient population. DPOAE frequency and level sweeps were measured in cystic fibrosis (CF) patients across four trials. Frequency sweeps were measured at discrete frequencies (f_2 = 8-16 kHz) with L_1/L_2 = 65/50 dB SPL. Level sweeps were measured with L_2 = 50 dB SPL and L_1 varied from 10-70 dB SPL at the two highest frequencies where DPOAEs were present. Preliminary data revealed decreased variability for the frequency sweep measures in those with CF in comparison to previous reports in non-patients over the same frequency range. This decreased variability could be due to the alternate calibration method (calibrated in a B&K 4157 ear simulator with insertion depth compensation – Siegel, 2009) used versus traditional calibration methods or genuine decreased variability in those with CF. Initial comparisons of DPOAE levels obtained with the level sweep in those with CF to non-patients tested one time revealed non-significant differences. With subsequent trials for the non-patient population we will discuss whether the calibration method or the CF population is contributing to the decreased variability found in HF DPOAE measures.

665 Reliability of Threshold and OAE Measurements Using Two Calibration Methods

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The reliability of measurements of hearing thresholds and otoacoustic emissions (OAEs) depends on calibration methods, especially at high frequencies. We have evaluated thresholds, distortion product OAEs, and stimulus frequency OAEs in a large population of human subjects, across five age groups. We compared results using two calibration methods: depth-compensated calibration in an IEC-711 ear simulator (Siegel, ARO Abstracts, 32:11-12, 2009) and forward pressure level (FPL) (Scheperle, et al, JASA, 124:288–300, 2008). All measurements were made over the full human hearing frequency range, in 1/3 octave bands. Both calibration methods appear reliable under conditions similar to those of a hearing clinic. We will present a statistical analysis to critically evaluate the relative performance of the two calibration methods.

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666 Monitoring Carboplatin Ototoxicity in Children with Distortion-Product Otoacoustic Emissions: A Feasibility Study

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Carboplatin is a common chemotherapy agent with potential ototoxic side effects that is used to treat a variety of pediatric cancers, including retinoblastoma. Retinoblastoma is a malignant tumor of the retina that is usually diagnosed in young children. Since children with retinoblastoma have visual impairment in one or both eyes, it is important to know if carboplatin is causing an additional sensory loss. This pilot study was designed to examine if distortion-product otoacoustic emission (DPOAE) tests performed before and after several courses of carboplatin could register a change in outer hair cell function in children due to the potential onset of carboplatin ototoxicity. Ten children (aged 3-72 months) diagnosed with unilateral or bilateral retinoblastoma were included in the sample. The children were tested before and after receiving 3-4 courses (cumulative dosage: 515-1548 mg) of carboplatin chemotherapy. DPOAEs were acquired from both ears of the children with 65/55 dB SPL primary tones (f2= 793-7996 Hz) and a frequency resolution of 3 points/octave. Comparisons were made between pre- and post-therapy mean DPOAE levels with paired-sample t tests. Evidence of ototoxicity in individual children was based on criterion reductions (≥6 dB) in DPOAE levels. Results indicated that significant differences ($p = 0.001$) in

pre- and post-therapy mean DPOAE levels were only observed at f2=7996 Hz. On an individual basis, children receiving higher doses of carboplatin exhibited criterion reductions in DPOAE level at several f2 frequencies. These findings suggest that DPOAE tests can provide useful information when monitoring children at risk of developing ototoxicity. This work as supported in part by grant CA 21765 from the U.S. Public Health Service and by the American Lebanese Syrian Associated Charities (ALSAC).

667 Auditory Peripheral Dysfunction in Tinnitus Subjects with Clinically Normal Audiograms

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The occurrence of tinnitus in people with clinically normal audiograms raises an important unanswered question: Is peripheral auditory dysfunction necessary for the development of tinnitus? We examined peripheral auditory function in 35 ears of 22 men (30-49 yrs), 10 of whom had tinnitus. All subjects had pure-tone thresholds ≤20 dB HL for all standard audiometric frequencies from 0.125-8 kHz. Measurements in each subject included some or all of the following: pure-tone thresholds from 9–16 kHz, distortion-product otoacoustic emissions (DPOAE; L1, L2=63, 60 dB SPL, f2/f1=1.2, f2=0.5–8 kHz, 14-28 pts/oct), DPOAE with and without 60 dB SPL contralateral broadband noise (L1, L2=55, 40 dB SPL, f2/f1=1.2, f2=1–8 kHz, 28 pts/oct), loudness discomfort levels (LDL), and stapedial reflex strength (ear canal pressure change to contralateral noise at 80 dB SPL). Measures were compared between tinnitus and non-tinnitus groups comprising subjects matched pair-wise in age and poorest threshold at or below 8 kHz.
1) Thresholds for both groups averaged 2-9 dB at and below 8 kHz, but indicated hearing loss above 8kHz. Thresholds were subtly (although not significantly) greater in the tinnitus group by approx. 3 dB. 2) DPOAE magnitudes for f2=2-4 kHz were significantly lower in tinnitus subjects. 3) The average reduction in DPOAE produced by contralateral noise was similar for the two groups. 4) LDL was not correlated with DPOAE magnitude (f2=2-4 kHz). 5) Stapedial reflex strength did not differ between the two groups. The data are consistent with diminished outer hair cell function and perhaps subtly greater deafferentation in the tinnitus subjects. These abnormalities may be causally related to tinnitus and/or correlate with additional pathology (e.g., loss of high-threshold afferents), which plays a causative role.
Funded by the Tinnitus Research Consortium

668 A Preliminary Report of Aging Effects on DPOAE Fine Structure, Components and MOC Reflex

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The 2f1-f2 DPOAE has been characterized as a dual-source response, comprised of at least two components, the generator (distortion) component originating near the f2 region and the CF component (reflection) from the 2f1-f2 site. The shifting phase relationship between these components as they combine in the ear canal produces the now-familiar pattern of DPOAE fine structure. Several DPOAE-based indices of peripheral auditory system function differ in human newborns and adults. However, little is known about how DPOAE fine structure and the relative contribution of individual DP components change across the human lifespan. This experiment is part of an ongoing investigation studying changes in: 1) DPOAE fine structure, 2) Component magnitude and phase and 3. DPOAE-based measures of medial olivocochlear activity from birth through old age. DPOAEs were recorded in 10 adolescents, 19 young adults and 11 middle-aged adults from 500 to 4000 Hz using primary tones swept at a rate of 8 sec/octave, with a fixed frequency ratio of $f_2 / f_1 = 1.22$ and stimulus levels of 65-55 dB SPL. Responses were recorded with and without broadband noise presented to the opposite ear. Preliminary analyses suggest that composite DPOAE level, as well as individual component levels are comparable in teens and young adults but reduced in middle-aged subjects. Generator component level was reduced more than the CF component. DPOAE fine structure was also more prevalent in the younger two age groups, in particular at high frequencies. There was a trend for young adults and teens to have stronger MOC reflex than middle-aged adults and teens generally had larger MOC-induced frequency shifts of fine structure maxima than either of the other two age groups. These results are preliminary as data collection is ongoing; nevertheless they suggest changes in auditory peripheral function between adolescence and middle age.

669 Relationship Between MOC Reflex Strength and Masked Thresholds

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Otoacoustic emission (OAE) amplitudes are often suppressed by the presence of contralateral acoustic stimulation (CAS). This effect is produced by the medial-olivocochlear (MOC) reflex. Past studies have shown that the MOC reflex is related to listening in noise and selective attention. In the present investigation, the relationship between the MOC reflex and masked thresholds was studied in 14 normally hearing adults, 18-30 years old. Detection thresholds were determined for a 1000Hz, 300-ms tone presented simultaneously with a 300-ms masker. Three masking conditions were tested: 1) broadband noise (BBN) 2) fixed-frequency 4-tone masker and 3) random-frequency 4-tone masker. While detection of the tone in all three masker conditions depends on the ability to listen

selectively at the target tone frequency, tonal maskers, and particularly random-frequency tonal maskers, are known to make greater demands on selective listening than broadband maskers. DPOAEs were evoked from 500-4000 Hz with primary tones swept in frequency at 8 sec/oct, using a fixed f2/f1 ratio of 1.22 at 65/55 dB SPL. The MOC reflex (difference between DPOAE level with and without CAS) was determined at frequencies corresponding to DPOAE fine structure maxima. The CAS-induced shift in fine structure peak frequency was also quantified. An inverse fast-Fourier transform (IFFT) was conducted to evaluate MOC effects on individual DPOAE components. Preliminary data analyses showed MOC activity to be correlated with masked thresholds for both indices of medial efferent function. Notably, higher masked thresholds in the BBN masker and the random-frequency 4-tone masker conditions were associated with a stronger MOC reflex for low- to mid-frequency DPOAEs. Correlations were also present between psychophysical thresholds and MOC measures calculated with either DPOAE distortion or reflection components. These results suggest that MOC activation plays a role in selective listening.

670 Lateral Asymmetry of Otoacoustic Emissions to Tonal and Broadband Stimuli in Children

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In neonates, transient OAEs have been shown to have larger amplitude when elicited from the right ear while DPOAEs, elicited with tonal stimuli, are larger in the left ear (Sininger & Cone-Wesson, 2004). The current study evaluates the asymmetry of processing based on stimulus type in school-aged children and measures tonal OAEs using a Stimulus-Frequency OAE which may be a more appropriate comparison (have a similar generation mechanism) to TEOAEs than DPOAEs. Thirty school-age children with otherwise normal auditory function were evaluated using Transient OAEs (60 dB SPL clicks) and SFOAEs at 1, 2 and 4k Hz (40 dB SPL probe level) with a Mimosa HearID system. In addition, all measures were taken with and without contralateral broadband noise (60 dB SPL) to evaluate the olivocochlear reflex (OCR). OAE measures were averaged for a fixed amount of time to insure low noise. Measures were performed in the left and right ears and the order of test conditions was randomized. As in neonates, school aged children show larger raw and SNR amplitude for TEOAEs in the right ear. The background noise of the TEOAE shows no asymmetry. The OCR is slightly larger in the right ear but this comparison does not reach statistical significance. In contrast, SFOAE amplitudes (raw, SNR and background noise) at all three frequencies are symmetrical, showing no ear advantage. The OCR as measured with SFOAEs is also symmetrical. Although no asymmetry is noted in the SFOAE, overall results continue to indicate that lateral asymmetry in the OAE of school aged children is based on stimulus type. Broadband stimuli elicit greater amplitudes in the right ear while tonal stimuli show no asymmetry. These findings could be indicative of a developmental trend demonstrating less clear asymmetry with aging. Only a suggestion of asymmetry in OCR was found leaving questions regarding the role of the olivo-cochlear system in auditory system lateral asymmetry.

671 The Importance of Considering Phase When Evaluating Efferent Function with DPOAE

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When efferent function in humans is evaluated by solely looking at changes in Distortion Product Otoacoustic Emissions (DPOAE) level evoked by contralateral acoustic stimulation (CAS), changes in the frequency and depth of DPOAE fine structure appears to produce both enhancement and suppression. Separating the two components provides consistent suppression of the components (Thompson et al., ARO Abstract 536, 2009). Vector subtraction of recordings with and without CAS (taking both amplitude and phase into consideration) also provides consistent suppression. Changes in the phase of ear canal recordings of low-frequency primaries provides a tool for determining whether the CAS evokes the middle ear reflex. The level at which the MEM muscles are activated depends not only on CAS level, but also on primary level and differs from subject to subject. This work was partially supported by Grant No. H133E03006 from the National Institute of Disability and Rehabilitation Reseach, U.S. Department of Education.

672 Fast and Slow Effects of Medial Olivocochlear Efferent Activity on Spontaneous Otoacoustic Emissions in Humans

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The medial olivocochlear (MOC) pathway, when activated either acoustically or electrically, decreases the electromotility of outer hair cells (OHCs), thereby inhibiting basilar membrane (BM) motion and auditory nerve (AN) activity. MOC activation has been shown to affect BM and AN activity on a fast (10-100 ms) and a slow (10-100 s) time scale. It is of practical importance to examine and understand the influence of the MOC pathway on otoacoustic emissions (OAEs), as OAEs provide a noninvasive assay of cochlear mechanics. Although the MOC effects on OAEs have been reported for spontaneous, transient evoked, stimulus frequency, and distortion product OAEs in a number of studies, none of these studies was designed to compare the fast and slow efferent effects. In this study, we explore the effects of MOC activation on spontaneous OAE by applying an acoustical approximation of the shock-burst paradigm used

to study the fast and slow efferent modulation of BM mechanics and AN activity. We demonstrate analogs of the fast and slow MOC effects seen in BM vibration and AN activity: spontaneous OAE amplitude is reduced by both the fast and slow MOC effects; however, fast MOC effects elevate while slow MOC effects reduce spontaneous OAE frequency. These data support the hypothesis that different mechanisms underlie the fast and slow MOC effects.

673 Analysis of Influence on Cochlear Activity for Auditory Attention from TEOAEs

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Our auditory system has the ability to focus one's listening attention on a single talker among a mixture of conversations and background noises, ignoring other conversations. This is called the cocktail party effect. And it's bound up with efferent system that carries impulse from the central nervous system to the periphery, the olivocochlear bundle (OCB). The activity of this bundle can be measured through otoacoustic emissions. In this study, we tried to demonstrate that increasing level of auditory attention to an ear elicits a significant reduction of OCB activation in the contralateral ear. Two groups of young adult subjects (n=10, 11) who had no history of auditory pathology participated in this experiment. There was the term of three months between the experiment of first group and the experiment second group. 6 people in the first group was the same people with the second group. TEOAEs were recorded in right ear at four test frequencies (1, 1.5, 2, 3 and 4k Hz). To induce the auditory attention, two types of contralateral stimuli were used: condition 1 (click stimuli) and condition 2 (mixed stimuli made of 3 pure tones (500, 2000, 4000 Hz)). While recording TEOAEs, subjects were instructed to count ipsilateral stimuli from TEOAEs or click stimuli in condition 1. In condition 2, they had to count only a specific pure tone (500 or 4000 Hz). As the results of the first group, in the case of auditory attention to contralateral stimuli, signal-to-noise (SNR) were reduced about 1.517 dB on condition 1 and 1.799 dB on condition 2 than in the case of auditory attention to ipsilateral stimuli. In the second group, SNR were reduced about 1.096 dB on condition 1 and 1.124 dB on condition 2. Different attenuation of peripheral auditory activity was observed both of on condition 1 and condition 2. These results demonstrate the correlation between the level of auditory attention and activation of OCB. Finally it is verified that increasing level of auditory attention to an ear elicits a significant reduction of OCB activation in the contralateral ear.

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674 Corticofugal Modulation of Peripheral Auditory Activity by Repetitive Transcranial Magnetic Stimulation of Auditory Cortex in Healthy Normal-Hearing Subjects: Preliminary Results of the MagOTO Study

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We previously provided evidence for a functional cortico-olivocochlear pathway in patients with refractory epilepsy, through the recording of otoacoustic emissions (OAE) during cortical electrical stimulation of auditory cortex. However, is it possible to activate this pathway in normal hearing subjects and through a non-invasive method of cortical stimulation, i.e. repetitive transcranial magnetic stimulation (rTMS)? A randomized, placebo-controlled, double-blind study, approved by Ethics committee, based on neuronavigated frameless stereotaxic rTMS of the anterior medial part of Heschl gyrus was designed. In 24 right-handed healthy adult informed volunteers, we performed a single 30-minute session of rTMS (1200 stimulations, intensity of 100% of rest motor threshold) under the following conditions: continuous 1 Hz-low frequency (LF) or intermittent trains of 10 Hz-high frequency (HF) stimulations, with either active or sham figure-eight coils. Transient evoked OAE (TEOAE) and distortion product OAE (DPOAE) responses as well as contralateral suppression of TEOAE (reflecting medial olivo-cochlear (MOC) functioning) were recorded in a counterbalanced order, before and immediately after the rTMS session, both ipsilaterally and contralaterally to the side of cortical stimulation. All measurements were repeated after 1, 24, and 48 hours on both ears. Preliminary results show that, after rTMS session, neither the group with sham stimulations nor the group with 1 Hz-LF active stimulations demonstrated any significant change of OAE responses. On the contrary, the group with active 10 Hz-HF rTMS showed a small but significant decrease in TEOAE and DPOAE amplitude as well as in MOC functioning, only in the contralateral ear. This bimodal effect disappeared after 48 hours. Our findings highlight, for the first time in healthy subjects and through rTMS exploration, a direct functional interaction between contralateral cortical auditory areas and the peripheral auditory organ.

675 High Throughput Analysis of Noise-Induced Protein Responses in Sensory, Vascular and Neural Components of Chinchilla Cochlea

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Noise-induced cochlear proteomic responses in three distinct regions of the cochlea were investigated in chinchillas exposed to a 0.5 - 8 kHz noise for 2 h at 112 dB SPL using antibody microarrays spotted with 725

antibodies. DPOAE testing indicated a permanent threshold shift 4 weeks after the noise exposure. Pathological examination revealed a widespread loss of outer hair cells with focal lesions in the second cochlear turn corresponding to the noise spectrum. Functional annotation analysis of the cochlear protein profile 2 h after noise exposure using "The Database for Annotation, Visualization and Integrated Discovery 2008" (DAVID) indicated the initiation of cell death in the sensory epithelium and the modiolus with no clear functional classification in the lateral wall. Bioinformatics pathway analysis using DAVID suggested the involvement of 'focal adhesion' and 'MAPK signaling' pathways in the sensory tissues while a 'long-term depression'-like mechanism was suggested to be involved in the neural tissue. A comparative analysis of the noise-induced proteomic responses indicated a positive correlation between the lateral wall and the modiolus suggesting the possibility of common biological responses. E2f3 and focal adhesion kinase (FAK) phosphorylated on Y-577 emerged as the proteins with major noise-induced increases in the sensory epithelium. The increase of these proteins assessed by immunolabeling corroborated with the increase observed using antibody microarray. Strong immunoreactivity of FAK was detected in the nuclei of inner hair cells and Hensen's cells, as well as in stereocilia, particularly in the regions of hair cell damage. In addition, an increased expression of E2f3 was detected in the noise-damaged outer hair cells. These results suggest that although stress or cell death seems to be a common outcome, the proteomic responses differ among the three regions. (We acknowledge support from NIH: R03DC010225, SJ; R01DC009091& R01DC00630, RS).

676 Developmental Expression in the Mouse Cochlea of PLZF, a Transcriptional Protein Involved in Protection from Acoustic Trauma

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PLZF (promyelocytic leukemia zinc finger protein) is a corticosteroid-responsive transcription factor found in the cochlea (and elsewhere in the body) both during development and in the adult. PLZF plays a number of context-dependent roles in development, including limb and skeletal formation, apoptosis and proliferation, expression of bmp and hox family proteins, and spermatogenesis. An analysis of early embryonic (up to E16) expression of PLZF indicated it was expressed in mesenchymal cells of the otocyst at E9.5- E10.5 (Avantaggiato et al, J Neurosci 1995). In the adult, Nagy et al (Hearing Res 2005) identified PLZF as an interaction partner for prestin. We have found PLZF to be essential in protecting the cochlea from acoustic trauma (Peppi et al, ARO abstracts 2010). To explore the potential roles of PLZF as a transcription factor in cochlear function, we quantified mRNA and we determined the localization of PLZF within the cochlea during development and in the adult using Bouins fixative, which optimizes immunohistochemical preservation of nuclear transcription factors. Levels of PLZF mRNA in the mouse cochlea increased until P30, and declined rapidly thereafter. At E15.5, spiral ganglion cells and mesenchyhmal cells near the cochlear duct were labeled. By P0 virtually all of the epithelial cells lining the cochlear duct showed nuclear expression of PLZF. The nuclei of inner and outer hair cells, as well as marginal cells of the stria vascularis were strongly labeled. By P15, epithelial cells lining the cochlear duct were only faintly labeled. PLZF label was now observed in the cytoplasm of pillar cells, Dieter's cells and fibrocytes in the spiral ligament. Nuclei of spiral ganglion neurons continued to label intensely. In the adult (P90), PLZF was predominantly located in the spiral ganglion cell nuclei, and in the cytoplasm of Dieter's cells, the heads and feet of the pillar cells, and in type 3 and 4 fibrocytes.

677 Involvement of P38 MAP Kinase and Sequestosome 1 Stress Protein in Acoustic Injury of the Cochlea

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This study evaluated the protective role of p38 mitogen-activated protein kinase (p38 MAPK) inhibitors and sequestosome 1 (Sqstm1/A170/p62), a stress-induced signal modulator, in acoustic injury of the cochlea in mice. Two weeks after the exposure of mice to acoustic stress, threshold shifts of the auditory brainstem response (ABR) from the pre-exposure level and hair cell loss were evaluated. The activation of p38 MAPK was observed in cochlea by immunostaining 4 h after acoustic stress. To examine the role of p38 MAPK in tissue injury, its inhibitors were intraperitoneally injected into male wild-type C57BL mice before the acoustic overexposure. The inhibitors SB202190 and SB203580 but not the inactive analogue SB202474 dose-dependently decreased the auditory threshold shift and outer hair cell loss induced by acoustic overexposure, suggesting the involvement of p38 MAPK in ototoxicity. We found that acoustic overexposure induced the up-regulation of Sqstm1 mRNA expression in the cochlea of wild-type mice and that SQSTM1-deficient mice exhibited an enhanced ABR threshold shift and hair cell loss, suggesting a role of SQSTM1 in the protection of tissue from acoustic stress.

678 The Expression of Proinflammatory Cytokines After Acoustic Overexposure

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Inflammation is originally protective reaction, however the excessive inflammation can result in damage of the tissues. Interleukin-6 (IL-6), Interleukin-1β(IL-1β), Tumor necrosisis factor-α (TNF-α) ,are molecules induced in inflammatory reaction. They are called proinflammatory cytokines. In present study, we examined the expression of proinflammatory cytokines in mice cochlea after acoustic overexposure.

The CBA/N male mice with normal Preyer reflex were used in this study. They were exposed to intense noise (130 dB SPL octave band noise) with a center frequency of 4 kHz for 3hours. Immeadiately 3hours after acoustic overexposure, bilateral cochleae were carefully removed from the skull base. Total RNA was isolated using Trizol Reagent. RT-PCR were carried out for proinflammtory cytokines（IL-6, IL-1β,TNFα）and β-actin.
Geranylgeranylacetone (GGA) granules were mixed with powdered rodent chow at concentrations of 0.5% which corresponding to 400-600mg/kg/day during the study. GGA food was administered to animals from 8 weeks of age for 2 months.
RT-PCR showed that in control groups , induction of IL-6 and IL-1β expression increased significantly after acoustic overexposure.
The levels of IL-6 and IL-1β repressed significantly in the GGA + acoustic overexposure group compared with in the acoustic overexposure only group .
The results of quantative real-time RT-PCR were approximately similar to results of RT-PCR.
The Auditory Brainstem Response (ABR) of all mice were assessed 7 days after noise exposure. Pretreatment of GGA for 2 months significantly suppressed the elevation of threshold after acoustic overexposure at 4,8,16kHz , in comparison to the control group.
These results indicate that proinflammatory cytokine repression in the cochlea played an important role as inner ear protection effect functionally.

679 Stretching Stress on the Organ of Corti Alters Adhesion Molecules and Induces the Degradation of the Cytoskeleton of Hair Cells

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Mechanical forces generated by acoustic overstimulation or electrode insertion during cochlear implantation cause stretching injury to the organ of Corti, leading to the interruption of cell-cell adhesion. To better understand the molecular mechanisms behind the stretching injury, we established an in vitro model of cochlear stretching injury. Using this model, a controlled stretching force was applied to the basilar membrane. Stretching the organ of Corti initiated rapid hair cell death. Dying cells manifested typical apoptotic phenotypes, including nuclear condensation, caspase-3 activation and cytochrome c release. Along the edge of hair cell lesions and around dying hair cells, there was strong immunoreactivity of E-cadherin, a key component of adherens junction, and its associated proteins, catenins, the bridge proteins linking E-cadherin to F-actin. The increase in the expression of these proteins preceded the onset of F-actin degradation, suggesting that disruption of cell-cell adhesion may serve as a triggering event for the degradation of the cytoskeletal structure of the hair cells. To determine whether acoustic trauma causes a similar pattern of damage, we exposed rats to intense noise at 120 dB SPL and found a rapid increase in E-cadherin expression. Again, the E-cadherin change preceded the onset of F-actin degradation. In addition to disruption of adherens junctions, we found the accumulation of Dextran-FITC fluorescence in the extracellular spaces between hair cells and Deiters' cells, suggesting the increase of the pericellular permeability, another indicator of disruption of cell-cell junctions. Taken together, the current study suggests that the disruption of cell-cell adhesion is an important early event in hair cell damage following mechanical stress to the organ of Corti.
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680 Caspase-2 Expression in the Inner Ear After Noise Insult

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Noise-induced hearing loss is caused by damage to the sensory cells in the inner ear. Much of the damage to the sensory cells is induced by metabolic stress. This stress triggers chemical cascades that initiate caspase-dependent apoptosis as well as other molecular events that result in cell death. There are at least 14 known caspases, broadly grouped as apoptotic initiators (caspase-8, -9, and -10) and apoptotic effectors (caspase-3, -5, -6, and -7). Caspase-2 is relatively unique in that it can act either as an apoptotic initiator or an apoptotic effector. Caspase-1, -3, -8, and -9, are clearly activated in the inner ear after noise exposure or other stressors. Expression has been observed in hair cells, spiral ganglion cells, stria vascularis and spiral ligament, and lateral wall. Evidence that caspase-5, -6, -7 and -10 are involved in apoptosis in the inner ear is also emerging. Although there is widespread evidence for caspase-dependant cell death in the inner ear after noise exposure, a role for caspase-2 in apoptotic cell death in the inner ear has not been explicitly evaluated.
Here, we present immuncytochemical evidence for caspase-2 detection in the inner ear after noise exposure. Guinea pigs were exposed to 115 dB SPL octave band noise (centered at 4 kHz) for 4 hours and euthanized subsequent to noise insult; control animals were not exposed to noise prior to euthanasia. Tissues were incubated with an antibody that binds to the larger of two caspase-2 splice variants (caspase-2 long). Caspase-2 long was detected in the supporting cells in tissues from animals exposed to noise, with less robust expression in control animals not exposed to noise stress. The primary antibody was omitted in negative control tissues. Taken together, the data suggest that caspase-2 long may play a role in early noise-induced cell death events in the inner ear.
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681 Overexpression of Pro-BDNF Leads to Increased Noise-Induced Hearing Loss

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In the central nervous system, proBDNF acts via p75-NTRand thetransmembrane protein sortilin to mediate neuronal apoptosis. A knock-in mouse was generated in which one *BDNF* exon was replaced with a *BDNF* sequence with a C-terminal hemagglutinin (HA) tag embedded in the prodomain cleavage site, resulting in overexpression of proBDNF (pro*BDNF-HA/+)*. We predicted that overexpression of proBDNF would accelerate apoptosis following noise exposure and worsen the severity of noise-induced hearing loss (NIHL). Pro*BDNF-HA/*+ mice were exposed to broadband noise for one hour (4–25 kHz, 110 dB). ABR and DPOAE analysis were performed at 24 hours, one week, and one month following noise damage. Histological analysis was performed at one month following noise exposure. At baseline, pro*BDNF-HA/*+ mice exhibited ABR thresholds no worse than wildtype littermates. Following noise damage, pro*BDNF-HA/*+mice demonstrated greater elevations in ABR thresholds at 24 hours and one month following noise exposure in comparison to $BDNF^{+/+}$littermates. High-frequency DPOAEs were significantly decreased in treated pro*BDNF-HA/*+ animals compared to wildtype at one month. In contrast, $BDNF^{+/-}$ mice demonstrated similar responses to noise exposure compared to $BDNF^{+/+}$ littermates. Immunostaining of serial sections of cochleas from pro*BDNF-HA/*+ and $BDNF^{+/+}$mice confirmed expression of p75 and sortilin within spiral ganglion neurons and marginal cells of the stria vascularis. ProBDNF immunolabelling was found in the basilar membrane of both genotypes. No alterations in gross cochlear anatomy were evident. In conclusion, proBDNF overexpression leads to increased NIHL following incomplete deafening with broadband noise.

682 Mitogen-Activated Protein Kinases: Differential Activation After Temporary and Permanent Hearing Loss in the Cochlea and the Auditory Brainstem

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Functional and morphological differences between transient and permanent hearing loss induced by acoustic trauma are well characterized whereas molecular correlates remain to be elucidated. A comparative analysis of the expression of the phosphorylated forms of extracellular signal-regulated kinase (ERK1/2), c-jun-N-terminal kinases 1/2 (JNK1/2) and p38 in the cochlea and the inferior colliculus (IC) after acoustic trauma of two different intensities resulting in either a temporary (TTS) or permanent (PTS) ABR threshold shift is presented. In a cochlea, an immediate up-regulation of phosphorylated p38, JNK1/2, and ERK1/2 was found after PTS while TTS resulted in a down-regulation of phospho-p38 with no change of pJNK and pERK. After a 24 h recovery the activation of JNK1/2 and ERK1/2 was detected in TTS group while the expression of phospho-p38 was still down-regulated. The reaction of all three MAPKs in the PTS group at 24 h post was different from the TTS since they all returned to the control levels. The expression of active forms of all three MAPKs was up-regulated at 30 min after PTS in IC whereas TTS resulted in an up-regulation of phospho-p38 only at 2 h after trauma. The level of brain-derived neurotrophic factor (BDNF), a potent otoprotective agent, was elevated in a cochlea for a longer duration after PTS compared to TTS. In contrast, the significant up-regulation of BDNF in IC was detected in PTS, but not in TTS group. The expression of BDNF receptor's TrkB (truncated form) was down-regulated in a cochlea only after PTS. No change of TrkB expression was detected in inferior colliculus. Thus, temporary and permanent hearing loss demonstrate different expression patterns and temporal aspects of MAPK, BDNF and TrkB in the cochlea and IC. The results of this study will help reveal the cellular mechanisms underlying hearing loss induced by acoustic trauma.

683 Expressions of Endothelin-1, Endothelin Receptor A, B in the Cochlea of Noise Induced Transient Threshold Shift Rat Model

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Recent research has shown that endothelin and its receptors have important role in blood flow regulation of many organs including cochlear. On the other hands, the causes of noise induced hearing loss are thought of direct mechanical effect and alteration of metabolism after blood flow reducing on cochlear. The aim of this study is to investigate expression of endothelin-1(ET-1) and endothelin receptor A,B(ETA, ETB) in the cochlear of noise induced transient threshold shift rat model.Normal twelve S-D rat were exposed to noise. Four of them were sacrificed at post-noise 1 day and another four animals were sacrificed at post-noise 4 day. The remainder four animals were sacrificed at post-noise 7 day. Four were normal controls that were not exposed to noise. Auditory function was evaluated with auditory brainstem responses and the expression of ET-1, ETA,ETB was examined by immunohistochemistry.The transient threshold shift was recovered at post-noise 4 day. Expression of ETA and ETB were changed in the course of time. It is suggested that there is some relation between recovery of noise induced, transient threshold shift and expression of ET-1, ETA, ETB in cochlea.

684 Cochlear Gene Expression in Mice Exposed to TTS and PTS Levels of Noise

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Proteins and protein networks associated with cochlear pathogenesis in the Ames waltzer (*av*) mouse, a model for deafness in Usher syndrome 1F (USH1F), were identified. Cochlear protein from wild-type and *av* mice at postnatal day 30, a time point in which cochlear pathology is well established, were analyzed by quantitative 2D-gel electrophoresis followed by mass spectrometry (MS). The analytic gel resolved ~ 2,300 spots; nearly 60 spots showed significant changes in intensity in the *av* cochlea compared to the control. The cochlin protein was identified in 20 peptide spots, most of which were up-regulated while others were down-regulated. Analysis of MS sequence data showed that, in the *av* cochlea, a set of full-length isoforms of cochlin was up-regulated, while isoforms missing the N-terminal FCH/LCCL domain were down-regulated. Protein interaction network analysis of all protein targets indicated as changing was performed with Metacore software. That analysis revealed a number of statistically significant candidate protein networks predicted to be altered in the affected cochlea. Quantitative PCR (qPCR) analysis of select candidates from the proteomic and bioinformatics investigations showed up-regulation of *Coch* mRNA and those of p53, Brn3a and Nrf2, transcription factors linked to stress response and survival. Increased mRNA of Brn3a and Nrf2 has been previously associated with increased expression of cochlin in human glaucomatous trabecular meshwork. Our report strongly suggests that increased level of cochlin is an important etiologic factor leading to the degeneration of cochlear neuroepithelia in the USH1F model. Since other mouse models for deafness in Usher type 1 (e.g. USH1B, 1C, and 1D) show a similar degenerative profile as the USH1F model, namely the loss of hair cells followed by the loss of spiral ganglion cells, the proteomic analysis reported here could have implications for Usher syndrome type 1.

685 Distribution of Adenovector Transfection After Sound Trauma in the Mouse

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Sound trauma has significant effects on many cell types in the inner ear. In previous studies we have shown that transfection of the cochlea with bcl-2 expressing adenovectors reduce the severity of sound trauma. In preparation for carrying out rescue and regeneration experiments we set out to determine if adenovectors could transduce the cells of Corti's organ after sound trauma. Adult C57Bl6 mice were exposed to a 16 kHz 115 dB tone for two hours. Ten days post sound trauma, animals were treated with Ad5gfp.11D, Ad5.gfap.gfp.11D or Ad28.gfp vectors delivered via the posterior semicircular canal. Animals were allowed to survive for 2 weeks and then processed for immunohistochemistry. Distribution of surviving hair cells was determined by cytocochleogram and compared to distribution of green fluorescent protein (GFP) distribution. Both the Ad5 and Ad28 based vectors were capable of delivering the gfp transgene to the damaged organ of Corti. Delivery of adenovector had no effect on the existing hearing loss induced by sound trauma.

686 Genetic Bases of Noise-Induced Endocochlear Potential (EP) Reduction in BALB/cJ Mice

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A single intense noise exposure causes reversible EP reduction in CBA/J and CBA/CaJ mice (Hirose et al., JARO 2003: Ohlemiller and Gagnon, HR 2007). We recently identified a major effect QTL, Nirep on Chr. 18, that accounts for most of this reduction (Ohlemiller and Gagnon, ARO 2009). We also reported that BALB/cJ mice show noise-induced EP reduction and cochlear pathology similar to that in CBAs. In BALBs, however, the trait appears recessively inherited versus C57BL/6, while it is dominant in CBA. This suggests that different alleles or loci may underlie similar traits in CBA and BALB. We are investigating the genetic bases of EP reduction in BALBs using N2 backcross mice to BALB and 13 recombinant inbred (RI) strains formed from B6 and BALB (CxB1-CxB13, JAX).

Thus far, 12 of 38 N2 backcross mice (32%) have shown EPs below 90 mV within 1-3 hrs after noise exposure (2 hr, 4-45 kHz, 110 dB SPL). This suggests that at least 2 loci are involved. Of 7 RI strains for which we have both control and noise exposure data, 2 show a significant decrease in EP after noise exposure, while 1 shows a significant increase. This suggests the existence of multiple loci that promote either EP decrease or increase after noise. These may interact to set the phenotype of some CxB RI strains.

In summary, BALB/cJ mice appear to carry autosomal recessive alleles at two or more loci that promote acute EP reduction after noise. The critical site of injury, as revealed by light microscopy, is within the lateral wall and not the organ of Corti. With sufficient N2 and RI data, it should be possible to map major QTLs for the noise EP phenotype using each model, and compare N2 and RI results for consistency. QTLs for EP reduction by noise may not affect noise-induced threshold shifts per se. They may, however, impact the long term stability of noise injury and how noise injury intersects with apparent presbycusis.

(NIDCD R01 DC08321 to KKO, P30 DC04665 to R. Chole)

687 Phosphoinositide Signaling in Acquired Hearing Loss

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Phosphatidylinositol 3,4,5-trisphosphate (PIP3) plays an important role in transducing signals from growth factors,

hormones and other extracellular activators to intracellular pathways. PIP3 signaling, in particular, is associated with the control of cell survival and cell death. PIP3 binds to and activates the phosphoinositide-dependent protein kinase-1 which, in turn, phosphorylates and activates the downstream target Akt which leads to phosphorylation of numerous downstream proteins targets. Akt is a major hub for intersecting pathways affecting cell growth, cell survival, and cell differentiation. It appears to be pivotal as an anti-apoptotic factor in many different cell death paradigms. We investigated this crucial survival pathway in three models of acquired hearing loss using CBA/J mice: kanamycin-induced deafness, noise trauma, and age-related sensorineural hearing loss. Immunostaining on cochlear cryosections revealed a rather wide-spread distribution of PIP3 in the cochlea which was markedly attenuated following any of the three insults. Consistent with a reduction of PIP3, the phosphorylation of the downstream target Akt at threonine 308 also significantly decreased in outer hair cells. Since the levels of PIP3 are reciprocally controlled by phosphoinositide 3-kinase and the phosphatase PTEN (phosphatase and tensin homologue deleted on chromosome ten) we began investigating the expression of theses enzymes. Preliminary results show increased PTEN activity in aging cochleae suggesting a decline of the survival capacity of traumatically affected outer hair cells via PIP3/Akt signaling due to an increase of PTEN.

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688 Transient-Receptor-Potential Channel TRPM3 Deficiency Leads to Noise Vulnerability and Progressive Hearing Loss in Mice

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Transient-receptor-potential channels (TRPs) are a heterogeneous family of transmembrane proteins that vary by their activation mechanisms and cation selectivities. Some of the TRPs have been proposed to be involved in cochlear function or to be candidates for proteins involved in the hair cell transduction process. However, the role of many other TRP variants for hearing is still unclear.

TRPM3 has not been described in the inner ear so far and the expression and function in the cochlea and auditory brain structures are unknown. Using TRPM3 specific primerpairs and riboprobes we could detect TRPM3-transcripts in the inner ear of rat and mouse. Within the inner ear, TRPM3 was abundant in the stria vascularis and spiral ganglion. RT-PCR-analysis showed TRPM3 transcripts also in the apical and basal turns of the organ of corti and in isolated inner and outer hair cells.

To examine the role of TRPM3 in auditory function we generated a TRPM3-deficient mouse line by deletion of the pore coding region of the TRPM3 gene and analyzed auditory evoked brainstem responses (ABR) and distortion product otoacoustic emissions (DPOAE). Hearing of young TRPM3-deficient mice was similar as compared to wildtype littermate controls although TRPM3-deficient mice were already significantly more vulnerable to acoustic overstimulation. In aged TRPM3-deficient mice, spontaneous hearing functions were significantly affected.

The pattern of hearing loss will be analyzed in respect to inner and outer hair cell function and correlated with the expression pattern and phenotype of the inner ear in TRPM3-deficient mice. We propose an important role of TRPM3 channels in the inner ear for retention of normal function and for the response to excitatory overstimulation.

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689 Susceptibility to Noise-Induced Hearing Loss in Two Congenic Mouse Strains

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In 1993 Erway et al. identified a single genetic locus (*Ahl*) that could explain the early presbycusis observed in the inbred C57BL/6J mouse strain. This locus was later shown to make this strain more vulnerable to noise-induced hearing loss (Davis et al., 2001). This locus is believed to code for cadherin 23.

Recently, Johnson has developed two relevant congenic mouse strains. In the congenic C57BL/6J strain, the *Ahl* locus has been replaced by the wild-type locus from the inbred CBA/CaJ strain (strain B6.CBA). In the congenic CBA/CaJ strain the wild-type locus has been replaced by the mutant *Ahl* locus (strain CBA.B6). The present study was designed to test these congenic strains for their vulnerability to noise-induced hearing loss.

Mouse hearing was tested pre-exposure by ABR at 8, 16 and 32 kHz. Groups of both strains were exposed to various levels of broadband noise between 98 and 118 dB for one hour. Seven to nine days later their hearing was again tested by ABR.

Generally, pre-exposure the B6.CBA congenics started out with more sensitive ABR thresholds than the CBA.B6s (strain differences were statistically significant at all frequencies pre-exposure.) This would be expected based on the fact that the congenic CBA.B6 mice genotypes included the mutant *Ahl* locus.

This presentation will compare ABR results in the two congenic strains as well as compare the present data with the previous inbred C57BL/6J and CBA/CaJ data.

690 PLZF-Deficient Mouse Mutants Do Not Generate Conditioning-Mediated Protection from Acoustic Trauma

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The cochlea can be "conditioned" to resist acoustic trauma via a corticosteroid-dependent process (Tahera et al, 2006). The amount of conditioning-related protection is remarkable; up to 40 dB of acoustic threshold shift can be prevented. The spectrum of damage in acoustic trauma ranges from excitotoxicity in afferent dendrites to apoptotic loss of hair cells, all of which can be prevented by conditioning. While many potential targets of corticosteroid activation have been analyzed in the ear, no compelling mechanism has yet been identified for its action. We have now identified a transcriptional protein, PLZF, which is present in the spiral ganglion, organ of Corti, and spiral ligament, all targets for acoustic trauma. PLZF mRNA is elevated in the mouse cochlea following acoustic stimulation, restraint stress, and corticosteroid treatment. PLZF appears to play an essential role in conditioning resistance to acoustic trauma: mice deficient in PLZF have hearing and responses to acoustic trauma similar to their WT littermates, but are unable to generate restraint-stress (conditioning) mediated protection against acoustic trauma.

691 Genes Contributing to Noise Resistance on Chromosome 17 in 129S6 Mice

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The inbred mouse strain 129S6/SvEvTac (S6) shows strong noise resistance (NR) when compared to CBA/CaJ (CB). S6 shows significantly less noise induced permanent threshold shift (PTS) than CB after identical exposure (8-16 kHz OBN, 103 dB SPL, 2 hr) when evaluated by ABR or DPOAE two weeks post-exposure. We have developed a quantitative trait locus (QTL) map for the trait of NR in S6. We generated F1 animals by intercrossing S6 x CB. N2 animals were generated by backcrossing the F1s to S6, the recessive parental strain. Approximately 250 N2 animals were tested for NR as described above. Chromosomal regions contributing significantly to NR were identified using the Rqtl analysis program. In S6, five QTL peaks were identified ($p<0.05$); nr1 on proximal mChr 17 had a LOD >7.0 ($p<0.001$) (Tempel et al., Abstr. 235, ARO, 2009).

We have used an integrative genomics approach to identify and evaluate candidate genes in nr1. First, the nr1 QTL region was narrowed using addition markers in the region. Second, consomic and congenic strains of mice were developed to further narrow the region and to determine if the nr1 locus would show noise resistance in isolation, i.e. when only one part of the genome was S6/S6. Third, presuming candidate genes would be encoded by S6, we identified regions within nr1 with ancestral S6 haplotypes. Fourth, we performed a systems analysis based on whole genome expression arrays comparing cochlear RNA expression in S6 vs CB, each when unexposed to noise, within one hour after noise exposure and at 24 hrs post exposure. Systems analysis algorithms identified genes whose expression profile changed coordinately over these time points and whose functions are related by the Gene Ontology database. Genes identified by systems analysis and localized to mChr 17 were identified. Finally, we sequenced candidate genes in the nr1 region to identify sequence differences between S6 and CB that might underlie noise resistance. We will present our molecular analysis the three leading candidate genes in the region and discuss our hypothesis for the functional role of the lead candidate gene in noise resistance.

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692 The Blood-Labyrinth-Barrier Repair After Loud Sound Injury Through Bone Marrow Cell Recruitment Mediated by a Local INOS Signal Pathway

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Sound trauma significantly impairs the cochlear blood–labyrinth barrier (BLB) and causes cochlear hypoxia and inflammation. However, neither the cellular process nor signaling pathways for wound repair in the ear are understood. In this study, irradiated C57BL/6J and B6.129P2-*Nos2*^{tm1Lau}/J mice were transplanted with GFP$^+$-BMDCs from C57Bl/6-Tg (UBC-GFP) mice. We then investigated the involvement of GFP$^+$-BMDCs in the region of the BLB in non-sound stimulated and in acoustic trauma conditions. We found irradiated and BM transplanted control mice have normal hearing thresholds either immediately after irradiation or two months after irradiation. No GFP$^+$-BMDCs were observed to migrate into the area of the BLB in the control mice following one month transplantation. Only small numbers of GFP$^+$-BMDCs were found to infiltrate into the area of the BLB two months after transplantation in the recipient mice. In contrast, when the animals were exposed to wide-band noise at a level of 120 dB for 3 hours/day for 2 consecutive days, robust BMDC migration was observed in the acoustic trauma cochlea. GFP$^+$-BMDCs migration was most prominent during the first week after acoustic trauma, and they accumulated significantly at two weeks in the stria vascularis. Some of the infiltrated BMDCs cells were positioned near capillaries and were seen to be fused with endothelial cells at four weeks. Most of the BMDCs expressed F4/80 and were identified as macrophages. Decreased CXCR4 (SDF-1α receptor) expression was found in iNOS$^{-/-}$and iNOS inhibitor treated mice. Moreover, the GFP$^+$-BMDCs recruitment was significantly reduced in the iNOS$^{-/-}$and iNOS inhibitor treated mice in comparison with GFP$^+$-BMDCs recruitment in the iNOS$^{+/+}$mice. These data suggest that the circulating bone marrow cells were

mobilized by acoustic trauma and migrated to the injured region. iNOS is an important signaling for SDF-1/CXCR4-mediated bone marrow cell recruitment and repair during acoustic trauma.

693 Bone Marrow Cells to Resident Tissue Macrophages in Relation to INOS-Derived Nitric Oxide in the Blood-Labyrinth-Barrier

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The blood-labyrinth-barrier (BLB) of normal cochlea contains a large population of perivascular cells (macrophages). The macrophages were identified by antibodies raised against cell surface molecules, such as F4/10 and CD68, expressed on macrophages. Confocal imaging showed the macrophages to be closely associated with microvessels, regularly distributed along the BLB, and structurally intertwined with endothelial cells and pericytes. Turnover and repopulation of these cells under normal and pathological conditions was studied in a model of lethally irradiated C57 BL/6J mice transplanted with GFP bone derived marrow cells (GFP+-BMDCs) from C57Bl/6-Tg (UBC-GFP) mice. Normal and acoustic traumatic mice were sacrificed at four, five, six, and eight weeks. The cochlear lateral wall tissues were then prepared. The resident macrophage population remained unchanged over the course of one month of observation in the cohort not exposed to loud sound. In contrast, resident macrophages in mice receiving 120 dB wide-band noise for 3 hours a day for 2 consecutive days reduced in number and migrated. Of significance, blood-derived macrophages with the GFP label were observed to be recruited immediately following acoustic injury and remained prominent the first week after acoustic trauma. Increased nitric oxide in the cochlear stria vascularis can act as one of the signals for the infiltration. Infiltration of GFP-labeled macrophages was significantly reduced in the animals pre-treated with an iNOS inhibitor and in the iNOS-/- mice. Our findings suggest that BMDCs are able to migrate across the BLB and replace resident tissue macrophages and that iNOS plays an important signaling role for driving BMDCS recruitment.

694 Cyclic AMP (CAMP) Signaling Plays Differential Roles in Spiral Ganglion Neuron (SGN) Axon Regrowth and Synaptogenesis on Inner Hair Cells (IHCs) Following Excitotoxic Trauma

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cAMP signaling has been shown to be important in axon growth, synaptic plasticity and modulation of AMPA currents. We developed an in vitro model of denervation and reinnervation of IHCs following excitotoxic trauma in neonatal rat cochlea. We have used this system to investigate the role of cAMP signaling in regeneration of the type I SGN peripheral axons and their synapses on IHCs. We quantify regeneration of peripheral axons and of synapses on IHCs by counting immunofluorescently-labeled peripheral axon-IHC contacts and the number of postsynaptic densities (PSDs) on each IHC. We previously showed that brief exposure to high level of the glutamate receptor agonist causes degeneration of 99% of type I SGN peripheral axons and IHC-SGN synapses. Reinnervation of IHCs occurs and new IHC-SGN synapses form. However, not all axons regenerate and the number of PSDs does not recover to the pre-trauma level. This in vitro model mimics aspects of noise-induced damage to the cochlea in vivo. We find that cAMP signaling has differing effects on axon regrowth and synaptogenesis. With regard to axon regrowth, elevation of cAMP signaling with a cell membrane-permeant cAMP analog, cpt-cAMP, increased axon regrowth in a protein kinase A (PKA)-dependent manner. With regard to formation of synapses, disruption of cAMP signaling reduces the number of PSDs, while H89, a PKA inhibitor enhanced the regeneration of PSDs. Thus, axon regrowth and synaptogenesis are distinct processes, regulated differently by cAMP signaling. We also examined a role for cAMP signaling in the initial degeneration of SGN afferent terminals caused by kainic acid (KA). Inhibition of PKA partially protected the afferent terminal from degeneration. In contrast, cpt-cAMP exacerbated excitotoxic damage to the type I SGN peripheral axons. Because PKA is a downstream target of the D1 dopamine receptor, this result may help determine the role of dopaminegic efferent systems in noise-induced trauma in the cochlea in vivo.

695 Noise-Induced Permanent Threshold Shift: Hair-Cell Loss Is Not the Whole Story!

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Noise-induced temporary ABR threshold shifts & DPOAE level shifts have been shown to be highly correlated with supporting-cell histopathology, especially in cases where there is little hair-cell loss. Permanent ABR threshold shifts (PTS) & DPOAE level shifts (PLS) have been attributed to losses of hair cells only. To determine the relation between cochlear pathology & functional deficits, we tested the hearing of 38 chinchillas with ABR thresholds and DPOAE levels for 0.5-20 kHz in 13 & 35 steps, respectively. Each animal was exposed to a 0.5- or 4-kHz OBN at 80–95 dB SPL for 24 h, or 108 dB SPL for 1-2 h. After a recovery time of 7–28 days, functional testing was repeated and the cochleae fixed in-vivo with OsO_4. The cochleae were prepared as plastic-embedded flat preparations. Organ of Corti (OC) length was measured & missing IHCs, OHCs & pillar cells were quantified. The size, type & location of focal lesions (≥ 50% concentrated loss of IHCs, OHCs or both, over a distance of at least 0.03 mm) were determined. Myelinated nerve fiber (MNF) loss in the osseous spiral lamina was estimated; pathology of pillars & Deiters' cells was graded. PTS & PLS were calculated at the frequency place of 148 lesions found in 46 OCs. These data were analyzed by lesion type for size & functional

loss. Findings were: PTS & PLS increased with size for all lesion types; small IHC lesions produced a PTS & no PLS while medium-large IHC lesions produced both; small-medium OHC lesions did not produce a PTS or PLS while large OHC lesions produced both; combined IHC/OHC lesions were larger & produced PTS & PLS; & MNF loss was associated with IHC loss. The magnitudes of PTS & PLS in medium-large lesions could not be accounted for by their size & % of IHC & OHC losses alone. The excess PTS & PLS was found to correlate with pathology of pillar & Deiters' cells. These observations suggest that: ABR thresholds are dominated by input from IHCs; MNF loss is generally secondary to IHC loss; & function in surviving IHCs & OHCs is compromised when supporting cells are permanently damaged & the tectorial membrane is uncoupled from the reticular lamina.

696 Ebselen Treatment for Noise Induced Hearing Loss Through Activation of Nrf2 Regulated Gene Expression

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Noise exposure leading to hearing loss is most commonly associated with loss of sensory cells (hair cells) and damage to non-sensory structures (i.e. stria vascularis, spiral ligament) in the inner ear. Ebselen, a small molecule mimic of glutathione peroxidase (GPx), has been shown to increase cochlear protein levels of GPx1 following low oral dosing in rats (4mg/kg) (Kil et al., Hear Res. 2007). The spiral ligament and stria vascularis appeared to be among the most affected structures within the noise exposed cochlea and had the highest level of GPx1 protein in both rats and mice. Ebselen induces gene expression changes through electrophilic activation of NF-E2-related factor 2 (Nrf2) (Sakurai et al., ChemResTox, 2006) and has been shown to reduce cisplatin induced ROS generation through Nrf2 activation in auditory cells (Kim et al., Hear Res, 2009). We are investigating changes in gene and protein expression in the cochlea of Nrf2 wt and ko mice exposed to noise with and without ebselen. ABR and DPOAE analysis in animals prior to and 1 hour, 1 day, and 1 month after noise exposure will be correlated with results from Affymetrix Mouse Gene 1.0ST array analysis and immunostaining for proteins whose expression is known to be regulated by Nrf2. Current results demonstrate that Nrf2 KO mice exhibit higher ABR threshold shifts than wt littermates following noise exposure. A detailed analysis of histological, gene and protein expression data will be discussed in the context of the contribution of Nrf2 in hearing loss and ebselen mediated otoprotection.

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697 Prevention of Noise-Induced Hearing Loss with an Inhibitor of NADPH Oxidase

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Toxic levels of reactive oxygen species (ROS) are key contributors to the lesion of dead outer hair cells (OHCs) seen in the cochlea after noise exposure. The current study was undertaken as an extension of Bielefeld et al. (2005) in which paraquat was used to demonstrate that NADPH oxidase is active in the cochlea and can contribute to cochlear ROS formation. NADPH oxidase is an enzyme that catalyzes a reaction in which NADPH donates an electron to molecular oxygen to generate the ROS superoxide. The hypothesis for the current study was that increased NADPH oxidase activity is partly responsible for the burst of ROS seen in the cochlea shortly after noise exposure. Therefore, blockade of NADPH oxidase would lead to reduced noise-induced ROS, OHC death, and hearing loss. NADPH oxidase was inhibited pharmacologically with AEBSF, a known inhibitor of NADPH oxidase activation (Diatchuk et al., 1997) delivered into the cochlea surgically through the round window membrane. The contralateral ears received distilled water as a vehicle control. Ten chinchillas were treated and exposed to a 4 kHz octave band noise at 106 kHz for 6 hours. Seven chinchillas were treated and exposed to 75 pairs of 155 dB pSPL impulses. ABR threshold shift and OHC loss data showed that the AEBSF treatment reduced cochlear damage from the impulse noise, but not from the continuous noise. Research supported by a New Investigator Research Award from the American Academy of Audiology.

698 Effect of Oral Administration of a Combination of 4-OHPBN and NAC on Noise-Induced Hearing Loss

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Acute acoustic trauma (AAT) results in oxidative stress to the cochlea through overproduction of cellular reactive oxygen, nitrogen, and other free radical species. Recently, a hydroxylated alpha-phenyl-tert-butylnitrone (4-OHPBN) alone and in combination with other antioxidant drugs such as N-acetyl-L-cysteine (NAC) and acetyl-L-carnitine (ALCAR) has been reported to reduce noise-induced permanent hearing loss by suppressing oxidative stress. In the previous study, chinchilla were treated with intraperitoneal injections of 4-OHPBN or in combination with NAC or NAC plus ALCAR. In this study, however we tested whether oral administration of 4-OHPBN plus NAC reduces permanent hearing threshold shift, distortion product otoacoustic emission (DPOAE), and outer hair cell loss in a dose-dependent manner.

Thirty-six chinchilla (six per group) were exposed to a 105 dB octave-band noise centered at 4 kHz for 6 hours and received the following treatments: 1) noise-exposed control group, 2) 4-OHPBN (10mg/kg) + NAC (20mg/kg), , 3) 4-OHPBN (20mg/kg) + NAC (50mg/kg) , 4) 4-OHPBN (50mg/kg) + NAC (100mg/kg) , 5) 4-OHPBN (75mg/kg) + NAC (200mg/kg) orally administrated beginning 4 h after noise exposure and twice daily for the next 2 days. Auditory brainstem response threshold shifts, DPOAE, and outer hair cell (OHC) loss were analyzed with ANOVA.

Oral administration of the combination of 4-OHPBN plus NAC decreased permanent threshold shifts, increased DPOAE amplitude, and reduced OHC loss in a generally dose-dependent manner similar to intraperitoneal administration. The present study provides significant and important information in the effective treatment of AAT.
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699 D-Methionine (D-Met) Provides Significant Outer Hair Cell and ABR Threshold Rescue from Noise Exposure

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For over a decade we have been investigating D-met as a protective agent against radiation- induced oral mucositis, and cisplatin-, aminoglycoside- and noise-induced hearing loss in animal studies. We now have documented significant protection from radiation-induced oral mucositis and cisplatin-induced hearing loss in 2 Phase II human studies without side effects. We earlier described that D-met can effectively prevent noise induced hearing loss (NIHL) even when first initiated 1 hour after noise cessation and have described pilot data suggesting that these time delays may be increased. A rescue agent for NIHL would have great clinical applicability because many noise exposures are unexpected. Further an individual may not be able to immediately access the protective agent (eg military and emergency personnel, car airbag deployment).
In this study, 5 groups of male chinchillas Laniger were individually exposed to a 105 dB SPL narrow band of noise centered at 4 kHz for 6 hours. In the 4 experimental groups, ip D-met (200 mg/kg per dose) was initially administered at 1, 3, 5 or 7 hours hour post noise exposure plus 4 additional doses BID (5 doses) at 12 hour intervals. The controls received equivalent volume saline.
Auditory brainstem response testing (ABR) was performed at baseline and on post-exposure days 1, 14 and 21. ABR thresholds were measured in response to tone bursts centered at the frequencies of 2, 4, 6, and 8 kHz.
At 21 days post noise exposure animals receiving D-met at the 1,3 and 5 hour time delays had significant ABR threshold protection at 2, 4, 6 and 8 kHz and outer hair cell (OHC) protection at the corresponding frequency regions. For the 7 hour delay, ABR threshold protection reached significance at 2, 4 and 8 kHz but not 6 kHz. We are still analyzing the OHC data for the 7 hour delay epoch. Although D-met was only given for 2 days after the noise, ABR thresholds showed a trend in improvement even from 14 to 21 days after the noise exposure.

700 Protective Effect of Calmodulin Blockers in Acoustic Injury of the Mouse

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Calmodulin blockers reportedly exhibit to have a protective effect from cell death of central nervous system. The purpose of the present study was to examine the protective effect of several types of calmodulinl blockers on the cochlea in acoustic injury. Female ddY mice of 8 weeks of age were used in this study. Animals were subjected to a 4 kHz pure tone of 128 dB SPL for 4 hours through an open field system inside a sound-exposure box. Auditory brainstem response (ABR) was examined before, one and two weeks after acoustic overexposure. After final ABR measured at two weeks after acoustic overexposure, whole mounts of organ of Corti were stained for the nucleus with propidium iodide, and missing hair cells (missing of staining with propidium iodide) were counted every 0.33 mm segments. Calmodulin blockers significantly improved the ABR threshold shifts and decreased hair cell loss two weeks after acoustic overexposure when it was administrated before acoustic overexposure. The present findings suggest that calmodulin blocker has protective effects against acoustic injury of the cochlea.

701 Genetic and Temporal Aspects of Protection Against Noise-Induced Hearing Loss by Kanamycin in Mice

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We recently described surprising protection against permanent noise-induced hearing loss (NIHL) by low-dose kanamycin (KN) in young mice (Fernandez et al., JARO in press). CBA/J mice were injected twice daily with a sub-clinical dose of KM (300 mg/kg, sc) or saline for 10 days beginning at 20 days of age, then exposed on day 11 to broadband noise (4-45 kHz, 110 dB SPL) for 30 s. Post-exposure ABR testing (5-40 kHz) was performed 10 days later. While saline-treated mice showed ~40 dB threshold shifts across frequencies and extensive OHC loss in the basal half of the cochlea, KM-treated mice showed thresholds statistically indistinguishable from unexposed controls, and no hair cell loss above the hook region. More recent experiments in our laboratory have addressed the following questions: 1) What is the minimal effective KM dosing paradigm? 2) Is protection by KM restricted to young mice? 3) Is protection restricted to CBA/J mice?
Reducing the dosing frequency of KM in young CBA/J to 1/day or 1 every 2 days was as effective as 2/day. KM every 3rd day was effective, but less so than more frequent dosing. A single dose was ineffective, whether administered 1 hr, 24 hr, or 48 hr prior to noise. Young KM-treated C57BL/6 (B6) mice exposed to 4 min of noise

were not significantly protected from noise when compared to saline-treated B6.
In summary, protection by low-dose KM against NIHL in young mice requires multiple doses, and appears to constitute a form of pre-conditioning. Mere presence of KM (as in single dose tests) is not sufficient, arguing against any simple action such as plugging OHC transducer channels. While it is possible that protection by KM in B6 mice requires different dose timing or levels than in CBA/J, aspects of the protective effects of KM in mice appear to vary with genetic background. It may be possible to use inter-strain differences to genetically dissect the critical biochemical pathways for protection.
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702 The Role of Prostaglandin E Receptor Subtypes EP2 and EP4 in Autocrine and Paracrine Functions of Vascular Endothelial Growth Factor in the Inner Ear

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Prostaglandin E1 (PGE1) is frequently used for the clinical treatment of acute sensorineural hearing loss (SNHL), however the mechanisms for the clinical effects of PGE1 have not yet been elucidated. The physiological effects of PGE1 and prostaglandin E2 (PGE2)are mediated by PGE receptor subtypes EP1, EP2, EP3 and EP4, the respective agonists for which have been purified. PGE1 and PGE2 can increase vascular endothelial growth factor (VEGF), particularly through EP2 and EP4. The biological effects of VEGF are mediated by the phosphotyrosine kinase receptors fms-related tyrosine kinase-1 (Flt-1) and fetal liver kinase-1 (Flk-1).
In this study, we examined the efficacy of a local EP4 agonist application to the cochlea for the treatment of SNHL. The protective effects of local EP4 agonist treatment before or after noise exposure were tested in guinea pigs using measurements of auditory brain-stem responses (ABRs) and histological analysis. Subsequently, we examined the efficacy of a local EP2 and EP4 agonist application on the production of VEGF proteins and mRNAs in mouse cochleae using an enzyme-linked immunosorbent assay and the real-time quantitative reverse transcription-polymerase chain reaction, respectively. We moreover investigated the localization of EP2, EP4, VEGF, Flt-1 and Flk-1 in mouse cochleae by immunohistochemistry.
The results demonstrated EP2 and EP4 expression in the cochlea, and showed pre- and post-treatment with the EP4 agonist significantly attenuated threshold shifts of ABRs, and significant attenuation in the loss of outer hair cells was found in local EP4 agonist treatment before noise exposure. The local application of the EP2 or EP4 agonist demonstrated an increase in VEGF protein and VEGF mRNA levels. Immunoreactivity for EP2, EP4, Flt-1 and Flk-1 was found in the cochlea. Furthermore, the intensity of the VEGF immunoreactivity in the spiral ganglion appeared to be increased by the local application of the EP2 or EP4 agonist.
These findings indicate local EP4 agonist treatment could attenuate acute SNHL, and demonstrate that EP2 and EP4 agonists stimulate VEGF production in the inner ear, particularly in the spiral ganglions. Moreover, the Flt-1 and Flk-1 expression observed in the present study suggests that VEGF has paracrine and autocrine actions in the cochlea. Thus, EP2 and EP4 might be involved in the mechanisms underlying the therapeutic effects of PGE1 on acute sensorineural hearing loss via VEGF production.

703 Blockade of Interleukin-6 Signaling Suppressed Cochlear Inflammatory Response and Improved Hearing Impairment in Noise-Induced Damaged Mice Cochlea

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Backgrounds: Hearing impairment or deafness can be the cause of serious socio-economic disadvantages. Recent studies have shown that inflammatory responses in the inner ear co-occur with various damaging conditions including noise-induced hearing loss. We reported that pro-inflammatory cytokine interleukin 6 (IL-6) was induced in the cochlea 6 hrs after noise exposure, but the pathophysiological implications of this are still obscure. To address this issue, we investigated the effects of IL-6 inhibition using the anti IL-6 receptor antibody (MR16-1) with a mouse hearing-loss model.
Methodology: Noise-exposed mice were treated with MR16-1 and evaluated. Improved hearing at 4 kHz as measured by auditory brainstem response (ABR) was noted in noise-exposed mice treated with MR16-1. Histological analysis revealed that the decrease in spiral ganglion neurons was ameliorated in the MR16-1-treated group, while no significant change was observed in the organ of Corti. Immunohistochemistry for Iba1 and CD45 demonstrated a remarkable reduction of activated cochlear macrophages in spiral ganglions compared to the control group when treated with MR16-1.
Conclusion: Thus, MR16-1 had protective effects both functionally and pathologically for the noise-damaged cochlea primarily due to suppression of neuronal loss and presumably through alleviation of inflammatory responses. Anti-inflammatory cytokine therapy including IL-6 blockade would be a feasible novel therapeutic strategy for acute sensory neural hearing loss including noise-induced hearing loss.

704 Acute Noise Induced Hearing Loss Is Reduced by Local Application of Steroids

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Noise exposure, despite protection measures, often leads to an acute noise trauma. The acute noise trauma quite frequently results in a permanent threshold shift (PTS). Treatments to limit the PTS show that corticosteroids may provide an effective medication. Here we investigated the use of corticosteroids in a preclinical animal model of acute noise trauma. Exposure to impact noise led to a permanent hearing loss in the entire frequency range. Hair cell loss was observed in the middle and apical region of the cochlea. Both the permanent hearing loss and hair cell loss was reduced after 2 week treatment with corticosteroids in a dose-dependent fashion. After noise trauma, local application of high-dose dexamethasone (1 and 4 mg/ml), prednisolone (25 mg/ml) or methylprednisolone (12.5 mg/ml) at the round window of the cochlea showed highest effects. With dexamethasone and prednisolone a statistically significant threshold improvement was observed. Dexamethasone also a clearly improved hair cell preservation compared to the untreated controls. To achieve high drug concentrations, effective to treat acute noise trauma, the application of steroids to the round window of the cochlea was necessary.

705 A Corticotropin-Releasing Factor System Expressed in the Cochlea Modulates Hearing Sensitivity and Protects Against Noise-Induced Hearing Loss and Oxidative Stress

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Little is known concerning the mechanisms establishing cochlear sensitivity and susceptibility to noise and drug-induced acoustic trauma. While the corticotropin-releasing factor (CRF) system is involved in activation of the classic hypothalamic-pituitary-adrenal axis, it is also involved in local physiological responses to stress in many tissues, and is expressed in the inner ear. We demonstrate that mice lacking the CRF receptor CRFR2, exhibit a significantly lower auditory threshold than wild type mice, but this gain of function comes at the price of increased susceptibility to acoustic trauma. We further demonstrate that glutamatergic transmission, purinergic signaling, and activation of Akt pathways within the cochlea are misregulated, which may underlie the enhanced sensitivity and trauma susceptibility observed in CRFR2-/- mice. Our data suggest that CRFR2 constitutively modulates hearing sensitivity and thereby may provide protection against noise-induced hearing loss. With respect to acoustic insults, generation of reactive oxygen species is a common denominator in many conditions that lead to cell death in the cochlea. While great effort has gone into discovering compounds that can prevent ROS-induced damage, less is known of the endogenous mechanisms in place to mitigate damage due to oxidative stress. We demonstrate that activity via the CRHR2 class of receptors protects against H2O2- and gentamicin-induced ROS and induced caspase-3 (pro-apoptotic) activity. We also use a shotgun differential proteomics approach to define changes induced by CRHR2 activity that may further explain the molecular mechanisms underlying its protective ability. These experiments demonstrate for the first time a role for CRH signaling in protecting the cochlea against oxidative stress, and suggest novel mechanisms other than induction of free radical scavengers that are involved in this protective mechanism.

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706 Connexin 43 Hemichannels Are Likely to Mediate Gentamycin Uptake in MDCK Cells

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Madin-Darby canine kidney (MDCK) cell has been used to study the cellular mechanism of aminoglycoside (AG) uptake and ototoxicity since it shares many features with cochlear strial epithelial cells. We have shown that Texas Red-tagged gentamicin (GTTR) can rapidly enter MDCK cells independently of endocytosis (Myrdal et al., 2005b), and the uptake is enhanced by low $[Ca^{2+}]_o$, suggesting membrane ion channels may mediate the uptake, although direct evidence for channel permeation remains elusive. As AGs are typically polyvalent cations, we hypothesize that AG uptake is mediated via multiple non-selective channels (NSCs). In this study, employing whole-cell and patch-clamp recording techniques, immunocytochemistry and GTTR confocal imaging analysis, we test the hypothesis that the big conductance connexin 43 (Cx43) hemichannels are present in MDCK cells to mediate the AG-uptake.

We found that: (1) confluent cells had a low input resistance (40 - 200 MΩ), large input capacitance (100 - 1000 pF) and multiple-term exponential capacitive transients, indicating a gap-junction mediated electrical coupling. 18β-glycyrrhetinic acid (18βGA, 30 μM) largely blocked this coupling. 2) With physiological solutions in the bath (high NaCl, 1.6 mM Ca^{2+}) and pipette (high K^+, buffered 118 nM free Ca^{2+}), single MDCK cells (plated <30 h) showed a resting potential of (-21 ± 1.1 mV, n=45). A nominal zero Ca^{2+} bath solution often increased conductance of whole-cell I/V curve in the voltage range tested (-140 to 100 mV) and the net current I/V had a reversal potential near 0 mV. Zero Ca^{2+} solution also enhanced open probability of unitary current that had a single-channel conductance of ~90 pS and a reversal potential near 0 mV in both whole-cell and outside-out patches. 3) This unitary current was suppressed by 30 μM Gd, 18βGA or 0.1-5 mM La, consistent with the feature of a hemichannel. 4) MDCK cells immuno-express Cx43 at the basolateral and apical membranes. 5) La (5 mM) and 18βGA (30 μM) both reduced GTTR uptake by 44 ± 32% and 51 ± 7.2%, respectively. We conclude that Cx43

hemichannels in MDCK cells play an important role in mediating aminoglycoside uptake.
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707 Identification of Ion Channel(S) Mediating Gentamicin Trafficking in Cochlear Strial Marginal Cells of Rodents

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Aminoglycosides (AG) are clinically important antibiotics but cytotoxic to inner ear hair cells causing hearing and balance deficits. Systemically applied gentamicin results in drug loading of perilymph, endolymph, the stria vascularis (SV), particularly marginal cells (MC), and hair cells. How AGs pass through blood-labyrinth barrier and into endolymph remains unknown. One hypothesis suggests that AGs are trafficked across marginal cells via multiple non-selective channels (NSC). We used whole-cell and patch recording and conventional intracellular recording (ICR) techniques on cells of *in vitro* stria vascularis (SV) of guinea pigs and a few mice, and connexin (Cx) immunocytochemistry, to test this hypothesis.
We found: (**1**) Whole-cell recording revealed at least three cell types with distinct membrane properties. First, presumably MCs, patched at the endolymphatic surface had a resting potential (RP) ~-40 mV, an input resistance (R_{in}) 0.5-1.5 GΩ, a single exponential capacitive current decay and an input capacitance (C_{in}) 20-80 pF. 18β-glycyrrhetinic acid (18βGA) changed R_{in} to >1 GΩ and C_{in} to <~30 pF, suggesting there was very limited intercellular coupling. Depolarizations beyond -40 mV from the holding potential (-60 mV) activated a slowly developing outward rectifying current in some cells, characteristic of IsK channels. More often, a robust TEA-sensitive delayed rectification and a La^{3+}-sensitive non-selective conductance were observed. Second, cells patched from edge or basal side of the SV showed a RP ~-80 mV, R_{in} ~0.2 GΩ and C_{in} ~300 pF, but this changed to ~1.5 GΩ and ~30 pF following application of 18βGA, suggestive of intermediate cells. Ba^{2+} depolarized this cell with two EC_{50}s of ~65 and 2800 µM from ICR. Whole-cell I/V curves showed robust inward and outward rectifications. The third cell type, possibly basal cells, showed a RP ~-20 mV, R_{in} ~1.5 G, C_{in} ~8 pF and a slight outward rectification I/V. (**2**) In outside-out MC apical patches and some whole-cell recordings, a 57 pS unitary K^+-current (reversal near E_K) with high P_o at depolarization, and a 105 pS unitary NSC current (reversal near 0 mV) were frequently observed. On-cell patches of MC apical membrane with high K^+ and low Ca^{2+} pipette often exhibited an IsK current and unitary currents of NSC and K^+-currents. (**3**) Cx43 immunofluorescence was observed within the MC cytoplasm, and as puncta near the basolateral and apical cell membrane, in addition to a rich expression in fibrocytes. We conclude that marginal cells express multiple NSCs, including hemichannels that are potentially AG-permissive.
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708 Do Infection-Mediated Vasoactive Substances Influence Cochlear Uptake of Aminoglycosides?

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Aminoglycoside antibiotics are essential for prophylaxis or treatment of bacterial sepsis, but are cytotoxic to inner ear hair cells causing hearing and balance deficits. During bacterial infection, serum levels of endogenous vasoactive substances such as histamine and serotonin are frequently elevated. These vasoactive substances also modulate the vascular permeability of endothelial cells lining the blood-brain and blood-labyrinth barriers. Do these vasoactive compounds increase the permeability of the blood-labyrinth barrier to aminoglycoside antibiotics?
We tested this hypothesis using intraperitoneal injection of fluorescently-conjugated gentamicin (GTTR) followed by intravenous injection of histamine (100 ul/ 10g of 10^{-6} M/l) or serotonin (100 ul/ 10g of 10^{-7} M/l) 15 minutes later in mice. For control, 10 ml/kg of saline was intravenously administered. Mice were euthanized by transcardiac perfusion of 4% paraformaldehyde at thirty minutes and one hour after injection of GTTR. Following labeling with Alexa 488-conjugated phalloidin, different cochlear turns were examined using confocal laser scanning microscopy and the distribution of GTTR were compared to animals treated without vasoactive substances.
In the control group, cochlear uptake of GTTR increased over time with an increasing apico-basal gradient in the stria vascularis. In histamine group, strial uptake of GTTR was decreased (by 23-42%) compared to the control group. Serotonin had a variable effect on GTTR uptake within the stria vascularis, ranging from 85 to 172% of control animals.
These pilot data indicate that individual systemic vasoactive substances can modulate cochlear uptake of gentamicin. Since permeability changes in the BLB by infection-mediated vasoactive substances is a dynamic process depending on the duration of treatment and the concentration of the vasoactive substances, further controlled and combination studies are necessary.
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709 Noise Exposure Facilitates Gentamicin Uptake in Outer Hair Cells in Vivo

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Noise exposure or aminoglycoside treatment is detrimental to hair cell morphology and function. When the two insults are combined, synergistic toxicity is evident both morphologically and physiologically (Gannon and Tso 1969, Ryan and Bone 1982). The underling mechanism of this synergism remains unknown. One hypothesis is that noise exposure enhances hair cell uptake of the cationic

aminoglycosides, e.g. gentamicin. We tested this hypothesis by assessing the degree of gentamicin uptake by murine hair cells in the presence or absence of moderate or severe sound exposure.
With prior moderate sound exposure (86 dB SPL, wideband noise, 72-96 hours), and a subsequent 30-minute systemic gentamicin administration, many outer hair cells display increased gentamicin immunofluorescence over that seen in control animals. The OHCs had hair bundles visualized by phalloidin labeling. With prior severe sound exposure (100 dB SPL, wideband noise, 3 hours), and subsequent 30-minute systemic gentamicin treatment, basal hair cells had little hair bundle survival and weak gentamicin immunolabeling. More apically-located hair cells, with surviving hair bundles display increased gentamicin immunofluorescence over control cells.
Noise is thought to increase the conductance of the endolymphatic surface of the organ of Corti by increasing the open probability of non-selective cationic channels, e.g., purinergic P2X receptors. P2X2 immunofluorescence revealed distinct OHC hair bundle and supporting cell apical labeling. We performed quantitative RT-PCR analysis of P2X2, P2X4 and P2X7 mRNA expression in whole cochlear extracts. After severe noise exposure (117 dB SPL, wideband noise, 20 hours), P2X2 expression increased by 1.8 fold, with no change for P2X7 and reduced expression for P2X4. After chronic moderate noise exposure, no change was observed for P2X2, P2X4, and P2X7 mRNA transcripts. Our data tentatively suggests that noise exposure increases OHC uptake of gentamicin, potentially by altering the open probability of P2X2 channels on the hair cell apical membrane, most likely due to increased ligand (ATP) availability.
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710 Aminoglycosides Rapidly and Selectively Enter Hair Cells, Largely Via Mechanotransducer Channels

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Previous work indicates that aminoglycosides can enter hair cells via mechanotransducer channels. We further tested this hypothesis by investigating aminoglycoside uptake and its sensitivity to channel blockers. Uptake was tested in rat organ of Corti explants using gentamicin conjugated to Texas Red (GTTR). GTTR could be directly imaged in live cells and the time course of its uptake compared to that of gentamicin by immunolabeling. GTTR was first tested in an in vitro model of toxicity where it was found to be considerably more potent than gentamicin alone, a likely consequence of the purification procedure. A two-photon microscope was used to perform time-lapse studies of GTTR uptake over 1 hour. Uptake was rapid and selective to hair cells. Cotreatment with mechanotransducer antagonists slowed uptake considerably suggesting that GTTR entry into hair cells was mediated by open MET channels. Parallel experiments using a gentamicin antibody on organotypic cultures treated with native gentamicin revealed similar temporal and pharmacological results. An unusual finding however, was the gentamicin antibody did not label tissue that was fixed immediately after treatment but required several hours post treatment to reveal specific immunolabeling. Although unusual, this result was quite robust and suggests either that a metabolite of gentamicin is recognized by the antibody or that the gentamicin entering the cell somehow has its epitope shielded (by gentamicin-binding proteins perhaps). Finally, NADH imaging during treatment with neomycin demonstrated a rapid change in metabolic state of hair cells, but not supporting cells, indicative of rapid selective hair cell uptake of aminoglycosides.
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711 The Differential Vulnerability Across Cochlear Turns in Gentamicin-Induced Hair Cell Damage Is Correlated with the Differential Uptake of Gentamicin

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Purposes : Basal turn hair cells (HCs) in the organ of Corti (oC) is most vulnerable to gentamicin(GM) followed by middle turns and apical turns. Moreover, the differential sensitivity of HCs appears to be closely related to differences in highly reactive oxygen species (hROS) formation (Choung YH, 2009). The purpose of this study is to evaluate whether the differential vulnerability across cochlear turns in GM-induced HC damage is correlated with the differential uptake of GM.
Materials and Methods : Explants of basal, middle or apical turn in organ of Cortis (oCs) from neonatal (p3) rats were cultured and exposed to GM(50uM, 35uM) for 48h. The uptake of GM was evaluated by immunohistochemistry using anti-GM antibody. These explants were co-labeled with MyoVIIa (hair cell staining) and DAPI (nucleus staining) for detection HC loss across cochlear turns. The intensities of GM fluorescence expressed in HCs were compared in each turns and rows and also analyzed comparing with the loss of hair cells.
Results : Basal turn HCs showed more intense GM fluorescence than middle turn HCs and very little GM uptake was observed in the apical turn when OC explants were exposed to GM 35uM for 48h. The differential uptake of GM across turns was not definite in the OC explants exposed to GM 50uM. The intensity of GM fluorescence was strongest in the 1st row of outer HCs, followed by 2nd and 3rd rows. The expression of GM was correlated with the damage of HCs in all cochlear turns and outer HC rows showing differential vulnerability of HCs. Interestingly, GM uptake was not expressed in HCs with weak staining of MyoVIIa and GM uptake was well correlated with the intensity of MyoVIIa expression..

Conclusions : The differential vulnerability across cochlear turns in GM-induced hair cell damage is correlated with the differential uptake of GM and GM uptake may be dependent on MyoVIIa expression in HCs.

712 Determination of the Apoptosis and Cell Survival Signal Transduction in the Rat Cochlea Following Neomycin Induced Deafness

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Sensorineural hearing loss is associated with loss of the hair cells in the cochlea. By the common hypothesis, cell death provoked by deafferentation of neurons may reflect deprivation of the neurotrophic factors. However, gene expression profiling in the auditory nerve (AN) of deafened rats revealed that artemin, brain- (BDNF) and glial cell line-derived neurotrophic factor (GDNF) were significantly upregulated in the rat cochlea 26 days following neomycin induced deafness. The goal of this project is the determination of the signalling of neurotrophic factors in the context of apoptotic mechanisms by gene expression analysis following 7, 14, and 28 days deafening. Differential gene expression of BDNF and GDNF, their corresponding receptors trkB, $p75^{NTR}$ and GFRα1, the pro-apoptotic signal molecules caspase 9, Bax and the anti-apoptotic signal molecules GLAST, Bcl-2 will be studied within this time interval by real time-PCR. Morphometric analysis of the AN degeneration was determined by spiral ganglion neuron (SGN) count following hematoxyline/eaosine staining of paraffin-embedded cochlea-sections. Total RNA was extracted from 20 AN of normal and deafened animal sacrificed after 7 days, respectively, and reverse transcribed. Following the establishment of the housekeeping gene Rplp2 (ribosomal protein) as the internal standard we found an increase in the gene expression of GDNF and the receptors GFRα1, p75 and trkB demonstrating an early upregulation of neurotrophic factor. Also, a significant 1.8fold decrease of the SGN was demonstrated following 7 days deafening indicating strong apoptosis process. In general, gene expression data cannot be related to function, however, they allow the verification of the neurotrophin hypothesis. As well, gene expression profiling may clarify the role of neurotrophic factors in the deafening process within the time intervall of deafness.

713 Genetic Analyses of Interactions with Eukaryotic RRNA Identify the Mitoribosome as a Target in Aminoglycoside Ototoxicity

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Aminoglycoside ototoxicity has been related to a surprisingly large number of effects exerted by these compounds on different cellular structures and metabolic pathways. The finding that patients with mutations in mitochondrial rRNA are hypersusceptible to aminoglycoside-mediated ototoxicity has indicated a possible role for mitochondrial protein synthesis. To study the molecular interaction of aminoglycosides with the eukaryotic ribosome we made use of the observation that the drug-binding site is a distinct domain defined by small subunit rRNA, i.e. the decoding A-site. Here, we altered the drug binding site in bacteria so as to mimic the small subunit decoding region (A-site) of human ribosomes and to study its molecular interaction with aminoglycoside antibiotics. Compared to hybrid ribosomes carrying the A-site of human cytosolic ribosomes, hybrid ribosomes with the A-site of human mitochondrial ribosomes showed a distinct pattern of aminoglycoside susceptibility which correlated with the relative cochleotoxicity of these drugs. Sequence alterations corresponding to disease-associated mitochondrial A1555G and C1494U mutations increased drug binding and rendered the ribosomal decoding site hypersusceptible to aminoglycoside-induced mistranslation and inhibition of protein synthesis. Our results provide experimental support for aminoglycoside-mediated dysfunction of the mitochondrial ribosome. We propose a pathogenic mechanism in which interference of aminoglycosides with mitochondrial protein synthesis exacerbates the drugs' cochlear toxicity, playing a key role in sporadic dose-dependent and genetically inherited aminoglycoside-induced deafness.

714 Long-Term Effects of Focal Gentamicin-Induced Lesions in the Vestibular Neuroepithelia

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We have previously reported that small doses of gentamicin delivered directly to the perilymph of chinchillas results in complete loss of afferent calyces without concomitant loss of all type I hair cells in specimens evaluated up to six months post-administration. These very small gentamicin doses also resulted in the drastic reduction of stimulus-evoked modulation of afferent neuron discharge, though spontaneous discharge was preserved. We have extended this investigation to specimens dosed with 1 µg gentamicin and that have been allowed up to 2 years post-administration recovery times. Vestibular epithelia were immunohistochemically processed with anti-calretinin to visualize a crucial critical subset of afferent neurons. Neuroepithelia were also processed with fluorescent Nissl stains and phalloidin. Electrophysiologic recording was conducted to document spontaneous and evoked discharge from vestibular afferent neurons. We found that, even after long post-administration periods, hair cell densities in the crista central and utricular peristriolar regions were similar to that found at shorter intervals. These regions remained devoid of afferent calyces. These findings demonstrate that the gentamicin-induced lesions persist well after one year following their induction, providing no evidence that adult vestibular neuroepithelia harbor capabilities for spontaneous recovery of hair cells or afferent calyces. As found at shorter intervals, most neurons exhibited spontaneous discharge with characteristics similar to that expected in an untreated sample. We did find, however, that the probability of

recording from afferents responding to rotational stimuli was modestly greater than found at shorter post-administration periods. This may be indicative of a limited capability for physiologic recovery after prolonged post-administration periods, and indicates that this preparation may be optimal for studies of inner ear neurorehabilitation.

715 Combined Cisplatin-Ethacrynic Acid Treatment Induces Widespread Apoptosis: Cochlea, Cochlear Nucleus, Hippocampus, Cortex

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Although ethacrynic acid (EA) does not cross the blood-brain barrier, it enhances the influx of ototoxic drug into the ear by disrupting the marginal cells lining the lateral wall of the cochlea. Consequently when cisplatin (Cis) was co-administered with EA, there was rapid and extensive damage to sensory and supporting cells throughout the cochlea. Since cisplatin is neurotoxic, it could conceivably diffuse out of the cochlea and damage other regions of the brain. To evaluate this hypothesis, we co-administered Cis (0.8 mg/kg, IP) with EA (40 mg/kg, IP) to chinchillas and monitored apoptosis throughout the brain and body by intravenous administration of FLIVO, a lipophilic tracer that crosses the blood-brain barrier, enters the cytoplasm and fluoresces in cells with activated caspases undergoing apoptosis. FLIVO was administered intravenously at various times following Cis/EA treatment. Afterwards, the animal was perfused intracardially with 10% formalin in PBS and the brain, cochlea and vital organs harvested for histological analysis. Little or no apoptotic labeling was evident in the cochlea 6 h post-treatment; however, many FLIVO-positive apoptotic sensory, neural and supporting cells were present throughout the cochlea 18 h post-treatment, consistent with our previous reports. Surprisingly, extensive apoptotic labeling was also seen in the ventral cochlear nucleus (CN), but not in the dorsal CN, 18 h post-treatment. Remarkably, moderate apoptotic labeling also appeared at more central loci such as the hippocampus and temporal cortex (auditory). At earlier post-treatment times (6 h), massive apoptotic labeling appeared in liver and kidney consistent the known hepatoxicity and nephrotoxicity of these drugs. These results indicate that Cis/EA treatment not only results in massive apoptosis in the cochlea, but also widespread neurotoxicity with heavy cell death in the ventral CN and moderate apoptosis in the hippocampus and temporal cortex. Supported by NIH grants R01DC006630 and R01DC009219

716 Cochlear Outer Hair Cell Degeneration in Mice Induced by Co-Administration of Cisplatin and Furosemide

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The expanding arsenal of transgenic mice has created a powerful model for investigating the biological bases of ototoxicity and other disorders of the inner ear. However, the uptake of ototoxic drugs such as cisplatin into the inner ear is limited by the blood-cochlear barrier. Consequently, high doses or prolonged treatments with cisplatin are needed to damage the inner ear; but these dosing strategies more often than not lead to nephrotoxicity and low rates of survival in mice. Furosemide, a loop diuretic that damages the stria vascularis and disrupts the lateral cochlear wall, has been shown to significantly enhance the ototoxicity of aminoglycoside ototoxicity. Therefore, we explored the use of furosemide to accelerate and enhance the ototoxicity of cisplatin in mice. Co-administration of furosemide plus cisplatin led to a significant and rapid loss of hearing as reflected in ABR and DPOAE measures. The hearing loss from cisplatin plus furosemide was accompanied by significant loss of outer hair cells. Research supported in part by NIH grant R01DC006630.

717 Ototoxic Effects of Carboplatin in Organotypic Cultures in Rats and Chinchillas

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Carboplatin, a second-generation platinum chemo-therapeutic drug, is considerably less ototoxic than cisplatin. While common laboratory species such as mice, guinea pigs and rat are highly resistant to carboplatin ototoxicity, the chinchilla stands out as highly susceptible. Moreover, carboplatin causes unusual gradient of cell death in chinchillas; moderate doses selectively damage type I spiral ganglion neurons (SGN) and inner hair cells (IHC) and the lesion tends to be relatively uniform along the length of the cochlea. Higher doses eventually damage outer hair cells (OHC), but the lesion follows the traditional gradient in which damage is more severe in the base than the apex. While carboplatin ototoxicity has been well documented in adult animals in vivo, little is known about its in vitro toxicity. To elucidate the ototoxic effects of carboplatin in vitro, we prepared cochlear organotypic cultures from postnatal day 3 rats and adult chinchillas. Chinchilla cochlear and vestibular cultures were treated with carboplatin concentrations ranging from 50 µM to 10 mM for 48 h. Consistent with in vivo data, carboplatin selectively damaged IHC at low concentrations (50-100 µM). Surprisingly, IHC loss decreased at higher doses and IHC were intact at doses exceeding 500 µM. The mechanisms underlying this nonlinear response are unclear but could be related to a decrease in carboplatin uptake via active transport mechanisms (e.g., copper). Unlike the cochlea, the carboplatin dose-response function

was dose-dependent with the highest dose destroying all chinchilla vestibular hair cells. Cochlear hair cells and auditory nerve fibers in rat cochlear organotypic cultures were unaffected by carboplatin concentrations <10 μM; however, both OHC and IHC were damaged once the dose reached 100 μM; 500 μM destroyed all the cochlear hair cells, but hair cell loss decreased at high concentration and nearly all the cochlear hair cells were present at the highest dose, 5000 μM. Unlike the nonlinear dose-response seen with cochlear hair cells, rat auditory nerve fiber and spiral ganglion losses increased with doses above 50 μM with the highest dose destroying virtually all type I neurons. The remarkable species differences seen in vitro suggest that chinchilla IHC and type I SGN posses some unique biological mechanism that makes them especially vulnerable to carboplatin toxicity.
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718 Cisplatin-Induced TLR Expression of Cochlea Deteriorates Hearing Impairment in the Presence of LPS

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Cisplatin is often related with reduction of circulating leukocytes, which allow the host vulnerable against pathogens including bacteria which is efficiently eliminated through immune surveillance system such toll-like receptors (TLRs) pathway. In the present study we investigated whether cisplatin induces the expression of TLRs in mouse cochleae explants as well as HEI-OC1 auditory cells. In parallel with ABR threshold shift, the expression level of TLR-2, TLR-4, and TLR-9 protein was markedly increased in several cochlea regions from C57BL/6 mice administered with cumulative doses of cisplatin (total 16 mg/kg body weight). Interestingly, the additional administration of LPS in mice treated with harmless dose of cisplatin resulted in significant hearing impairment. We further demonstrated that a direct interaction between TLR and LPS, and the exasperating mechanisms of LPS in the cisplatin-injected mice including NF-kB and pro-inflammatory cytokine expressions. Taken together, our results suggest that activation of TLRs cascades including infection with Gram-negative bacteria may further deteriorate hearing impairment caused by platinum-containing chemotherapeutic agents.
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719 Cochlear Distribution of Texas Red-Conjugated Cisplatin

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The anti-neoplastic drug cisplatin is used clinically to treat epithelial and metastatic cancers in more than 1 million patients in North America and western Europe. Sixty percent of patients receiving multiple doses of cisplatin experience some degree of hearing loss, neurotoxicity and/or nephrotoxicity.
Direct tissue identification of cisplatin has used radiolabeled cisplatin, positron emission tomography, or chromatography. However, these techniques cannot provide a cellular distribution of cisplatin in a heterogeneous cellular structure like the cochlea. We have developed a Texas Red-cisplatin conjugate whose uptake by cells can be modulated, and is trafficked within the cochlea in vivo.
Cisplatin was conjugated in DMF at room temperature to a commercially-availableTexas Red isomer mixture using a di-amine bridge and isolated with 70% yield. Cisplatin-Texas Red (DDP-TR) is toxic to zebrafish neuromast hair cells at doses similar to the native ligand, cisplatin. In vitro uptake of DDP-TR in MDCK cells is modulated by extracellular gentamicin and calcium. Intravenous injections of DDP-TR in rats revealed cytoplasmic uptake in marginal cells and intra-strial tissues of the stria vascularis compared to fibrocytes in the lateral wall. Cytoplasmic DDP-TR fluorescence was also observed in linguinal receptors, and as diffuse and punctate (endocytotic) fluorescence in kidney proximal tubule cells and hepatocytes.
By determining which cells in the cochlea take up cisplatin and the mechanisms by which cisplatin enters cells, we can begin to develop new strategies to prevent cochlear uptake of cisplatin and subsequent cisplatin-induced ototoxicity. This will allow clinicians to use cisplatin more efficaciously while preserving auditory function, which is especially important in pediatric patients who are acquiring language skills.
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720 Cisplatin Ototoxicity Causes Structural Changes in Inner Ear Supporting Cells and Reduces the Numbers of Resident Macrophages

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Cisplatin is a chemotherapeutic agent that is highly effective in the treatment of several types of solid tumors. Ototoxicity is a common side effect of cisplatin therapy, often leading to permanent hearing loss. The ears of nonmammalian vertebrates are able to regenerate after acoustic trauma or aminoglycoside ototoxicity, but our recent studies have demonstrated that the avian ear cannot regenerate after cisplatin injury. Since regenerated hair cells arise from epithelial supporting cells, our observations suggest that cisplatin has direct effects on supporting cells. The present study examined changes in the morphology and structure of supporting cells after cisplatin treatment. Utricles from chicks and mice were placed into organotypic culture and treated for 24 hours with 10 uM cisplatin. Specimens were then rinsed and maintained in vitro for an additional 1-7 days in cisplatin-free medium. Extensive hair cell injury was observed at 7 days after cisplatin treatment. Notably, we also observed

a significant disruption of the actin cytoskeleton in surviving supporting cells, which was accompanied by reduced levels of N-cadherin at cell-cell junctions. Similarly, cisplatin treatment caused a nearly-complete loss of ZO-1 expression, suggesting that epithelial tight-junctions had been disrupted. We also examined the effect of cisplatin on resident macrophages. Chick cochleae and utricles were cultured for 24 hr in either 10 uM cisplatin or 1 mM streptomycin. Specimens were then rinsed and maintained for an additional 48 hours in ototoxin-free medium, and tissue macrophages were identified using the KUL01 antibody. Numerous macrophages were present in the streptomycin-treated specimens, while cisplatin appeared to reduce the numbers of tissue macrophages by about 10-fold. Finally, we performed comparable experiments with utricles from CX3CR1-GFP transgenic mice, which express GFP in all macrophages. Treatment with 10 uM cisplatin resulted in the near-elimination of tissue macrophages. Taken together, these data show that the ototoxic effects of cisplatin are not limited to hair cells. Rather, cisplatin also causes maintained changes to inner ear supporting cells and resident macrophages, leading to major structural defects in the sensory epithelium.
(Supported by the NIDCD and the NOHR)

721 Oxaliplatin Ototoxicity in Rat Cochlear Organotypic Cultures

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Oxaliplatin is platinum-based, FDA-approved chemo-therapy drug typically used in combination with fluorouracil and leucovorin to treat colon cancer. Oxaliplatin is similar to cisplatin in terms of its anti-tumor mechanisms and efficacy, but is less nephrotoxic and ototoxic. However, oxaliplatin is extremely neurotoxic, suggesting that it might preferentially damage the spiral ganglion neurons in the inner ear. To gain insights into its ototoxic profile, oxaliplatin was applied to postnatal day 3 rat cochlear organotypic cultures for 24, 48 or 78 h at doses ranging from 1 to 5000 μM. Hair cell damage was evaluated using fluorescently-labeled phalloidin and spiral ganglion and nerve fiber degeneration were assessed by immunolabeling with an antibody that recognizes the 200 kD neurofilament protein. Consistent with it neurotoxic propensity, oxaliplatin selectively damaged nerve fibers at a very low dose 1 μM. In contrast, the dose required to damage hair cells and spiral ganglion neurons was 50 fold higher (50 μM). These results are consistent with oxaliplatin's neurotoxic profile. Unexpectedly, a complete does-response assessment with oxaliplatin revealed a nonlinear function with surprisingly little damage at very high doses. Hair cell and spiral ganglion destruction only appeared when the oxaliplatin doses were in the 50 to 500 μM range; hair cells and spiral ganglion neurons were intact when the dose exceeded 1000 μM. Nerve fibers exhibited a similar nonlinear dose-response function; damage was first observed at 1 μM, but nerve fibers appeared normal at the 5000 μM dose. The mechanism responsible for this nonlinear response are unclear but could be related to reduced uptake of oxaliplatin via active transport mechanisms. Research supported in part by NIH R01DC006630.

722 Ouabain-Induced Cochlear Degeneration in Rat in Vitro and in Vivo

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Ouabain is a selective and potent inhibitor of the plasma membrane Na+/K+-ATPase pump. In vivo studies in gerbil and mice have found that round window application of ouabain selectively destroys type I spiral ganglion neurons, but has little effect on hair cells possibly due to the fact that the round window membrane lies adjacent to the spiral ganglion in the basal turn of the cochlea. To elucidate the neurodegenerative effects of ouabain in vitro, we treated postnatal day 3 rat cochlear organotypic cultures with ouabain concentrations ranging from 250 μm to 1 mM for 24 h to 72 h. Damage was evaluated with phalloidin to label the hair cells, an antibody against neurofilament 200 kD to label neurons, and annexin V, which binds to phosphatidylserine exposed on the membrane outer leaflet of cells undergoing apoptosis. Consistent with in vivo studies in adult animals, low concentrations of ouabain only damaged spiral ganglion neurons; however, high doses destroyed both spiral ganglion neurons and cochlear hair cells. Neuronal and hair cell death in vitro occurred by apoptosis. To evaluate the ototoxic effects in vivo, 1 mM or 10 mM of ouabain was applied to the rat round window membrane. The auditory brain stem response (ABR) and distortion product otoacoustic emissions (DPOAE) were evaluated before and after ouabain treatment to explore the changes in auditory function. Surface preparations, plastic embedded thin sections, frozen sections, and TUNEL staining were used to assess cochlear pathology. Application of 1 mM of ouabain to the rat round window membrane did not significantly alter ABR thresholds or DPOAE amplitudes. In contrast, 10 mM of ouabain significantly increased ABR thresholds and decreased DPOAE amplitudes at high frequencies. Consistent with the physiological results, both spiral ganglion neurons and hair cells were missing or damaged in the basal turn of the cochlea and both hair cells and neurons were TUNEL-positive indicative of apoptotic cell death. Supported in part by NIH grants R01DC006630 and R01DC009219

723 Salicylate Induces Spiral Ganglion Neuron Degeneration

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High doses of salicylate (aspirin) can cause up to 40-50 dB of hearing loss as well as intense tinnitus. Salicylate treatment known to cause tinnitus in animals raises salicylate concentration in CSF to 1.4 mM. While salicylate-induced hearing loss and tinnitus are thought to be completely reversible, some data suggest that prolonged treatment, might damage the inner ear. To

investigate this possibility, we treated cochlear organotypic cultures from postnatal day 3 rats with salicylate doses between 1 and 10 mM for 48 h. Spiral ganglion neurons (SGN) showed obvious salicylate-induced histopathologies characterized by a dose-dependent shrinkage of SGN size; significant size reductions were seen with doses as low as 1 mM. Pathological changes were restricted to SGN since hair cells in the same culture showed no signs of damage even with doses as high as 10 mM. Salicylate-treated SGN showed positive TUNEL staining, an indication of DNA fragmentation in cells undergoing apoptosis. Salicylate treatment also induced caspase-labeling implicating the caspase pathways in SGN cell death. To test the hypothesis that salicylate induces SGN degeneration by enhancing NMDA currents, we blocked NMDA receptors with high concentrations of magnesium. The salicylate-induced decrease in SGN size was only partially blocked by magnesium treatment. These results indicate the high doses of salicylate can induce caspase-mediated SGN degeneration in postnatal cochlear cultures. SGN degeneration appears to be mediated in part by the influx of calcium through NMDA receptor; however, other cell death signaling pathways are likely to be involved.

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724 Developing an Ototoxicity Screen in the Zebrafish Lateral Line: Results of the BIOMOL FDA-Approved Drug Library Screen

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The zebrafish lateral line is a powerful system for studying hair cells and hair cell death. Hair cells can be easily labeled and imaged in vivo with fluorescence microscopy. We have previously described a screening system to rapidly assess drugs for possible ototoxic effects (Chiu et al., 2008). Our original screening protocols used one drug concentration (100 µM) and one exposure duration (one hour). More recent experience has demonstrated that some ototoxins such as gentamicin and cisplatin require longer durations to cause hair cell damage. This has led to modification of our exposure parameters for ototoxicity screening.

We have now screened the BIOMOL FDA-Approved Drug Library, a library of 640 FDA-approved drugs, for ototoxic effects. We hypothesize that there are drugs with previously undescribed ototoxic effects within this library composed entirely of FDA-approved drugs. Five days post-fertilization zebrafish larvae were labeled with the fluorescent dye YO-PRO-1 and then exposed to drugs from the drug library in 96-well tissue culture plates. Fish were exposed to drugs at two different durations: 1 hour and 6 hours. Preliminary results identified known ototoxic aminoglycosides, as well as potentially novel ototoxic drugs such as nicorandil and doxazosin. Novel drugs that were observed to have an ototoxic effect on the lateral line were retested and dose-response curves were established.

725 Protective Effect of Gangliosides GM1 and GM3 Against Gentamicin-Induced Hair Cell Loss of the Rat Cochlea

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Recent studies have suggested that apoptotic cell death is considered to play a key role and c-Jun N-terminal kinase(JNK) pathway is involved in gentamicin-induced cochlear hair cell loss. Ganglioside GM1 is glycosphingolipid that reportedly protected neural cells against different insults. Although initially its effects were reported in neural cells, but recently it has been revealed that ganglioside GM1 can protect other cell lines. Ceramide is a sphingolipid that acts as a mediator of apoptosis. Conversely sphingosine 1-phosphate(S1P), which is a metabolite of ceramide, acts against apoptosis. Ganglioside GM1 is derived from ceramide and has been shown to induce the synthesis of S1P. Ganglioside GM3 is also the metabolite of ceramide that lies upstream of ganglioside GM1. This study was designed to investigate the possible involvement of ganglioside GM1, ganglioside GM3, ceramide and S1P in hair cell death due to gentamicin. Organ of Corti explants from basal turn of p3 or p4 rats were maintained in tissue culture and were exposed to 35 microM gentamicin for up to 48 hours. Effects of gangliosides GM1 and GM3 on gentamicin-induced hair cell loss were examined by counting outer hair cells after the gentamicin exposure. Gangliosides GM1 and GM3 decreased gentamicin-induced hair cell loss. JNK activation was seen during the gentamicin exposure, which was suppressed by gangliosides GM1 and GM3. Addition of membrane-permiable C2-ceramide alone had no effect without gentamicin, but its coadministration with gentamicin had synergistic effect. Sphingosine 1-phosphate decreased gentamicin-induced hair cell loss as well as gangliosides GM1 and GM3 did. The results indicate that gangliosides GM1 and GM3 act to decrease hair cell loss in gentamicin ototoxicity. In addition, regulation of sphingolipid metabolism plays a possible role in gentamicin-induced cochlear hair cell loss.

726 The Effect of a Src Inhibitor (KX1-004) on Cisplatin Toxicity and Antineoplastic Activity

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The Src inhibitor (KX1-004) has previously shown to protect against noise-induced hearing loss (Harris et al., 2005; Bielefeld et al., 2005). Since cisplatin is known to induce forms of stress on the cochlea that are similar to noise (oxidative stress, hair cell apoptosis), the hypothesis

of the current pilot study was that the Src inhibitor could be used effectively to prevent cisplatin-induced hearing loss, while maintaining or enhancing cisplatin's chemotherapeutic effects. In this pilot study, athymic rats had HT-29 tumor cells implanted in each hip. After a 7-day incubation period, the rats were either allowed to continue to grow their tumor; were given cisplatin, or were given cisplatin + KX1-004. The cisplatin + KX1-004 rats compared to cisplatin alone had less weight loss, less hearing loss and outer hair cell loss, less severe kidney pathology, fewer significant protein changes in the hippocampus, and greater tumor suppression. The results illustrate a novel approach to preventing the toxic effects of cisplatin using a Src inhibitor.

727 Round Window Administration of TAT-FNK Protein, a Bcl-XL Derivative, Immersed in Gelatin Sponge Prevents Aminoglycoside-Induced Cochlear Damage

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Apoptosis may result from cochlear sensory hair cell injury caused by many kinds of oto-toxic insults including acoustic trauma, loss of trophic factor support, ischemia-reperfusion, as well as exposure to ototoxins such as aminoglycoside antibiotics and the anti-neoplastic agent cisplatin. The application of agents that protect cochlear cells from apoptosis would be a useful clinical intervention for patients suffering from deafness. Protein therapeutics has the advantage of rapidly delivering effective substances, although entry of the protein to the cochlear, whether administered systemically or topically, may be limited by its high molecular weight.

In the current study, we constructed a powerful artificial cytoprotective protein, TAT-FNK, from an anti-apoptotic member of the BCL-2 family, Bcl-XL, by fusing FNK with the protein transduction domain, TAT, of the HIV/TAT protein. When gelatin sponge soaked with TAT-FNK was placed on the round window membrane (RWM), this protein penetrated through the membrane and was found to be diffusely distributed throughout the cochlea. The distribution was most prominent in the hair cells (HCs) and supporting cells, and the expression was greatest 6hr after injection and continued up to 24hr postinjection. We then examined whether this protein could attenuate cochlear damage induced by an ototoxic combination of kanamycin sulfate (KM) and ethacrynic acid (EA) in vivo. Gelatin sponge soaked with PTD-FNK or vehicle was placed on the RWM 1hr before the ototoxic insults. Auditory brainstem responses (ABR) and HC loss were evaluated 14 days later. In comparison with vehicle-administered controls, the TAT-FNK protein significantly attenuated ototoxic drug-induced ABR threshold shifts and the extent of HC death. Cochlear organotypic cultures were prepared from postnatal day 5-7 rats and treated with a medium containing TAT-FNK or vehicle. 2hr after exposure the medium was changed to one containing a combination of KM and EA with TAT-FNK or vehicle for 12 hr. Similarly to the in vivo study, this in vitro study demonstrated that TAT-FNK significantly attenuates HC death and reduced caspase-9 expression. These findings indicate that TAT-FNK topically applied to the RWM is successfully delivered to the cochlea where it can be effective in preventing apoptotic cell death of the cochlear HCs induced by KM and EA via the mitochondrial caspase-9 pathway..

728 Celastrol Inhibits Aminoglycoside-Induced JNK Activation and Hair Cell Death

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Sensory hair cells are susceptible to death caused by noise, aminoglycoside antibiotics, cisplatin and ageing. Aminoglycoside antibiotics are widely used in the treatment of bacterial infections, but serious side effects include irreversible hearing loss. We have previously demonstrated that induction of heat shock proteins (Hsps) inhibits aminoglycoside-induced hair cell death and hearing loss. Celastrol is a small bioactive molecule that has been identified as a pharmacological Hsp inducer. Our data indicate that celastrol is protective against aminoglycoside-induced hair cell death in the adult mouse utricle in vitro. Here we have examined the mechanisms underlying celastrol's protective effect. Treatment with celastrol results in robust upregulation of Hsp70 and Hsp32 expression. JNK phosphorylation is an early apoptotic signaling event in aminoglycoside ototoxicity. Therefore we analyzed JNK phosphorylation by western blot. Exposure to neomycin resulted in JNK phosphorylation, and celastrol inhibited neomycin-induced JNK phosphorylation. We next analyzed whether the protective effect of celastrol requires the major heat shock transcription factor Hsf1. Utricles from Hsf1 -/- mice and their wild-type littermates were cultured in celastrol and then exposed to gentamicin. Celastrol was protective against gentamicin-induced hair cell death in wild-type mice, and this protective effect was retained in utricles from Hsf1-/- mice (Three-way ANOVA, $F_{1,59} = 10.48$, $p < 0.001$, $n=67$). These data indicate that Hsf1 is not required for celastrol's protective effect. mRNA analysis revealed that celastrol induces Hsp32 expression (but not Hsp70 expression) in utricles from Hsf1 -/- mice. These data suggest that Hsp32 may be an important mediator of celastrol's protective effect against aminoglycoside-induced hair cell death. This work was supported by NIDCD 5R01 DC07613, DC07613-S1, and F31DC10559.

729 Hsp70 Inhibits Aminoglycoside-Induced Activation of JNK and Downstream Signaling

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Exposure to aminoglycosides results in hair cell death that is mediated by specific apoptotic proteins, including c-Jun N-terminal kinase (JNK). Overexpression of heat shock protein 70 (Hsp70) inhibits aminoglycoside-induced cochlear hair cell death and hearing loss in vivo. In order to examine the molecular mechanism(s) underlying the

protective effect of Hsp70 against aminoglycoside-induced hair cell death, we have analyzed the effects of Hsp70 on aminoglycoside-induced JNK activation. Utricles from adult CBA mice were heat shocked in vitro and were exposed to neomycin for 12 hours. Protein analysis indicates that neomycin exposure results in robust activation (phosphorylation) of JNK. In addition, heat shock inhibits this neomycin-induced JNK phosphorylation. In order to determine if Hsp70 inhibits JNK phosphorylation, utricles from mice that constitutively overexpress Hsp70 (and their wild-type littermates) were treated with neomycin for 12 hours. Western blot data indicate that Hsp70 overexpression inhibits neomycin-induced JNK phosphorylation. We have begun to examine downstream targets of JNK, and our data indicate that at least three known specific targets of JNK are phosphorylated in utricles treated with neomycin. Specific substrates for activated JNK include the transcription factors c-Jun (phosphorylated by JNK on Ser63) and ATF-2 (phosphorylated by JNK on Thr71) and the pro-apoptotic Bcl-2 family member BimEL (phosphorylated by JNK on Ser65). Our recent data indicate that Hsp70 overexpression inhibits neomycin-induced BimEL phosphorylation. In addition, heat shock inhibits neomycin-induced c-Jun activation. Taken together, these data suggest that the protective effect of Hsp70 against aminoglycoside-induced hair cell death is mediated in part by inhibition of JNK and downstream BimEL activity. This work was supported by NIDCD 5R01 DC07613.

730 Heat Shock Inhibits Cisplatin-Induced Activation of P53 and STAT-1 in Adult Mouse Utricle

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Cisplatin is an effective chemotherapeutic drug used to treat a wide variety of cancers. However, a proportion of patients who receive cisplatin develop significant permanent hearing loss. The ototoxic effects of cisplatin result in part from damage to sensory hair cells. We have previously shown that heat shock inhibits cisplatin-induced hair cell death in the adult mouse utricle in vitro. The molecular mechanisms underlying cisplatin-induced hair cell death are poorly understood. Previous studies have implicated the pro-apoptotic molecules p53 and STAT-1 as key players in cisplatin-induced hair cell death. p53 is a key mediator of the DNA damage response in cells, resulting in upregulation of specific pro-apoptotic proteins in the face of irreparable DNA damage. p53 is activated in neonatal rat cochlea and utricle in vitro following cisplatin treatment, and chemical inhibition of p53 inhibits cisplatin-induced hair cell death (Zhang et al. 2003). STAT-1 is a transcription factor that controls the expression of pro-apoptotic proteins in response to cellular stresses, including genotoxicity and ROS. STAT-1 is required for cisplatin-induced hair cell death in organ cultures of adult mouse utricle (Schmitt et al. 2009). In order to examine the mechanisms underlying the protective effect of heat shock against cisplatin-induced hair cell death, we have analyzed the effect of heat shock on the activation (phosphorylation) of both p53 and STAT-1. Our data indicate that heat shock protein 70 (Hsp70) and Hsp32 each inhibit cisplatin-induced hair cell death. Western blot analyses reveal that cisplatin treatment results in activation of both p53 and STAT-1, and that heat shock inhibits activation of both molecules. We are currently investigating the roles of Hsp70 and Hsp32 in the inhibition of cisplatin-induced activation of p53 and STAT-1. Supported by NIDCD 5R01DC07613 and F30DC010522.

731 Hydrogen Protects Auditory Hair Cells from Cisplatin-Induced Ototoxicity

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Cisplatin is a widely used chemotherapeutic agent for the treatment of various malignancies including head and neck cancers. However, its application is sometimes limited by nephrotoxicity, neurotoxicity, and ototoxicity, which is partly mediated by oxidative stress. Recent findings show that cisplatin ototoxicity appears to involve the production of reactive oxygen species in the inner ear by activating enzymes unique to the cochlea. Molecular hydrogen was recently established as an antioxidant that selectively reduces the hydroxyl radical, and has been reported to protect the central nervous system, liver and kidney. We have reported that molecular hydrogen acts as an efficient antioxidant and protect cochlear hair cells from reactive oxygen species (Kikkawa et al. 2009). The purpose of this study was to evaluate the potential of molecular hydrogen to protect auditory hair cells against cisplatin toxicity. We used cochlear explants obtained from postnatal day 2 ICR mice. After 24 h incubation, the explants were transferred to the medium containing various concentrations of cisplatin (0, 10, 20, 40μM). We then examined the effects of hydrogen gas which was dissolved directly into the media. Following 48 h incubation, the tissue samples were fixed and provided for histological analysis to evaluate hair cell survival. Presence of intact auditory hair cells was assayed by phalloidin staining. Cisplatin caused hair cell loss in a dose-dependent manner. The addition of hydrogen gas significantly increased the numbers of remaining inner hair cells and outer hair cells. These data suggest that molecular hydrogen has the capability to protect cochlear hair cells against cisplatin toxicity. The supplementation of molecular hydrogen may contribute to reduction of cisplatin ototoxicity.

732 The Protection of Vestibular Hair Cells with Resveratrol

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Recently, It was reported that many kinds of polyphenol could protect tissue against the stress. Resveratrol is well

known as the polyphenol abundant in grapes. This molecule has demonstrated reactive oxygen species (ROS) scavenger activity. In this present study, we studied the effect of resveratrol on vestibular hair cell death induced by aminoglycoside.

Cultured utricles of CBA/N mice were used. Cultured utricles were divided to three groups (Control group, Neomycin group, Neomycin + Resveratrol group). In the Neomycin group, utricles were cultured with neomycin (1 mM) to induce hair cell death. In Neomycin + Resveratrol group, utricles were cultured with neomycin and resveratrol (100 - 1.0 µM). Twenty-four hours after exposure to neomycin, the cultured tissues were fixed with 4% paraformaldehyde. To label hair cells, immunohistochemistry were performed using anti-calmodulin antibody. The rate of survival vestibular hair cells was evaluated with the fluorescence microscope. The survival rate of hair cells in Neomycin + resveratrol group was significantly more than that in Neomycin group.

The results indicated that resveratrol protects sensory hair cells against neomycin-induced death in mammalian vestibular epithelium. Reesveratrol can be used as the protective drug in the inner ear.

733 Minocycline Protection of Hearing Loss in Gerbils Treated with Neomycin

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We investigated the efficacy of the antibiotic minocycline as a therapeutic agent for amelioration of hearing loss in gerbils treated with the ototoxic aminoglycoside, neomycin. Minocycline is multifaceted in that it exhibits anti-inflammatory, antibiotic and anti-apoptotic properties in several neural and non-neural tissues. We hypothesized that as an anti-apoptotic agent, minocycline would inhibit apoptosis of sensory cells within the inner ear when exposed to neomycin.

Baseline Auditory brainstem Responses (ABR) measurements were made on gerbils prior to experimental treatments. Gerbils then received a single trans-tympanic injection of 40mM neomycin in the left ear and Ringer's balanced lactate in the right ear and either 0, 1.2 or 1.5 mg/kg intraperitoneal injection of minocycline in normal saline daily for five days. Day 1 injection was given at commencement of the baseline ABR measurement.

Four weeks post-treatment ABR measurements were made and animals sacrificed for histological preparation of the cochleae. Analysis of pre and post treatment ABR measurements demonstrated minocycline amelioration of hearing loss. Quantification of spiral ganglion neurons is under way to determine if there is a correlation with the ABR measurements. Minocycline used in combination with other otoprotective agents is envisioned as a realistic means of preventing hearing loss due to aminoglycoside treatment.

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734 Aminoglycoside Ototoxicity Ameliorated with Mechanotransducer Channel Blockers

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Aminoglycosides are the most widely used antibiotics worldwide despite having the major side effects that lead to deafness and kidney failure. To test the hypothesis that hair cell susceptibility to aminoglycosides is due to these drugs rapidly and selectively accumulating in hair cells by passing through mechanotransducer channels, an in vitro model of toxicity was developed. Rat organ of corti cultures were prepared at either P2 or P4 and treated with gentamicin at doses ranging from 0.05-1mM, for time periods ranging between 1-24hrs followed by post incubations in normal medium for 0-48 hrs. A paradigm for toxicity was established where organ of corti tissue was prepared at P4, and treated on P5 with 0.1 mM gentamicin for 1 hr. The tissue was then washed and remained in culture until P7 at which time it was fixed with 4% paraformaldehyde and stained for phalloidin and parvalbumin 3 (to identify hair cells). Cells were imaged with a Zeiss LSM confocal microscope at 63x where cell counts were made. Loss of both hair bundle and cell body constituted cell loss. A tonotopic gradient in damage was observed with base being highly susceptible, losing more than 50% of their hair cells and apex less. Mechanotransducer channel blockers, curare, amiloride, quinine and AM1-43 were applied at the same time as gentamicin to test for protection. AM1-43 alone was toxic. When cultures were allowed to recover till P9 (4-5 days post treatment), both curare and quinine, open channel blockers, were protective, while amiloride was not. Endocytosis blockers had no effect on toxicity. Together this data support the argument that aminoglycosides rapidly accumulate in hair cells because of their ability to pass through mechanotransducer channels. Mechanotransducer channel blockers were also protective against dihydrostreptomycin and neomycin, suggesting a common mechanism of entry.

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735 Intracochlear Infusion of Brain-Derived Neurotrophic Factor Combined with Electrical Stimulation Affects Function and Morphology of Spiral Ganglion Neurons in Cats Deafened as Neonates

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Both administration of neurotrophins and electrical stimulation from a cochlear implant have been shown to prevent degeneration of spiral ganglion (SG) neurons after deafness in several animal models. In this study, electrophysiological and histological data were evaluated to assess the effects of brain-derived neurotrophic factor (BDNF) combined with electrical stimulation (ES) in the developing auditory system of cats. Kittens were deafened as neonates by systemic neomycin injections and

implanted unilaterally at 4-5 weeks of age with a scala tympani array containing six wires for electrical stimulation and an integrated drug-delivery cannula for infusion of neurotrophic agents from an osmotic pump. Animals received 10 weeks of BDNF treatment and chronic electrical stimulation for an additional 10 to 18 weeks (BDNF+ES).

Electrically-evoked auditory brainstem responses (EABR) were recorded every two weeks to evaluate functional thresholds and set current levels for chronic ES on two bipolar channels. A third electrode pair remained non-stimulated, but provided longitudinal EABR data. In a previous study, 10 weeks of BDNF infusion resulted in a significant decrease in thresholds, whereas no threshold shift was observed with infusion of artificial perilymph. In the present study, animals that received combined BDNF+ES exhibited a significant decrease ($P = 0.034$) in the mean EABR thresholds for electrode pairs used in chronic stimulation, whereas the thresholds on the non-stimulated channels showed no significant shift.

Histological studies of cochleae from BDNF+ES animals showed larger SG cell size, improved SG cell survival, greater density of radial nerve fibers in the osseous spiral lamina, and marked sprouting of fibers into the scala tympani. A substantial number of spouted fibers were present several months after cessation of the BDNF treatment. This finding suggests that these ectopic fibers, which may contribute to the decreased thresholds, are maintained by electrical stimulation.

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736 Screening for Hair Cell Death Inhibitors in the Zebrafish Lateral Line

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Several current drugs, including aminoglycoside antibiotics and the chemotherapeutic agent cisplatin, exhibit ototoxicity as a prominent side effect, often directly or indirectly by killing hair cells. While research during the past decade has uncovered key features of drug-induced cell death *in vitro*, our knowledge of hair cell death pathways is still incomplete. Recently, the zebrafish lateral line system has been developed to use as a tractable model for conducting chemical and genetic screens for inhibitors and facilitators of hair cell death. This screening approach bypasses the need for *a priori* hypotheses about specific death pathways. As a step toward understanding cell death signaling in drug-exposed hair cells we conducted a small-scale screen of a custom cell death inhibitor library of 60 compounds from Calbiochem. 5-6 day old zebrafish were incubated for 1 hr in a single inhibitor at concentrations that were based on published literature. Fish were then incubated in inhibitor along with ototoxin concentrations and exposure times selected as the minimum concentration and time that results in substantial hair cell death: either 200 µM neomycin (30 minutes treatment with 1 hr recovery), 50 µM gentamicin (6 hrs), 200 µM kanamycin (6 hrs), or 500 µM cisplatin (6 hrs). Hair cell survival was assessed with the mitochondrial potentiometric dye DASPEI. Inhibitors of multiple pathways, including a proteasome inhibitor and an NF-κB activation inhibitor, protect hair cells from each of the three aminoglycosides but not from cisplatin. In contrast, we find that two serine protease inhibitors, FUT-175 and antipain, protect hair cells from all four ototoxins, suggesting that serine protease activity occurs downstream in convergent cell death pathways. These results highlight several hair cell death pathways and provide abundant opportunities for further pathway characterization.

737 Heterogeneous Distribution of the Calcium Binding Protein Calretinin in Murine Spiral Ganglion Neurons

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The firing patterns of spiral ganglion neurons in response to sound stimuli can be categorized into distinct rate-intensity classes that are associated with spontaneous rate and threshold characteristics (Winter et al, *Hear Res*. 1990). As a first step to determine whether neuronal features could contribute to these diverse electrophysiological firing patterns we characterized the distribution of calcium binding proteins, which are important regulators of neuronal excitability (Gall et al *J. Neurosci.* 2003).

Using immunochemistry, we observed that isolated spiral ganglion neurons *in vitro* displayed three separate categories of anti-calretinin antibody luminance. Frequency histograms of normalized fluorescence intensity measured from a single experiment were best fitted with the sum of three Gaussians. Five experiments revealed consistent patterns with three means of 0.042±0.008, 0.142±0.021, 0.319±0.038 (error represented by SEM). In order to determine whether any of the three categories were attributable to type II spiral ganglion neurons, we examined preparations co-labeled with type II-specific anti-peripherin antibody. Putative type II neurons were only lightly labeled with anti-calretinin luminance, the frequency histogram of which was best fit with single Gaussian (mean 0.052±0.026, n=5). The remaining 95% of the cells, putative type I neurons, were still best fit by the sum of three Gaussians with comparable means (0.048±0.011, 0.150±0.026, 0.315±0.034, n=5). These observations were consistent in neuronal cultures from different tonotopic regions and in more intact organ of Corti culture preparations.

Our results suggest that calretinin might be associated with spiral ganglion neuron excitability. Future experiments will compare anti-calretinin fluorescence intensity of spiral ganglion neurons along the tonotopic axis and with neuronal firing patterns. Supported by NIH NIDCD R01 DC-01856.

738 Characterization of Voltage-Gated Calcium Channel α-Subunits in Spiral Ganglion Neurons

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Voltage-gated calcium channels (VGCC) are important regulators of neuronal signaling. As previous work from our laboratory has shown, spiral ganglion neurons are intrinsically unique; each consists of their own distinct endogenous ion channel composition that contributes to specific electrophysiological phenotypes. In order to understand how calcium channels may contribute to the underlying mechanism of these diverse neuronal firing patterns, we have taken molecular and immunocytochemical approaches to examine their gene expression and localization in the spiral ganglion.

We began by systematically surveying the VGCC mRNA profile. Of the Ca_v1 members, $Ca_v1.2$ and $Ca_v1.3$ mRNA transcripts are present (C_t ratio = 0.663 ± 0.028; 0.656 ± 0.032, respectively), but not $Ca_v1.1$ and $Ca_v1.4$. Immunostaining shows that $Ca_v1.2$ α-subunits are expressed in neurons and satellite cells; whereas $Ca_v1.3$ α-subunits are predominantly localized in the neurons. Of the Ca_v2 members, $Ca_v2.1$ and $Ca_v2.3$ α-subunits have higher amounts of mRNA transcripts (C_t ratio = 0.734 ± 0.029; 0.729 ± 0.031, respectively) than that of $Ca_v2.2$ α-subunit (C_t ratio = 0.634 ± 0.030). Elevated $Ca_v2.1$ and $Ca_v2.3$ mRNA levels may be due to the fact that they are expressed in both neurons and satellite cells; whereas $Ca_v2.2$ α-subunit is restricted to the neurons. $Ca_v3.1$, $Ca_v3.2$, and $Ca_v3.3$ mRNA transcript levels are as follows: C_t ratio = 0.741 ± 0.030; 0.697 ± 0.030; 0.590 ± 0.021.

Our results suggest that multiple VGCC may play a role in regulating firing pattern of spiral ganglion neurons. Future experiments will evaluate the distribution of Ca_v3 α-subunits, and compare type I and type II neurons in regions of the spiral ganglion to further understand auditory afferent processing. Supported by NIH RO1 DC01856.

739 The Role of the Auxiliary Ca2+ Channel Alpha2delta-3 Subunit for Signal Transmission in the Auditory Brainstem and Acoustic Startle Reflex Pathway

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Voltage-gated Ca^{2+} channels consist of a pore-forming α_1 subunit (SU) and auxiliary SUs β and $\alpha_2\delta$. Co-assembly of an α_1 SU with a β and an $\alpha_2\delta$ SU is fairly specific, giving rise to various Ca^{2+} channels with distinct cell-specific properties. The $\alpha_2\delta$-SU is required for trafficking and stabilizing α_1 SUs in the plasma membrane, thereby defining the amplitude and biophysical properties of Ca^{2+} currents. Four genes, *CACNA2D*1-4, are known that code for $\alpha_2\delta$1-4.

Mice deficient for the *CACNA2D3* gene coding for $\alpha_2\delta$-3 (Jackson Laboratories) have slightly increased ABR hearing thresholds over the entire frequency range, show reduced amplitudes and increased latencies of ABR waveforms but normal DPOAEs. Additionally, $\alpha_2\delta$-$3^{-/-}$ mice (ko) show a reduced acoustic startle response whereas their tactile startle response is markedly enhanced.

Analyzing the origin for the impaired hearing and startle response, we recorded presynaptic Ca^{2+} currents in inner hair cells (IHC). Currents were slightly different between wild-type (wt) and ko IHCs but this difference cannot account for the ko phenotype. LacZ reporter staining and in-situ hybridization revealed expression of $\alpha_2\delta$-3 in spiral ganglion (SG) and cochlear root neurons (CR). To test if presynaptic Ca^{2+} channels along the hearing and startle response pathways were affected, we performed immunohistochemistry for P/Q and N-type Ca^{2+} channels. P/Q channel immunoreactivity in the soma of SG and CR neurons and their presumptive synaptic terminal region was reduced in $\alpha_2\delta$-$3^{-/-}$ mice. Similarly, we observed a reduction of P/Q and N-type expression in brainstem cochlear nuclei but an increased expression in pontine giant neurons. In conclusion, the hearing and startle deficits of $\alpha_2\delta$-$3^{-/-}$ mice are likely caused by an altered presynaptic expression and function of Ca^{2+} channels in SG, CR neurons and further up in the brainstem along the hearing and startle pathways.

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740 Hair-Cell Spontaneous Activity in the Zebrafish Lateral-Line Organ

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Our ability to discriminate Brahms from Beethoven is owed in part to the highly tuned spiking of auditory nerves following hair cell (HC) activation by sound waves. This tuning is due to high fidelity neurotransmitter release at specialized ribbon synapses on HCs. In the absence of stimuli, auditory nerves display spontaneous spikes that are likely the product of vesicle release from HCs. The apparent mechanism for this spontaneous neurotransmission is the transient gating of Cav1.3 calcium channels that occurs at the HC’s resting potential (V_{rest}). Both the determinants of V_{rest} and the physiological role for spontaneous activity itself are not well understood. Here we used an intact preparation to study the activity of posterior lateral-line (PLL) neurons in day 5 zebrafish larvae. Initially, we observed that spontaneous spiking was completely abolished in mutant larvae where either of the HC-specific proteins, vesicular glutamate transporter 3 (Vglut3) or L-type calcium channel (Cav1.3a), were absent. However, mutant larvae that lacked functional mechanoelectrical transduction (MET) machinery still displayed spontaneous activity. To quantify the MET-contribution to spontaneous activity we applied saturating concentrations of the transduction channel blockers amiloride and dihydrostreptomycin to WT larvae.

Consistent with a contribution of MET currents to V_{rest}, we found that the average ISI for spontaneous spikes increased from approximately 70 ms to 140 ms. Previous work on HCs describe the contribution of I_h currents (among others) to HC spontaneous spiking. To determine whether I_h contributed to HC output and afferent spiking, we utilized the specific blocker ZD 7288. Interestingly, saturating concentrations of ZD reduced PLL spiking by 90% and the remaining activity was composed of bursts of relatively normal spiking followed by large periods (seconds to minutes) of inactivity. Taken together, our data describe spontaneous activity in acousto-lateralis neurons that is HC-dependent, requires Cav1.3a channels, and is generated by a combination of MET current, I_h current and additional source(s) that we are currently investigating.

741 Ionic Mechanisms That Regulate Murine Spiral Ganglion Neuron Firing Excitability

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In addition to the contribution of middle ear mechanics (Rosowski, 1991), elevated hearing sensitivity to mid-frequency sounds may be partially attributed to the spiral ganglion neurons' endogenous membrane properties. In a previous study we found that neurons isolated from mid-cochlear regions demonstrated the highest firing sensitivity when compared to their apical and basal counterparts (Liu & Davis, 2007). Studies are currently underway to examine the contribution of individual classes of low voltage activated ion channels to the regulation of neuronal excitability.

We examined two different components that could contribute to neuronal excitation. The first component, resting membrane potential, was affected by the I_h current (Liu & Davis, 2008). The second component we examined was absolute threshold voltage. With perfusion of 10 mM tetraethylammonium (TEA), prolonged action potential latency (Δ=4.37±1.38 ms, n=3) and broadened duration (Δ=1.20±0.31 ms, n=3, $p<0.05$) were observed without any demonstrable effect on threshold (Δ=-1.02±0.62 mV, n=3). Application of 0.2 mM 4-aminopyridine (4-AP), on the other hand, had a significant effect on threshold (Δ=-10.61±4.51 mV, n=3) in addition to prolonged latency (Δ=62.00±27.28 ms, n=3) and broadened duration (Δ=2.83±1.32 ms, n=3). Studies using the more specific blocker, 20 nM α-dendrotoxin (DTX) also resulted in significant changes in threshold (Δ=-12.02±4.09mV, n=5, $p<0.05$), suggesting that those changes resulting from 4-AP application were related to α-DTX sensitive, shaker-related channels. We will test whether low voltage activated A-type current via K_V4 channels are also involved in threshold regulation by utilizing $K_V4.1$ / $K_V4.2$ specific blockers.

In summary, two firing sensitivity related membrane properties appear to be separately regulated through precisely controlled ion channel composition to generate accurate neuronal codes to a wide range of stimulus intensity levels. Supported by NIH NIDCD R01 DC-01856.

742 Pre- And Postsynaptic Properties Determine the Firing Pattern of Developing Primary Auditory Neurons

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Neurons in the auditory pathway exhibit spontaneous electrical activity before the onset of hearing. This activity is required for proper maturation of auditory circuits, yet the mechanisms that initiate this activity, and the means by which it influences development, are poorly understood. Our previous studies indicate that supporting cells in the pre-hearing cochlea periodically release ATP, which depolarizes nearby inner hair cells (IHCs) and initiates bursts of action potentials in spiral ganglion neurons (SGNs). Action potentials within bursts occur as a highly stereotyped sequence of "mini-bursts" containing 2–6 action potentials separated by 5–10 ms, which repeat every 100–300 ms. Paired IHC-SGN recordings indicate that each SGN mini-burst is triggered by a single Ca^{2+} spike in the presynaptic IHC, with the interval between mini-bursts determined by the interval between successive Ca^{2+} spikes. To determine how single Ca^{2+} spikes trigger repetitive firing of SGNs and the factors that pattern action potentials within mini-bursts, we recorded from SGNs in cultured and acutely-isolated pre-hearing rat cochleae. Whole-cell recordings from SGN cell bodies and afferent dendrites revealed that each Ca^{2+} spike in the presynaptic IHC triggered repetitive bouts of multivesicular release, leading to prolonged EPSCs with complex waveforms. In current-clamp recordings, individual EPSCs summed to produce large, compound EPSPs that brought auditory nerve fibers above spike threshold for over 10 ms – long enough to initiate multiple action potentials. To determine the maximum firing rate of SGNs we focally applied the non-NMDA receptor agonist kainate (100 µM) to SGN dendrites at the base of IHCs. Kainate application evoked prolonged bursts of up to 15 action potentials that occurred at a maximum frequency of 200 Hz. Together, these results indicate that the pattern of action potentials within mini-bursts is determined by the frequency of multivesicular release events, with the maximum firing rate determined by the refractory period of the dendrite. This clustering of activity into discrete mini-bursts may facilitate propagation of spontaneous activity through developing auditory circuits, and promote the formation and maintenance of tonotopically arranged connections in auditory centers of the brain.

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743 Responses of Ferret Auditory Nerve Fibres to Tones

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The ferret is increasingly used for behavioral and physiological studies of auditory function. However, while sophisticated paradigms are being employed in behavioral tests and in physiology at the midbrain and cortical level, there is a distinct lack of information about basic auditory processing at the lower levels of the auditory system.

While it is a reasonable assumption that, as a mammal, the ferret will have auditory nerve responses qualitatively like those of other mammals there have been notable quantitative differences in such important variables as the width of tuning and the high-frequency limit of phase locking across commonly used mammalian animal models. Here we have recorded from the auditory nerve of the anaesthetized ferret. We measured the responses to a 50-ms pure tone over a wide range of sound levels and frequencies. We used these data to estimate the frequency response area from which we measured the sharpness of tuning as the quality factor (Q10 dB: the characteristic frequency divided by the width at 10 dB above minimum threshold). We computed period histograms of the responses to estimate the strength of phase locking using the vector strength measure. The minimum threshold corrected to dB SPL matched the audiogram in the high-frequency region, but deviated significantly from the audiogram below 1 kHz. This discrepancy, for which we as yet have no explanation, is consistent with previously published threshold data from the auditory cortex of the ferret. The tuning width was generally somewhat broader than that of the cat, rarely exceeding a Q10 dB value of 7, and overlapped extensively with similar guinea pig data from the literature. The upper limit of phase locking was consistent with previously published data from the guinea pig which begins to decline at 1 kHz and is non-existent at 3.5 kHz, about an octave lower than comparable data from the cat. Our analyses thus far indicate that auditory nerve responses in the ferret are quantitatively closer to those of the rodent than to those of another carnivore (the cat).

744 Neural Tuning Measured with Forward-Masked Compound Action Potentials

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Frequency selectivity is one of the most fundamental cochlear properties (Robles and Ruggero, 2001). Recent behavioral and otoacoustic measurements suggest that it is sharper in humans than in laboratory animals (Shera et al., 2002), but this is disputed based on comparisons of behavioral and electrophysiological measurements across species (Ruggero and Temchin, 2005). There are no direct neural recordings from human single auditory nerve fibers, but gross potentials may enable tighter comparisons across species. We search a protocol that is minimally invasive and gives good correspondence with single fiber selectivity and can be applied to humans.

Oxenham and Shera (2003) argued that forward masking of a brief signal probe is more suitable to study frequency selectivity than simultaneous masking, because it is less confounded by cochlear nonlinearities. We combined the forward masking paradigm of the latter study with compound action potential (CAP) recordings at the round window of chinchillas and cats - two species for which extensive single fiber data are available. Brief probe tones were presented at a level fixed at 10-15 dB above the unmasked CAP threshold, and the level of a notched noise masker causing a criterion reduction in CAP amplitude (typically 50%) was determined. By varying notch width, Q10 values (ratios of signal frequency to 10 dB notch bandwidth) were obtained.

We found better agreement between Q10 values of forward-masked CAPs and single fibers than previous studies using simultaneous masking. Q10 values increased with probe frequency; straddled the values for single fibers; and were higher for cat than for chinchilla. These results suggest that forward-masked CAPs may enable electrophysiological assessment of human cochlear frequency selectivity.

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745 Subharmonics and Auditory Nerve Tuning Curves in Gerbil

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In the literature, an auditory nerve tuning curve obtained from single unit recording typically has a tail and a tip. Responses beyond the tip in the well supra-CF frequency region had never been documented. In a study designed to explore that region specifically, we found plateau responses in the supra-CF region of several auditory nerve tuning curves at very high sound pressure levels (~120dB SPL). (Huang&Olson, ARO 2009, poster #623) However, a complicating issue at high sound pressure levels is the generation of subharmonics, which are likely generated in the eardrum (Dallos & Linnell 1966 JASA 40(3):561-564). Indeed, we found subharmonics in a subset of ear canal pressure we measured in gerbils, and they might have contributed to the supra-CF responses.

Here we present auditory nerve tuning curves in which supra-CF neural responses were present while subharmonics were beneath the noise floor in the ear canal pressure. Thus these detections of supra-CF neural responses did not seem to suffer from subharmonic "contamination." To probe this further, we delivered loud tones and compared ear canal pressure and intracochlear pressure at subharmonics frequencies, and the quantitative relationship between the two reinforced that subharmonics were probably not responsible for the supra-CF neural responses we measured.

It is conceivable that this subharmonic finding has at least two clinical implications. First, eardrum produced subharmonics might occur with very high power hearing aids. This could pose a problem to patients with band limited or high frequency hearing loss, since subharmonics that are within their normal-hearing frequencies could be perceived to be "louder" than the fundamental. Second, we found that the eardrum (the putative source of the subharmonics) was a reasonably effective sound radiator. Thus hearing aids that drive the eardrum directly might produce feedback.

746 Amplitude and Phase in the Apical Turn of the Gerbil Cochlea Estimated from Auditory Nerve Recordings

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Mechanical measurements of the mammalian cochlea have predominantly focused on the high frequency region, which is located in the basal turn. Data from the mid and apical turns of the mammalian cochlea, representing mid and low frequencies, are sparse, due to their relatively poor accessibility. Moreover, the interpretation of cochlear-mechanical data from the extreme apex is problematic due to unknown effects of surgical trauma.

An alternative method to assess mechanical properties of the mammalian cochlea is to use electrophysiological data from the auditory nerve (AN). In this study we present data obtained from the AN in Mongolian gerbil. Stimuli were irregularly spaced tone complexes ("zwuis") that consisted of several primary components of known phase and amplitude. This enabled us to extract relative amplitude and relative phase from the response of an AN fiber. When extending the measurements to the phase locking regime, absolute phase can be established. The resulting "filter shapes" could be used to reconstruct vibration of the basilar membrane at the site of innervation of the associated AN fiber.

We evaluate to what extent cochlear processing in the apex differs from that in the base. For AN fibers with a characteristic frequency (CF) below 5 kHz we classify different types of filter shapes for different CFs. We also describe changes in amplitude and phase as sound intensity is varied.

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747 Mapping Auditory Nerve Density Using Chirp Stimuli and High-Pass Noise Masking

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A critical barrier to future implementation of regenerative treatments for sensorineural hearing loss is the lack of clinical tools that can specify the target(s) within the cochlea and auditory nerve for delivery of therapeutic agents. Our recent research in a gerbil model with auditory nerve lesions suggests that the amplitude of the compound action potential (CAP) to high-level toneburst stimuli is a good predictor of overall auditory nerve survival but does not pinpoint the cochlear location of neural damage. A location-specific estimate of nerve damage may be possible with high-level stimuli by systematically limiting the region of nerve fibers contributing to the CAP with high-pass noise masking. The goal of this initial study in normal-hearing gerbils is to determine the ideal stimulus to trigger synchronous neural firing along a large portion of the cochlea.

CAPs were evoked with low frequency (2 kHz) tonebursts and with rising frequency tonebursts (chirps) at 90 dB SPL during simultaneous masking with white noise high-passed at 1/3 octave intervals between 0.5 and 50 kHz. Masker cutoff frequency was converted to distance along the cochlea and N1 amplitude was plotted for each stimulus as a function of distance from apex. The derivatives of the normalized amplitude functions yield density functions that are assumed to represent the distribution of nerve fibers along the cochlea that discharge synchronously to generate a CAP.

Results for both stimulus types indicated that N1 amplitude decreased and N1 latency increased as the masker cutoff frequency decreased. Neural density functions for chirp stimuli were twice as broad as the neural density functions for 2 kHz tonebursts. These data suggest that the location of synchronous neural firing is shifted proportionally by the masker cutoff frequency, and that high-level chirps trigger synchronous firing across a greater portion of the cochlear partition than high-level low frequency tonebursts.

748 Neural Modulation Sensitivity Is Determined by One-Dimensional, Nonlinear Maps

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The temporal fluctuations of speech within a spectral channel encode important information. In cochlear implants, these fluctuations are represented by the time-varying amplitude of the stimulating current pulses. The sensitivity of the neural response to modulation of the pulse amplitude, however, depends upon the condition or dynamical state of the spike generator. For example, it is well known that an intense pulse train places the spike generator in a dynamical state which is insensitive to modulations of the pulse amplitude, with the result that the neural discharge rate and the synchrony do not encode the applied modulation. Our recent work has shown that a number, the Lyapunov exponent, calculated in biophysical models from the unmodulated response, quantifies the sensitivity of the spike generator to applied modulations (O'Gorman, 2006; O'Gorman et al., 2009). In this poster, we show that the Lyapunov exponent to ongoing pulsatile stimulation can be found without explicitly solving the complicated nonlinear differential equations that govern spike generation. In particular, we show that a family of one-dimensional curves or maps determines the Lyapunov exponent of Hodgkin-Huxley-type models to a wide range of pulse amplitudes and pulse rates. Analysis of the geometry of these maps may allow for cochlear implant stimuli to be optimized for the conveyance of temporal information in speech.

749 Spatial Profiles of Correlation in Spike Timing to Broadband Noise Across Auditory Nerve Fibers

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The cat auditory nerve (AN) contains ~50000 neurons innervating ~2500 inner hair cells across the cochlear basilar membrane. We are interested in the extent to which

these neurons carry correlated temporal patterns, which depends on both the acoustic stimuli and intrinsic properties of the auditory periphery. Due to cochlear filtering, even Gaussian broadband noise – for which adjacent frequencies have random phase - can produce correlated responses in fibers originating from nearby cochlear positions. We studied the spatial profile and extent over which correlated responses to broadband noise are found in the AN.

Neural responses to repeated presentations of a single token of broadband noise were obtained from all fibers encountered in a given nerve. Spike trains from each fiber ("reference fiber") were compared with spike trains of all other fibers ("test fibers") in order to obtain normalized same-stimulus cross-correlograms (SCC). As reported earlier (Joris et al., 2006), such SCCs show maxima at delays that depend on the distance between cochlear positions of the two fibers. We measured correlation in two ways: a) as the height of the largest peak of the SCC ("PH") and b) as the SCC value at zero delay ("T0").

The observed spatial profiles show broad regions of correlation centered on the reference fiber. Using the PH metric, the half-widths of spatial profiles were large for apical cochlear regions and decreased towards the base. Using the T0 metric, half-widths were much smaller and increased towards the base, so that at the most basal locations half-widths were similar for PH and T0. The results indicate that, if the CNS has monaural delays available to compare timing across fibers of different CFs at different delays, the spatial bandwidth of correlation is much broader than when such delays are not available.

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750 Time-Intensity Trading: Ongoing Temporal Coding of Broadband Noise in the Auditory Nerve as a Function of Intensity

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Human azimuthal sound localization relies on two cues: interaural level differences (ILDs) and interaural time differences (ITDs). Processing of these cues is thought to occur in separate brainstem circuits, but psychophysical experiments have shown that under some conditions ILDs and ITDs can be traded, which could reflect interaction between the cues at a low anatomical level. The latency hypothesis (Jeffress 1948) postulates that a decrease in response latency with increasing stimulus intensity accounts for this interaction. Previous physiological studies examined and to some extent supported the hypothesis by studying onset responses of binaural neurons, mostly using transient stimuli. However, human time-intensity trading has been observed to occur at low frequencies and to both transient and ongoing stimuli. Also, the timing of onset and ongoing responses depends on sound pressure level (SPL) in different ways. We tested the basic tenet of the latency hypothesis by studying the effect of changes in SPL on the ongoing timing of auditory nerve (AN) responses of cats.

We compared spike times in response to broadband noise, obtained from single AN fibers at multiple SPLs, using a crosscorrelation analysis. We found that changes in SPL cause small but systematic shifts in the ongoing timing in the AN, resulting in longer delays between stimulus onset and neural response at lower SPLs. The size of the shifts depended on characteristic frequency (CF). Also, for fibers with CFs between 1 and 5 kHz, paradoxical shifts were seen, with high intensities causing delayed spike times. These results show that a limited and rather complex time-intensity trade exists at the most peripheral neural level. Comparison of correlograms with revcors suggests that these effects reflect changes in cochlear filtering with SPL rather than changes in latency as traditionally conceived.

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751 Ensemble Responses of the Auditory Nerve to CV Syllables Spoken by Multiple Talkers

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Simultaneous, cross-frequency cues are important for speech recognition. Earlier studies showed that these cues appear to create fixed patterns in ensemble responses at the auditory periphery for well-articulated normal speech and noise vocoded speech (Clarey et al., 2004; Loebach and Wickesberg, 2006). A limitation of the two previous studies, however, is the very few speakers used to generate the speech stimuli. The purpose of the current study was to determine whether the ensemble responses produced by the auditory nerve display the same fixed temporal patterns for CV stimuli spoken by multiple speakers.

Five versions of the CV pairs /pa/, /pe/, /pi/ and /pæ/ spoken by a total of 7 female and 8 male speakers were selected from the LDC speech corpus. The five versions of each CV pair were presented 20 times in train at 70 dB pe SPL. Responses were recorded from a total of 101 individual auditory nerve fibers in 32 ketamine anesthetized chinchillas. Ensemble responses were created by using the CFs of fibers to allocate individual peristimulus time histograms to one of twenty critical bands used to calculate the articulation index. Within each band, histograms were averaged, normalized, and the resulting histograms were averaged across bands (Loebach and Wickesberg, 2006).

For a number of the speakers, the temporal patterns in the ensemble responses to the stop consonant /p/ replicated the three peak pattern found in the two previous studies. These versions of /p/ were well-articulated stimuli with broad spectral content. Other speakers' versions of /p/ elicited temporal patterns that were not as clear or displayed only a single peak. These stimuli lacked the broad spectral content of the well-articulated CV pairs. Our initial analysis indicates that the divergence of a response pattern from the three peak standard correlated with an under representation of fibers whose characteristic

frequencies fell within the major spectral components of the stimulus.

752 Within and Across Fiber Temporal Fine Structure Coding in Auditory Nerve Following Noise Induced Hearing Loss

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Recent perceptual studies suggest that hearing impaired listeners have reduced ability to use temporal fine structure (TFS) cues. Although it has been proposed that reduced phase locking ability in auditory nerve (AN) fibers following sensorineural hearing loss (SNHL) might underlie these perceptual deficits, their neural correlates remain unknown. The present study characterized within and across fiber TFS coding in chinchilla AN fibers responding to pure tones, broadband noise (BBN) and speech. Responses of an individual AN fiber ('Base CF') to 9 frequency-shifted stimuli were used to predict the responses of 9 AN fibers with effective characteristic frequencies (CFs) spanning an octave range around Base CF using a spectro-temporal manipulation procedure. Phase transitions were computed across the 9 effective CFs for a pure tone presented at Base CF. Shuffled cross-correlogram analyses were used to quantify across-CF TFS coding in terms of a neural cross-correlation coefficient and a characteristic delay. Pure-tone phase transitions across CF were shallower in the SNHL population. Characteristic delay increased with increasing CF separation in both normal and SNHL populations, but estimated traveling wave delays between CFs were reduced following SNHL. Across CF neural correlation decreased with increasing CF separation for BBN and speech, with the SNHL population showing a wider range of CF separations over which significant correlated activity existed. These results are consistent with broadened tuning following SNHL. In contrast to these two observed effects of SNHL on across-fiber coding (increased correlation and decreased characteristic delay), within fiber strength of TFS coding for BBN and speech was not degraded following SNHL. Based on spatiotemporal theories of auditory perception, degradation in across fiber rather than within fiber TFS coding might explain reduced ability of hearing impaired listeners to use TFS cues.
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753 Predicted Effects of Sensorineural Hearing Loss on Across-Fiber Envelope Coding in the Auditory Nerve

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Cross-channel envelope correlations have been hypothesized to influence speech intelligibility, particularly in adverse conditions. Acoustic analyses suggest that speech envelope correlations differ for syllabic and phonemic modulation bands. The present study examined the influence of cochlear filtering by predicting cross-channel envelope correlations in different speech modulation bands for normal and hearing-impaired (HI) conditions.
Neural cross-correlation coefficients computed from shuffled correlograms have been used to quantify cross-stimulus similarity in envelope responses. These metrics were extended by segregating neural envelope responses into three modulation bands: 0-5 Hz (syllabic), 5-64 Hz (phonemic) and 64-300 Hz (periodicity). The neural cross-correlation coefficients were used to quantify across-fiber envelope coding in each modulation band. Spike trains were generated from a physiologically based auditory-nerve model responding to a speech sentence. Correlations were also computed for a HI model version that included outer-hair-cell (OHC) damage.
Cross-channel neural predictions revealed that syllabic envelope was highly correlated across a wide range of spectral channels. Phonemic and periodicity envelopes were correlated mainly between pairs of adjacent channels. Evaluation of the effect of characteristic-frequency (CF) separation revealed that neural cross-correlation for envelope decreased with increases in CF separation for all modulation bands, with greater syllabic-envelope correlation than phonemic or periodicity correlation at each CF separation. OHC impairment was predicted to increase the degree of cross-CF envelope correlation for all modulation bands, similar to the effects of sensorineural hearing loss on across-fiber coding of temporal fine structure. These results have important implications for predicting the effect of sensorineural hearing loss on speech intelligibility.
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754 Pre-Synaptic and Post-Synaptic Auditory Neuropathy

WITHDRAWN

755 Identification of Inputs to Olivocochlear Neurons Using Transneuronal Labeling with Pseudorabies Virus (PRV)

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Olivocochlear (OC) neurons receive inputs from a variety of sources including the cochlear nucleus, the inferior colliculus, and the auditory cortex. In each of these nuclei, however, the identity of the cell types providing the inputs is not known. We investigated the identity of neural inputs to MOC neurons using transneuronal transport of the Bartha strain of Pseudorabies virus (PRV) according to the methods of Horvath et al. (2003) Eur. J. Neurosci. 18:1439. After injection of PRV into the cochlea of guinea pigs, retrogradely labeled OC neurons were visible after 1 day survival time but there was no anterograde labeling in auditory nerve fibers or in the cochlear nucleus. Both medial and lateral OC neurons were retrogradely labeled

but the ratio was variable from case to case. At 2-3 day survival times, labeling persisted in OC neurons and also appeared in the cochlear nucleus, as if PRV had passed across synapses and retrogradely labeled the cell bodies of neurons providing inputs to OC neurons. Labeling appeared mainly in the AVCN and PVCN where the numbers of labeled neurons on the injected side were about twice those of the opposite side. Most neurons were multipolar cells in the core of the nucleus and extensive dendritic filling enabled us to identify some as planar multipolar neurons. There was little labeling of bushy or octopus cells. These data suggest that multipolar neurons provide the intermediate limbs of the OC reflex pathways. Labeling for 2-3 day survival times was also present in the pontine dorsal raphe and the locus coeruleus. Longer survival times resulted in labeling in the inferior colliculus, auditory cortex, and other nuclei, confirming that these sources provide descending inputs to OC neurons. (Supported by NIDCD RO1 DC01089.)

756 Cochlear Nucleus Multipolar Cell Projections to MOC Neurons in the Ventral Nucleus of the Trapezoid Body

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Medial olivocochlear (MOC) neurons receive inputs from the ventral cochlear nucleus (VCN) that drive their response to sound as part of the MOC reflex. The identity of the cochlear nucleus cell type providing these inputs is not known. We tested the hypothesis that cochlear nucleus multipolar cells project to MOC neurons. We injected biotinylated dextran amine (BDA, 10k MW) into the dorsal cochlear nucleus (DCN) of mice. These injections labeled ventral acoustic stria fibers (from VCN multipolar neurons that have collaterals to the DCN). Labeled axons presumably from planar multipolar neurons were relatively thin (1-3 µm diam.) and gave off branches to the ipsilateral lateral superior olive (iLSO) and the contralateral ventral nucleus of the trapezoid body (cVNTB) before continuing on to the contralateral inferior colliculus (cIC). Some individual axons branched to both nuclei, while others gave off only a branch to the iLSO or only a branch to the cVNTB. In the cVNTB, labeled terminals were en passant and terminal boutons of small size (< 3 µm diam., < 10 μm^2area). In several cases, MOC neurons in the cVNTB (labeled by a second separate cochlear injection of Fluorogold) were contacted by these boutons on their dendrites and cell bodies. These data suggest that planar multipolar neurons provide the intermediate limb of the MOC reflex pathway. In addition, our injections labeled the dorsal acoustic stria (DAS). These thick crossing fibers projected primarily to the cIC with occasional branches to the cDCN. Surprisingly, some DAS fibers also branched to the contralateral superior olivary complex (cSOC), but their terminations were biased more caudal and dorsal relative to the location of MOC neurons. Supported by: NIDCD RO1 DC01089.

757 Malformation of the Rat Superior Olivary Complex in an Animal Model of Autism

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Autism is a complex neurological disorder that affects social development and is associated with auditory deficits including deafness, increased thresholds to tones, intolerance for ordinary sound levels and difficulty hearing in the presence of background noise. Additionally, we have reported a consistent and significant disorganization of neurons within the medial superior olive in postmortem human autistic specimens. Taken together, these observations suggest that abnormal morphology in the lower auditory brainstem may contribute to the auditory deficits observed in autism and provide the foundation for a systematic examination of the auditory system in a controlled model for autism. Moreover, prenatal exposure to valproic acid (VPA) has been associated with autism in humans and reproduces autism-associated malformations in rodents. To investigate the integrity of the rat superior olive, we exposed timed pregnant female albino rats to a single intraperitoneal injection of valproic acid on day 12.5 of gestation. Animals were divided into two groups: prenatal exposure to VPA (n=8) or control (n=4). All nuclei within the rat SOC were included in an analysis of cell body neuronal morphology and neuronal number and revealed highly significant differences between control and experimental animals. These findings are in line with descriptions of the auditory brainstem in autistic individuals.

758 Characterization of Glycinergic Inhibition to MNTB Principal Cells

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Neurons in the medial nucleus of the trapezoid body (MNTB) receive strong, well-timed and fast excitatory input via the highly specialized calyx of Held. The extremely large synaptic currents produced by the calyx have sometimes led to the view of the MNTB as a failsafe relay nucleus. However, the observation that MNTB neurons also receive substantial glycinergic inhibition is inconsistent with the view of a relay nucleus. Previous work has shown that the conductances of the glycinergic inhibitory currents compare to the conductances of the extremely large calyceal excitatory inputs, and that glycinergic inhibition can suppress firing of MNTB neurons. However, the functional role of this inhibition is not well understood.

We performed whole-cell patch clamp recordings from gerbil MNTB brain slices to study the features of the inhibition in more detail. We were specifically interested in the synaptic properties of the inhibition under conditions that match those of the intact brain as much as possible. Besides using animals well past hearing onset and performing all recordings at physiological temperature, we re-introduced various levels of background activity into the brain slice to simulate spontaneous activity present in the auditory brainstem in-vivo.

We isolated glycinergic inputs to MNTB by blocking excitatory receptors as well as GABAa-receptors and stimulated glycinergic inputs electrically in the immediate vicinity of the neuron. With this method, we readily observed inhibitory postsynaptic currents (IPSCs) in every principal cell we tested. Currents could always be blocked by the glycine-antagonist strychnine. When the effects of 'spontaneous' background activity for prolonged periods of time was tested, substantial depression of IPSCs could be observed. Transmission remained phasic even with long-term stimulation. High frequency activity revealed tonic components in the IPSC trace.
O.A. and F.M. contributed equally.

759 Function of KCNQ Channels in the Calyx of Held

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The medial nucleus of the trapezoid body (MNTB) of mammals is a prominent component of the medullary auditory system that effectively relays signals required for sound localization. Fast transfer of signals from globular bushy cell in ventral cochlear nucleus to MNTB principal cell is facilitated by the calyx of Held, a giant glutamatergic nerve terminal. Using patch clamp techniques, we have identified in the calyx a slow-activating, weakly inactivating K^+ current with properties well suited to a role in regulation of subthreshold properties of synapses. Recordings were made from calyces in brainstem slices of P8-12 rats. In the presence of extracellular TTX, $CdCl_2$, TEA and 4-AP, voltage pulses from -100 mV to -30 mV induced a slowly activating outward K^+ current. This current was blocked by XE991 and linopirdine, antagonists of KCNQ (Kv7) family K^+ channels. Consistent with this identification, the outward currents were potentiated by flupirtine, a KCNQ activator. The XE991-sensitive K^+ conductance began to activate just positive to -80 mV, suggesting that it could play a role in the resting properties of the calyx. Indeed, application of XE991 or linopirdine depolarized the terminal by several mV and decreased resting conductance, while flupirtine had the opposite effects. Furthermore, XE991 markedly increased calyceal excitability and decreased spike-frequency adaptation. XE991 facilitated transmitter release by enhancing vesicle release probability, as assessed by measurements of mEPSC frequency as well as evoked EPSCs and paired-pulse ratios. Our data are consistent with immunohistochemical data that revealed that the KCNQ5 subunit is expressed in the calyx of Held (Caminos et al., 2007). We thus propose that KCNQ5 channels control multiple aspects of presynaptic function including resting properties, excitability and transmitter release.

760 A Comparison of in Vivo Synaptic Transmission in the Mouse and Gerbil MNTB

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The medial nucleus of the trapezoid body (MNTB) provides inhibitory input to two binaural nuclei in the superior olivary complex: the lateral superior olive (LSO) and the medial superior olive (MSO). Their relative sizes vary considerably across species, and this variation is linked to the relative importance of interaural level differences and time differences in sound localization. In mouse, a high-frequency specialist, LSO is relatively large, whereas in gerbil, a species with good low-frequency hearing, MSO is well developed. We studied whether the different relative sizes of LSO and MSO are reflected in the coding of sounds by the MNTB in these two species. As the MNTB has been proposed to provide well-timed inhibition to MSO, we were particularly interested in the timing and security of MNTB cells. We performed whole-cell and loose-patch recordings of principal cells in the MNTB of anesthetized adult mice and gerbils to test whether synaptic transmission and temporal coding of auditory information in the MNTB differs between the two species. In both species, MNTB responded only to contralateral auditory stimulation. More importantly, although postsynaptic action potential failures were observed in both animals, these failures made a relatively small contribution to tone adaptation. In addition, in both species, no evidence for the presence of short-term depression was observed. Interestingly, gerbil MNTB neurons with low characteristic frequencies could show strong phase-locking to the auditory stimulus. These high-synchrony cells yielded vector strengths up to 0.98 in response to low-frequency tones. Our results suggest that in both species the calyx of Held synapse primarily acts as a fast auditory relay, and that strong phase-locking in gerbil globular bushy cells of the cochlear nucleus is preserved in the gerbil MNTB.

761 Hyperbilirubinemia Causes Hearing Loss and Impairment of Synaptic Transmission at the Calyx of Held Auditory Synapse

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Severe cases of neonatal jaundice are associated with deafness and disorders of the central nervous system such as Kernicterus. The mechanism of the auditory deficit is unclear, but auditory brainstem response (ABR) recordings suggest involvement of the auditory brainstem (Shapiro, 1988). We have employed the Gunn rat model of hyperbilirubinemia to raise free bilirubin and have investigated changes in synaptic transmission in the Medial Nucleus of the Trapezoid Body (MNTB) using in vivo and in vitro methods.
ABRs revealed a significant loss of sound-evoked brainstem activity after 18 hr exposure of homozygous jj-Gunn rats to elevated bilirubin levels. In vitro extracellular field potential recording from the MNTB using the MED64 showed an increased delay and reduced amplitude of synaptic responses. Whole-cell patch-clamp recording from principal neurons of the MNTB confirmed that postsynaptic action potentials were similar to control animals (Wistar rats). However stimulation of the calyx of Held axons failed to elicit EPSCs in jj-Gunn rats exposed to elevated bilirubin levels, whilst control rats showed large calyceal EPSCs following stimulation. Anterograde

labelling of the calices by transport of dextran-rhodamine from the aVCN and multi-photon imaging, revealed degeneration of the calyceal terminals of bilirubin-exposed jj Gunn rats. Electron microscopic examination confirmed these pathological changes in axons and synaptic terminals. This evidence clearly shows that one central mechanism of bilirubin toxicity is through degeneration of the calyx of Held synapse and subsequent presynaptic failure.

762 Endocannabinoid Receptor Expression in the LSO and MNTB of the Developmental Rat

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Neurons in the lateral superior olive (LSO) receive tonotopically aligned excitatory and inhibitory inputs, an organization which is important for frequency-specific computation of interaural intensity differences. During development, the inhibitory, glycinergic pathway from the medial nucleus of the trapezoid body (MNTB) becomes tonotopically refined by the silencing of most initially-formed MNTB-LSO synapses. Because endocannabinoids (EC) are important retrograde messengers that can decrease the strength of inhibitory synapses in an activity-dependent manner, we investigated whether EC signaling is present in the developing LSO.

The expression of CB1 receptor (CB1-R) was investigated in rats aged postnatal day (P)3 (n=5) and P12 (n=5) using immunohistochemistry. At both ages, CB1-R labeling was present in the LSO and as well as MNTB. In the MNTB, labeling was present around cell bodies consistent with its expression in the calyces of Held, as previously reported (Kushmerick, et al., 2004). CB1-R labeling was also present along MNTB axons as punctae in the LSO. Fluorescent double labeling with SV2 antibodies suggests a presynaptic expression.

To test whether CB1-R can mediate synaptic depression of MNTB-LSO synapses whole-cell patch clamp recordings were performed from LSO neurons in slices obtained from neonatal rats. Electrical stimulation of MNTB inputs (15 stimuli at 20 Hz for 5 min) elicited strong synaptic depression (n=7) which was blocked by the specific CB1-R antagonist AM251 (10 µM, n=6) or by inclusion of the calcium chelator BAPTA (20 mM, n=6) in the recording pipette.

Together these results demonstrate the presence of CB1-R and functional endocannabinoid signaling in the developing MNTB- LSO pathway and suggest that endocannabinoids play a role in the tonotopic sharpening of this inhibitory sound localization circuit.

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763 A Novel Form of Synaptic Plasticity Observed at Excitatory Inputs to the LSO

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LSO principal neurons process azimuthal cues by comparing sound level differences between the two ears, with the ipsilateral ear providing glutamatergic excitation via the cochlear nucleus, and the contralateral ear providing GABA/glycinergic inhibition via the medial nucleus of the trapezoid body (MNTB). Inputs from the CN and MNTB converge onto LSO neurons in a precise, frequency-specific manner (Sanes and Rubel, 1988). In neonatal animals, initial projections from the MNTB (Kim and Kandler, 2003) and CN (Sanes and Wooten, 1987) are exuberant, and are refined during early postnatal development. This suggests that excitation and inhibition are refined 'in tandem,' with changes in the activity of one pathway influencing the maintenance or elimination of synapses in the opposed pathway. Here we report a novel form of plasticity in the glutamatergic CN-LSO pathway in which hyperpolarization of postsynaptic LSO neurons facilitates LTP. When LSO neurons in slices from neonatal mice (P4-P9) were voltage clamped near resting potential (-70 mV), stimulation of CN inputs (5 x 100Hz x 1s) elicited LTD (EPSC amplitude post induction = 86.9 ± 3.7% pre-induction, n=5). In contrast, if LSO neurons were held at -90 mV, the same induction protocol elicited LTP (EPSC amplitude post induction = 145.5 ± 8.8% pre-induction, n=4). These differences in the polarity of potentiation were also observed in experiments in which plasticity was induced in current clamp at potentials of -70mV and -90mV). Thus, CN inputs to the LSO are selectively potentiated when they co-occur with LSO neuron hyperpolarization. This suggests that during development, CN inputs are potentiated if their activity coincides with inhibition elicited by co-active MNTB inputs. Ongoing experiments are determining whether inhibition supplied by MNTB inputs is able to provide this LTP-inducing hyperpolarization. References: 1)Kim and Kandler, Nat. Neurosci (2003); 6(3): 282-90. 2) Sanes and Wooten, J.Neurosci (1987); 7(11): 3803-11. 3) Sanes and Rubel, J.Neurosci (1988); 8(2): 682-700. Supported by the NIDCD (DC04199 (KK) and T32 MH 18273 (JBC).

764 Physiological Heterogeneity in the Avian Superior Olivary Nucleus

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The superior olivary nucleus (SON) provides the majority of inhibitory input to the auditory nuclei responsible for ITD processing in birds. Acoustic and electrical properties of SON cells were characterized by *in vivo* and *in vitro* electrophysiological recordings. *In vivo*, SON cells could be broadly categorized into two types: cells that were excited by acoustic stimulation, and cells that were suppressed. The majority of SON cells belonged to the excitatory category. Of these, 54% were sensitive to interaural intensity differences (IID's), 46% were sensitive to interaural timing differences (ITD's), 31% were sensitive

to both, and 23% were monaural responders. The suppression type cells had a high rate of spontaneous discharges that were suppressed by acoustic stimulation. We also investigated intrinsic properties of SON neurons using whole cell current clamp recordings. Similarly, multiple physiological profiles were observed from SON cells. Some cells had a phasic firing response to depolarizing current injection (firing one or two spikes at the onset of current injection) while others fired tonically throughout current injection. These results suggest that SON neurons are functionally specialized as "timing" or "integrating" cells. Since Kv1 and Kv3 family potassium channels contribute substantially to "timing" properties in other auditory neurons, we investigated the distribution of Kv1.1 and Kv3.1 K+ channels in the SON using immunohistochemistry. Unexpectedly, Kv1.1 and Kv3.1 channels are expressed rather uniformly throughout the nucleus. These studies demonstrate that the SON contains a heterogeneous population of physiological types, which suggests that SON neurons may have various functional roles in auditory processing.

765 Increased Temporal Precision of Envelope Coding in the Intensity-Coding Pathway of the Barn Owl

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Owls principally use two variables to determine sound source location: interaural time difference (ITD), which indicates the azimuth coordinate and interaural level difference (ILD), which cues the vertical coordinate. These variables are processed in two parallel pathways in the brainstem, which converge on the inferior colliculus (IC), where they give rise to space-specific neurons in the external nucleus of the inferior colliculus (ICx). Neurons in nuclei of the auditory brainstem preceding the lateral shell of the core of the inferior colliculus (ICcl) are narrowly tuned to frequency. As neurons' space specificity emerges, their frequency tuning expands and their spectrotemporal receptive fields (STRFs) become more complex. The STRFs of neurons in the ascending ITD pathway preceding ICcl have been shown to be simple and highly separable in frequency and time. However, STRFs in the intensity pathway have not been characterized and may contribute to IC neurons' spectrotemporal complexity. We have thus recorded neurons in nucleus angularis (NA) and the pars posterior of the lateral lemniscus (LLDp), two nuclei belonging exclusively to the ILD pathway. In these neurons we found highly separable STRFs comparable to those found in the ITD pathway. We also measured the reproducibility and temporal precision of the neural response to the stimulus envelope in both pathways. We found that neurons of the ILD pathway exhibit greater temporal precision in their response than neurons belonging to the ITD pathway which are able to phase lock to frequencies up to 9 kHz. Thus, although neurons of the ILD pathway lack the temporal accuracy to sustain phase locking, they are able to more precisely encode envelope timing.

766 Modeling the Effect of SAM-Tone Modulation Frequency on Average Response Rates of LSO Cells

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The lateral superior olive (LSO) is a brainstem nucleus that mainly encodes information in high frequency sounds. Previous studies have shown that LSO cells are sensitive to envelope interaural time difference (ITD) in sinusoidally amplitude-modulated (SAM) tones. It has also been shown that the average response rate of many LSO cells in response to ipsilateral SAM tones decreases dramatically with modulation frequency above a few hundred Hertz. This low-pass feature is not directly inherited from the inputs since the response rate of most LSO afferents does not change much with increasing modulation frequency. In the modeling study described here, an LSO cell model is developed to investigate mechanisms that would lead to the rate decrease of LSO cells described above. One contributing factor explored is the low-threshold potassium channel (KLT) that is believed to be present in LSO cells (Barnes-Davies et al, European Journal of Neuroscience, 2004). When this channel is included in the model, the empirically observed rate decrease is shown by the model. In addition, the model shows that the membrane time constant also plays an important role in the rate decrease. However, in contrast to available data, model responses to pure tones show low rates of response, comparable to those with high modulation frequencies. The effects of other types of channels on these properties are being explored.

767 Inhibition Shapes the Temporal Discharge Patterns of Units in the Lateral Superior Olive: A Modeling Study

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Most studies of single-unit responses in the lateral superior olive (LSO) have been conducted in anesthetized preparations; two have sampled responses in unanesthetized, decerebrate animals. In anesthetized cats, LSO units usually respond to ipsilateral best-frequency tone bursts with a chopper-type discharge pattern, characterized by regularly spaced peaks of activity initially time-locked to the stimulus onset. In contrast, LSO units in decerebrate cats often display less regular, primary-like discharge patterns. Barbiturates are known to alter the balance of excitation and inhibition, and thus may explain these differences.

The goal of the present modeling study was to investigate the role of inhibition in shaping the temporal discharge patterns of LSO units. Consistent with the known anatomy, model LSO units receive excitatory inputs on their distal dendrites from model spherical bushy cells in the ipsilateral cochlear nucleus, which in turn receive inputs from model auditory nerve fibers. LSO cells also receive inhibitory inputs on their soma from globular bushy cells in the contralateral cochlear nucleus via a synapse in the (not explicitly modeled) ipsilateral medial nucleus of the

trapezoid body. In the current set of simulations, the input was strictly monaural, thus the contralateral inhibitory inputs simply provided tonic (spontaneous) inhibitory drive. Simulation results show that model LSO units display chopper-type discharge patterns when the inhibitory inputs are weak, which become primary-like when the inhibitory synaptic strength is increased. These results thus suggest that inhibition plays a substantially larger role in shaping response properties in decerebrate than anesthetized preparations. Supported by NIDCD grant R01 DC 05161.

768 Rebound Spiking in the Mouse Superior Paraolivary Nucleus

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The superior paraolivary nucleus (SPON) is a prominent structure in the superior olivary complex that receives predominantly contralateral excitatory input from the cochlear nucleus and a glycinergic feed-forward inhibitory input from the ipsilateral medial nucleus of the trapezoid body. One of the current hypotheses regarding the SPON function is that it contributes to the encoding of sound duration. This is based on the fact that most of the SPON neurons fire action potentials transiently at the offset of pure tones or broadband noise stimulation (e.g. Kadner et al., 2006 J Neurophysiol. 95: 1499-1508). Using the patch clamp technique and calcium imaging, we have characterized the basic membrane properties of SPON neurons before and after hearing onset in mice brainstem slices. Furthermore, we have tested the hypothesis that the physiological correlate to the offset response demonstrated in vivo is generated by a post-inhibitory rebound depolarization. The results demonstrate that these neurons express specific membrane properties, such as a low membrane time constant and large voltage-dependent rectification, that appear to accommodate fast and temporally precise electrical signalling. Another prominent biophysical property of all recorded SPON neurons is a rebound depolarization with one or a few anodal spikes following a hyperpolarization. A pharmacological characterization of the rebound depolarization in these neurons reveals an intricate collaboration of the hyperpolarization-activated current Ih and the low voltage-activated Ca current of T-type. These currents act in concert to trigger a reliable rebound depolarization response, most probably driven by the feed-forward inhibition. The functional role of this type of rebound firing could well correlate to the offset spiking seen in vivo upon tone stimulation.

769 Are Chopper-Like Offset Responses of SPN Neurons Mediated by a Hyperpolarization-Activated Cyclic Nucleotide-Gated Current (I_H)?

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The superior paraolivary nucleus (SPN) is involved in encoding of sound duration and provides precisely timed GABAergic inhibition to the inferior colliculus. Following acoustic stimulation *in vivo*, three types of action potential (AP) firing have been described for the SPN: chopper, onset and offset firing patterns. The SPN receives bilateral excitatory input from the cochlear nuclei, but AP firing is suppressed during a sound by inhibitory input from the medial nucleus of the trapezoid (MNTB) and once released from this inhibition, SPN neurons respond with rebound APs. Here we use whole-cell patch recording in mouse brainstem slices to analyze the contribution of voltage-gated K^+ channels to the excitability and AP response patterns of SPN neurons. On depolarization under current-clamp, SPN neurons fire trains of large, fast APs (peak amplitude: 66.7 ± 4.3 mV; halfwidth: 0.32 ± 0.02 ms, n=14) with firing rates proportional to the increasing depolarization. Hyperpolarizing current injection showed the characteristic slow sag of I_H, and a depolarizing off-response, triggering a burst of APs following the current step. Voltage-clamp showed inward I_H current activated by hyperpolarizing voltage steps from -50 mV; it was half-activated at -90.7 ±6.1 mV, with a peak conductance around 20nS, and activation was fit with two time-constants: 39.9 ms (62.5%) and 315ms (37.5%) measured on stepping to -120mV (n=11). Outward K^+ currents were dominated by Kv3 channels, since >50% of the current was blocked by 3 mM tetraethylammonium (TEA). Immunolabelling was used to confirm that Kv3 and HCN subunits were expressed in the SPN. We postulate that the inhibitory MNTB inputs which suppress firing during sound also activate I_H; its slow deactivation then triggers the off-response AP firing on cessation of the sound.

770 Responses to Temporal Sound Features in the Inferior Colliculus Are Influenced by the Superior Paraolivary Nucleus

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The superior paraolivary nucleus (SPON) is a major source of GABAergic inhibition to the auditory midbrain. The ability of SPON neurons to signal the occurrence of discontinuities within ongoing stimuli makes them well-suited to encode temporal features of sound. In particular, SPON neurons detect silent gaps in pure tone stimuli with great sensitivity, and synchronize to low modulation rates of sinusoidal amplitude-modulated (SAM) tones with high vector strengths. Despite our growing knowledge of SPON unit responses to various stimuli, it remains unclear how this nucleus contributes to the processing of acoustic information in its main synaptic target, the inferior colliculus (IC). One approach to examine the influence exerted by the SPON on processing of temporal sound information in the midbrain is to compare single unit responses in the IC to gap and SAM stimuli, both in the presence and absence of SPON activity. Thus, we used iontophoretic delivery of the GABAA receptor agonist muscimol to reversibly silence SPON activity while conducting simultaneous recordings in the IC. Inactivation of SPON inputs was accompanied by an increase in gap detection thresholds and a decrease in the synchronicity of responses to SAM stimuli in IC neurons. In addition, the range of SAM tone modulation

frequencies over which IC neurons phase-locked was lower when the SPON was inactivated. These preliminary findings suggest that the SPON likely contributes to the encoding of gap and SAM stimuli by IC neurons. Supported by NIDCD RO1 DC02266

771 Effect of Sampling Frequency on the Measurement of Phase-Locked Spikes

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Temporal information of sound is often coded by phase-locked neural activity in the auditory system. In order to quantify the degree of phase-locking, the metric called vector strength (VS) has been widely used. Since VS is derived from spike timing information, precise measurement of spike occurrence is important in calculating VS. In electrophysiological experiments, spike timing is measured with finite temporal precision, which is determined by the sampling frequency. Intuitively, sampling rate should be as large as possible to reduce the error in the calculation of VS. In practice, however, the maximum sampling rate is usually determined by technical limitations. Choice of sampling rate is important for animals such as the barn owl, where phase locking has been observed up to 8kHz. How high a sampling rate is sufficient to obtain good measurement of VS? We calculate errors in VS assuming several different types of sampling effects and compare our theoretical results with data from in vivo recordings of auditory brainstem neurons. We show that error in VS is well estimated by an equation: Error = 1-sin(πR)/(πR), where R is the signal frequency divided by the sampling frequency. This means that the error in VS calculation due to sampling is expected to be less than 0.5% if the sampling frequency is set 20 times larger than the signal frequency. Our results provide a practical guideline for choosing the proper sampling frequency for the measurement of VS.

772 Properties of the Low-Frequency Components of the Auditory Neurophonic in the Barn Owl's Nucleus Laminaris in Response to Clicks

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The auditory neurophonic potential is a local field potential appearing in nuclei where phase locking is observed. In response to acoustic clicks, a complex oscillatory neural response occurs in the nucleus laminaris with a component that reflects the best frequency measured with tonal stimuli, the high-frequency component, and one or more low-frequency components. We have already reported that the high-frequency component was better fitted by a Gaussian function than by a Gammatone function and that a frequency glide is presented in the carrier of the high-frequency component (Wagner et al., J Neurophysiol 102: 1227 (2009)). This report deals with the low-frequency components obtained after separation from the high-frequency component by low-pass filtering. The envelopes of these components were obtained from the analytic signal created by using the Hilbert transform. The time courses of the envelope and carrier waveforms were characterized by fitting them with filters. The envelope was better fitted with a Gammatone function than with a Gaussian function. The carrier was better fitted with a frequency glide than with a constant frequency. Additionally, the Gammatone function provided a better fit for the carrier than the Gaussian function. Thus, some properties of the impulse response of the low-frequency components of the neurophonic potential in the nucleus laminaris of barn owls differ from properties of the high-frequency components. The low-frequency components, however, reflect many characteristics also observed in responses of the basilar membrane and auditory nerve in mammals, most notably that the shape can well be fitted by a Gammatone function.

773 Endogenous GABA Sharpens Interaural Time Difference Coding in the Chicken Nucleus Laminaris

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GABAergic inhibition activated exogenously improves coincidence detection in the avian nucleus laminaris (NL), but the role of endogenous GABA is poorly understood. We therefore investigated the characteristics and functional significance of synaptically released GABA in NL neurons. Whole-cell recordings were performed in slice preparations obtained from late chicken embryos. Under voltage clamp recordings, a GABAaR antagonist SR95531 (10 μM) not only abolished spontaneous inhibitory postsynaptic currents (sIPSCs), but also consistently caused an outward current, indicating a tonic GABAaR-mediated current in NL neurons. Evoked inhibitory postsynaptic currents (eIPSCs) expressed different temporal patterns depending on the stimulus frequency. At 1 Hz, discrete eIPSCs were clearly distinguishable. At 5 Hz, eIPSCs began to summate, and a smooth current plateau formed at stimulus frequencies above 100 Hz. Under current clamp recordings, we stimulated the ventral and dorsal excitatory glutamatergic inputs to NL neurons to mimic the process of interaural time difference coding, and evaluated the effects of synaptically released GABA on coincidence detection. Stimulation of the GABAergic pathway at a frequency of 100 or 200 Hz sharpened both evoked excitatory postsynaptic potentials and the time window for coincidence detection. Conversely, blockade of GABAaRs broadened both parameters. Our results show that a tonic GABAaR-mediated conductance is present in NL neurons, and endogenous GABA sharpens interaural time difference coding.

774 Burst Firing of MSO Neurons *In Vitro* Prior to Hearing Onset

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Spontaneous action potential activity and in particular burst firing is a prominent feature of developing auditory neurons prior to hearing onset, but the mechanisms controlling this activity are not well understood. We addressed this question in the medial superior olive (MSO) by making whole-cell current clamp recordings from MSO neurons in gerbil brainstem slices before and up to hearing onset (P8-P12). At ages <P12, suprathreshold step depolarizations generated a fast, overshooting action potential followed by a train of more slowly rising spikes that could be graded in amplitude and were typically associated with a slow subthreshold membrane oscillation. In some cases, an initial burst of 1-4 spikes from 80 to 400 Hz was observed. In paired somatic and dendritic recordings, all spikes were detected first at the soma, presumably reflecting an axonal site of initiation. However, both bursting and repetitive spiking was eliminated in the presence of 10 µM nifedipine or 3 µM mebifradil, indicating contributions from L and T-type voltage-gated calcium channels (VGCCs). All of these features could be observed up until P11/12, after which MSO neurons fired a single spike at the onset of current steps. In response to synaptic stimulation of ipsilateral or contralateral excitatory inputs, EPSPs recorded in MSO neurons at <P12 exhibited a strong, slowly rising late component that typically triggered a bursting firing pattern. This late component was abolished both by membrane hyperpolarization and the presence of 50 µM L-AP5, indicating that it was mediated by NMDA receptors. Taken together, our findings suggest that prior to hearing onset, the firing activity of MSO neurons is dominated by a bursting phenotype, which is driven through the cooperative activation of both NMDA receptors and VGCCs. Such activity may be important for the normal development of the MSO and its targets.

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775 GABAB Receptor Activation as a Possible Mechanism for Maintaining Precise ITD Selectivity Across Varying Stimulus Conditions: An in Vitro Study

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Interaural time disparities (ITDs) are the primary cues for localization of low frequency stimuli. ITDs are computed by coincidence detecting neurons in the medial superior olive (MSO) in mammals. Behavioral studies show localization performance is largely independent of stimulus intensity. However, a large increase in synaptic input as stimulus intensity increases may degrade ITD selectivity in the absence of compensatory mechanisms. Thus, it is of great interest to determine what factors contribute to the maintenance of precise ITD processing. Here we show in P7-P24 neurons that excitatory and inhibitory synaptic currents to the MSO are modulated in both amplitude and kinetics by activation of GABAB receptors (see Burger et al. 2007). We hypothesize that GABAB dependent suppression of synaptic strength is one mechanism by which ITD precision may be maintained over a wide range of stimulus intensities. We utilized three complimentary approaches to test the potential impact of GABAB activation on ITD encoding in MSO neurons. 1) Current clamp recordings from MSO neurons were made while pharmacologically isolated excitatory inputs were bilaterally stimulated using pulse trains that simulate ITDs in vitro. MSO neurons showed strong selectivity for bilateral delays. Application of 0.1mM baclofen, a GABAB agonist, sharpened tuning in 22/25 cells, decreasing half-width by an average of 25.6%. 2) To probe more subtle features of GABAB's influence, we simulated synaptic inputs using dynamic clamp. This allowed for the independent control of multiple input properties (e.g. input number, kinetics, etc.). GABAB activation degraded selectivity for simulated low intensity stimuli, but improved precision at higher intensities. 3) We confirmed and extended these results in a computational model modified from Zhou et al. 2005. Our studies suggest that in vivo modulation of synaptic input by GABAB receptors may act to preserve ITD selectivity across various stimulus conditions.

776 Axonal Delay Lines from Bushy Cells to the Medial Superior Olive: A Reexamination

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In the Jeffress model (1948) a binaural cell is maximally active when the external acoustic delay (interaural time differences: ITDs) is compensated by an internal delay, bringing the inputs from left and right ears in coincidence. By arranging axonal branching patterns of the monaural input fibers in systematic and opposite ways for the ipsi- and contralateral inputs, a range of length differences is created so that ITD is transformed into a spatial activation pattern along the binaural nucleus.

Two anatomical studies in the cat support the existence of delay lines, particularly for the bushy cell inputs from the anteroventral cochlear nucleus to the contralateral medial superior olive (MSO) (Smith et al., 1993; Beckius et al., 1999). Reconstructions in Beckius et al. quantitative but were based on gross injections without physiology, while Smith et al. labeled and physiologically characterized single fibers but made largely qualitative reconstructions. We therefore reexamined the latter material, tracing and digitizing 16 axons on a Neurolucida system (Microbrightfield, Colchester, VT).

The reconstructions largely confirm the observations of the two studies. For most contralateral axons, a ladder-like, caudally directed pattern is seen. For ipsilateral axons the

pattern is more complex with smaller rostrocaudal length differences in a direction opposite to the contralateral pattern, but for 2/7 ipsilateral fibers the rostrocaudal pattern ran in the same direction as the contralateral fibers. Surprisingly, the tonotopic distribution of the afferents indicated that low CFs are under- rather than overrepresented in the MSO (Guinan et al., 1972).
Comparison of our reconstructions with binaural physiological data indicates that the suggestive anatomical patterns cannot account for the distribution of best delays as a function of CF in the cat (Hancock & Delgutte 2004; Joris et al., 2006). Some other mechanism must contribute to or be responsible for the internal delays.

777 Reassessing the Intrinsic and Synaptic Properties of MSO and LSO Principal Neurons in Light of Their Interaural Time Difference Sensitivity

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Sensitivity to interaural time differences (ITDs) has been recorded from neurons in the medial and lateral superior olive (MSO and LSO) alike, despite their apparently different tonotopy, presumed function in hearing and differences in patterns of innervation. In order to explore intrinsic neural mechanisms that might account for some of the possible variations in ITD sensitivity observed between the MSO and LSO, we explored how intrinsic properties of MSO and LSO neurons are distributed across the tonotopic axis in and between the two nuclei, using sinusoidal current injections of linearly increasing/decreasing frequency (ZAP). The ZAP current injection is commonly employed to measure a neuron's input impedance and any resonant peak thereby encountered can be associated with that neuron's preferred input-frequency selectivity. The effects of a variety of conditions, including the application of drug XE991 to block channels of the KNCQ family, were examined. The average frequency at which resonant peaks were observed in pMSO neurons of guinea pig was 171 ±89 Hz (mean ±S.D. n=9) whereas in pLSO neurons of the same species, it was 83.3 ±28 Hz (mean ±S.D. n=3). Results also stress the importance of a neuron's holding potential in maintaining a resonant peak. In order to clarify the contribution of presynaptic inputs, we have explored the possibility of using calcium imaging as a means of monitoring networks in vitro after bilateral stimulation of trapezoid body fibres. However before this goal is realised, proof that a calcium signal is induced by action potentials must be first demonstrated in principal neurons followed by a "calibration of the system". Towards these ends, pMSO and pLSO neurons were patched with a pipette containing the calcium sensitive dye, Oregon Green 488 BAPTA-1, and current steps of minimal amplitude threshold to evoke action potentials were injected somatically. Initial results suggest that a conspicuous calcium signal is elicited after multiple action potentials are evoked and that this technique merits further development.

778 A Comparison of Correlation Sensitivity and Bandwidth at Multiple Anatomical Levels in the Interaural Time Difference Processing Pathway

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The interaural time difference processing pathway consists of different monaural and binaural stages. Previously, we measured bandwidths (BW) and correlation sensitivity at two stages in this pathway: the monaural auditory nerve (AN) and the binaural inferior colliculus (IC). We showed that while BW depends on characteristic frequency (CF), comparisons within the same CF range showed no difference in the mean BWs of these two structures (Mc Laughlin et al, J Neurophysiol, 2008). This suggests that there is little frequency convergence at the level of the IC and that the frequency selectivity determined in the cochlea is preserved at higher levels. Unexpectedly, this study revealed that AN and IC neurons can be similar in CF and BW yet respond differently to changes in correlation.
Here, we applied the same coincidence detection analysis to calculate pseudo-binaural correlation and noise delay functions (NDFs) from trapezoid body (TB) data recorded in cats. We quantified BW and correlation sensitivity using the same curve fitting methods as Mc Laughlin et al 2008. We find that BWs are similar to those in the AN and IC. However, TB fibers are more sensitive to changes in correlation than AN fibers, consistent with TB fibers being more temporally precise. In contrast, IC neurons are less sensitive to correlation than both TB and AN fibers. Thus, the high temporal precision of the monaural stages is not present in the IC. To examine whether the loss of precision takes place before or after the coincidence detection stage in the medial superior olive (MSO) we quantified a relationship, across the AN, TB and IC, between correlation sensitivity and NDF damping. This allowed us to predict correlation sensitivity in MSO neurons based on damping of published NDFs (Yin and Chan, J Neurosci, 1990). Surprisingly, MSO correlation sensitivity is similar to that in the IC, indicating that the precise temporal information available monaurally may never be fully exploited binaurally.

779 Binaural Interaction and Internal Delays: The Intrinsic Disparity Hypothesis

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The binaural system compares sound waveforms at the two ears, and in that comparison the brain uses internal delays, so that the inputs from the two ears are compared at different temporally offsets. By virtue of the internal delay between its inputs, a binaural neuron is maximally responsive to a certain ITD – the best delay – which is equal to and opposite in sign to the internal delay, because at that ITD the inputs are coincident.
Several mechanisms have been proposed to underlie internal delays. Common to all models is a reliance on

temporal circuit features, e.g. axonal delays, cochlear disparities, or timed inhibition. Here, a new hypothesis is advanced which puts the source of internal delay at the site of binaural interaction: the medial superior olive (MSO).
The hypothesis is based on two premises. First, intrinsic properties of MSO neurons constitute a neural form of "DA conversion", so that coincidence detection is not on the incoming spike trains per se but on analog waveforms. Spiketrains are transformed into waveforms in a frequency (interval) dependent way, making the integration time longer for low than for high stimulus frequencies. Second, intrinsic properties are asymmetrical between contra- and ipsilateral compartments, so that the effective input at the point of binaural interaction is more sluggish contralaterally than ipsilaterally. "Intrinsic" is defined loosely: the asymmetry could be in morphology, innervation pattern, ion channel distribution, voltage dependence, etc.
It is proposed that these mechanisms effectively convert the statistics of the input spike trains into frequency-dependent best delays, akin to a phase shift. By slowing the rise time of contra- relative to ipsilateral events, internal delays arise and generate binaural tuning favoring sounds leading at the contralateral ear, with frequency-dependent best delays smaller than half a stimulus period.
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780 Modeling Binaural Response in the Auditory Brainstem to Electric Stimulation of the Auditory Nerve: Effects of Membrane Properties on ITD Sensitivity

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The neural sensitivity to interaural time difference (ITD) is limited with bilateral electric stimulation of the auditory nerve compared to the ITD sensitivity observed in normal hearing. Specifically, ITD-sensitive neurons in the binaural pathway show tuning to ITDs of electric stimulation, but only in a narrow range of conditions. A network of model neurons was used to study the binaural response to electric stimulation. A descriptive model of an auditory nerve fiber (ANF) was implemented and used to provide input to a model of binaural processing in the ascending auditory pathway. Acoustic and electric responses were simulated with models incorporating variations in neural mechanisms and compared to each other and to empirical data. We found that ITD sensitivity is dependent on the model's membrane channels, synaptic inputs, and properties of the input stimulation. A model with slow membrane time constants and weaker synaptic inputs shows good ITD sensitivity at low stimulation rates but no ongoing activity at high rates. Stronger synaptic inputs and faster membrane are necessary for sustained ITD sensitivity at high stimulation rate. This is consistent in both acoustic and electric stimulations, although the dependency on neural and stimulus parameters is stronger in electric responses. In the model with the slower membrane and weaker inputs, amplitude modulation restores the ongoing ITD response to high-rate electric pulse trains. In contrast, amplitude modulation of high-rate electric pulse trains impairs the ITD sensitivity in the model with fast membrane and stronger inputs, reflecting saturated response near the best ITDs of the model cell and monaurally driven responses outside the best ITDs. We suggest that the rate-limitation of the ITD sensitivity to electric stimulation can be attributed to neural mechanisms in the central auditory pathway.
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781 Prediction of Precedence Effect Phenomenon by a Network of Coincidence Detector Cells

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In a reverberant environment a sound source approaches the listener through several paths. The first arriving wave front dominates many aspects of perception, even though each of the following wave fronts (echoes) is audible in separation. This phenomenon of echo suppression is known as the precedence effect.
In order to predict the experimental results, we designed a model that consists of a multi layered network architecture of coincidence detector (CD) cells. The model includes the following brainstem nuclei: Cochlear Nucleus (CN); Superior Olivary Complex (SOC); Ventral Nucleus of the Lateral Lemniscuses (VNLL); and the Inferior Coliculus (IC). Each nucleus includes either Excitatory-Excitatory (EE) or Excitatory-Inhibitory (EI) cells.
The inputs to the model are simulated spike trains arriving from the auditory nerve fibers. The stochastic behaviour of those spike trains can be described as non-homogenous Poison Process (NHPP). According to a method developed by Krips and Furst (Neural Computation, 21, 2524–2553, 2009), the output of every CD cell in the network also behaves as NHPP and its instantaneous rates (IR) can be derived
We demonstrate prediction of one aspect of the precedence effect, the ability to suppress an echo in a lateralization experiment. This type of psychoacoustical test consists of two clicks, with varying inter click interval (ICI). The subject's task is to indicate the direction the second click. Experimental results demonstrated maximum degradation in performance at ICI of 2 msec. We have successfully predicted this phenomenon using the Cramer-Rao lower bound at the model's output.
This approach was previously proved to be successful in other binaural phenomena such as BMLD, JND of ITD and ILD and MAA.

782 Mapping Tinnitus in Auditory Related Brain Regions Across Two Rat Models Using Manganese Enhanced MRI

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There are no reliable biomarkers for diagnosis and treatment of tinnitus, although advances in rapid tinnitus screening have recently gained ground. Combining gap inhibition of the acoustic startle reflex (gASR) with the manganese-enhanced MRI (MEMRI) method we contrast brain region activity prior to and following the onset of tinnitus across two different rat models. The gASR was used to determine tinnitus status, while brain regions associated with the perception of tinnitus in awake freely moving subjects was shown using MEMRI. Tinnitus was induced either with repeated doses of salicylate or a single noise exposure. Salicylate induced tinnitus resulted in wide spread changes in brain activity when compared to noise induced tinnitus which showed much more restricted changes in activity. However, there were regions of overlap suggesting that a fundamental component of tinnitus may be identifiable/common regardless of the manner in which the tinnitus was induced. Therefore, we demonstrate that the combination of gASR and MEMRI provides a non-invasive, sensitive metric for using changes in activity level to identify tinnitus related brain regions specific to the method of tinnitus induction as well as across methods in animal models of tinnitus. Our results provide the foundation for future studies aimed at correlating the severity and longevity of tinnitus with neuronal activity, the role of hearing loss and evaluation of the efficacy of treatment across paradigms.

783 Sound-Evoked Forward Suppression of Spontaneous Firing in Auditory Neurons May Relate to Residual Inhibition in Tinnitus

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Recently, we observed that sounds lasting a few milliseconds can suppress spontaneous firing in inferior colliculus neurons for several hundreds of milliseconds. The aim of this study was to extend this research by determining whether the duration of this suppression depends on sound duration. Extracellular responses of inferior colliculus (IC) neurons exhibiting spontaneous firing were recorded in awake CBA/CaJ mice. Stimuli were presented as pure tones at the characteristic frequency for IC neurons or broadband noise. Duration of the stimuli ranged from 50 ms to 1500 ms, and they were presented once every 30 sec. The vast majority of neurons (93%) showed spontaneous firing ranging from 0.4 to 43 spikes/second (sp/s) with median of 5.2 sp/s. Neurons could be divided into two groups based on their response pattern. First, a large population of neurons (74%) exhibited an onset or/and offset response pattern. A relatively small population of neurons (26%) fired during the entire duration of the stimulus. The vast majority of neurons from both populations (82%) showed sound-evoked suppression of spontaneous firing; however, the suppression duration was remarkably different for these populations. The neurons exhibiting an onset or/and offset response pattern also exhibited a short suppression lasting a few tens of milliseconds right after on-off responses occurred. Neurons that fired during the duration of the sound stimulus also showed suppression of spontaneous firing as long as several seconds. Duration of this suppression increased with an increase in the duration of the stimulus.

Hyperexcitability or elevated spontaneous activity in the central auditory system often correlates with the perception of tinnitus in humans; thus we hypothesize that sound-evoked suppression of spontaneous firing may underlie residual inhibition, the phenomenon whereby tinnitus is suppressed by long duration sounds. Supported by NIH R01 DC00537.

784 Salicylate-Induced Tinnitus: Alterations in Neuronal Activity in the Inferior Colliculus of Tranquilized Mice

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Tinnitus, the perception of a phantom sound, affects ~14% of the population. Sodium salicylate (SS), the active component of aspirin, induces transient tinnitus in humans and animals, and is often used to investigate its neural mechanisms. The purpose of this study was to investigate changes in neuronal firing patterns across the tonotopic axis of the central nucleus of the inferior colliculus (ICC), a midbrain auditory region which receives inputs from many other brain regions and is a gateway for processing both spectral and temporal properties of sounds. Extracellular recordings were made from the ICC of tranquilized mice (CBA/CaJ) using vertically oriented 16-channel Michigan probes, which allowed for sampling of multi-unit (MU) activity across the entire tonotopic axis of the ICC. Recordings were made from the same location pre-SS, and 0.5, 1, 2, and 3 h post-SS administration (250 mg/kg IP). Frequency-response area (FRA) maps were generated from the MU response to brief tones (1-72 kHz; 0-80 dB SPL) from a speaker placed 30° in the contralateral hemifield. FRA maps showed a progressive increase in minimum threshold (MT) following SS, ultimately reaching ~40 dB SPL shift by 3 h post-SS, result consistent with SS-induced outer-hair cell dysfunction. In addition, for many units FRAs underwent significant SS-induced changes presumably due to alterations in the normal balance of excitation and inhibition. Neural correlates of temporal processing were assessed using the gap-in-noise paradigm (1-96 ms in duration) and the minimum gap threshold (MGT). Following SS administration, there was a significant increase in the mean MGT from 2 ms pre-SS to 4 ms at 30 m post-SS ($p < 0.001$), and 5 ms at the 1, 2 and 3 h post-SS time points ($p < 0.001$). Finally, spontaneous rates (10 min recording) across the entire population of units decreased following

SS. Inter-spike intervals and k'-1, a measure of neural synchrony using the cross-correlations of units across different frequency bands, were also assessed. The significant increase in MGT and the change in FRA shapes of ICC neurons following acute SS intoxication may reflect an alteration in the normal balance of excitation and inhibition known to sculpt many of the response properties of ICC neurons. Supported in part by grants from the NIH (R03DC008685, R01DC009219, R01DC009091)

785 Mechanisms Underlying Plasticity of Rate-Level Functions in the Inferior Colliculus

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We are investigating the role of input convergence and local neural circuitry in the coding of sound intensity by single neurons in the inferior colliculus (IC). Rate-level functions (RLFs) in IC neurons range from monotonic, wide dynamic range to nonmonotonic, narrow dynamic range functions, and RLF shapes can change when tone repetition rate changes. Our previous studies indicate that monotonic RLFs in the IC are generated by local circuit dynamics, while nonmonotonic RLFs reflect extrinsic inputs. In the current study, we are examining the effect of changing tone repetition rate on local circuit activation, and the consequent changes in RLF shape and dynamic range.

Neuronal firing rates at different sound intensities are recorded extracellularly in the IC of awake mice, in control conditions and with local circuits blocked by pressure injection of high-divalent cations. Pure tones are delivered to the contralateral ear at the cell's characteristic frequency, with repetition rates ranging from 1/s to 16/s, and RLFs constructed over a 0-90 dB SPL intensity range.

Preliminary data suggests that the peak firing rate of nonmonotonic units increases with tone repetition rate, but the intensity corresponding to the RLF peak remains invariant. The width of the nonmonotonic RLF increases asymmetrically with repetition rate, broadening only at higher intensities. Increases in both peak height and RLF width appear to correlate with changes in spike count during successive tones, increasing as repetition rate increases. The asymmetric nature of this correlation implicates an intensity dependent effect on the plasticity of RLFs. Our data suggests that intensity tuning and selectivity in the IC is affected by plasticity in the synaptic and network interactions in the IC.

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786 $GABA_A$ Receptors Mediate Stimulus-Specific Adaptation (SSA) in the Auditory Midbrain

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Some neurons in the auditory system adapt to a repeated sound but resume firing when a rare one is presented. This phenomenon is called stimulus-specific adaptation (SSA) and has been demonstrated to occur in the auditory cortex (AC), and more recently in the inferior colliculus (IC) and auditory thalamus. The demonstration of SSA subcortically raised the important question of whether SSA is created de novo subcortically or whether it is inherited from the AC via descending projections. The functional mechanisms underlying stimulus-specific adaptation are not known, but intracellular recording has shown that excitation in IC neurons is often followed by long-lasting hyperpolarization, possibly due to synaptic inhibition (Covey et al., 1996) and it is well known that GABAergic inhibition shapes many response properties in the IC. To determine whether local inhibition contributes to the SSA exhibited by IC neurons, we recorded from 45 single units in the IC using an oddball stimulation protocol similar to that of Ulanovsky et al. (2003) and Malmierca et al. (2009), while applying microiontophoretic injections of gabazine, a $GABA_A$ receptor blocker. Our results demonstrate that in a majority of units (about 75%), injections of gabazine significantly reduced the degree of SSA by at least 20%. In another 17 units we tested SSA under conditions of low repetition rate (ISI = 2000 ms) and small frequency contrast (Δf = 0.10), which are thought to elicit SSA only at the AC level. Our results demonstrate that some IC neurons also display SSA under these extreme conditions. Taken together, these results suggest that SSA in the IC is at least partially mediated by GABAergic inhibition, and that it is not merely inherited through excitatory descending projections from the AC.

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787 Stimulus-Specific Adaptation in Neurons in the Rat's Dorsal Cortex of the Inferior Colliculus

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The dorsal cortex of the inferior colliculus (ICd) is one of the major subdivisions of the mammalian auditory midbrain. Neurons in this structure display stimulus-specific adaptation by generating strong spiking to initial presentations of repetitive tone bursts but weak or no spiking to subsequent sound presentations. Responses of neurons showing stimulus-specific adaptation can be restored by changes in sound intensity and/or frequency.

In order to obtain better understanding of stimulus-specific adaptation in ICd neurons and its contribution to auditory novelty detection, we conducted extracellular recordings from single neurons in this structure using the rat as a model species. Stimulus-specific adaptation was evaluated by comparing responses of a neuron to the following two acoustic stimuli. The first one consisted of multiple blocks of repetitive tone bursts. Each block had 20 sweeps of a single tone burst presented at a constant rate of 1 /sec. Tone bursts in different blocks had different frequencies but a fixed intensity as well as duration and rise/fall times. The second type consisted of 20 different randomized sequences of tone bursts. Each sequence was composed of the entire set of tone bursts used for creating repetitive tone burst blocks, with each tone burst presented only once. Tone bursts were presented at a constant rate of 1 /sec. Our results revealed that most neurons in the ICd responded with stronger firing to randomized sequences than repetitive blocks of tone bursts. Neurons showing strong stimulus specific adaptation, i.e., those with large differences between responses to repetitive blocks and randomized sequences of tone bursts, had onset firing patterns. These results suggest that auditory novelty detection in the ICd is dependent on a specific subset but not the entire population of neurons.
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788 Adaptation to Stimulus Statistics in the Perception and Neural Representation of Auditory Space

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Sensory systems are known to adapt their coding strategies to the statistics of their environment, but little is still known about the perceptual implications of such adjustments. We investigated how the processing of auditory space adapts to stimulus statistics by presenting human listeners and anesthetized ferrets with noise sequences in which interaural level differences (ILD) rapidly fluctuated according to a Gaussian distribution. The mean of the distribution biased the perceived location of a subsequent stimulus, whereas the distribution's variance changed the listeners' spatial sensitivity. Neurons in the inferior colliculus can account for these perceptual phenomena. Their ILD preference adjusted to match the stimulus distribution mean, resulting in large shifts in their rate-ILD functions, while their gain adapted to the stimulus variance, producing pronounced changes in discriminability. Our findings suggest that processing of auditory space is geared towards representing relative spatial differences rather than absolute positions.

789 The Time Course of Binaural Masking in the Inferior Colliculus of Guinea Pig

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Psychophysical studies show a slower response to changes in the binaural input than to changes in the monaural input (binaural sluggishness). However, depending on the nature of the experiment there is disagreement about the time course. Tracking changes in a target yield fast time constants, whilst detecting a constant target against a varying background yield the slowest. Changes in the binaural properties of a target are tracked up to high rates by binaural inferior colliculus cells (Joris, et al., 2006: J. Neurosci. 26, 279-289, Siveke, et al., 2008: J. Neurosci. 28, 2043-2052). Indeed, cells respond rapidly to a step change and then the firing rate slowly adapts (Ingham and McAlpine, 2004: J. Neurophysiol. 91, 632-645). These experiments, though, are analogues of those psychophysical experiments which give the faster time constants. Sluggishness should be more apparent physiologically in a binaural masking paradigm, detecting a short tone in a noise masker with a step change in correlation, since the small change in firing rate due to the signal must be detected against the adapting firing rate change caused by the step change in the binaural masker. However, in preliminary results in 24 inferior colliculus cells in the anaesthetised guinea pig, tracking target threshold across a transition in a binaural masker (e.g. from No So to Nπ So) provided little evidence of sluggishness within individual cells, despite masking level differences in these cells comparable with previous data. The failure to find any evidence for sluggishness in this direct analogue of the psychophysical paradigm and our binaural masking level difference findings (Jiang, et al., 1997: J. Neurosci. 17, 9331-9339) suggests an hypothesis that sluggishness may result from a change in focus between different populations of cells signalling threshold in different binaural configurations, rather than within the intrinsic properties of the cells themselves.

790 Adaptation of IC Neurons to Repeated Presentation of a Harmonic Complex

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In recent years the phenomenon of stimulus specific adaptation has been the subject of interest amongst computational modellers trying to replicate the features of this adaptation by modelling the properties of the underlying neural mechanisms. To support this effort we are investigating the properties of these mechanisms by examining adaptation to different stimuli presented using different protocols. Here we present data gathered using repeated presentations of harmonic complexes. Gerbils were anaesthetised with vapourised isoflurane delivered in carbogen. Stimuli included 100 repeated presentations of a harmonic complex (fundamental frequency of 800Hz and 12 components). Neural responses were recorded using platinum-iridium electrodes, amplified and visualised on an oscilloscope then recorded through the microphone input of a laptop computer. Electrolytic lesions were made in recording tracks to confirm electrode locations histologically. Spikes were threshold discriminated offline using MATLAB software. Responses were measured as spike counts over the time period 0-350ms after stimulus onset summed across 20 stimulus presentations. Adaptation to the repeated stimulus was assessed by

varying the range of repetitions over which these responses were summed from the first 20 to the last 20. 8/28 inferior colliculus neurons examined responded with similar spike numbers across stimulus presentations. The response of 12/28 neurons decreased greatly over the first ~30 repetitions and then stabilised at a lower value for the remaining presentations. 4/28 increased their responses with more stimulus presentations. 2/28 neurons maintained consistent responses until declining after ~60 repetitions. One neuron greatly increased responses after ~10 repetitions but decreased again after ~30. These results show the variety of adaptation/facilitation time courses within the inferior colliculus and of the underlying mechanisms that give rise to them.

791 Inferior Colliculus Neurons and Psychophysical Performance: Perceptual Enhancement Reflects Central Computations

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Previous sound stimulation can increase a listener's sensitivity to a subsequent signal. For example, thresholds for a probe tone centered in a spectral notch are lower when the probe-bearing complex is preceded by a band-reject sound (the conditioner). Several lines of evidence point to a central origin for this effect. The purpose of this study was to compare human psychophysical sensitivity to the representation of these stimuli in single neurons of the marmoset inferior colliculus (IC) using nearly identical parameter spaces. Four normal-hearing human listeners exhibited robust enhancement for the detection of a 4-kHz probe sound; the magnitude of the effect was subject-specific and depended on both the conditioner notch width (NW, varied from 0 to 2 octaves) and the duration of the masker-probe complex (25, 50, and 100-ms durations were tested). Responses from 101 neurons in the central nucleus of the IC of two marmoset monkeys were also recorded. A wide range of response properties were observed across neurons, including both suppression and enhancement of the probe response by the conditioner that could adapt, build up, or remain static over the course of the probe. Despite the diversity of both psychophysical and physiological measures of enhancement, several common features emerged. On average, the perceptual and neural data both reveal a similar best NW for eliciting enhancement (~0.5 octaves) and a comparable maximum shift in detection threshold given the presence of the conditioner (~10 dB, with shifts larger than 15 dB in individual subjects and neurons). Analysis of the neural data using a varying time window shows that while enhancement is the largest near the probe onset, discriminability of the probe responses with and without the conditioner remains relatively constant because of a decrease in across-repetition variability associated with the use of longer spike-counting windows. (Supported by NIH grants DC00115 and DC9164).

792 Corticofugal Modulation of Adaptation to Sound Level Statistics in the Inferior Colliculus

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Neurons in the inferior colliculus (IC) adapt rapidly to the distribution of sound levels in a manner that improves neural coding around the mean level. Employing cryoloops bilaterally to cool and thus reversibly inactivate the auditory cortices of anaesthetised guinea pigs, we examined whether descending, or corticofugal, input to IC from auditory cortex influences this adaptation. Responses of IC neurons were recorded to different sound level distributions before, during and after cortical cooling. We find that the rapidity and extent of adaptation to sound level statistics is affected by the descending pathways. It appears that cooling the cortex modulates adaptation time constants in both directions, either slowing or speeding the adaptation process. In addition, whilst adaptation of rate-level functions to stimulus statistics persists in the presence of cooling, cooling leads to impairment of the matching of neural coding to mean sound level. These data suggest that adaptation at the level of the IC, whilst primarily dependent on ascending input and intrinsic mechanisms, is open to modulation by the corticofugal system, which appears to increase the capacity of adaptation to reconfigure neural responses in accord with environmental demands.

793 Changes in C-Fos Expression in the Inferior Colliculus After Unilateral Auditory Cortex Ablation in the Adult Rat

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The Inferior Colliculus (IC) is the main auditory midbrain center, and receives convergent ascending projections from multiple auditory brainstem nuclei, commissural projection, and descending projections from the Auditory Cortex. In this work, we studied the effects induced by unilateral ablation of the auditory cortex over the c-Fos immunoreactive neurons of the IC. We analyzed, a control-silence group (acoustically isolate), a control group acoustically stimulated (open field, during one hour, with 5 kHz and 80 dB), and groups with cortical ablation and on-stimulated with survival times postlesion (PL) of 15 and 90 days. We used stereological techniques to evaluate changes in the number of IC cells c-Fos positives. We also applied densitometric analyses to assess alterations in the intensity of the immunoreaction and size of the positive c-Fos-neuronal nuclei.

The stereological results show an increased in the number of c-Fos immunoreactive neurons in the IC of control stimulated-group. At 15 days PL we noted a 60% decrease in number of neurons when compared with control stimulated-group. At 90 days PL the reduction was only of 35%. In the control stimulated-group, densitometric and morphometrical analysis revealed a significant increase for both, the gray level and perimeter, of c-Fos positive nuclei.

At 15 days PL, we found an increase of gray level, but also a decrease of nucleus perimeter. However at 90 days PL, the gray level was unaffected, but an increase of nucleus perimeter was detected.
The results indicate that the deafferentation of the Corticocollicular Projection significantly affects the expression of c-Fos in the IC. These alterations seem to be restored after long-term PL periods, suggesting that the IC have capacity for compensating the damage.
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794 Tonotopy Within the Human Inferior Colliculus: Is It Fixed or Plastic?

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Tonotopy is accepted as a fundamental feature throughout the central auditory system although direct evidence in humans is scarce and numerous findings on auditory plasticity argue against a hard-wired frequency organization. Through investigation of a new deep brain stimulation prosthesis that is currently implanted within the central nucleus of the inferior colliculus (ICC) of one deaf patient, we have identified for the first time in humans the existence of a systematic pitch organization across the ICC consistent with what has been inferred from human anatomical data and functional animal studies (i.e., low frequency regions superficially and higher frequency regions within deeper locations). However, this pitch organization was not initially present until after 2 to 4 months of daily electrical stimulation across the ICC. Interestingly, within the first 2 months of stimulation all pitch percepts were perceived as being low, comparable or lower to the subjective pitch level of a man's voice. Considering that the patient had only low frequency residual hearing (<1 kHz) prior to complete deafness and then electrically stimulated after 6 years of deafness, we believe that predominant activation of low frequency regions prior to deafness induced plastic reorganization of higher perceptual centers to code predominantly for low frequency information. Restoration of a systematic pitch organization due to ICC stimulation was not simply due to a re-learning effect of matching the incoming pitch information to the location of stimulation since we did not have the correct frequency-to-electrode processor mapping until after 10 months of stimulation. In other words, "non-tonotopic" spatial activation across the ICC was sufficient to restore the original pitch organization or at least a spatial order consistent with what has been inferred from animal studies. These findings raise the possibility that although pitch organization may be perceptually altered through a reorganization in higher centers (e.g., in the primary auditory cortex), there still exists some fixed tonotopic organization within the ICC that can be grossly activated to restore tonotopy within those perceptual centers. This type of fixed-plastic framework could explain how the brain is capable of appropriately adapting to varying and meaningful spectral environments without losing its fixed frame of reference. *Research supported by Cochlear Ltd.*

795 Neural Coding of ITD with Bilateral Cochlear Implants: Effects of Auditory Experience

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Human bilateral cochlear implant users do poorly on tasks involving interaural time differences (ITD), a cue which provides important benefits to normal–hearing listeners, especially in challenging acoustic environments. Yet the precision of neural ITD coding in acutely-deafened, bilaterally-implanted cats is essentially normal (Smith and Delgutte, J. Neurosci. 27:6740-6750). One explanation for this discrepancy is that neural plasticity induced by the extended periods of binaural deprivation typically experienced by cochlear implant users may degrade neural ITD sensitivity. To test this hypothesis, we recorded from single units in inferior colliculus (IC) of two groups of bilaterally-implanted, anesthetized cats: acutely-deafened cats, which had normal binaural hearing until experimentation, and congenitally deaf white cats, which received no auditory inputs until the experiment. Rate responses of only half as many neurons showed significant ITD sensitivity to low-rate pulse trains in congenitally deaf cats compared to acutely-deafened cats. For neurons that were ITD sensitive, ITD tuning was broader and best ITDs were more variable in congenitally deaf cats. A signal detection model constrained by the physiology supports the idea that the degraded neural ITD coding resulting from deprivation of binaural experience contributes to poor ITD discrimination by human implantees.
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796 Central Auditory Processing Following Exposure to an Augmented Acoustic Environment: Is There a Critical Period?

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Congenital sensorineural hearing loss affects thousands of infants each year and can result in significant delays in speech and language development. Previous studies by Turner and Willott have shown that early exposure to an augmented acoustic environment (AAE) limits outer hair cell death and maintains peripheral auditory thresholds in a mouse model of congenital hearing loss. The current study assesses the developmental time course of AAE and asks the questions: i) what are the effects of delaying the start of the exposure on peripheral and central auditory function and ii) what are the effects of limiting the duration of the exposure?
Mice of the DBA strain were exposed to the AAE stimulus for 60 days. In addition two other groups were examined:

mice exposed for 30 days followed by 30 days of no exposure, and mice unexposed until P30 followed by 30 days of exposure. Auditory brainstem responses (ABRs), distortion product otoacoustic emissions (DPOAEs) and extracellular physiology were used assess peripheral and central auditory function. To assess the effects on central auditory processing we recorded neural activity from a 16-channel vertical multi-channel electrode placed in the inferior colliculus (IC). Frequency response areas (FRAs) were measured from 2 to 64 kHz and from 0 to 85 dB SPL, from which we derived each units best frequency, threshold and tuning sharpness. To assess temporal processing we recorded responses to a gap-in-noise stimulus and determined the minimum gap threshold (MGT) and recovery from the neuronal response to carriers present at 60, 70 and 80 dB SPL.

Significant improvement in peripheral function was seen in all three exposure groups, with the greatest decreases in ABR thresholds and increases in DPOAE amplitudes seen in mice exposed for a full 60 days. In the IC, delaying the start of exposure by 30 days resulted in similar best frequency increases (12.8 vs 10.7 kHz), threshold decreases (42.0 vs 57.0 dB) and Q10 increases (3.9 vs 2.4) compared to controls. Additionally, the mean MGT was shorter (12.9 vs 29.1 ms) compared to controls. While improvements were also seen in the mice only exposed from birth to P30, the magnitude of these improvements was smaller. These results suggest that the time of onset of the AAE exposure is of little importance in improving auditory function, however, continued exposure is essential to maintain the beneficial effects and limit the functional loss. These experiments pave the way for possible therapeutic intervention in children suffering congenital sensorineural hearing loss.

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797 Serotonin Release in the Auditory Midbrain of Females During Acute Stress

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Male mice exposed to acute stress due to restricted movement respond by releasing serotonin into the auditory midbrain. This suggests modulation of auditory processing during stressful situations. Given that males and females cope with stress differently, we exposed female CBA/J mice to the same restricted movement stressor to determine if serotonin would also increase in females. Subjects were placed in a restricted arena, which prevented horizontal locomotion, but allowed for circling and rearing. Circling was defined as continuous movement in either clockwise or counterclockwise direction within the restricted arena. An animal was considered to be rearing when only the hind limbs were in contact with the floor of the arena. During behavioral testing serotonin was measured in the inferior colliculus (IC) with carbon fiber voltammetry using nafion-coated electrodes, which exclude the metabolites of serotonin. In females there was no increase in serotonin levels in the IC in response to acute stress, in contrast to the 15% increase observed in males in a previous study. Preliminary behavioral analysis suggests that females are less active in the restriction arena than males, providing a potential correlation between behavioral vigor and serotonin increases in the IC. These data suggest that auditory processing is modulated differently between males and females during acute stress.

798 Tone Processing in the Higher-Order Auditory Cortical Field DP of Awake and Anesthetized Female Mice - Mothers and Naïve Females in Various Estrous Cycle Phases

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Secondary fields of the left auditory cortex in mammals are considered to play a major role in perception and recognition. There are only few electrophysiological studies about sound representation in these fields so far. Here we examined the representation of pure tones (PT) in the left dorsoposterior (DP) field of the house mouse (Mus musculus) auditory cortex. Single- and multi-unit recordings from cortical layers III and IV were carried out in anesthetized (ketamine/xylazine) and awake mothers and naïve females during all various estrous phases.

In line with previous studies on primary cortical fields, we also observed in the higher-order field DP lower spontaneous activity and longer response latencies in anesthetized animals compared to awake animals. In addition, neurons in awake mothers had shorter latencies and a sharper tuning (Q10- and Q40-values) than those of awake naïve females. The higher auditory field DP did not indicate significant differences with regard to the effects of anesthesia on neuronal response types and evoked rate at best frequency.

Interestingly, the neurons of the awake naïve females showed significantly shorter latencies and sharper tuning (Q10-values) in the estrus phase compared to the other phases of the estrous cycle.

This study provides further evidence that the anesthetic state significantly and specifically influences neuronal responses especially in secondary fields of the auditory cortex. For the first time we show that this influence is modulated during the estrous cycle and, thus, depends on hormonal changes. It becomes evident that neuronal data from awake animals provide the relevant basis for studying cortical mechanisms that directly relate to auditory perception and recognition in high-order fields of the auditory cortex.

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799 Measures of Backward Masking in Auditory Cortex of Awake Gerbils

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One measure of temporal processing, which contributes to many auditory tasks, is backward masking, where a brief tone is masked by a subsequent noise. The neural substrate of backward masking has never been examined in auditory cortex (ACx) or awake animals. We have addressed this issue by recording from single neurons in the ACx of awake restrained gerbils. A brief (10ms) ramped tone at each neuron's best frequency (BF) was followed immediately by a constant-amplitude narrowband noise masker (500 ms) centered at BF. First, firing rate (FR) was measured in the window (~20ms) where the tone alone elicited the maximal response for each cell. A decrease in FR was observed in the presence of the noise masker despite greater total signal energy. Second, to assess each cell's tone-evoked threshold with and without noise, FR was obtained over the entire stimulus period (500ms). The thresholds of noise+tone were referenced to noise-alone trials, while the thresholds of tone-alone were referenced to spontaneous activity. On average, backward masking produced higher thresholds. Importantly, both these analyses examine a particular neural response window, assuming that signal onset and duration are known. However in the absence of a cue (e.g., in a natural environment or non-cued detection task), the animal would only be able to rely on its neural activity to discriminate between signals. We thus chose the first spike as the beginning of the analysis window and used a spike-distance analysis to compare resulting spike trains. Spike trains to noise-alone were compared to spike trains to noise+tone, producing measures of detection for a tone emerging from noise. Where chance detection is 50%, this metric showed that tones were detectable at 70%. Our data thus indicate that backward masking is visible in ACx at the level of individual neurons by several measures, even under conditions of stimulus uncertainty.

800 Wireless Multi-Channel Single Unit Recordings from Freely Roaming and Vocalizing Marmosets

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The common marmoset (Callithrix Jacchus), a New World primate, is highly vocal when unrestrained. This makes it an ideal model for studying neural mechanisms for vocal production, sensori-motor interaction and auditory feedback. However, neurophysiological studies in naturally behaving primates have posed a range of technical challenges to neuroscientists and engineers. Here we present a wireless neural recording technique that is capable of collecting multi-channel single-unit data from chronically implanted freely roaming and vocalizing marmosets. A light weight, low power and low noise head stage is attached to a 16 channel electrode array placed in the pre-motor area of adult marmosets. The head stage is capable to transmitting single-unit neural data with Signal to Noise ratio comparable to a tethered system for upto 3 hours while the marmoset is engaged in interactive vocal behaviors. In order to minimize RFI / EMI corrupting the neural data, the experiments are conducted within a large (20' x 10' x 8') RF and acoustically shielded chamber. This combination of wireless neural recording technique with interactive vocal behavior enables us to study neural mechanisms underlying vocal communication. [Supported by NIH grant DC005808 (X.W.)]

801 Simultaneous Neural and Behavioural Assessment of Pitch Discrimination in Freely Moving Ferrets

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We have established methods for obtaining electrophysiological recordings from the auditory cortex of freely moving ferrets whilst they perform a listening task. Neural data were obtained using chronically implanted microelectrodes, which were implanted into the auditory cortex of ferrets previously trained to perform a pitch discrimination task. Ferrets were trained in a two-alternative forced choice paradigm that required them to judge whether the second of two artificial vowel sounds (the "target") was higher or lower in pitch than a preceding "reference" sound (Walker et al 2009). Bilateral electrode arrays (Neuralynx, WARP-16 devices) were implanted following methods adapted from those used by Eliades and Wang (2008). By implanting arrays bilaterally we were able to make simultaneous recordings from up to 32 (2 x 16) independent tungsten electrodes. Each electrode may be independently advanced into the brain to allow serial recordings from different cortical depths. Neural signals were recorded from animals while they performed the pitch discrimination task in their twice-daily testing sessions, over the course of a year. Over this time period the electrodes were advanced approximately every two weeks in increments of 50-200 μm. The locations of the electrode implants varied slightly between animals but aimed to target the ventral borders of primary auditory cortex and the dorsal parts of the posterior fields. These areas typically contain neurons with low best frequencies and high sensitivity to changes in stimulus fundamental frequency (Bizley et al., 2009). Local field potential and spike data were recorded both while the animal was performing the behavioural task, and afterwards when the animal was passively listening. In many cases neurons either did not respond during the behavioural condition or responded in a way that was neither informative about the pitch of the target sound, nor predictive of the animal's response. In a small proportion of recordings neural responses were modulated by the fundamental frequency of the target sound. Because we measured both neural and behavioural signals we were able to examine single neuron "choice probabilities" in order to assess what contribution any single neuron might have made to the animal's behavioural judgment.

802 Contrast Normalisation in Auditory Cortex

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Contrast normalisation [Heeger, 1992] is a well established theory which accurately describes the responses of neurons in primary visual cortex. According to this theory, the responsiveness of each neuron is modulated by a divisive 'gain control' mechanism. The responses of neurons with similar receptive fields are summed to produce a contrast signal, which acts as the denominator in the gain control mechanism.

Contrast normalisation is thought to be a fundamental process in visual object recognition, because it makes neural responses invariant to changes in contrast (which are relatively unimportant for object recognition), while emphasising the variations in structure which distinguish one object from another.

In this study, we asked whether contrast normalisation is present in the responses of neurons in auditory cortex. We recorded neuronal responses to dynamic random chord (DRC) stimuli. Each DRC comprised a sequence of 25ms chords, each comprising pure tones chosen randomly from a probability distribution. By changing the probability distribution, we produced a set of DRCs in which contrast and level were independently varied.

To determine the effects of contrast and level on the gain of each neuron, we first estimated a single spectrotemporal receptive field (STRF) which describes the tuning of each neuron to the whole set of DRCs. Then, for each contrast/level condition, we estimated a sigmoidal transfer function which describes the relationship between the response predicted by the STRF and the actual responses of the neuron in that condition. We find that the gain of auditory cortex neurons is negatively correlated with both level and contrast, and that the effect of contrast is broadly consistent with the contrast normalisation theory.

These results indicate that contrast normalisation operates in auditory as well as visual cortex, and suggest that it may be a general principle of sensory coding.

803 Neural Correlate of Stream Segregation on the Basis of Phase Relations Between Harmonics in Complex Sounds in the Bird Auditory Forebrain

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When successive sounds with different features such as frequency or temporal pattern are presented, they are integrated into one stream or segregated in separate streams according to their perceptual similarity. It has been suggested that segregated streams are represented by separate populations of neurons in the brain. Here, the representation of streams of harmonic-complex sounds with three different phase relationships between harmonics was studied using multi-unit recording in the European starling forebrain. These sounds differ in the temporal waveform only, but not in their amplitude spectrum. Neuronal responses were recorded using the "ABA-" triplet paradigm (for details see Itatani & Klump 2009). The frequency components of the A-signals were always in cosine phase (C). The frequency components of the B-signal were either in cosine phase (CCC- triplets), or they had alternating phase (CAC- triplets) or their phase was random (CRC- triplets). The fundamental was 100, 200 or 400 Hz. Stimulus duration was 125ms, and stimuli were either presented at a high or at a low repetition rate. The tuning characteristics of the recording sites were determined and components of the complex were chosen to fit the neurons' tuning.

Harmonic complexes with different phase relations between components evoked different numbers of spikes. The second sound in the triplets evoked the lowest response rate if in cosine phase whereas the rate responses were the highest in random phase. Recording sites with a characteristic frequency (CF) above 1 kHz preserved more temporal information than those with a lower CF, showing in part highly regular phase-locking patterns corresponding to the fundamental of the stimuli. Forward suppression in the responses to successive sounds was also observed. The results indicate that harmonic complexes with different phase relations may be represented by separate neuronal populations.

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804 Responses to Synchronous Vs. Alternating Tone Sequences in Monkey Primary Auditory Cortex: a Crucial Test of a Neural Model of Auditory Stream Segregation

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According to a neural model of auditory stream segregation in primary auditory cortex (A1), alternating ABAB tone sequences are perceived as a single stream when tonotopically organized neurons in A1 respond to both 'A' and 'B' tones, and as two separate streams when neurons respond exclusively to 'A' tones or to 'B' tones of the ABAB sequences (Fishman et al., 2001). In the latter case, which occurs when their frequency separation is large or their presentation rate is fast, 'A' and 'B' tones activate separate frequency channels along the tonotopic map in A1, thus paralleling their perceptual segregation. While supported by several neurophysiological studies, this model was recently challenged in a study of A1 in ferrets that compared responses to sequences where 'A' and 'B' tones were presented either synchronously or in alternation (Elhilali et al., 2009). Under synchronous (SYN) and alternating (ALT) conditions, the sequences are perceived as a single stream and as two separate streams, respectively. Accordingly, if the model of stream segregation is correct, then a difference in tonotopic response patterns should be observed under these two stimulus conditions, with a significantly larger 'dip' in neural activity occurring in-between tonotopic locations tuned to frequencies of the 'A' and 'B' tones in the ALT condition than in the SYN condition. Contrary to predictions of the

model, no significant difference in the depth of the 'dip' was observed between ALT and SYN conditions. The present study attempted to replicate these results in macaque A1, and thereby subject the model to a crucial test. In contrast to findings of Elhilali et al. (2009), a significantly larger 'dip' was observed under ALT than under SYN conditions, thus supporting the original model of stream segregation. Explanations for the discrepancy in results are considered. In accord with Elhilali et al. (2009), findings are still consistent with a role for temporal asynchrony in streaming.

805 Examining a Temporal Mismatch Between Spike- And Current-Source-Density-Receptive Fields in Ferret Auditory Cortex

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Local field potentials (LFPs) are receiving renewed interest as a measure of subthreshold population activity. Such activity may not easily be detected through spike trains because the LFP and spike records can contain quite different information. Thus it is important to compare the stimulus selectivity of LFP and spiking activity. Studies in the visual [Liu et al. 2006] and auditory [Kayser et al. 2007] cortex have explored such differences in tuning between LFPs and spikes. LFPs can be rendered more informative by deriving from them current source densities (CSDs). CSDs reveal the spatial distribution of currents through the extracellular medium, specifically those due to the synaptic activity of excitatory cells. Here, we investigated the relationship between the stimulus selectivities of CSD and spiking activity in primary auditory cortex (A1) of medetomidine/ketamine-anaesthetised ferrets. We presented dynamic random chords, estimated spectrotemporal receptive fields (STRFs) for CSDs and spikes, and compared the two. We find that the time course of the spike and CSD STRFs differ considerably. Spikes depend on the previous 100-200 ms of the dynamic stimulus, while the CSDs integrate over considerably longer periods (~400 ms). We propose three hypotheses to account for this mismatch. First, during continuous stimulation of the anaesthetised A1, thalamic drive to cortex may last considerably longer than spiking responses. Alternatively, there may be low-pass filtering of the LFP/CSD by the extracellular medium, as has been suggested by Destexhe et al. (2009). A third possibility is that stimulus integration takes place in A1 over far longer periods than spike-STRFs suggest, yet most of these dynamics are subthreshold, and hence appear in synaptic current flows and not in spike trains. We are currently critically evaluating these hypotheses by measuring responses to the same stimuli in inferior colliculus and thalamus.

806 A Cortical Population Code for Sound Locations

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The auditory cortex is involved in sound localization behavior. The spatial receptive fields (SRF) of cortical neurons tend to be large. There is no evidence for a systematic one-to-one space map in the cortex. This suggests that the cortex uses population codes to represent space. However, the nature of such codes remains unknown. Auditory SRFs are shaped by binaural sensitivity and frequency-specific ear directionality. A consistent observation in the cortex is the clustered representation of binaural cues. How binaural clusters underlie population codes for space remains unclear.

These issues were addressed in the cortex of the pallid bat. The pallid bat uses passive hearing to localize prey-generated noise. Its auditory cortex contains a region selective for noise. This noise-selective region contains two binaural clusters based on sensitivity to IID. One cluster contains binaurally inhibited neurons with a systematic map of IID sensitivity. The second cluster contains neurons with peaked IID sensitivity with a systematic map of preferred IID. These clusters predict a population code in which the extent of activated cortex changes systematically with azimuth. This prediction was tested in this study. Using single unit recordings and sequential dichotic and free-field stimulation, the IID sensitivity, frequency tuning and azimuth SRF were mapped across the noise-selective region. We present evidence for a systematic change in the extent of activated cortex as sound moves from ipsilateral to contralateral locations. In the binaurally inhibited cluster, the region of activated cortex starts in lateral cortical locations to systematically include more medial location. In the cluster of neurons with peaked IID, the activity is maximal for sounds from frontal locations. The activity patterns do not change with sound intensity. These data provide cues on how neurons with a broad range of spatial selectivity act together to represent space.

807 Nonlinear Interactions in Cat Auditory Cortex

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We applied Wiener-Volterra system identification methods to characterize the nonlinear response properties of auditory cortical neurons in the ketamine-anesthetized cat. Specifically, we use the so-called "Poisson-Wiener" (PW) approach, which is based on an analysis of responses to Poisson-distributed impulse train inputs. In our initial study (Pienkowski et al., 2009, J. Neurophysiol), we showed that including second-order temporal interactions in a PW model gave reasonably accurate predictions of neuronal temporal modulation transfer functions.

Here we continue with this computational framework to investigate two additional issues: 1. second-order spectral interactions *within* auditory cortical multi-units, and, 2. second-order contributions to the correlation of spontaneous firing *between* auditory cortical multi-units.

1. We observed that facilitory cross-frequency interactions in multipeaked spectrotemporal receptive fields (STRFs) were weak relative to suppressive auto-frequency interactions (i.e., the well-documented "forward suppression" between similar excitatory frequencies). In particular, no unit with a multipeaked STRF showed a supralinear sensitivity for the combination of sound frequencies represented in that STRF. Thus, contrary to common assumption, it is doubtful that individual units with multipeaked STRFs function as combination-sensitive feature detectors, at least in auditory generalists such as the cat.
2. We observed a strong (on average, 3- to 5-fold) increase in the probability of synchronous firing between a pair of units in auditory cortex (both within and across areas AI, AAF and PAF), when the trigger unit fired not a single spike but two (or more) spikes separated by <5 ms. Thus, spontaneous activity in auditory cortex can be substantially more synchronized than it appears on the basis of linear cross-correlation analysis.

808 Across Frequency Binaural Level Comparisons in Auditory Cortex

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Interaural level differences (ILDs) are initially computed at levels relatively low down in the ascending auditory system (e.g. the lateral superior olive). At early auditory nuclei, frequency tuning is not much wider than that of auditory nerve fibres. In the auditory cortex, much wider frequency tuning can be observed, presumably reflecting a convergence of inputs from lower nuclei, having different frequency tuning. If ILD processing occurs predominantly in the brainstem, then interaural level comparisons will only be computed for relatively small frequency differences between the two ears. Thus interaural-level comparisons should be made over narrow bandwidths and then integrated into broadly tuned cortical neurons. Taken together these two assumptions would suggest that binaural interactions in cortical units should be 'frequency specific'. Frequency specificity has been shown in the adaptation of cortical responses to sequences of tones, and to a lesser extent in the inferior colliculus and thalamus.
This hypothesis that binaural interactions in the auditory cortex are frequency specific was tested by presenting a fixed contralateral tone, often evoking an excitatory response, and measuring the ipsilateral frequency tuning curve, which normally evoked an inhibitory response. This measure was repeated at a number of different frequencies and the changes in frequency tuning assessed. The results suggest that across ear interactions are to a degree frequency specific in some cortical neurons. However, across the population these effects were weak. Inhibitory tuning was generally broadly tuned and stronger for contralateral tones near to the unit CF, suggesting that the dominant interaction is between on-CF excitatory contralateral inputs and a broad range of ipsilateral inhibitory frequencies. We additionally frequently observed excitatory ipsilateral tuning when units were weakly driven contralaterally. These results may have implications for the way we understand integration of across frequency spatial information in cortical neurons.

809 Temporal-Integration Mechanism of Bone-Conducted Ultrasonic Speech Sound

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Some profoundly deaf can perceive amplitude modulated bone-conducted ultrasound (AM-BCU) and recognize the speech signals through the AM-BCU. This characteristic of AM-BCU provides the possibility of the creating a bone-conducted ultrasonic hearing aid (BCUHA) which is the alternative to a cochlear implant. However, the characteristic of AM-BCU has not been enough studied and the perceiving mechanism of AM-BCU still has been unclear. To investigate the temporal integration systems of AM-BCU, we evaluated the growth of N1m for duration changed AM-BCU using magnetoencephalography (MEG). Eight Japanese subjects with normal-hearing took part in this study. The Japanese vowels /a/ with 6 degrees (10, 15, 30, 40, 60 ms) of duration were presented by air-conduction and bone-conduction with a 30 kHz carrier. Intensity level of air-conducted speech stimuli was 30dB above the sensitive level of 60 ms vowel /a/. Intensity level of AM-BCU was presented the intensity that the loudness of AM-BCU matched that of air-conducted stimuli. MEG recordings were performed with a 122-channel whole-head neuromagnetomater (Neuromag-122, Neuromag). The N1m amplitudes for AM-BCU increased with the increments of the durations and found the significant main effect for duration like the N1m amplitudes for air-conducted speech sound. These results suggest that the temporal integration system also functions for AM-BCU. However, there was a difference the saturation point of N1m for stimulus duration between AM-BCU and air-conducted speech sound, which may reflects the difference of perceiving mechanism between AM-BCU and air-conducted.

810 Objective Assessments of Bone-Conducted Ultrasonic (BCU) Hearing by Neuromagnetic Measurements

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Bone-conducted ultrasound (BCU) is perceived even by the profoundly sensorineural deaf. We have been developing a novel hearing-aid using BCU perception, that transmits a 30-kHz bone-conducted carrier that is amplitude-modulated by speech.
In this study, the fundamental capabilities of our BCU hearing-aid (BCUHA) were objectively assessed by magnetoencephalography (MEG) and neuronal source analyses in normal-hearing and profoundly deaf subjects. Frequency- and temporal-resolutions were assessed by

measuring mismatch fields (MMFs), which generally reflect the perceptual properties of sound discrimination. As well, the capability to discriminate between two-channel BCUs presented to the left and right mastoids was verified by the laterality of N1m, the most prominent auditory evoked brain magnetic deflection peaking about 100 ms after sound onset.

In the frequency-discrimination tests, the magnitudes of MMFs for amplitude-modulated BCU (AM-BCU) are larger than those of air-conducted sound (AC) when frequencies of the standard-stimuli are below 0.125 kHz or above 6.0 kHz. However, BCU and AC showed almost the same MMF-magnitudes when the standard-stimuli were between 0.25 and 4.0 kHz. Undersampling might have occurred at the higher frequencies; however, we have no evidence to explain the low discriminability at the lower frequencies. These results objectively indicated that our BCUHA has a practical frequency-discrimination capability.

In the temporal-discrimination tests, MMFs for changes of stimulus-duration were measured. Magnitudes of MMFs for AM-BCUs reached more than 80% of those for air-conducted sounds (ACs), indicating the practical temporal resolution of the BCUHA.

Also, in the laterality-discrimination tests, N1m evoked by contralateral stimuli were larger in amplitude and shorter in latency than those evoked by ipsilateral stimuli for AM-BCUs and ACs. These results agree with earlier reports of AC auditions, and suggest that two-BCU channels presented to the left and right mastoid were separately localized, i.e, each BCU channel entered the ipsilateral auditory pathway before the superior olivary nucleus. This finding also gives a rationale for the development of a multi-channel (at least two) BCUHA.

811 Hemispheric Lateralization of Cortical Responses in Children Using Bilateral Cochlear Implants

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We examined cortical activity evoked by right versus left ear stimulation in children using bilateral cochlear implants (CIs). CIs stimulate the auditory nerve using electrical pulses, promoting auditory development in children who are deaf. Because unilateral hearing is known to cause reorganization along the auditory pathways, we hypothesized that: (1) children using unilateral CIs prior to bilateral CI use will show abnormal patterns of auditory activity across the cortical hemispheres and (2) the degree of deviation from normal will be associated with the period of unilateral CI use.

Electroencephalographic responses were measured from 64 scalp locations in 15 children. Some received a second CI after a long (> 2 years) or short (6-12 months) period of unilateral CI use (n=9 and n=4, respectively). Another 2 children received both CIs simultaneously. All had used bilateral CIs for 3-4 years. Data were compared with responses to pure tones in age-matched children with normal hearing. Locations of neural activity in the cortex were identified using beamformer analysis. We measured the difference in activity between left and right cortical hemispheres (cortical lateralization) for each evoked response. Changes in cortical lateralization between left and right side evoked responses were calculated.

Normal hearing children showed lateralization from left ear stimulation to the right hemisphere and a contralateral shift in lateralization with right ear stimulation. Similar results were found in children who received bilateral CIs simultaneously or after a short period of unilateral CI use. In contrast, children who had long periods of unilateral CI use showed little change in cortical lateralization when stimulation was switched from the first to the second CI.

Results suggest that normal patterns of activity in the auditory cortices can be promoted by bilateral CI use but are disrupted if this experience is preceded by extended periods of unilateral CI stimulation.

812 First-Spike Latency in the Congenitally Deprived Auditory Cortex

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In the central nervous system, spike timing, in addition to firing rate, is thought to carry information on the sensory stimulus (also on its non-temporal features). First spike latency (FSL) is one possible measure of such representation (e.g. Chase and Young 2007, PNAS 104:5175-80). To demonstrate the functional relevance of such central “time code” requires also evidence that it depends on sensory experience. The present study is aimed to compare FSL and its variability between hearing competent and congenitally deaf cats (CDCs). Multiunit responses to binaural cochlear implant stimulation from 4 CDCs and 4 hearing controls (HCs) were recorded in the field AI. All animals were electrically stimulated by pulse trains (500Hz, 3 pulses) at intensities of 0-12 dB above response thresholds with varying interaural level and time differences. FSL was computed as a latency of the first spike that could not be explained by a Poisson process using the spontaneous rate observed in the prestimulus time window (Chase and Young, 2007). In HCs, significantly higher FSL variance was found with changing stimulus features (ITD and ILD), and FSL e.g. systematically decreased with increasing electrical stimulus level in HCs. In HCs a significantly higher proportion of recorded units showed a (negative) correlation between FSL and firing rate. Compared to hearing animals, FSL was substantially less dependent on stimulus configuration in CDCs. In conclusion, without sensory experience FSL does not show a systematic relation to stimulus features. These results support the functional relevance of FSL for central representation of auditory stimuli.

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813 Cortical Development and Re-Organization in Children with Auditory Neuropathy/Dys-Synchrony

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Auditory Neuropathy/Dys-synchrony (AN/AD) is a recently described type of hearing impairment, affecting nearly 10% of the hearing impaired population, that preserves outer hair cell function but disrupts afferent processing in the inner hair cells/auditory nerve and/or brainstem. Abnormalities in subcortical afferent processing disrupt normal cortical maturational processes, however, there is virtually no information regarding cortical development and plasticity in children with AN/AD. We have begun an investigation of timelines & mechanisms for central auditory deprivation, plasticity and re-organization in children with AN/AD. We have a large dataset of cortical maturational data from over 100 infants and children with AN/AD. In this study, we report on results from an initial analysis of 20 children from that dataset. We examined cortical activity (using dipole source analyses and sLORETA), intra-subject neurophysiology variability (using analysis such as ICA, PCA & matching pursuit) and morphology, latency and amplitude of the P1 cortical auditory evoked potential (CAEP). The P1 CAEP is generated within the auditory cortex and is considered a biomarker of central auditory development (Sharma et al, 2007). Results reveal that infants and children with AN/AD can be categorized as exhibiting normal, delayed and abnormal patterns of cortical maturation and organization. Further, children who showed normal cortical development also had the highest scores on measures of speech perception relative to children who showed delayed and abnormal maturation. In addition, we find that children with AN/AD who have abnormal cortical development also show high levels of intra-subject cortical variability underlying cortical responses. Finally, we present data that support the possibility that cortical potentials may be useful clinical biomarkers for guiding intervention choices and assessing the efficacy of intervention in children with AN/AD. Supported by NIH

814 Conductive Hearing Loss Produces Changes in Cortical Inhibition That Persist to Adulthood

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Hearing loss during early life can lead to persistent deficits in auditory processing that may be due, in part, to altered synapse function within the central auditory system. We have previously found that one week of sensorineural hearing loss during early development profoundly weakens inhibitory synapses in the auditory cortex (Kotak et al., 2008). However, it is unknown whether more moderate forms of hearing loss also produce these changes. More importantly, it is not known whether these synaptic changes are transient or whether they persist into adulthood. To assess the effects of moderate conductive hearing loss (CHL), the malleus was removed bilaterally in postnatal day (P) 10 gerbils, elevating auditory thresholds by about 35-45 dB (Tucci et al., 1999; Xu et al., 2007). Animals were reared for either one week (P17-22) or to adulthood (P60-100), and synaptic inhibition was then assessed in brain slices containing the auditory cortex using whole-cell voltage clamp recordings. Spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded in cortical layer 2/3 pyramidal cells in the presence of ionotropic glutamate receptor antagonists. At one week after CHL induction, sIPSCs were significantly smaller than age-matched controls (sIPSC amplitude: CHL=-17$\pm$2 pA, n=7; control=-28$\pm$4 pA, n=7, t=2.2, p=0.04). The magnitude of this change was similar to that previously reported in animals with sensorineural hearing loss. When CHL animals were reared to adulthood (P60-100), sIPSCs remained profoundly smaller than adult controls (sIPSC amplitude: CHL=-12$\pm$2 pA, n=10; control=-28$\pm$6 pA, n=11, χ^2=8.3, p=0.004). Together, these results indicate that CHL leads to changes in inhibitory synapses that persist into adulthood. Therefore, alterations to cortical synaptic inhibition may contribute to sound processing deficits in adults that can accompany moderate hearing loss.

815 Blast and Acoustic Trauma-Induced Tinnitus: Auditory and Non-Auditory Aspects

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Tinnitus has complex etiology, involving both auditory and non-auditory factors. Although efforts have centered around the auditory contribution to tinnitus, less attention has been paid to the non-auditory aspects. In this study, we investigated the auditory and non-auditory attributes of tinnitus following blast and noise exposures. In the first experiment, 9 Long-Evans rats were blasted in a shock tube at 14 psi (194 dB SPL). Behavioral manifestation of tinnitus and hearing loss using startle reflex paradigm and MRI diffusion tensor imaging (DTI) in auditory and non-auditory structures were evaluated following blast exposure. Our results showed that blast exposure induced temporary tinnitus and hearing loss, as well as significant changes in the dorsal cochlear nucleus and the corpus callosum. In the second experiment, we investigated the relationship between tinnitus and non-auditory function such as memory and anxiety. In this study, 14 Long-Evans rats were exposed to a 10 kHz tone at 120 dB SPL for 2-3 hours. Using the same procedures, rats were behaviorally tested for tinnitus and hearing loss. After tinnitus development, they were tested for spatial memory and anxiety using Morris Water Maze (MWM) and Elevated Plus Maze (EPM), respectively. Our results showed that tinnitus positive animals performed better at various stages

in the MWM than tinnitus negative animals. No significant difference in EPM was found between the two groups. The results point out that enhanced memory function may be necessary to sustain the behavioral manifestation of tinnitus. Taken together, our data supports previous findings that tinnitus is closely related to hearing loss and trauma-induced plastic changes in auditory and non-auditory brain structures. The enhanced memory function in tinnitus positive animals suggests that maintenance of tinnitus may entail increased input from the limbic system, and may not unilaterally impair cognitive and other limbic functioning.

816 Influence of Intensity and Quantity of Noise Exposure on Neuronal Activity in Central Auditory Structures

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Noise exposure leads beside cochlear hair cell loss to profound long-term changes within the central auditory pathway. A modified spontaneous activity, changes in neuronal cell density and neurotransmitter action and tonotopic map reorganization were reported for several auditory structures. Some of these changes were already present after exposure to moderate sound levels, which does not lead to a permanent threshold shift. It seems to be highly important to investigate the underlying mechanisms leading to long-lasting effects in the central auditory system. Therefore, the calcium-dependent activity was observed by manganese-enhanced (ME) MRI to monitor changes in activation of neural tissue. In this study, normal hearing mice were exposed to moderate (90 dB SPL) or high-level (115 dB SPL) broadband noise (5-20 kHz) for three hours under anaesthesia. A further group was exposed twice at 115 dB SPL with an interval of 7 days between the treatments. 7 or 14 days after noise trauma, hearing thresholds were determined by measurements of the frequency specific auditory brainstem response. Calcium-dependent neural activity was measured by 7T-MRI scanning 24 hours after injection of a manganese chloride solution. Signal strengths in several auditory structures (DCN, VCN, SOC, IC, MGB and AC) were measured in all treatment groups and compared with those of normal hearing controls. The data of the present study indicate that the effect of noise on auditory brain areas (i.e. calcium-dependent activity) could be enhanced by an increased intensity or a repeated exposure. However, activity changes also occurred after the application of moderate sound and were strengthened with increasing noise intensity (especially on higher auditory structures). The results show that the effect of noise on the central auditory system depends on the quality as well as on the quantity of the exposure (i.e. the integration of sound intensity over time).

817 Long-Term Reorganization of Mature Primary Auditory Cortex by Passive Exposure to Moderate-Level Sounds

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Recent work in our lab has shown that long-term (6-week), passive exposure of mature cats to moderate-level (68 dB SPL), band-limited (2–4 kHz or 4–20 kHz) tone pip ensembles can profoundly decrease neural activity in primary auditory cortex (AI) to sounds in the exposure frequency range, and increase neural activity to sounds outside that range. The concomitant reorganization of the AI tonotopic map resembles that following restricted lesion of the sensory epithelium, although in our case, no signs of cochlear trauma can be detected. Exposure-induced plasticity is largely reversible over the long-term, although it appears that several more subtle changes can persist at least for months. Intermittent exposure (12h-on/12h-off) has qualitatively similar albeit weaker effects compared to continuous exposure, a finding with implications for those in noisy work/quiet living environments, even at levels considerably below those presently considered harmful to hearing.

Similar effects of two-octave-wide (but noise) exposure have recently been demonstrated in AI of developing animals. However, it is known that exposing developing animals to tone pips and narrowband FM sweeps produces an *increase* in AI responsiveness at the exposure frequencies. Recent data with third-octave-wide exposures (centered at 4, 8 and 16 kHz) in mature animals will be compared to these developmental data. We will revisit the potential mechanisms underlying exposure-induced AI plasticity in developing and mature animals.

818 Role of GABAergic Activity in Auditory Cortex Gain Control

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High doses of salicylate, which cause peripheral hearing loss, can enhance sound-evoked response in the central auditory system (CAS). This enhancement phenomenon suggests that the CAS increases the central gain to compensate the reduced peripheral input. Abnormal increases in central gain may contribute to hyperacusis and tinnitus. To explore the neural mechanisms underlying central gain changes, we examined the effects of GABA receptor agonists and antagonists on AC neural responses. Bicuculline, a GABA-A receptor antagonist, significantly enhanced the firing rate of neurons in AC. In contrast, muscimol, a GABA-A receptor agonist and S-baclofen and R-baclofen, GABA-B agonists, strongly suppressed the firing rate or sound-evoked local field potential. Consistent with previous results, salicylate treatment (systemic injection or local application on the AC) caused a significant increase in sound-evoked AC neural activity and also significantly enhanced the amplitude of the startle reflex, a behavioral manifestation of increased gain. S-baclofen or r-baclofen, GABA-B receptor agonists, reduced the salicylate-induced

enhancement of AC firing rate. Similarly, vigabatrin, an anti-seizure drug that increases GABA concentration in the brain, also reduced the salicylate-induced AC response enhancement. Consistent with the central gain hypothesis, vigabatrin and baclofen also reduced the exaggerated startle reflex response induced by salicylate. Our results suggest that salicylate reduces GABA inhibition in the CAS leading to enhancement of the AC response. Increasing GABA-medicated inhibition with baclofen or vigabatrin reduces the central gain and reverses the exaggerated neural and behavioral responses evoked by high dose of salicylate (Supported by RNID, AFAR and NIH).

819 Auditory Processing in a Mouse Model of Fragile X Syndrome

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Fragile X syndrome (FXS) is a genetic disorder that is associated with autism, and manifests through symptoms including repetitive behavior, social anxiety, loss of communicative ability, and audiogenic seizures. *Fmr1 KO* mice represent a genetic model for studying FXS which replicates many of the symptoms seen in FXS patients. Previous work done in *Fmr1 KO* mice has demonstrated disregulation of excitatory and inhibitory input to the cortex, including a decrease in excitatory input to fast-spiking inhibitory interneurons along the thalamocortical pathway. The loss of excitation led to a decrease in activation of inhibitory cells. Whether this trend extends to the auditory cortex has not yet been determined. In this study, we used single unit extracellular electrophysiology to assess possible differences in sensory processing between control and *Fmr1 KO* mice. We considered frequency representation (tonotopy, tuning bandwidth) in the primary auditory cortex, as well as representation of frequency modulated (FM) sweeps and inhibitory sidebands in control and *Fmr1 KO* mice. We found *Fmr1 KO* mice show reduced representation of slow upward frequency modulated sweeps. Abnormalities in the bandwidth or time to onset of inhibitory sidebands in *Fmr1 KO* mice may explain the differences seen in FM sweep processing. Given that slow upward sweeps are prominent in mouse vocalizations, the observed results suggest impaired vocalization perception in *Fmr1 KO* mice.

820 Background Strain Influences Behavioral Hearing-In-Noise Assessment

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The olivocochlear (OC) efferent system provides feedback to the cochlea, and likely acts as a gain control mechanism that enhances signal-in-noise processing. We plan to test this hypothesis directly, by using genetically-engineered mice lacking either their medial (MOC) system ($\alpha 9$ nAChR null mice) or mice lacking a portion of their lateral (LOC) system (CGRP-null mice). We and others have shown that in these mouse lines the absolute thresholds and DPOAE response magnitudes are unaffected by these genetic alterations. However, the background strains of these genetically-engineered mice differ, and may exhibit a variety of baseline behaviors. We have begun to study this variation across inbred mouse strains (CBA/CaJ, C57BL6/J, 129SvEv, and C57 x 129SvEv hybrids) in a behavioral test that assesses hearing-in-noise abilities. Our testing makes use of the acoustic startle response (ASR) and the ability of prepulses to inhibit the ASR [ie, prepulse inhibition (PPI)] to assess the mouse's ability to detect prepulse signals presented in quiet or embedded in masking noise. The ASR was measured in response to brief 80-130dB SPL noise bursts, delivered in quiet or in the presence of a 70dB SPL background noise. We determined a startle threshold and an optimum level for maximal ASR with all strains exhibiting ASR thresholds near 90dB SPL. Likewise all animals showed PPI when prepulses were presented in quiet. Finally, prepulses were delivered 48-72 dB SPL in 3 dB steps in the presence of a continuous 60dB SPL broadband masker. These data reveal a masked threshold, below which there is no PPI and above which PPI grows with increasing prepulse level. CBA mice had the lowest masked threshold, -6dB S/N, while 129SvEv as a group have the highest at 0 dB S/N. These experiments pave the way for behavioral hearing-in-noise assessment using genetically-engineered mice.

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821 Block of Norepinephrine Reuptake by Nortriptyline Improves Sustained Auditory Attention and Decreases Impulsivity

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Sustained attention is essential for maintaining focus on routine tasks, including those requiring inhibition of response. The inability to inhibit inappropriate responses has been linked to disorders like attention deficit hyperactivity disorder (ADHD), a neurobehavioral disorder characterized by impaired performance in a sustained attention task. ADHD occurs probably due to a combination of dysfunction of the prefrontal and lateral cortices of the brain, which are affected by subcortical modulatory neurotransmitters including acetylcholine and catecholamines with mechanisms not yet completely clear.

To test whether and how tonic activation of noradrenergic system affects auditory attentional processes, we developed a rat behavioral system and protocol to estimate the time-span of attention. The system is endowed with a pellet dispenser, a retractable lever and a loudspeaker for sound delivery as a motion trigger, in a cage in which a rat must withhold a response to a short tone presented at random time intervals (from 5 to 80 seconds) after presentation of lever, to obtain a food reward. We assessed the time span of rat auditory attention by the decay time of exponential curve best fitting the success rate as a function of random trigger time presentation.

We found that nortriptyline, a tricyclic antidepressant known to block the reuptake of norepinephrine and, to a

lesser extent, serotonin, greatly improves the time span of attention, increasing the success rate from 25 ± 10 s in control to 42 ± 14 seconds in nortriptyline. Nortriptyline also decreases impulsivity, measured as % of premature lever press (PLP) at a fixed interval of t = 20 s from 49% in control to 26% after nortriptyline.
Our data corroborate the findings that upregulation of noradrenergic function improves the span of auditory attention while simultaneously decreasing impulsivity, and represents a valuable animal model for assessment of effectiveness of new drugs aimed to prolong attention span.

822 Nicotinic Modulation of Attentional Shift in the Auditory System

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Numerous studies have demonstrated cognitive improvements resulting from the application of nicotine, especially in those tasks aimed at measuring attention. More extensively, nicotine's effect on attentional performance has been shown in chronic cases of nicotine use in humans as well as with patients suffering various neurological disorders, such as schizophrenia and Alzheimer's disease. While the neuro-pharmacological relationship between nicotine and acetylcholine-driven attentional processes has been examined, studies tend to focus on the duration of time in which a subject can attend to a specific stimulus or series of stimuli rather than on the subjects' adaptive attentional capabilities. The present study addresses the possibility that the cholinergic agonist nicotine could improve performance on a task testing the ability to shift attention between sensory modalities under both normal and pharmacologically impaired conditions.
In a first set of experiments, we tested the effects of decreasing cholinergic neurotransmission by systemic administration of the muscarinic antagonist atropine, in a cross modal experimental task designed to tax both the auditory and visual systems. Atropine significantly impaired performance in auditory shift trials and perseverative trials, while significantly increasing the overall response latency. In a second set of experiments, we tested the effects of nicotine on a second group of rats, on the same experimental task. Nicotine significantly improved performance on both auditory and visual trials, under repetitive trial conditions, and significantly decreased overall response latency. In a third set of experiments we tested the effect of nicotine within the impaired model. Systemic administration of nicotine significantly improved performance in auditory and visual shift trials, while showing moderate improvements in response latency and perseverative trial conditions. These results indicate the potential therapeutic use of nicotine as a cognitive enhancer, as well as provide evidence for *receptor type compensation*.

823 Salicylate-Induced Tinnitus: Alterations in Neuronal Activity in the Auditory Cortex of Anesthetized Rats

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Neuroplastic alterations and increased synchronization of neuronal discharges in the auditory cortex (AC) and related brain regions may be related to the phantom sensation of sound, tinnitus. Our previous study revealed that a high-dose salicylate injection in rats induced tinnitus-like behavior around 16 kHz and enhanced local field potentials (LFP) in the tinnitus frequency-region (Yang et al., 2007, Hear. Res. 226, 244-253). This experiment was designed to further investigate alterations in neural activity in rat auditory cortex following salicylate injection (300 mg/kg). Spontaneous unit discharges, response maps, and peri-stimulus time histograms of neurons in the AC were assessed at different times before and after the salicylate treatment along with LFPs. Sixteen -channel microelectrodes were used to record AC activity and single microelectrodes were used for recording LFPs from the hippocampus (HC). This report will present the salicylate-related alterations in neuronal discharges and cross correlations between neuronal discharges and LFPs from the AC and the HC. Interestingly, salicylate-treatment caused many neurons to expand and shift their response maps towards the tinnitus region (i.e. high-frequency neuron maps expanded towards lower frequencies and the low-frequency neuron maps expanded towards higher frequencies) leading to concentration of units tuned to where tinnitus-like behavior was observed. Map-expansion appeared to emerge prior to the threshold shift. The mechanisms underlying the salicylate-related re-organization are currently unclear, but may be related to diminished GABAergic-signaling reported by others.
This study was supported by grants from NIH (R01DC009091; R01DC009219) and Tinnitus Research Initiative to Salvi

824 Salicylate-Induced Modulation of Gene and Protein Expression in Rat Auditory Cortex Correlates with Behavioral Phenotype of Central Tinnitus

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Tinnitus, the perception of a phantom auditory sensation, can be highly disturbing causing anxiety, depression, stress, and sleep disturbance. Administering large dose of sodium salicylate is a highly reliable and effective method for inducing temporary tinnitus in humans and animals and this pharmacologic approach has been used extensively to investigate the neural and biological mechanisms that give rise to tinnitus. While the peripheral auditory system may be an important neural generator of tinnitus, central auditory pathways are thought to play a major role, possibly by down regulating centrally-mediated inhibition. Considerable effort has gone into identifying the neural

correlates of salicylate-induced tinnitus at multiple sites along the auditory pathway, but few studies have examined the molecular mechanisms that give rise to salicylate-induced tinnitus. We have used a large antibody microarray targeting 725 proteins to screen for significant changes in protein expression occurring in the auditory cortex (AC) of Sprague-Dawley rats with salicylate-induced tinnitus. Salicylate treatment (250 mg/kg, IP) induced significant ($p<0.05$) protein expression changes of 15-25% in 23 proteins ($p<0.05$). Expression of 7 proteins decreased compared to saline-treated controls while expression increased in the remaining 16. Subsequent gene expression results with a custom qRT-PCR array yielded results consistent with the proteomic data. Salicylate-induced tinnitus was associated with significant changes in the expression of 3 functional groups AC proteins; those involved with (1) neuronal maturation and differentiation (Inexa, Smn1, Rab6ip2, Hnrnpu, Cnp, Myd88, Cttn), (2) cell cycle and (Cdc2, Ccna2, Prmt6, Cdc14a) (3) glucose metabolism (Pdia3, Grb2). Ppp2ca and Ppp2r2c appear in all three classifications. Another, GSK3b, functions both in metabolism and synaptic signaling. Research supported in part by NIH grants R01DC009091 and R01DC009219.

825 Characterization of Tinnitus Treatment Drugs in Pentylenetetrazole-Induced Activity Using an in Vitro Model of Auditory Cortex Networks

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Tinnitus or “ringing in the ears” affects approximately 30 million people in the USA alone, with 10 million being highly debilitated by the malady. In this study, we have used an in vitro model to test the efficacy of drugs that attempt to treat tinnitus. Dissociated neurons from auditory cortex of mouse embryos were grown on photoetched microelectrode arrays (MEA) with 64 transparent indium-tin oxide electrodes. The proconvulsant drug pentylenetetrazole (PTZ) was applied on spontaneously active auditory cortex networks (ACNs). Following this, Gabapentin, Linopirdine or L-Carnitine was administered at various concentrations to determine if the activity could be reduced to the pre-PTZ level. Results indicated that a concentration of 1.0 mM PTZ significantly increased neuronal activity, presumably mimicking tinnitus. The selective Ca2+ channel blocker Gabapentin, the potassium channel blocker Linopirdine, and the antioxidant L-Carnitine, reduced the activity in ACNs to or below their pre-PTZ condition. The potency of the drugs was Linopirdine > Gabapentin > L-Carnitine at concentration ranges comparable to clinical levels. This in vitro system is a unique platform for screening drugs to study their efficacy in controlling the induced increase in neuronal firing that may mimic tinnitus-like neural activity in humans.

826 Effects of Conditioner Position on Detection Thresholds in the Owl

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In the natural acoustical environment, ambient noise levels fluctuate, and the auditory system must adapt to maintain its sensitivity. In our experiments with barn owls (Tyto alba), we asked whether the location of a conditioning stimulus affects the ability to detect a probe stimulus at a different location. Our experiments were carried out in virtual auditory space using individualized head transfer functions that were equalized for the average binaural levels across loci. In owls (and humans), the pupil dilates when some feature of the auditory environment changes. Here, we use this pupillary dilation response (PDR) to assess the detectability of a test probe presented after different conditioning stimuli. Responses to the test probe are compared to responses to a habituating probe. Habituating probes were 100 ms epochs of silence. The test probe consisted of 100 ms bursts of broadband noise, and the conditioning stimulus was an otherwise continuous broadband noise that was interrupted by the test or habituating probes. We found that detection thresholds scaled with the level of the conditioning stimulus for all conditioner locations. However, detection thresholds obtained when conditioner and probe were spatially separate were lower than the thresholds obtained when conditioner and probe were co-localized, thus suggesting that the adaptation may be spatially specific in the owl.
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827 Contextual Shifts in Sound Localization Induced by an a Priori-Known Distractor

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A previous study of sound localization with a preceding distractor found that the responses were biased away from the distractor location even on the interleaved baseline trials on which the target was preceded by no distractor [Kopco et al., JASA, 121, 420-432, 2007; Tomoriova et al., ARO Abstract #1019, 2009].
The current study measured the dependence of this contextual plasticity on the distractor characteristics. Subjects localized 2-ms frozen noise bursts presented either in the left (-11° to -79°) or the right (11° to 79°) hemifield of the frontal horizontal plane, accompanied on some trials by an distractor. The distractor either preceded or followed the target (by up to 400 ms), it varied in its spectro-temporal characteristics (single click, multiple clicks, noise), or in its location. Performance was compared to baseline blocks that only contained no-distractor trials.
Contextual shifts of 5° away from the distractor location were induced for targets near the distractor, independent of whether the distractor preceded or followed the target. The effect was modulated by the distractor type and location. This pattern of results suggests that the contextual plasticity is likely caused by multiple factors, including distractor-induced short-term adaptation of the

spatial representation, perceptual anchoring of the a priori known distractor location, and/or an interaction between the perceived target and distractor locations.
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828 Does Temporal Weighting of Interaural Level Differences Include Both Onset and Offset-Specific Effects?

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In a previous study [Stecker 2007, *ARO Abs* 30:910], we examined the ability of listeners to detect dynamic interaural differences of time (ITD) and level (ILD) when the cues were manipulated over the duration of a 16-click train from a maximum at stimulus onset to zero (diotic) at offset or vice versa. Consistent with "onset dominance" at high rates, discrimination of dynamic ITD at 2 ms inter-click interval (ICI) was good when onsets carried ITD but poor when onsets were diotic and peak ITD occurred at offset. ILD discrimination, in contrast, was equally sensitive to peak ILD occurring at stimulus onset or offset, a result at odds with studies suggesting suboptimal integration of ILD information with stimulus duration [Hafter et al. 1983, *JASA* 73:1708-13]. To address this discrepancy, the current study tested listeners in a dynamic ILD discrimination task with ILD applied to the onset/offset or to middle portions of the stimuli. ILD was imposed on Gabor click trains centered at 4000 Hz in four dynamic binaural conditions, with the peak ILD occurring at onset (condition R000), at offset (000R), at both onset and offset (R00R), or midway through the stimulus (0RR0). Threshold values of peak ILD were obtained using a 2-down 1-up adaptive procedure, then compared against thresholds obtained in four control conditions: static ILD (RRRR), single click (R), and monotic presentation of dynamic intensity cues in conditions R00R and 0RR0. Preliminary results featured lower threshold ILD in condition R00R than 0RR0, suggesting uneven (but temporally symmetric) temporal weighting for ILD. This result is consistent with past studies of sensitivity to dynamic and static ILD for stimuli varying in duration (e.g., Hafter et al. 1983), although not strongly supported by studies that have measured temporal weighting functions for ILD directly [Brown and Stecker 2008, *ARO Abs* 32:914; van Hoesel 2008, *JASA* 124:3861-72].
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829 Does the Onset "Tip the Scale" in the Franssen Effect?

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Lateralization studies were conducted to explore the factors that influence onset dominance in a headphone facsimile of the Franssen illusion. The primary purpose was to determine whether, as suggested by Buell et al. (JASA, 1991) and by Freyman et al. (JASA, 1997), the role of the onset is to tip the scale in favor of one of multiple interaural time delays (ITDs) present in the ongoing sound, or whether lateral position established early in the stimulus persists regardless of the specific ITDs in the ongoing sound. Target sounds consisted of successive binaural pairs of 1-ms noise bursts that alternated in ITD between left-leading and right-leading with a 2-ms interburst interval. This alternating ITD pattern was repeated every 4 ms for a total duration of 250 ms. The attenuation of the initial burst was varied parametrically from 0 to 24 dB. For classic precedence effect stimuli consisting of two pairs of bursts, initial burst attenuation produces a continuous change in perceived lateral position that increasingly favors the second burst as attenuation is increased. For the longer Franssen-type stimuli used here, it was unknown whether lateralization would be dominated by the first burst until a certain critical attenuation is reached and by the second burst beyond that critical attenuation, or instead whether the lateral position moves gradually with increasing attenuation of the initial burst, similar to what is observed for brief pairs of bursts. Preliminary results indicate that lateral position varies continuously with increasing attenuation, suggesting that the onset ITD does not, in the general case, "tip the scale" between competing ongoing ITDs [Work supported by NIH DC01625].

830 A Two Source Reference for Determining When a Simulated Echo Becomes Localizable

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Despite their prevalence in nature, echoes are not perceived as events separate from sounds arriving directly from an active source, until the echo's delay is long. This division between the perception of one fused sound-source location and two separate locations is considered echo threshold. The stimulus parameters that can cause fusion or echo threshold to be reached are, nevertheless, often contradictory. Echo threshold can be reached, for example, even when the echo's delay is short, provided that the length of time during which the echo is present alone, after the offset of the leading sound, is sufficiently long (i.e., the lag-alone segment). Similarly, humans can experience fusion when the delay is long, provided that the lag-alone segment is short. Fusion can also be experienced when the sounds are gated so that the lead- and lag-alone segments are both removed, provided that the temporally superposed segment that remains is sufficiently long (> ~50 ms). To resolve these (and other) apparent contradictions, we propose using two uncorrelated sounds as a reference for determining when fusion breaks down and lag stimuli start to become localizable. In our current experiment, two uncorrelated noise bursts (30 ms) were presented concurrently and interspersed amongst lead/lag sound pairs having similar durations. Subjects were asked to indicate two locations if two distinct auditory images were detected or a single location if only one image was detected. Consistent with the phenomenon of fusion, a single image was usually reported when the delay was short (< ~3 ms), corresponding to the hemisphere from which the leading sound was spatialized. A single image at locations that varied from trial to trial was also reported, however, when two uncorrelated sounds were presented. Because two

locations were reported more frequently as the lead/lag delay was increased (> 3 ms), we propose that two uncorrelated noise bursts, presented concurrently, may serve as a natural reference for determining when fusion breaks down and lag stimuli start to become localizable. [Work supported by grants from NIDCD F32-DC008267 and RO1-DC03925].

831 More Modeling of Temporal Weighting Functions for Interaural Time and Level Differences

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Numerous studies have investigated the time-course of binaural cue processing by estimating temporal weighting functions (TWFs). TWFs describe spatial-cue sensitivity over the durations of brief stimuli, and have been estimated variously by measuring threshold interaural differences for a brief "probe" stimulus embedded in a longer diotic "fringe" [Zurek 1980, *JASA* 67:952-64; Akeroyd and Bernstein 2001, *JASA* 110:2516-26], by measuring improvements in discrimination with increasing duration [Hafter and Dye 1983, *JASA* 73:644-51; Hafter and Buell 1990, *JASA* 88:806-12], and by multiple regression of localization responses onto spatial variation applied independently to multiple temporal divisions of the sound [Saberi 1996, *Percept Psychophys* 58:1037-46; Dizon et al. 1998, *ARO Abs* 21:42; Stecker and Hafter 2002, *JASA* 112:1046-57; van Hoesel 2008, *JASA* 124:3861-72; Macpherson and Wagner 2008, *ARO Abs* 31:301].

For both broadband and narrowband rapidly modulated sounds, TWFs have revealed varying degrees of "onset dominance," defined by high onset weight and reduced weight for later parts of the stimulus. This effect is stimulus dependent, and likely reflects the contributions of multiple physiological mechanisms. In a previous presentation [Stecker and Brown 2009, *JASA* 125:2523], we investigated those contributions by estimating TWFs on the basis of model responses of the auditory periphery, binaural coincidence detection, and temporal integration. In the current study, we evaluated several empirically and theoretically derived TWFs by using them to generate predictions for binaural discrimination and lateralization tasks discussed in the literature. The results of this modeling suggest that multiple forms of TWF may be consistent with any given set of behavioral data, and that conclusions regarding the underlying dynamic mechanism may depend strongly on the analytical assumptions used to derive the TWF.

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832 Localization Dominance Depends on Envelope Correlation

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In natural, echoic environments, listeners localize the source emitting the sounds instead of their reflections, which frequently overlap in time with the sound arriving directly from the source. The delay between the onsets of the direct and reflected sounds is thought to be critical for differentiating the leading and lagging waveforms. Studies in humans and barn owls (Tyto alba), however, show preferential localization of the direct sound even for a synchronously gated lead/lag pair (the onset and offset delays are removed). We hypothesize that the "ongoing delay" between corresponding envelope peaks in the lead and lag waveforms provides a cue for distinguishing the direct from the reflected sounds. We have already shown in owls that the preferential localization of the direct sound requires envelope modulations (Nelson & Takahashi, ARO 2008). Here we tested another prediction of the envelope hypothesis, i.e., that the preferential localization of the lead requires that the envelopes of the leading and lagging sounds be correlated. Owls trained to make head saccades to speakers were presented with correlated lead/lag pairs (2ms ongoing delay) and envelopes that were 100% correlated or 0% correlated. Owls preferentially localized the lead sound when the envelopes were correlated but showed little or no bias when the envelopes were uncorrelated, thus supporting the envelope hypothesis. When the envelopes are fully correlated, a peak in the lead is likely to overlap with the corresponding peak in the lag, obscuring the onset of the rising edge of the lag peak. Neurons in the owl's auditory space map, which guide its saccades, are highly sensitive to rises in envelopes, and as a result, discharge preferentially to the lead over the lag for instances of high correlation. When the envelopes are uncorrelated, peaks in the lead and lag are equally likely to obscure one another, making either sound equally likely to evoke a saccade. [Supported by NIDCD RO1-DC003925]

833 Investigation of the Precedence Effect in Ferrets: Do They Experience a Build-Up of Echo Suppression?

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Listening in complex, echoic environments is an everyday task whose neuronal basis is poorly understood. A simple way of simulating echoes is by presenting two stimuli from different locations with a short delay between them. Human psychophysical studies have shown that for inter-stimulus delays (ISDs) of up to ~1 s, the two sounds are fused and their perceived location lies between the two locations. At slightly longer delays, the second stimulus is suppressed and the perceived location is dominated by the actual location of the first sound. Studies of the precedence effect in humans have shown that echo suppression changes with the number of stimulus repetitions. As the number increases, the delayed sound fades away so that only the leading sound is heard. The neuronal basis of this so-called "build-up" effect is not yet understood.

In this study, we sought to demonstrate the precedence effect in ferrets, and thus develop an animal model to investigate the neuronal basis of the build-up effect. We trained ferrets in a free-field 2AFC task to discriminate the direction of a sound as coming either from the left (-90°) or

from the right (+90°). Stimuli were paired noise bursts of 5 ms duration presented via the left and right speaker at different ISDs. Ferrets were rewarded for approaching the speaker of the leading noise burst in the pair. Localization accuracy of the leading sound peaked at low to intermediate ISDs, suggesting that the precedence effect is indeed present in ferrets. At longer ISDs, performance deteriorated, implying that the leading and lagging sounds may have both been perceived. We are now using behavioural and neurophysiological methods to determine whether build-up of the precedence effect can be observed. Preliminary behavioural data suggest that the number of stimulus repetitions and the ISD both affect performance in the localization task. This might be indicative of the presence of a build-up effect in ferrets.

834 Does Multi-Second Monaural Adaptation Reduce Sensitivity to Interaural Time Differences in Human Listeners?

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In a previous study (Kuznetsova et al. 2008), we demonstrated that *in vitro* stimulation of neurons in chicken nucleus magnocellularis (NM) causes adaptation of spike-timing precision that develops over 5-10 seconds. Because interaural time difference (ITD) processing is subserved by inputs from NM to binaural coincidence detectors in nucleus laminaris (NL), a critical consequence of this adaptation is a reduced sensitivity to ITD among NL neurons (Kuznetsova et al., in preparation). We hypothesized that similar adaptation occurring in the human binaural pathway could similarly reduce ITD sensitivity in binaural neurons, and that such reduction would result in corresponding changes in psychophysical ITD sensitivity. Under this hypothesis, monaural adaptation caused by sustained stimulation would exert a frequency-specific reduction in sensitivity to an ITD target, independent of any interaural cues present in the "adapter" stimulus. We tested this prediction psychophysically in human subjects by measuring ITD discrimination for tone bursts following either (1) a period of silence (2) a train of adapter tone bursts of the same frequency carrying randomly fluctuating ITDs or (3) a train of tone bursts of a different frequency, also carrying random ITDs. Preliminary data suggest a frequency-dependent reduction in discrimination performance following exposure to the adapter train. [Supported by NIH F31-DC91763, VA Merit Review, and NIH R03-DC009482.]

835 Rate Adaptation in Binaural Detection

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In order to describe binaural-masking level differences (BMLDs) in Gaussian and low-noise noise, Bernstein et al. [J. Acoust. Soc. Am., 106, 870-876 (1999)] used a cross-correlation model that incorporated a stage of peripheral compression known to occur at the basilar membrane for sine tones. Recent work by Recio-Spinoso et al. [J. Assoc. Res. Otol. (2009)] has shown that the basilar membrane responds in a quasi-linear way for white noise stimuli. Given this recent result, an alternative explanation must be found for the difference in BMLDs between Gaussian and low-noise noise. One possible explanation is that binaural low-noise noise stimuli may be affected by binaural rate adaptation (i.e., a decrease in binaural sensitivity with increasing pulse rate), which is known to occur in interaural time difference sensitivity for pulse trains with equal-amplitude pulses [Hafter and Dye, J. Acoust. Soc. Am., 73, 644-651 (1983)].

This study aims to determine whether Gaussian and low-noise noise incoherence detection and BMLDs are better described by a model with basilar membrane compression or binaural rate adaptation. To do this, the envelope of 10-Hz and 50-Hz bandwidth stimuli was presented to listeners by using Gaussian-envelope tones at different rates, center frequencies, and bandwidths. Such stimuli are similar to those presented to cochlear-implant users, and the results of this work have implications for cochlear-implant users understanding speech in noisy situations.

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836 Concurrent Development of the Head and Pinnae and the Acoustical Cues to Sound Location in the Chinchilla

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Propagating sound waves are filtered in a spatial- and frequency-dependent manner by the head and pinna giving rise to the acoustical cues to sound source location. These spectral and temporal transformations are dependent on the physical dimensions of the head and pinna. Therefore, the magnitudes of binaural sound location cues - the interaural time (ITD) and level (ILD) differences - are hypothesized to systematically increase while the frequency ranges of ILD production are expected to decrease due to the increase in head and pinna size during development. The frequency ranges of the spectral notch cues to source elevation are also expected to decrease. This hypothesis was tested here by measuring directional transfer functions (DTFs), the directional components of head-related transfer functions, and the linear dimensions of the head and pinnae for chinchillas from birth through adulthood. Dimensions of the head, pinnae width and pinnae height increased by factors of 1.7, 1.7 and 2.5, respectively, reaching adult values by ~8 weeks. From the DTFs, the ITDs, ILDs and spectral shape cues were computed. Maximum ITDs increased from ~180 µs in 1 week olds to 250µs in adults. ILDs depended strongly on source location and frequency exhibiting a shift in the frequency range of substantial ILD (>10dB) from higher to lower frequencies with increasing head and pinnae size. Similar trends were observed for the spectral notches which were present for frontal sources for frequencies from ~10-16 kHz at 1 week to ~6-10 kHz in adults. The development of the spectral notch cues, the

spatial- and frequency-dependent distributions of DTF amplitude gain, acoustic directionality, maximum gain and the acoustic axis were systematically related to the dimensions of the head and pinnae. These monaural acoustical properties of the head and pinnae in the chinchilla are mature by ~6 weeks. Support: NIDCD R01-DC6865 (DJT) and T32NS007083 (HGJ).

837 Acoustic Cues for Sound Localization Measured in Humans and a Mini-Basketball

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The human head and pinnae shape the sounds that reach the ear canals. The transfer functions representing signal transformation between the source and each ear canal, is called the head related transfer function (HRTF). Measurements of HRTFs have typically been done in two dimensions, azimuth and elevation whereas much less attention has focused on the 3rd dimension, distance.

Human recordings were made in an anechoic chamber, from miniature microphones placed deep in each ear canal. An acoustic point source was placed at 15º increment of azimuth at distances between 10 and 160 cm in half doubling steps. The sound was a logarithmically swept broadband chirp producing a constant level within ±12 dB over 0.1 – 20 kHz. From the HRTFs we computed the following candidate acoustic cues: interaural time difference (ITD) and interaural level difference (ILD) as a function of frequency, distance and azimuth. For comparisons we also obtained the HRTFs from a mini-basketball, chosen since the diameter is similar to that of a human head.

We found in humans that: 1) ITD changed with frequency and distance in a complex manner. At frequencies below 0.4 kHz, ITD decreased with decreasing distance, whereas above 0.5 kHz, ITD increased with decreasing distance. At far distances, ITD increased with decreasing frequency. 2) ILD generally increased with decreasing distance and increasing frequency. Although ILD was minimal at low frequencies for far distances (>80 cm), it increased substantially for close distances (<28 cm). 3) The spectral patterns in each ear changed with sound source location, a potential monaural cue of sound localization. The changes in ITD and ILD with frequency and distance seen in humans were reproduced, in general, in the mini-basketball but were larger and more complex in humans.

838 Responses of Inferior Colliculus Neurons in the Unanesthetized Rabbit to Virtual Auditory Space Stimuli

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We investigated the neural coding of sound source location using virtual auditory space (VAS) stimuli. We generated the VAS stimuli based on individual rabbits' head related transfer functions (HRTFs) measured in an anechoic chamber at source locations with azimuths of ±150º in 15º steps and distances between 10 and 160 cm in half doubling steps (Bishop et al., 2009 ARO abstract). We used, wide band (0.2 – 20 kHz) or band limited (0.2 – 1.25, 1.25 – 5, 5 – 20 kHz) noise stimuli that were filtered by the HRTFs. These VAS stimuli were 100 msec presented every 700 msec and were delivered through earphones coupled to custom-fitted ear molds where the end of the sound delivery tube was approximately at the position where the microphone had been placed during the ear canal measurements. Neural recordings were made with tungsten-in-glass microelectrodes lowered in a dorsal-ventral direction with a microdrive. Our approach was to determine the neuron's best frequency (BF) and use this information to choose one of three band limited noises. Time permitting, we also used the wide band noise stimulus. At a given distance, we tested the neuron's azimuthal sensitivity by presenting the azimuths in random order. In order to elucidate the underlying mechanism, we presented the VAS stimuli with altered binaural cues as well as presenting the VAS stimuli monaurally.

To date, the bulk of our neurons had BFs above ~3 kHz. We found that most of these neurons were tuned to azimuths on the contralateral side (re: recording site) with best azimuths ranging from 0 – 90º. This tuning tended to broaden with stimulus level although the degree of level tolerance differed among neurons. In many neurons the azimuthal tuning was similar regardless of whether the VAS stimuli were presented binaurally or only to the contralateral ear. Setting ILD to zero and flattening the spectrum yielded mixed results implying that the ILD and spectral cue contributed in different ways in different neurons.

839 Frequency Mismatch in Low-Best Frequency Neurons in the Barn Owl Argues Against Cochlear Delays as a Coding Mechanism for Interaural Time Difference

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Recent analyses have suggested that cochlear delays may play a role in the representation of interaural time difference (ITD) (Joris et al., PNAS 103: 12917 (2006)). In the barn owl, cochlear delays do not play a role for frequencies above 3 kHz (Pena et al., J Neurosci 21: 9455 (2001)). However, the effect of cochlear delays for frequencies below 3 kHz has not been examined. Theory predicts a frequency mismatch between the left and right inputs to a binaural, ITD-sensitive neuron that depends on the ITD represented by the neuron. We extracellularly recorded from neurons in the core of the central nucleus of the inferior colliculus. For a given neuron we first varied the interaural time difference, accumulated an ITD-tuning curve and obtained the best ITD. Afterwards, an iso-intensity frequency-response curve for both the left and right inputs independently as well as for binaural stimulation at the best ITD were recorded. Our preliminary sample consists of 36 recording sites. The best ITDs ranged from -720 µs to 1050 µs. The best frequency, measured binaurally, covered a range from 400 Hz to 2900 Hz. To test whether cochlear delays may explain our data, predictions for the frequency differences in the left and right inputs were calculated from the best ITD and the best

frequency, measured binaurally. We compared these predictions with the measured frequency differences. The mean measured frequency differences were only 33% of the predicted frequency differences. In six neurons, we observed no frequency mismatch between both inputs, although the absolute best ITD varied between 120 µs and 500 µs. Moreover, a paired t-test did not reveal a significant difference (p=0.79) in the monaural best frequencies. We therefore conclude, that it is very unlikely, that the coding of ITDs can be explained by cochlear delays in the barn owl also in the low-frequency range.

840 The Range of Interaural Time Differences in the Barn Owl Is Almost Independent of Frequency

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Barn owls can localize sound targets with an accuracy of up to 3 degrees in both the horizontal and vertical planes. The hearing range of barn owls covers frequencies from about 200 to about 12000 Hz. Azimuthal localization is mediated by interaural time differences (ITD), whereas interaural level differences (ILD) are a major cue for elevational localization.

The influence that the head, body and outer ear (the facial ruff) have on the localization cues (ITD, ILD and frequency spectrum) is described by the so-called head-related transfer functions. The barn owl's facial ruff and the asymmetrically-arranged ears influence sound differently depending on the frequency. The dependence of ITD and ILD on sound source position are well understood in the high-frequency range (>2 kHz) (Campenhausen and Wagner, J comp Physiol 192:1073 (2006)). Because of the high attenuation in the interaural canal, the two ears work practically independently in the high-frequency range.

Much less is known about the relationship between ITD and frequency in the low–frequency range. The attenuation in the interaural canal is much weaker for lower frequencies so that the ears may act as pressure-difference receivers. Since recent modelling suggested that the ITD range widens considerably in pressure-difference receivers (Vossen and van Hemmen, personal communication), we compared the ITD span (the difference between the most positive and the most negative ITD) in the high- and low-frequency ranges for the barn owl. Calculation was done in 1/3 octave filters. We found that a) the ITD span was around 600 µs for frequencies between 4 and 10 kHz. It dropped to 500 µs for 3 kHz, and then increased again to 600 µs for frequencies below 1 kHz. Differences between individuals were small. Thus, our data suggest that the ITD range of the barn owl's hearing system is not broader in the low-frequency range than in the high-frequency range.

841 The Representation of ITD Detectors in Mammals: No Evidence for 'Jeffress-Like' Distributions

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A major cue for sound localisation is the difference in the time of arrival of sound at the two ears, the interaural time difference (ITD). In nuclei in the brainstem and primary auditory cortex, ITD-sensitive neurons respond with a highest spike rate to a particular ITD - the best ITD. The distribution of best ITDs in any one species, and it's role in the representation of auditory space, remains a contentious issue. Most models of ITD representation, generally instantiations of the classic Jeffress model, assume a uni-modal distribution of best ITDs centered around zero (i.e. midline spatial positions). Furthermore, best ITDs are assumed to reside largely within the range of ITDs naturally encountered (the physiological range). An alternative model, the optimal coding model, predicts that each species employs different best ITD distributions, dependent on sound frequency and their interaural distance. For example, species with small interaural distances should employ bimodal distributions of best ITDs with the majority of best ITDs beyond the physiological range. By testing predictions of the Jeffress model on new and previously-published data sets, recorded from the brains of a wide range of mammals, we find that the classic model generally fails to predict the form of the distribution of best ITDs. In contrast, the optimal-coding model provides a good qualitative description of the distributions of ITDs seen in different animals. This includes the majority of best ITDs lying beyond the physiological range below a species-dependent sound frequency, and sound frequency dependent multi-modal distributions of best ITDs.

842 Closed-Field Measures of Behavioral Sensitivity to ITDs and ILDs in the Ferret

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Spatial hearing depends upon a number of acoustical cues, including interaural time differences (ITDs) and interaural level differences (ILDs). To assess the relative importance of these cues for localizing broadband sounds, previous investigations have presented stimuli over headphones. A number of studies of auditory localization have now been carried out in ferrets, which are particularly easy to train using positive conditioning techniques. To date, these studies have been based on the free-field presentation of sounds from a bank of loudspeakers. Although estimates of neural sensitivity to ITDs and ILDs have been obtained by recording from neurons in the midbrain and auditory cortex of anesthetized ferrets, corresponding estimates of behavioral sensitivity are lacking. To address this issue, 5 ferrets were trained to lateralize broadband and tonal stimuli presented over

customised headphones attached to a previously implanted head-post. Psychometric ITD and ILD functions were assessed by a two-alternative forced-choice (2AFC) task using the method of constant stimuli, in which one cue was varied whilst keeping the remaining cue fixed in value. Probit analysis was then used to estimate ITD and ILD thresholds for stimuli of varying duration and level. Behavioral sensitivity to ITDs and ILDs was found to be broadly similar to that observed in humans, confirming the ferret as an excellent model for understanding the neural mechanisms underlying the processing of binaural spatial cues.

843 Correlation of Individual Performance in Monaural and Binaural Temporal Detection Tasks

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Auditory performance in psychoacoustical tests often varies markedly among listeners, but the mechanisms responsible for the variation are usually unclear. This study focused on an interaural time disparity (ITD) detection and explored the source of its inter-listener variability. We hypothesized that the ITD detection performance is determined mainly by the efficiencies of two types of mechanisms: One is a "monaural" mechanism that encodes stimulus temporal structure by means of the neural phase-locking. This mechanism can be thought to be the common basis for various auditory abilities that rely on temporal fine structure of stimuli. The other is a "binaural" mechanism that compares temporal information between the two ears.

We tested listeners' performance in periodicity-pitch discrimination and ITD detection. Stimulus for the pitch discrimination task was presented monaurally. It was a harmonic complex with a fundamental frequency of 100 Hz, consisting of components with nominal harmonic numbers of 8-14. Thresholds for detecting a common frequency shift imposed on all the components were measured. This test can be thought to be sensitive to the efficiency of the monaural mechanism [Moore and Moore, JASA. 113, 977-85 (2003)]. Essentially the same stimulus was used also for the ITD detection task, but was presented binaurally.

Thresholds for either of the tasks differed significantly among listeners. The thresholds of individual listeners for the two tasks showed a significant *negative* correlation. If inter-listener variability of the ITD detection performance were due to the variability in the monaural mechanism, one would expect a *positive* correlation. Thus, our results reject this possibility. An interpretation of the observed negative correlation is that mechanisms for pitch and ITD processing share and compete for limited "resources" at a certain stage in the auditory pathway.

844 Spatial and Temporal Unmasking in a Symmetric Stimulation Paradigm

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Spatial unmasking has been explored by separating the spatial positions of signal and masker, typically along the horizontal plane. The first experiment is designed to question whether this spatial separation und the resulting release from masking results from the signal and masker being processed in independent spatial channels generated by i.e. an array of coincidence detector as proposed in the Jeffress model. In a series of free field detection experiments with a horizontal loudspeaker array, we quantified spatial unmasking with two uncorrelated band-stop noise maskers symmetrically arranged, both in space and frequency, around the signal. Spatial unmasking of a band-pass signal presented from 0 deg azimuth and elevation was at maximum 3 dB with a 90 degree spatial separation between the frontal signal and the two lateral maskers. The small degree of spatial unmasking despite the large spatial separation argue against a multi-channel spatial processing, but in favour of a two channel system as recently proposed for mammals. In the second experiment, we investigated the interaction of spatial with temporal unmasking in a similar symmetric paradigm. The signal was a train of eight 800 Hz pips, the maskers were 12 trains of eight pips, with 1/3 octave frequency spacing between 200 and 3200 Hz. Unmasking was quantified as a function of both the spatial and temporal distribution of the masker pips relative to the signal pips. When the masking pips were simultaneous to the signal pips, spatial unmasking was equally small as in Experiment 1. Introducing a temporal jitter into the masker pip trains increased unmasking considerably; indicating an additive relationship between spatial and temporal unmasking. These results argue against a detrimental effect of a presumed binaural sluggishness in spatio-temporal unmasking. Instead, the data are in agreement with the hypothesis that spatial and temporal unmasking are optimally combined to solve a demanding detection task.

845 Maximum Sensitivity to Interaural Incoherence: At the Peak or the Slope of ITD-Tuning Curves

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The auditory system shows a remarkable ability to detect differences in the sound waves reaching the two ears. Such interaural incoherencies, which occur naturally in reverberant environments and when independent sounds emanate from different locations simultaneously, also underlie the phenomenon of binaural unmasking, an important component of the cocktail party effect. Recent physiological data from binaural midbrain neurons (Shackleton & Palmer, 2006) showed lower sensitivity to interaural incoherence than to interaural time differences (ITDs). Analyses were based on changes in mean spike rate. Our computer simulations demonstrate greater sensitivity based on spike rate variance, due to ITD

fluctuations inherent to interaurally incoherent stimuli. As for static ITD, sensitivity to varying ITD is greatest on the tuning curve's slope. Thus, two neural ITD channels, one tuned to positive, one to negative ITD, in such way that their slopes a located at zero ITD, provide for maximum sensitivity to reductions in interaural incoherence from a diotic reference stimulus. Such ITD channels are shown to exist in either side of the brain (McAlpine et al., 2001). Whereas the neural responses of these two ITD channels are known to co-vary in response to diotic stimulus variations, they behave in a complementary manner to interaural differences. Hence, the ratio between both populations normalises diotic intensity fluctuations in the stimulus.

Our model is based on the non-normalised cross-correlation between left and right signals, filtered by 500-Hz gammatone filter. It has two parameters: population spike count (determining the "internal" Poisson noise) and neural integration time constant (i.e. the smoothing of ITD fluctuations). The latter was determined by trading incoherence sensitivity with ITD sensitivity (taken from literature: 0.98 and 15 microseconds, respectively). The resulting 10-ms value supports the emerging view that the binaural system has a higher temporal resolution than perceptual sluggishness suggests. When pooled over approx. 200 neurons, the model shows that the resulting increase in variance in hemispheric balance can account for the astonishing behavioural sensitivity to interaural incoherence. As well as suggesting a common mechanism by which changes in interaural parameters might be detected, the model also explains the sharp reduction in sensitivity to changes from already incoherent reference stimuli.

846 Processing of Interaural Temporal Disparities at High Frequencies: Which Metrics Work and Which Do Not?

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In several recent studies, we showed that threshold interaural temporal disparities (ITDs) measured with high-frequency auditory stimuli depend on the temporal signatures of the envelopes of the stimuli. Recently, we have used "raised-sine" stimuli to try to determine which temporal features of the envelopes of high-frequency waveforms enhance ITD processing. Raised-sine stimuli permit independent variation of the modulation frequency, modulation depth, and "dead-time/relative peakedness" of stimulus envelopes, while also suitably restricting spectral content. At the 2009 Midwinter meeting we reported threshold-ITDs measured while varying the peakedness of the envelope for stimuli having modulation frequencies between 32 and 256 Hz. Graded increases in the peakedness led to graded decreases in envelope-based threshold ITDs. An interaural correlation-based model was generally able to account for the thresholds. In this presentation, we present a quantitative evaluation of how well specific envelope-based metrics including interaural correlation, fourth moment, or peakwidth might also account for the data. It will be seen that, of the three candidate metrics, only the interaural correlation of the envelope provides accurate predictions of the threshold-ITDs and it does so provided that peripheral auditory processing is taken into account. Work supported by NIH DC-04147 from the National Institute on Deafness and Other Communication Disorders, National Institutes of Health.

847 The Normalized Cross-Correlation Function Including Peripheral Compression: A Case of Mistaken Identity?

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In an experiment with reproducible noise-pairs, listeners discriminated target stimuli with small amounts of interaural incoherence from stimuli that were interaurally coherent. The stimuli were chosen so that the detection data may determine whether the binaural system uses a decision statistic that is similar to the peak of the cross-correlation function (without or with peripheral compression), or a statistic related to the instantaneous interaural phase difference (IPD) and interaural level difference (ILD). It was found that neither type of model could describe the detection data well for any listener, which is in contrast to previous similar experiments [Goupell and Hartmann, J. Acoust. Soc. Am., 122, 1029-1045 (2007)]. Subsequent statistical analysis of the individual noise-pairs was performed. It was found that the fluctuations (standard deviation over time) in the IPD were correlated to the fluctuations in the ILD for small amounts of incoherence, but became more uncorrelated for larger amounts of incoherence. The fluctuations in the ILD were correlated with the value of the cross-correlation function with compression for small amounts of incoherence, but became more uncorrelated for larger amounts of incoherence. The fluctuations in the IPD were correlated with the value of the cross-correlation function with compression, which was independent of the amount of incoherence ($r = -0.8$). It appears that the fluctuation in the IPD is essentially the same statistic as the value of the cross-correlation function with compression. This finding explains the inability of the models to describe the data in this experiment and previous experiments, and also has implications for future binaural modeling efforts.

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848 A Spiking Neural Model of Binaural Sound Localization

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We present a spiking neuron model of binaural sound localization based on coincidence detection in frequency bands. We use measured human HRTFs to simulate realistic binaural input to the auditory system, which we model as a series of band-pass filters followed by neural filtering, spike generation and coincidence detection in each frequency band. The binaural neurons detect the similarity between transformed monaural inputs, similarly

to the Jeffress model. In this model, the direction of a sound source results in the activation of a specific neural assembly. We find that the identity of the activated neural assembly is specific to source direction and invariant to the nature of sound (tones, instruments, speech). Assembly identity can be used to estimate elevation, but only away from the median plane, consistent with psychophysical performance for low-frequency sounds (<3 kHz). In our model, we find that the distribution of best interaural delays varies with characteristic frequency: for lower frequencies (<1 kHz), best delays are larger (up to 1 ms) and their distribution is bimodal, with fewer best delays near the center (0 μs); for higher frequencies (>1 kHz) best delays are smaller and more central. This frequency dependence of delays, which is qualitatively consistent with simple geometrical head models, explains the performance of the model in estimating elevation without high frequency spectral cues.

849 Cisplatin Ototoxicity in the CBA Mouse Model of Presbycusis

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Cisplatin is well recognized as an ototoxic agent. Previously published investigations of cisplatin-induced ototoxicity have focused on young animal models. Since 60% of all newly diagnosed malignancies occur in individuals over the age of 65, in this study we investigated cisplatin-induced ototoxicity in the CBA/NIA mouse model of presbycusis. We recorded auditory brainstem evoked response (ABR) thresholds in aged mice before and after cisplatin administration. Our first goal was to determine an optimal dose for inducing ototoxicity (i.e., producing measurable hearing loss associated with an acceptable mortality rate). Our second goal was to assess ABR threshold changes of the aged mice as a function of stimulus frequency. These results were compared to those of the young CBA mice (Hill et al. Otology & Neurotology 2008; 29:1005-1011). 23-24-month-old CBA/NIA mice were used. ABRs were recorded using a TDT system in response to 4 msec (1 msec rise/fall) tone pips presented at 8, 16, 24 and 32 kHz in 5 dB steps between 90 and 50 dB SPL. At 16 mg/kg, cisplatin induced 100% mortality. At 14 mg/kg mortality rate was 33%. These rates were higher than those in young mice (40 and 20%, respectively). Eight days after treatment with 14 mg/kg cisplatin, the four-tone average mean threshold shift was 6.4 dB, with a range of 3.5-8.3 dB. ABR threshold increases at each individual stimulus frequency were statistically significant (one-tailed t-test, $p < 0.005$), except at 16 kHz. These results suggest that 14 mg/kg is the optimal cisplatin dose for investigating ototoxicity in both young and old CBA mice. The absolute magnitude of cisplatin-induced threshold changes in older animals was smaller than those in young mice. When the age-related elevation of ABR thresholds is taken into account, the present results imply significant impairment of auditory function in aged mice after cisplatin administration.

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850 The Change of Aggregations with Heat Shock Protein 70 in the Cochlea During Aging

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Mechanisms of age-related hearing loss have not been elucidated as aging processes are extremely complex. Although oxidative stress and apoptotic cell death are involved in progression of ARHL, number of trial to treat ARHL is limited. Heat shock response is characterized by induction of heat shock proteins (HSPs) in response to stresses such as heat shock. HSPs act as molecular chaperones which stabilizes denatured proteins, facilitates their removal or repair, and some HSPs also inhibit apoptotic pathways. Several studies demonstrated that heat shock response diminishes during aging (Morley et al., 2004) and chaperone activity is reduced in age-associated disorders (Soti et al., 2002). There, however, is few report which indicates the correlation with aging and HSPs in inner ear. Previously, we demonstrated that HSPs expression is altered during aging and administration of an inducer of heat shock response suppresses age-related hearing loss (ARHL) known as “presbycusis” and hair cell loss in DBA/2J known to be one of ARHL mouse model (Mikuriya, et al., Brain Research, 2008). In this study, we found some aggregate with Hsp70 were increased in cochlea cells. In inner ear, however, the report that the relation between ARHL and aggregation is few. Here, we report that the result about immunohistochemistry of Hsp70 and aggregates in aged cochlea with other ARHL models.

851 Can Hydrogen Water Attenuate Age-Related Hearing Impairment?

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Aging is the most common cause for hearing loss. Previous studies have demonstrated involvement of reactive oxygen species (ROS) in mechanisms of age-related hearing impairment. Several anti-oxidants have been demonstrated to attenuate cochlear damage due to aging in animal models. Recently, molecular hydrogen has been paid considerable attention as an anti-oxidant. Previously, we have reported that molecular hydrogen rescue cochlear hair cells from ROS-induced injury. We therefore investigated protective effects of hydrogen water against age-related hearing impairment. We used DBA/2J mice as a model for age-related hearing impairment because of its early onset in this strain. The animals were divided into two groups, Hydrogen group, of which animals were supplied hydrogen water and Control group, in which

animals supplied normal water instead of hydrogen water. We monitored auditory function by recording ABRs. The endocochlear potential (EP) was also measured at 16 weeks of age. The animals survived 16-40 weeks and were sacrificed after collection of blood samples, which were used for the analysis of serum levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) that is a product of oxidatively damaged DNA. The cochlear specimens were provided for histological analyses. The results in ABR measurements showed that hydrogen water significantly attenuated ABR threshold shifts. The effect of hydrogen water was most prominent at 16 weeks of age. No significant decrease of EP values was observed in either group. The serum levels of 8-OHdG in Hydrogen group were significantly higher than those of Control group. These findings suggest that hydrogen water may delay the onset of age-related hearing impairment. This work was supported by I□from Phrmacheutical CO.,Ltd (Tokyo, Japan).

852 Involvement of the Neural Cell Adhesion Molecule (NCAM) in Age-Related Hearing Loss as Assessed in NCAM Null Mutant Mice

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Introduction: The neural cell adhesion molecule (NCAM) plays a crucial role in neuronal migration, neurite outgrowth and fasciculation, synaptogenesis, and synaptic plasticity in the developing and mature nervous system. Interestingly, NCAM interacts with neurotrophic factor receptors, such as the FGF receptor and GFRα1, which are important for neuronal survival in the spiral ganglion of the inner ear in vitro and in vivo. Here we tested the hypothesis that NCAM null mutant mice are more prone to develop age-related sensorineural hearing loss.

Methods: Frequency specific auditory evoked brain stem responses (ABRs) and distortion product oto-acustic emissions (DPOAEs) were measured every 3-4 weeks in NCAM null mutant (KO) and matched wild type (WT) littermate male and female mice from 7 weeks to 36 weeks of age (n = 6-8 animals per age group). At 36 weeks, the animals were sacrificed and inner ears were subjected to histological analysis. Spiral ganglion cell number, whole ganglion size and spiral ganglion neuron density were determined in H&E stained 8μm frozen sections.

Results:NCAM KO mice had significantly higher ABR thresholds than age matched WT mice at all tested frequencies from 4 to 32kHz. Hearing thresholds as determined by ABR were steadily rising in NCAM KO and WT mice with increasing age. The rise of hearing thresholds occurred at an earlier age and increased more rapidly in NCAM KO than in WT mice. Spiral ganglion neuron cell body counts, spiral ganglion size and spiral ganglion neuron density were comparable in NCAM KO and WT in the respective cochlear turns.

Discussion: The combined results indicate that expression of NCAM is not required for the ontogenetic development of normal hearing function. In young mice, NCAM KO mice show similar hearing capabilities. However, our data suggest that the absence of NCAM leads to an accelerated age-related sensorineural hearing loss. This phenotype of the mutant mice does not appear to be due to increased loss in number of spiral ganglion neurons, but may be related to abnormal synaptic contacts with ensuing altered synaptic activity that negatively affects hearing properties of the NCAM KO mice when compared the WT mice.

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853 Comparison of Cochlear Morphology and Apoptosis in Mice Models of Presbycusis

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Morphological study on presbycusis, or age-related hearing loss has been performed in several different strains of mice which showed hearing loss with auditory pathology. C57 mouse has been known to be a mouse model for early onset presbycusis while CBA mouse is characterized by relatively late onset hearing loss. To further understand the early onset hearing loss that could be related with aging process of the cochlea in C57 mouse strain, we compared cochlear pathology and accompanying apoptotic process within the cochlea of C57 mice to those of CBA mice in their younger age period.

Auditory brainstem response (ABR) recordings demonstrated the higher threshold at 32 kHz even at the age of P3mo of C57 compared to the CBA mice. Cochlear pathology of P1mo C57 and CBA mice did not show any big differences in organ of Corti, spiral ganglion and stria vascularis. However, C57 mice showed the pathologic organ of Corti at the basal turn from P3mo onward. Number and shape of the spiral ganglion cells and morphology of stria vascularis of C57 were different from those of CBA mice. Immunohistochemistry studies with apoptotic markers also showed different apoptotic process of the cochlea between two strains.

Since cochlear pathology and apoptotic process have been observed even at P3mo, C57 mouse could be an excellent animal model for early onset sensorineural presbycusis. Further studies to investigate the intrinsic or extrinsic etiologic factors that lead to early degeneration of cochlea in C57 mice would give us more understanding of the possible pathomechanism of early onset presbycusis.

854 Age-Related Changes in Cochlear Histopathology of Rhesus Macaque Monkeys

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Age-related hearing loss (ARHL) is attributed to histopathological changes in the peripheral organ (i.e., cochlea) and/or changes in central auditory processing that lead to spectral and temporal processing deficits. Schuknect (1964) described multiple histopathological patterns of ARHL, which were classified as sensory HL,

neural HL, conductive HL, and metabolic/strial HL. Our research examining the incidence of ARHL in macaque monkeys revealed several ABR threshold patterns, which suggests the existence of multiple peripheral pathologies. The present study examines the cochlear histopathology from temporal bones of young, middle aged and geriatric macaque monkeys.

Temporal bones from six Rhesus macaque monkeys (15-35 years of age), were obtained from medical cull animals at the California National Primate Research Center (CNPRC) at Davis, CA. Animals were included in this study if they had a tone-evoked ABR within one year of the cull date. Each animal was transcardially perfused with 4% paraformaldahyde and 0.25% gluteraldahyde in 0.9% saline. Following brain removal, the temporal bone was removed and then stored in karnovsky fixative until tissue processing. Cochleae from each animal were then processed for both scanning electron microscopy and light microscopy to reveal the associated elements of the organ of Corti.

Preliminary results show age-related changes in cochlear histopathology similar to that described by Schuknect (1964). Interestingly, hair cell and stereocilia loss were marginal. Whole mount osmium fixed preparations reveal innervation by myelinated nerve fibers through the osseous spiral lamina from the base to apex in all animals examined. Age-dependent changes and declines were identified in the stria vascularis and spiral ganglion cells. These results suggest that macaque monkeys experience multiple forms of ARHL, and that they have similar cochlear changes to those reported in humans.

855 Prevention of Age-Related Hearing Loss by MB-12066

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Age-related hearing loss (ARHL) is the predominant neurodegenerative disease of ageing. Currently, it has still to be elucidated the mechanism of ARHL and the effective way to prevent ARHL. Mouse models of ARHL have been used frequently in ageing research. MB-12066 is an activator that can increase the cellular NAD+/NADH ratio by accelerating NADH oxidation to NAD+, which is mediated by NAD(P)H:quinone oxidoreductase (NQO1). Cumulative studies have suggested that the ratio of NAD+ to NADH could be critical factors in ageing process by regulating SIRT, PARP-1, and oxidative stress. Therefore, we investigated the effect of MB-12066 on ARHL in C57BL/6 mice which undergo natural ageing process with gradual auditory dysfunction. In the present study, C57BL/6 mice were randomly divided into 3 experimental groups. First group of C57BL/6 mice (Control group) were fed with regular dietary chow. Second group was fed with dietary supplement of MB-12066 (MB group: 0.06 % of regular chow weight) to the regular chow. Third group was fed with 70% of regular chow as a calorie restricted (CR) group. The feeding regimens began at an age of 12 months old and auditory brainstem response (ABR) was measured at every 3 month from age of 12 months old to age of 24 months. Compared to 2 months old age C57BL/6, the ABR thresholds of control group mice significantly elevated even at 12 months old, and then reached the total deafness level at 24 months old. In case of MB-12066 supplementation with regular dietary chow, there were no significant changes of hearing threshold up to 15 months compared with age-matched control mice. However, MB-12066 supplementation improved hearing threshold in 8, 16 and 32 kHz at both 18 and 21 months old compared with age-matched control mice. Surprisingly, these mice showed significantly improved ABR thresholds (more than 35dB) in all tested frequencies (4-32 kHz) at 24 months old compared with age-matched control mice. Interestingly, CR group improved ABR thresholds up to 21 months old, but rapidly increased ABR threshold and thereby no significant differences were apparent at 24 months old compared with age-matched control mice. We confirmed the involvement of several cellular molecules such as NQO1, AMPK, and SIRT1 in NAD+/NADH metabolisms and mitochondrial biogenesis and role of pro-inflammatory cytokines. Taken together, our data provide the first candidate and its protective mechanisms of MB-12066 in prevention of ARHL.

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856 Mitochondrial Dysfunction and Proinflammatory Cytokines Sign Ageing Cochlea and Hearing Impairment

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Age-related hearing loss affects more than half of people over 60 years of age, making presbycusis a major health problem Due the lack of reliable animal models, no efficient treatment is actually available (with the exception of hearing aids).. Senescence-accelerated mice (SAM) had been produced to investigate the deleterious effects of ageing in the brain and cardiovascular system. In this study, we analyzed the time course, the morphological and the molecular correlates of age-related hearing loss in senescent-accelerated prone 8 (SAMP8) mice. Functional and ultrastructural data demonstrated that SAMP8 displayed same pathological features as reported in temporal bones from patient with presbycusis. Molecular analysis revealed mitochondrial dysfunction leading apoptosis in the organ of Corti, spiral ganglion and in the stria vascularis. Senescence also regulate macrophages activation in the cochlear scalae and increased pro-inflammatory cytokines expressions. Selective autophagic vacuoles into the spiral ganglion neurons was confirmed by the increased expression of the autophagic marker microtubule light chain 3 (LC3). Accumulation of protein aggregates (lipofisin), demonstrates that spiral ganglion

cell death shares common mechanisms with other degenerative pathologies such Alzheimer and dementia.

857 Mature Mammal Hearing Loss: A Natural Experiment in Presbycusis

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For humans, we have extensive data on hearing loss from congenital defects, aging, noise exposures, traumatic events, and disease. By contrast, natural, non-experimentally induced hearing loss in other species, particularly presbycusis and NIHL, is not commonly studied. Concern for anthropogenic acoustic impacts in marine species became particularly acute in the last decade. While we have substantial information on the basic hearing ability of many marine mammals, we know little about the incidence and nature of natural hearing loss.

This paper presents data from computerized tomography (UHRCT), histology, and electron microscopy of 38 marine mammal ears, including 15 ears from mature captive bottlenose dolphins (Tursiops truncatus) for which audiometric data were available. Specimens were collected postmortem, scanned, and mapped via light microscopy to determine the nature and sites of inner and middle ear pathologies.

The data show marine mammals sustain hearing loss from multiple etiologies, including labyrinthitis ossificans, parasitic infestations, trauma, chronic multi-stage otitis, and aging. Older dolphins and seals develop degenerative pathologies (neural, hair cell, support cell, and mineralization loss) paralleling presbycusic changes in older humans. In the captive cases, the location and severity of inner ear pathologies were consistent with losses in frequency and sensitivity consistent with their hearing records. Of note, mature females displayed comparatively little hearing loss while mid to older male animals all had extensive high frequency losses. All species also demonstrated distinct, consistent losses in the same topological region of the basal turn at a point coincident with the 4 kHz notch position in human cochleae. This study demonstrates that some loss elements, such as sexual differences in presbycusic onset and "notches", may have both structural and biochemical foundations common to many species. [Supported by ONR and NIH/NIDCD]

858 Semi-Quantitative Analysis of the Expression of Markers in Different Fibrocytes of the Spiral Ligament of CD-1 Mice

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Fibrocyte cultures are useful in the development of cellular transplantation therapy to ameliorate strial presbyacusis. Five main types of fibrocyte are distinguishable in the spiral ligament by their morphology, location and relative expression of caldesmon, S-100 and Na+/K+/ATPase. Suko et al. (Hear Res, 140, 137-144; 2000) characterized fibrocytes cultured from mice lateral wall immunocytochemically as type I on the basis of these markers. However, we have cultured fibrocytes from rat and CD-1 mice that expressed all the marker proteins immunocytochemically. Given that labelling efficacy can vary and immunofluorescence is difficult to quantify, we have investigated the relative distribution of markers in the spiral ligament of CD-1 mice in vivo by quantitative immunogold labelling to provide a means of more accurately identifying fibrocyte types in culture. Cochleae were fixed in 4% paraformaldehyde and embedded in LR White resin at 50oC or fixed in 4% paraformaldehyde and 0.1% glutaraldehyde and embedded in Lowicryl HM20 resin at –80oC. Ultrathin sections were labelled using rabbit primary antibodies to each of the three proteins followed by secondary antibodies conjugated to 15 nm gold particles and the density of the labelling was quantified. All three proteins were localized in all of the different types of fibrocyte but caldesmon expression was significantly greater in type III than in the other types, S-100 was significantly greater in types I, II and V and Na+/K+/ATPase was significantly greater in types II and V. Caldesmon when present was primarily cytoplasmic, S-100 was localized to the cytoplasm and nucleus and Na+/K+/ATPase was predominantly in the plasma membrane, being higher over the membrane of the surface processes than over the cell body membrane. In conclusion, on the basis of these data, quantitative analysis of relative expression of markers using a combination of antibodies to these three proteins can be used to characterise cultured fibrocytes.

859 Efferent Nerve Degeneration Associated with Alpha-Synuclein Expression in Mouse Cochlea: a Possible Cause of Early Onset Presbycusis

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Efferent nerve under outer hair cells plays a role in the protection of the outer hair cells from loud stimuli. Previously, we have shown that α-synuclein was expressed within the cochlea and localized to the efferent auditory synapses at the base of the outer hair cells. We also found the predominant early onset hearing loss and efferent auditory deficit in the mice lacking α-synuclein. To further prove our hypothesis that efferent nerve deficit related with lacking α-synuclein might be the leading cause of early onset hearing loss, here, we performed another study of comparing the morphology of efferent nerve endings and α-synuclein expression within the cochlea in different mice models of presbycusis.

C57 mouse strain which is a well known model of early onset hearing loss and CBA mouse strain, a model of relatively late onset age-related hearing loss were used in this study. Auditory brainstem response (ABR) using clicks and tone burst stimuli were measured. Cochlear morphology of the two strains was compared at different ages. Whole mount cochlea immunohistochemistry with synaptophysin and α-synuclein has been performed. Western blot and RT-PCR studies for α-synuclein protein and mRNA expression in the cochlea or different sites of brain have been added.
The C57 mice which showed the earlier high frequency hearing loss demonstrated more severe pathologic changes within the cochlea, especially at the basal turn compared to CBA mice. Expression of α-synuclein in the efferent nerve endings at the base of outer hair cells was more prominent and stronger in CBA mice than in C57 mice.
Together, this study results support our hypothesis that efferent nerve dysfunction or dysmorphism which is related to α-synuclein expression could be a possible causal factor of early onset presbycusis. Further study to elucidate the pathomechanism in the cell level would be necessary.

860 Changes in the Expression Pattern of Estrogen Receptors in the Central Auditory System of Pre-Pubertal, Young Adult and Aged Mice

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Estrogens affect many biological functions, exerting their effects mainly via the two estrogen receptors (ERs) alpha and beta. There is both experimental and clinical evidence of the influences of the sex hormones on the auditory system and ERs have been mapped in the cochlea as well as in many non-auditory brain areas. We have examined the distribution pattern of these two proteins in the central auditory system of the mouse using immunohistochemistry. Pre-pubertal (4 weeks), young adult (3 months) and aged (28 months) CBA male and female mice were studied. Both ER alpha and ER beta were found to be present in the mouse central auditory pathway, localized in a number of auditory nuclei of the brainstem, including the dorsal and ventral cochlear nucleus, superior olivary complex, medial nucleus of the trapezoid body, nuclei of the lateral lemniscus, and inferior colliculus. In contrast, rostral centers of auditory processing such as the medial geniculate nucleus and auditory temporal cortex were far less immunoreactive for ERs. Expression patterns were found to be divergent between the two proteins. Expression pattern comparisons between males and females showed no major differences in either age group. Differences in the levels of protein expression were observed in different age groups. There was no uniform change in the expression of the two types of estrogen receptors with age. Some areas showed increases, whereas other central auditory nuclei showed a down-regulation, and others displayed no change. These results provide an anatomical basis for further mechanistic studies that will promote our understanding of estrogens' actions at different levels of the auditory processing.

861 Effects of Age on Pre-Pulse, Variable and Fixed Gap Inhibition of the Acoustic Startle Response Amplitude in HET4 Mice

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Age-related hearing loss and auditory processing problems are common conditions. A hearing loss of sufficient magnitude to cause difficulty in understanding conversational speech occurs in close to 50 percent of people 75 and older. The incidence of central auditory processing disorders (CAPD) and tinnitus increase in this population. The mechanisms underlying CAPD and tinnitus are unknown, although loss of synaptic strength from inhibitory amino acid transmitters might be involved. There is a lack of animal models describing changes in auditory processing with age. Here we incorporate a behavioral response measure with the auditory brainstem response in an outbred strain (HET4) of middle-aged (9-16 mon) and geriatric (22-24 mon) mice.
The acoustic startle reflex (ASR) is a sound-induced movement from the animal that can be measured and used as an unbiased metric. This startle response can be inhibited by a pre-pulse or a gap in an ongoing noise. We now use this metric to characterize the ASR amplitude I/O functions in quiet and in nine different background noise conditions that vary by center frequency and level. Pre-pulse inhibition (PPI) and gap inhibition (GI) using the same nine noise conditions are also compared in the middle-aged vs. old mice.
The preliminary findings show that the middle-aged mice, compared to the old, show lower ASR thresholds and larger startle amplitudes in quiet. In quiet, startle amplitudes reach a maximum around 100dB SPL. In the presence of background noise, startle thresholds are elevated but maximum startle amplitudes are increased compared to the quiet condition. In the older mice, startle amplitudes are roughly correlated with ABR thresholds. In preliminary results, geriatric mice with low ABR thresholds, compared to the middle-aged mice with comparable thresholds, tend to have less inhibition from a preceding gap, indicating a possible presence of tinnitus or CAPD. We will present data from variable-width gap assays to further quantify effects of CAPD and/or tinnitus in geriatric mice.
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862 Effect in CBA/CaJ Mice of Varying Signal Intensity and Age on the Inhibition of the Acoustic Startle Reflex Produced by a Change in Sound Location Along the Azimuth

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Increasing the level of a test signal enhances the detection of a change in its location, an effect found in psychophysical judgments in humans and notably in spike

count and latency of cortical neuronal responses to variation in suprathreshold sound levels in monkeys (Woods et al. 2006, J. Neurophysiol. 96:3323-3337). Here we examine the effect of varying suprathreshold carrier levels on the "prepulse inhibition" (PPI) produced by changes in sound location in young adult and in near senescent mice. Old mice differ from young mice mostly in their requiring a longer time interval between the change in location and the ASR before PPI is evident: it is possible that an increase in signal level would "normalize" this apparent PPI deficit in old mice, as this may enhance their encoding of signal onsets. In these experiments the startle stimulus was a burst of WBN (20 ms duration, 130 dB SPL), the signal carrier a WBN set at various levels. Three experiments were conducted in which: (A) In 6 month old mice shifts in angular separation of the sound between 22.5^o and 180^{o}preceded the ASR at lead times of 10, 50, and 200 ms, with 5 levels between 40 and 78 dB SPL; (B) In 7 month and 24 month old mice, 45^o shifts in location preceded the ASR by 10 and by 100 ms, with levels of 40, 60, and 78 dB SPL; (C) In 7 month old and 22-24 month old mice 45^o shifts in sound location preceded the ASR by lead times varying between 5 and 150 ms, with levels of 40, 60 and 78 dB. Overall, PPI increased with wider angular separation and independently with greater noise level. Both the effect of angular separation and the PPI deficit in old mice were most pronounced with brief lead times. This performance deficit in old mice was diminished but not eliminated with higher carrier levels. The generality of these effects across a broader range of angular separations and carrier levels remains to be determined.

863 Exposure to Noise in Early Ontogeny Affects the Auditory Startle Reflex in Adulthood in the Rat

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Several studies have demonstrated abnormalities in the processing of sound frequency and intensity in rat pups exposed to noise. In the present study, changes in the acoustic startle reflex (ASR) and the prepulse inhibition (PPI) of ASR were used as behavioral indicators of changes in the auditory system caused by noise exposure during the sensitive developmental period. Rats (strain Long-Evans) were exposed to noise (125 dB SPL, for 8 min) on the 14th postnatal day; later, at the age of 3-6-months, the ASR and PPI of ASR were examined and compared to those of age-matched control animals. The ASR responses to noise or tone bursts (2-32 kHz) with an intensity of 70-120 dB SPL and the PPI of ASR elicited by 2-32 kHz tone pips with an intensity of 10-80 dB SPL were investigated. In addition, hearing thresholds were measured using auditory brainstem responses (ABR). ABR hearing thresholds did not differ significantly between the noise-exposed and control animals. ASR amplitudes were systematically lower in the exposed animals. In both groups of animals, high frequency tones evoked considerably lower ASR amplitudes than low frequency stimuli. However, in exposed rats a significant decrease in ASR amplitudes occurred already at 8 kHz, whereas in the controls the pronounced decrease appeared only at 16 kHz. A different shape of the PPI functions was found in the exposed rats in comparison with controls: a steep increase of ASR inhibition at lower prepulse intensities (20-30 dB SPL) was followed by a small non-significant increase at higher intensities in exposed rats, while in control rats a gradual decrease of ASR amplitude was found across the full intensity range of prepulse stimuli. The results indicate that acoustic trauma in the early developmental period that does not induce auditory threshold shifts may result in a deterioration of intensity perception in addition to abnormalities in the processing of sound frequency (Grecova et al., Eur J Neurosci, 2009).

864 Tinnitus: From Rats to Humans - Validation of the Acoustic Gap Startle Paradigm

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Background: The acoustic startle reflex is a primitive reflex produced by a sudden and unexpected loud sound. It can be inhibited by inserting a weak sound (a prepulse sound) a few milliseconds before the startle sound. A modified version of prepulse modulation was recently developed to model tinnitus in animals, by using a silent gap rather than a prepulse. In normal rats, the gap decreases the startle reflex, similar to a prepulse. In contrast, in rats with salicylate- or noise- induced tinnitus, there is no inhibition of the startle reflex, presumably because the gap is filled with the tinnitus sound. The lack of inhibition is specific to background noise of frequency close to tinnitus frequency, and is therefore used to objectively demonstrate the presence of tinnitus. We propose that, transposed to human subjects, the startle reflex may provide an effective paradigm to measure tinnitus objectively.

Objectives: Our objectives were to 1) validate the efficiency of the gap to inhibit the acoustic startle reflex, using high- and low- frequency background noise, in normal-hearing human participants, and 2) examine how it compares with inhibition induced by a prepulse.

Methods: Young participants without hearing loss or tinnitus were recruited. STARTLE-only trials consisted of a 50ms white noise 105dB(A) burst. GAP trials consisted of a 50ms silent gap inserted into either high- or low-frequency background noise set at 65dB(A), followed by a startle sound. PREPULSE trials consisted of either 50ms bursts of low- or high- frequency noise at 65dB(A) presented in quiet, followed by a startle noise. Electromyographic responses of eyeblink amplitude to the three types of stimuli were measured.

Results: Preliminary results show that prepulse sounds were very effective in inhibiting the acoustic startle reflex, by as much as 2-3 fold, a finding consistent with previous studies in human subjects. The gap, however, was not as effective in inhibiting the reflex, especially in the high frequency range.

Conclusion: Although the gap-in-background noise paradigm may not be as effective as the prepulse paradigm in inhibiting the acoustic startle reflex in normal-

hearing subjects, it is a promising approach to objectively measure tinnitus in humans.

865 Gap Induced Reduction of the Acoustic Startle Response as a Behavioural Test for Noise Induced Tinnitus in Guinea Pigs

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Gap induced reduction of the acoustic startle response has now been used reliably to assess tinnitus in rats (Turner et al., Behav. Neurosci, 2006). This test, which is based on the assumption that if a background acoustic signal was qualitatively similar to the rat's tinnitus, poorer detection of a silent gap in the background would result. Here, we used a short gap (20ms) in a continuous background noise to assess the usefulness of this technique in guinea pigs. Guinea pigs were exposed to a unilateral, 97dB SPL RMS, 1/4 octave band noise with a center frequency of 7kHz for two hours. In some animals this noise exposure was repeated 2 weeks after the first exposure. A control group of animals received a sham noise exposure (normal background sound level inside the sound booth). Auditory brainstem responses (ABRs) were recorded before and after noise exposures to quantify the immediate effect of the exposure, which was a 30-90dB shift with a maximum at 8 or 9kHz. ABRs were recorded weekly after the exposure to document the recovery from exposure, which in most animals was complete one week after the exposure. After noise exposure the gap induced reduction of the startle response was diminished for the 8-, 12- or 16 kHz noise bands, which is interpreted as a behavioural correlate of tinnitus at those frequencies. The same animals were also tested for a prepulse-induced reduction of the startle response, which is assumed to test the hearing threshold, however in our data set this did not correlate with the results of ABR recordings.

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866 Baclofen and the Role of GABA Inhibition on Salicylate and Noise Induced Tinnitus

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Some forms of tinnitus are believed to arise from abnormal central activity following peripheral ear injury. In the present study, we used Gap Prepulse Inhibition of the Acoustic Startle (GPIAS) in rats to evaluate the hypothesis that GABA mediated inhibition could play an important role in modulating either transient or persistent tinnitus. In untreated rats, brief silent gaps presented before a loud startling stimulus significantly suppress startle responses. When animals were treated with high doses of salicylate (250 mg/kg) or exposed to intense narrow band (123 dB SPL, 16 kHz, 100 hz BW) unilateral noise, GPIAS was significantly reduced at 16-20 kHz, suggesting the presence of tinnitus in this frequency region.

After rats showed evidence of tinnitus following high doses of salicylate, treatment with the baclofen-S enantiomer caused a dose-dependent decrease in the post-salicylate exaggerated startle response and a partial reversal of gap prepulse inhibition in roughly half the animals tested. In contrast the baclofen-R enantiomer which is stereospecifically active on GABA-B receptors failed to suppress the exaggerated startle response and had little effect on the reduced gap prepulse inhibition.

Two weeks after unilateral noise trauma (16 kHz NBN, 123 dB SPL, 1 h) a subset of rats showed impaired GPIAS 12-24 kHz consistent with the presence of tinnitus. When rats were treated with baclofen-S there was significant reversal of impaired GPIAS 12-16 kHz but 20 kHz remained abnormal. These results suggest that baclofen-S partially reduced the tinnitus percept. In contrast, baclofen-R was ineffective at reducing the presence of tinnitus at all frequencies tested.

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867 Variability in Tinnitus Suppression Via Electric Stimulation

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Electric stimulation of the cochlea has been shown to suppress tinnitus, but the parameters of an effective electric stimulus remain unexplored. Furthermore, a clear distinction needs to be drawn between tinnitus masking and suppression, as they utilize different mechanisms. Previously, high-rate pulse trains (4800pps) delivered to the cochlea produced substantial or complete tinnitus suppression in a number of patients (Rubenstein et al. 2003), whereas low-rate (20-200pps) pulse trains completely suppressed tinnitus in a patient with debilitating tinnitus (Zeng et al. presentation at 2007 ARO).

Here we explored a large parametric space of electric stimuli and measured the effects on tinnitus suppression. Stimulation rate, electrode place, and loudness of the stimuli were varied, and electric stimuli were delivered to the cochlear implant for a total of six minutes. Subjective loudness of tinnitus, as well as loudness of the stimulus, was assessed in thirty (30) second intervals. A total of twelve cochlear implant subjects with tinnitus have participated in our study to date.

Eight out of the twelve subjects achieved tinnitus suppression greater than 30% (<30% suppression could be attributed to a placebo effect), with six subjects experiencing complete suppression during electric stimulation. Preliminary analysis of the data, however, indicates no trends across stimulation rate, place, or level. The present results suggest that electric stimulation via a cochlear implant can be an effective therapeutic for those suffering from tinnitus; however, the effective suppression stimulus varies greatly among individuals.

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868 Temporal Interaction of Pulses in Cochlear Implants

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Speech coding strategies in cochlear implants suffer from channel interaction and temporal interaction of pulse sequences. Earlier studies showed that simultaneous stimulation produces large interaction effects but even non-simultaneous stimulation has a significant effect. The influence of inter pulse distance (IPD) on interaction was previously attributed to refractory effects and investigated in masking experiments.

In this study, we investigated temporal interaction of two individual biphasic pulses at variable distance in cochlear implant patients. The first of the two pulses was subthreshold with a fixed charge in relation to the channel's threshold. This first pulse preconditions the auditory fibers for the following next pulse, the probe. This double pulse constellation with different IPDs allows us to evaluate temporal interaction in the range from 20 µs up to 20 ms. We tested the interaction by threshold adjustment of the probe amplitude (6 subjects on apical and / or middle electrode, pulse phase 40 µs, single pulses 4 Hz).

The main result is that a subthreshold pre-pulse significantly reduces the probe threshold (up to 16% ± 6%, pre-pulse amplitude 20% below threshold, IPD=20 µs). The interaction between two successive pulses lasts at least 300 µs. It grows with increasing (subthreshold) pre-pulse amplitude and decreases with IPD.

In summary, our measurements revealed significant temporal interaction effects from sub-threshold pulses. This interaction will impact coding strategies, therefore it is important to predict and correct not only simultaneous interactions but also temporal interactions. We expect that mostly neuronal dynamics are responsible for the observed temporal interaction effects, which we will analyze in the next step with model calculations.

869 Are All Syllables Perceived Equally? A Comparative Analysis of Song Syllable Perception in Zebra Finches (Taeniopygia Guttata) and Budgerigars (Melopsittacus Undulatus)

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The ability to identify a target signal within ambient noise is crucial for individuals of any species living in a clamorous environment. Determining how these individuals extract target signals embedded in noise is crucial to understanding how these signals are perceived when conditions are less than ideal. Humans have been shown to focus primarily on the first phoneme of a word and can even predict the remaining rest of the word from this limited information. The current study examines the importance of syllable position in birds in identifying a target song embedded in chorus noise. One zebra finch song was used as the target stimulus and a chorus of three other zebra finch songs was used as the masker for four zebra finch and four budgerigar subjects trained on a 2-AFC operant conditioning task. After successfully training the birds to distinguish the chorus masker (Masker) from the combined chorus and target zebra finch song

(Target), the birds were given probe trials on 15% of all trials. These probe trials consisted of individual syllables and sequential combinations of zebra finch song syllables. Subjects were always rewarded for responding to probe stimuli. The first syllable elicited more 'target' responses compared to the other syllables, suggesting that the first syllable is the most important one in the perception of birdsong by these birds, similar to speech perception in humans. Further, the sequential combination of syllables resulted in a gradual increase in 'target' bird song responses, indicating that as more of the song is presented, the ability to hear the birdsong improves.

870 Female and Male Mice Vocalize in Response to Auditory Playbacks of Mouse Ultrasonic Vocalizations

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Although previous studies have found that both male and female mice produce ultrasonic vocalizations (USVs) under a variety of circumstances, the exact function of these USVs remains unclear. Male mice will readily produce many USVs when in the presence of a female mouse or when placed in female-soiled cage shavings. Female mice in diestrus are also known to vocalize to other females, and a social role has been suggested for these vocalizations. It is not known, however, if either male or female mice would emit ultrasonic vocalizations in response to auditory playbacks alone, as is found in many other animals. In the present study, solitary male and female CBA/CaJ mice were placed into a sound-proof booth with testing beginning approximately three hours after lights off. The mice were kept in their own cage and bedding, to avoid introducing any novel olfactory stimuli. The estrus cycle for each female was determined visually and each female was tested twice during estrus and twice during diestrus. To keep the number of playback sessions constant for all of the animals, the males were also tested in four three-minute sessions. Previously recorded USVs from male and female conspecifics were played back to the mice through an ultrasonic speaker on a continuous loop, but separated by several seconds of silence. The stimulus recordings used for the playbacks were taken both from males and from females in estrus and diestrus. These recordings were edited so that each contained the same number of calls per three-second interval. A microphone was placed over the mouse's cage and recorded any vocalizations by the subjects in response to these playbacks. Both the males and females called to

these mouse recordings, even without any additional visual or olfactory cues, demonstrating that USVs can be elicited solely by auditory cues and suggesting that they are important in mouse interactions, possibly even facilitating reproductive and social behavior.

871 Recovering Sound Sources from Acoustic Invariance

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Natural auditory environments present organisms with mixtures of sounds. Segregating individual sound sources from these mixtures is thought to require prior knowledge of source properties, yet these presumably cannot be learned unless sources are segregated first. In previous work, we found that the auditory system could bootstrap its way around this problem by tracking invariant structure in the acoustic input. We developed a simple method for generating novel naturalistic sounds; listeners heard mixtures of these sounds and had to judge whether a subsequent probe sound had been present in the mixtures. Sounds were difficult to recognize when heard in single mixtures, but were readily identified when they occurred more than once across different mixtures.

Here we report the results of additional experiments that clarify the nature of this effect. First, listeners could readily discriminate target sounds heard in mixture sequences from time-reversed probe sounds, suggesting that performance in multiple mixture conditions is not simply due to listeners recognizing the long-term average stimulus spectrum. Second, listeners could recognize a target sound presented repeatedly with the same distractor sound, so long as the temporal relation between the two sounds was varied across presentations. Third, we found that the target recognition advantage for multiple over single mixtures also occurred for sounds that were processed to isolate the effects of either energetic or informational masking.

Our results indicate that listeners distinguish the feature configurations belonging to single sources from those that accidentally occur in mixtures by tracking those that occur repeatedly in the auditory input. By tracking acoustic invariances, listeners can segregate sources if they occur repeatedly over multiple mixtures, even without the aid of bottom-up segregation cues or top-down knowledge of sounds. [Supported by NIDCD grant R01 DC 07657.]

872 Influences of Interaural Time Differences in Grouping of Ambiguous Auditory Scenes

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In everyday settings, listeners must segregate sounds of interest using acoustic grouping cues. Interaural time differences (ITDs) are one such cue, and can enhance performance on many scene analysis tasks. However, ITDs alone do not permit segregation of sound elements if other, non-spatial cues indicate that different elements belong to the same sound source. These findings suggest that binaural cues can influence auditory object formation, but only when grouping is otherwise ambiguous.

McDermott and colleagues recently examined sound segregation with novel noise stimuli that were constructed to share some statistical properties of everyday sound sources, but that lacked some of the structural regularity underlying common acoustic grouping cues (harmonicity, strong common onset, etc.). Listeners could reliably segregate a repeating target sound from a distractor sound only if the distractor varied with each target repetition. This varying-distractor condition provided subjects with multiple unique glimpses of the repeated target, allowing them to identify it.

Here, we used similar stimuli and procedures to test the utility of ITD for sound segregation in the absence of other grouping cues. As in the previous study, we presented subjects with a repeating target mixed with distractors that either repeated or varied within each sequence; in addition, target and distractor ITD either were the same (diotic condition) or differed (spatial condition). We found that ITD cues enhanced segregation of target and distractors (performance tended to be better in the spatial than in the diotic condition). However, the magnitude of this "spatial benefit" depended on how well a particular listener was able to segregate the target in diotic conditions. Our results support the idea that when other grouping cues are ambiguous, ITDs can help segregate simultaneous sound elements.

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873 How Object Formation Can Influence Speech Perception

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Listeners automatically group sound into auditory objects, a process that allows them to selectively attend to and process one source when there are multiple competing sources. However, many of the processes of object formation are obligatory, and can therefore interfere with perception. Here, we manipulated speech to test this idea, extracting two spectral bands far apart in frequency. We hypothesized that the ability to understand the speech would be degraded when the two bands were grouped into different objects, but enhanced when they were grouped together. In contrast, we hypothesized that perception of the location of an individual band would be degraded when the two bands were grouped together, but enhanced when the bands were perceived in different objects.

Native English-speaking subjects were asked to identify a monosyllabic word that was band-pass filtered into two frequency bands centered at 2.5kHz (high) and 500Hz (low). When the high and low bands were presented simultaneously, performance was better than for either band alone, but when the low band was "captured" in a

stream of the same repeated token, intelligibility was degraded. In a second experiment, using the same stimuli, the high band was presented with a left- or right- leading 300 μs ITD and subjects were asked to do left/right discrimination. When presented simultaneously with the diotic low band, discriminability of the ITD was degraded compared to when the high band was presented in isolation, presumably due to obligatory grouping. Performance recovered when the low band was instead captured by the repeating stream.
Together these experiments illustrate that automatic grouping processes operate on speech objects, influencing the perception of different attributes such as identity and location. The effects of grouping can either degrade or enhance performance, depending on the task.
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874 Interaction Between Vocal Tract Length and Fundamental Frequency on Vowel Segregation

WITHDRAWN

875 Influence of Voice Continuity on Selective Auditory Attention

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A recent study showed that when listeners selectively attend to a sequence of spoken digits in the presence of other competing digits, identification is better when the location of the target sequence is fixed than when the location changes between consecutive digits. This study explored whether listeners are better at focusing selective auditory attention when target voice quality, rather than spatial location, is continuous over time. We hypothesized that there would be a cost of switching attention from one voice to another and that this cost would be greatest when the time between digits was short.
Subjects identified a five-digit target sequence while ignoring three simultaneous five-digit masker sequences that were time reversed (rendering them unintelligible). The target voice was either fixed or randomly varied across digits. The inter-digit delay was either 0 ms or 500 ms.
Performance was better overall in the fixed-voice trials, where identification was not significantly affected by the inter-digit delay. In the fixed-voice trials, listeners were more likely to make a correct identification if they had correctly identified the previous digit in the sequence. Performance in the random-voice trials was worse overall, but was better at the longer delay than the shorter delay. For the random-voice trials, performance on the previous digit had only a weak effect on performance for the subsequent digit.
Results suggest that voice continuity facilitates selective attention: 1) by allowing listeners to maintain rather than switch attention when processing an ongoing target (an effect most pronounced at short delays) and 2) by enhancing the ability to select a subsequent sound that is similar to the sound in the attentional foreground (an effect present for both rapid and slow sequences).
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876 Perceptual Discontinuities Between Sequential Sounds Differing in Ear of Presentation

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In several studies of between-ear gap detection in our lab it was noted that a sequential pair of noise bursts presented without an intervening delay was generally perceived to be discontinuous. We have suggested that these perceptual discontinuities might have contributed to the elevated gap-detection thresholds observed when the leading and trailing markers of the gap were presented to different ears under two-alternative, forced-choice conditions. Since both the standard (no gap) and comparison (gap) signals are perceived to be discontinuous, listeners are forced to perform a gap discrimination task (i.e., which discontinuity was longer). Here we describe an attempt to determine the duration of these perceptual discontinuities. On each trial, our inexperienced listeners were presented with a single, sequential pair of noise bursts having some delay between them, and were asked to make a simple decision about whether the sequence was perceived to be continuous or not. The delays ranged from those sufficient to produce clear discontinuities (+40 ms) to those in which the sound onsets were (or were almost) coincident. Separate groups of participants were tested at one of three leading sound durations (50, 100 or 300 ms) and stimuli were presented at 40 dB (SL) via headphones. As expected, the inexperienced listeners generally perceived sequences without physical delays to be discontinuous. Moreover, listeners required less temporal overlap in the sequences to yield 50% continuous judgments at the shorter than the longer leading sound durations. These findings provide additional evidence that the ability to perform relative-timing operations on sounds presented to the two ears can be influenced by the temporal features of those sounds.

877 Context Effects in Auditory Stream Segregation: Adaptation of Frequency Shift Detectors or Criterion Shifts?

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It is well known that perception of two streams (or "streaming") in a repeating ABA- sequence is increased when the frequency separation (Δf) between A and B tones is large. More recently, it has been shown that streaming is reduced when the Δf of a prior ABA- sequence is large (Snyder et al., 2008, 2009). One interpretation of this contrastive context effect is that neural units tuned to large Δf's undergo adaptation, decreasing the perceived Δf of subsequent sequences, and resulting in less streaming, much like a perceptual after-effect. An

alternative interpretation, based on signal detection theory, is that prior exposure to a large Δf results in an increase in the decision criterion toward the large-Δf end of the internal continuum, thereby decreasing later perception of two streams. In support of the criterion-shifting explanation, we show that the effect of prior Δf can be accounted for both qualitatively and quantitatively by across-trial adjustments of the internal criterion that listeners use when deciding between "one stream" and "two streams". We further show that exposure to continuous frequency glides with large or small Δf's does not affect the perception of a subsequent ABA- pattern. Considering that frequency glides are likely to activate and adapt Δf detectors, we conclude that the effect of prior Δf on streaming is most likely to result from an effect at the decision level, rather than from a sensory-level mechanism such as neural adaptation of frequency-shift detectors. [Work supported by NIH RO1 DC 007657]

878 Pattern Specificity in the Effect of Prior Δf on Auditory Stream Segregation

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During repeating sequences of low tones (A), a high tone (B), and a silence (-) in an ABA- pattern, perception of two separate streams ("streaming") increases with greater frequency separation (Δf) between the A and B tones; in contrast, a prior ABA- context pattern with a large Δf results in less streaming during a subsequent ABA- test pattern (Snyder et al., 2008). Recently, we showed that the context and test patterns do not need to be presented in the same frequency range for a large prior-Δf effect to occur (Snyder et al., 2009). The purpose of the present study was to investigate what aspects of the context pattern *are* necessary to facilitate an effect on perception of the test pattern. Presenting a -B-- context with a variable-frequency tone did not affect perception during an ABA- test, suggesting that the change in B-tone frequency from context to test per se is not what caused the effect of prior Δf and that a melodic change between A and B tones is necessary. We further investigated the extent to which the context and test patterns need to have similar rhythmic (xxx-xxx-) and melodic (up-down-flat-up-down) structures, and found that when the test pattern was ABA- and the context pattern was AB-- or -BA-, a smaller effect of prior Δf occurred compared to when the context and test were both ABA- patterns. Replicating this effect of pattern and ruling out that simply the greater the number of melodic changes the larger the effect of prior Δf, we found that when the test pattern was AB--, larger effects of prior Δf occurred when the context was AB-- or -BA-, compared to when the context was ABA-. These results suggest that a maximal prior-Δf effect occurs when the rhythmic pattern of the context and test are similar, regardless of the direction of melodic change. In conclusion, the effect of prior Δf on streaming depends on the presence of 1) at least one melodic change in the context, and 2) similar rhythmic patterns in the context and test.

879 Auditory Streaming by SAM Determined Using the Drunken Horse Paradigm

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Signals in nature are grouped or segregated when the auditory system analyses the acoustic scenes based on their acoustic characteristics. Van Noorden (1975) has developed a paradigm (the ABA_ paradigm) to determine the effect of different stimulus characteristics on the perceptual separation or integration of successive signals ("auditory streaming"). Subjects can either be asked to report their subjective perceptual state (i.e., one or two stream percept), or stream segregation can be determined experimentally using the "drunken horse" paradigm (e.g., Cusack and Roberts, 2000). This method assumes that the detection of a temporal shift of the B-tone in the ABA_ sequence is easier if perceiving one stream than if perceiving two streams. The following research focuses on the shift detection in a series of successive sinusoidally amplitude modulated (SAM) tones to evaluate streaming by SAM.

Four human subjects participated in the experiment using the ABA_ stimulus paradigm (stimulus duration 125ms, presented at a rate of 4 Hz, _ indicates a silent interval). Two carrier frequencies (1000, 4000Hz) and three amplitude modulation frequencies of the A-tone (30, 100, 300Hz) were used. The modulation frequency of the B-tone was adjusted relative to that of the A-tone to evoke one stream, two streams or an ambiguous percept, respectively. A shift of the B-tone was introduced within 1-7s after starting of the observation period. In the GO/NOGO experiment with the ABA_ stimulus being continuously presented the subject indicated the shift detection via a touch screen. Stimulus sequences evoking a subjective one-stream percept allowed detecting significantly smaller shifts than sequences evoking an ambiguous or two-stream percept. The shift detection threshold sequences resulting in a two-stream percept was significantly larger than in the other sequences. The modulation frequency significantly affected the shift detection threshold.

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880 Behavioral Measures of Auditory Streaming in Ferrets

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The ability to parse sound sequences into "streams" that correspond to different sound sources (e.g., predator or prey) in the listener's environment likely plays a crucial adaptive role in most animal species. While this auditory "streaming" ability has been studied extensively in humans, comparatively few studies have been devoted to measuring the phenomenon in other species, including birds, fish, and monkeys. In this study, we adapted two auditory-perception tasks, which have been used in recent psychophysical studies in humans, to obtain behavioral measures of auditory streaming in ferrets. One task

involves the detection of shifts in the frequency of tones inside an alternating tone sequence. The other task involves the detection of a stream of regularly repeating target tones embedded inside a randomly-varying multi-tone background. In both tasks, performance was measured as a function of various stimulus parameters, which previous psychophysical studies in humans have shown to influence auditory streaming. Ferret performance in the two tasks was found to vary as a function of these parameters, in a way that is qualitatively consistent with the human data. These results expand on earlier investigations of auditory streaming in animals by providing evidence that the phenomenon also occurs in ferrets. In addition, they provide two new approaches for its behavioral measurement which could also prove valuable in future physiological investigations of the neural correlates of streaming.

881 Acoustic Factors Influencing Auditory Streaming in Budgerigars and Zebra Finches

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The ability to accurately distinguish between sounds that originate from multiple sources in the environment is crucial for the survival of many animals, impacting behaviors from reproduction to locating food. As such, we would expect a high degree of conservation of auditory stream segregation across many different species. To date, evidence of auditory streaming has been shown in a wide range of organisms; however, these studies typically use simple stimuli emitting from only one location. The current experiment utilizes birdsong and a classification paradigm to determine the role of intensive, spatial, temporal, and spectral cues on streaming in budgerigars (*Melopsittacus undulatus*) and zebra finches (*Taeniopygia guttata*). Both species were trained, using operant conditioning procedures, to differentially peck keys in response to either a synthetic zebra finch song consisting of five syllables ("whole song") or to the same song with the fourth syllable omitted ("broken song"). Probe trials were then inserted, where potential cues for streaming were tested by altering characteristics of the fourth syllable, and the birds responded to the probe trials with either the "broken song" response or the "whole song" response. When the birds report hearing a whole song, it suggests that the altered syllable has become perceptually fused with the rest of the syllables to form one auditory stream, or object. Preliminary results show that the birds are most likely to report hearing a whole song when the replaced syllable in the probe trials is played closer in space, at the same intensity, and with the same spectral characteristics as the rest of the song, suggesting that these cues are important for auditory streaming in these birds.

882 A Test of Gestalt Auditory Grouping Principles in Cope's Grey Treefrog (*Hyla Chrysoscelis*): Perceptual Restoration or Rule-Based Sensory Biases?

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Investigating auditory illusions in humans has brought insight into the processing of sounds and the principles of auditory scene analysis. For example, humans experience a perceptual restoration when listening to a degraded speech signal in which speech segments are replaced with noise. This phenomenon, known as temporal induction, accounts for the perceptual restoration of missing sounds and the so-called 'continuity illusion'. Whereas the stimulus parameters eliciting this illusion are well understood, the underlying mechanisms of the illusion remain poorly known. Functionally this illusion appears to be an adaption for hearing in multi-source acoustic environments in which behaviorally relevant signals can be masked by other sounds. In three mammalian and one avian species, this illusion has been demonstrated and first neural mechanisms processing it could be investigated. Our study was conducted to test whether this illusion has relevance in a wider range of taxa. We conducted two-alternative, non-forced choice phonotaxis experiments to test whether the illusory perception of degraded mating calls is experienced in Cope's gray treefrog (*Hyla chrysoscelis*). Whereas a gap-filling noise restored some of the attractiveness of pulsatile calls with missing pulses, we found little evidence that the frogs perceived illusory pulses in the gap-filled portions of the call. Instead the noise appears to function as an acoustic ornament that results in a greater attractiveness of some stimuli than others and seems to exploit a sensory bias in the gray treefrog. The expression of this sensory bias depended on the temporal structure of added noise and the number and distribution of the discrete pulses. This study was supported by DFG GRK 591 to FS, NSF 0342183 to JJS, and NIDCD R03-DC008396 to MAB.

883 Influences of Aging on Memory for Intensity

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Previous research (ARO 2009; Abs. 215) found that intensity discrimination for brief (50 ms) narrowband noise bursts was impaired by presenting to-be-discriminated bursts in sequences created by the presence of two off-frequency flanking noise bursts, each preceded by 150 ms of silence. A correlation was found between aging and performance in tasks involving the identification of sequences based on intensity differences but not in tasks involving the comparison of two sequences in order to detect intensity differences. In the current experiments,

groups of younger and older listeners with and without mild hearing impairment were asked to make intensity comparisons in a set of conditions that similarly varied comparison and identification tasks. In one condition, the standard was presented before the target sequence (a pre-cue comparison condition), while in another, the standard appeared after the sequence (a post-cue comparison condition). No age effects were predicted for these two conditions, based on the use of immediate comparisons. In a second set of conditions, similar groups of listeners performed a sequence identification task using similar sequences and changes in intensity. Results will be discussed in terms of the hypothesis that there exist distinct influences of aging on immediate and delayed recall for intensity. [Supported by NIH/NIDCD R03 DC008395 and VA RR&D CD2 C4963W]

884 Reverberation Disrupts Spatial Selective Auditory Attention

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In a complex setting, listeners can selectively attend to a desired speech target using spatial cues like interaural time differences (ITDs). However, reverberation smears out fine temporal information, reducing the reliability of ITDs. Given this, it is likely that reverberant energy degrades spatial selective auditory attention. Moreover, anecdotal reports suggest that the ability to focus spatial auditory attention varies across individuals and degrades with age, effects that may be exacerbated by the presence of reverberation.

To test these ideas, adult subjects across a range of ages (18 – 65) were asked to report a stream of digits spoken from a source simulated from directly ahead (azimuth 0°;) while ignoring competing digit streams spoken by the same male talker from simulated locations 15°; to the left and 15°; to the right. Thus, to perform the task, listeners had to focus attention on the target location while ignoring the distracting digit streams. The three source locations were simulated using a rectangular room model and KEMAR head-related transfer functions. The level of reverberant energy was varied by changing the model's wall absorption coefficients, producing anechoic, moderate-reverberation, and high-reverberation conditions. Overall, performance was best in the anechoic condition and worst in the high-reverberation condition. Listeners nearly always reported a digit from one of the three simulated locations (showing that reverberation did not render the digits unintelligible); however, the probability of reporting one of the masker digits increased with increasing reverberation. Inter-subject differences were very large, as expected. The relation between performance on this task and age, memory span, and other basic abilities will be discussed. Future work will explore the interaction of reverberation and hearing loss on spatial auditory attention.

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885 Voluntary and Obligatory Influences of Visual Spatial Cues for When and Where to Listen

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We tested whether listeners can ignore the location of a visual cue that provides information about when to listen if they know the visual cue location is uninformative or misleading.

Subjects listened for a target sentence that randomly came from either left (-40˚) or right (+40˚). The target occurred at a random time in a stream of similar sentences from the same location, making it difficult to detect. Three masking streams of similar sentences were presented on each trial (from -90˚, +90˚, and either +40˚ or -40˚, opposite the target location). In VALID trials, a visual cue came from the target location. In INVALID trials, the visual cue was from the location opposite the target. Three different block types were used: VALID-KNOWN (all trials valid), INVALID-KNOWN (all trials invalid), and RANDOM (trials randomly chosen to be either VALID or INVALID). The visual cues either conveyed location (a light presented prior to the start of the target and masker streams), location and timing (a light presented at the target onset), or location, timing, and lip-reading information (a video of the target talker presented at target onset).

When visual cues for where to listen preceded the acoustic stimuli, listeners made use of the cues (VALID-known and INVALID-known trials) or ignored them (RANDOM trials), as appropriate. When the visual cue occurred on at the onset of the target sentence, its location always influenced performance: performance was better in VALID-known than in INVALID-known trials and better in VALID-RANDOM than in INVALID-RANDOM trials. Lip-reading cues aided intelligibility, but performance remained better for VALID-RANDOM than INVALID-RANDOM video trials.

Results suggest that when a visual cue provides information about when to listen, cue location has an obligatory draw on spatial auditory attention, even when listeners know they should ignore visual location.

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886 Interplay of Stimulus Detection and Eye Movements in an Alternative-Forced Choice Paradigm: A Combined EEG/EOG and Psychophysical Study

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Sound detection near absolute threshold is a chance event, but the contributions of peripheral and central factors are not understood. We developed a physiologically motivated peripheral "bottom-up" model which accounts for the dependence of absolute threshold SPL on stimulus shape and duration, up to about 200 ms. Beyond this limit, thresholds are slightly higher than predicted. Our model is based on short-time constant (1-2 ms) leaky integration of the stimulus amplitude envelope and formation of detection events from three independent

sub-events, followed by probability summation. It accounts very well for the dependence of the latency of auditory-nerve fiber responses on the envelope and duration of tones (Neubauer & Heil 2008; Heil et al. 2008).
To explore the contribution of central mechanisms to this discrepancy, we measured the EEG and EOG of human listeners while they had to detect tones (532 ms duration) in an adaptive 3I-3AFC-paradigm. Observation intervals were labelled visually by pop-up windows on a computer screen. As expected, the potentials evoked by the near-threshold tones were rather small and not well suited for analysis. However, wavelet analyses revealed differential relationships between the timing of visual labels and of the auditory stimulus and the timing and frequency or amplitude of eye movements and of cortical activity at frontal electrodes during correct detections and misses.
Our data suggest that the visual label triggers a period of enhanced attention, lasting some 200 ms and reflected in reduced eye-movements. Interestingly, this period matches the 'temporal window of integration' known from other perceptual phenomena. Interplay of a visually triggered span of attention with the auditory stimuli could account for the discrepancy between predicted (by peripheral mechanisms) and observed absolute thresholds for long-duration tones.
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887 Effect of Juvenile Auditory Training on Adult Perception

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A general theory of sensory development holds that early experience can influence central nervous system function, thereby shaping adult perceptual skills. However, support for this idea is based largely on the neural effects of sensory deprivation or continuous exposure to a limited sensory environment. A more cogent test of this theory would provide sensory training to juvenile animals and ask whether their perceptual skills were affected in adulthood. Here, we trained juvenile gerbils (P25-40) on an amplitude modulation (AM) detection task, and determined whether their performance in adulthood was better than naïvely trained adults (P70). Juvenile animals were trained on a conditioned avoidance task for a total of 10 days. When they returned as adults, initial AM thresholds were obtained, followed by 10 days of additional testing. Adult animals trained as Juveniles displayed similar initial thresholds as naïve Adults (31.7± 4.4% depth for Juvenile-trained-Adults; 27.8 ± 5.1% for Naïve-Adults). However, the performance on the final 3 days of testing was significantly better in Adults trained as Juveniles as compared to naïve Adults (Juvenile trained: 14.7 ± 3.1% depth; Adult naïve: 22.4 ± 5.5%). Similar differences were obtained when considering the last 2 thresholds, the last threshold, or the single best threshold. As a control, a group of animals trained in Adulthood were re-tested after one month to determine whether the effect of training depends on age. AM thresholds did not improve in these adult animals. These results suggest that adult behavioral performance can be improved by early sensory training, even before perceptual skills are mature.

888 Changes in Accuracy with Interval Order During Auditory Development and Learning

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Several lines of evidence suggest that both the later maturation of human hearing (between 6-12 years) and auditory perceptual learning are the result of enhanced auditory cognition rather than improved peripheral or sensory coding of sounds. We analysed the extent to which target sound identification/discrimination accuracy in each interval of sequential, 3I-3AFC psychophysical tasks changed during development and learning. The development study involved 1469 children tested on 5 different tasks of temporal and frequency resolution and discrimination (FD). Highly significant developmental changes were seen between and within tasks. On most tasks, accuracy on the first interval improved with age to surpass the other intervals, for which accuracy increased only slightly, or was unaffected by age. For FD, in contrast, a massive and stable advantage of the third interval in early life was gradually equalised by the first interval by 11 years. At initial test in a learning study, we also found significant interval effects in 58 adults training to 'discriminate' between three identical tones, but asked to perform either FD or intensity discrimination (ID). During ID 'training', a greatly improved accuracy was found in the first interval with little change in the other intervals. This improvement was due to a reduction in false positive responses during learning that also saw decreased responding in the first interval, but increased responding in the third interval across training blocks. Together, these results support the view that procedurally similar, or even physically identical tasks, can involve very different patterns of maturation in children, and listening strategies in both children and adults.

889 The Emergence of Sound Localization Abilities in Children Who Use Bilateral Cochlear Implants

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Cochlear implants (CIs) successfully promote monaural hearing abilities such as speech perception. However, spatial hearing abilities such as sound localization are challenging with one CI, and often are better with bilateral CIs (BICI). BICIs in young children provide a unique opportunity to study spatial hearing in deaf children who undergo auditory deprivation prior to the onset of hearing. Previous findings from our lab suggest that minimum audible angle thresholds in children with BICIs are lower (better) than in children who use a single CI and a hearing aid in the contralateral ear. However, sound localization accuracy with multiple source locations is a more functionally relevant task. Earlier studies in our lab with bilateral children (5-14 years) who had extended periods of

deafness in the second-implanted ear indicated that 11/19 had improved sound localization accuracy with BICI compared with one CI. In the present study, we investigated (a) the effect of bilateral experience on performance, and (b) whether earlier bilateral activation might lead to the development of sound localization accuracy at a younger age and in a larger proportion of subjects. Children 4-9 years old were tested in BICI mode, at one of five bilateral experience intervals: 3-6, 12-15, 24-27, 36-42, or 42+ months. Several children returned for a follow-up visit at their next consecutive interval. Stimuli were spondaic words (60 ±4 dBA), presented randomly from one of 15 loudspeakers positioned in the horizontal plane at 10-degree increments. Results indicated that RMS errors ranged from 19.2 to 49.5 degrees. Overall, localization accuracy improved and group variance decreased with increased bilateral experience. Preliminary findings from those with second visit follow-up testing indicate that all children showed decreases (improvement) in RMS error as the amount of experience with the second implant increased.

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890 Learning Owl Ears

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Human sound localization results primarily from the processing of binaural differences in sound level (ILDs) and arrival time (ITD) for locations in the horizontal plane (azimuth) and of spectral shape cues generated by the head and pinnae for positions in the vertical plane (elevation). This particular correspondence of cues to different dimensions is typical for many species, but the barn owl is a notable exception. Due to a vertical asymmetry in the ears of this highly-specialized, nocturnal hunting species the two types of binaural cues correspond to different dimensions: ITDs vary with azimuth, while ILDs vary mainly with elevation. It is assumed that the specific relationship between sound location and sound cues is learned, both by owls and humans. However, since the dimensionality of the cues within a species never changes, it is not known whether the relationship between sound dimension and sound cues can be learned. To study this, we applied molds to human's ears that removed elevation-information contained in the spectral cues, while introducing ILDs that varied with sound elevation, effectively providing subjects with owl-like localization cues. Results show that acute sound localization was severely impaired, yet after several weeks, subjects regained normal localization performance. Our findings suggest that the dimensionality of the cues is not hard-wired, but can be learned.

891 The CI-MUSHRA Method to Assess Musical Sound Quality: A Study of Bass Frequency Perception in Cochlear Implant Users

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Cochlear implant (CI) users commonly report subjectively poor sound quality during music perception. One such example is the loss of low frequency (bass) response in CI users, attributable to the lack of apical cochlear stimulation. The purpose of this study was to design a method to objectively quantify this loss of musical sound quality in CI users. A version of the Multiple Stimulus with Hidden Reference and Anchor (MUSHRA) method was modified for use in CI subjects (CI-MUSHRA). This method allows for subjective rating of a series of related musical stimuli that have been parametrically altered, together with an unaltered original stimulus ("reference") and a highly altered version ("anchor"). Subjects were asked to assign a quality rating score between 0 (poor) and 100 (excellent) for each stimulus, and were required to give a score of 100 to at least one stimulus in the series, providing a form of inter-subject calibration. We included 7 versions of 25 original stimuli that included 5 high-pass filtered (cutoff frequencies at 200 Hz, 400 Hz, 600 Hz, 800 Hz, 1000Hz) versions with decreasing low frequency information, in addition to the anchor and reference. Six CI users and ten normal nearing (NH) control subjects participated in the study. In NH subjects, a statistically significant ($p<0.001$, ANOVA) difference was observed between ratings for the reference, high-pass filtered, and anchor stimuli. Quality ratings correlated significantly with the amount of low frequency information present. CI subjects, by comparison, were largely unable to distinguish differences between any of the stimuli (including the anchor), and displayed a random distribution of quality ratings ($p=0.32$, ANOVA). These findings support the use of the CI-MUSHRA as a method to evaluate quality of music perception in CI users, and provide objective confirmation of the poor perception of bass frequencies in CI users.

892 Auditory Preference of Children with Autism Spectrum Disorders

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Recent research on children with Autism Spectrum Disorders (ASD) suggests differences from typically-developing (TD) children in the preference for 'social' versus 'nonsocial' sounds. However, these studies have been based largely on indirect measures which assume a strong association between preference and time spent orienting to a sound, as judged by an independent observer. In the present study preference was assessed 'directly' by measuring the frequency with which the child independently initiated different sounds as a form of play. Four categories of sounds were investigated ranging from highly social (prosodic speech) to highly nonsocial sounds

(repetitive/monotone mechanical sounds). Participants were sixteen children with ASD and fourteen TD children with ages 24-47 months. Contrary to the results of past studies, both groups displayed a strong preference for the highly social sounds (prosodic speech). The discrepancy in outcome is attributed to differences between children with ASD and TD children in their interactive versus reactive response to stimuli. [Research supported by NIDCD].

893 Spatial Release from Masking in Children: Symmetric and Asymmetric Masker Distribution in the Horizontal Plane

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Spatial release from making (SRM) is improvement in speech intelligibility measured when target and maskers are spatially separated, as opposed to when they are co-located. When the target is front and the maskers are on one side of the head (asymmetrical), the effect can be as large as 12 dB in normal-hearing (NH) adults. SRM occurs due to combination of monaural head-shadow and binaural interaction effects. To better study these effects, we recently measured SRM when maskers are symmetrically distributed in the horizontal plane to the right and left, such that monaural head-shadow is obliterated. We recently found that in NH adults there can be substantial SRM for symmetrical maskers. The present study focused on SRM for symmetrical versus asymmetrical masker locations in NH children and in children who use bilateral cochlear implants (BICI), all 4-9 years of age. Speech intelligibility was measured using a 4-AFC task in which an auditory label is matched to a visual target [Litovsky, R.Y. (2005) J Acoust Soc Am. 117:3091-9]. Results showed in NH children, SRM varied with most measures falling between 4-7 dB in asymmetrical conditions, and slightly lower yet still positive in symmetrical conditions. The latter suggests that in the absence of monaural cues such as head-shadow, NH children are able to benefit from spatial separation of target speech and maskers. In contrast, for children who are deaf and use BICIs, SRM is, on average, 1.37dB in asymmetrical and negative in symmetrical conditions, suggesting that symmetrical maskers lead to worse performance relative to conditions in which maskers are co-located with the target. Group comparisons made by matching children for "hearing age" suggest that the amount of time children have spent with exposure to auditory stimulation is not the determining factor. Alternatively, the fact that BICIs are not coordinated and lack fine-structure cues may account for the reduced SRM compared with NH children.

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894 The Role of Interaural Time and Level Cues in Spatial Release from Masking and Localization Abilities for Cochlear Implant Users

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Cochlear implant (CI) users demonstrate a bilateral benefit for both speech perception in noise and sound localization. Considerable evidence suggests that these patients have poor sensitivity to interaural time differences (ITD), but normal or near normal sensitivity to interaural level differences (ILD), suggesting that bilateral benefits may be dominated by ILD sensitivity. The purpose of this study was to determine whether results from speech perception in noise tests involving spatial release from masking (SRM) and those from sound localization tests largely reflect ILD sensitivity. We used head-related transfer functions (HRTFs) based on the microphone response specific to CI processor models, allowing us to independently manipulate ITD and ILD cues. Six bilateral CI users were tested, and their performance was compared to normal hearing (NH) subjects listening to stimuli processed with HRTFs corresponding to the patients' CI processors. SRM was measured using HINT. Localization was measured using a test in which participants located the sound of a gun shot originating from one of twelve locations separated by 15°. There were three conditions for both tasks: 1) Both ITD and ILD cues present; 2) Only ITD cues present; and 3) Only ILD cues present. With both cues present, the CI users' localization was roughly comparable to the NH group, but their SRM was poorer. With only ITD cues, CI users performed poorly for both localization and SRM. With only ILD cues, the CI users' SRM was roughly comparable to that of the NH group, but most CI participants' localization was significantly better than that of the NH group. These results suggest that ILD cues are the dominant source of CI users' bilateral benefit for both SRM and sound localization. The CI users' superior localization, when only ILD cues were available, suggests a potential learning effect, possibly facilitated by reduced access to ITD cues.

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895 Binaural Unmasking with Multiple Masking Electrodes in Bilateral Cochlear Implant Users

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There is growing evidence to suggest that bilateral cochlear implant (BICI) users gain an advantage from a second implant in noisy situations. However, there is a gap in their performance relative to normal hearing (NH) listeners, possibly because in clinical fittings left and right processors are not coordinated. When stimulation is coordinated, binaural masking level difference (BMLD) is seen as the improvement in signal detection threshold when target and noise differ in binaural cues. BMLD is

~9dB when single pairs of binaurally pitch-matched electrodes are activated, but only ~1.5dB when speech stimuli occur in sound field, contrasting with NH listeners who show similar BMLDs for tones and speech. A possible explanation is that channel interactions between electrodes limit BMLD in CI users. To test this idea, in the present study we examined how BMLD for a given pair of electrodes is affected by the addition of stimulation using adjacent (masking) electrodes. The use of multiple pairs of electrodes represents a more realistic stimulation condition compared to single electrode pairs used in prior studies. Five BICI users were tested using concurrent stimulation of multiple sites. Baseline BMLD (single pair) averaged 8.86±1.01 dB (mean±s.e.). In contrast, average BMLD for various permutations of adjacent noise masker locations was 2.10±0.68 dB, which is similar to speech BMLD levels found in previous studies. These results suggest that binaural unmasking cues were still present despite interactions from neighboring electrodes. Adding masking noise directly to the signal channel increased detection thresholds and BMLDs. Increasing the number of masking electrodes increased thresholds, but reduced BMLDs. Neural response telemetry data showed a loose correlation between the amount of channel interaction and BMLD. The present result suggests that reduction of channel interactions in bilateral CI users may improve their binaural hearing performance.

896 The Benefits and Perceptual Mechanism by Bilateral and Bimodal Cochlear Implant Users

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The present study was designed to investigate the benefits and perceptual mechanisms of combining two cochlear implant (CI) devices or cochlear implant and hearing aid (HA) device. Consonant recognition was measured in a steady-state speech shaped noise as a function of signal-to-noise ratio (SNR) plus quiet condition in one bilateral cochlear implant user and one bimodal (CI+HA on contralateral ear) user. Confusion matrices were measured in the following conditions: for bimodal listeners, (1) CI alone and (2) CI + HA; for bilateral CI users, (1) right CI alone, (2) left CI alone, and (3) CI + CI. The confusion matrices were thoroughly analyzed to understand two specific questions: (1) how many dB signal-to-noise ratio (SNR) is benefited by bilateral/bimodal condition, relative to that by monolateral condition, (2) does speech processing mechanism (perceptual confusions) differ between bilateral/bimodal and monolateral conditions. The results show that regardless of listening conditions the recognition scores of stop consonants were significantly poorer than those of fricatives and nasals. The average amount of benefits in bilateral or bimodal listening is approximately 5dB SNR, but such a benefit highly depends on individual sounds and device. The detailed analysis of confusion matrices also revealed that there are usually two major competitors for each given target, and such competitors are consistent over listening conditions. The data suggest that perceptual cues for the target and competitors are heard and processed similarly between two listening conditions, but utilization of the perceptual cues are more limited in the monolateral condition than in bilateral/bimodal condition. Such confusions were not resolved at higher SNR, even in quiet, indicating that adding another CI or HA may not increase the clarity of perceptual cues for these confusions, particularly place of articulation.

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897 A Sensitive Period for Cortical Development and Plasticity in Children with Sequential Bilateral Cochlear Implants

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In the last few years there has been an increase in the number of children who have one implant and who have received (or wish to receive) a second implant at a later age in childhood. In children it appears to be the case that a major motivation for bilateral implantation is the expectation that children will acquire good speech perception skills in the second implanted ear, and by listening with two ears, they will improve their speech understanding ability in quiet and in noise. However, mixed outcomes have been reported in behavioral studies. In this study, we explored neurophysiology as a means for better understanding the conflicting behavioral outcomes in sequentially implanted children. We examined cortical development and plasticity following bilateral cochlear implantation in children who receive second implants at varying ages in childhood and in early adulthood. In 38 sequentially implanted children, we assessed speech perception in quiet and in noise, as well as cortical activity (using dipole source analyses and sLORETA), in relation to the morphology and latency of the P1 cortical auditory evoked potential (CAEP). The P1 CAEP is generated within the auditory cortex and is considered a biomarker of central auditory development (Sharma et al, 2007). Variables including age of first implant, second implant, and interval between implants were considered. Results showed that latency, morphology and cortical activation varied systematically in an age-related manner in sequentially implanted children. Speech perception scores in quiet and in noise were strongly correlated with the electrophysiologic results. We find a sensitive period in early childhood during which cortical plasticity is preserved (allowing for bilateral stimulation of central auditory pathways), resulting in good speech perception outcomes after late sequential implantation in congenitally deaf children. Research Supported by NIH.

898 Ipsilateral Simultaneous Masking Between Acoustic and Electric Stimulations

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Residual acoustic hearing can be preserved in the same ear following cochlear implantation (CI) with either soft surgical techniques or short electrode arrays. The combined electric-acoustic stimulation (EAS) significantly improves cochlear implant performance, particularly speech recognition in noise. The present study investigates simultaneous masking between acoustic pure tones and electric pulse trains to probe the underlying psychophysical mechanisms.

Several CI subjects, with acoustic hearing preserved at low frequencies in their implanted ear, participated in the study including 1 subject with a fully inserted 24 mm Nucleus Freedom array and 5 Iowa/Nucleus Hybrid subjects with 6 mm short arrays. Masking data of the CI subject with the 24 mm array showed that stimulation from the most apical electrodes produced threshold elevations of 10-14 dB for 500 to 750 Hz tones, but no elevation for 125 and 250 Hz tones. The Iowa/Nucleus Hybrid subjects did not exhibit masking from electric stimulation. Conversely, when pure tones were used to acoustically mask electric stimulation, the subject with the 24 mm array showed electric threshold elevation up to 58% dynamic range, while the short array subjects showed elevations up to 27% dynamic range at only the apical electrodes.

The present data are consistent with the place-theory of masking. To determine whether the observed masking has a central origin, contralateral masking was measured between the implant ear and the opposite unimplanted ear. Preliminary data showed comparatively absent or reduced amounts of masking. The present results may help improve the complementary programming of hearing aids and cochlear implants.

899 Effects of Experience on Electric Pitch Perception in Hybrid and Long-Electrode Cochlear Implant Patients

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Recent experiments indicate that pitch perceived through a Hybrid (short-electrode) cochlear implant can shift after 1-5 years of experience with the implant, by as much as 2 octaves (Reiss et al., 2007). We hypothesize that these changes are driven by spectral discrepancies between acoustic and electric inputs introduced by the patient's cochlear implant speech processor frequency-to-electrode allocation, or MAP. In other words, long-term experience with a cochlear implant + residual acoustic hearing may cause a patient to adapt pitch sensations from each electrode to align with the allocated MAP frequencies, which are also the acoustic tone frequencies most often stimulated simultaneously with that electrode through the acoustic hearing.

Here we compare updated electric-to-acoustic pitch match data from Hybrid cochlear implant patients, and new data from long-electrode cochlear implant patients with residual acoustic hearing in the non-implanted ear. Data was obtained both in patients that had just been hooked up, and patients with several years of experience with the implant. Preliminary findings suggest that electric pitch adaptation also occurs in some, but not all, long-electrode cochlear implant users. Similarly to Hybrid users, long-term pitch matches in some long-electrode users are more closely aligned with the corresponding MAP frequencies than early pitch matches. This variability in electric pitch adaptation across subjects may be due to differences in hearing aid use and the type of hearing aid in the non-implanted ear.

Overall, these results suggest that long-electrode patients may also undergo changes in electric pitch with experience, and that these changes may be determined by not just the MAP frequency, but also by the type of and presence of amplification in the non-implanted ear.

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900 Anisochronous Beat Detection: A Study of Rhythmic Clocking in Cochlear Implant Users

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The concept of internal rhythmicity is referenced in myriad processing models that deal with the interface between perception and action. Within these models, rhythmic clocking (beat perception and synchronization) refers to the extrapolative expectancy that is established with as few as three isochronous beats. Rhythmic clocking is an integral concept in both music and spoken language that may provide a basis for the observed correlations in performance for these disparate tasks. In this study, we sought to design a task of rhythm perception for cochlear implant (CI) users that went beyond simple pattern recognition or tempo differentiation. We devised a test of anisochronous rhythm perception in which subjects were presented with four percussive beats, the first three of which were perfectly isochronous. The fourth beat was presented either anisochronously (both early and late conditions) or isochronously. The stimuli were presented at three different tempos, corresponding to slow, moderate and fast. The fourth beat was presented in three deviations for each the early and late conditions, corresponding to a shift of 1/16, 1/8 and 3/16 of a beat, respective to the tempo. Subjects were asked to identify whether the fourth beat occurred early, late or in time with respect to first three isochronous beats. We hypothesized that CI users would demonstrate reduced performance on this test of internal rhythmic clocking in comparison to normal hearing (NH) control subjects. We also included a third subject group, that of highly trained conservatory musicians (MUS), to examine the effects of musical training on internal rhythmicity. We further hypothesized that musicians would perform significantly better than both NH controls (despite their normal hearing) and CI users.

The CI group (n=12) performed comparably to normal hearing participants (n=12) in all anisochronous rhythm detection tasks (CI = 56.45 ± 13.93%; NH = 51.49 ± 13.82%; p=0.14). Musicians (n=7) outperformed CI subjects but also NH controls (MUS = 70.92 ± 5.95%; NH vs MUS - p<0.0001, CI vs MUS - p<0.0001), suggesting that musical training may improve CI user performance on tasks for which they already perform at normal hearing levels.

901 Pitch Perception of Regular-Interval Noise Stimuli in Cochlear Implant Users

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Cochlear mechanisms of pitch perception rely upon a combination of temporal ('rate-pitch') and spectral ('place-pitch') processing in individuals with normal hearing. For individuals with cochlear implants (CI), place-pitch mechanisms are limited by a combination of variable location and fixed-spacing of electrode placement vis-à-vis the cochlear tonotopic axis, while rate-pitch mechanisms are limited by altered phase-locking and reduced stochasticity of the auditory nerve. As a result, CI users demonstrate severe limitations in pitch processing that provide a major obstacle for both music and tonal language perception. In this study, we evaluated the ability of CI users to perceive pitches generated using regular-interval noise (RIN) stimuli. RIN pitches were created from white noise that was repeatedly delayed and added to itself, creating a pitch percept whose frequency is related to the reciprocal of the delay yet whose signal contains no spectral peaks. During the experiments, normal hearing subjects and CI users were presented with one test of pitch ranking (higher vs. lower) and one test of isochronous melody recognition using both pure tones and RIN pitches that were high-pass filtered to eliminate resolvable spectral cues. Normal hearing listeners demonstrated no observable difference during perception of pure tones or RIN pitches. In contrast, CI users demonstrated greater accuracy during pitch ranking and melody recognition for RIN pitches than for pure tones. These preliminary data support the use of RIN stimuli to assess and possibly improve musical pitch perception in CI users.

902 Bandpass Filtering Can Improve Cochlear Implant Users' Music Perception

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Individual cochlear implant (CI) users' melodic contour identification (MCI) has been shown to be differently affected by instrument timbre. For these CI users, "pre-processing" the acoustic input signal may remove conflicting and/or ambiguous pitch cues produced by some frequency components. In this study, MCI was measured in 8 CI users using piano samples processed by 4 bandpass filters: low (310-620 Hz), middle (620-2480 Hz), high (2480-4960 Hz) and full (310-4960 Hz). The lowest note of each contour was D#3 (F0=312 Hz), and the spacing between successive notes in the contours was 1, 2, or 3 semitones; thus, all F0s fell within the lowest filter range. Mean performance was best with the middle band and poorest with the high band. Some CI subjects performed equally well with the low, middle and full bands. Others performed best with the middle band and much poorer with the low, high and full bands. For these subjects, removing the low and/or high frequency components improved melodic pitch perception.

While bandpass filtering may optimize melodic pitch cues for some CI users, instrument timbre perception may require the full input frequency range. In a second experiment, musical instrument identification (MII) was measured in the same subjects, using the same bandpass filters as in the MCI test; subjects were asked to choose among six instrument response choices: piano, clarinet, violin, organ, trumpet, and glockenspiel. Different from the MCI data, mean performance was best for the full band, with the middle band producing nearly equivalent MII performance.

These results suggest that individual CI users may benefit from optimizing the acoustic input for music. Removing potentially conflicting low and high frequency cues seemed to improve most CI users' melodic pitch perception with little decrement in timbre perception, at least as measured by these relatively simple MCI and MII tasks.

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903 Chronic Neurotrophin Infusion and Electrical Stimulation in the Deaf Cochlea: Implications for Cochlear Implant Spatial Selectivity

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The application of exogenous neurotrophins to the cochlear fluid prevents the degeneration of spiral ganglion neurons (SGN) following the loss of cochlear hair cells. The SGN peripheral nerve fibers also resprout in an abnormal disorganized manner following neurotrophin treatment [1]. This study aimed to investigate the extent of disruption of auditory nerve cochleotopic organization with regards to the spatial selectivity of electrical stimulation. Two weeks after ototoxic deafening, adult guinea pigs were given intracochlear neurotrophins or artificial perilymph via an osmotic pump. Half of each group also received chronic intracochlear electrical stimulation (ICES) from a banded electrode array and clinical speech processor. Following a four week treatment period multi-unit spike clusters were recorded across the inferior colliculus in response to ICES on different bipolar electrode pairs to determine the sharpness of spatial tuning. Chronic ICES resulted in significantly broader spatial tuning (Two-way ANOVA, p<0.03) across different stimulation sites and over a range of intensities up to 3.5dB above threshold. Neurotrophin treatment did not have a significant effect on tuning curve width (p>0.05). Therefore, neurotrophin treatment does not reduce the

spatial selectivity of cochlear implant electrode arrays with designs based on current clinical models.
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904 Neurotrophic Effects of Exogenous Brain-Derived Neurotrophic Factor (BDNF) and Electrical Stimulation in Cats Deafened as Neonates

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The postnatal development and survival of spiral ganglion (SG) neurons depend upon both neurotrophic support and neural activity. Our earlier studies in cats deafened at birth (neomycin, 60 mg/kg SQ SID for 16-21d) indicated that intracochlear electrical stimulation (ES) only partly prevents SG degeneration in this animal model of congenital deafness. Thus, neurotrophic agents that might be combined with ES to further enhance neural survival are of interest. This study assessed the effects of intracochlear infusion of BDNF after early deafness. Kittens were deafened as neonates, implanted at 4-5 weeks of age with scala tympani electrodes with an integrated drug-delivery cannula connected to a mini-osmotic pump. BDNF (94 µg/ml; 0.25 µl/hr) was infused continuously for 10 weeks.

In BDNF-treated cochleae, SG cell somata were normal adult size and significantly larger than cells on the contralateral side (mean 77% of normal; $p<0.024$). We used a physical dissector stereological method to accurately determine the numerical density of SG cells *independent of cell size*, by counting nucleoli in serial 5 µm plastic sections. After BDNF infusion, mean SG density was 79% of normal and was significantly higher than in the contralateral ears (mean 62%; $P=0.001$; $n=5$), indicating that BDNF was effective in promoting survival of SG neurons in these developing animals.

Additional experiments are exploring whether this SG survival advantage is maintained when BDNF infusion is followed by several months of ES. Initial results suggest that enhanced SG neural survival is largely maintained (BDNF+ES = 71% of normal; control side =44%), but normal cell size is not (BDNF+ES = 92% of normal; control = 81%). Other notable findings with BDNF+ES include higher density and larger size of myelinated radial nerve fibers in the osseous spiral lamina, sprouting of fibers into the scala tympani, reduction in EABR thresholds on stimulated channels, and maintenance of apparently normal central tonotopic organization in the inferior colliculus.

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905 Towards a Measure of Neural Survival in Recipients of Cochlear Implants: Focused-Stimulation Thresholds, Speech Understanding, and Electrode Locations

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Cochlear implant (CI) focused-stimulation thresholds vary greatly depending on cochlear place of stimulation. Focused thresholds give a measure of the efficacy of stimulation for a given channel and may give insight into the local state of the neural tissue. For example, a high threshold could indicate (1) poor neural survival near a channel, (2) a longer electrode-to-modiolus distance, or (3) that other factors are limiting current flow to the neural tissue. It is likely that a combination of factors underlies the observed variability in focused-stimulation thresholds across cochlear place. One of these factors, the electrode-to-modiolus distance, can be directly measured from high-resolution CT scans.

In an attempt to better understand the factors determining focused-stimulation thresholds, we analyzed high-resolution CT scans of eleven CI-implanted cochleae of CIs users who have also undergone extensive psychophysical and speech testing. In particular, focused-stimulation thresholds have been measured for all functioning electrodes.

We have observed that up to 60% of the within-subject variance of focused Phased-Array (PA) thresholds is accounted for by electrode-to-modiolus distance. In addition, 74% of the variance in CNC Word Scores across subjects is accounted for by the amount of within-subject variability in PA Thresholds (N=6). Other psychophysical tests and analyses are ongoing.

We aim to determine if combinations of focused thresholds, other psychophysical tests, and electrode-to-modiolus distance can act as predictors of neural survival. Knowledge of the neural state near each electrode could guide fitting strategies and potentially improve the transmission of information across the electrode-neural interface.

906 Intracochlear Monitoring of Acoustically-Generated Potentials During Cochlear Implantation in Gerbils with Normal-Hearing and Noise Induced Hearing Loss

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Preservation of acoustic hearing after cochlear implantation improves speech recognition and discrimination, especially in the presence of background noise. Presently, residual hearing is often lost or compromised during cochlear implantation. To understand this process we used normal-hearing gerbils to identify electrophysiological markers of cochlear damage during electrode advancement. Similar techniques can be

applied to animals with high-frequency hearing loss, a condition often observed in patients eligible for cochlear implants.

All experiments were conducted in urethane-anesthetized gerbils. Animals were either normal-hearing or had a noise induced hearing loss (high-pass noise with varying cut-off frequencies). Tungsten-iridium electrodes were placed at the round window and recordings of the cochlear microphonic (CM) and compound action potential (CAP) were taken in response to free-field tone bursts with varying frequencies and intensities. The electrode was advanced in incremental steps and data at each step was compared to that obtained at the round window. Following electrophysiological analysis, the cochlea was fixed and prepared for histological review.

Results from normal-hearing animals indicated that contact with intracochlear structures was associated with an increase in thresholds and loss of CAP and CM amplitude. Loss of activity first occurred in the CAP rather than the CM. Once a small but significant reduction in CAP suprathreshold amplitude was identified, withdrawal of the electrode was associated with full or partial restoration of response amplitude. Preliminary results from hearing impaired animals showed reductions in the CM and CAP at the round window consistent with high-frequency hearing loss.

These results indicate that a reduction of intracochlear potentials can be used as an early marker of imminent cochlear damage. Recordings from noise exposed animals suggest that CAP and CM are reliable markers in the clinically relevant condition of high-frequency hearing loss. Importantly, these measurements are potentially available with current cochlear implants and recording capabilities. Therefore, surgeons could use these markers to prevent loss of residual hearing, thus optimizing combined electric and acoustic stimulation of the auditory system.

907 Identification of Electrophysiological Markers of Damage in the Gerbil Cochlea During Electrode Implantation Using a Limited Stimulus Set and Direct Endoscopic Visualization

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In cochlear implant recipients with residual hearing, the ipsilateral combination of electric and acoustic hearing (electric acoustic stimulation or EAS) has been shown to provide improved speech discrimination scores, especially in background noise. However, retention of residual hearing is problematic, as it is often destroyed during implantation of the electrode. In previous animal experiments (see Suberman, Campbell et al., this volume), intracochlear potentials (CM and CAP) were recorded using a wide range of stimuli as an electrode was advanced through the scala tympani. Small and reversible reductions in the potentials were identified as the electrode impinged on cochlear structures. While these experiments were informative, time limitations during human cochlear implantation make use of a wide range of stimuli impractical. Consequently, we tested whether similar physiological effects could be obtained using a reduced stimulus set. To better understand the correlation between physiologic effects and electrode positioning, a micro-endoscope was utilized for direct visualization of the electrode as it contacted cochlear structures.

Experiments were conducted in urethane-anesthetized, normal-hearing gerbils. Through an intact round window, a 0.3 mm diameter endoscope was used to visualize the tungsten electrode as it was advanced towards the basilar membrane through a cochleostomy. At intervals during advancement, the CM and CAP were recorded at a single frequency and intensity. Once a decrease in response magnitude was observed, the electrode was withdrawn to determine if the effects were reversible. Cochlear damage, if present, was then identified histologically.

Electrophysiologic damage due to electrode impingement on intracochlear structures was readily detectable at a single frequency and intensity. In most cases, the damage was reversible when the electrode was withdrawn. The site of electrode contact visualized with the endoscope correlated well with histological evidence of intracochlear damage. These results indicate that a small stimulus set with potential intraoperative application can accurately identify electrophysiologic markers of damage. The endoscopic imaging reinforces the idea that these markers are highly sensitive to impending intracochlear damage.

908 Anatomical Considerations of Cochlear Morphology and Its Implications for Insertion Trauma in Cochlear Implant Surgery

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Insertion trauma in cochlear implant surgery is a feared surgical risk, potentially causing neural degeneration and altered performance of the implant. In literature insertion trauma is reported to occur at specific locations (at the base, at about 180º and in deep insertions over 400º). This has been ascribed to surgical technique and electrode design in relation to the size of the scala tympani. This study investigates whether there is an underlying anatomical substrate serving as a potential source for insertion trauma at these specific locations.

Eight human temporal bones have been harvested and preserved in formaline. A SkyScan-1076 micro-CT scanner (Aartselaar, Belgium) was used for data collection. The 3 dimensional path of the cochlear spiral was determined by segmentation, skeletonization, distance mapping and wave propagation technique applied on the micro- CT images. The most likely positions along this path at which a cochlear implant might induce pressure on cochlear structures, such as the basilar membrane and the wall of the scala tympani, due to deviation of the cochlear duct

from a smooth trajectory were estimated with linear regression.
The cochlear lumen shows an non-continuous spiraling path. Certain similarities in the shape and pattern of the central luminal paths in 8 different bones, strongly suggest that these potential pressure points occur more frequently at certain specific points along the cochlear lumen. It turned out that potential pressure points during cochlear implantation exist at the basilar membrane in the region of 180-225° (12-14mm) and 725° (22-26mm) and at the floor of the scala tympani around 0-90°, 225-270° and 405-450° ($p<0.00001$).
Our data favour the idea that the intrinsic 3-dimensional cochlear morphology contributes to the risk for insertion trauma during cochlear implantation at specific locations.

909 Factors Associated with Incomplete Insertion of Electrodes in Cochlear Implant Surgery: A Histopathologic Study

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Atraumatic and complete insertion of the electrode array is a stated objective of cochlear implant surgery. Forty temporal bones from patients who in life had undergone cochlear implantation were evaluated. Specimens were serially sectioned and reconstructed by two-dimensional methods. Two electrode metrics were determined for each bone: the inserted length (IL) and the active electrode length (AEL). The ratio of these two metrics (IL/AEL) was used to split the temporal bones into two groups: those with incomplete insertion (n=27, IL/AEL<1.0) and those with complete insertion (n=13, IL/AEL≥1.0). Seven possible histopathologic indicators of resistance to insertion of the electrode were evaluated. Obvious obstruction by abnormal intracochlear bone or soft tissue accounted for only six (22%) of the 27 partial insertions. Of the remaining 21 bones with incomplete insertions and 13 bones with complete insertions, dissection of the spiral ligament to the lateral cochlear wall was the only histopathologic indicator of insertion resistance identified with significantly higher frequency in the partial-insertion bones than in the complete-insertion bones ($p=0.003$). An observed trend for the percentage of complete insertions to decrease with the number of times the electrode penetrated the basilar membrane did not reach significance. In the bones without an obvious obstruction, the most frequently observed indicator of insertion resistance was dissection of the spiral ligament (with no contact of the lateral cochlear wall) identified in 67% (14/21) of partial-insertion bones and in 92% (12/13) of complete-insertion bones. These results are consistent with the view that (1) the electrode contact with cochlear structures resulting in observable trauma to the basilar membrane, osseous spiral lamina and/or spiral ligament does not necessarily impact the likelihood of complete insertion of the electrode array and (2) once contact trauma to the spiral ligament reaches the point of dissection to the cochlear wall, the likelihood of incomplete insertion increases dramatically.

910 Physiological and Anatomical Changes in the Auditory Nerve Fibers of Chronically Deaf Cats

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We have described the probabilistic and temporal response properties of feline auditory nerve fibers (ANFs) stimulated by electric pulse trains (Zhang et al., JARO 8: 256-72, 2007; Miller et al. JARO 9: 122-37, 2008). The data were obtained from acutely deafened cats and are considered normative. We are amidst a study of the responses of ANFs from chronically deaf cats and preparing each cochlea for histological analysis of spiral-ganglion survival and axon morphometry. ANF anatomical measures will be assessed at the level of the whole-nerve trunk and osseus spiral lamina (OSL) for evaluation of central and peripheral axons to determine whether or not a peripheral-to-central gradient of deafness-related change is evident. This presentation will provide an early look at differences between acute-deaf and chronic-deaf data sets. Data from the chronically deaf cats were obtained after a post-deafening survival period of 12-15 weeks after deafening with systemic kanamycin and ethacrynic acid.
The most notable change in the temporal responses of the "chronic" ANFs was significantly greater spike-rate adaptation in response to pulse trains. That is, a greater proportion of ANFs (in one animal, nearly all fibers tested) had PST histograms characteristic of the "strong adapters" of normal ears described in Zhang et al. (see above). At this time, the most evident changes in the anatomy of ANFs are the packing density (due to neuronal death) and reduction in axon cross-sectional areas. While the cross-sectional area of myelin also decreased, the decreases in axon cross-sectional area were greater. Also, signs of axonal shrinkage and cell death were evident both at the peripheral (OSL) level and the nerve trunk. One issue that we hope to address is whether or not the smaller-diameter fibers from the "chronic" cats are non-functional or necrotic or "down-regulated" viable fibers with small diameters.
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911 Polarity-Dependent Sensitivity of the Electrically Stimulated Human Auditory System in Users of Three Makes of Cochlear Implant and at Different Cochlear Sites

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Cochlear implants typically use symmetric biphasic pulses in order to maintain charge balance and hence avoid cochlear damage. This makes it hard to assess which phase is the more effective. However, experiments using pseudomonophasic pulses, in which a "short high" pulse of one polarity is followed by a "long low" pulse of the

opposite polarity and equal charge, have shown that less current is needed to achieve the same loudness when the short-high pulse is anodic than when it is cathodic (Macherey et al, 2006, JARO). Combined with other findings, this suggests that, contrary to the results of most animal experiments, the anodic polarity is more effective, at least at moderately loud levels. Here we extend those findings, obtained with the Advanced Bionics (AB) device, by using asymmetric pulse shapes that can be implemented in other devices. In all cases we used 400-ms 99-pps pulse trains in monopolar mode, with a phase duration of 32 μs/phase. For the AB and MedEl devices we used triphasic pulses in which the central phase had twice the amplitude of the 1st and 3rd phases. For the Nucleus device, we used pairs of biphasic pulses with opposite leading polarity, each with an inter-phase gap of 58 μs, and separated by 8us; this resulted in two adjacent phases of the same polarity in the centre of the waveform. All cases showed that, at comfortable level, the current level needed was about 1-2 dB lower when the central portion was anodic than when it was cathodic. This occurred for electrodes in several different cochlear positions, including electrode 1 of the MedEl device, which is inserted deep into the cochlear apex - suggesting that the polarity sensitivity happens at a wide range of sites. Previous results have also shown that, by using asymmetric pulses in bipolar mode, polarity differences can affect the site of excitation, and we will report applications of this finding to different devices and cochlear sites.

912 Extending the Range of Pitch Perception by Cochlear Implant Listeners

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Pitch can be conveyed to cochlear implant (CI) listeners along two dimensions corresponding to the locus of excitation ("place pitch") and to the repetition rate of the waveform ("temporal pitch"). Both cues suffer from limitations. First, the range of place pitches is limited by the fact that electrodes are usually not inserted fully into the apex of the cochlea. Second, most studies of temporal pitch reveal an "upper limit" of about 300 pps, beyond which changes in repetition rate do not produce an increase in pitch. Here, we show that both of these limitations can be partially overcome by modifying the electrical pulse shape.

Seven Advanced Bionics CI users took part. Experiment 1 compared the place pitches evoked by several narrow bipolar "BP+1" stimuli. The stimuli were symmetric biphasic pulses in which both phases had the same duration and amplitude, or were "pseudomonophasic" pulses (second phase longer and lower in amplitude than the first). The lowest pitch was obtained for pseudomonophasic pulses in which the first phase was anodic on the most apical electrode ("PS-A" pulses). Experiment 2 measured temporal pitch discrimination in a two-interval forced-choice task using the optimally efficient mid-point comparison procedure (Long et al., Ear & Hearing, 2005). Subjects ranked single-channel pulse trains with rates ranging from 105 to 1156 pps. This task was repeated at several intra-cochlear stimulation sites and using both symmetric and pseudomonophasic pulses. The upper limit of temporal pitch for PS-A stimuli presented to apical electrodes was significantly higher than all other conditions, averaging approximately 700 pps. Measures of discriminability obtained using the method of constant stimuli indicated that this pitch percept was probably weak. However, a multidimensional scaling study showed that the percept associated with a rate change, even at high rates, was orthogonal to that of a place change.

913 Quantifying Perceptual Effects of Different Levels of Current Focusing

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Large current spreads yielding channel interactions potentially limit cochlear implant (CI) performance. Tripolar stimulation (current focusing) has been used as a tool to reduce current spread and channel interactions. Anecdotal reports suggest that tripolar stimulation provides a cleaner sound. In the following study, we quantify what (if any) perceptual differences can be attributed to a change in focusing. Patients with Advanced Bionics CII or HiRes 90k CIs were asked to either rate or discriminate different levels of current focusing with partially tripolar (pTP) stimuli. Stimuli consisted of 300 ms pulse trains with 226 μsec phase durations at 1000 pps. The pTP focusing coefficient (σ) ranged from 0.0 to 0.75 or 0.875 in 0.125 steps. All stimuli were loudness balanced to a comfortable level.

In the discrimination task, all pairs of σ values were compared 30 times using a 3IFC task. Two intervals contained one level of σ while the remaining interval contained a different level of σ. Stimuli were jittered ±0.5 dB to mask loudness cues. In the rating task, each stimulus used in the discrimination task was presented one at a time. Subjects were asked to rate the stimulus by clicking on a bar how well the stimulus was described by 1 of 10 adjectives ("clean", "pure", "high", "full", "flute-like", "dirty", "noisy", "low", "thin", and "kazoo-like".) The procedure was repeated 15 times for each pTP stimulus and all ten adjectives.

Preliminary data suggests that patients are often able discriminate values of $\sigma > 0.5$ from each other while it tends to be difficult to discriminate smaller values of σ. Patients with good discrimination between levels of focusing report that high values of σ are described as "clean", "pure", "high", and "flute-like" while lower values of σ are described as "dirty", "noisy", "low", "kazoo-like" as well as simultaneously "thick" and "thin." Complete data will be presented.

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914 Discrimination Between Simultaneous and Sequential Virtual Channels

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In cochlear implants (CIs), virtual channels (VCs) can be created by simultaneous or rapid sequential stimulation of adjacent electrodes. VCs typically produce a pitch intermediate to the component physical electrodes. While both simultaneous and sequential VCs may produce intermediate pitch percepts, it is unclear whether simultaneous and sequential VCs are perceptually equivalent. In this study, discrimination between simultaneous and sequential VCs was measured in 7 users of Advanced Bionics CII or HiRes 90K CI devices. To see the effect of neural overlap, VC discrimination was measured for both monopolar (MP) and bipolar+1 (BP+1) stimulation modes. All VCs were 300 ms bi-phasic pulse trains, with 100 μs phase duration and 250 pulses per second per electrode stimulation rate; For MP VCs, equal current was delivered to each component electrode (i.e., α=0.5). For BP+1 VCs, equally loud current was delivered to each component electrode. For sequential VCs, the inter-pulse interval (IPI) between the offset of the first pulse and onset of the second pulse varied between 0 and 1.8 ms. All stimuli were loudness balanced at a comfortably loud listening level. VC discrimination was measured using a 3-interval forced-choice procedure; ± 0.5 dB jitter was applied to stimuli to reduce loudness cues.

For MP stimulation, there was a small but significant perceptual difference between simultaneous and sequential VCs. For BP+1 stimulation, perceptual differences between sequential and simultaneous VCs were stronger and seemed to increase with IPI. This suggests that the amount of neural overlap and refractory effects influenced the perceptual differences between sequential and simultaneous VCs. The results suggest that simultaneous and sequential VCs may produce similar percepts with broad stimulation modes, and that narrow stimulation modes may not provide the neural overlap needed to support sequential VCs.

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915 Improving Virtual Channel Discrimination with Current Focusing in a Multi-Channel Context

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Simultaneous in-phase stimulation on adjacent electrodes creates current peaks between the two electrodes. The intermediate current peaks provide pitch sensations between the two electrodes and are commonly called virtual channels (VCs). To distinguish these VCs from other types of VCs, they will be called Monopolar Virtual Channels (MPVCs). QPVCs are current-focused VCs using 4 simultaneously stimulated electrodes: the two center electrodes are used to steer current while the outer two electrodes are used to focus current. We recently showed that Quadrupolar Virtual Channels (QPVCs) provide more intermediate pitch percepts between two electrodes than MPVCs when presented in isolation, possibly because QPVCs have sharper peaks and reduced current spread. Although QPVCs provide increased resolution in isolation compared to MPVCs, it is unknown whether this benefit will carry over to multi-channel stimulation. We hypothesized that QPVCs provide improved VC discrimination compared to MPVCs when VCs are presented with distracters on nearby electrodes.

Stimuli consisted of sequentially interleaved pulse trains comprised of 3 channels. One channel (the signal) is either a MPVC or a QPVC, steered to 3 VC locations within 1 mm. Discrimination between the 3 loudness-balanced VC signals was measured using a 3IFC task. The other two channels (distracters) are either MPVCs or QPVCs symmetrically positioned on either side of the signal and were presented at varying distances (in mm) from the signal. The task was repeated with distracters at varying levels, from zero to maximal comfort. Distracters were loudness balanced in each stimulation condition.

Preliminary evidence suggests that QPVC signals are beneficial to VC discrimination compared to MPVC signals in the presence of nearby distracters. The improvement of VC discrimination in a multi-channel context could indicate benefits of QPVC stimulation in a speech processing strategy.

916 Relation Between the Shape of the Tripolar Intracochlear Electrical Field and Psychophysical and Physiological Spread of Excitation

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To reduce channel interactions from monopolar stimulation, more focused electrode configurations, such as tripoles, are considered. Tripoles require higher current levels to achieve the same loudness as monopoles, but how much extra current is needed differs between subjects. In this study we will relate the distribution of the intracochlear Electrical Field Image (EFI) to a physiological and a psychophysical measure of the Spread of Excitation: eCAP-SOE and Psychophysical Forward Masking (PFM-SOE).

In an earlier study (Berenstein et. al., ARO 2008), the loudness of the tripolar stimulus could be explained in 6 out of 10 subjects by the individually estimated peak magnitude of the EFI (assumed to occur at the location of the central active electrode). This “Equal Peak” hypothesis is most easily understood as explaining when a stimulus reaches detection threshold.

In a subsequent study (Berenstein et. al., CIAP 2009), the EFI peak predicted PFM thresholds in a majority of subjects, but less so the eCAP threshold, which suggests that the EFI peak indeed can predict the increase in stimulus amplitude needed to achieve equal loudness compared to a monopolar stimulus. However, loudness above threshold is often assumed to be determined by the width of the excitation along the basilar membrane. The second study also showed that focusing tended to sharpen

the field as well decreased PFM-SOE, but less so eCAP SOE.
Obviously, both the peak and width of the field will increase with increasing current level. The goal of this study is to test to what extent the peak and width are independent predictors of supra-threshold loudness growth. If independent it will be investigated if the combined contributions from peak and width on loudness can be explained as the effect of one quantity, namely surface of the stimulus.
We will discuss the relation between the three geometric quantities peak, width and surface, calculated from EFI (intracochlear), eCAP-SOE (peripheral), and PFM-SOE (central) as a function of focusing across the electrical dynamic range.

917 Poor Electrode-Neuron Interface Demonstrated by Steep Growth of Loudness with the Partial Tripolar Configuration

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The partial tripolar (pTP) configuration allows for systematic variation of electrical field size for cochlear implant stimulation while maintaining a constant locus of activation centered at the active electrode. By varying the fraction of return current delivered to the flanking electrodes, the configuration can be systematically changed between 0 (MP) and 1 (TP). Previous studies have demonstrated that as the electrical field becomes more focused, channel-to-channel variability in threshold increases. High TP thresholds are indicative of a poor electrode-neuron interface, and we have shown previously that such channels have broad tuning. In the present study we test the hypothesis that channels suspected to have a poor interface to nearby neurons will have abnormal growth of loudness with large pTP fractions.
In adult listeners with Advanced Bionics implants, thresholds were measured with TP to identify the low-, median-, and high-threshold channels. Growth of loudness functions were then measured for various pTP fractions in each of these channels. Subjects rated the loudness of each stimulus on a scale from 0 ("can't hear it") to 100 ("too loud"). Also for each of these channels, equal loudness contours for 50% of the MP dynamic range were measured with various pTP fractions.
Results indicate that as the pTP fraction is increased, the growth of loudness functions become shallower. These functions are steepest for high-threshold channels when a focused stimulus is used. Also for focused stimuli, higher absolute thresholds appear to be related to steeper loudness growth functions. Equal loudness contours across electrode configurations are also steepest for high-threshold channels. Together these findings support the hypothesis that channels with high TP thresholds have a poor electrode-neuron interface.
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918 Directed Spiral Ganglion Axon Growth on Photopolymerized Micropatterns

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Hair cell damage results in degeneration of spiral ganglion neurons (SGNs) and their peripheral axons. Directed regrowth of SGN peripheral axons to approximate or contact cochlear implant stimulating electrodes would allow for improved spatial and temporal resolution and increased performance. Here we examined the ability of micropatterned channels (50-200 micron ridge periodicity) induced by photopolymerization to direct SGN neurite growth. Biocompatibility of methacrylate polymers with different cross-link density was evaluated using dissociated SG cultures from P5 rat pups by measuring cell density, neuron survival, and neurite length. Photopolymerization was used to create micropatterned channels from methacrylate monomers with varying polarity, mechanical properties, and cross-link density. Micropattern topology depended strongly on polymer processing conditions and composition. Explants and dissociated SG cultures were grown on micropatterned polymers, unpatterned polymers, glass, and plastic and immunostained with neuron and Schwann cell (SC) specific antibodies. Digital images were analyzed for neurite growth directed along polymer micropatterened channels versus growth patterns seen in controls. Neurite growth was radial on unpatterned surfaces, whereas micropatterned channels directed SGN neurite growth parallel to the pattern. The angle of the initial versus terminal segment of neurite relative to the pattern is significantly different between patterned and unpatterned conditions, demonstrating turning of the neurites to parallel the pattern. SCs also aligned with the pattern in the presence and absence of neurites. By contrast, fibroblast outgrowth was restricted and did not align to the pattern. These results demonstrate the ability of photopolymerized micropatterns in biocompatible materials to direct SGN neurite growth in vitro.

919 In Vivo Effects of Surface Patterned Cochlear Implant Electrode Arrays

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To reduce fibrous tissue growth around the cochlear implant (CI) electrode carrier after cochlear implantation surface patterning could be one promising approach. Linear structures of a width of 5 µm that were proven to reduce fibroblast growth in vitro were transferred to platinum electrode contacts and the moulding dies of CI electrodes by means of femtosecond laser. Currently we are investigating these surface patterned electrodes in vivo.

Hearing guinea pigs were implanted with an 8-channel CI electrode for 4 weeks. Hearing thresholds were verified before implantation and before sacrifice acoustically and after implantation and once per week electrically. Impedances were monitored every day during the first two weeks after implantation and later on a weekly basis with Custom Sound EP software. Additionally, frequency specific impedance measurements were carried out always after determination of the electrical stimulation threshold. After sacrifice, cochleae were harvested, fixed and embedded in resin with the electrode in situ. The cochleae were grinded and documented to evaluate the tissue growth around the electrode array.
Typically, four contacts of the array could be inserted into the cochlea. Impedances at these contacts increased from about 2 kOhms after implantation to 8-10 kOhms after 4 weeks with no influence of the silicone structure. Acoustic hearing thresholds increased after implantation indicating loss of hearing in most animals. EABR thresholds increased during the first week after implantation and came then back to the initial values. Electrodes with surface patterned silicone surfaces showed higher stimulation thresholds than control electrodes.
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920 Adhesion Force Measurements of Fibroblasts on Micro Structured Surfaces by AFM

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The adhesion of cells on the surface is a crucial prerequisite for the interaction of the surrounding tissue with implants. A post operative growth of connective tissue leads in cochlear implants to an increase of impedance. By modifying the surface topography in the micrometer range, the interaction between the implant and surrounding tissue is selectively controlled and adhered cells are influenced [1,2]. The aim of this study was to compare measured adhesion forces on diverse silicone and silicon micro structures.
By means of fs-laser and nanosecond (ns) lasers microstructures in silicon (Si) (spike structure) and silicone (molded of spike structure, disordered microstructure) have been generated. For all structures, the surface topographies were mapped by SEM and AFM and the hydrophobicity was determined by measuring contact angle. The measurement of the adhesion of fibroblasts (NIH 3T3) in the first tow minutes was performed by AFM (JPK NanoWizard II) using tipless cantilevers (Nanoworld). The measured maximum force of adhesion ($F_{.max}$) and the number of the membrane tethers were correlated with the surface structure.
We demonstrated that the adhesion of cells to the surface topographies in the range of micrometer depends strongly on the geometry of the interaction area between the cells and the topography. There was also a big difference between adhesion forces of different cells of the same passage and with the same dimension. The measured maximum force of adhesion ($F_{.max}$) and the count of the observed membrane tethers showed similar values for all silicone substrates. The non structured silicon (Si) showed in comparison to all other surfaces sig. the largest number of membrane tethers.

921 Dual Channel Optical Stimulation of the Guinea Pig Cochlea

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Optical stimulation has been developed over the last several years as a means of increasing the spatial selectivity of artificial neural stimulation. Our group is investigating optical stimulation in the cochlea as a means to increase selectivity of stimulation in cochlear implants. Some of the limitations of modern cochlear implant performance, including sequential rather than simultaneous stimulation, are attributed to the spread of electric current through the cochlea. In contrast, optical radiation does not spread significantly. This can allow for stimulation of more discrete neural populations and adjacent neural populations simultaneously. Here, we examine two-channel, simultaneous optical stimulation of the cochlea, via multichannel recordings from the inferior colliculus.
Normal adult, pigmented guinea pigs were used for the experiments. The left cochlea was accessed via the bulla for CAP threshold recordings. A 16-channel, penetrating electrode or a single tungsten wire electrode was inserted stereotactically into the contralateral inferior colliculus (IC), perpendicular to the frequency planes. Two cochleostomies were made in the basal turn of the cochlea, with separations varying between 0.5 – 3 mm. One optical fiber (200µm diameter) was inserted into each cochleostomy. Each fiber was connected to an independent optical source, operating at 1860nm, 100µs pulse duration, and 10Hz repetition rate. The animal was deafened, and the IC responses to each optical source - individually and the two optical sources combined - were recorded via Plexon software. Spatial maps and temporal patterns of the IC responses were analyzed.
Pilot data indicate that optical fibers need to be separated by a minimum of 1/4 octave, or 0.5 mm, in the guinea pig cochlea for the IC responses to be measured by two adjacent electrodes. Using optical stimulation, it is possible to simultaneously stimulate adjacent populations of neurons in the cochlea.
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922 Temporal Properties of Inferior Colliculus Recordings During Stimulation with an Infrared Laser

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The motivation for using infrared lasers to stimulate auditory neurons relates to the spatial selectivity of neural stimulation. To develop intelligent coding strategies, it is important to characterize patterns of neuronal activity to stimulation. With the present study we define a parameter space for the laser stimuli that results in a similar pattern of activation in the central nucleus of the inferior colliculus (ICC) of acutely deafened guinea pigs, to that observed with acoustical stimulation in normal hearing animals.

Healthy guinea pigs were used. A cochleostomy was drilled into the basal cochlear turn. The animals were acutely deafened. An optical fiber was placed inside the cochleostomy to stimulate spiral ganglion cells. A multichannel recording electrode was placed in the ICC to record neural responses. Peri-stimulus histograms (PSTHs) and inter spike interval histograms (INTHs) were constructed to determine temporal response properties.

PSTHs of IC recordings show several maxima of neural activity following optical pulses. Characteristic is a single maximum at 5.55±0.41 ms (N=18) after the stimulus at low levels just above threshold. With increasing stimulus levels, multiple maxima appear and the delay time for the first maximum decreases. For 10 of the 18 neurons, a second maximum could be observed at 7.22±0.45 ms. A third maximum appeared in about half of the neuron clusters (N=10/18). The delay time was 9.24±0.18 ms. Changing the pulse duration did not change the response pattern at the inferior colliculus electrode. The pulse repetition rate has been changed from 10 to 300 Hz. The delay time for the first maximum at about 5.6 ms changes little with increasing repetition rate. With increasing stimulus rates the second maximum disappeared and the delay time for the third maximum increased by about 0.5 ms.

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923 The Spread of Excitation in Inferior Colliculus for Optical Cochlear Stimulation

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We have investigated the spread of excitation in the central nucleus of inferior colliculus (ICC) in response to optical stimulation of the cochlea. Recent work has shown that optical stimulation of neural tissue is spatially selective and may allow for an increased information transfer across the neural interface. A practical cochlear implant based on optical stimulation should transfer a maximum amount of information using a minimum amount of energy. The amount of energy delivered depends upon the size of optical fiber that couples to the laser. It is critical that individual fibers are small enough so that an optical implant made of multiple fibers can be safely inserted into the scala tympani. To determine the optimal size of optical fiber and the spread of excitation in ICC, we compared the energy required to evoke compound action potentials in the cochlea and compared responses in the central nucleus of inferior colliculus (ICC) using different sized optical fibers. A multi-channel recording electrode was placed in the ICC to record neural responses. Acoustic stimulation confirmed the tonotopic frequency mapping across the multiple electrode contacts. We investigated the spread of excitation in the ICC using fibers of diameter 50, 100, 200 and 400 µm. Input-output curves of responses of ICC neurons were measured by varying optical radiation energy. Spatial tuning curves measured in ICC indicate that optical stimulation with 200 µm fiber provides tonotopically-restricted stimulation of cochlear neurons and sufficient dynamic range.

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924 Cochlear Stimulation by Pulsed Infrared LASER-Light: Direct and Indirect Stimulation

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LASER stimulation of neural tissues has been reported by several groups within the last years. They stimulated the sciatic, facial and vestibulocochlear nerve of rodents. The studies reported neural action potentials (APs) triggered by pulsed low-infrared LASER-light. Until today the underlying mechanism of LASER generated APs remains unknown. Thermal and mechanical effects have been discussed. Previous reports demonstrated opto-acoustic phenomena during LASER stimulation at very high LASER energies. In the present study we investigated whether opto-acoustic phenomena can be measured at energies that are used for neural stimulation, including stimulation of the cochlea. Using radiant energies of 1.4-648 µJ at 30-1000 µs pulse duration (fiber diameters 200, 400 and 600 µm), focused acoustic waves were detected in front of the glass fiber. The sound pressure level was up to 63 dB SPL. When the optical fiber was placed near an undeafened rat cochlea, compound action potentials could be recorded at the round window niche even if the LASER was not oriented towards the cochlea. LASER induced soundwaves and CAPs were analyzed for different laser settings using microphone recordings in water and air, in humid and in dry conditions. The acoustic phenomena were not detectable in the environment of 100% N2O, indicating that absorption of energy by water vapors could be responsible for these acoustic phenomena.

When analyzing CAP responses, however, two different LASER-evoked CAP components were observed: one easily masked by noise, the other not easily masked. The latter component was not observed if the tip of the fiber did not aim at the cochlea. This indicates that in natural conditions, LASER stimulation can evoke two different phenomena: an indirect stimulation via acoustic phenomena ("optophony"), and a direct stimulation, possibly mediated via direct excitation of cells within the cochlea or auditory nerve.

925 Effects of Green Light Application at Ear Drum and Middle Ear Level

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Green laser light is a source of energy that can effectively activate the cochlea, as we have presented in our previous reports (Wenzel et al 2008, 2009). The goal of this follow-up study was to assess the potential of green light stimulation for an improved hearing aid.

Auditory brainstem responses (ABRs) were recorded preoperatively in anesthetized guinea pigs to confirm normal hearing. We applied green laser pulses using a 50-µm core-diameter optical fiber, to the tympanic membrane and into the opened middle ear on various bony structures, as well as towards the round window membrane. The laser stimulation consisted of 532 nm, 10 ns laser pulses with a level range of 0.6-23 µJ/pulse (frequency-doubled Nd:YAG-laser, Quantel Brilliant B, France). We recorded both optically-induced ABRs (OABRs) and neural spike activity within the inferior colliculus central nucleus (ICC) to laser pulse stimulation.

OABRs were elicited with single pulse stimulation within the outer as well as the middle ear. The OABR peaks increased with energy level and varied in magnitude depending on the location of stimulation. At low energy levels, we also elicited spike activity localized to specific best frequency regions depending on the location of stimulation on the ear drum and within the middle ear. As the energy level was increased, greater spike activity appeared across the tonotopic gradient of the ICC.

Our findings demonstrate that visible light can be used to activate the peripheral hearing organ when applied to the ear drum or on bony structures within the middle ear that can transmit vibrations to the cochlea. It is also possible to elicit localized neural activation within the ICC that appears to depend on stimulation location. We propose that this novel, non-contact laser stimulation method could be used to improve implantable and non-implantable hearing aids as well as for research purposes.

926 Techniques to Improve the Efficiency of Active Middle Ear Implants: Effect of Different Coupling Methods to the Ossicular Chain

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Active middle ear implants (AMEIs) provide advantages over hearing aids in the rehabilitation of sensorineural hearing loss. Efficient transfer of energy from the AMEI to ossicular chain (OC) is desired to maximize output and optimize gain. The aim of this study was to objectively assess the performance of an AMEI using five methods of coupling to the OC. Six temporal bones were prepared to expose the OC. The AMEI (Otologics Gen II MET-V™) was coupled to the OC via: 1) direct contact on the body of the incus (baseline condition); 2) insertion of transducer tip into a laser-generated hole in the body of the incus; 3) an àWengen clip to the long process of the incus; 4) a 0.5 mm diameter cylinder to the long process of the incus; and 5) a bell-shaped prosthesis in contact with the stapes. AMEI performance was assessed by stapes velocities (H_{EV}) and the maximum equivalent ear canal sound pressure levels (L_{Emax}) were computed. The acoustic stapes velocity transfer function (H_{TV}) before and after loading the transducer was measured to determine loading effect. Data were analyzed in three frequency ranges: low (0.25-1 kHz) medium (1-3 kHz) and high (3-8 kHz). The AMEI in contact with the incus without a laser hole produced L_{Emax} of 112, 126 and 122 dB in low, medium and high frequency ranges, respectively, while the change in H_{TV} was -4, -2 and 2 dB. Seating the tip in a laser hole significantly improved L_{Emax} by 6 dB at high frequencies. The àWengen clip significantly improved L_{Emax} by 14, 11 and 19 dB by frequency range compared to baseline without negative loading effects. Cylinder tip coupling improved L_{Emax} by 9, 10 and 11 dB in the respective frequency ranges, without negative loading effects. Coupling via the bell-shaped tip improved L_{Emax} by 22, 17 and 16 dB in the respective frequency ranges. Stapes velocity generated by the AMEI transducer is influenced substantially by the coupling technique between transducer tip and the OC. Support: Otologics Education Grant

927 Can Stapes Velocity Be Used to Estimate the Efficacy of Mechanical Stimulation of the Round Window of the Cochlea with an Active Middle Ear Prostheses?

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Mechanical stimulation of the round window membrane (RW) with active middle ear prostheses (AMEP) has shown some functional benefit in clinical reports in patients

with mixed hearing loss. There are published standards for objective measurement of AMEP performance based on stapes velocity in human cadaver temporal bones for AMEPs when coupled to the ossicular chain. Here, stapes velocity is a reasonable measure of the input to the cochlea via the AMEP because the system is driven in the forward direction. However, these standards may not be applicable when estimating the efficiency of the RW drive with an AMEP because the stapes velocities in this case are expected to be more complex due to driving the cochlea in the reverse direction and the potential effects of "third windows". Here we test this hypothesis by measuring the cochlear microphonics (CM) and the stapes velocities in response to both acoustic stimulation (forward direction) and RW stimulation with an AMEP (reverse direction). Seven ears in five chinchillas were studied. For each stimulus frequency, the amplitude of the CM was measured separately as a function of intensity (dB SPL or dB mV). Equivalent vibrational input to the cochlea was estimated by equating the acoustic and AMEP CM amplitudes at a constant value. For the same CM amplitude output we assume that the same vibrational input to the cochlea was present regardless of the route of stimulation. The stapes velocities for these equivalent outputs were not significantly different for low and medium frequencies (0.25-4 kHz) but the velocities for AMEP-RW drive were significantly lower for higher frequencies (4-14 kHz). The results suggest that the measured stapes velocities when driving the RW underestimate the actual mechanical input to the cochlea. At frequencies above 4 kHz, stapes velocity underestimated the RW input by ~20 dB. The possible influences of cochlear third windows are discussed as well as alternative methods to assess AMEP performance when driving the RW. Support: Otologics Education Grant

928 Direct Mechanical Stimulation of the Chinchilla Malleus with the MET Middle Ear Implant

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Understanding resultant ossicular velocities from both acoustic and artificial stimulation, is important in the design and application of middle ear implants. Accurate measurements of lever ratios have been difficult because of anatomical limitations to the approach angle for laser Doppler vibrometry. We improved the accuracy of these measurements in a chinchilla animal model by driving the malleus directly with the MET (Otologics LLC, Boulder, Colorado, USA).

Nine chinchillas were anaesthetized following the UCHSC animal welfare guidelines. KTP laser was used for ossicular chain exposure. After obtaining 'closed-field' baseline acoustic transfer function data, the tympanic membrane was excised. The MET with 0.5mm ball-tip was attached to the malleus umbo with cement. Calibrated sinusoidal stimuli (0.25-14kHz) were delivered to the MET in intervals up to ~900mVRMS. Ball, malleus, incus and stapes velocities were recorded by LDV via the external auditor canal i.e. in the pistonic plane.

The stapes velocities for MET malleus drive demonstrated maxima at 2-3 kHz - consistent with MET resonance. This was quite unlike the normal acoustic TF for the chinchilla which has a typical minima, or notch, around 2.6 kHz. The electromechanical transfer function resembled more the typical human transfer function. The stapes velocity was proportional to measured MET ball velocity at all frequencies and stimulus voltages, indicating that the chinchilla ossicular chain behaves linearly in this test range. The calculated peak equivalent sound pressure (LEmax) produced by malleus stimulation at the peak rated driving voltage for the MET (1VRMS) was 90-140 dB.

This reference data has been useful to compare efficacy of other points of ossicular stimulation including the round window. Our study supports the validity of clinical direct malleus stimulation tests (per canal) to assess patient suitability for middle ear implantation.

929 Electrocochleographic Measurements in Chinchilla with Unilateral Deafness and Fitted with a Bone Anchored Hearing Aid

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Patients suffering from unilateral deafness can be fit with a Bone Anchored Hearing Aid (BAHA) which is positioned at the impaired side. The vibrations from the BAHA output are transmitted transcranially to the contralateral healthy cochlea. The rationale is to reduce the head-shadow effect for sound sources on the impaired side. Despite successful outcomes in humans, little detailed objective data is available in terms of the physiologic performance. This study explored the physiologic responses induced by a BAHA (Divino, Cochlear Bone Anchored Solutions, Molnlycke, Sweden) fitted on the side of the unilateral deaf ear by destruction of cochlea in five chinchilla lanigeras. Electrocochleographic signals (cochlear microphonic, CM) were recorded. Measurements were done with and without the BAHA with free–field stimulation in three positions: loudspeaker ipsi to the deaf ear, on the side of the normal ear, and along the midline. The stimuli were tones ranging in frequencies from 0.25 to 4 kHz. With the device turned ON, CM thresholds were decreased. The mean decreases in CM thresholds were 10 dB for sources ipsi to the deaf ear (ipsi to BAHA), 14 dB at the midline, and 5 dB for sources contra to the deaf ear. The CM amplitudes at any particular sound level were increased for ipsilateral sources side by turning the BAHA ON. There was no significant change observed in sensitivity of CM amplitude measured by slopes (µV/dB SPL) with the hearing aid ON or OFF. The observed changes in CM amplitude and threshold were sound location dependent. These results were confirmed by presenting stimuli with known interaural time difference (ITD) or interaural level

differences (ILD). The CM amplitudes were modulated with ITDs or ILDs when BAHA input was leading or with higher ILD. This study suggests that BAHA fitting provides mechanical inputs to the contralateral cochlea that could potentially explain a “pseudo stereophonie” in patient with only one functional cochlea.

930 Round Window Position of the Vibrant Soundbridge Middle Ear Implant: How Coupling Is Influenced by Placement and Orientation of the FMT Demonstrated in an Animal Study

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The Vibrant Soundbridge (VSB) a semi-implantable middle ear device transmits electro-mechanically induced vibrations generated by a floating mass transducer (FMT) to the inner ear. To alleviate conductive as well as mixed hearing loss the FMT is attached to the ossicular chain or directly to the oval or round window (RW) or even to an artificially drilled window. The main challenge of transducer positioning is to maximise the transfer of vibration energy to the cochlea to achieve an optimal outcome. So far attempts to accomplish this were restricted to intra-operative trials including variations of transducer positions and the use of fascia. In the present study these parameters were investigated for the RW position in a more systematic manner in acute cat experiments.

Normal hearing cats were stimulated acoustically or mechanically via the FMT with Gaussian shaped tone pips or clicks. Auditory brainstem responses (ABRs) and compound action potentials (CAPs) were recorded at different stimulus intensities. N1P1 amplitudes were analysed and I/O functions were plotted. Pictures of all FMT placements were taken for later reconstruction of positions and orientations of the FMT in order to correlate them with hearing outcome.

CAPs could be elicited by placement of the FMT at the RW, at the bony edge of the RW or even at a drilled hole in the bulla far distant from the RW but with clear variations of amplitude and thresholds dependent on the placement of the FMT. Contact with the RWM directly or via a fascia mostly evoked high amplitudes and low thresholds. Although orientation of the device is not critical as long as it stays in contact to the RWM an advantage for a perpendicular orientation was observed. Fascia can enhance coupling but also decrease it if it is too thick. However, fascia is beneficial in keeping the FMT in place.

In conclusion, a direct placement at RWM oriented perpendicular to the RWM with or without fascia mostly leads to optimal outcomes.

931 Experimental Output Determination in Direct Cochlea Stimulation with the DACS PI

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Introduction The Direct Acoustic Cochlea Stimulator Partial Implant (DACS PI, Phonak Acoustic Implants) is intended to provide direct stimulation to the cochlea by circumventing the middle ear. The approach of direct cochlea stimulation with an actuator may substitute middle ear transmission, but the determination of the actual actuator output remains challenging. Here the actuator output to an artificial 3rd window is compared experimentally to the natural sound input to the middle/inner ear.

Methods To determine the stimulation efficiency of the actuator the ASTM standard (F2504.24930-1) method for the output determination of implantable middle ear hearing devices (IMEHDs) in human cadaver temporal bones (TBs) was adapted. First the stapes footplate displacement in response to sound applied to the external ear canal was determined. TBs fulfilling the ASTM criteria were implanted with actuator driven pistons next to the footplate, leaving the oval window unchanged. Then stapes footplate vibration in response to actuator stimulation was measured. From these two measurements the achieved output of the transducer at 1Vrms input voltage was calculated in terms of equivalent sound pressure level at the tympanic membrane.

Results In 29 fresh temporal bones the stapes footplate response to sound was measured. Only data from 11 TBs were found within the acceptance range of the ASTM standard and contributed to further analysis. Comparison of sound evoked stapes footplate vibration with actuator driven lead to a sound equivalent sound pressure level of approx. 114-115 eq dB SPL at nominally $1V_{rms}$ input. Analysis of subgroups with Ø .6mm (N=8) and Ø .8mm (N=3) pistons indicate a higher efficiency of larger pistons.

This result demonstrates that pronounced sensorineural hearing loss components can be sufficiently treated with the DACS PI. The treatment of combined hearing losses that are currently difficult to treat becomes a realistic option by bypassing the middle ear.

932 The Effect of Cochlear Electrode Insertion on the Mechanics of the Middle Ear

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Hypothesis: It is thought that the insertion of a cochlear electrode might result in a change of the mechanical properties of the middle-inner ear interface (cochlear impedance) that leads to an alteration of the vibration pattern of the stapes.

Background: Preservation of residual low-frequency hearing with addition of electrical stimulations can improve the speech perception in noisy environments of cochlear implant users. The technique by which electrical and acoustic stimulation are combined is known as Electro-Acoustical-Stimulation (EAS). In order to utilize EAS, an intact middle ear mechanism is required. The aim of the current study is to investigate the effects of electrode insertion on the magnitude and phase of stapes vibratory pattern across the human hearing frequency range, with special attention to the changes that might occur at low frequencies.
Methods: 9 fresh frozen human temporal bones were used. Stapes displacement was measured by a Laser Doppler Vibrometry before and after insertion of a cochlear implant electrode under conditions of half, full insertion and post removal of the electrode. Middle ear was stimulated by a bank of tones in the 0.1 kHz – 10 kHz range with a total 90 dB SPL.
Results: A slight increase of as much as 8 dB was observed at middle and high frequencies for the displacement and 60° for the phase. Change at low frequencies was negligible in all temporal bones.
Conclussions: Cochlear insertion produces a small change in the stapes vibratory pattern at middle and high frequencies and no change at low frequencies. With the appropriate surgical techniques, EAS is feasible with cochlear implants. In-vivo experiments of the same nature need to be carried out to validate these results with data from intra-operative measurements.
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Presenter: Antonio G. Mirón

933 Electrical Suppression of Tinnitus: A Neuromodulation Approach

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Tinnitus is a phantom sound that occurs in the absence of external acoustic stimulation. If chronic, tinnitus can have debilitating effects on patients and it has a significant economic impact on society due to its large prevalence. Although a number of treatment methods have been used, so far there are still no reliable therapies. Recent clinical studies have demonstrated that auditory cortex electrical stimulation (ACES) can suppress tinnitus in patients. However, the large variability in the efficacy of ACES-induced suppression of tinnitus across individuals has hindered its development into a reliable therapy. Due to ethical reasons, many issues cannot be comprehensively addressed in patients. We have developed an animal model of tinnitus suppression through ACES and neuromodulation. In this model, behavioral testing and electrophysiological recordings were conducted following noise exposure to test for behavioral manifestation of tinnitus and its neural correlates. Electrical stimulation of the AC was implemented to determine if ACES suppresses tinnitus and modulates its neural correlates. Our data showed that noise exposure induces tinnitus and the induced tinnitus is significantly suppressed by ACES, which is in line with clinical observations. In addition, we found that ACES induces complex neural responses (inhibition, excitation and no change) in the dorsal cochlear nucleus and inferior colliculus, structures that have been implicated in the etiology of tinnitus. Furthermore, we found that ACES modulates neural interactions across auditory brain structures, suggesting that suppression of tinnitus may involve balancing neural activity or adjusting neural information flow at least along the auditory axis. We expect that an in-depth understanding of the underlying mechanisms of ACES-induced tinnitus suppression will further clinical investigations and stimulate development of specialized neural prosthesis for effective tinnitus suppression.

934 Auditory-Tactile Integration Enhances Cochlear Implant Speech Perception in Noise

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A cochlear implant (CI) restores speech perception in a quiet environment, but its performance is limited in background noise. Studies have shown that low-frequency acoustic stimulation (<500 Hz) can enhance CI performance in noise, but it is not available to most CI users. Because tactile sensation operates in this frequency range, we hypothesize that a tactile aid, combined with a cochlear implant, can improve speech recognition in noise, similar to the CI and hearing aid combination. Recognition of IEEE sentences in a speech-spectrum-shaped was evaluated under CI, Tactile, and CI+Tactile conditions. Fundamental frequency (F0) information was extracted from the target sentences and delivered through a tactile aid attached to the subject's finger tip. Speech reception thresholds (SRT) were measured using a one-down, one-up adaptive procedure. The mean thresholds are 10.82 dB for CI only condition and 8.05 dB for CI+Tactile condition, tactile stimulation, with the highest difference at over 7 dB. The present results suggest that tactile stimulation compensates for the lack of low-frequency information in cochlear implants. Although tactile aids had been abandoned thanks to advances in cochlear implants, the novel use of a tactile aid in combination of a cochlear implant may give the tactile aid new life in today's auditory rehabilitation.

935 Impact of SmartFocus Control on Aided Speech Intelligibility in Background Competition

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Many hearing aid signal processing features are designed to overcome the reduced speech intelligibility in

background noise characteristic of most hearing aid wearers. Most of these features are automatically deployed, yet studies have shown that wearers can reliably control and prefer to have some control of the processing in their own hearing aids. Here we investigated the potential advantage of providing wearers direct access to several adaptive signal processing parameters that can be manipulated by a single control. The SmartFocus control of the Unitron Passport hearing aid allows wearers to simultaneously modify noise reduction, speech enhancement, gain, and microphone directivity to improve either comfort or speech clarity. Speech intelligibility and sound quality were evaluated in 44 subjects with sensorineural hearing loss. Test conditions included aided listening with the SmartFocus feature disabled and three conditions with SmartFocus enabled (maximum comfort, maximum clarity, and an intermediate setting). Based on audiometric thresholds, subjects were fit bilaterally either with standard BTE (Cohort A; n = 22) or open-fit BTE (Cohort B, n = 22) hearing aids. Target speech was presented from a speaker in front (0 degrees) of the listener at 65 dB SPL. Background competition was presented from speakers at 0, 90, 180, and 270 degrees. Performance improved significantly in the SmartFocus maximium clarity condition relative to the SmartFocus disabled condition. Sentence recognition thresholds (50% correct) using the HINT with continuous speech-shaped noise improved by 2.0 and 1.5 dB SNR for Cohorts A and B. Sentence recognition using the BKB-SIN test in multi-talker babble improved by 3.8 and 2.8 dB SNR for Cohorts A and B. There was no significant change across 8 sound quality attributes. These results indicate that optimizing several signal processing features can have a significant synergistic effect above and beyond the impact of directional microphones.

936 Speech Recognition for Bone-Conducted Ultrasound

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According to previous studies, Ultrasound can be perceived through bone-conduction and some profoundly deaf subjects as well as normal hearing subjects can discriminate bone-conducted ultrasound (BCU) whose amplitude is modulated by different speech sounds. These findings suggest the usefulness of developing a bone-conducted ultrasonic hearing aid (BCUHA). However, the characteristics of BCU are still poorly understood. The aim of this present study was to compare BCU and air-conducted audible sound (ACAS) in terms of their associated speech perception tendency and to investigate the different perceptual characteristics of BCU and ACAS. In this study, speech discrimination tests using Japanese 20 monosyllables were performed with normal hearing subjects at both BCU and ACAS. BCU and ACAS were compared for intelligibility and hearing confusion. With BCU, the maximum percent correct totaled about 75%. Our comparison of the hearing confusion with ACAS and BCU according to the individual syllabic nuclear group showed a clear difference in the incorrect rates. In addition, the stimulus nuclear groups were often perceived in other nuclear groups in BCU. Our study showed that it is possible to transmit language information using BCU in normal hearing subjects. Our results suggested the possibility of a difference in speech recognition between BCU and ACAS.

937 Whole-Cell Recordings from Calyx Endings in the Turtle Posterior Crista

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A split preparation of the turtle posterior crista allows the visualization of individual calyx endings. Whole-cell recordings are made from the base of calyces, which typically innervate multiple type I hair cells. Fluorescent dye diffusing out of the patch electrode and visualized post-experiment confirms that recordings are made from calyx endings. Typical values are resting potentials of -70 to -75 mV, resistances of 35 Mohms; and capacitances of 35 pF. In voltage clamp, spontaneous synaptic events appear as fast, inward currents (mEPSCs). Consistent with their being mediated by AMPA receptors, mEPSCs are blocked by CNQX and prolonged by cyclothiazide. Quantal rates decline in the presence of Cd2+. Deconvolution is used to detect the start of individual mEPSCs, whose amplitude and timing can then be determined. Inward currents typically average 20 – 60 pA in peak amplitude and show considerable size variability (cv = 0.5). mEPSC shapes of large and small events are virtually identical. Interevent times are exponentially distributed. Spontaneous rates are low (<10/s), but can be increased to 100/s by high bath K+ without affecting mEPSC amplitude or shape. mEPSPs recorded in current clamp are large and can average > 2 mV. The calyx terminal also displays a repertoire of voltage-gated currents including outward delayed rectifiers and Ih. The latter is identified by its time-dependent activation during hyperpolarizing steps and its sensitivity to the antagonist ZD7288. TTX-sensitive Na+ currents are evoked by depolarizing currents and can give rise to repetitive trains of action potentials.

938 Postnatal Development of Conductances in Rodent Type I Vestibular Hair Cells and Calyces

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The rodent vestibular system is immature at birth and during the first postnatal week vestibular type I and type II hair cells start to acquire their characteristic morphology and afferent innervation. In the mouse utricle hair cells begin to transduce mechanical signals before birth, but basolateral conductances that shape the receptor potential continue to develop postnatally (Geleoc and Holt 2003, Rusch et al. 1998). We are studying postnatal changes in the membrane properties of immature hair cells, type I hair cells and afferent calyx terminals acutely isolated from the semicircular canals of gerbils and rats using whole cell

patch clamp with the goal of better understanding development of the type I hair cell/calyx synapse. At postnatal day (P) 5 immature hair cells expressed a delayed rectifier potassium current which activated at potentials above ~ -50 mV in both species. Most type I hair cells also expressed a tetrodotoxin-insensitive transient sodium current with a mean half-inactivation of ~ 90 mV. At P6 in rat and P7 in gerbil a low voltage activated potassium current ($I_{K,l}$) was first observed and conferred a low input resistance, typical of adult type I hair cells. $I_{K,l}$ expression increased markedly during the second postnatal week. $I_{K,l}$ was present in all rat type I hair cells by P14 and in all gerbil type I hair cells by P19, which correlated with the time of eye opening in both species. During the third postnatal week, sodium current expression declined and sodium currents were no longer detected by the fourth postnatal week in rat and the sixth postnatal week in gerbils. From P6 onwards calyces from both species expressed large sodium currents, inactivating potassium currents and spontaneous excitatory postsynaptic currents. Understanding the ionic changes associated with hair cell and afferent maturation should help elucidate development and regeneration mechanisms in the inner ear.

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939 Distribution of Efferent Terminals in Adult Mouse Vestibular Neuroepithelia

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The utricle of the inner ear vestibular labyrinth is essentially a flat sheet of sensory epithelium that best encodes linear head movements (tilt and translation) relative to Earth gravity. Important insights into its sensory-specific organization have recently been made indicating that the medial striola harbors characteristics of its hair cells and afferent neurons that have important contributions to spatial head movement coding. Through the present study we queried whether the distribution of efferent terminals exhibits parallel heterogeneities in utricles from adult mice (C57Bl/6 background). Efferent boutons were identified using immunocytochemical markers for vesiculated choline transporter (VChT) and choline acetyltransferase (ChAT). Terminals in which VChT and ChAT were colocalized were quantified in 7 utricular regions, using phalloidin labeling of the stereocilia and identification of hair cell morphologic polarization to navigate the utricular topography. The areas investigated were rostral, central, and caudal regions of medial and lateral striola, and the medial extrastriola. Efferent terminal distributions were computed as densities (i.e. terminals per 100 μm^2), and were evaluated in the context of hair cell densities. VChT labeling was extensive throughout mouse vestibular neuroepithelia, and was confirmed to represent efferent terminals through colocalization with ChAT. We found efferent terminal densities to be highest in the medial striola, slightly higher than medial extrastriola and approximately twice that of lateral striola. Hair cell densities were lowest in the medial striola and approximately equal in lateral striolar and medial extrastriolar regions. These data combine to indicate that efferent innervation density (efferent terminals per hair cell) is highest in the medial striola and lowest in the lateral striola. These data indicate that centrifugal projections to the utricle are heterogeneously distributed with respect to its topography.

940 Efferent Actions Differentially Affect Afferent Sensitivity to Sinusoidal Indentation in the Turtle Posterior Semicircular Canal

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In the turtle posterior crista, afferents are classified according to their discharge regularity, gain and phase of their response to sinusoidal indentation, as well as their response to efferent stimulation. In fact, afferent responses to efferent stimulation alone are used to predict the afferent's morphology and position within the crista. For instance, bouton afferents near the planum (BP) are weakly excited, those near the torus (BT) are strongly inhibited, and those more medially-located (BM) show mixed inhibitory-excitatory responses. Calyx/dimorphic (CD) units respond with a large excitation consisting of both fast and slow components. These different efferent actions are principally attributed to activation of three distinct ACh receptors: alpha9/alpha10 nicotinic ACh receptors (α9/10nAChRs) on type II hair cells, putative α4/β2nAChRs on bouton and CD units, and muscarinic AChRs on CD afferents. We are studying how each of these different efferent mechanisms alters the response of each afferent class under various combined efferent/afferent stimulation paradigms. Activation of either group of nAChRs in BT/BM or CD units during efferent stimulation reduces the afferent's sensitivity (i.e. gain) to sinusoidal indentation. Yet, activation of mAChRs on CD afferents results in a gain enhancement. Phase relationships appear unaffected in either case. In BT/BM units, attenuation of afferent gain is observed at shock frequencies as low as 2-5 Hz while increasing to 20-50 Hz often completely blocks the afferent's indenter response. Here, efferent stimulation also linearizes the afferent's response to larger indenter stimuli. Reduction of afferent gain in CD units requires much higher shock frequencies which seldom completely suppress the responses of CD afferents to the indenter stimulus. Interactions among the various efferent synaptic mechanisms likely account for the differential effects of efferent stimulation on afferent gain. (Supported by NIH DC008981).

941 Activation of Muscarinic ACh Receptors Underlies Efferent-Mediated Slow Excitation in Calyx-Bearing Afferents of the Turtle Posterior Semicircular Canal

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In the turtle posterior semicircular canal, electrical stimulation of efferent neurons results in several well-characterized effects on afferent discharge. In fact, an afferent's response to efferent stimulation can be used to regionally locate its ending within the neuroepithelium. Bouton units near the planum are weakly excited whereas bouton afferents near the nonsensory torus are strongly inhibited. Bouton units more medially-located (BM) show mixed inhibitory-excitatory responses, and finally calyx/dimorphic (CD) afferents, restricted to the central zone, show a large excitation often consisting of both fast and slow components. Fast excitation in CD units peaks in tens of milliseconds, lasts for 1-1.5 seconds, and is attributed to direct activation of nicotinic ACh receptors (nAChRs) located on afferent terminals. Slow excitation in CD afferents typically takes seconds to develop and persists for several minutes. The kinetics of the slow response suggests that it is likely maintained by the activation of intracellular signaling pathways initiated either by activation of fast nAChRs or through a metabotropic receptor. Using several pharmacological tools, we show that slow excitation persists following the blockade of the nAChRs suggesting that fast and slow excitation are generated independently. Secondly, efferent-mediated slow excitation is likely driven by muscarinic AChRs as it is antagonized by nanomolar concentrations of the muscarinic antagonist atropine and can be mimicked by the application of muscarine. Furthermore, electrophysiological data suggest that slow excitation also involves closing potassium channels in the calyx ending. Possible candidates include KCNQ channels which are expressed in CD afferents and whose closure can be mediated by mAChR activation. Pharmacological experiments using antagonists of KCNQ channels are being performed to determine their possible role in efferent-mediated slow excitation. (Supported by NIH DC008981).

942 Efferent-Mediated Excitation of Turtle Calyx-Bearing Afferents Does Not Involve α9/10nAChRs

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In vestibular end organs, cholinergic efferent neurons can provide synaptic input to type II hair cells, bouton afferents, and afferent calyces. This synaptic connectivity varies among vertebrates, as do the resultant efferent actions on afferent discharge during stimulation of efferent neurons. In the turtle posterior semicircular canal, all three efferent inputs are present and efferent actions are diverse including both rapid inhibition of bouton afferents and rapid excitation of calyx/dimorphic (CD) afferents. Efferent-mediated inhibition invariably involves the activation of alpha9/alpha10-containing nicotinic ACh receptors (α9/10nAChRs) on type II hair cells. Calcium influx through α9/10nAChRs activates small-conductance, calcium-dependent potassium channels that subsequently hyperpolarize hair cells and decrease transmitter release. In contrast, the efferent-mediated excitation of CD units is generated by activating nAChRs located on calyceal terminals that then directly depolarize the afferent. Previous electrophysiological data using α9/10nAChR antagonists and the nicotinic antagonist DHβE suggested that about 70% of the nAChR-mediated excitation in CD afferents was pharmacologically distinct from α9/10nAChRs. Sensitivity to low concentrations of DHβE and previous immunocytochemical studies suggest that α4β2-containing nAChRs might be responsible for this response. However, given that a smaller fraction of the response (~30%) was sensitive to α9/10nAChR antagonists, a role for α9/10AChRs could not be completely ruled out. To address this question, we have recently characterized the effects of several pharmacological agents with putative selectivity towards α4β2nAChRs. At least three of these compounds completely block excitation in CD afferents without interacting with α9/10nAChRs on hair cells. These new data further support a role for α4/β2nAChRs alone in efferent-mediated excitation of afferent discharge. (Supported by NIH DC008981).

943 Regulation of Cellular Calcium in Vestibular Supporting Cells by Otopetrin 1

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Otoconia are complex calcium carbonate ($CaCO_3$) biominerals that are required for the sensation of gravity. Degeneration of otoconia is thought to contribute significantly to balance disorders and to the displacement or ectopic formation of otoconia that occur in patients suffering from benign positional vertigo (BPV). In addition, commonly used aminoglycoside antibiotics can lead to disruption of otoconial structure and function. Despite the prevalence of balance disorders, little is known about the mechanisms regulating the development and pathology of the vestibular mechanosensory apparatus.

Tlt and *Mlh* mice have a severe balance disorder due to the congenital absence of otoconia. By positional cloning we identified mutations in *Otopetrin 1* (*Otop1*) as the genetic etiology of the *Tlt* and *Mlh* mouse phenotype. Knockout of the *Otop1* gene, by insertion of *beta-galactosidase,* also resulted in otoconia agenesis and provided a tool to track expression of *Otop1* and examine its molecular and cellular function *in vivo*.

Otop1 is a multi-transmembrane domain protein which is essential for mineralization of otoconia. The mechanism driving mineralization of otoconia is poorly understood, but it has been proposed that supporting cells and a mechanism to maintain high concentrations of calcium are critical. Using *Otop1* knockout mice and a utricular

epithelial organ culture system, we show that Otop1 is expressed and functions at the apex of supporting cells to increase cytosolic calcium in response to purinergic agonists, such as ATP. This is achieved by blocking mobilization of calcium from intracellular stores in an extracellular calcium-dependent manner and by mediating influx of extracellular calcium. These data suggest a model in which Otop1 functions to sense extracellular calcium concentrations near supporting cells and to respond to ATP in the endolymph to increase intracellular calcium levels during otoconia mineralization.

944 Notch Signaling in the Normal and Traumatized Mouse Utricle

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The mammalian vestibular epithelium has a limited capacity for spontaneous hair cell (HC) regeneration. The mechanism underlying the regeneration is not well understood and the extent of cell repair is unclear. Because the Notch signaling pathway mediates the formation of the HC mosaic during ear development, we tested whether it plays a role in HC regeneration in the mature mammalian vestibular epithelium. We examined Notch signaling molecules and their mRNA expression levels by immunohistochemistry (IC) and quantitative real-time polymerase chain reaction (qRTPCR), respectively, in normal and traumatized adult mouse utricles. We induced a unilateral lesion by infusing streptomycin into the posterior semicircular canal. Using IC, we detected Jagged1 in supporting cells in both normal and lesioned utricles. Atoh1 was absent in the normal tissue, but could be detected 3 days after the ototoxic trauma. A larger number of Atoh1-positive cells were found at 1 week and remained at 4 weeks after the lesion. Double-labeling for Atoh1 and myosin VIIa (a HC marker) identified three kinds of cells: Atoh1-positive/myosin VIIa-negative, Atoh1-negative/myosin VIIa-positive, and cells positive for both. We speculate that Atoh1 expressing cells are at different stages of regeneration and that Atoh1-negative/myosin VIIa-positive cells are previously existing HCs that survived the trauma. Notch1, Hes1 and Hes5 fluorescence was not detected in normal or lesioned tissues. qRTPCR data showed a decrease for *Hes5* and an increase in *Atoh1* after the lesion. Thus, HC regeneration in the mouse utricle was associated with down-regulation of *Hes5* and up-regulation of *Atoh1*. These results suggest that Notch signaling participates in the response of cells in the utricle to ototoxic trauma.

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945 Gravity Receptor Function Is Impaired in *Triobp* Knockout Mice

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Mutations of TRIOBP cause DFNB28, a recessively inherited nonsyndromic form of human deafness. TRIOBP is expressed in stereocilia of cochlear and vestibular hair cells and a knockout (KO) mouse model is profoundly deaf by postnatal day 25 (P25). *Triobp* KO mice demonstrate normal balance behaviors suggestive of normal vestibular function. Therefore, the purpose of the present study was to quantitatively characterize the vestibular phenotype of *Triobp* knockout mice using vestibular evoked potentials (VsEPs). VsEPs directly assess the inner ear gravity receptor organs (i.e., utricle and saccule). *Triobp* KO mice were tested at three ages: P40, P80 and 15 months of age. Three VsEP response parameters were quantified and compared between +/- and -/- genotypes: 1) threshold, 2) P1 latencies, and 3) P1-N1 amplitudes. At all ages, *Triobp* +/- mice had normal VsEP response parameters with thresholds averaging -10.5 dB re: 1.0g/ms, P1 latencies averaging 1.23 ms, and P1-N1 amplitudes of 0.81 µV. Despite profound hearing loss at an early age, *Triobp* -/- mice had measurable VsEPs until at least P80. At P40, -/- mice had significantly elevated thresholds (-3.3 dB re: 1.0g/ms), significantly prolonged P1 latencies (1.56 ms) and significantly smaller P1-N1 amplitudes (0.24 µV). By P80, thresholds remained elevated (-5.5 dB) while P1 latencies were more prolonged (1.87 ms) than at P40. At 15 months, all -/- mice had absent VsEPs while *Triobp* +/- mice had normal VsEPs. Prolonged latencies are consistent with a deficit in sensory transduction that delays or affects neurotransmitter release and thus delays the activation of primary afferents. Measurable VsEPs at P40 and P80 are consistent with normal behaviors for the *Triobp* -/- mice. Since the VsEPs do not disappear entirely until sometime after 80 days of age, balance behaviors remain normal well into adulthood. Work supported by NIH R01DC006443 to SMJ and Z01 DC000048 to TBF.

946 Morphological Assessment of an Epitympanic Approach for a Transmeatal Selective Neurectomy of the Lateral and Anterior Ampullary Nerve

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Introduction: Despite a high success rate of surgical treatment of benign paroxysmal positional vertigo, some patients still suffer from a cupolithiasis of the canal cristae of the lateral and anterior semicircular canals. A transmeatal approach to the osseous canal of the lateral and anterior ampullary nerve was assessed morphologically.

Materials and Methods: 80 halves of human heads being divided into 2 groups by 40 halves per group, all preserved with Thiel's method were investigated. For group 1, the

osseous canal of the nerves innervating and the common ampulla of the lateral and anterior semicircular canal were probed firstly to be found the surgeon. Group 2 represented specimens where the surgeon tried to reach the nerve without touching the membranous labyrinth before anatomical assessment by dissection.
Results: Group 1 showed direct hits of the osseous canal of the two ampullary nerves in 5 cases. In 28 cases the surgeon had to open the common osseous ampulla to reach ampullary nerves. In seven cases the nerves were inaccessible because of topography of the lateral semicircular canal and the osseous canal of the ampullary nerves to facial nerve. Group 2 showed 3 direct hits of the osseous canal of the ampullary nerves, in 35 cases the surgeon needed to open the osseous ampulla. In two cases the ampullary nerves were not accessible.
Conclusions: The epitympanic approach to the anterior and lateral ampullary nerves is mainly feasible via the common osseous ampulla of the semicircular canals, rarely by direct hits of the osseous canal.

947 The Impact of Low-Voltage Activated K^+ Currents on Spike-Timing Regularity in Mammalian Vestibular Afferent Neurons

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Differences in the regularity of inter-spike intervals may be important for the sensory code used by vestibular afferent neurons. To study whether the afferents' own ion channels determine spike timing, we made whole-cell patch clamp recordings from the isolated somata of vestibular afferent neurons from young rats and mice. Using depolarizing current steps we previously identified two types of vestibular afferent neurons: transient neurons fired one or two onset spikes and sustained neurons fired trains of spikes or a spike followed by large voltage oscillations. By injecting simulated excitatory postsynaptic currents (pseudo-EPSCs) at pseudo-random intervals we previously showed that the same EPSC trains evoked more regular firing in sustained neurons than in transient neurons, suggesting that the afferent's own ion channels can set spike timing. Here we show that the transient firing pattern correlated with a low-voltage-activated potassium current (I_{LV}), suggesting that this current may be important for controlling spike timing. We tested the idea with modeling and pharmacology. In addition to variable amounts of I_{LV}, the model neuron had a transient Na^+ current, a high-voltage activated K^+ current, and a leak current. The modeling suggests that many differences between transient and sustained neurons (e.g., resting potential, input resistance, and membrane time constant) are well captured by varying the density of I_{LV}. However, response features such as post-spike and sub-threshold oscillations were not well captured. To pharmacologically study the impact of I_{LV} on spike timing, we applied α -dendrotoxin (α-DTX, 100 nM), recently reported to block I_{LV} in vestibular afferents. In our sample, α -DTX had more modest effects: it only partly blocked I_{LV} and did not convert transient firing patterns into sustained firing patterns These preliminary results suggest that I_{LV} flows through more than one kind of ion channel.
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948 Phospholipase C-Mediated Inhibition of the M-Potassium Current by Muscarinic-Receptor Activation in the Vestibular Primary-Afferent Neurons of the Rat

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The vestibular system efferent-cholinergic input to afferent neurons is mediated by cholinergic synapses that activate both muscarinic and nicotinic receptors. Previously we had shown that the muscarinic-acetylcholine-receptor (mAchr) activation modulates the low-voltage-activated M-type potassium current in the vestibular afferent neurons (Pérez et al., 2009). In this work we studied the second messenger system mediating the inhibition of the M-current after mAchr activation. For this, voltage clamp recordings were obtained in the cultured vestibular-afferent neurons of the rat and the M-current measured in the deactivation. The intracellular perfusion of the phospholipase C (PLC) inhibitor U73122 significantly attenuated the inhibitory action of the mAchr receptor agonist oxotremorine-M. Its inactive analog U73343 produced no significant action. The use of the phosphatidylinositol 4,5 bis-phosphate (PIP2) scavenger poly-L-lysine led to a significant rundown of the M-current. Our results show that mAchr activation modulates the M-current via PLC activation and subsequent PIP2 reduction in the vestibular afferent neurons.
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949 Can Inner Ear Application of Rolipram, a P-CREB Up-Regulator, Induce Phosphorylation of CREB in Vestibular Ganglion Cells?

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Phosphorylation of the transcription factor cAMP responsive element-binding protein (CREB) is thought to play a key role in neurogenesis. In our previous study, phosphprylated form of CREB (p-CREB) –like immunoreactivities were observed in vestibular ganglion cells after unilateral surgical labyrinthectomy and unilateral TTX infusion. These results indicate that vestibular ganglion cells may have a potential of neuronal plasticity. We thought the possibility that up-regulation of p-CREB might facilitate neuronal plasticity.
Rolipram, a phosphodiesterase (PDE) 4 inhibitor, increases cAMP levels and leads to up-regulate the

phosphorylation of CREB. The aim of the present study was to evaluate the changes of p-CREB in vestibular ganglion cells after local application of rolipram into unilateral inner ear in the guinea pig by osmotic pump.
Hartley white guinea pigs with normal tympanic membranes and normal Preyer reflexes were used in this study. A tiny hole was made adjacent to the round window in the right ear, at the end of the 12-h saline infusion, the pump then infused 0.2 mg/ml rolipram continuously. We examined p-CREB –like immunoreactivities in vestibular ganglion cells 12 h and 36 h after the beginning of rolipram infusion.
P-CREB –like immunoreactivities were observed in bilateral vestibular ganglion cells 12 h and 36 h after the beginning of rolipram infusion. In our previous study, after any kinds of peripheral vestibular lesions, p-CREB-like immunoreactivities were disappeared within 8 h. These data indicated that inner ear application of rolipram might induce continuous phosphprylation of CREB in vestibular ganglion cells.

950 Inhibiting Vestibular Schwannoma Cell Growth with Lapatinib and AG825 (Tyrphostin), a Comparative Study

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Recent evidence suggests that growth and/or proliferation signaling in VS offer opportunities to identify molecular therapeutic targets. Primary human VS tumors from both sporadic and NF2 patients have been demonstrated by immunohistochemistry and co-cultures to express EGFR (ErbB1) and ErbB2 (Her2). Furthermore, schwannomas express ErbB2 in the activated, phosphorylated form. Our results indicate that human VS express EGFR/ErbB2 heterodimers in an activated state. ErbB3 receptor expression is also demonstrated, and forms heterodimers with both EGFR and ErbB2, but the predominant identity of receptors in ErbB heterodimer pairs in VS appear to be comprised of EGFR and ErbB2.
Recently, a study showed that Erlotinib, a small molecule inhibitor of EGFR, retards growth of VS xenografts in nude mice. Lapatinib (Tykerb), a dual small molecule inhibitor of EGFR and ErbB2, is used to treat advanced metastatic ErbB2-positive breast cancer patients. Lapatinib binds the ATP-binding site located in the kinase domain of EGFR and ErbB2. This effectively prevents auto-phosphorylation, and inhibits subsequent activation of downstream signaling cascades.
In this study, we examine the effect of small molecule ErbB inhibitors on VS. Primary VS cell cultures and HEI193, an immortalized VS cell line derived from an NF2 patient, were treated with Lapatinib and AG825, a selective inhibitor of ErbB2. We performed Annexin-V cell death assays, and cell cycle and cell viability assays using propidium iodide (PI) staining. We found a robust, dose-dependent inhibition of VS and HEI193 cellular growth with Lapatinib. AG825 also inhibits growth, but to a lesser extent than observed with Lapatinib. Our results indicate that dual inhibition of EGFR and ErbB2 is more effective at reducing VS cellular growth than inhibition of ErbB2 alone. These findings compliment our lab's results from co-immunoprecipitation assays, which predominantly demonstrate EGFR and ErbB2 heterodimer pairs in VS.

951 Persisitent C-Jun N-Terminal Kinase Activity Contributes to the Survival of Human Vestibular Schwannoma Cells by Suppressing Accumulation of Mitochondrial Superoxides

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Vestibular schwannomas (VSs) result from inactivating mutations in the *merlin* tumor suppressor gene. The merlin protein suppresses a variety of progrowth kinase signaling cascades including extracellular regulated kinase/mitogen activated protein kinase (ERK/MAPK), c-Jun N-terminal kinase (JNK), and phosphatidyl-inositol 3-kinase (PI3-K)/Akt. Recent studies indicate that ERKs and Akt are active in human VSs and here we show that JNKs are also persistently active in human VS cells. Using cultures from human VSs, we investigated the contribution of each of these signals to the proliferative and survival response of VS cells. Inhibition of ERK or Akt signaling reduced VS cell proliferation, but did not increase apoptosis, whereas inhibition of JNK with pharmacological or peptide inhibitors reduced VS cell proliferation and survival by inducing apoptosis. By contrast, inhibition of JNK prevents apoptosis in normal Schwann cells. Inhibition of JNK increased the fluorescence intensity of VS cells loaded with 5-(and-6)-chloromethyl-2',7'-dichlorodihydrofluorescein diacetate (H_2DCFDA), a fluorescent probe for reactive oxygen species (ROS). Further, ebselen, a ROS scavenger, rescued VS cells with suppressed JNK from apoptosis suggesting that JNK activity protects VS cells from apoptosis by limiting accumulation of ROS. VS cultures treated with JNK inhibitors demonstrated significantly higher levels of MitoSOX Red fluorescence implying that persistent JNK activity specifically suppresses superoxide production in the mitochondria. Overexpression of superoxide dismutase 2 (MnSOD, mitochondrial SOD) prevented apoptosis in VS cells with suppressed JNK signaling. Taken together these results indicate that persistent JNK activity enhances VS cell survival, at least in part, by suppressing accumulation of mitochondrial superoxides.

952 Inhibitory and Excitatory Vestibular Afferent Responses Induced by Infrared Light Stimulation of Hair Cells

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Recent work has illustrated the feasibility of in vivo neural activation with pulsed infrared light. Here, we investigated the activation of hair cells in the vestibular organs with infrared light. Experiments were done in the oyster toadfish, Opsanus tau and responses of single afferent fibers were recorded. The dynamic responses of horizontal canal afferents were characterized using transepithelial electrical stimulation and/or mechanical stimulation. Control experiments provided the frequency-dependent gain (spikes/sec per deg/sec) and phase (deg re: peak stim) of the afferents. The same afferents were recorded while focusing pulsed infrared light on the sensory epithelium of the horizontal semicircular canal. Results showed a mix of inhibitory and excitatory responses induced with the infrared light. The inhibition and excitation correlated with the type of response observed during mechanical stimuli. Velocity sensitive afferents were observed to increase their discharge rate with incident infrared light while acceleration sensitive afferents reduced their discharge rate. These afferent classes have some analogy to regular and irregularly discharging units in other species. A subset of afferents phase-locked their discharge with the pulsed infrared stimulation. Some highly phase-advanced afferents were driven into complete inhibition following infrared stimulation and they returned to their baseline discharge once the infrared source was turned off. The data indicate differential sensitivity of different hair cell types to infrared stimulation depending upon the mix of neurotransmitters, receptors and channels expressed. The pulsed infrared stimulation offers new means to selectively activate hair cells and to investigate mechanism(s) of activation.

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953 Neurometric Thresholds of Vestibular Afferents

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The means by which primary vestibular afferents provide a signal to central vestibular circuits to guide vestibular responses is not well defined. The vestibular system has two major types of neurons: regular and high-gain irregular neurons; and low-gain, or phase-led, irregular neurons. These are anatomically and physiologically distinct, but their relative contribution to vestibular signal processing remains controversial. We approached this problem by determining neurometric curves for vestibular-nerve afferents in mice. We recorded horizontal semicircular-canal afferents during 40 deg/sec peak velocity sinusoidal rotations about an earth-vertical axis at 0.5 Hz, measuring firing rates over time periods from 0.01 to 0.5 sec. We found that regular afferents were equally sensitive at detecting rotations over all durations of time except 0.01 sec. The 79% threshold at the longer periods of time was approximately 20 deg/sec. The thresholds for irregular afferents were more consistently dependent on time, with the best thresholds obtained when counts were measured over longer periods of time. These results indicate the ability of afferents to carry information is dependent in part in the decoding mechanisms of postsynaptic cells. The duration of the decoding operation, in particular, determines in part the cell's ability to contribute to downstream vestibular function.

954 Effects of Click Intensity and Duration on Sound-Evoked Vestibular Responses in Rats

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Since sound-evoked vestibular responses offer simplicity and the capacity to stimulate each labyrinth separately, their potential value as a test of vestibular function has been widely recognized. In order to develop better vestibular tests based on this phenomenon, the extent to which sound activates each of the five vestibular end organs needs to be determined. In an earlier study (Zhu et al., Program 72.2, 2009 Neuroscience Meeting Planner. Chicago, IL: Society for Neuroscience, 2009. Online.), we studied the responses of a large group (677) of vestibular afferents to acoustic clicks (0.1ms duration, 130dB peak SPL). In contrast to previous studies that focused on the saccular sensitivity to sound, we identified a significant number of canal afferents that were click sensitive. In the present study, we further examined the effects of intensity and duration on click-evoked responses in rat vestibular afferents. Thus far, we have studied the responses of 156 vestibular afferents (106 canal units and 50 otolith units) to 0.1ms duration clicks at 5 intensity levels (130, 120, 110, 100, 90 db peak SPL) and 113 vestibular afferents (77 canal units and 36 otolith units) to 130 db SPL click at 4 durations (0.1, 0.2, 0.5 and 1ms). Our ongoing recording and data analysis will provide new information on how clicks activate each of the five vestibular end organs and provide the neural basis to interpret sound-evoked vestibular responses.

955 Information Processes in the Lateral Semicircular Canals: A Mathematical Analysis

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The analysis of the information process in the semicircular canals of the vestibular system in the form of transfer function was developed by Fernandez and Goldberg (1971). In this work we further elaborate a mathematical

system in which the description of information processes in the semicircular canals in the state space was used. The model developed is a compartmental-type model in which we considered all the stages of the sensory activation process in the biosensor of angular acceleration. The functional and numerical parameters of the model have been identified by physiological and morphological experiments. Mechanical stimulus as a trapezoidal change of the angular velocity that corresponds to the head turning around the vertical axis was used (input signal). We obtained a description of the changes in the firing frequency of the primary afferent neurons of the lateral semicircular canal (output signal) in response to the mechanical stimulus, indicating that output signal gives information about the angular velocity or the angular acceleration. The model results indicate that there are fundamental differences in the output signals from the left and right lateral semicircular canals to the mechanical stimulus.

We propose that the model thus obtained and its results may contribute to vestibular prostheses development and particularly, the conclusion that no single plane canal simulating device can be used, and at least two canal-like devices must be used to obtain an adequate reproduction of vestibular system bidirectional response capabilities.

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956 Spontaneous Discharge Variability Alone Does Not Predict Detection Threshold and Information Transmission Properties in Vestibular Afferents

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Sadeghi et al. (*J Neurosci*, 2007) found that, in the macaque, 'regular' horizontal semicircular canal (HSC) afferents (i.e. those with a low normalized coefficient of variation (CV*) of spontaneous interspike intervals), despite having lower gains than irregular (CV*≥0.15) HSC afferents at all but the lowest frequencies, had lower detection thresholds (v_T) in response to sinusoidal stimuli and higher mutual information densities (MI) in response to broadband stimuli. They suggested that, in general, lower power in the spontaneous discharge of regular afferents in the stimulus frequency band would result in lower noise during stimulation and hence a higher signal-to-noise ratio (SNR) in the responses of regular afferents compared to irregular afferents, thereby leading to a lower v_T and higher MI. We tested the generality of these conclusions by examining the relationship between CV*, v_T and MI in bullfrog HSC afferents using similar stimuli and analyses. We discovered that bullfrog afferents with higher CV* could actually have lower v_T and higher MI, despite their spontaneous discharge having higher power than more regular afferents in the stimulus frequency band. These findings suggest a more subtle role of CV* in determining vestibular coding properties than Sadeghi and colleagues envisioned. In general, the SNR of an afferent response is determined by a combination of intrinsic properties (inter-trial response variability and response sensitivity) and stimulus properties. Sensitivities in irregular frog afferents have a more dominant effect on the SNR than response variability, leading to a high SNR despite a high CV*. Based on our results, we conclude that variability of spontaneous discharge alone cannot in general serve as a predictor of neural coding properties. In the vestibular system in particular, individual regular afferents will not necessarily have lower detection thresholds and/or higher mutual information densities than their irregular counterparts.

957 Colocalization of 5-HT1F Receptor and Calcitonin Gene-Related Peptide in Rat Vestibular Nuclei

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The aim of this study was to determine whether CGRP (calcitonin gene-related peptide) colocalizes with 5-HT_{1F} receptor in rat vestibular nuclei using a double immunohistochemical staining procedure. The frequent co-occurrence of migraine and balance disorders suggests a pathophysiologic link between the two. However, the mechanism of migrainous vertigo has not been elucidated, though serotonin (5-HT) and its receptors are believed to involve in the pathogenesis of migrainous vertigo. Furthermore, 5-HT_{1F} receptor agonists and CGRP receptor antagonists have recently attracted attention as potential treatments for migraine, and CGRP release from trigeminal neurons has been associated with migraine. This study demonstrates the colocalization of 5-HT_{1F} receptor and CGRP in the rat vestibular nuclei, which suggests that 5-HT_{1F} receptor regulates the release of CGRP from vestibular nuclei. This finding indicates that 5-HT_{1F} receptor agonists may ameliorate migrainous vertigo by attenuating elevated levels of CGRP release from vestibular nuclei.

958 Membrane Excitability of Cerebellar Purkinje Cell After Unilateral Vestibular Deafferentation: A Preliminary Report

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Vestibular primary and secondary afferents are distributed to the vestibulocerebellum which also has extensive connections with vestibular nuclei. Purkinje cell in the vestibulocerebellum respond to vestibular stimulation and impose adaptive guidance of vestibular coordinate system. During vestibular compensation after unilateral labyrinthectomy (UL) some neurons in medial vestibular nucleus develop a sustained increase in their intrinsic excitability. But the role of vestibulocerebellum in vestibular compensation is still in controversy.

We investigated possible changes of intrinsic properties of Purkinje cell in lobule X following the UL and during the

course of vestibular compensation. Using patch clamp technique, passive and active properties of Purkinje cell were evaluated in slices prepared from rats 12 h or 72 h after UL and control animals. Only maximal firing frequency and interspike interval among evaluated parameters were significantly different between ipsilesional 12 h after UL and control.
Our results suggest that unilateral vestibular deafferentation may affect the intrinsic membrane properties involved in Purkinje cell firing.

959 Changes in Calbindin Expression Within the Flocculus After Unilateral Labyrinthectomy in Rats

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The role of the flocculus in vestibular compensation is still a longstanding controversial issue. Calbindin regulates intracellular signaling and can consequently alter the sensitivity of Purkinje neurons to synaptic signals. Thus it has recently been reported to be a reliable marker of human Purkinje cell. In the present study, temporal changes in expression of calbindin were examined in the cerebellar flocculus after loss of the peripheral vestibular sensory organ. Unilateral labyrinthectomy (UL) was performed in rats, and expression of calbindin was tested using immunohistochemistry at 2, 6, 24, 48 and 72 h following UL. Both the staining intensity and number of calbindin-positive Purkinje cells in the ipsilateral flocculus to the lesion side decreased 6 h after UL compared to the control and contralateral side. Forty-eight hours after UL, the expression of calbindin returned to control levels and asymmetric expression in bilateral flocculus also subsided. These results suggested that transient reduction of calbindin expression in the ipsilateral flocculus to the lesion side may reflect a decrease in the GABAergic inhibition of the floccular Purkinje cell projections to the ipsilateral vestibular nuclear complex during vestibular compensation.

960 Clinical Trial for Local IGF-1 Treatment for Acute Sensorineural Hearing Loss

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The objective of the study was to test the efficacy of local IGF-1 application via biodegradable hydrogels for the treatment of acute sensorineural hearing loss that did not respond to systemic glucocorticoid treatment.
We designed an interventional phase I-II clinical trial to examine the safety and efficacy of local IGF-1 treatment for glucocorticoid-resistant acute sensorineural hearing loss. The primary outcome were set to evaluate the recovery of average hearing levels in pure-tone audiometry 12 weeks after the treatment. Secondary outcomes included rates for the occurrence of adverse effects including middle ear inflammation and vertigo. Trial size was planned to be 25.
Twenty five cases had been registered and received the application of IGF-1-releasing hydrogels on the round window membrane. Twenty three cases finished the hearing test 12 weeks after the application. Among these 23 cases, 11 cases (48%) showed improvement of average hearing levels more than 10 dB. No severe adverse effects were found in all the registered patients. The best recovery were observed in a 23-year-old female who received local IGF-1 treatment on day 18 after the onset. Her average hearing levels of 5 frequencies improved from preoperative 79 dB to 30 dB 4 weeks later. These results suggested the therapeutic potential and the safety of local IGF-1 treatment.

961 Is a Tinnitus Seminar a Successful Treatment Modality?

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This study's aim was to determine if a one-time tinnitus seminar could provide patients significant relief from distress caused by their tinnitus. Measurements were made using the tinnitus reaction questionnaire (TRQ) and by comparing a waitlist control group to a treatment group. The percent of improvement chosen as clinically significant for this study was 20 %. The benefit was determined by analyzing the decrease in scores between pre and post treatment TRQs. The tinnitus reaction questionnaire was chosen because it has been shown to have good internal consistency (cronbachs alpha = .96), test-retest reliability (r = .88), and has good correlation values to findings from clinicians (Wilson et al., 1991). There were 33 participants total (10 participants in the waitlist group and 23 participants in the treatment group). Of the 33 participants, 30 completed the study, a 91% completion rate.
There was not a significant difference between pre-post tinnitus reaction questionnaires when comparing the waitlist control group to the treatment group. A one way ANOVA was used to determine the difference between total change by group, [F(1,28)=1.39, P=0.246)]. A one way ANOVA performed for 3 different test scores as a function of group was also found to not be statistically significant [F(1,28)=0.605, P=0.44). It may be that statistically significant results were not found between the control and treatment groups due to the small sample size. Other studies using similar criteria with larger group sizes have been able to produce statistically significant results (Henry et al., 2008).
A second questionnaire designed by the researchers to determine perception of the seminar demonstrated positive findings. Further analysis was carried out by dividing the seminar participants into one group demonstrating clinically significant changes and another that did not. The mean score for the participants with clinically significant improvement was 5.75 out of 8. The non-clinically significant group also had a mean score of 5.29 out of 8, (0.046 % difference). Every participant marked that they would recommend the seminar to other tinnitus sufferers,

while 95% found the seminar helpful, and followed at least one of the seminar's recommendations. The vast majority of participants, 90% also felt they had a better understanding of their tinnitus following the seminar.
As many as 50 million people suffer from tinnitus in this country and many of them may be told by health professionals that no treatment options are available to help them. Tinnitus seminars offer a potential benefit to both patients disturbed by their tinnitus as well as the medical community's approach to procedures for tinnitus at an excellent cost/benefit ratio.

962 Combined Temporal and Prefrontal Transcranial Magnetic Stimulation for Tinnitus Treatment

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Low-frequency repetitive transcranial magnetic stimulation (rTMS) of the temporal cortex has been shown to be an effective treatment strategy for patients with chronic tinnitus. Clinical effects are probably mediated by modulation of thalamocortical processing. Inhibitory thalamic function is also modulated by the dorsolateral prefrontal cortex. Therefore, we have investigated a new rTMS treatment strategy for tinnitus patients consisting of a combination of high-frequency prefrontal and low-frequency temporal rTMS.
100 patients with chronic tinnitus were randomized to receive either standard treatment with low-frequency temporal rTMS or a combination of high-frequency prefrontal and low-frequency temporal rTMS. Treatment effects were assessed with a standardized tinnitus questionnaire (TQ).
There was an improvement of the TQ-score after rTMS treatment for both groups. The combined frontotemporal rTMS treatment was more effective than temporal treatment alone. These results suggest that the effects of low-frequency rTMS for the treatment of tinnitus can be enhanced by prior high-frequency rTMS of the dorsolateral frontal cortex. Further research is needed to identify the mechanisms by which frontal rTMS may enhance low frequency rTMS effects.

963 Tinnitus Suppression by Low-Rate Modulated Sounds

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Tinnitus affects 10% to 20% of the population and can adversely impact quality of life. Numerous treatments have been attempted to remedy tinnitus, but a definitive treatment does not exist. Acoustic stimulation has been used to mask tinnitus, but this is usually effective only when the tinnitus is relatively soft. In our study, we aimed to find an effective and low-cost means of suppressing tinnitus via modified acoustic stimulation. Suppression differs from masking in that effective tinnitus suppressors are often lower in level and with different temporal and spectral properties than the perceived tinnitus, whereas maskers are often presented at an equal level and share similar temporal and spectral properties as the perceived tinnitus.
A prospective, observational study design was undertaken to identify customized and patterned acoustic stimuli in a group of patients whose tinnitus could not be controlled by current conventional methods. We focused on low-rate amplitude-modulated (AM) or frequency-modulated (FM) sounds because they have been shown to produce sustained and highly synchronized cortical responses in the brain. In addition to AM and FM tones, we investigated pure tones, band-pass noise, and white noise, totaling 5 different sound therapy approaches. These were delivered acoustically via headphones to 25 subjects with tinnitus.
Amplitude, frequency, and shape of the modulation were modeled alone and in combination with other subject factors to predict suppression and determine which combination of parameters provided the best response. More than half of the subjects experienced suppression with either an amplitude- or frequency- modulated tone. Often, the tones were high-frequency and near the frequency region of the matched tinnitus. No subject experienced total suppression with white noise, the traditional sound therapy approach. The present result suggests that sound therapy is an underexplored but promising area of research for tinnitus treatment.

964 Repetitive Transcranial Magnetic Stimulation (RTMS) for Treatment of Chronic Tinnitus

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[Background and purpose] There is compelling evidence that tinnitus is associated with functional alterations in the central nervous system. Repetitive transcranial magnetic stimulation (rTMS) is a potent tool for modifying neural activity at the simulated area and at a distance along functional anatomical connections. Depending on stimulation parameters, cortical networks can be functionally disturbed or modulated in their activities. Low-frequency rTMS has been shown to result in a decrease in cortical excitability. The technique can alleviate tinnitus by modulating the excitability of neurons in the auditory cortex. We aimed to investigate effects of low-frequency rTMS in the patients and determine the factors that predict a beneficial outcome with rTMS treatment.
[Method] Sixteen patients (male 10, female 6) with chronic tinnitus underwent low-frequency (1Hz) rTMS (intensity: 110% motor threshold, number of stimuli: 1200) to their left auditory cortex. The treatment outcome was assessed with a visual analog scale (VAS) of loudness, annoying and

duration, loudness balance test, and tinnitus handicap inventory (THI). Therapeutic success was studied according to the patients' clinical characteristics.
[Results] A significant reduction in the VAS (loudness and annoying) occurred immediately after rTMS, which gradually returned to pretreatment levels following 7 days. The patients with normal hearing and age-related hearing loss have a tendency to benefit more from rTMS treatment.
[Conclusion] These results support the potential of rTMS as a new therapeutic tool for the treatment of chronic tinnitus. Because this study was performed with a small sample sizes and showed high interindividual variability of treatment effect, further development of the technique is needed before it can be recommended for use in clinical routine.

965 Ameliorative Effect of Customized Sound Stimulation on Sensorinerual Hearing Loss

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Sound onditioning (i.e., prior exposure to low-level sounds) has been known to protect hearing ability against damage by traumatic noise in a number of mammalian species, including humans. It has also been reported that acoustic stimuli can slow progressive sensorineural hearing loss. Thus, exposure to a moderately augmented acoustic environment could delay the loss of auditory function in mice. In addition to these protection and retardation effects, long-term sound conditioning could enhance cochlear sensitivity in normal hearing guinea pigs. In this study, we applied customized sound stimuli to 30 patients with sensorineural hearing loss and compared the hearing thresholds before and after sound stimulation to investigate if the sound stimulation could change hearing ability. The results showed that acoustic stimuli could significantly improve hearing ability.

966 Tinnitus Spectrum and Its Real-Time Visualization Based on a 134 Band Cochlear Model

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The existing method for matching tinnitus relies on the paradigm for listening single pure tone or band pass filtered noise. There are many types of tinnitus spectrum in accordance with hearing loss regions; 1) single pure tone, 2) multiple pure tones (harmonic or non-harmonic complex tone), 3) single filtered noise, 4) multiple filtered noise, and 5) the combination of 1) ~ 4). In this respect, a new method has been designed to achieve accurate performance for finding various types of tinnitus spectrum. This study introduces two inventions; 1) tinnitus taxonomy based on 10 types of tinnitus spectrum and 2) a method for visualization of tinnitus spectrum using a 3D cochlear model with 134 band (1/24 Oct) frequency resolution.

967 Tinnitus Retraining Therapy Using Portable Music Player

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The aim of this study is to evaluate the tinnitus retraining therapy (TRT) using a portable music player with environmental sounds as the sound generator.
The patients exist that can have no therapeutic benefit due to the difficulty of volume adjustment of tinnitus control instrument (TCI) and the tinnitus spectra above 6 kHz. When the tinnitus spectra are higher than 6 kHz, the tinnitus can not be covered with TCI or hearing aid. The patients using environmental sounds recorded in portable music player as a substitute for TCI or hearing aid in TRT were recommended to use the sound of water or waterfall. We analyzed environmental sounds including the sound of water or waterfall acoustically and examined the effectiveness of the environmental sounds in TRT.
The subjects were twenty-six patients who had tinnitus as a chief complaint and were followed in the specialized outpatient department for tinnitus in Kagawa University Hospital between January 2005 and February 2008. They were divided into three groups according to the type of devices; TCI group, hearing aid group and portable music player group. The sound of the murmur of a stream (one of the water sounds) contained a wide-frequency band and a constant strength, whereas the wave sound showed inconstant strength. Therefore, we mainly used the sound of murmur of a stream in the portable music player group. The mean score of Tinnitus handicap Inventory (THI) in portable music player group was decreased one year after the treatment. The efficacy ratio in portable music player group was almost equal to the mean THI score in TCI group and hearing aid group.
In conclusion, TRT using portable music player had good efficacy similar to TCI and hearing aid. The sound of the murmur of a stream was one of the most effective sounds in TRT. TRT using portable music player as sound generator can provide the most cost-effective treatment option for tinnitus patients.

968 The Relation Between the Tinnitus Spectrum and the Tone Audiogram

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The tinnitus spectrum is a representation of the frequency content of subjective tinnitus.
The subjects' tinnitus ear or ears were presented with a number of tones of varying frequency, and the subjects were asked to rate the degree to which the tone is present in his tinnitus between 0 to 100. This results in a tinnitus spectrum, that displays the "likeliness" vs. frequency. We

studied the correspondence between the tinnitus spectrum and the tone audiogram, recorded of 81 subjects.
The subjects were assigned to one of six groups based on their hearing loss. Patients in groups 1 through 4 had hearing thresholds of 20dB or better up to 500Hz, 1kHz, 2kHz and 4kHz, respectively. Subjects in group 5 had no hearing loss, and group 6 contained the subjects that did not qualify for groups 1 through 5. For each group the average tinnitus spectrum was determined. The average tinnitus spectrum increased with the frequency for groups 1-4. For group 4, the spectrum also increased rapidly but only from 3kHz and up. The group without hearing loss showed an increase of the likeliness from 1kHz up.
One hypothesis relates tinnitus to neuronal de-afferentiation, arising from peripheral hearing loss. This hypothesis predicts that the pitch content of a tinnitus percept is related to the shape of the tone audiogram. The presented data generally match this hypothesis. However, the de-afferentiation hypothesis does not account for the origin of the tinnitus in normal-hearing subjects. An increase in synchronicity across neuronal networks has also been proposed as a cause for tinnitus. Neural synchronicity (phase-locking) primarily occurs in response to low-frequency stimuli (upper limit 2.5kHz). Synchronicity across high-frequency neurons may be particularly unusual in healthy subjects. Hence, it is conceivable that it easily leads to an abnormal percept, i.e. high-frequency tinnitus in normal hearing individuals.

969 Vowel Perception of Cochlear Implant Users: Listening Vs. Lip Reading

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As speech processing devices or coding strategies have progressed, speech perception with cochlear implant (CI) has become significantly improved. Some users, however, use signs or lip reading to communicate with others because of the limited auditory information. Speech perception with lip reading may help to get information on degree of visual dependence, but it is difficult to unify test conditions. In Japanese, most syllables end with vowels and thus vowel perception is highly involved in speech intelligibility. We prepared vowel perception test to analyze vowel processing in CI uses both in visual and auditory aspects.
We enrolled 28 adults and 22 children who received CI as well as 10 normal hearing adults as controls. The auditory stimuli were five Japanese vowels (/a/, /e/, /i/, /o/ and /u/) separately uttered by a female speaker. The visual stimuli were apparent motions that were succession of pictures of woman□fs face, and six images were used: neutral lip form without any movement and five lip forms articulating Japanese vowels. Three test conditions used were as follows: 1) AV, the matched pairs of visual and auditory stimuli (e.g. lip form /a/ and voice /a/); 2) AV-mismatch, the inconsistent pairs of stimuli (e.g. lip form /a/ and voice /i/); and 3) A-only, neutral lip form and auditory stimuli.
Normal hearing subjects did not present any differences in correct rate or reaction time among the three test conditions. In CI users, correct rate was lower and reaction time was longer in AV-mismatch condition compared to the other conditions. Inverse correlation was observed between the monosyllable score and error rate in AV-mismatch condition, in which they were taken in by the visual information. Response pattern peculiar to CI users elicited degree of visual dependence, although it was a very easy task for normal hearing people.

970 Delayed Cochlear Implantation in Adult Patients with Prelingual Severe to Profound Hearing Loss

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Objectives: To examine the audiologic performance of adult patients with prelingual severe to profound hearing loss after cochlear implantation and to evaluate which patient factors influence their postoperative performance.
Material and methods: Thirty-two subjects with severe to profound hearing loss which developed before the age of 4 were included in this study. Subjects were implanted at a mean age of 28(range,16-44) with Nucleus CI24(n=18,56%), Clarion HiRes 90K(n=11,34%), and Medel PULSA(n=3,10%) device. Details of etiology, duration of deafness, hearing aid history, hearing thresholds before operation, communication mode and educational environment were investigated. Speech perception tests were performed preoperatively and 12 months after the operation. Statistical analyses were conducted to identify associations between test scores and other patient factors.
Results: The results showed significant improvement in open set speech perception(sentence) scores after the implantation(mean scores from 6.45 to 45.4%, $p<0.05$). Preoperative hearing of better ear and preoperative speech perception scores correlated with postoperative performances(pearson correlation coefficient -0.65 and 0.43, respectively). Education and communication mode were also closely related to postoperative performances. No significant correlations with postoperative scores were detected when analyses were performed by duration of deafness, etiology, and hearing aid history. In the group with poorer performances, preoperative hearing thresholds were significantly worse than those with better performances, and larger portion of patients attended special schools and used sign languages.
Conclusion: Cochlear implantation for prelingually deafened adults must be determined carefully and sufficient preoperative counseling about postoperative effect should be performed especially when the patient has poor residual hearing and has been educated with sign language in special schools.

971 Auditive Outcome in Cochlear Implant Users with GJB2(Connexin26)-Associated Congenital Hearing Loss

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OBJECTIVES: A controversy exists in the literature regarding better auditory performance of cochlear implanted children with mutations in the gap-junction-protein beta2 (GJB2) gene. This is partially due to insufficient numbers, lack of proper statistics etc. As this represents a very important issue for the counselling clinician, the aim of the study was to analyze auditory performance in GJB2-related hearing loss in relation to other causes and review the literature.

DESIGN: Retrospective study. 44 children with unilateral cochlea implants were assigned into 3 groups according to their cause of deafness: group A contained 13 patients with GJB2-related deafness, group B 15 with hereditary hearing loss and negative GJB2-screening, and group C 16 with a nonhereditary cause. The auditory outcome performance was evaluated by pure-tone audiograms (PTA), the Monosyllabic-Trochee-Polysyllabic-Word Test (MTP) and the Listening Progress Profile (LiP) according to the EARS-protocol (Evaluation of Auditory Response to Speech) pre- and postoperatively up to 6.5 years. Additionally the educational setting was considered. Statistical analysis included direct comparison by using mixed models and computing the variance to test for homogeneity.

RESULTS: A trend towards better performance for the GJB2 group was observed regarding PTA, even more pronounced for LiP / MTP. However, a significant p-value was not reached. A high homogeneity, expressed by a significant variance of MTP and LiP was observed in the GJB2 group. Differences in educational setting were not significant.

CONCLUSIONS: The results of the study seem to support a better auditory performance of GJB2-patients with cochlea implants due to a cochlear origin of the defect. The significant homogeneity for this group is of paramount issue for the counselling clinician and a very important observation.

972 Predictors of Language and Auditory Outcomes in Children After Two Years of Cochlear Implant Use

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Early cochlear implantation (CI) in profoundly deaf children has allowed them to develop auditory and language skills much closer to those of hearing peers than previously possible. Despite the impressive skills achieved by numerous children with CI's, variability in performance remains a significant concern. The purpose of this study was to determine predictors of language and auditory outcomes two years after implantation. Clinical data of six tests regarding language and hearing development in children up to seven years of age were included in this retrospective study. Participants were a large sample (N=125) of Dutch and Flemish children who received their implant before the age of five (M=02;00y, SD=00;11y). The results of two standardized language tests, the Reynell Development Language Scales (RDLS) and Schlichting Expressive Language Test (SELT), were analyzed. In addition, the verbal skills were evaluated by filling out two questionnaires; the Meaningful Use of Speech Scale (MUSS) and the Speech Intelligibility Rating (SIR). Finally, the Meaningful Auditory Integration Scale (MAIS) and the Categories of Auditory Performance (CAP) were used to evaluate the auditory skills. The collected data exists of 123 scores on the RDLS, 115 scores on the SELT, 82 completed MAIS and 79 completed MUSS questionnaires. The SIR was filled out for 39 children and the CAP for 43 children. Although age at implantation is a significant predictor ($p<0.05$) of language test scores, a lot of variability (±90%) remains unexplained. This finding led to the inventory of five additional factors as possible causes of this variability; (1) the presence of other disabilities, (2) the etiology of deafness, (3) the age at diagnosis, (4) multilingualism and (5) whether the child is uni- or bilaterally implanted. Combining this information in a multiple regression model might clear out the contribution of each individual factor and possible interactions with regard to the overall variability.

973 Humming in Tune: Sex Recognition by Mosquitoes on the Wing Through Acoustic Distortion

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Hearing by the "World's most dangerous animal" is mediated by the amazingly sensitive Johnston's organ at the base of the antenna. For decades it has been known that males use their Johnston's organ for listening for females but we had no idea what the females did with theirs. Surprisingly, females actually listen to the males. By listening to each other, individuals of the opposite sex of the predatory mosquito Toxorhynchites brevipalpis recognise each other's sex through wing-beat frequency matching. In this species, the wing-beat frequencies of males and females are similar. Frequency matching occurs also in mosquito species (Culex, Aedes and Anopheles) that are major vectors of disease, where the wing-beat frequencies of males and females differ considerably. In these species the males and females match harmonics of their flight tones. This matching occurs beyond the frequency range of the Johnston's organ but within range of the mechanical responses of the antennae. We show that the Johnston's organ is able to detect difference tones in the harmonics of the antennal vibrations. Acoustic

distortion in hearing organs exists usually as an interesting epiphenomenon. Mosquitoes, however, appear to use acoustic distortion as a sensory cue.

974 Recent Advance in Induced Pluripotent Stem Cells

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Reprogramming of somatic cells into pluripotent stem cells has been reported by several combinations of transcription factors (Oct3/4, Sox2, Klf4, c-Myc, Nanog, and Lin28) and chemical compounds in mouse and human. They are similar to ES cells in morphology, proliferation and differentiation potential. The patient-specific iPS cells will contribute to the fields of elucidation of pathogenesis, drug discovery, toxicology study, and cell transplantation therapy in the future. Although iPS cells were established in 2006, a lot of studies have already reported. In this meeting, we want to discuss current progress on iPS research.

975 Strategies Toward CNS-Regeneration Using IPS Cell Technology

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It had been long believed that adult mammalian central nervous system does not regenerate upon their injury. However, we have been trying to challenge this dogma by taking advantage of stem cell technologies. Above all, there is an increasing interest of induced pluripotent stem (iPS) cells as a source of neural stem/progenitor cells (NS/PCs), which can be obtained from patients' own somatic cells. In order to obtain safeness of iPS cells-derived NS/PCs, we examined various factors who could affect the tumorigenic ability of iPS cells-derived NS/PCs, including the origin of iPS cells, usage of cmyc transgene or usage of genetic selection using drug resistant gene. As a result, we found that the origin of the iPS cells is the crucial factor for predicting the safety issue and that the content of undifferentiated cells (i.e. differentiation-resistant cells) within NS/PCs highly correlated with their tumorigenic activity. When we examined the therapeutic ability of NS/PCs-derived from pre-evaluated non-tumorigenic iPS cells for injured spinal cord using mouse model, we found that Pre-evaluated iPS-derived NS/PCs and ES-derived ones show similar therapeutic effects upon SCI. The detailed histological analysis has indicated that increased 5-HT positive fibers and Myelinated fibers obtained by the transplantation of iPS-derived NS/PCs could have contributed to the functional recovery. Furthermore, we could verify functional recovery of NOD/SCID mouse SCI model by the transplantation of human iPS-derived NS/PCs. In the this talk, future direction of iPS cell research will be discussed.

976 Pros and Cons of Different Stem Cell Types for Future Clinical Use

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Previous studies have demonstrated the potential of cell-based therapy for the treatment of hearing loss. Stem cells isolated from various sources appear to possess the potential to generate hair cell-like cells and spiral ganglion neurons, which holds the promise for restoration hearing by cell-based therapy. However, several obstacles to be overcome still remain before practical application. The methods for otic induction of stem cells have not been established distinctly. The feasibility of harvesting stem cells is critical considering clinical application. It remains a challenge to realize functional restoration of cochleae by cell transplantation. In this presentation, I will focus on the final issue, development of the transplantation approach using stem cells. Particularly for clinical applications, bone marrow-derived stromal cells are an attractive potential cell source. I will present data on the efficacy of transplantation of bone marrow-derived stromal cells for regeneration of the spiral ligament and spiral ganglion. An interesting aspect of these experiments is the fact that several studies have indicated that paracrine effects of stromal cells could play a central role in regeneration of various tissues. Hence, we also examined whether paracrine effects of stromal cells are involved in the repair of the cochlea. In an accompanying study, we investigated the prospective of induced pluripotent stem (iPS) cells as a source for regeneration of spiral ganglion neurons. Neurally induced iPS cells exhibited the capability for survival and differentiation into glutamatergic neurons after transplantation into the cochlea similarly to ES cells. However, differences in the proliferative activity among cell lines tremendously vary in iPS cells differed from ES cells. On conclusion derived from these studies is that the selection of appropriate iPS cell lines is critical for avoiding tumorigenesis.

977 Functional Hair Cell-Like Cells from Embryonic and Induced Pluripotent Stem Cells

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Mechanosensitive sensory hair cells are the linchpin of our senses of hearing and balance. The inability of the mammalian inner ear to regenerate lost hair cells is the major reason for the permanence of hearing loss and certain balance disorders. Here we present a stepwise guidance protocol starting with pluripotent mouse embryonic stem (ES) and induced pluripotent stem (iPS) cells, which were directed toward becoming ectoderm capable of responding to otic-inducing fibroblast growth factors. The resulting otic progenitor cells were subjected to varying differentiation conditions, one of which promotes the organization of the cells into epithelial clusters displaying hair cell-like cells with stereociliary bundles. Bundle-bearing cells in these clusters responded to

mechanical stimulation with currents that were reminiscent of immature hair cell transduction currents.

978 Stem Cells from the Organ of Corti as a Drug Screening Tool

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Sensorineural hearing loss is often caused by damage and subsequent loss of sensory hair cells in the auditory sensory epithelium. Several research strategies have been devised to induce hair cell regeneration in mammals. These strategies have identified and validated target molecules for drug development. A new option among these approaches has been opened by the isolation of progenitor/stem cells from the early postnatal organ of Corti. These progenitor/stem cells can self-renew and differentiate into hair cell and supporting cell like cells. Their potential for self-renewal and differentiation can be applied to the development of drug screening assays that allow for the identification of drug-like molecules with regenerative effects. Compounds identified in these screening assays can then be taken to further analysis at the organ level.

979 Identifying Properties of Progenitor Cells That May Account for the Disparate Regeneration Capacities of Mammalian and Non-Mammalian Hair Cell Epithelia

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During development and in response to manipulation, epithelial cells can convert into mesenchymal cells by losing their intercellular junctions and other epithelial characteristics through a process known as Epithelial-Mesenchymal Transition (EMT). Mesenchymal-Epithelial Transition (MET) also occurs. Cells dissociated from avian ears readily undergo EMT and can be expanded to large numbers, frozen, thawed, and induced to undergo MET to form hollow epithelial spheres and develop into hair cells in vitro. Differences in composition of the intercellular junctions that play key roles in EMT and MET also may contribute to the disparity in the regeneration capacities of mammalian and non-mammalian hair cell epithelia. As postnatal mammals mature, their vestibular supporting cells express increased levels of E-cadherin, the prototypic epithelial adhesion molecule, and they develop unique and substantial thickening of the circumferential belts of F-actin at their intercellular junctions. These changes in mammalian hair cell epithelia strongly correlate with declines in progenitor cell proliferation and other measures of repair processes. In contrast, it has been reported that little or no E-cadherin is expressed in the vestibular epithelia of birds, and the circumferential F-actin belts in avian supporting cells remain thin throughout life. Avian vestibular epithelia also show no declines in progenitor proliferation capacity and no change in epithelial wound repair even when utricles from 1-year-old birds are compared against those from hatchlings. These observations suggest that the epithelial junctions of mammalian supporting cells may be suitable targets for treatments designed to lift limits to regeneration in mammalian ears.
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980 Inner Ear Progenitor/stem Cells: Gone with the Wnt?

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The early postnatal mammalian cochlea contains a population of undifferentiated cells that can self-renew as free-floating clonal spheres and differentiate into various cell types including hair cells and supporting cells. However, this cell population rapidly disappears during the first 3 weeks of life, the mechanism underlying this phenomenon remains unclear. Because Wnt signaling plays a role in maintaining stem cell populations in other organ systems, we assumed that the canonical Wnt pathway is involved in the maintenance of cochlear progenitor/stem cells. We further hypothesized that changes in Wnt pathway-related gene expression will coincide with loss of stemness in the neonatal organ of Corti. Here we present evidence that in the mouse cochlea, the expression of several Wnt pathway factors decreases during the first three weeks. Using the Axin2-lacZ mouse line as a reporter, we have identified Wnt responsive cells residing in the sub-basilar membrane region and Kolliker's organ in the early postnatal period. This population of Axin2-positive cells decreases over the first three weeks. When isolated via flow cytometry, these Axin2-positive cells have the ability to self-renew by forming clonal spheres and form hair cell-like cells *in vitro*. Ongoing studies including gain- and loss-of-function manipulations of the Wnt pathway are performed to further characterize the Axin2-positive cells, which appear to be a candidate cochlear stem/progenitor cell population.
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981 Spatial Information Required for Normal Sound Localization and Training-Induced Plasticity Is Distributed Across Auditory Cortex

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The contribution of the auditory cortex to normal sound localization and its recalibration by experience was explored behaviorally in adult ferrets by reversibly inactivating different cortical areas by subdural placement of a polymer that released muscimol over a period of weeks. The polymer was placed bilaterally over the anterior, middle or posterior ectosylvian gyrus (EG), so that different regions of the auditory cortex were inactivated in different animals. The extent of the inactivation was verified electrophysiologically. Sound localization abilities were tested by measuring both the initial head orientation and the approach-to-target responses made by the animals, which were trained by positive conditioning to

localize broadband sounds from loudspeakers spanning the full 360 degrees of azimuth. The animals were still able to localize sound, as assessed by either of the response measures, following inactivation of any region of the EG, and their performance was comparable in accuracy at most sound durations to that measured before inactivation. We also examined localization plasticity by reversibly plugging one ear. In control animals, monaural occlusion impaired localization accuracy, particularly on the ipsilateral side, but, with appropriate behavioral training, performance recovered over the next few days. This was the case for both approach-to-target and head orienting responses. We found that inactivation of any of the three main regions of the EG resulted in less complete and slower recovery than in controls. The effects on approach-to-target response plasticity were similar regardless of the areas inactivated, suggesting that the integrity of each of these regions of the auditory cortex is needed to achieve complete adaptation. In contrast, head orienting responses were affected in different ways depending on the cortical areas inactivated, implying some specialization in the processing of spatial information across the auditory cortex.

982 Differential Effects of Task on Spatial Sensitivity in Cortical Areas A1, DZ and PAF in Awake Behaving Cats

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Previously we have reported that neurons in various auditory cortical areas in unanesthetized cat differ in their sensitivity to sound location. Neurons in area A1 tend to have broad spatial tuning, with spatial receptive fields ranging from hemifield to omnidirectional. In area DZ, neurons tend to respond best to stimuli near the frontal midsagittal plane. In area PAF, neurons with sharp spatial tuning exhibit best areas more evenly distributed across space. Here we compare the behavioral task dependence of spatial sensitivity in these three areas. We recorded extracellular spike activity with chronically implanted 16-channel probes. In all conditions, the spatial receptive field of each neuron was assessed by 80 ms broadband noise-burst probe stimuli played from free field speakers in the horizontal plane. We compared neuronal responses under three behavioral conditions: 1) idle -- exposed to probe stimuli without engaging in behavior tasks; 2) discrimination -- detecting a change from the probe stimulus to a click train, regardless of the location of the sound; and 3) localization -- distinguishing a shift in stimulus elevation to 40 degree above horizontal plane. Overall, we found that neurons in A1 exhibited stronger task dependent modulation than did neurons in DZ and PAF. During the localization task, many A1 neurons reduced their spatial receptive fields from omnidirectional to hemifield by suppressing their responses to non-favored stimuli. In areas DZ and PAF, the localization condition was associated with an increase in spatial acuity in a measure of two point resolution and an increase in tonic firing to favored stimuli. The differential task-dependent modulation of spatial sensitivity among these three areas suggests that these three cortical areas might play distinct roles in auditory spatial processing.

983 Behavioral Measures of Spatial and Pitch-Based Selective Auditory Attention

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In a complex listening environment, we can selectively attend to an object of interest based on either its location or another feature, like its pitch. The current study explored this ability and the performance cost associated with switching between attending to location and attending to pitch.

Subjects were instructed to report one of two simultaneously presented digits, spoken by the same talker, that were embedded in broadband noise. On each trial, the two monotonized digits differed in pitch by 6 semitones and differed in location by 60˚ (-30° and +30° azimuth). Dichotic broadband noise was presented to the subject continuously throughout the experiment. In different trials, statistically identical stimuli were presented; however, an arrow on a computer screen instructed listeners whether to attend to location or pitch. Trials were blocked based on the consistency of the instructions from trial to trial. In “space-only” blocks, left and right arrows indicated whether the listener was to report the target digit from the left or from the right, respectively, on a given trial. In “pitch-only” blocks, up and down arrows indicated whether the target digit on a given trial had the higher or lower pitch, respectively. In “mixed” blocks the arrow on each trial indicated whether listeners should attend space (left or right) or pitch (up or down). Finally, by varying the inter-stimulus interval, we explored the time required to switch attention from one feature to another.

Subjects were more accurate at reporting the target digit when they attended to location compared to when they attended to pitch. Moreover, the reaction time was shorter for space trials than for pitch trials, suggesting that it was easier to perform the task when attending to space compared to when attending to pitch. Results suggest that the cost of switching attention from pitch to location was greater than that of switching from location to pitch.

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984 MEG and EEG Measures of Spatial and Pitch-Based Selective Auditory Attention: Frontal Eye Fields Activation

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The frontal eye fields (FEF) are located in the premotor cortex, which is part of the frontal cortex of the primate brain. In vision, converging evidence identifies the FEF as an important source of top-down spatial attentional control in addition to its well-known role in saccade production. Microstimulation of the arcopallial gaze fields (a region analogous to the mammalian FEF) of the barn owl sharpens the spatial selectivity of neurons in the deep layers of the midbrain optic tectum (analogous to the superior colliculus of humans). A recent fMRI study showed the dorsal precentral sulcus (a human anatomical site of FEF) was more activated when subjects attended to the spatial features of competing auditory streams than a non-spatial feature. These results suggest that FEF is engaged by auditory spatial attention.

Here, we studied the role of FEF in auditory spatial attention in humans, exploiting the high temporal resolution of both magneto- and electro-encephalography (M/EEG). In each trial, we presented a cue followed by two simultaneous digits that differed in pitch and in spatial location. Statistically identical stimuli were presented in two blocks: one in which subjects were instructed to attend to the cue location and one in which they attended the cued pitch.

Results confirm that the FEF is differentially more activated when subjects attend to location than when they attend to pitch. Furthermore, this FEF activation is engaged before the onset of the digits, suggesting a top-down modulation in preparation for the auditory stimuli. By comparing results to a control saccade task, we explore whether the FEF involvement is more akin to an inhibition for reflexive gazes towards a source of interest, or whether the FEF guides top-down spatial attention to both visual and auditory objects.

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985 A Positron Emission Tomography (PET) Test for an Enhanced Role for the Cerebellum During Pitch Discrimination

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Recent years have seen a growing debate concerning the function of the cerebellum. We have proposed that the cerebellum may be more involved in sensory than motor function, and have specifically predicted that its activity should be particularly enhanced during sensory discrimination tasks. Consistent with this hypothesis we have shown using functional Magnetic Resonance Imaging (fMRI) that 'finger' regions of the human cerebellum are more active during tactile discrimination tasks than during finger movement alone (Gao et al., 1996, Science 272:545). In this report we extend our investigation to auditory pitch discrimination using PET in healthy humans. Auditory studies remove the confound of movement inherent in studies of other sensory systems, including the somatosensory system.

In the current study 10 normal subjects were trained to discriminate deviant tones presented with a slightly higher pitch than a standard tone, using a Go/No Go paradigm. To ensure that discrimination was performed at equivalent levels of performance for each subject, individual psychometric curves were assessed beforehand using a two-step psychoacoustic procedure. Subjects were then scanned under several conditions including: while resting in the absence of any sounds; while passively listening to the standard tones; and while detecting deviant tones slightly higher in pitch among these standard tones at four different performance levels.

Analysis of regional cerebral blood flow (rCBF) data outlined activation during the passive listening condition in both the auditory cortices and the cerebellum. In the cerebellum, however, the discrimination conditions were associated with significant increases in both activation volumes and magnitudes. These results are consistent with our previous neuroimaging findings in the somatosensory domain, and extend to an additional sensory system evidence that the cerebellum is, in fact, more active during discriminative than passive sensory conditions.

986 A Behavioral Measure of the Cochlear Changes Underlying Temporary Threshold Shifts

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Temporary threshold shift (TTS) refers to the increase in absolute threshold following exposure to intense sound. It has been suggested that TTS is a consequence of dysfunction of the outer hair cells (OHCs) in the cochlea. The present study tested this hypothesis using a behavioral measure of OHC gain. The signal was an 8-ms, 4-kHz pure tone. OHC gain was estimated as the difference in the masker level required to mask the signal between forward maskers at 4 kHz and 1.6 kHz. 18 normal-hearing participants took part. Testing was conducted before, shortly after, and several days after participants attended various loud music venues. Personal dosemetry readings at the venues had a mean level of 99 dB (L_{Aeq}). Average TTS for the signal measured shortly after attendance was 10.8 dB, similar to the average gain reduction estimate of 11.5 dB measured at the same time. This suggests that the threshold increase was caused mainly by OHC dysfunction, although an analysis of the off-frequency masker thresholds suggests a small

contribution from inner hair cell dysfunction. [Supported by BBSRC grant BB/D012953/1.]

987 Psychoacoustical Evidence of Spectro Temporal Modulation Filters

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Several physiological studies indicate that the auditory system is sensitive to spectro-temporal amplitude modulations. The present study investigates if such a sensitivity is also observed psychoacoustically in humans. The modulation depth at thresholds of a sinusoidal spectro-temporal target modulation is measured in a generalized masked threshold pattern paradigm, i.e., in the presence of a spectro-temporal masker modulation centred at various combinations of spectral and temporal modulation frequencies. The data is consistent with the hypothesis of a spectro-temporal modulation filter. Such a modulation frequency selectivity was already proposed on the basis of psychoacoustical data for purely temporal amplitude modulation and purely spectral amplitude modulations. However, the current data is not in line with the assumption that the frequency selectivity to spectro-temporal modulations can be modelled as a combination of a purely spectral and a purely temporal modulation filter. Such an approach would imply that masking is only determined by the absolute values of the spectral and temporal modulation frequencies. In contrast, the data show that a temporally downward drifting spectral ripple envelope (i.e., both temporal and spectral modulation frequencies are positive) is much less effective in masking a target modulation if the target modulation is an upward drifting ripple (negative temporal modulation frequency and positive spectral modulation frequency). A model is proposed, being able to account for the sensitivity to the direction of the spectro-temporal modulation as well as the residual masking which is observed when masker and target envelope ripples drift in opposite directions.

988 Loudness Gating Beyond the Primary Auditory Cortex

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Recent human and animal studies have begun to reveal cortical regions involved with awareness gating and novelty detection. There appears to be both automatic and conscious manipulation of coding mechanisms that enable detection of specific and meaningful sound features especially when embedded within a complex masking environment. Through investigation of a new deep brain stimulation prosthesis implanted within the auditory midbrain of five deaf patients, we have been able to present electrical pulse trains with different rates and assess how loudness and auditory cortical activity change as a function of time and pulse rate. Cortical activity was measured by recording electrically-evoked middle latency responses (eMLR, high forehead referenced to back of neck), which generally corresponds to synchronized neural activation from the midbrain up to the primary auditory cortex (A1). For most regions, continuous pulse stimulation elicited some degree of loudness adaptation in which the loudness percept decreased to a softer sustained level. However, for continuous stimulation of the dorsal cortex of the inferior colliculus (ICD), the loudness decayed to an inaudible level within a few seconds. Interestingly, ICD stimulation elicited eMLR responses similar in shape to those elicited with acoustic stimulation and remained stable even as the stimulus became inaudible. Although electrical stimulation elicits artificial activation of the ICD, we were still able to elicit typical eMLR responses indicating synchronized activation up to A1. However, the fact that the eMLR responses remained stable over time while the loudness percept decayed to an inaudible level suggests that ICD stimulation does not appropriately activate regions beyond A1 to maintain a stable loudness percept. These findings suggest a loudness gating mechanism beyond A1 that requires specific neural input (in addition to synchronized activity associated with eMLRs) for perceptual awareness of sound. The question remains if such a loudness gating mechanism underlies the brain's ability to ignore insignificant ascending information and allocate limited processing resources for more meaningful and relevant sound features. *Research supported by Cochlear Ltd.*

989 The Effects of the Catechol-O-Methyltransferase (COMT) Val^{158}Met Polymorphism on Auditory and Visual Bistable Perception

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Dissociations between physical inputs and subjective experience are useful for examining temporal dynamics of perceptual organization. However, there is still some controversy as to whether the formation of percepts in different modalities is implemented in common or distributed mechanisms in the brain. The present study examined effects of the catechol-O-methyltransferase (COMT) polymorphism on spontaneous switching of percepts in auditory streaming and visual plaids. The COMT is related to the metabolism of released dopamine: the activity of the enzyme containing methionine (Met) is lower than that containing valine (Val); levels of COMT expression are greater in the temporal cortex than in the occipital cortex. The COMT polymorphism of 72 participants was genotyped and the participants were classified into three groups: 13 Met/Met, 32 Val/Met, and 27 Val/Val carriers. Participants were instructed to listen to a triplet-tone sequence passively in the auditory streaming task, whereas they were asked to observe two moving gratings in the visual plaid task. In the two tasks (five 90-s runs for each), participants pressed a button whenever perceiving subjective changes between single coherent and two distinct streams or upward grouped and sideward

split motion. The number of perceptual switches was larger for the Met/Met group than for the Val/Met and Val/Val/ groups in the auditory streaming task, but was not in the visual plaid task. In both tasks, however, the rhythmicity of perceptual switches did not differ among the three groups. These results indicate that the formation of percepts in auditory streaming is more susceptible to the efficiency of synaptic transduction in the dopaminergic system. Our findings support the idea that auditory and visual perceptual organization depends on different neural mechanisms.

990 Evidence for a Common Pitch Processor for the Perception of the Residue Pitch from Binaural and Diotic Pitch Components

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This study investigated whether the processes determining the residue pitch of a harmonic complex tone can combine a component pitch derived solely from binaural interaction (Huggins pitch) with a component pitch for which no binaural processing is required.

In a two-alternative forced-choice task, subjects indicated which of two complex tones had the higher pitch. Complex tones consisted of two "harmonics", and had a fundamental frequency of 400 Hz (1st and 2nd harmonics at 400 and 800 Hz), 266.7 Hz (2nd and 3rd harmonics at 533.3 and 800 Hz), or 160 Hz (4th and 5th harmonics at 640 and 800 Hz). Each stimulus was compared with each other. The lower and upper harmonics were, respectively: (i) both Huggins pitches; (ii) both narrowband noises; iii) a Huggins pitch and a narrowband noise; (iv) a narrowband noise and a Huggins pitch.

When the harmonics were either both narrowband noises or both Huggins pitches ("single mode" conditions), 10 out of 15 subjects mostly perceived the residue pitches of the complexes rather than the component pitches, i.e., they listened synthetically rather than analytically. This was indicated by the finding that, for a given pair of harmonics, they consistently judged the complex with the higher F0 as higher in pitch even though the lower harmonic of that complex had a lower frequency. Importantly, their response pattern was not significantly different in the "mixed-mode" conditions, where a Huggins-pitch harmonic was combined with a narrowband-noise harmonic.

The results indicate that the mechanism which derives residue pitch does not differentially process component pitches of different origin (whether or not they require binaural processing). This supports the idea that there exists one single mechanism for the derivation of residue pitch from binaural components and from spectral components, and that this mechanism operates at or after the level of the medial superior olive.

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991 Auditory Interactions in FM Direction Discrimination in Humans

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Auditory processing in a real-world acoustic environment involves very often listening to sequences of acoustic events such as human speech or species-specific vocalizations in vocal animals. The auditory context as it is created by the acoustic events occurring before or after a given signal in such a sequence might change the way the signal is perceived and recognized. Such contextual interactions could, in general, improve or impair the recognition of important signal components.

We found such effects of perceptual interference in a paradigm (4 experiments, 10 subjects) consisting of an unattended (ignored) pure tone presented before a frequency-modulated tone (FM) that was the test stimulus in a discrimination task on FM modulation direction. Performance in the discrimination task depended on the occurrence of the pure tone, its temporal distance from the target tone, and its frequency. Improved performance occurred for a continuous alignment of frequency components of the two tones in one direction, while a discontinuous alignment of frequency components (i.e. including a change in the direction of frequency change) resulted in deteriorating performance. This interference was obvious in upward and in downward modulated FM stimuli, both, for frequencies that covered the FM modulation range, and for interfering pure tones separated in frequency by up to 1.5 octaves and in time by up to 1200 ms.

This novel type of interfering interaction in auditory processing demonstrates long-lasting implicit memory-like representation of recently presented stimuli. The interference might have a general importance in pattern-based recognition processes, as used, for example, in speech perception. One aspect of such interference has recently been termed "informational masking" (Gutschalk et al., 2008, PLoS Biology). Extending this concept, we were also able to find such masking, but "informational priming" as well. The representation of these interactions at the cortial level is currently under investigation.

992 Disruption of Frequency-Discrimination Learning by a 30-Minute Break

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For perceptual learning to occur across multiple sessions, a sufficient amount of training has to be provided within each session (Wright and Sabin, 2007). We hypothesize that the effect of training within each session must accumulate to reach a learning threshold to induce lasting performance improvements. Here we asked how this cumulative process is affected by a break during training. We trained normal-hearing adults to discriminate frequency deviations from 1 kHz using a pair of brief tone pips. During each of 7 daily training sessions, trained listeners completed 720 two-interval-forced-choice trials with a 30-min break inserted after the first 360 trials. We thus provided a total amount of within-session training that

is known to yield significant across-session learning on this condition (720 trials), but interrupted this training after an amount that is known to be insufficient to generate such learning (360 trials). The trained listeners failed to improve between pre- and post-training tests (p=0.9), indicating that the break disrupted learning. Thus, across-session learning on this frequency discrimination condition requires not only a sufficient amount of training in each session, but also no marked interruption in the progression of that training. We speculate that, when training stops, the accumulated effect of prior practice starts to decline if the learning threshold has not been reached. In practical terms, the current results indicate that, for perceptual learning to occur, requirements for both training amount and time schedule need to be met. [Supported by NIH/NIDCD]

993 Exploring the Acoustic Basis of Consonance Using Individual Differences

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Some combinations of musical notes are pleasing, or consonant, while others are unpleasant, or dissonant. Although this distinction is central to music, its origins remain controversial. The dominant contemporary theory posits that dissonance is due to beating between frequency components, and that consonant chords are those that minimize beating. An alternative view is that consonant chords derive their pleasantness from their resemblance to single notes with harmonic spectra. It has also seemed plausible that consonance might not be rooted in acoustics at all, and that listeners simply learn to like specific chords that are prevalent in the music of their culture. We utilized individual differences to distinguish between the candidate theories.

Subjects were first tested for their aversion to pure-tone beats, and to inharmonic spectra of single complex tones. Consistent individual differences were observed in the magnitude of subjects' aversion to beats and inharmonicity, but the two effects were uncorrelated across subjects, suggesting independent factors. Subjects then rated the pleasantness of musical intervals and three-note chords. If particular acoustic factors determine consonance, stronger sensitivity to those factors should predict stronger musical preferences. Across over 250 subjects, only the preference for harmonic spectra was consistently correlated with preferences for consonance over dissonance - the aversion to beats showed no such correlation. Harmonicity preferences were also correlated with the number of years subjects had spent playing a musical instrument, whereas the aversion to beating was not. The results suggest that much of consonance perception is rooted in the basic acoustic property of harmonicity. Harmonicity is evidently predictive of important structures in Western music, and with exposure to music, listeners learn to like it. [Supported by NIH grant R01DC05216.]

994 Modeling Neural Responses and Perceptions of Complex Sounds: Introduction

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The goal of this symposium is to provide members of the ARO community with a relatively comprehensive introduction and update on recent progress in computational modeling of the ascending auditory system. The model presentations will 'ascend' the auditory system, starting with models for both acoustical and electrical responses of auditory-nerve fibers. Models for the responses of brainstem, midbrain, and cortical neurons will follow. The final presentation will feature a physiologically based model for predictions of psychophysical responses to speech stimuli, in listeners with and without hearing loss. The styles and levels of these models are quite diverse, from cellular-level models that focus on ion-channel dynamics, to phenomenological models that focus on signal-processing representations of neural mechanisms, to models that include the influence of behavioral relevance on neural responses. Some of the models are motivated by questions related to basic neural mechanisms, such as adaptation and sensitivity to the rate-of-change of input signals, and their effects on coding of information. Other models attempt to explain phenomena related to auditory responses to complex sounds, such as spectral and temporal processing of information related to the location and content of complex sounds, including spectrally and temporally complex maskers. These presentations will highlight questions that can be successfully addressed using computational modeling approaches, and introduce several powerful computational tools that are available to the hearing science community.

995 A Phenomenological Model of the Auditory Nerve: Long-Term Adaptation with Power-Law Dynamics in the IHC-AN Synapse

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Accurate models of normal and impaired neural representations of sound are useful tools in studying how acoustic stimuli are encoded in the brain, as well as for testing our understanding of the underlying physiological processes of the auditory system in ways that might not be feasible in physiological experiments. Recently power-law adaptation, which possesses a long memory with no privileged timescales, has drawn a lot of attention in describing the dynamics of biological systems. There is growing evidence that the dynamics of biological systems that appear to be exponential over short time courses are in some cases better described over the long term by power-law dynamics. In this talk we review some recent developments in modeling the neural responses in the auditory nerve (AN), especially the implication of including power-law dynamics in the model for the inner hair cell-AN

synapse. Our most recent model of the AN has incorporated most of the nonlinearities seen at the level of the AN, and thus the model responses are consistent with a wide range of physiological data from both normal and impaired ears for stimuli presented at levels spanning the dynamic range of hearing. The model can now be used as a front-end in many research areas, such as speech recognition in noisy environments, computational modeling of auditory scene analysis, modeling of neural circuits in the auditory brain-stem, design of hearing-aid amplification schemes, and design of speech processors for cochlear implants.

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996 Modeling Temporal Response Properties of Electrically-Stimulated Auditory Nerve Fibers

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Several physiological studies have shown that auditory nerve fibers in deafened ears exhibit complex patterns of stimulus-dependent adaptation and facilitation in their spike rate in response to electrical stimulation from cochlear implants (CIs). Such temporal response properties cannot be explained by models of the auditory nerve membrane that only include the fast-sodium and delayed-rectifier potassium channels of Hodgkin & Huxley. In this talk I will describe a stochastic Hodgkin–Huxley-type model that can incorporate a number of different voltage-gated ion channels that could be contributing to some key characteristics of the temporal response properties of auditory nerve fibers to CI stimulation. The code for this model is available publically for CI researchers to investigate the effects of these ion channels on the coding of acoustic features in CI stimulation.

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997 Investigating the Response of the Wide-Band Inhibitor Model of Comodulation Masking Release

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Comodulation masking release (CMR) is an across-frequency process where coherent amplitude fluctuations across a wide region of auditory filters enables the detection of an otherwise masked signal. A computational neural circuit has been produced, which shows how lateral inhibition may explain CMR exhibited by single units in the ventral cochlear nucleus. The circuit consists of a transient chopper unit (narrow-band) inhibited by an onset-chopper (wide-band) unit. Both these units were modelled using as an input a revision of the Sumner et al. 2003 guinea-pig auditory nerve (AN) model which incorporates the dual-resonance non-linear (DRNL) model of basilar membrane frequency selectivity. Dentritic filtering was approximated using a first-order Butterworth digital filter, and a Hodgkin-Huxley type single compartment somatic cell model (Rothman & Manis, 2003) was used for the neural cell unit response. All models were implemented in the development system for auditory modelling (DSAM; http://dsam.org.uk). The dependency of the circuit's CMR response on the spectral relationship between the two unit types was explored and showed in particular how the characteristics of the wide-band unit plays a major role. The model responses were investigated using a variety of CMR paradigms and the model has allowed a detailed investigation of the CMR process which would not have been possible from in-vivo recordings.

998 Slope Sensitivity Under Noise: How Does a Phasic Brainstem Neuron Model Encode Slow Inputs

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Phasic-firing neurons in the auditory brainstem can detect input slopes --- they do not respond to ramp currents with slopes below certain values (McGinley and Oertel D, Hear Res, 2006). However, our simulations with an auditory brainstem neuron model (Rothman and Manis, J Neurophysiol, 2003) show that added noise lowers the slope threshold. We investigate how noise enables phasic neurons to detect slow signals, which alone cause no response, by lowering the neuron's slope threshold. Meanwhile, sensitivity to input slope is still present in the noise-gated response, in both averaged firing rate and temporal patterns. Instead of firing to the peak of a low-frequency signal, the phasic model fired on the rising and falling phases. Our findings can be applied to responses of medial superior olivary (MSO) neurons driven by high-frequency sound with slow amplitude modulations (AMs) (e.g., < 100 Hz). We hypothesize that noise helps MSO neurons encode slow AMs by detecting the AM's rising phase, thereby making the neurons sensitive to timescales ranging from tens of microseconds to tens of milliseconds. Two cellular mechanisms, a low-voltage activated potassium current and the low-voltage inactivation of sodium current, contribute to the slope sensitivity of MSO neurons. (Supported by NIH/NIDCD-008543)

999 Neural Modeling of the Spectral Processing Pathway

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Psychophysical tests have shown that our ability to determine the elevation of a sound source depends on the filtering properties of the head and pinna, which add direction-dependent notches to the spectra of sounds. Physiological and behavioral data in cats suggest that the dorsal cochlear nucleus (DCN) initiates a pathway specialized to process this cue, and that type O units in the inferior colliculus (ICC) serve as the midbrain component of the spectral processing pathway. Using electrical circuit models, the goals of our work are to understand how spatial information is encoded in the periphery and how the directional code is modified at the level of the midbrain.

DCN principal cells (type IV units) are inhibited by spectral notches at their best frequency (BF). The traditional conceptual model of the DCN suggested that this sensitivity was shaped mainly by inhibitory inputs from wideband inhibitors (WBIs), which received auditory nerve inputs over a wide frequency range and inhibited type IV units over a narrow range. Recent results have shown however that WBIs are unresponsive to stimuli with wide notches and thus have narrower input bandwidths than previously assumed. New modeling results suggest that making the output bandwidth of WBIs wide while keeping their input bandwidth narrow can explain the notch responses of type IV units.
In contrast, type O units show a tuned excitatory response for spectral notches located just below BF. The current conceptual model suggests that type O units acquire their notch selectivity by the action of near-BF inhibitory inputs: that is, the selective excitatory response occurs because the inhibitory input is weakened when energy is removed from this frequency range. Modeling results support this hypothesis and suggest that a population of type O units could provide a labeled-line representation of spectral notches and thus a directional code for sound source localization within the ICC. Supported by NIDCD grant R01 DC05161.

1000 Cortical Representation of Complex Auditory Scenes

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Auditory cortical responses encode spectral features and dynamics that closely reflect the percept of the sound. These responses are also adaptive during performance of auditory tasks, becoming modulated by the attentional demands of the task, its rules and objectives, and its level of difficulty. In this talk, I shall review computational models of the cortical receptive fields the give rise to these responses, and of the manner they change when animals engage in different behaviors. I shall also describe how prefrontal (PFC) cortical responses could potentially provide the source of some of these top-down influences and what we know about the direct anatomical projections and physiological interactions between the PFC and the auditory cortex.

1001 Modeling Individual Hearing Impairment with a Physiologically-Based Auditory Perception Model

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Recently, an auditory signal processing model was developed, which could simulate psychoacoustic data from a large variety of conditions related to spectral and temporal masking in normal-hearing listeners (Jepsen et al., 2008). The model includes the dual-resonance non-linear (DRNL) filterbank (Lopez-Poveda and Meddis, 2001) to simulate the non-linear cochlear signal processing, and is otherwise similar to the modulation filterbank model by Dau et al. (1997). In the present study, the model parameters were modified at different processing stages to simulate hearing impairment. The modifications of the model were based on individual data from notched-noise and forward masking, intensity discrimination and modulation-depth discrimination experiments. The same model was used to predict error patterns in consonant recognition in a binary recognition task with synthesized stimuli. The model helps understanding the perceptual consequences of hearing impairment in individual listeners and can be useful for the evaluation of hearing-instrument signal processing.

1002 New Genes Involved in Deafness from Large-Scale Mouse Screens

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Deafness is common in the human population, but for the majority of affected individuals we do not know the molecular basis, especially in the case of progressive hearing loss. New deafness genes will identify new molecules essential for normal auditory function. To identify genes underlying deafness, we started a new programme using the growing resource of targeted mouse ES cells to generate mouse lines with mutations in known genes. We aim to generate 250 new mutants each year and screen them for signs of many diseases, including deafness and balance defects.
To detect hearing impairment, we record ABRs from anaesthetised mice using pin recording electrodes on the scalp and calibrated freefield broadband clicks and tonebursts delivered in 5dB steps. We test four mutants for each line, and the measurement takes 15-20 minutes per mouse. Waveforms are analysed to give thresholds, wave amplitudes and latencies.
So far we have completed screening of 143 lines and have preliminary data for a further 30 lines. Of these 173 genes, nine represented mutants that were known to have a hearing impairment beforehand and were included as positive controls. All nine showed raised thresholds as expected. Among the remaining lines, we have detected one with severe deafness (*Spns2*), two with moderate hearing impairment (*Mcph1, Lrig1*), and four with mild hearing impairment (*Matn1, Dusp3, Phf20, Psd95*), plus three with decreased amplitudes of response (*Socs7, Tpd52l2, Psmb2*) and one with prolonged latencies of response (*Adam17*). Of these eleven, only one would have been detected by the Preyer reflex (earflick in response to sound), emphasising the value of ABR analysis. None of these genes were expected to be involved in hearing impairment prior to screening. We are following up the most interesting mutants with more detailed analysis of the pathology in order to understand the underlying biological mechanisms as well as establish their role in deafness in the human population.

1003 Next Generation Sequencing Approaches to Identify Novel Genes Critical for Hearing

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In studies of genetics of deafness, families with inherited hearing loss have been extremely valuable, providing paths to discovery of genes essential to mechanisms of hearing. Until now, such studies have depended on Sanger sequencing of genes in candidate genomic linkage regions. Constraints of PCR-based sequencing have largely limited gene discovery to conventional mutations in known genes in relatively small regions of linkage. The advent of next generation sequencing technology has the potential to dramatically accelerate the pace of gene discovery.

We have undertaken three approaches with next generation sequencing to discover novel genes responsible for hearing loss in families, regardless of whether the phenotype is dominant or recessive and for any size family. (1) For families in which hearing loss maps to linkage regions of less than 3 megabases, we tile the entire genomic interval on capture arrays. DNA fragment libraries from the hearing impaired probands are hybridized to the arrays and the enriched portion of the genome is sequenced to approximate 30 fold coverage to reveal the critical allele. (2) For small nuclear families in which hearing loss maps to one or more very large linkage regions, we capture the entire coding region or 'exome' and approximately 400 miRNAs with in-solution oligonucleotides, sequence to approximately 30 fold coverage, and evaluate all functional variants co-inherited with the phenotype. (3) In order to identify all classes of structural variants – inversions, translocations, duplications, or deletions – in families unresolved by other methods, we construct large-insert mate-pair libraries from the proband and sequence the entire genome to high clone coverage. Structural mutations can be resolved from discordant mate-pairs, in which orientation, strand location, or distance apart differs from expected values. The results emerging from these studies indicate that many more novel genes for hearing loss will be discovered in the near future.

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1004 Two Novel Deafness Genes Identified by Homozygosity Mapping in Dutch Families

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The locus heterogeneity of autosomal recessive nonsyndromic hearing loss (arNSHI) is large. So far about 30 genes have been identified but for more than 50 loci, the causative genes are still elusive. Since loci are generally determined in one or a few large consanguineous families, their size is often 10-30 Mb and although next generation sequencing enables the simultaneous sequencing of exons and regulatory regions of all genes within a region, strategies to delimit the critical region and "intelligent" candidate gene selection remain attractive for disease gene identification. In the Netherlands, there are a number of well known genetic isolates. Although the remaining part of the population is regarded to be mixed there are quite a number of regions with limited migration until about 1950. Therefore, we followed a strategy of homozygosity mapping with high density SNP arrays in 125 patients with putative arNSHI from 77 families to delimit the critical region of known deafness loci and/or identify novel loci. Mutations in GJB2 had been excluded prior to the analysis. Homozygous regions smaller than 1 Mb were not further investigated. We were able to delimited the critical region for DFNB25 to an 0.8 Mb interval harbouring exons of two genes. Also, we obtained indications for a novel locus of 3.2 Mb with 11 known and predicted genes. Mutation analysis of all exons in the DFNB25 interval and selected candidate genes in the novel locus revealed putatively pathogenic mutations in four families. The DFNB25 gene was found to be mutated in two additional families of Pakistani origin with linkage or suggestive linkage to the interval. The phenotype associated with DFNB25 can be progressive and childhood onset of vestibular dysfunction can occur. Our results indicate that the demographic structure of the Dutch population is favourable for this method which is likely to be the case in more European countries.

1005 A Novel Mutation in a PDZ-Containing Protein in Black Swiss Mice Causes Outer Hair Cell Defects and Progressive Hearing Loss

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As early as 4 weeks of age Black Swiss (BLSW) mice show a hearing deficit with ABR threshold shifts of 30-40 dB SPL for all four tested stimuli (click, 8, 16, 32 kHz) and reduced DPOAEs. Whole mount preparations of the organ of Corti at postnatal day 3 though postnatal day 7 revealed a stereociliary outer hair cell bundle defect in these mice. By 12 months BLSW mice are almost deaf and there is an extensive degeneration in the inner ear suggestive of a progressive phenotype. By analysis of a (BLSW x Cast/Ei) x BLSW backcross we mapped a quantitative trait locus (QTL), *ahl5*, on MMU10, as the major contributor to the hearing deficit. Using *ahl5* interval specific congenic lines we delimited the *ahl5* QTL to a 2Mb region on MMU10. To map the *ahl5* mutation, coding exons within the interval were sequenced and a single base substitution, resulting to an amino acid change, was identified in a gene (*Ahl5*gene) encoding a PDZ-containing protein, which is expressed in both inner and outer hair cells. Protein alignment among different mammalian species showed that the mutation occurs in a highly conserved region within the PDZ domain of the protein most likely causing inability to bind through its PDZ domain to form complexes. Using *Ahl5* transgenic mice, we confirmed that the *Ahl5* gene mutation is responsible for the hearing loss of the BLSW mice. In addition to their hearing deficit, white noise at the level of 90-110dB, generate audiogenic seizures (AS), which appear to reduce in severity with age. Since all *ahl5* specific congenic lines displayed the AS phenotype and no other mutation was identified within the 2Mb region we are currently investigating whether the Ahl5 mutation is responsible for this susceptibility to AS and the potential link between the two phenotypes. So far there has been no record linking this gene to any hearing impairment phenotype elucidating the function of the mutated protein will unravel new pathway(s) involved in the complex process of hearing.

1006 Otolaryngologic Manifestations of FGF3 Mutations

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Fibroblast growth factors are a family of proteins encoded by 22 different FGF genes, some of which have been implicated in vertebrate inner ear development. Recessive mutations of fibroblast growth factor 3 (FGF3) have been associated with LAMM syndrome [OMIM 610706], characterized by complete labyrinthine aplasia, microtia and microdontia. These features were fully penetrant in all previously reported subjects with FGF3 mutations (Tekin et al, 2007; Tekin et al., 2008; Alsmadi et al., 2008). Whereas these patients have no detectable inner ear structures, Fgf3 knockout mice are reported to have normal or mildly abnormal inner ears with variable penetrance and expressivity (Alvarez, 2003; Hatch, 2007, Mansour, 1993). Here we describe the clinical phenotype of additional families segregating hearing loss linked to chromosome 11q13.3-q13.4 in which the FGF3 gene resides. Ten affected individuals from three large families segregating FGF3 mutations were studied with CT and/or MRI scans to detect inner ear abnormalities. One family segregated a previously reported mutation (p.R104X) and two families segregated novel mutations (p.R95W; p.R132GfsX26) of FGF3. All individuals homozygous for p.R104X or p.R132GfsX26 had fully penetrant features of LAMM syndrome. However, all individuals homozygous for p.R95W showed hearing loss without microtia or prominent microdontia. Moreover, partial development of the labyrinth was observed in two p.R95W homozygotes, including one with cochlear basal turn, vestibule, and posterior semicircular canal, while the other four p.R95W homozygotes had no detectable inner ear structures. Therefore, we conclude that the manifestations of FGF3 mutations can range from nearly non-syndromic deafness with variable inner ear structural development to LAMM syndrome.

1007 Hypomorphic Mutation of Barttin Is Associated with Nonsyndromic Deafness DFNB73

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Antenatal Bartter syndrome comprises a heterogeneous group of autosomal recessive salt-losing nephropathies. Mutations in *BSND*, in contrast to the other genes for Antenatal Bartter syndrome, are associated with sensorineural deafness and significant renal abnormalities. *BSND* encodes barttin which is an accessory subunit of two human ClC-K channels that are essential for chloride absorption along the distal nephron, and for endolymph formation in the inner ear. We mapped a recessively inherited nonsyndromic deafness phenotype in four Pakistani kindreds to a 4.04-cM locus (*DFNB73*) on chromosome 1p32.3. We identified the cause of isolated hearing loss segregating in these families as a novel mutation in *BSND* resulting in the substitution of a threonine for a conserved isoleucine (p.I12T). Moreover, in one sibship of family PKDF815, p.I12T in compound heterozygosity with p.E4X, a loss of function mutation, results in mild renal failure in addition to hearing loss. We characterized the functional consequence of p.I12T on ClC-K/barttin channel properties. p.I12T barttin associates with ClC-K, promotes surface insertion and switches the anion channel to an active form. p.I12T leaves ion conduction and gating unaffected, but does interfere with chaperone function of barttin in intracellular trafficking resulting in a small but significant reduction in ClC-K/barttin currents. Our results demonstrate that p.I12T is a hypomorphic allele of Barttin sufficient for renal function but not sufficient for normal hearing.

1008 Partial Loss-Of-Function Mutations in the PRPS1 Gene Cause Non-Syndromic X-Linked Sensorineural Deafness (DFN2)

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Non-syndromic deafness is highly heterogeneous with the vast majority of cases associated with mutations in autosomal genes. The contribution of X-linked cases has been estimated between 1% and 5%. Four X-linked non-syndromic deafness loci have so far been mapped. The DFN2 locus was mapped to Xq13-q24, and defined by three different families. Here, we report a five generation Chinese family (GZ-Z052), affected by a postlingual profound sensorineural deafness. All phenotypes of this family are clinically consistent with non-syndromic sensorineural hearing loss (DFN2). By haplotype analysis, we placed the locus within a 5.4 cM genetic interval defined by markers DXS8020 and DXS8055, overlapping with the DFN2 locus. Mutations screening of the phosphoribosyl pyrophosphate synthetase 1 (PRPS1) gene in family GZ-Z052 and in the three originally reported DFN2 families led to the identification of four different missense mutations in PRPS1. None of the affected individuals in family GZ-Z052 had been diagnosed with gout. They have neither hyperuricemia nor hyperuricosuria. We found no difference in expression of PRPS1 assessed by quantitative PCR, in which the average expression of the gene from affected males was compared with the average expression from unaffected male members and from control individuals. Furthermore, our data showed that in erythrocytes from affected males, the PRPS1 activity was significantly lower than that determined in the control cells. Finally, by in situ hybridization we presented evidence that Prps1 was expressed in both vestibular and cochlea hair cells, with continuous expression in hair cells and postnatal expression in the spiral ganglion cells. Overall, our findings emphasize the need for tight regulation and balance of PRPP synthetase activity and that decrease activity can lead to non-syndromic deafness. As the metabolic enzyme critical for nucleotide biosynthesis, the PRPP synthetase 1 is a promising target for drug in prevention and treatment of hearing loss.

1009 Genome-Wide Screening for Genetic Loci Associated with Noise-Induced Hearing Loss

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Abstract Noise-induced hearing loss (NIHL) is one of the more common sources of environmentally induced hearing loss in adults. In a mouse model, Castaneous (CAST/Ei) is an inbred strain that is resistant to NIHL, while the C57BL/6J strain is susceptible. We have used the genome-tagged mice (GTM) library of congenic strains, carrying defined segments of the CAST/Ei genome introgressed onto the C57BL/6J background, to search for loci modifying the noise-induced damage seen in the C57BL/6J strain. NIHL was induced by exposing 6-8-week old mice to 108 dB SPL intensity noise. We tested the hearing of each mouse strain up to 23 days after noise exposure using auditory brainstem response (ABR). This study identifies a number of genetic loci that modify the initial response to damaging noise, as well as long-term recovery. The data suggest that multiple alleles within the CAST/Ei genome modify the pathogenesis of NIHL and that screening congenic libraries for loci that underlie traits of interest can be easily carried out in a high-throughput fashion.

1010 A Knock-In Mouse Model for DNFA20 Deafness

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Ten dominant missense mutations in gamma-actin (ACTG1) have been identified as the cause of hearing loss in DFNA20 families. Although the mutations are located in different functional domains of gamma-actin, the end result is a progressive form of non-syndromic sensorineural hearing loss beginning in the high frequencies with an onset in the second to third decade of life. This shared phenotype is indicative of a common functional deficit in mutant gamma-actin protein function (Zhu et al 2003). To address the question of whether these mutations cause hearing loss via a loss of function versus a dominant negative mode of action, we generated a knock-in mouse model for the P264L mutation.

Mice harboring the P264L allele of ACTG1 in both the heterozygous and homozygous state born at the expected Mendelian ratios, remain viable, and do not have noticeable vestibular deficits. Initial characterization of hearing shows high frequency loss in mice heterozygous for the P264L allele by 6-8 weeks of age. Mice homozygous for the P264L allele exhibit a nearly complete hearing loss by 6 weeks of age that corresponds with a loss of outer hair cells in the apical turn and a complete loss of all hair cells in the basal turn. Compared to the previously characterized ACTG1-null mice which have reduced viability and muscular myopathy (Belyantseva et al 2009), the P264L mutation produces a much earlier hearing loss in mice and fewer pleiotropic effects are observed. Our data suggest a dosage-dependent, dominant negative mode of action for this mutation.

1011 Otitis Media in a Mouse Model for Down Syndrome

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The Ts65Dn mouse shares many phenotypic characteristics of human Down syndrome. Here, we report that otitis media, characterized by effusion in the middle ear and hearing loss, was prevalent in Ts65Dn mice. Of the 53 Ts65Dn mice tested, 81.1% had high auditory-evoked brainstem response (ABR) thresholds for at least one of the stimulus frequencies (click, 8 kHz, 16 kHz and 32 kHz), in at least one ear. The ABR thresholds were variable and showed no tendency toward increase with age, from 2 to 7 months of age. Observation of pathology in mice, aged 3-4 months, revealed middle ear effusion in 11 of 15 Ts65Dn mice examined, but only in two of 11 wild-type mice. The effusion in each mouse varied substantially in volume and inflammatory cell content. The middle ear mucosae were generally thickened and goblet cells were distributed with higher density in the epithelium of the middle ear cavity of Ts65Dn mice as compared with those of wild-type controls. Bacteria of pathogenic importance to humans also were identified in the Ts65Dn mice. This is the first report of otitis media in the Ts65Dn mouse as a model characteristic of human Down syndrome.This work was supported by NIH grants R01DC007392,R01DC009246 and N01HD73265.

1012 Proteomics and Bioinformatics Analysis Reveals Up-Regulation of Cochlin in the Cochlea of Usher 1F Mouse Model

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Proteins and protein networks associated with cochlear pathogenesis in the Ames waltzer (*av*) mouse, a model for deafness in Usher syndrome 1F (USH1F), were identified. Cochlear protein from wild-type and *av* mice at postnatal day 30, a time point in which cochlear pathology is well established, were analyzed by quantitative 2D-gel electrophoresis followed by mass spectrometry (MS). The analytic gel resolved ~ 2,300 spots; nearly 60 spots showed significant changes in intensity in the *av* cochlea compared to the control. The cochlin protein was identified in 20 peptide spots, most of which were up-regulated while others were down-regulated. Analysis of MS sequence data showed that, in the *av* cochlea, a set of full-length isoforms of cochlin was up-regulated, while isoforms missing the N-terminal FCH/LCCL domain were down-

regulated. Protein interaction network analysis of all protein targets indicated as changing was performed with Metacore software. That analysis revealed a number of statistically significant candidate protein networks predicted to be altered in the affected cochlea. Quantitative PCR (qPCR) analysis of select candidates from the proteomic and bioinformatics investigations showed up-regulation of *Coch* mRNA and those of p53, Brn3a and Nrf2, transcription factors linked to stress response and survival. Increased mRNA of Brn3a and Nrf2 has been previously associated with increased expression of cochlin in human glaucomatous trabecular meshwork. Our report strongly suggests that increased level of cochlin is an important etiologic factor leading to the degeneration of cochlear neuroepithelia in the USH1F model. Since other mouse models for deafness in Usher type 1 (e.g. USH1B, 1C, and 1D) show a similar degenerative profile as the USH1F model, namely the loss of hair cells followed by the loss of spiral ganglion cells, the proteomic analysis reported here could have implications for Usher syndrome type 1.

1013 Interaction of Aminoglycosides with Human Mitochondrial 12S Ribosomal RNA Carrying the Deafness-Associated Mutation

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Mitochondrial 12S rRNA A1555G mutation is one of the important causes of aminoglycoside-induced and nonsyndromic hearing loss. Here, we employed a RNA-directed chemical modification approach to understand pathogenesis of aminoglycoside-induced hearing loss. The patterns of chemical modifications in the RNA oligonucleotides carrying the A1555G mutation by dimethyl sulphate were distinct from those of wild-type version, in the presence of aminoglycosides. I n the RNA analogue carrying the A1555G mutation, the reduced reactivity towards DMS occurred in the bases G1555 as well as C1556 and A1553 in the presence of paromomycin, neomycin, gentamicin, kanamycin, tobramycin and streptomycin. In particular, the base G1555 exhibited the marked but similar levels of protection in the presence of 0.1 microM to 100 microM of neomycin, gentamicine and kanamycin. By contrast, the levels of protection in the base G1555 appeared to be correlated with the concentration of paromycin, tobramycin, and steptomycin. Furthermore, the increasing reactivities toward these probes in the presence of these aminoglycosides were observed in the bases A1492, C1493, C1494 and A1557 in the RNA analogue carrying the A1555G mutation. These data suggested that the A1555G mutation altered binding properties of aminoglycosides at the A-site of 12S rRNA and led to local conformation changes in the 12S rRNA carrying the A1555G mutation. The interaction between aminoglycosides and 12S rRNA carrying the A1555G mutation provides new insight into pathogenesis of aminoglycoside ototoxicity.

1014 Genetic Protection Against Deafness Caused by Hypothyroidism

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Congenital hypothyroidism causes hearing impairment in humans and mice. Thyroid hormone (TH) has pleiotropic effects on cochlear development, and genomic variation influences the severity of the hearing problem. *Prop1*^{df} and *Pou1f1*^{dw} mutant mice lack pituitary thyrotropin, which causes severe TH deficiency and variable hearing impairment, depending on the genetic background (Karolyi et al., 2007). DW-*Pou1f1*^{dw} mutants are profoundly deaf and exhibit delayed development of the organ of Corti, permanently reduced potassium channel gene expression and function, and other abnormalities (Mustapha et al., 2009). In contrast, DF-*Prop1*^{df} mutants have very mild hearing impairment. Because the critical period for TH replacement is late gestation and early neonatal life, we assessed the contribution of the maternal environment by transferring mutant eggs to surrogate mothers. The vastly different effects of hypothyroidism on hearing in *Prop1*$^{df/df}$ and *Pou1f1*$^{dw/dw}$ mice were maintained in the progeny born from surrogates, suggesting that genetic background effects are intrinsic to the fetus, rather than an influence of maternal TH during gestation or lactation. To assess the genetic complexity of protective effects, an F1 intercross was generated between *Pou1f1*^{dw} carriers and an inbred strain with excellent hearing, *Mus castaneus*. Approximately 16% of the mutant progeny had normal hearing. A genome scan of these individuals revealed a locus on chromosome 2, named modifier of dw hearing, *Mdwh*, that rescues hearing despite persistent hypothyroidism. This chromosomal region contains a modifier of *Tubby* hearing (*Moth1*) that encodes a protective allele of the microtubule-associated protein *Mtap1a* (Ikeda et al., 2002). We crossed *Pou1f1*^{dw} carriers with two strains that carry protective alleles of *Mtap1a* and found that 129/Ola is protective for dw hearing, while AKR is not. Thus, protective alleles of *Mtap1a* are not sufficient to rescue dw hearing. Microarray analysis identified cochlear gene expression changes caused by hypothyroidism in *Pou1f1*^{dw} mice. Some of these are positional candidates for the modifier gene. We expect that identification of protective modifiers will enhance our understanding of the mechanisms of hypothyroidism-induced hearing impairment.

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1015 Knockdown of Cochlear NADPH Oxidase Isoform (NOX3) by SiRNA Attenuates Cisplatin Ototoxicity

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Cisplatin is used to treat solid tumors. Dose limiting side effects include nephrotoxicity and ototoxicity. Recent studies indicate that generation of reactive oxygen species (ROS) via the cochlear-specific NADPH oxidase isoform,

NOX3, contributes to cisplatin ototoxicity. Also, knockdown of NOX3 by short interfering (si) RNA reduced cisplatin-induced apoptosis of an organ of Corti cell line, UB/OC-1. We tested the effect of NOX3 knockdown by trans-tympanic injection of siRNA on cisplatin-induced ototoxicity in the rat. Different concentrations of NOX3 siRNA were administered 48 h prior to cisplatin (11mg/kg, i.p). Hearing loss was measured by auditory brainstem responses (ABRs) and scanning electron microscopy (SEM) was used to assess hair cell integrity. Cisplatin increased ABR thresholds by 30-40 dB by 72 h. Trans-tympanic administration of NOX3 siRNA significantly reduced the magnitude of hearing loss 3, 5 and 7 days post cisplatin administration. Cisplatin produced ~50% loss of outer hair cells (OHC) in the organ of Corti, which was substantially reduced (~5% loss) by NOX3 siRNA. Thus, pretreatment of the cochlea with trans-tympanic NOX3 siRNA can reduce cisplatin ototoxicity. Previous studies have implicated several genes, such as transient receptor potential (TRPV1), NOX3, kidney injury molecule 1 (KIM1) and inducible nitric oxide synthase (iNOS) in cisplatin ototoxicity. Immunohistochemical studies indicate an increase in NOX3, TRPV1, KIM1 and iNOS proteins by ~2-3 fold in OHC's, stria vascularis and spiral ganglion cells and qRT-PCR studies showed increased mRNA encoding NOX3 (~6 fold), TRPV1 (~5.5 fold), KIM1 (~3.8 fold) and iNOS (~6 fold), which were attenuated by NOX3 siRNA, indicating that NOX3 siRNA also reduces molecular correlates of cisplatin ototoxicity. Knockdown of NOX3 expression by trans-tympanic siRNA provides an effective method of delivery. This could provide otoprotection in cancer patients treated with cisplatin-based chemotherapy.

1016 The Role of CTR1 in Platinum-Induced Ototoxicity

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This effort was aimed toward the determination of the role of an influx transporter, CTR1, in cisplatin-induced ototoxicity. The application of RT-PCR, quantitative RT-PCR (qPCR), Western blot and immunohistochemistry studies revealed that mouse CTR1 (Ctr1) is abundantly expressed and highly localized at the primary sites of cisplatin toxicity in the inner ear, mainly outer hair cells (OHC), inner hair cells (IHC), stria vascularis (SV), spiral ganglia (SG) and surrounding nerves of the mouse cochlea. A CTR1 substrate, copper sulfate decreased uptake and cytotoxicity of cisplatin in HEI-OC1, a cell line expressing molecular markers reminiscent of OHCs. siRNA-mediated knockdown of Ctr1 in this cell like caused a corresponding decrease in cisplatin uptake. Intratympanic administration of copper sulfate 30 minuntes before intraperitoneal administration of cisplatin was found to prevent hearing loss at click and 8, 16 and 32 kHz frequencies. These results suggest that chemical modulation of CTR1 function through inhibitors such as copper sulfate can be useful in ameliorating the cochlear uptake and hence the ototoxicity of cisplatin. The possibility of local administration of CTR1 inhibitors during cisplatin therapy as a means of otoprotection is raised.

1017 Role of P53 Signaling in Cisplatin Ototoxicity

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Despite tremendous efforts and over 30 years of drug development, the ototoxic drug, cisplatin, continues to be a widely used agent for treating solid tumors. In order to search for effective otoprotective treatments that may be administered before or with cisplatin, it is important to determine early cellular responses. Previously, we identified several p53 transcriptional targets up-regulated in rat cochleae only 48 h after cisplatin administration. Here, we report results of antibody microarray proteomic screening at 24 h. Promoter analysis of genes coding for proteins whose expression was altered by cisplatin at 24 and 48 h suggest that transcription factors p53, c-jun and NF-kB may dominate gene regulation at these early time points. Several cisplatin-induced proteins were identified that were not previously associated with the onset of ototoxicity. One, cellular prion protein, a p53 substrate, is of particular interest for its involvement in metal binding, cell survival and apoptosis. The data indicate the importance of the regulation of the p53-mediated transcriptome. We acknowledge support from the National Organization for Hearing Research Foundation (DC), Deafness Research Foundation (DC), and NIH (R01DC006630, R01DC00909101, RS).

1018 Activation of Transient Receptor Potential Vanilloid 1 in the Cochlea Induces Hearing Loss in the Rat by Activating STAT 1

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Previous studies have implicated the transient receptor potential vanilloid 1 (TRPV1) in mediating cisplatin ototoxicity. Activation of TRPV1 by cisplatin increased generation of reactive oxygen species (ROS) and intracellular Ca^{2+} accumulation, contributing to outer hair cell (OHC) death in the cochlea. In addition, activation of TRPV1 is believed to facilitate the entry of cisplatin into the OHCs. As such, knockdown of TRPV1 by short interfering (si) RNA protects against cisplatin ototoxicity by suppressing ROS/Ca^{2+} pathway and/or the entry of cisplatin into the cell. In the current study, we examined the consequence of direct activation of TRPV1 on cochlear function in the rat. Trans-tympanic injections of capsaicin to the rats produced transient hearing loss, as assessed by ABR threshold shifts (~25dB) at 24 h and returned to baseline at 72h. Scanning electron microscopy showed no OHC damage with capsaicin treatments. In *in vitro* cultures, we observed that capsaicin increased intracellular Ca^{2+} accumulation, ROS generation and activated signal transducer and activator of transcription 1 (STAT1). The

activation of STAT1 was dependent on ROS generation mediated via the NOX3 isoform of NADPH oxidase, since knockdown of this enzyme by short interfering (si) RNA attenuated capsaicin-mediated STAT1 activation. To examine a possible role of STAT1 in capsaicin-mediated transient hearing loss, transtympanic injections of STAT1 siRNA were performed. Protection from temporary capsaicin induced hearing loss was seen in rats treated with siSTAT1. In addition, STAT1 siRNA treatment also abrogated cisplatin induced hearing loss in rats. STAT1 siRNA also reduced the degree of inflammation in the cochlea, as demonstrated by reduced levels of capsaicin-stimulated inducible nitric oxide synthase (iNOS), and cyclooxygenase-2 (COX-2). Taken together, these results support a role of TRPV1 activation in mediating cisplatin ototoxicity by activating a STAT1-dependent inflammatory process in the cochlea.

1019 Screening for Ototoxicity in Anti-Cancer Drugs Using the Zebrafish Lateral Line

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Anti-cancer drugs, while effective at killing cancer cells, often have additional toxicities to other cells in the body. In the inner ear, ototoxic effects are well known for anti-cancer drugs such as cisplatin, vincristine and vinblastine. These drugs were identified after anecdotal reports of hearing loss led to more systematic review. Currently, there is no standard drug screen for ototoxic effects during drug development.

We hypothesized that there are likely other cytotoxic anti-cancer drugs currently used on humans that have occult ototoxic effects. Previous studies have demonstrated the utility of the zebrafish lateral line in screening for ototoxic drugs (Chiu et al. 2008). This study describes a screen of a National Cancer Institute (NCI) library composed of FDA-approved anticancer drugs (Approved Oncology Drugs Plated Set consisting of 88 drugs) for drugs that cause hair cell death in the zebrafish lateral line. The goal of the screen is to identify potential novel ototoxic drugs, and possibly establish "ear-safety" in the drugs not identified by the screen. Five days post-fertilization zebrafish (Danio rerio) larvae of the AB wild type strain were labeled with YO-PRO-1 to selectively label hair cell nuclei of the lateral line. Labeled fish were then exposed to anti-cancer drugs for 1 or 6 hours followed by fluorescence microscopy imaging. Preliminary screening results identified 6 out of 7 well-established ototoxins (well documented hearing loss or hair cell loss), and 4 out of 6 less-established ototoxins (single case reports of hearing loss). Dose-response relationships were established for novel potentially ototoxic drugs.

1020 A New *In Vivo* Mouse Model of Cisplatin Ototoxicity

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Cisplatin is a chemotherapeutic drug that is ototoxic. Several model systems have been established for investigating cisplatin-induced hair cell death, including *in vivo* models in rats and guinea pigs. To investigate the roles of specific genes in cisplatin ototoxicity, an *in vivo* mouse protocol would allow for the use of transgenic and knockout animals.

We developed a cisplatin treatment protocol that induces reproducible hearing loss and hair cell death in adult mice. Baseline auditory brainstem response (ABR) thresholds were measured in female 129S6 mice (age 6-8 wks.). Mice were then treated with 16 mg/kg cisplatin or saline (controls) by intraperitoneal (IP) injection. Boluses of sterile saline (0.3-0.5 ml) were given IP at 90 min. prior to cisplatin and 24 and 48 hours later. Repeat ABR testing and tissue collection were performed 72 hrs. after cisplatin injection. This treatment protocol resulted in statistically significant ABR threshold shifts, with a mean threshold shift of 19 dB (± 4 SE) in response to clicks. For pure-tone stimuli, there were mean threshold shifts of 14 dB (± 4 SE) at 16 kHz and 24 dB (± 3 SE) at 32 kHz. There were no significant threshold shifts in control animals. Cisplatin-treated mice showed high variability in their response to anesthesia at 72 hrs. following cisplatin treatment, requiring anywhere from 60 to 200% of the usual dose for ABR testing (ketamine 130 mg/kg, xylazine 8.8 mg/kg). However, the required dose did not correlate with the degree of hearing loss. Histologically, cisplatin caused a 45% loss of outer hair cells in the mid-base of the cochlea while outer hair cells in the apex were preserved, and inner hair cells were preserved throughout. There was no hair cell loss in control animals. Mortality was 0%. This new model system can be used to investigate cisplatin ototoxicity in genetically manipulated adult mice.

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1021 Dosage Dependent Effects of Neomycin on Spiral Ganglion Cells in a Guinea Pig Model

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After severe and/or long term hair cell (HC) loss, neurites recede from the auditory epithelium, and spiral ganglion cells (SGC) may degenerate. The SGC degeneration is more common and severe in animal models than in humans. Thus, current animal models are less than optimal for studies of SGC survival and cochlear implant function. In an attempt to generate a model where HCs are lost but SGCs survive, we tested whether the loss of neurons is a direct effect of an aminoglycoside or

secondary to the loss of HCs. Neomycin, which eliminates HCs and most SGCs when injected into the cochlea at a 10% solution, was used to determine if lower concentrations would kill HCs and spare SGCs. We injected neomycin into the right cochlea through the round window membrane of 22 pigmented guinea pigs in 4 dosage groups: 10% (N=3), 5% (N=7), 1% (N=8) and 0.5% (N=4). The left ears were used as non-deafened controls. All subjects were sacrificed at 7 days. Some cochleae were labeled for neurofilament and actin and prepared as whole mounts; others were decalcified, embedded in plastic, and sectioned for SGC counts. The 0.5% neomycin did not induce HC loss. Higher concentrations produced flat epithelium without HCs or differentiated supporting cells (SC) in at least the first (basal) turn. Survival of HCs and SCs in more apical turns decreased as dosage increased. Peripheral neurites were absent from flat epithelia, but persisted in scarred or intact epithelia. 1% neomycin produced some loss of SGC, but significantly less than 5% or 10% ($p<0.01$). Also, 1% neomycin resulted in significantly greater SGC loss in the 1st turn than in the 3rd ($p<0.05$). Thus, some SGCs may survive low doses of neomycin for at least a short time after loss of HCs. Additional long-term studies are needed to determine whether SGCs can survive in the absence of HCs following 1% and 5% neomycin.

1022 Aminoglycoside Induced Hair Cell Death in Neonatal Rat Cochlea Explants Is Dependent on the Extracellular Calcium Concentration

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In mammals aminoglycosides antibiotics cause irreversible deafness by triggering hair cell death. The primary entry route for aminoglycosides is thought to be the transduction channel and Ca^{2+} competes for that entry (Gale et al 2001, Marcotti et al 2005). In the zebrafish lateral line the concentrations of external Ca^{2+} ($[Ca^{2+}]_{ext}$) and Mg^{2+} modulate aminoglycoside-induced hair cell death (Coffin et al 2009). Here we show that aminoglycoside-induced cochlear hair cell death depends upon $[Ca^{2+}]_{ext}$. We exposed cochlear cultures from 3-4 day old rats to neomycin (1mM for 6 hours) in varying, 0.1 to 10 mM $[Ca^{2+}]_{ext}$. We assayed cell death by staining DNA with DAPI and confirmed pyknosis in hair cells by double-labelling with anti-MyosinVIIa. The amount of hair cell death was dependent upon $[Ca^{2+}]_{ext}$. Reducing $[Ca^{2+}]_{ext}$ to 0.1 mM caused a 24-fold increase in neomycin-induced hair cell death (inner and outer hair cell combined). However, in 0.1 mM Ca^{2+} controls (without neomycin) we observed pyknosis in a small but significant number of cells (5-fold increase compared to 1.25mM Ca^{2+}). Moreover, it was clear that many hair cells were damaged after 6 hours in 0.1 mM Ca^{2+}. It is possible that reducing $[Ca^{2+}]_{ext}$ below a certain threshold (between 0.5 and 0.1 mM) affected *inter*cellular junctions, and/or by reducing store-operated Ca^{2+} entry, negatively impacted on *intra*cellular Ca^{2+}. Finally, we co-stained all preparations with fluorescent phalloidin. We observed prominent f-actin-rich, ring-like structures surrounding pyknotic hair cell nuclei. 3D projections of confocal stacks revealed these structures to be basket, or calyx-like. We quantified these actin-rich structures in our neomycin experiments and found a high correlation coefficient (0.95) with the number of pyknotic nuclei. The actin did not colocalise with myosinVIIA indicating that it was supporting-cell rather than hair-cell actin. Thus, in agreement with live-imaging data from the chick utricle (Bird et al, ARO abstract 2008), supporting cells in the mammalian cochlea are able to phagocytose hair cells and their nuclei. In summary, these data support the hypothesis that in hair cells the primary route of aminoglycoside-entry is via the transduction channels.

1023 Aminoglycosides Enter Hair Cells Via Both Endocytosis and the Transduction Channel

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The mechanism by which aminoglycosides enter hair cells (HCs) is a matter of debate. There is evidence in other cell types that these antibiotics bind to one or more receptors on the cell surface and are internalized during receptor endocytosis. It has been proposed that a similar mechanism operates in HCs. Alternatively, others have suggested that aminoglycosides bind to the mechanoelectrical transduction (MET) channel of the HC, and transit the channel to enter the cell. Procedures that block the MET channel have previously been reported to reduce aminoglycoside toxicity. We explored whether the MET channel or endocytosis provide routes of entry for aminoglycosides into the HC. We determined the effect of inhibitors of transduction channel opening, or of three different forms of endocytosis, on the toxicity of gentamicin to neonatal mouse HCs in vitro. A dose of gentamicin comparable to that observed in perilymph in vivo was employed. Brief treatment with either BAPTA or elastase was used to disrupt HC tip links and to close the MET channel. Chlorpromazine was used to inhibit clatherin-mediated endocytosis, the form most associated with receptor endocytosis. Filipin was used to inhibit lipid raft-mediated endocytosis, also associated with the internalizetion of receptors as well as phosphoinositol-linked proteins and certain toxins via either calveolin-dependent or calveolin-independent processes. Endocytosis inhibitors were used in concentration ranges ending in the highest dose that did not produce damage to the HCs when used alone. HCs were pre-treated with an endocytosis inhibitor, then treated with gentamicin plus inhibitor for 72 hours. BAPTA and elastase treatment was limited to 10 minutes per day, followed by rinsing and replacement of the media. Treatment with either BAPTA or elastase significantly reduced the toxicity of gentamicin, but did not eliminate it. Treatment with chlorpromazine also partially, but significantly, protected HCs. Treatment with

filipin had no effect on gentamicin toxicity. The results suggest that aminocglysides can enter HCs both through the transduction channel and via endocytosis.

1024 Too Much of a Good Thing: Long-Term Treatment with Salicylate May Strengthen Outer Hair Cell Function But Damage Auditory Sensitivity

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Aspirin has been extensively used in clinical settings. Its side effects on auditory function, including hearing loss and tinnitus, seem only a temporary event. A recent promising finding is that a chronic treatment with a high-dose salicylate (the active ingredient of aspirin) enhances expression of the outer hair cell (OHC) motor protein (prestin), resulting in a strengthened OHC electromotility and distortion product otoacoustic emissions (DPOAEs). We have used salicylate in our lab for two different purposes: (1) to strengthen prestin expression for restoration of cochlear function; and (2) to induce tinnitus in an animal model. Unexpectedly, a permanent loss of cochlear sensitivity was often observed after the chronic salicylate treatment. In the current experiment, rats of different strains and ages were injected with salicylate at a dose of 200 mg/kg/day for 5 days per week for 3 weeks or at higher dose levels (250-350 mg/kg/day) for 4 days per week for 2 weeks. The salicylate treatments induced a permanent reduction of auditory brainstem response (ABR), cochlear compound action potential (CAP), and cochlear microphonics (CM), and also occasional OHC loss, while the DPOAEs were enhanced or remained at the pre-exposure levels. Interestingly, with an increased interval between injections from 1/day to once or twice per week, a similar amount of salicylate treatment as above enhanced DPOAEs, but did not show a salicylate-related ototoxicity. The mechanisms underlying the permanent salicylate ototoxicity are discussed. Hearing restoration may be achieved by using an optimized salicylate treatment paradigm.

1025 Protection of Hair Cells from Aminoglycoside Antibiotic Exposure in the *persephone* Mutant Zebrafish

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The death of mechanosensory hair cells underlies many forms of noise-, ototoxin- and age-induced hearing loss. One approach to prevent hearing loss is to look for modulators that prevent hair cell death. We previously reported a genetic screen identifying zebrafish mutants that retain lateral line hair cells following treatment with the ototoxic drug neomycin *(Owens et al, PLOS Genetics, 2008).* One of these mutants, *persephone (pers),* carries a fully penetrant recessive mutation that confers almost complete protection from neomycin- and gentamicin-induced hair cell death. Active fluorescently-labeled gentamicin readily enters *pers* hair cells, demonstrating that the mutation does not confer resistance by blocking drug uptake. Altered intracellular conditions therefore likely underlie aminoglycoside protection in *pers* mutants. The *pers* mutation maps to an uncharacterized protein referred to here as SLC4A1b--a homolog of the conserved SLC4A1 and SLC4A2 bicarbonate/chloride cotransporters. Mutant SLC4A1b in *pers* carries a missense mutation in the putative second transmembrane domain. Transiently expressed wild-type SLC4A1b tagged with GFP is readily transported to the plasma membrane of zebrafish embryo cells, while the majority of the mutant form is retained in the endoplasmic reticulum. We are further characterizing the trafficking of mutant SLC4A1b, and targeting genetically encoded indicators to the ER, mitochondria, and cytosol in wild-type and *pers* embryos. These indicators should reveal whether intracellular conditions of *pers* hair cells are altered by aberrant activity of the SLC4A1b transporter. We speculate that perturbations in intracellular bicarbonate or chloride inhibit cell death responses induced by aminoglycoside exposure in the *persephone* mutant.

1026 Protective Effect of Paracrine Factors Secreted by Adipose Tissue Derived Stromal Cells Against Aminoglycoside Ototoxicity

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Autologous stem/progenitor cells transplantation is emerging as a novel therapeutic option for hearing loss. Recent reports indicated that adipose tissues could supply pluripotent cells, adipose tissue derived stromal cells (ADSCs). ADSC transplantation was reported to show beneficial effect on ischemic diseases. It is thought that the effects of the therapy are due to not only the puluripotency of the cells but growth factors and chemokines the cells secrete. When we take such a paracrine effect into consideration, it is easier for us to use pluripotent cells derived from its own individual than ES cells in terms of carcinogenesis and immune response. First we determined whether ADSCs protect auditory hair cells via paracrine effects. ADSCs were harvested from C57BL/6 mice. Postnatal day 3 mouse cochlea epithelia ware placed on culture-mesh inserts and transferred to serum-free medium containing 1 mM neomycin or ADSC conditioned medium containing 1mM neomycin. After 24 hour incubation, histological evaluation was performed. As a result we observed that ADSC-conditioned medium significantly reduced hair cell loss due to aminoglycoside toxicity. Next we determined what kinds of factors are

produced by ADSCs. ADSCs were cultured in serum-free medium for 3 days. At the end of the culture period, the supernatant was collected for analyses. The levels of growth factors were measured using ELISA. As a result we observed that HGF, IGF-1, VEGF, NGF, TGF-β1 and MCP-1 are detectable in ADSC conditioned media. It has been reported that HGF, IGF-1, TGF-β1 have protective effects on inner ear hair cells against their injury. In conclusion we demonstrated that ADSCs have the potential of HC protection via paracrine of multiple growth factors and chemokines *ex vivo.*

1027 Adenoviral Transfection of Supporting Cells with Hsp70 Inhibits Hair Cell Death

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Ototoxic drugs such as aminoglycosides and cisplatin cause death of mechanosensory hair cells. We have previously shown that heat shock inhibits both aminoglycoside- and cisplatin-induced hair cell death in the adult mouse utricle. Heat shock protein 70 (Hsp70) is necessary for this protective effect, and overexpression of Hsp70 protects hair cells against both aminoglycoside and cisplatin toxicity. Taken together, these data indicate that Hsp70 induction is a critical stress response in the inner ear that can promote survival of hair cells exposed to major stressors. Recent analyses using confocal microscopy indicate that heat shock-induced Hsp70 is localized primarily in supporting cells, with little induction in hair cells. These data suggest that supporting cells mediate the protective effect of Hsp70 against hair cell death. We have recently developed a method of efficient and exclusive transfection of supporting cells of the adult mouse utricle using adenovirus. Using adenovirus type 5 (dE1/E3) to deliver GFP under the control of the CMV promoter, we find that adenovirus specifically transfects supporting cells. Supporting cell transfection efficiency is 43.6 ± 14%, and hair cells are not transfected. Importantly, we find that adenoviral transfection of supporting cells does not result in toxicity to hair cells or supporting cells, as cell counts in Ad-GFP transfected utricles are equivalent to those in non-transfected utricles. To begin to examine the role of supporting cells in mediating the protective effect of Hsp70 against ototoxic hair cell death, we transfected supporting cells with adenovirus containing Hsp70 driven by the CMV promoter (Ad-Hsp70). Utricles were treated with Ad-Hsp70 for 24 hours and then cultured in media containing neomycin (2mM) for an additional 24 hours and fixed. Transfection of supporting cells with Ad-Hsp70 inhibited neomycin-induced hair cell death. Ad-GFP served as a control and was not protective against neomycin-induced hair cell death. Because hair cells are not transfected with adenovirus, these data indicate that Hsp70 in supporting cells protects hair cells against ototoxic death. These data therefore suggest that supporting cells directly mediate hair cell survival. Supported by NIDCD R01DC07613.

1028 Bucillamine Attenuates Cisplatin-Induced Ototoxicity in HEI-OC1 Auditory Cells and Balb/c Mice

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Bucillamine is a dithiol-containing compound which has been used clinically in treatment of rheumatoid arthritis. In present study, we first tested the protective effects of bucillamine against cisplatin. In vitro studies, treatment with bucillamine significantly inhibited the apoptotic death, intracellular redox state and oxidative stress induced by cisplatin. The activity of caspase-3 and caspase-8 significantly increased in cisplatin-treated cells. However, pretreatment with bucillamine significantly inhibited the activity of caspase-3 and caspase-8. Also, bucillamine pretreatment significantly suppressed the generation of ROS such as hydroxyl radicals, nitric oxide, and superoxide anion in cisplatin-treated cells. It markedly increased the expression of mRNA and protein levels of phase II detoxifying or antioxidant enzymes, including heme oxygenase-1, NAD(P)H:quinine oxidoreductase, and superoxide dismutases through transactivation of Nrf2-ARE pathway. Intracellular antioxidant glutathione (GSH) contents significantly increased in bucillamine-treated cells through the induction of intracellular GSH biosynthesis enzymes, including γ-glutamylcysteine synthetase and glutathione synthetase. Current results with bucillamine applied in organotypic cultures of Corti's organ effectively protected the hair cell death of cochlear and markedly decreased the activation of caspase-3 induced by cisplatin. In vivo study, auditory brainstem evoked response (ABR) was determined in adult Balb/C mice receiving a single intraperitoneal injection of 16 mg/kg cisplatin with or without treatment with 100 mg/kg bucillamine for 3 days beginning 12 h before the cisplatin administration. ABR threshold was markedly increased hearing threshold shifts in response to various stimuli (click and tone bursts at 4, 8, 16 and 32 kHz) in cisplatin-injected mice. However, pretreatment with bucillamine significantly prevented the cisplatin-induced ABR threshold shift in vivo.
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1029 Is There a Physiological Third Window? Measurements of Human Cadaveric Intracochlear Differential Pressures for Round Window Stimulation with Fixed Stapes

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It has been hypothesized that the inner ear is a rigid structure filled with incompressible fluid, with only two flexible windows to produce differential pressure across the partition for transduction of sound. This hypothesis

has been supported with experiments in the past for the case of a normal inner ear with forward stimulation through the oval window. However, this evidence does not rule out the existence of a normal physiological third window that is small in conductance compared to the oval and round windows, such that the small third window influence is insignificant under normal conditions. In the case of reverse round window (RW) cochlear stimulation, fixation of the stapes should prevent cochlear transduction if the hypothesis held true. However, it has been reported that RW stimulation has improved hearing in patients with fixed stapes (Beltrame et al 2009). RW stimulation has potential as a treatment for stapes fixation when stapedectomy is contraindicated. Our experimental results in cadaveric temporal bone preparations show that reverse RW stimulation after fixation of the stapes resulted in higher pressures in both scalae, which were similar (within 1 dB) in magnitude. However, the calculation of the complex differential pressure revealed significant pressure difference, indicating achievable hearing. These results suggest that a clinically-relevant physiological third window likely exists in the scala vestibuli compartment. Possible fluid release mechanisms include the endolymphatic duct, neural and vascular channels, as well as the possibility that the bone surrounding the inner ear is not infinitely rigid throughout, and/or that the combination of having unequal volumes in scala vestibuli and scala tympani with slight fluid compressibility results in a physiological third window.

1030 Analysis of Power Flow in the Cochlea Based on Measurements with Optical Coherence Tomography

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With Optical Coherence Tomography (OCT) it is possible to display the optical reflectivity of the various layers of biological tissue. One step further is to measure vibrations, in our case of movements of several tissue layers inside the cochlea. With the OCT technique we have measured sound-induced vibrations of the basilar membrane (BM) and the Reticular Lamina (RL) in the basal turn of the cochlea of the guinea pig. With sinusoidal stimulation both responses are frequency-selective and nonlinear around the “best frequency” of approximately 18 kHz. For weak and moderately strong stimuli the RL response is consistently larger than the BM response, and the “best frequency” of the RL is somewhat higher than that of the BM. These findings force us to reconsider the dynamics of the central channel in the system, i.e., the channel of the Organ of Corti (OoC). With a certain degree of flexibility we have considered the Organ of Corti (OoC) as a channel closed at both ends, and on both sides bounded by the RL and the BM. By using “standard” hydrodynamics it proved possible to compute the power produced by the OHCs. In the peak region of the response we found that positive power is delivered to the BM, thus confirming the concept of cochlear amplification.

1031 Measuring the Backward Wave Propagation in the Cochlea in Vivo

WITHDRAWN

1032 Autoregressive Moving-Average Models of Basilar Membrane Responses to Broadband Noise

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Basilar-membrane responses to white Gaussian noise were recorded using laser velocimetry at basal sites of the chinchilla cochlea with characteristic frequencies near 10 kHz. First-order Wiener kernels were computed by cross-correlation of the noise stimuli and the response vibrations. Autoregressive moving-average (ARMA) filters, i.e., filters containing spectral zeros and poles, were fitted to the kernels in either the time or frequency domain. ARMA filters with transfer functions including zeros outside the unit circle, implying non-minimum-phase behavior, provided excellent fits to the kernels and accurately predicted basilar-membrane responses to other noise stimuli presented at the same level as the stimulus for the kernel computation. All-pole (autoregressive) minimum-phase filters provided less accurate fits.

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1033 Measurement of the Viscoelastic Mechanical Properties of the Tectorial Membrane at Acoustic Frequencies

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The tectorial membrane (TM) has been shown to have a critical role in hearing sensitivity and frequency selectivity. The main role of the TM in hearing is determined by its mechanical interaction with the tips of the stereocilia of the OHC. Therefore, a number of studies have used specialized probes such as Atomic Force Microscopy (AFM) or Magnetic Tweezers to characterize the mechanical properties of the TM. However, all these studies have been performed at quasi-static or moderate frequency regimes (0.1 to 9 kHz), only partially overlapping the frequency range of mammalian hearing. We have developed a novel technique to measure the viscoelastic mechanical properties of the TM at acoustic frequencies. A cantilever with an attached microsphere is forced to oscillate at tens of nanometers above the TM. The elastic modulus and viscosity are estimated by measuring the frequency-dependence of the phase lag between the oscillating microsphere and the driving piezo at various heights above the TM. Oscillations at ~30 kHz were used and samples of TM of guinea pig were measured at the base, middle and apex. Measured values for the Young’s modulus were 3.3 ± 2.3 kPa (apex), 5.7 ± 3.3 kPa (mid) and 14 ± 16 kPa (base), whereas values for the effective

viscosity were 0.0068 ± 0.0064 Pa-s (apex), 0.01 ± 0.008 Pa-s (mid) and 0.03 ± 0.04 Pa-s (base). These results provide evidence that the graded mechanical properties of the TM are conserved at acoustic frequencies. Moreover, incorporation of the viscoelastic properties of the TM at acoustic frequencies into quantitative models of cochlear mechanics will help understand the underlying mechanisms of hearing.

1034 Comparison of Basilar Membrane Measurements in Fresh and Frozen-Thawed Inner Ear Preparations

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Soft tissues in the middle and inner ear typically degrade rapidly post mortem. This makes it challenging to collect reliable data from cochleae harvested from endangered species or human cadavers. In these species there is usually little or no ability to perfuse specimens or perform measurements immediately post mortem. As a result, samples are often frozen and subsequently thawed for measurement. Since freezing and thawing can change the mechanical properties of soft tissues, we have investigated how freezing and thawing of intact temporal bones affects the mechanical properties of inner ears used in cochlear research. Quasi-static measurements were made of stiffness in fresh and frozen then thawed basilar membranes from Mongolian Gerbils (*Meriones unguiculatus*) and bottlenose dolphins (*Tursiops truncatus*). A force probe was fabricated using a glass micropipette, piezoelectric actuator, and piezoelectric force sensor. The probe tip was a pulled glass micropipette with a flattened tip 10 micrometers in diameter. The probe was used to measure the stiffness of the basilar membrane while applying a series of static transverse displacements. Measurements were made on gerbil ears less than two hours post mortem and on bottlenose dolphin ears less than 24 hours post mortem. Frozen tissue was prepared by surgically isolating and freezing the gerbil bulla within one hour after death, or for the dolphin bulla, within 24 hours. In the basal turn of gerbil the stiffness decreased 24 percent after one freeze-thaw cycle. In the basal turn of the bottlenose dolphin, the stiffness decreased 36 percent after one freeze thaw cycle.

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1035 How Does Sound Leave the Cochlea by Internal Excitation of the Basilar Membrane?

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In normal operation, sounds enter the cochlea via the stapes and generate forward traveling waves on the fluid-loaded basilar membrane (e.g., Bekesy, McGraw-Hill, 1960). Kemp (J. Acoust. Soc. Am., 64(5)) discovered that sound is emitted by the cochlea in a way that is physiologically vulnerable. These sounds are called otoacoustic emissions (OAE). Because of their relation to the health of the cochlea, OAE have evolved as a tool for both noninvasive diagnosis and understanding cochlear mechanics (Probst, J. Acoust. Soc. Am., 89(5)). However, OAE are not yet completely understood. OAE are hypothesized to arise from active processes in the cochlea. The path that intracochlearly generated sound takes when emitted from the cochlea – as a slow coupled fluid-structure wave or a fast mainly fluid born wave (Wilson, Physics Today, April 2008) - has yet to be established. This work uses mechanical-electrical-acoustic finite element model to predict the path of sound propagation due to internal excitation of the basilar membrane. In addition to the response in frequency domain, basilar membrane responses at spatial domain are examined as well, which gives a clear picture about the direction of wave propagation. Results show that the direction of wave propagation depends not only on the health state of a cochlea, but also on the relative value of the excitation frequency and the best frequency of the excitation place. The study of the simulations of the internal excitation of the basilar membrane may inspire the understanding of the mechanism of OAE.

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1036 Results from Merged TWAMP and Sandwich Cochlear Models

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We constructed a model of the cochlea by merging the Sandwich model (de Boer, 1990) with the TWAMP model (Hubbard, 1993). This construction was motivated by the finding that the tectorial membrane (TM) behaves as a damped transmission line that has a relatively-slow propagation velocity (Ghaffari, et al., 2007), like the one assumed to exist, and which was coupled with a second, frequency-selective transmission line in the TWAMP model. We modeled active stereocilia force as dependent force sources coupling between the TM and the reticular lamina, which is the “top” part of the sandwich model. When the coupling factor is zero, the combined model is simply the Sandwich. When the coupling factor is increased, we found that the basilar membrane response to tonal input can be increased by up to 20 dB, depending on the initial gain settings that represent the strength of somatic forces in the Sandwich.

1037 Modeling the Effects of Organ of Corti Cytoarchitectural Modifications

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Due to various factors, such as high intensity noise or ototoxic drugs, as well as therapeutic techniques for repair or regeneration, the organ of Corti in the mammalian cochlea can assume a configuration that deviates substantially from “normal.” The present objective is to examine theoretically the effects of such structural deviations on the excitation of the remaining hair cells. The approach involves using the Fast4 computer program, with which most cross-sectional details of the organ of Corti can be included. However, the longitudinal motion of tissue and

fluid is neglected, so the results are valid only for the long wavelength response, i.e., for frequencies less than the "best frequency" (BF), or the frequency at which the maximum response is produced for a particular section. The response of this model agrees with measurements of the normal organ of Corti under mechanical and electrical loading, and has offered an explanation for the various phases and the peak splitting seen in neural responses (Steele and Puria, 2005). The modifications tested using this technique include removing Dieters' rods, removing phalangeal processes, removing pillar cells, removing or stiffening the tectorial membrane, and removing hair cells or spacing them irregularly. A new Matlab-based interface program is used to simplify the task of modifying the Fast4 geometry and material properties. As an example of how structural deviations can affect function, a change in the orientations of 15-50% of first row outer hair cells, as found in albino guinea pigs, was shown by Yoshida and Liberman (1999) to cause a 5-10 dB reduction in the threshold of hearing. With a change in polarity of the electromotility of the first row of outer hair cells, the model shows a maximum of 15 dB reduction in the displacement of IHC cilia, which appears to be consistent with the experimental observation. [Work supported by grant DC007910 from the NIDCD of NIH.]

1038 Active Force Transmission in the Organ of Corti Micromachine

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The outer hair cell (OHC) is a force generator in the mammalian cochlea to amplify faint sound signals. It is believed that the organ of Corti (OC), a highly organized microstructure accommodating the OHCs, functions to optimize the force transmission from the OHC to the basilar membrane and the inner hair cell. We aimed to understand how the OHC force contributes to the OC mechanics. Many recent studies reveal that the OC can no longer be considered as a rigid body and has a complex mode of deformation. We developed a full 3-D finite element model of the OC to dissect its mechanics. Detailed geometric information was taken from a gerbil cochlea at 2 and 10 mm from the stapes. The cochlear partitions of several hundred micrometers long were modeled. The model includes all structurally significant components: OHCs, pillar cells, Deiters cells, reticular lamina. The model was validated by reproducing several experimental results: point stiffness and longitudinal space constant measured at the basilar membrane, response to step current through the OC. Then we simulated how two different active forces from the OHC (the OHC somatic force and the stereocilia-based force) deform the OC. With the stereociliary-based force of 0.03 and 0.23 nN per hair bundle at the apex and base, the basilar membrane was deformed by 12 and 0.9 nm at respective location. With the OHC somatic force of 3.3 nN, the basilar membrane was displaced by 84 and 2.7 nm. While the deformation of the OC by the stereociliary-based force was monotonic, the deformation by the OHC somatic force was complex in two ways: 1) the basilar membrane was deformed non-monotonically, 2) the reticular lamina was displaced in opposite phase to the basilar membrane. We conclude that even though the stereocilia-based force is orders of magnitude smaller than the OHC somatic force, it still can deform the basilar membrane to serve as a cochlear amplifier. Funded by NIH RO1 DC01362

1039 Time Domain Solutions of a Non Linear Nonlocal Feed-Forward Cochlear Model

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The equations representing the cochlea from the micromechanical point of view are intrinsically non linear. The presence of nonlinearity, as a physical non-perturbative property of the system requires a time domain solution. On the other hand, the time domain solution, with adequate spatial and temporal resolution, is a cumbersome problem from the numerical point of view.

Elliott et al. (2007) proposed a finite differences method to solve the propagation equations of the traveling wave along the basilar membrane in a 1-d linear model. In this work a nonlinear non-local feed-forward cochlear model is used to simulate otoacoustic emissions. The model represents the outer hair cells system as a non linear filter whose response is proportional to the total force acting on the basilar membrane. The equations are solved in the time domain by means of finite elements discretization techniques. Optimization procedures have also been used to reduce the calculation time, which, otherwise, would increase dramatically with increasing number of discrete elements. Roughness functions were introduced in the cochlear parameters, smoothly distributed along the basilar membrane, and the presence of otoacoustic emissions was verified by means of time-frequency analysis technique of the response at the stapes.

The basilar membrane transfer function profile and the response at the stapes were studied in different response saturation regimes. The experimental time-frequency behavior of the transient otoacoustic emissions is well matched by the model. If two stimulus tones, f1 and f2 with f2/f1=1.22, are introduced, the model also reproduces correctly the distortion product (DP) 2f1 – f2 and 2f2 – f1 in particular as regards the motion of the basilar membrane places that are tonotopically resonant for the three frequencies involved.

The proposed model seems to be a useful tool to simulate the cochlear functionality in different ranges of the characteristic parameters.

1040 Compliance Profiles of the Basilar Membrane and the Basal-Apical Dichotomy of Sound Processing

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The geometry and material properties of the basilar membrane (BM) form the basis of the mapping of frequency to place along the cochlea. The details of the interaction of their components remain largely unknown. Here, we use a finite-element analysis to explore the impact of geometry, boundary conditions and elastic material properties on the spatial characteristics of BM point compliance. The analysis is based on anatomical and stiffness data from the BM in guinea pig. The arcuate zone (AZ) and the pectinate zone (PZ) are treated individually, with the AZ defined as being elastically isotropic and the PZ as orthotropic. Based on anatomical data, it is argued that the radial component of Young's modulus of the PZ decreases exponentially with distance from base to apex. The model yields a space constant for this exponential of 2.1 mm for a tapered BM thickness and 1.9 mm for a constant BM thickness. The model suggests that: 1) the longitudinal stiffness gradient depends primarily on the basal-apical reduction of the effective BM thickness, 2) the gradients in longitudinal and radial coupling depend mainly on widening of the BM from base to apex, 3) coiling exerts its greatest influence in the apical third of the BM, 4) the greatest effect of the boundary conditions is on coupling, 5) exponential decrease of the radial component of Young's modulus in the PZ from base to apex causes widening of the compliance profile and lateral shift of its centre, which becomes noticeable apical to about the 1-kHz place. Shift and expansion are not observed for linear rather than exponential decrease of the radial component of Young's modulus. This spatial change of the compliance profile suggests that mechanical excitation in the apical region of the organ of Corti might be different to that in the basal region. Evidence for a base-apical dichotomy in mechanical excitation mechanisms has already been reported for both mechanical and neuronal responses to sound stimulation.

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