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REVIEW

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Wiring the senses: Factors that regulate peripheral axon pathfinding in sensory systems

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Abstract

Sensory neurons of the head are the ones that transmit the information about the external world to our brain for its processing. Axons from cranial sensory neurons sense different chemoattractant and chemorepulsive molecules during the journey and in the target tissue to establish the precise innervation with brain neurons and/or receptor cells. Here, we aim to unify and summarize the available information regarding molecular mechanisms guiding the different afferent sensory axons of the head. By putting the information together, we find the use of similar guidance cues in different sensory systems but in distinct combinations. In vertebrates, the number of genes in each family of guidance cues has suffered a great expansion in the genome, providing redundancy, and robustness. We also discuss recently published data involving the role of glia and mechanical forces in shaping the axon paths. Finally, we highlight the remaining questions to be addressed in the field.

KEYWORDS

axon guidance, cell signaling, cranial ganglia, placodes, sensory systems

1 | INTRODUCTION

The survival of a living organism depends on its capacity to sense and respond to the environment. The cranial sensory systems (visual, auditory/vestibular, olfactory, somatosensory, and gustatory) allow organisms to respond to external and internal stimuli. Except for the retinal neurons that derive from the central nervous system (CNS), the rest of the cranial sensory cells arise from neural crest cells or the cranial placodes, specialized neural regions of the ectoderm adjacent to the central nervous system.

These sensory systems are composed of specialized cells with receptors that capture external sensory inputs, which are then transmitted to the brain by either the same receptor cells or first-order sensory afferent neurons. In the case of olfactory neurons, their somas reside in the olfactory epithelium, whereas the rest of cranial afferent neurons' cell bodies reside in the cranial ganglia [V (trigeminal ganglion), VII (geniculate ganglion), VIII (cochleovestibular ganglion), IX (superior and petrosal ganglia), and X (jugular and nodose ganglia)]. Sensory neurons vary across systems: olfactory and auditory/vestibular neurons are bipolar and extend a dendrite and an axon and two axons, respectively. The other cranial sensory neurons are pseudo-unipolar, and they extend a single axon with two branches: one innervating the target sensory tissue and the other directed to second-order neurons of the CNS (Figure 1).

The patterns of innervation of cranial afferent neurons are stereotyped but highly complex. The axons' long route to reach their targets is guided by the ability of the

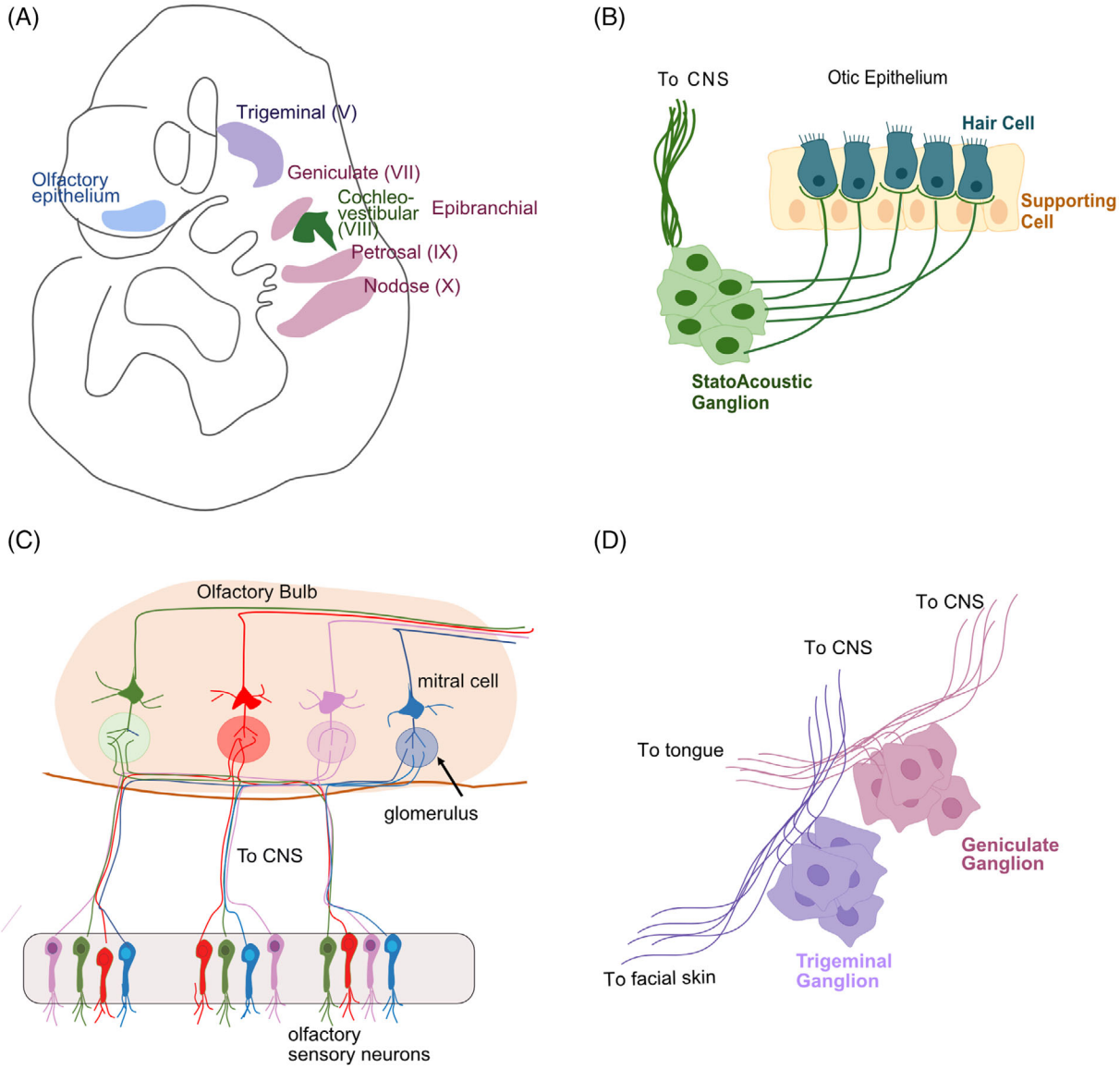

FIGURE 1 Depiction of cranial ganglia and associated sensory organs. (A) In the mouse embryo, the neural crest and the cranial placodes (olfactory, otic, trigeminal, and epibranchial) give rise to sensory cells and neurons. The somas of olfactory neurons reside in the olfactory epithelium, whereas the rest of the sensory somas reside in the trigeminal, cochleovestibular, geniculate, petrosal, and nodose ganglia. (B) The sensory epithelium of the inner ear contains hair cells and supporting cells. The bipolar neurons that innervate the hair cells cluster in the statoacoustic ganglion (SAG), from which they extend one axon toward the hair cells in the otic epithelium and another one directed to the brainstem. (C) Olfactory sensory neurons (OSNs) in the olfactory epithelium are surrounded by supporting cells. OSNs are bipolar: they extend dendrites to the lumen of the olfactory epithelium to sense odor information and an axon toward the olfactory bulb. OSN axons converge in glomeruli in the olfactory bulb, a specialized forebrain structure, that serves as the first relay station in the pathway toward the CNS. (D) The sensory neurons of the trigeminal and geniculate ganglia are pseudo-unipolar. They extend a single axon with two branches: one innervating the sensory target (facial skin and tongue, respectively) and one branch directed to second-order neurons in the CNS

growth cone to sample signals in the environment. Many decades of work in *Drosophila* and vertebrate models have permitted uncovering the main molecular mechanisms and cues orchestrating the growth of axons toward their innervation sites (for reviews[1-3]). Extending neurites navigate under the influence of chemoattractant and chemorepellent cues of either long or short-range that modify cytoskeletal elements on the growth cone to regulate its dynamics and pathway. The molecular nature of these guidance cues is diverse and elicits particular signaling transduction cascades and gene transcription, but a local activation of calcium at the growth cone seems to be a common feature.[4,5] Throughout the years, it has been difficult to functionally assess the roles of these

molecules as they often work in concert and redundantly, leading to mild or no clear phenotypes when abrogated. Some of the identified molecular mechanisms are shared between CNS and PNS; however, while CNS axons navigate mainly between neurons, glial cells, and endothelial cells, PNS axons encounter a greater variety of cells surrounding them, such as neural crest cells, mesodermal cells, glia, endothelial cells, and other neurons.

Here we will discuss data obtained in mouse, chick, and zebrafish studies to summarize the main molecules involved in PNS afferent sensory axon pathfinding and targeting, describe the similarities and differences in the establishment of peripheral innervation of the different sensory systems and species, and provide some directions for future axon guidance studies.

2 | INNERVATION OF THE AUDITORY AND VESTIBULAR SYSTEM

The inner ear, a highly complex three-dimensional organ of our head, is the sensory organ responsible for hearing and balance. Sensory information is captured in specialized sensory patches: the ventral organ of Corti detects auditory stimuli, the saccule, and the utricle sense linear movements, and the three cristae perceive rotation. Each of these sensory patches contains hair cells which are mechanosensory transducers and supporting cells that provide a structural scaffold to enable the function of hair cells. Neurons from the statoacoustic ganglion (SAG) that innervate both hair cells of the inner ear and neurons of the brainstem[6] are located anteroventrally and outside of the otic epithelium (Figure 1B).

The inner ear development begins with the establishment of the otic placode that invaginates and pinches off from the ectoderm to form a hollow ovoid structure named the otic vesicle. During this period, a neurogenic domain emerges at the anteroventral domain of the otic vesicle in which neuronal progenitors become specified. Otic neuroblasts then delaminate out of the otic vesicle to coalesce in the SAG.[7-10] As the otic vesicle grows and matures, sensory specification follows. Sensory progenitors emerge initially in a broad domain shared with the neurogenic domain that later splits into individual sensory patches. New sensory patches not coming from previous neurogenic domains also appear as described in cell lineage experiments in chicks and mouse[11,12] (Figure 2A).

In adult mice, auditory neurons are located ventrally and relatively near the organ of Corti of the cochlea in the Rosenthal canal, while vestibular neurons are located more dorsally and project to the anterior, lateral, or posterior crista of the saccule or utricle (Figure 2A). There are distinct subpopulations of fibers within auditory neurons. Type I auditory neurons are more abundant, innervate the inner hair cells, and are responsible for encoding sound.[13] Type II auditory neurons innervate the outer hair cells and are proposed to play a role in sensing damage.[14] The chick's auditory sensory organ is known as the basilar papilla. It is located in the cochlear duct and is functionally equivalent to the mammalian organ of Corti.[15] Fish do not contain a specialized hearing organ analogous to the organ of the Corti or the basilar papilla. Instead, the sacculus and the lagena are involved in the hearing function, in addition to their vestibular roles.[16] Moreover, fish also have an external mechanosensory system called the lateral line. It is formed by cycles of progenitor cell migration that arrange in clusters along with the lateral line named neuromasts. Each neuromast contains support cells, mechanoreceptors (hair cells), and mantle cells.[17]

2.1 | Initial auditory and vestibular peripheral outgrowth

Early work suggested that the first afferent sensory neurons forming the SAG send projections back to the otic vesicle by re-tracing the migratory path used by neuroblasts after delamination from the otic vesicle.[18] However, an improved understanding of lineage relationships between neurogenic and sensory (hair cell forming) territories indicates that not all sensory patches harboring hair cells have generated neuronal precursors previously.[19,20] Hence, axonal projections cannot re-track previous paths but need novel guidance cues emanating from the target cells.

Initially, the axon guidance events of neurons begin independent of hair cells, as axons reach the proximity of their target days before hair cells are fully differentiated.[21] Accordingly, null mice for the genes Pou4f3, Atoh1, or FGF10 that fail to either differentiate hair cells or develop the posterior crista, still receive normal innervation.[22-24] At this initial period of neurite outgrowth (E4-6 in the chick, E11-14 in the mouse and rat), axonal growth is most probably favored by trophic factors secreted by the otocyst. Two cytokines, the macrophage migration inhibitory factor (MIF), and the monocyte chemotactic protein 1 (MCP1) promoted directed axon outgrowth toward the otic tissue in in vitro assays, and knockout (KO) mice lacking MCP1 or MIF displayed hearing deficits.[25-28] Other morphogens such as Shh, Wnt, BMP4, and several FGFs have also been shown to promote directed neurite outgrowth in chicken SAG explants.[29]

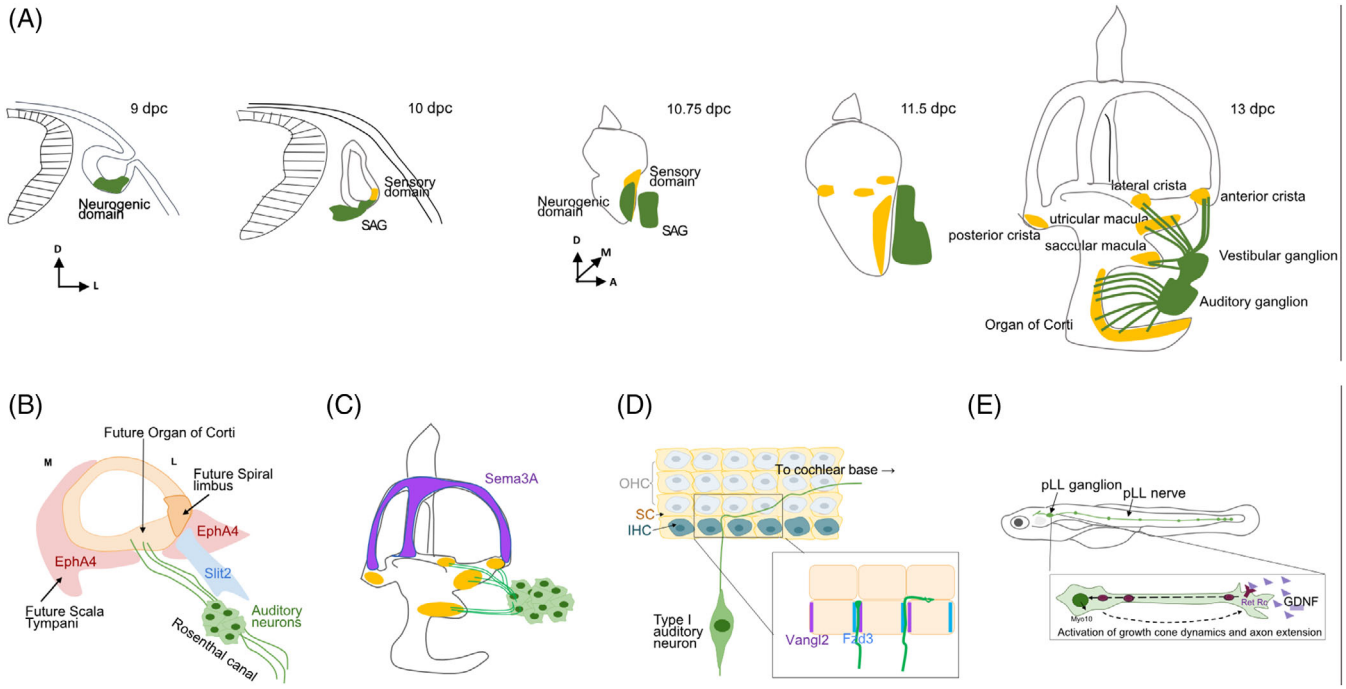

FIGURE 2 Innervation of the auditory and vestibular system. (A) Development of the mouse inner ear. The otic placode arises as a thickening of the ectoderm that invaginates to generate the otic vesicle. Within the vesicle, a population of neuroblasts from the neurogenic domain located anteroventrally delaminate and coalesce into the statoacoustic ganglion (SAG). Sensory progenitors that will give rise to the hair cells in the inner ear arise in a broad domain shared with neurogenic cells to then split into individual sensory patches. Sensory neurons distribute into the auditory and vestibular ganglia, from which they extend axons to innervate the hair cells in the different sensory patches of the inner ear. (B) SAG axons directed to the organ of Corti penetrate the sensory epithelium between two walls of EphA4-expressing cells. Slit2 secreted by the spiral limbus restricts the position of auditory neurons in the Rosenthal canal to ensure proper targeting into the otic epithelium. (C) In the vestibular sensory patches, Sema3A halts axons that target the anterior crista, so they do not overgrow past this area. (D) Type II auditory neurons innervate the cochlea's three rows of outer hair cells. Axons travel past the row of inner hair cells to make a 90° turn toward the base of the cochlea and ascend to reach the outer hair cells. Noncanonical Wnt signaling promotes Vangl2 and Fzd3 protein asymmetries in opposite basolateral walls of supporting cells, which establish a planar polarity axis that instructs the turn of the axon toward the cochlear base. (E) The vestibular system of fish also contains the lateral line. Pioneer axons extending from the posterior lateral line ganglion (pLLG) set the path for follower axons forming the posterior lateral line (pLL) nerve. Guidance of pioneer axons occurs through GDNF/Ret51 signaling that evokes a transcriptional response that upregulates Myo10 in the growth cone and results in the subsequent regulation of filopodia dynamics

Recently, Druckenbrod and colleagues have examined the colocalization of auditory neurons and glial precursors during the initial steps of auditory axon projection. They showed that glial precursors establish a scaffold for SAG axons to extend along them. Neurons from the rear of the SAG are the first ones to contact glial precursors and extend along them. In contrast, auditory neurons closer to the border of the ganglion use the rear glial-axon projections as a scaffold to grow toward the otic epithelium.[30] Interestingly, cochleae depleted of glial cells still receive innervation, albeit less efficiently, due to neurite-neurite fasciculation.[31,32] Thus, glial precursors seem to synergize with neurons to improve the efficiency of innervation. In zebrafish, neural crest cells have also been shown to guide inner ear afferent pioneer axons to the neural tube.[33]

2.2 | Axon navigation toward the otic epithelium

Once neurites have begun extending, classic chemoattractants and repellants contribute to actively guiding axons to the hair cells present in sensory patches. The Eph-ephrin molecules are involved in short-range attraction or repulsion. In mice, the SAG neurites penetrate the sensory area of the organ of Corti through a channel between two walls of cochlear duct EphA4-producing cells.[34] EphA4 might repulse ephrin-B2+ axons to promote their fasciculation and make them advance straight through the EphA4-free channel while preventing their growth in the wrong direction[35] (Figure 2B). Hence, in this context, EphA4 acts as a repulsive signal. On the other hand, SAG neurons also express EphA7. Since its

loss reduces the number of type I fibers and inner hair cells afferent targeting, it is suggested that EphA7 acts as a promoter of axon outgrowth.[36] It remains to be established if the Eph-ephrin system is also relevant for targeting other species such as zebrafish, in which unpublished data describe that EphA7 is also expressed.

Other guidance cues are Netrins, Slits, and Semaphorins. The Netrin receptor, Deleted in Colorectal Carcinoma (DCC), expressed in SAG neurons, seems to guide axons to their targets in the cochlea and vestibular patches. In mouse embryos mutant for DCC, axons are misrouted and show disrupted bifurcation patterns.[37,38] However, the expression of Netrin in the cochlea is weak, and KO mice show normal auditory innervation. Therefore, it remains to be determined whether other secreted molecules could serve as DCC ligands in the cochlea.

In chick, the chemorepellents Slit2 and Slit3 are expressed in the sensory patches of the otocysts during neurite extension (E2-E7.5), while their receptor Robo2 is expressed in neurons of the SAG. It has been proposed that when axons encounter a sensory patch that is still not their target, Slits emanating from the incorrect sensory patch induce the activation of Robo2 in SAG neurons. This process may (1) make the axons unresponsive to the positive cues deriving from the sensory patches or (2) alter the balance of attracting and repelling cues favoring the repulsion and bypassing these sensory domains to reach farther sensory organs to innervate.[39,40] In mice, Slit2 expression in the spiral limbus of the organ of Corti seems to prevent the dispersal of auditory neurons outside the Rosenthal canal (Figure 2B). Mice lacking Slit2 and Robo1/2 display disrupted coalescence of auditory neurons and fail to innervate hair cells.[41]

Another set of chemorepulsive cues, Semaphorins, signal through multimeric receptor complexes formed by Plexins and Neuropilins (Npn) and provide a stop signal to growing neurites. Mice lacking the Neuropilin receptor show steering errors among their SAG neurons. Vestibular axons fail to stop when they reach their target in the anterior and lateral cristae, then traverse the Sema3A-expressing epithelium, and grow dorsally to reach the skin[42-44] (Figure 2C). In the cochlea, restricted inhibitory expression of Sema3F in the outer hair cells activates Npn2 in type I auditory neurons, which causes the retraction of axons contacting outer hair cells so they can be directed to their correct target in the inner hair cells.[45] Axons reaching the sensory epithelia then undergo a process of branch refinement whereby auditory neurons lose extra axon branches to form unramified synaptic contacts with specific inner hair cells. Instead of playing the classical role of axon repulsion, in this case, Sema5B expressed by immature hair cells acquires a novel function and acts as a crucial regulator of axon debranching. Activation of its receptor PlexinA on type I fibers leads to fewer neurons that bear longer terminal branches, whereas Sema5B KO mice show an abundant number of neurons but shorter terminal branches. This finding emphasizes the role of Sema5B-PlexinA1 signaling in limiting auditory neuron terminal debranching without causing axonal repulsion, so the correct number of synapses in the cochlea is ensured.[46]

In the organ of Corti, type II auditory neurons innervate the three rows of outer hair cells. To reach their targets, they extend axons past a single row of inner hair cells to then make a 90° turn toward the cochlear base[47] and ascend apically to reach the different rows of outer hair cells (Figure 2D). This turn takes place between the basolateral walls of the supporting cells surrounding the outer hair cells, and it is not altered when classical guidance cues or neurotrophins are absent.[47]

Planar cell polarity (PCP) signaling, which regulates the polarity of cells along the axes of tissues, has been previously linked to a variety of developmental events, including axon outgrowth and guidance.[48-51] In the cochlea, intercellular signaling via PCP core proteins seems to have a crucial role in promoting type II auditory axons' turn to innervate the three layers of outer hair cells. In a model established by Ghimire and colleagues in 2019, it was proposed that noncanonical Wnt signaling through the receptors Fzd3 and Fzd6 promotes protein asymmetries in the supporting cells of the cochlear duct that are relayed between neighbors: the protein Vangl2 is distributed along the basolateral wall of supporting cells but is enriched on the side of cells facing the cochlear apex[52] in opposition to Fzd3 which is enriched on the side facing the cochlear base.[53] This establishes a planar polarity axis that provides directional information to the incoming growth cone (Figure 2D). When Vangl2 is absent in a KO mouse, SGN fibers turn incorrectly toward the apex, emphasizing the role of Vangl2 in axon guidance.[52] The same phenotype is seen when all Wnt secretion and signaling are disrupted by targeting the gene Porcn—encoding for an O-acyltransferase that is required for Wnt palmitoylation, secretion, and biologic activity—in the cochlea or when the receptors Fzd3 and Fzd6 are double-knocked out, which results in the loss of the Vangl2 asymmetric positioning and axon turning errors.[53] Altogether, this PCP signaling pathway seems crucial for correct type II auditory axon turning; however, it is unknown how the axon detects this asymmetry and can respond to it. It would be worth investigating whether the axon contains complementary PCP proteins that interact with the supporting cells' to receive the information to turn, or if the polarization of supporting cells changes the subcellular distribution of membrane-bound axon guidance cues such as Ephrins or Semaphorins that the axon can detect and respond to.

2.3 | Targeting and wiring of auditory and vestibular axons

Neurotrophins have mainly been involved in axonal survival in many systems. However, a role in axon guidance in the inner ear has also been postulated. In the inner ear, genetic manipulation of Neurotrophin expression does not perturb peripheral axon growth but compromises the correct wiring of their specific targets. In vivo experiments revealed that once the first fiber projections are established via the other guidance cues, there is an upregulation of Neurotrophin expression that enables them to perform their role in axon targeting.[54] Brain-derived neurotrophic factor (BDNF) and Neurotrophin-3 (NT3) are the main Neurotrophins involved in late guidance and trophic support of SAG axons in the mammalian inner ear. BDNF is prominently expressed in the vestibular organ, while its expression in the cochlea follows an apex to base gradient.[55,56] Conversely, NT3 expression follows a base to apex gradient in the cochlea and shows very low expression in the vestibular organ. In the cochlear basal turn, NT3 precedes the expression of BDNF, which later on invades the base of the cochlea. Interestingly, mice expressing BDNF under the control of the NT3 promoter display exuberant projection of vestibular neurons to the base of the cochlea, suggesting a role of BDNF in axon guidance.[54]

In the zebrafish, pioneer axons from the posterior lateral line ganglion are guided by the posterior lateral line primordium. The primordium expresses the neurotrophic factor glial-derived neurotrophic factor (GDNF),[57] which exerts action in the pioneer sensory axons through its receptor Ret51. GDNF/Ret51 signaling elicits a transcriptional response mediated by *Jip3* that results in the upregulation of *Myo10* in the growth cone, which regulates filopodia formation and thus pioneers axon extension[58] (Figure 2E). BDNF is also expressed in the hair cells of the neuromasts[59] but whether it functions as a trophic factor only or also has a guidance role is unknown.

3 | INNERVATION OF THE OLFACTORY SYSTEM

The olfactory system is responsible for our ability to sense odors. It is composed of the olfactory epithelium, which is found in the nasal cavity and contains the somas of the olfactory sensory neurons (OSNs); and the olfactory bulb, a specialized structure of the forebrain that acts as a first relay station in the pathway toward the CNS[60] (Figure 1C). OSNs in the olfactory epithelium project in spherical neuropil structures called glomeruli, localized in the olfactory bulb (Figure 1C). Each OSN expresses a specific olfactory receptor[61,62] whose expression defines to which odorants the neuron responds and in which glomerulus of the olfactory bulb it assembles. Same olfactory receptor-expressing OSNs are not ordered in the epithelium and are heterogeneously fasciculated in their pathway[63]; however, they end up converging in the olfactory bulb, where they segregate to innervate the glomeruli distributed in the three dimensions of the bulb, establishing a topographic map.[64]

3.1 | Initial outgrowth of olfactory axons

At the time of axon extension, the tissues surrounding and in contact with olfactory axons suffer large morphogenetic processes, such as folding, convergence-extension, migration, polarization, and division, among many others. Lately, there has been an interest in understanding the influence of mechanical cues on axonal growth or guidance.[65-67]

In vivo imaging studies in the zebrafish have shown that a combination of cell movements occurring in the olfactory placode promotes the elongation of olfactory pioneer axons through a process called retrograde extension. Unlike the classical paradigm of the axon growing forward to connect the neural tube, the cell bodies of olfactory pioneer axons are pulled away from the axon tips that remain attached to the brain surface (the olfactory bulb in the telencephalon).[68] First, olfactory placode cells move in parallel to the brain, actively converge toward the center of the placode, and squeeze out central neurons from the brain surface (Figure 3A). Then, the evagination of the developing optic cup in the proximity of the olfactory placode exerts lateral traction forces on the olfactory placode cells, pushing the neurons' cell bodies away from the brain (Figure 3A). Consequently, when the optic cup does not form in eyeless *rx3* mutant embryos, placode movements are reduced, and axons are shorter and less stretched.[69] Thus, pioneer axon elongation is mediated by mechanical forces but not by guidance or trophic molecules. While these findings have not been confirmed in other species, these movements could also explain pioneer olfactory axon formation in mammals and humans. Another study using explants of mouse olfactory epithelia has quantified the force of axon-axon adhesion during fasciculation.[70] Hence, new biophysical studies are shedding light on the biomechanics behind axon growth and adhesion.

As in the inner ear, glial cells also play an important role in guiding extending OSNs. Olfactory ensheathing cells are the glia from the primary olfactory system.[71] During both development and regeneration, these cells migrate ahead of the axons and establish the path over

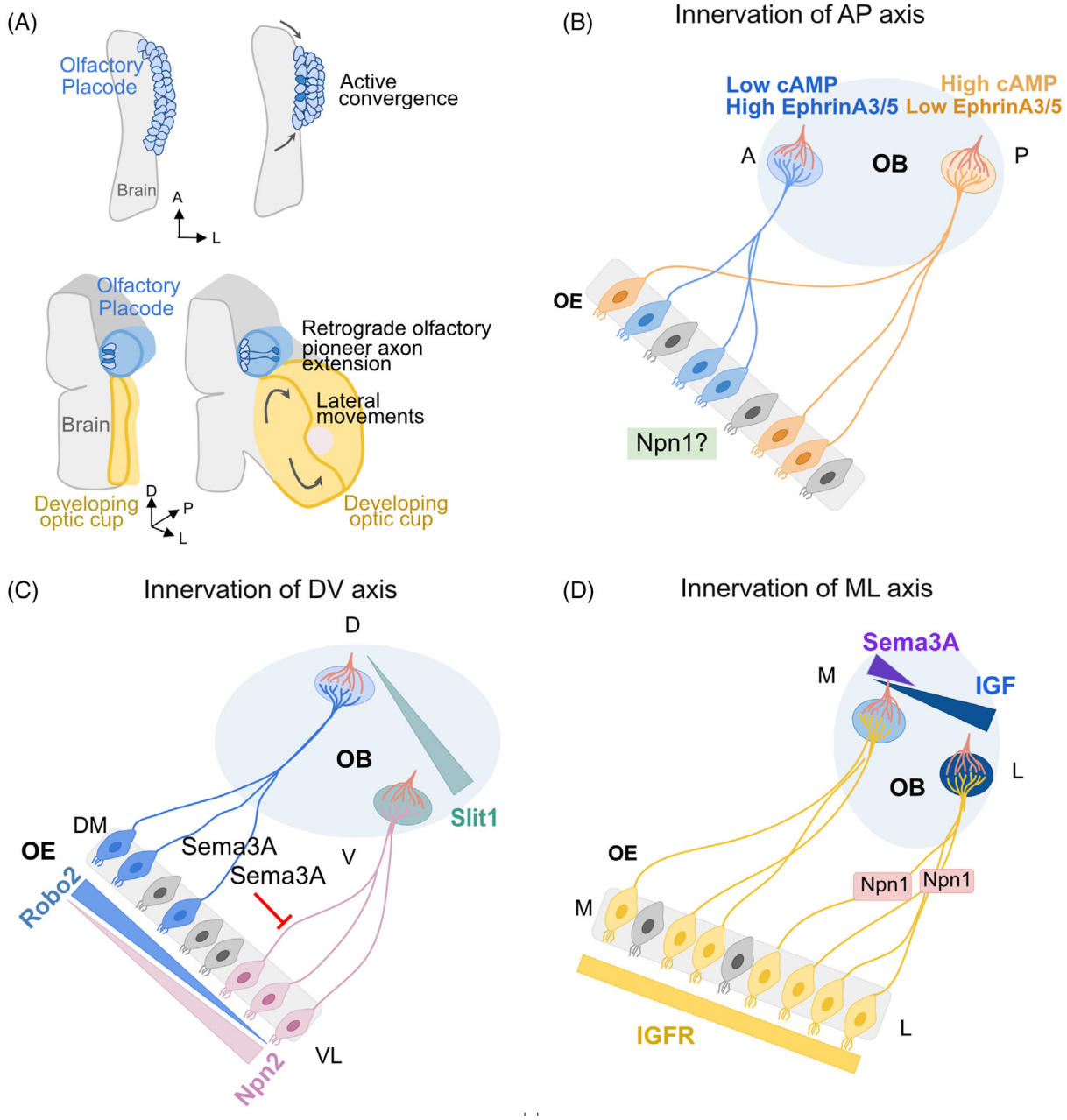

FIGURE 3 Establishment of the olfactory innervation. (A) Zebrafish pioneer olfactory axons form by retrograde extension. Extrinsic convergent forces and lateral movements emanating from the developing optic cup pull the cell bodies away from the axon tips attached to the brain region of the future olfactory bulb. (B) Activation of same-olfactory receptor-expressing axons (orange) leads to high levels of cAMP and low levels of EphrinA3 and EphrinA5, which drives olfactory axons to converge in posterior glomeruli. Axons with low olfactory receptor activity (blue) have low levels of cAMP and high EphrinA3 and EphrinA5 expression, which drives axons to converge in anterior glomeruli. Npn1 increases position variability, but its net effect on olfactory axons is unknown. (C) Neurons in the dorsal region of the olfactory epithelium express high levels of Robo2 and target the dorsal region of the olfactory bulb where Slit levels are low. The first established dorsal axons secrete Sema3A, which repels newly extending axons expressing high levels of Npn2, driving them to the ventral region of the olfactory bulb. (D) High levels of IGF in the lateral region of the olfactory bulb attract IGFR+ axons. Sema3A expressed in the midline of the olfactory bulb steers Npn1+ axons toward lateral positions, whereas Npn1-axons seem to target medial regions of the olfactory bulb by default. A, anterior; D, dorsal; DM, dorsomedial; L, lateral; OB, olfactory bulb; OE, olfactory epithelium; P, posterior; V, ventral; VL, ventrolateral

which the axons will extend to reach their targets in the olfactory bulb.[72] It is possible that olfactory ensheathing cells also use the pioneer olfactory axons to migrate ahead and create a permissive substrate for later-born OSNs; however, this idea still has to be addressed experimentally. Some studies also suggest that olfactory

ensheathing cells could secrete specific neurotrophic factors[73,74] contributing to olfactory neurite growth and guidance. One of these factors seems to be Frzb, a secreted Wnt inhibitor expressed in olfactory ensheathing cells abutting the olfactory bulb and whose deletion in mice disrupts olfactory axon targeting and maturation.[75] How Frzb guides OSNs or the identity of other olfactory ensheathing cell-secreted molecules that may participate in OSN axon pathfinding remains to be elucidated.

3.2 | Innervation along the anteroposterior axis of the olfactory bulb

Besides odor detection, olfactory receptors have an instructive role in axon targeting along the anteroposterior (AP) axis of the olfactory bulb.[76-79] Olfactory receptors are G-protein-coupled receptors that, when activated, induce cAMP production.[80] Since ablation of adenylyl cyclase compromises olfactory receptor-specific axon convergence in the AP axis of the bulb, a model was proposed in which cAMP levels regulated the transcription of genes for distinct axon guidance molecules such as Npn1,[81] EphrinA5, and EphrinA3.[82] This model entailed that high cAMP levels in OSN axons promoted the expression of high levels of Npn1 and low levels of EphrinA5, which guided axons to innervate glomeruli in more posterior regions of the olfactory bulb and vice versa (Figure 3B).

The proposed influence of Ephrin levels in axon convergence was consistent with experiments in which overexpression of EphrinA5 and EphrinA3 produced an anterior shift in the glomeruli location.[82] However, another study cast doubt on the established role of Npn1 in olfactory AP patterning. The work concluded that the conditional absence of Npn1 in OSN axons did not result in a simple anterior shift of the M71 glomerulus; instead, the switch had different direction components.[83] Therefore, while it is true that Npn1 absence increases the positional variability of glomeruli, this variance does not show a clear set of rules (Figure 3B). The timing could also be essential for the axons' ability to respond to Npn1. In an in vivo mice study of OSN targeting, it was observed that deletion of Npn1 signaling at early stages produces an anterior shift of the M71 and M72 glomeruli, whereas deletion of Npn1 at later stages promotes OSNs targeting dorsally. These findings suggest that young OSNs might be more sensitive to Npn1 expression than older ones.[84] However, more OSNs populations should be studied to elucidate the exact role of Npn1 in axon guidance and targeting along the AP axis of the olfactory bulb.

In summary, olfactory receptor signaling seems to be a critical determinant for innervation along the AP axis of the olfactory bulb. However, other sets of molecules contribute to establishing glomeruli's position along the dorsoventral and mediolateral axis.

3.3 | Innervation along the dorsoventral axis of the olfactory bulb

The location of the soma of an OSN in the olfactory epithelium determines the dorsoventral (DV) position of the glomerulus that its axon innervates in the bulb.[63,85,86] Olfactory receptor expression in the epithelium is continuous, which rules out its role in DV targeting.[87,88] Thus, spatial information may be governed by graded expression of signaling cues and receptors across the DV axis of the olfactory epithelium.

One of the cues expressed in a graded manner is the Slit-Robo system. Robo2 is expressed in a high dorsomedial to low ventrolateral gradient in the olfactory epithelium during development, whereas its ligand Slit1 is expressed in the ventral region of the olfactory bulb[89,90] (Figure 3C). OSNs expressing high levels of Robo2 target the dorsal region of the olfactory bulb, where Slit1 levels are low. Mutant mice for both of these molecules show dorsal axons misrouted toward the ventral aspect of the olfactory bulb.[89,90] Therefore, Slit1-Robo2 repulsive interactions stimulate the dorsoventral segregation of axons in the olfactory bulb by restricting the access of dorsally targeting axons into the ventral region. Nonetheless, only a subset of axons is affected by Robo2 and Slit1 mutations, which indicates that other factors may act in combination to guide the OSN axons toward their target. One candidate seems to be Robo1, provided by associated olfactory ensheathing cells to the OSN axons that repel the Slit ligands from the ventral region of the olfactory bulb.[91] How exactly OSNs are instructed by olfactory ensheathing cell-expressed Robo1 is still unknown.

The receptor Neuropilin2 (Npn2) expression in the olfactory epithelium follows the opposite gradient to Robo2: high on the ventral region of the olfactory epithelium and low on the dorsal aspect (Figure 3C). Its ligand, Sema3A, is secreted by the axon terminals of dorsally projecting OSNs that have previously innervated the olfactory bulb.[92-94] Later born high expressing Npn2 axons avoid contact with Sema3A in the dorsal aspect of the bulb and direct their axons toward the ventral region[94] (Figure 3C). In zebrafish, Npn2a/b, together with Sema3fa, also regulates olfactory bulb targeting.[95] Thus, OSN axon convergence along the DV axis is influenced by complementary chemorepellent gradients of Slit1 and Sema3A, being the mechanism conserved among vertebrates.

3.4 | Innervation along the mediolateral axis of the olfactory bulb

Similar to the DV axis, there seems to be a light correlation between the position of an OSN in the olfactory epithelium and the place of the glomerulus it innervates in the mediolateral (ML) axis of the olfactory bulb. Neurons located in lateral or medial regions of the epithelium innervate the lateral or medial areas of the olfactory bulb, respectively.[96]

During development, insulin growth factor (IGF) is expressed at the anterior part of the olfactory bulb in a low medial to high lateral gradient, while IGFR (its receptor) is found in OSNs (Figure 3D). Disruption of genes encoding for IGF ligands (IGF1 and IGF2) or the receptor (IGFR) leads to severe misrouting of lateral targeting OSN axons toward an ectopic medial location.[97] However, the fact that all OSNs express IGFR[97] does not explain the different responsiveness of some axons to IGF. One possibility is that the default option for axons is to innervate the medial region; therefore, only axons exposed to high levels of IGF will innervate the lateral side. However, other unknown mechanisms could be involved in the ability of OSNs to respond to IGF.

Furthermore, the chemorepellent Sema3A, expressed in the ventral midline of the olfactory bulb (Figure 3D), is capable of steering subsets of Npn1+ OSN axons toward lateral areas. In mice carrying a Sema3A null mutation, the mediolateral sorting of Npn1+ axons is lost,[94,98,99] highlighting its role in ML guidance of axons in the olfactory bulb. In summary, the ML organization of OSN axons along the olfactory bulb is controlled by the laterally attractive cue IGF and the medially repulsive expression of Sema3A (Figure 3D).

3.5 | Axon convergence in the different glomeruli

The combination of AP, DV, and ML patterning establishes the coarse topography of olfactory innervation. However, the olfactory map in the olfactory bulb is discrete: each glomerulus receives innervation from same-olfactory receptor-expressing OSNs. The process of homogeneous axon fasciculation is intrinsic to the OSNs and does not rely on cues secreted by the olfactory bulb.[100-102] Instead, similar to the establishment of the AP axis, the refinement to constitute the discrete map also depends on olfactory receptor activity. However, it takes place at later stages.

Neuronal activity in the OSNs through the olfactory receptors regulates the expression of genes coding for adhesion and guidance molecules at the axon termini. These molecules include Kirrel2, Kirrel3, and EphA5-EphrinA5.[77,103] Axons with low neuronal activity express high levels of Kirrel3, which promotes their homotypic adhesion and fasciculation. Conversely, axons with high neuronal activity express high levels of Kirrel2 and fasciculate together.[77] On the other hand, heterotypic axons—activity-low vs activity-high—segregate from each other through the expression of repulsive EphrinA5 and EphA5, respectively.[77,82,104] Other adhesion molecules such as BIG2 have also been found to be regulated by olfactory receptor activity. However, the corresponding binding molecule for BIG2 has not been found yet, and, therefore, the function of BIG2 in local OSN axon sorting remains to be determined.[105]

In summary, homogeneous axon convergence into a single glomerulus seems to be governed by discrete olfactory receptor-activity-dependent adhesion and guidance cues. This process ensures that a single type of OSNs innervates each glomerulus.

4 | INNERVATION OF THE FACIAL SOMATOSENSORY SYSTEM (TRIGEMINAL)

The vertebrate trigeminal system is responsible for receiving facial somatosensory stimuli such as touch, temperature, pressure, vibration, and proprioception. The different regions of the face are innervated by first-order neurons whose cell bodies reside in the trigeminal ganglion (TG), from which they extend central projections toward the brainstem. Peripherally, trigeminal axons segregate in three branches—the ophthalmic, maxillary, and mandibular nerves—which follow specific routes to innervate mechanoreceptors, thermoreceptors, and nociceptors in the face, oral cavity, and nasal cavity.[106]

4.1 | Initial trigeminal axon outgrowth

Newly growing trigeminal axons seem to rely on chemorepulsion to acquire the peripheral direction. In fish, the chemorepellent Sema3D expressed in the roof plate repulses axons to the periphery.[107] Even though this mechanism has not been confirmed in other vertebrates, the expression of semaphorins in the chick TG such as Sema3C in the early stages of axon growth[108] could indicate the action of a similar system to direct trigeminal axons outside the TG, toward the periphery and away from the brain.

There is debate on whether or not neurotrophins are required for the early stages of trigeminal neurite outgrowth. While some studies showed that neurites could

emerge from TG explants when cultured in a neurotrophin-free medium,[109-111] Bax/NGF KO experiments indicated that some neurons depend on nerve growth factor (NGF) signaling at the early stages of peripheral axon outgrowth.[112] Nonetheless, this requirement seems to affect only specific subpopulations of trigeminal neurons. For years, it was thought that BDNF and NT3 emanating from maxillary tissues acted as a long-range chemoattractant for maxillary trigeminal axons. However, the KO mice for these molecules showed no effect on trigeminal axon guidance.[113] This finding and the fact that BDNF and NT3 are both expressed in the target epithelium and in the pathway mesenchyme point to the role of these molecules as short-range attractants.

In the search for a long-range attractant candidate, a recent study examined the role of Neuregulin 4 (Nrg4) in trigeminal axon outgrowth.[114] Nrg4 was able to promote maxillary fiber outgrowth independent of neurotrophins in vitro, being most responsive at E12. In vivo, this stage corresponds to the day after the earliest trigeminal neurites reach the maxillary cutaneous tissue. At E12, $Ngr4^{-/-}$ mice showed diminished maxillary innervation in vivo. The neurites' path was correct, but they had just not grown as far. Therefore, Nrg4 from the skin has no influence in guiding the direction of growth, ruling out its function as a long-range attractant. Since the axon growth-promoting effect of Nrg4 is transient and does not persist further than E16, it is possible that some fibers, like the maxillary ones, use Nrg4 to sustain their initial outgrowth until they reach an area closer to the target where they can begin to depend on target-derived neurotrophins[114] as development advances. Therefore, neurotrophin dependence of trigeminal axons varies across axon types and timeframes.

4.2 | Trigeminal axon navigation to and targeting

Once axons have begun extending, pathway regionalization of trigeminal axons seems to be governed by contact guidance. In chick, the TG is bilobed: one lobe contains the neurons comprising the ophthalmic branch, and the other lobe contains the neurons from the maxillary and mandibular branches. Axons from the ophthalmic branch differentially express EphA3, while maxillary and mandibular axons have low levels of EphA3. EphrinA5 and EphrinA2 repress ophthalmic axons expressing high levels of EphA3 from entering the first branchial arch mesenchyme (the pathway for maxillary and mandibular axons). Therefore, repulsive Eph-Ephrins interactions act as an obstacle to prevent the entrance of high EphA3 expressing ophthalmic axons in improper regions[115] (Figure 4A).

In the innervation of the rodent whisker pad by maxillary axons, Ephrin signaling has a different function. Maxillary neurites access the developing whisker pad from a caudal to a rostral direction. As axons enter the whisker pad, they spread into fascicles that divide into five-row nerves (rows A to E; Figure 4B) to then envelope developing hair follicles (whiskers).[116] EphA4 is expressed in the ventral region of the peripheral whisker pad. $EphA4^{-/-}$ mice exhibit a perturbed whisker patterning in which the dorsal rows show expected axon invasion (rows A to D), but the whiskers and axons from the most ventral row (E) are lost (Figure 4B). In the mutant mice, ventral axons defasciculate abnormally and do not associate with follicles.[117] Thus, EphA4 seems to provide a repulsion cue for axons approaching the ventral whisker pad to prevent them from prematurely dissociating so that accurate terminals innervate each whisker at the correct time (Figure 4B).

During axon navigation toward the target, Sema3A and Sema3F, expressed following a unique pattern in the rodent face, restrict inappropriate innervation areas for trigeminal PlexinA3+ and PlexinA4+ axons (Figure 4B). Accordingly, the KO for these molecules results in the defasciculation of trigeminal axons, more prominently from the ophthalmic branch, leading to the invasion areas from which they are usually blocked.[118] In the mandibular branch, which innervates the teeth, Sema3A appears to be responsible for coupling tooth innervation and morphogenesis by restricting the time axons can access their innervation fields. Mice deficient in Sema3A show premature innervation of the presumptive dental mesenchyme.[119]

BMPs are other signaling cues that, in addition to patterning, also have a role in axon guidance.[120] BMP4 is expressed in the developing facial region, acts as a repulsive cue,[121] and defines a specific permissive field for ophthalmic axons to advance in the right direction in between BMP-expressing regions (Figure 4B). Trigeminal ophthalmic axons can sense this path and mediate a response through the expression of Megf8, a modifier of BMP signaling. Loss of Megf8 prevents axons from sensing the BMP4 inhibition and leads to ophthalmic axon defasciculation and reduced growth.[122]

4.3 | Trigeminal axon branching

When axons arrive in the vicinity of the skin, axon branching is vital to spread out terminals across the facial area. Although Slit/Robo signaling is usually repulsive, Slit proteins seem to promote somatosensory axon

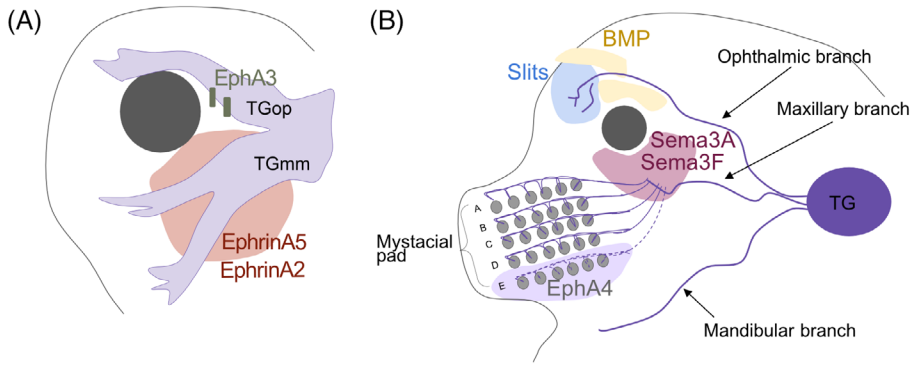

FIGURE 4 Facial trigeminal innervation. (A) Ophthalmic branch regionalization. In chick, the TG is bilobed. The ophthalmic portion of the TG extends axons that innervate the area surrounding the eye. These axons express EphA3, which prevents them from entering the first branchial arch mesenchyme that expresses high levels of repulsive EphrinA5 and EphrinA2 and defines the path for maxillary and mandibular axons. (B) Facial trigeminal innervation in the mouse embryo. The ophthalmic branch of the trigeminal nerve is repulsed from inappropriate areas by Sema3A and Sema3F interaction with PlexinA4 receptors in ophthalmic axons. BMP4 expression defines a permissive channel for ophthalmic axons to advance in the right direction. Finally, Slits promote axon branching around the eye. The rodent maxillary branch innervates the five rows of whiskers. In the most ventral row (E), Eph-Ephrin interactions prevent the premature defasciculation of axons, ensuring each whisker's correct innervation at the appropriate time. TGop, ophthalmic portion of the trigeminal ganglion; TGmm, maxillary and mandibular portion of the trigeminal ganglion

branching. In zebrafish, overexpression of Slit2 leads to expanded branching and growth of trigeminal axons. This action, however, requires the action of the semaphorin receptor PlexinA4.[123] This novel function appears to be conserved in mammals: Slit2/Slit3 or Robo1/Robo2 double mutant mice show reduced axon branching around the eye[124] (Figure 4B).

Contrarily, Semaphorins exert a negative effect on axon branching. Mutant Sema3A mice exhibit enhanced trigeminal axon branching. Interestingly, this action requires the activation of axonal Npn1, and Plexin co-receptors as the KO for Npn1 abolishes Sema3A-mediated axon repulsion.[43]

5 | INNERVATION OF THE GUSTATORY SYSTEM

The gustatory system is responsible for the sense of taste. Gustatory information from the environment is perceived by specialized taste receptor cells in the taste buds that reside in papillae and are distributed over the tongue, soft palate, and epiglottis. The taste receptor cells are innervated by primary gustatory neurons whose cell body is located in the geniculate, the petrosal, or the nodose ganglia. Studies of axon pathfinding in the gustatory system have been more prominently focused on projections from the geniculate ganglion that innervate the fungiform papillae in the anterior tongue through the chorda tympani nerve. Due to this reason and space constraints, we will discuss the main findings regarding geniculate taste innervation in this section.

5.1 | Initial geniculate neurite extension

The initial outgrowth of gustatory axons depends on neurotrophins. In vitro studies showed that the extension of geniculate axons requires a neurotrophin in the culture media. However, only BDNF, Neurotrophin4 (NT4), and GDNF can support this process, whereas NT3 and NGF cannot.[125] Even though some neurotrophin level is

needed for axons to extend, none of them is exclusively required. Ablation of BDNF or NT4 does not prevent geniculate axons from reaching the tongue *in vivo*,[126,127] indicating that these neurotrophins can act redundantly to support axon extension. BDNF and NT4 do not seem to work as long-range chemoattractants either. Even though the mammalian tongue is known to promote directed axon extension,[128] in vitro experiments have demonstrated that neither the absence of BDNF nor NT4 affect the chemoattractive properties of the tongue.[128] Therefore, future studies should be directed toward isolating said long-range chemoattractant in the developing tongue.

Because there are different gustatory neuron populations with a distinct expression of TrkB and dependence on neurotrophins,[129] it has been proposed that the action of BDNF and NT4 on the guidance of geniculate neurites could be exerted by binding to the p75 neurotrophin receptor. p75 has been found to be important in regulating gustatory axon guidance, and tongue innervation as p75 null mice showed defects in this process and lost half the geniculate neurons.[130] However, the phenotype resulting from $p75^{-/-}$ mice is not the same as the ones observed in $Bdnf^{-/-}$ and $Ntf4^{-/-}$ mice, and when p75 is removed from gustatory neurons only, mutant mice show normal taste and no loss of geniculate neurons.[131] These findings indicate that p75 does not mediate the role of BDNF and NT4 in gustatory axon guidance[130] and that the developmental function of p75 is exerted by the expression of this receptor in other cell types of the neuronal circuit toward the tongue. Deciphering the identity of these p75-expressing cells could enable us to find novel cell nonautonomous mechanisms important for the establishment of innervation in different sensory systems.

5.2 | Axon navigation toward the lingual epithelium

In mice, gustatory axon navigation toward the tongue epithelial surface occurs between E11.5 and E13.5, and axon penetration into the epithelium and targeting occurs between E14 and E16.5. To ensure proper and accurate innervation of fungiform papillae, these two stages have different neurotrophin requirements. While the first part depends mainly on epithelial NT4, the second relies on BDNF expressed in both the lingual epithelium and the fungiform papillae.

Gustatory axons from the chorda tympani nerve reach the surface of the tongue epithelium by E11.5, and position lateral and caudal to the papillae that they will innervate[125,132,133] (Figure 5). As the tongue expands between E12 and E13.5, axons grow within the tongue surface epithelium from lateral/caudal to medial/rostral areas of the developing tongue[134] (Figure 5). On E12.5, there is a peak of NT4 expression in the tongue epithelium, whereas BDNF levels are low.[135] Then, the expression of NT4 decreases right before axon penetration in the epithelium[135] (Figure 5).

As previously mentioned, NT4 has a growth-promoting effect on geniculate neurites earlier in development. However, at this stage, NT4 KO mice lose very few fungiform papillae,[136,137] and axons target normally,[138] dismissing the role of NT4 as a geniculate axon attractant *in vivo*.[139] Instead, it seems that NT4 has a crucial role in preventing axons from penetrating the lingual epithelium too early,[139] as not only high doses of NT4 can stunt geniculate axon growth *in vitro*[139] but also overexpression of NT4 in the lingual epithelium during axon targeting inhibits geniculate branching and exploration within the epithelium.[140] These findings and the fact that NT4 expression in the lingual epithelium is reduced right before targeting—when axons have to penetrate the epithelium and reach the fungiform placodes—indicate that NT4 plays a dual role in tongue innervation: first, it promotes the outgrowth of geniculate axons toward the tongue mesenchyme and then it prevents the premature penetration of geniculate fibers into the fungiform placodal epithelium.[139]

Nonetheless, neurites are still attracted to the tongue because the developing taste epithelium also expresses BDNF, which acts as the main short-range chemoattractant in the tongue. By E13.5, BDNF expression has allowed axons to reach the vicinity of fungiform placodes to almost touch them, but NT4 prevents their premature contact[139] (Figure 5).

5.3 | Axon penetration and targeting in the fungiform papillae

On E14.5, fibers from the chorda tympani nerve start to defasciculate and penetrate the epithelial lingual surface to innervate the gustatory papillae. This process depends mainly on BDNF, the only chemoattractant identified in the tongue.

BDNF is expressed in the taste placodes before they receive the innervation, starting at E12.5.[141-143] Then, as the fungiform papillae mature, restricted focal expression of BDNF promotes the growth of gustatory axons toward the BDNF source[144] (Figure 5). Mice lacking BDNF show abnormal innervation patterns with increased branching,[138,145] which indicates that BDNF is both necessary and sufficient for guiding geniculate fibers to innervate their correct targets during development. Moreover, target-derived BDNF also allows geniculate axons to

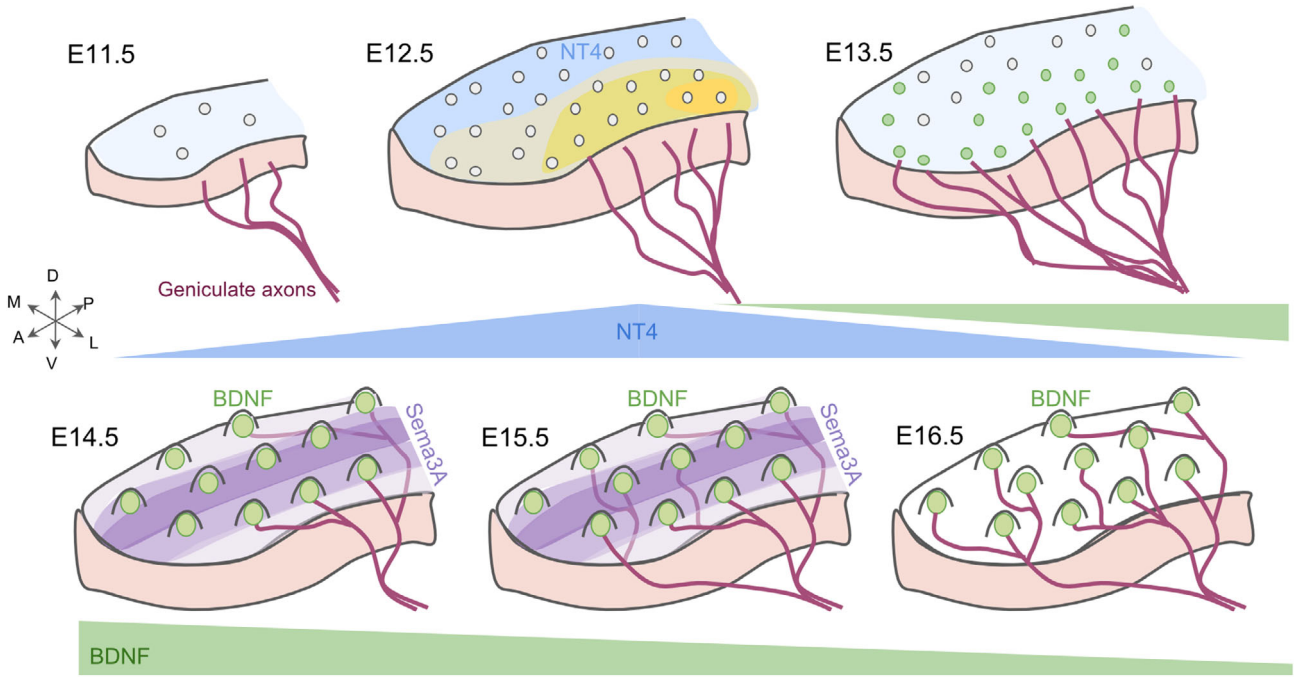

FIGURE 5 Development of the innervation of the mouse tongue. On E11.5, the first geniculate axons arrive at the surface of the tongue epithelium. At this point, axons become dependent on NT4. As the tongue expands, axons grow lateral/caudal to medial/rostral (yellow shows the direction of progression). A peak of NT4 in the lingual epithelial prevents axons from penetrating the taste placode epithelium too soon. On E13.5, NT4 expression in the lingual epithelium decreases, and BDNF expressed in the taste placodes attracts axons. Reduced inhibition by NT4 and increased attraction by BDNF direct axons toward the vicinity of the developing gustatory papillae. On E14.5 geniculate axon penetration takes place. Axons defasciculate to penetrate the epithelial lingual surface to innervate the BDNF-expressing fungiform papillae. The medial to lateral gradient of Sema3A, regulates the lateral to the medial progression of innervation by preventing the premature access of axons to medial papillae. By E15.5, geniculate axons have reached the center of the papillae and the taste bud primordia. On E16.5, there is the maximum proportion of innervated papillae. Blue and green bars represent axon dependence on NT4 and BDNF, respectively

distinguish between taste and nontaste epithelia, as overexpression of BDNF in the entire lingual epithelium results in the innervation of nontaste filiform papillae that ectopically express BDNF.[135] However, in absence of BDNF, a few gustatory axons still reach and innervate fungiform placodes, albeit 4 days after these neurons would normally innervate their targets, at E18.5.[138] This finding suggests the presence of another chemoattractant expressed in the tongue. Novel transcriptomic studies should focus on identifying this additional chemoattractant.

Further tuning and regulation of geniculate axon targeting are achieved by chemorepellants. At this stage, Sema3A is expressed in the lingual epithelium[146] in a decreasing medial to lateral gradient[125,133] (Figure 5). Because tongue innervation has been seen to proceed in the opposite direction—from lateral to medial—it appears that Sema3A regulates the order and progression of geniculate innervation by preventing the premature growth of taste sensory afferents into the epithelium. Accordingly, in Sema3A$^{-/-}$ mutant mice, geniculate axons reach and penetrate the medial regions of the tongue epithelium too early.[128,147] Another semaphorin, Sema3F is also expressed in the tongue epithelium; however, its function remains to be elucidated.[128]

More recently, a study has described how cell-contact guidance cues also play a role in tongue innervation. Right after the initial penetration, EphrinB2 is expressed in fungiform papillae, while the receptors EphB1 and EphB2 are found in geniculate axons.[148] Double KO mice for EphB1 and EphB2 mice show a significant decrease of innervation in the tongue that resembles the one observed in the mouse whiskers in the absence of EphA4.[148] This indicates that Ephrin signaling in the tongue might regulate temporal axon arborization and innervation of the taste epithelium. Nonetheless, while it is known that Eph/Ephrin signaling is definitely needed for correct gustatory innervation, is not known the specific action it has over axons: whether or not it halts axon growth, or if the phenotype observed answers to the lack of Eph/Ephrin signaling in other stages where its function may be more relevant. The use of conditional mutants might elucidate this issue in the future.

TABLE 1 Summary of the main axon guidance molecules involved in peripheral sensory axon pathfinding

Family	Molecule	Sensory system	Expression	Ligand/receptor expression	Function	Species	Reference
Eph-Ephrins	EphrinA2	Trigeminal system	First branchial arch mesenchyme	EphA3 in ophthalmic trigeminal axons	Axon *repulsion* to prevent ophthalmic axons from invading the maxillary and mandibular pathways	Chick	115
	EphA3	Trigeminal system	Axons of the ophthalmic branch of the trigeminal nerve	EphrinA5 and EphrinA2 in first branchial arch mesenchyme	Axon *repulsion* to prevent ophthalmic axons from invading the maxillary and mandibular pathways	Chick	115
	EphrinA3	Olfactory system	Olfactory sensory neurons	EphA receptors in target cells of the OB	Axon convergence along the AP axis of the OB *(not known if axon repulsion or attraction)*	Mouse	82
	EphA4	Inner ear	Fibroblasts of the spiral ligament and the spiral lamina	Maybe Ephrin-B2 and Ephrin-B3 in cochlear neurons	Axon *repulsion* to ensure the path to Organ of Corti	Mouse	34.35
		Trigeminal system	Ventral region of the peripheral whisker pad	EphrinA4 in trigeminal maxillary axons	Axon *repulsion* to regulate proper timing of axon defasciculation and innervation of the whiskers	Mouse	117
	EphrinA5	Olfactory system	Olfactory sensory neurons	EphA receptors in target cells of the OB	Axon convergence along the AP axis of the OB *(not known if axon repulsion or attraction)*	Mouse	82
			Olfactory sensory neurons	EphA5 receptors in different-OR expressing olfactory neurons	Axon *repulsion* to converge in different glomeruli	Mouse	77,104
		Trigeminal system	First branchial arch mesenchyme	EphA3 in ophthalmic trigeminal axons	Axon *repulsion* to prevent ophthalmic axons from invading the maxillary and mandibular pathways	Chick	115
	EphA7	Inner ear	SAG neurons	Undetermined	Axon *attraction*-promotion of axon outgrowth	Mouse	36

TABLE 1 (Continued)

Family	Molecule	Sensory system	Expression	Ligand/receptor expression	Function	Species	Reference
	EphrinB2	Gustatory system	Lingual epithelium	EphB2 in geniculate axons	Probably axon *repulsion* to regulate proper timing of axon defasciculation and innervation of the taste epithelium	Mouse	148
Semaphorins	Sema3A	Inner ear	Epithelium of extending semicircular duct walls	Npn1 in SAG neurons	Axon *repulsion* to prevent SAG neurites to travel past their targets in the cristae	Mouse	42-44
		Olfactory system	Olfactory sensory neurons that have innervated the dorsal aspect of the OB	Npn2 in a gradient ventral to dorsal in the OE	Axon *repulsion* to direct olfactory axons to the ventral region of the OB	Mouse	92,93
			Ventral midline of the OB	Npn1 in olfactory axons	Axon *repulsion* to steer olfactory axons to lateral areas of the OB	Mouse	94,98,99
		Trigeminal system	Developing facial skin around the eye	Npn1 and PlexinA3 and PlexinA4 co-receptors in trigeminal axons	Ophthalmic axon *repulsion* to avoid premature defasciculation and invasion of improper areas	Mouse	118
					Regulation of axon *branching*	Mouse	43
			Presumptive dental mesenchyme	Npn1 in mandibular trigeminal axons	Mandibular axon *repulsion* to ensure proper timing of dental mesenchyme innervation	Mouse	119
		Gustatory system	Medial to lateral gradient in the developing tongue	Maybe Npn2 in geniculate axons	Axon *repulsion* to prevent premature geniculate axon growth and penetration of the lingual epithelium	Mouse	128
			Bitter TRCs	Undetermined	Presumably bitter geniculate axon *attraction* toward bitter TRCs	Mouse	153

(Continues)

TABLE 1 (Continued)

Family	Molecule	Sensory system	Expression	Ligand/receptor expression	Function	Species	Reference
	Sema3C	Trigeminal system	Trigeminal ganglion	Undetermined	Axon *repulsion* might shoot trigeminal axons outside the TG and to the periphery	Chick	108
	Sema3F	Inner ear	Outer hair cells	Npn2 in type I auditory neurons	Axon *repulsion* to direct type I auditory neurons to their targets in inner hair cells	Mouse	45
		Trigeminal system	Developing facial skin around the eye	PlexinA3 and PlexinA4+ in trigeminal axons	Ophthalmic axon *repulsion* to avoid premature defasciculation and invasion of improper areas	Mouse	118
	Sema5B	Inner ear	Immature hair cells	PlexinA1 on type I auditory neurons	Regulation of axon *debranching*	Mouse	46
	Sema7A	Gustatory system	Sweet TRCs	Undetermined	Presumably sweet geniculate axon *attraction* toward sweet TRCs	Mouse	153
Slits	Slit1	Olfactory system	Ventral region of the OB	Robo2 in olfactory neurons of the OE in a dorsal to ventral gradient	Axon *repulsion* to restrict the access of dorsally targeting axons into the ventral region of the OB	Mouse	89,90
	Slit2	Inner ear	Sensory patches of the otocysts	Robo2 in SAG neurons	Axon *repulsion* to allow axons to travel past attractive sensory patches and reach farther ones	Chick	40
			Spiral limbus of the organ of Corti	Robo1/2 in SAG neurons	Spatial restriction of auditory neurons in the Rosenthal canal	Mouse	41
		Trigeminal system	Possibly facial skin	PlexinA4 and Robo2 in trigeminal axons	*Branching* and growth of trigeminal axons	Zebrafish	123
	Slit3	Inner ear	Sensory patches of the otocysts	Robo2 in SAG neurons	Axon *repulsion* to travel to farther sensory patches	Chick	40
Netrins	DCC	Inner ear	SAG neurons	Undetermined	Axon *attraction* to cochlea and vestibular sensory patches	Mouse	37

TABLE 1 (Continued)

Family	Molecule	Sensory system	Expression	Ligand/receptor expression	Function	Species	Reference
Neurotrophins	BDNF	Inner ear	Vestibular sensory patches; cochlea in a apex to base gradient	TrkB in SAG neurons	Axon *attraction* toward the vestibular sensory patches and the apex of the cochlea	Mouse	54
		Gustatory system	Fungiform papillae	TrkB in geniculate axons	Axon *attraction* toward the gustatory papillae	Mouse	135,144
	NT3	Inner ear	Base of the cochlea	TrkB in SAG neurons	Axon *survival*	Mouse	54
	NT4	Gustatory system	Lingual epithelium	TrkB in geniculate neurons	Axon *repulsion* to regulate the proper timing of axon penetration	Mouse	126,139
	GDNF	Lateral line	Lateral line primordium	Ret51 in pioneer sensory axons	Axon *growth* through enhancement of Myo10 and growth cone dynamics	Zebrafish	58
	NGF	Trigeminal system	Facial skin	TrkA in trigeminal axons	Axon *attraction* early stages of outgrowth	Mouse	102

5.4 | Innervation of specific taste receptor cells in the taste buds

Finally, once taste afferents have reached the taste buds in the fungiform papillae, they establish connections with the multiple taste receptor cells. Each taste receptor cell recognizes and relays information about one of the basic taste qualities: bitter, sweet, sour, salty, and umami.[149] Similarly, most ganglion neurons are also tuned to single taste qualities.[150] Given that different types of taste receptor cells are intermingled in the taste buds, how can axons distinguish their precise target taste receptor cell? Unlike the olfactory receptors in the olfactory system, taste receptors are not indispensable for ensuring the connection between taste receptor cells and their targets.[151,152] Lee et al.[153] determined that Sema3A and Sema7A had a prominent role in wiring bitter and sweet axons, respectively. Deletion of Sema3A from bitter taste receptor cells in mice led to almost 50% of bitter-responsive neurons responding to other qualities. Misexpression of Sema3A in sweet taste receptor cells was able to redirect bitter axons to sweet taste receptor cells. When examining Sema7a, researchers found similar results but in the sweet taste pathway.

Overall, these data emphasize the role of Sema3A and Sema7A as guiding cues for establishing the bitter and sweet taste pathways. However, the receptors for Sema3A and Sema7a on the axons of ganglion neurons remain undetermined. Moreover, the fact that in combined deletion and misexpression of Sema3A, 30% of bitter-responsive axons were able to reach their correct targets[153] points to the presence of other possible undescribed signals contributing to the establishment of the specific cellular target.

6 | CONCLUSION AND FUTURE PERSPECTIVES

Santiago Ramon y Cajal, in the XIXth century, already proposed that axons would grow under the influence of molecules from surrounding tissues.[154] Since the identification of the first axon guidance molecules, a vast amount of studies has expanded the repertoire and has revealed that the molecular system is rather redundant to ensure robustness in the wiring of the nervous system. From this review, it is apparent that the different sensory systems share most of the guidance cues families

(Semaphorins, Eph/Ephrin, Slits, and Neurotrophins) with small variations in their action and their combinatorics (Table 1). For instance, Sema3A is used in the olfactory bulb and Sema3F in the cochlea to repulse axons and prevent incorrect targeting. However, it remains to address whether these two guidance cues are completely interchangeable or if each one triggers specific signaling events. Most functional studies in sensory systems have been carried out in mice, but the zebrafish model provides a new tool to assess time axonal growth in real-time and discriminate between defects in extension, fasciculation, targeting, or survival. The combination of multiplexing gene editing and live imaging techniques can provide novel data of the precise temporal and spatial action of the guidance cues, the identification of novel ones, and improve quantitative analysis.

Besides cell signaling and transcriptional mechanisms, a new focus has been put on biophysical forces exerted by tissues. As demonstrated in the zebrafish olfactory placode, pushing and pulling forces and cell movements affect sensory axon formation and extension. Further studies in this direction are needed.

Moreover, it remains to be addressed if all these mechanisms described in animal models are conserved in humans. Due to ethical concerns, the human embryo cannot be studied at the time when sensory axon targeting occurs. However, recent advances in the stem cell research field, namely the use of organoids, have begun tackling the inaccessibility problem for many embryonic organs and tissues. So far, human pluripotent stem cell-derived organoids have been described for some sensory systems, including the inner ear,[155] and the skin.[156] In addition, lingual organoids have been produced using rat stem cells,[157] and the translation of this protocol to human stem cells could be assessed in the future. Since organoids maintain organ cellularity and architecture, this system can serve as a valuable tool to study human axonal growth and targeting.

All in all, a proper sensory function is crucial for the survival of an organism. Further understanding of the rules of sensory innervation in embryonic and adult tissues will benefit the regeneration of sensory nerves after damage.

AUTHOR CONTRIBUTIONS

Gemma Nomdedeu-Sancho: Investigation (lead); writing – original draft (lead); writing – review and editing (equal). **Berta Alsina:** Conceptualization (lead); funding acquisition (lead); supervision (lead); writing – original draft (supporting); writing – review and editing (lead).

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REFERENCES

1. Tessier-Lavigne M, Goodman C. The molecular biology of axon guidance. *Science*. 1996;274(5290):1123-1133. doi:10.1126/science.274.5290.1123
2. Goldberg J. How does an axon grow? *Genes Dev*. 2003;17(8):941-958. doi:10.1101/gad.1062303
3. McCormick L, Gupton S. Mechanistic advances in axon pathfinding. *Curr Opin Cell Biol*. 2020;63:11-19. doi:10.1016/j.ceb.2019.12.003
4. Stoeckli E. Understanding axon guidance: are we nearly there yet? *Development*. 2018;145(10). doi:10.1242/dev.151415
5. Hong K, Nishiyama M, Henley J, Tessier-Lavigne M, Poo M. Calcium signalling in the guidance of nerve growth by netrin-1. *Nature*. 2000;403(6765):93-98. doi:10.1038/47507
6. Kelley M. Regulation of cell fate in the sensory epithelia of the inner ear. *Nat Rev Neurosci*. 2006;7(11):837-849. doi:10.1038/nrn1987
7. Abello G, Alsina B. Establishment of a proneural field in the inner ear. *Int J Dev Biol*. 2007;51(6–7):483-493. doi:10.1387/ijdb.072343ga
8. Riley B, Phillips B. Ringing in the new ear: resolution of cell interactions in otic development. *Dev Biol*. 2003;261(2):289-312. doi:10.1016/s0012-1606(03)00245-8
9. Bok J, Chang W, Wu D. Patterning and morphogenesis of the vertebrate inner ear. *Int J Dev Biol*. 2007;51(6–7):521-533. doi:10.1387/ijdb.072381jb
10. Alsina B, Whitfield T. Sculpting the labyrinth: morphogenesis of the developing inner ear. *Semin Cell Dev Biol*. 2017;65:47-59. doi:10.1016/j.semcdb.2016.09.015
11. Fekete D, Muthukumar S, Karagogeos D. Hair cells and supporting cells share a common progenitor in the avian inner ear. *J Neurosci*. 1998;18(19):7811-7821. doi:10.1523/jneurosci.18-19-07811.1998
12. Raft S, Koundakjian E, Quinones H, et al. Cross-regulation of Ngn1 and Math1 coordinates the production of neurons and sensory hair cells during inner ear development. *Development*. 2007;134(24):4405-4415. doi:10.1242/dev.009118
13. Spoendlin H. Innervation patterns in the organ of Corti of the cat. *Acta Otolaryngol*. 1969;67(2–6):239-254. doi:10.3109/00016486909125448
14. Flores E, Duggan A, Madathany T, et al. A non-canonical pathway from cochlea to brain signals tissue-damaging noise. *Curr Biol*. 2015;25(5):606-612. doi:10.1016/j.cub.2015.01.009
15. Hirokawa N. The ultrastructure of the basilar papilla of the chick. *J Comp Neurol*. 1978;181(2):361-374. doi:10.1002/cne.901810208
16. Whitfield T, Riley B, Chiang M, Phillips B. Development of the zebrafish inner ear. *Dev Dyn*. 2002;223(4):427-458. doi:10.1002/dvdy.10073
17. Gasanov E, Rafieva L, Korzh V. BDNF-TrkB Axis regulates migration of the lateral line primordium and modulates the

maintenance of mechanoreceptor progenitors. *PLoS One*. 2015;10(3):e0119711. doi:10.1371/journal.pone.0119711

18. Carney P, Silver J. Studies on cell migration and axon guidance in the developing distal auditory system of the mouse. *J Comp Neurol*. 1983;215(4):359-369. doi:10.1002/cne.902150402
19. Satoh T, Fekete D. Clonal analysis of the relationships between mechanosensory cells and the neurons that innervate them in the chicken ear. *Development*. 2005;132(7):1687-1697. doi:10.1242/dev.01730
20. Raft S, Koundakjian E, Quinones H, et al. Cross-regulation of Ngn1 and Math1 coordinates the production of neurons and sensory hair cells during inner ear development. *Development*. 2007;134(24):4405-4415. doi:10.1242/dev.009118
21. Whitehead M, Morest D. The development of innervation patterns in the avian cochlea. *Neuroscience*. 1985;14(1):255-276. doi:10.1016/0306-4522(85)90177-0
22. Xiang M, Maklad A, Pirvola U, Fritzsch B. Brn3c null mutant mice show long-term, incomplete retention of some afferent inner ear innervation. *BMC Neurosci*. 2003;4(1):2. doi:10.1186/1471-2202-4-2
23. Pauley S, Wright T, Pirvola U, Ornitz D, Beisel K, Fritzsch B. Expression and function of FGF10 in mammalian inner ear development. *Dev Dyn*. 2003;227(2):203-215. doi:10.1002/dvdy.10297
24. Fritzsch B, Matei V, Nichols D, et al. Atoh1 null mice show directed afferent fiber growth to undifferentiated ear sensory epithelia followed by incomplete fiber retention. *Dev Dyn*. 2005;233(2):570-583. doi:10.1002/dvdy.20370
25. Bianchi L, Daruwalla Z, Roth T, et al. Immortalized mouse inner ear cell lines demonstrate a role for chemokines in promoting the growth of developing Statoacoustic ganglion neurons. *J Assoc Res Otolaryngol*. 2005;6(4):355-367. doi:10.1007/s10162-005-0013-8
26. Shen Y, Thompson D, Kuah M, et al. The cytokine macrophage migration inhibitory factor (MIF) acts as a neurotrophin in the developing inner ear of the zebrafish, *Danio rerio*. *Dev Biol*. 2012;363(1):84-94. doi:10.1016/j.ydbio.2011.12.023
27. Bank L, Bianchi L, Ebisu F, et al. Macrophage migration inhibitory factor acts as a neurotrophin in the developing inner ear. *Development*. 2012;139(24):4666-4674. doi:10.1242/dev.066647
28. Barald K, Shen Y, Bianchi L. Chemokines and cytokines on the neuroimmunoaxis: inner ear neurotrophic cytokines in development and disease. Prospects for repair? *Exp Neurol*. 2018;301:92-99. doi:10.1016/j.expneurol.2017.10.009
29. Fantetti K, Fekete D. Members of the BMP, Shh, and FGF morphogen families promote chicken statoacoustic ganglion neurite outgrowth and neuron survival in vitro. *Dev Neurobiol*. 2012;72(9):1213-1228. doi:10.1002/dneu.20988
30. Druckenbrod N, Hale E, Olukoya O, Shatzer W, Goodrich L. Neuronal processes and glial precursors form a scaffold for wiring the developing mouse cochlea. *Nat Commun*. 2020;11(1):5866. doi:10.1038/s41467-020-19521-2
31. Morris J, Maklad A, Hansen L, et al. A disorganized innervation of the inner ear persists in the absence of ErbB2. *Brain Res*. 2006;1091(1):186-199. doi:10.1016/j.brainres.2006.02.090
32. Mao Y, Reiprich S, Wegner M, Fritzsch B. Targeted deletion of Sox10 by Wnt1-cre defects neuronal migration and projection in the mouse inner ear. *PLoS One*. 2014;9(4):e94580. doi:10.1371/journal.pone.0094580
33. Zecca A, Dyballa S, Voltes A, Bradley R, Pujades C. The order and place of neuronal differentiation establish the topography of sensory projections and the entry points within the hindbrain. *J Neurosci*. 2015;35(19):7475-7486. doi:10.1523/jneurosci.3743-14.2015
34. van Heumen W, Claxton C, Pickles J. Expression of EphA4 in developing inner ears of the mouse and Guinea pig. *Hear Res*. 2000;139(1–2):42-50. doi:10.1016/s0378-5955(99)00158-6
35. Coate T, Raft S, Zhao X, Ryan A, Crenshaw E, Kelley M. Otic mesenchyme cells regulate spiral ganglion axon fasciculation through a Pou3f4/EphA4 signaling pathway. *Neuron*. 2012;73(1):49-63. doi:10.1016/j.neuron.2011.10.029
36. Kim Y, Ibrahim L, Wang S, et al. EphA7 regulates spiral ganglion innervation of cochlear hair cells. *Dev Neurobiol*. 2015;76(4):452-469. doi:10.1002/dneu.22326
37. Kim Y, Wang S, Tymanskyj S, Ma L, Tao H, Zhang L. Dcc mediates functional assembly of peripheral auditory circuits. *Sci Rep*. 2016;6(1):23799. doi:10.1038/srep23799
38. Salehi P, Myint A, Kim Y, et al. Genome-wide association analysis identifies DCC as an essential factor in the innervation of the peripheral vestibular system in inbred mice. *J Assoc Res Otolaryngol*. 2016;17(5):417-431. doi:10.1007/s10162-016-0578-4
39. Fekete D, Campero A. Axon guidance in the inner ear. *Int J Dev Biol*. 2007;51(6–7):549-556. doi:10.1387/ijdb.072341df
40. Battisti A, Fekete D. Slits and robos in the developing chicken inner ear. *Dev Dyn*. 2008;237(2):476-484. doi:10.1002/dvdy.21429
41. Wang S, Ibrahim L, Kim Y, et al. Slit/Robo signaling mediates spatial positioning of spiral ganglion neurons during development of Cochlear innervation. *J Neurosci*. 2013;33(30):12242-12254. doi:10.1523/jneurosci.5736-12.2013
42. Miyazaki N, Furuyama T, Takeda N, Inoue T, Kubo T, Inagaki S. Expression of mouse semaphorin H mRNA in the inner ear of mouse fetuses. *Neurosci Lett*. 1999;261(1–2):127-129. doi:10.1016/s0304-3940(98)00988-4
43. Gu C, Rodriguez E, Reimert D, et al. Neuropilin-1 conveys Semaphorin and VEGF signaling during neural and cardiovascular development. *Dev Cell*. 2003;5(1):45-57. doi:10.1016/s1534-5807(03)00169-2
44. Katayama K, Imai F, Suto F, Yoshida Y. Deletion of Sema3a or plexinA1/plexinA3 causes defects in sensory afferent projections of Statoacoustic ganglion neurons. *PLoS One*. 2013;8(8):e72512. doi:10.1371/journal.pone.0072512
45. Coate T, Spita N, Zhang K, Isgrig K, Kelley M. Neuropilin-2/Semaphorin-3F-mediated repulsion promotes inner hair cell innervation by spiral ganglion neurons. *Elife*. 2015;4:e07830. doi:10.7554/elife.07830
46. Jung J, Zhang K, Wang Z, et al. Semaphorin-5B controls spiral ganglion neuron branch refinement during development. *J Neurosci*. 2019;39(33):6425-6438. doi:10.1523/jneurosci.0113-19.2019
47. Zhang K, Coate T. Recent advances in the development and function of type II spiral ganglion neurons in the mammalian inner ear. *Semin Cell Dev Biol*. 2017;65:80-87. doi:10.1016/j.semcdb.2016.09.017
48. Zou Y. Wnt signaling in axon guidance. *Trends Neurosci*. 2004;27(9):528-532. doi:10.1016/j.tins.2004.06.015
49. Wang Y, Nathans J. Tissue/planar cell polarity in vertebrates: new insights and new questions. *Development*. 2007;134(4):647-658. doi:10.1242/dev.02772

50. Goodrich L, Strutt D. Principles of planar polarity in animal development. *Development*. 2011;138(10):1877-1892. doi:10.1242/dev.054080
51. Tissir F, Goffinet A. Shaping the nervous system: role of the core planar cell polarity genes. *Nat Rev Neurosci*. 2013;14(8):525-535. doi:10.1038/nrn3525
52. Ghimire S, Ratzan E, Deans M. A non-autonomous function of the core PCP protein VANGL2 directs peripheral axon turning in the developing cochlea. *Development*. 2018;145. doi:10.1242/dev.159012
53. Ghimire S, Deans M. Frizzled3 and Frizzled6 cooperate with Vangl2 to direct Cochlear innervation by type II spiral ganglion neurons. *J Neurosci*. 2019;39(41):8013-8023. doi:10.1523/jneurosci.1740-19.2019
54. Tessarollo L, Coppola V, Fritzsch B. NT-3 replacement with brain-derived neurotrophic factor redirects vestibular nerve fibers to the cochlea. *J Neurosci*. 2004;24(10):2575-2584. doi:10.1523/jneurosci.5514-03.2004
55. Schecterson L, Bothwell M. Novel roles for neurotrophins are suggested by BDNF and NT-3 mRNA expression in developing neurons. *Neuron*. 1992;9(3):449-463. doi:10.1016/0896-6273(92)90183-e
56. Fritzsch B, Tessarollo L, Coppola E, Reichardt L. Neurotrophins in the ear: their roles in sensory neuron survival and fiber guidance. *Prog Brain Res*. 2004;146:265-278. doi:10.1016/s0079-6123(03)46017-2
57. Schuster K, Dambly-Chaudière C, Ghysen A. Glial cell line-derived neurotrophic factor defines the path of developing and regenerating axons in the lateral line system of zebrafish. *Proc Natl Acad Sci USA*. 2010;107(45):19531-19536. doi:10.1073/pnas.1002171107
58. Tuttle A, Drerup C, Marra M, McGraw H, Nechiporuk A. Retrograde ret signaling controls sensory pioneer axon outgrowth. *Elife*. 2019;8:e46092. doi:10.7554/elife.46092
59. Hashimoto M, Heinrich G. Brain-derived neurotrophic factor gene expression in the developing zebrafish. *Int J Dev Neurosci*. 1997;15(8):983-997. doi:10.1016/s0736-5748(97)00017-8
60. Maresh A, Rodriguez Gil D, Whitman M, Greer C. Principles of glomerular Organization in the Human Olfactory Bulb—implications for odor processing. *PLoS One*. 2008;3(7):e2640. doi:10.1371/journal.pone.0002640
61. Zhao H, Ivic L, Otaki J, Hashimoto M, Mikoshiba K, Firestein S. Functional expression of a mammalian odorant receptor. *Science*. 1998;279(5348):237-242. doi:10.1126/science.279.5348.237
62. Zhang X, Firestein S. The olfactory receptor gene superfamily of the mouse. *Nat Neurosci*. 2002;5(2):124-133. doi:10.1038/nn800
63. Miyamichi K, Serizawa S, Kimura H, Sakano H. Continuous and overlapping expression domains of odorant receptor genes in the olfactory epithelium determine the dorsal/ventral positioning of glomeruli in the olfactory bulb. *J Neurosci*. 2005;25(14):3586-3592. doi:10.1523/jneurosci.0324-05.2005
64. Potter S, Zheng C, Koos D, Feinstein P, Fraser S, Mombaerts P. Structure and emergence of specific olfactory glomeruli in the mouse. *J Neurosci*. 2001;21(24):9713-9723. doi:10.1523/jneurosci.21-24-09713.2001
65. Ahmed W, Li T, Rubakhin S, Chiba A, Sweedler J, Saif T. Mechanical tension modulates local and global vesicle dynamics in neurons. *Cell Mol Bioeng*. 2012;5(2):155-164. doi:10.1007/s12195-012-0223-1
66. Koser D, Thompson A, Foster S, et al. Mechanosensing is critical for axon growth in the developing brain. *Nat Neurosci*. 2016;19(12):1592-1598. doi:10.1038/nn.4394
67. Thompson A, Pillai E, Dimov I, Foster S, Holt C, Franze K. Rapid changes in tissue mechanics regulate cell behaviour in the developing embryonic brain. *Elife*. 2019;8:e39356. doi:10.7554/elife.39356
68. Breau M, Bonnet I, Stoufflet J, Xie J, De Castro S, Schneider-Maunoury S. Extrinsic mechanical forces mediate retrograde axon extension in a developing neuronal circuit. *Nat Commun*. 2017;8(1):282. doi:10.1038/s41467-017-00283-3
69. Monnot P, Gangatharan G, Baraban M, et al. Intertissue mechanical interactions shape the olfactory circuit in zebrafish. *EMBO Rep*. 2021;23(2):e52963. doi:10.15252/embr.202152963
70. Šmít D, Fouquet C, Pincet F, Zapotocky M, Trembleau A. Axon tension regulates fasciculation/defasciculation through the control of axon shaft zippering. *Elife*. 2017;6:e19907. doi:10.7554/elife.19907
71. Tennent R, Chuah M. Ultrastructural study of ensheathing cells in early development of olfactory axons. *Dev Brain Res*. 1996;95(1):135-139. doi:10.1016/0165-3806(96)00091-0
72. Chehrehasa F, Windus L, Ekberg J, et al. Olfactory glia enhance neonatal axon regeneration. *Mol Cell Neurosci*. 2010;45(3):277-288. doi:10.1016/j.mcn.2010.07.002
73. Pellitteri R, Spatuzza M, Russo A, Stanzani S. Olfactory ensheathing cells exert a trophic effect on the hypothalamic neurons in vitro. *Neurosci Lett*. 2007;417(1):24-29. doi:10.1016/j.neulet.2007.02.065
74. Wang Y, Teng H, Gao Y, Zhang F, Ding Y, Huang Z. Brain-derived neurotrophic factor promotes the migration of olfactory Ensheathing cells through TRPC channels. *Glia*. 2016;64(12):2154-2165. doi:10.1002/glia.23049
75. Rich C, Perera S, Andratschke J, et al. Olfactory ensheathing cells abutting the embryonic olfactory bulb express Frzb, whose deletion disrupts olfactory axon targeting. *Glia*. 2018;66(12):2617-2631. doi:10.1002/glia.23515
76. Wang F, Nemes A, Mendelsohn M, Axel R. Odorant receptors govern the formation of a precise topographic map. *Cell*. 1998;93(1):47-60. doi:10.1016/s0092-8674(00)81145-9
77. Serizawa S, Miyamichi K, Takeuchi H, Yamagishi Y, Suzuki M, Sakano H. A neuronal identity code for the odorant receptor-specific and activity-dependent axon sorting. *Cell*. 2006;127(5):1057-1069. doi:10.1016/j.cell.2006.10.031
78. Lewcock J, Reed R. A feedback mechanism regulates monoallelic odorant receptor expression. *Proc Natl Acad Sci USA*. 2004;101(4):1069-1074. doi:10.1073/pnas.0307986100
79. Shykind B, Rohani S, O'Donnell S, et al. Gene switching and the stability of odorant receptor gene choice. *Cell*. 2004;117(6):801-815. doi:10.1016/j.cell.2004.05.015
80. Imai T, Sakano H. Odorant receptor-mediated signaling in the mouse. *Curr Opin Neurobiol*. 2008;18(3):251-260. doi:10.1016/j.conb.2008.07.009
81. Imai T, Suzuki M, Sakano H. Odorant receptor-derived cAMP signals direct axonal targeting. *Science*. 2006;314(5799):657-661. doi:10.1126/science.1131794
82. Cutforth T, Moring L, Mendelsohn M, et al. Axonal Ephrin-as and odorant receptors. *Cell*. 2003;114(3):311-322. doi:10.1016/s0092-8674(03)00568-3
83. Zapiec B, Bressel O, Khan M, Walz A, Mombaerts P. Neuropilin-1 and the positions of glomeruli in the mouse

olfactory bulb. *eNeuro*. 2016;3(5):ENEU16.2016. doi:10.1523/eneuro.0123-16.2016

84. Assens A, Dal Col J, Njoku A, et al. Alteration of Nrp1 signaling at different stages of olfactory neuron maturation promotes glomerular shifts along distinct axes in the olfactory bulb. *Development*. 2016;143:3817-3825. doi:10.1242/dev.138941
85. Astic L, Saucier D. Anatomical mapping of the neuroepithelial projection to the olfactory bulb in the rat. *Brain Res Bull*. 1986;16(4):445-454. doi:10.1016/0361-9230(86)90172-3
86. Sauciert D, Astic L. Analysis of the topographical organization of olfactory epithelium projections in the rat. *Brain Res Bull*. 1986;16(4):455-462. doi:10.1016/0361-9230(86)90173-5
87. Ressler K, Sullivan S, Buck L. A zonal organization of odorant receptor gene expression in the olfactory epithelium. *Cell*. 1993;73(3):597-609. doi:10.1016/0092-8674(93)90145-g
88. Vassar R, Chao S, Sitcheran R, Nuñez J, Vosshall L, Axel R. Topographic organization of sensory projections to the olfactory bulb. *Cell*. 1994;79(6):981-991. doi:10.1016/0092-8674(94)90029-9
89. Cho J, Lepine M, Andrews W, Parnavelas J, Cloutier J. Requirement for Slit-1 and Robo-2 in zonal segregation of olfactory sensory neuron axons in the Main olfactory bulb. *J Neurosci*. 2007;27(34):9094-9104. doi:10.1523/jneurosci.2217-07.2007
90. Cho J, Kam J, Cloutier J. Slits and Robo-2 regulate the coalescence of subsets of olfactory sensory neuron axons within the ventral region of the olfactory bulb. *Dev Biol*. 2012;371(2):269-279. doi:10.1016/j.ydbio.2012.08.028
91. Aoki M, Takeuchi H, Nakashima A, Nishizumi H, Sakano H. Possible roles of robo1+ ensheathing cells in guiding dorsal-zone olfactory sensory neurons in mouse. *Dev Neurobiol*. 2013;73:828-840. doi:10.1002/dneu.22103
92. Cloutier J, Giger R, Koentges G, Dulac C, Kolodkin A, Ginty D. Neuropilin-2 mediates axonal fasciculation, zonal segregation, but not axonal convergence, of primary accessory olfactory neurons. *Neuron*. 2002;33(6):877-892. doi:10.1016/s0896-6273(02)00635-9
93. Walz A, Rodriguez I, Mombaerts P. Aberrant sensory innervation of the olfactory bulb in Neuropilin-2 mutant mice. *J Neurosci*. 2002;22(10):4025-4035. doi:10.1523/jneurosci.22-10-04025.2002
94. Taniguchi M, Nagao H, Takahashi Y, et al. Distorted odor maps in the olfactory bulb of Semaphorin 3A-deficient mice. *J Neurosci*. 2003;23(4):1390-1397. doi:10.1523/jneurosci.23-04-01390.2003
95. Cheng R, Dang P, Taku A, et al. Loss of Neuropilin2a/b or Sema3fa alters olfactory sensory axon dynamics and protoglomerular targeting. *Neural Dev*. 2022;17(1):1. doi:10.1186/s13064-021-00157-x
96. Levai O, Breer H, Strotmann J. Subzonal organization of olfactory sensory neurons projecting to distinct glomeruli within the mouse olfactory bulb. *J Comp Neurol*. 2003;458(3):209-220. doi:10.1002/cne.10559
97. Scolnick J, Cui K, Duggan C, et al. Role of IGF signaling in olfactory sensory map formation and axon guidance. *Neuron*. 2008;57(6):847-857. doi:10.1016/j.neuron.2008.01.027
98. Schwarting G, Kostek C, Ahmad N, Dibble C, Pays L, Püschel A. Semaphorin 3A is required for guidance of olfactory axons in mice. *J Neurosci*. 2000;20(20):7691-7697. doi:10.1523/jneurosci.20-20-07691.2000
99. Schwarting G, Raitcheva D, Crandall J, Burkhardt C, Puschel A. Semaphorin 3A-mediated axon guidance regulates convergence and targeting of P2 odorant receptor axons. *Eur J Neurosci*. 2004;19(7):1800-1810. doi:10.1111/j.1460-9568.2004.03304.x
100. Bulfone A, Wang F, Hevner R, et al. An olfactory sensory map develops in the absence of normal projection neurons or GABAergic interneurons. *Neuron*. 1998;21(6):1273-1282. doi:10.1016/s0896-6273(00)80647-9
101. St. John J, Clarris H, McKeown S, Royal S, Key B. Sorting and convergence of primary olfactory axons are independent of the olfactory bulb. *J Comp Neurol*. 2003;464(2):131-140. doi:10.1002/cne.10777
102. Ardiles Y, de la Puente R, Toledo R, Isgor C, Guthrie K. Response of olfactory axons to loss of synaptic targets in the adult mouse. *Exp Neurol*. 2007;207(2):275-288. doi:10.1016/j.expneurol.2007.06.022
103. Vaddadi N, Iversen K, Raja R, et al. Kirrel2 is differentially required in populations of olfactory sensory neurons for the targeting of axons in the olfactory bulb. *Development*. 2019;146. doi:10.1242/dev.173310
104. Sakano H. Neural map formation in the mouse olfactory system. *Neuron*. 2010;67(4):530-542. doi:10.1016/j.neuron.2010.07.003
105. Kaneko-Goto T, Yoshihara S, Miyazaki H, Yoshihara Y. BIG-2 mediates olfactory axon convergence to target glomeruli. *Neuron*. 2008;57(6):834-846. doi:10.1016/j.neuron.2008.01.023
106. Lumpkin E, Caterina M. Mechanisms of sensory transduction in the skin. *Nature*. 2007;445(7130):858-865. doi:10.1038/nature05662
107. Liu Y, Halloran M. Central and peripheral axon branches from one neuron are guided differentially by Semaphorin3D and transient axonal glycoprotein-1. *J Neurosci*. 2005;25(45):10556-10563. doi:10.1523/jneurosci.2710-05.2005
108. Chilton J, Guthrie S. Cranial expression of class 3 secreted semaphorins and their neuropilin receptors. *Dev Dyn*. 2003;228(4):726-733. doi:10.1002/dvdy.10396
109. Davies A, Lumsden A, Slavkin H, Burnstock G. Influence of nerve growth factor on the embryonic mouse trigeminal ganglion in culture. *Dev Neurosci*. 1981;4(2):150-156. doi:10.1159/000112751
110. Davies A, Lumsden A. Relation of target encounter and neuronal death to nerve growth factor responsiveness in the developing mouse trigeminal ganglion. *J Comp Neurol*. 1984;223(1):124-137. doi:10.1002/cne.902230110
111. Buchman V, Davies A. Different neurotrophins are expressed and act in a developmental sequence to promote the survival of embryonic sensory neurons. *Development*. 1993;118(3):989-1001. doi:10.1242/dev.118.3.989
112. Patel T, Jackman A, Rice F, Kucera J, Snider W. Development of sensory neurons in the absence of NGF/TrkA signaling in vivo. *Neuron*. 2000;37(1):183. doi:10.1016/s0896-6273(02)01176-5
113. O'Connor R, Tessier-Lavigne M. Identification of maxillary factor, a maxillary process-derived chemoattractant for developing trigeminal sensory axons. *Neuron*. 1999;24(1):165-178. doi:10.1016/s0896-6273(00)80830-2

114. Howard L, Wyatt S, Davies A. Neuregulin-4 contributes to the establishment of cutaneous sensory innervation. *Dev Neurobiol*. 2021;81(2):139-148. doi:10.1002/dneu.22803
115. Jayasena C, Flood W, Koblar S. High EphA3 expressing ophthalmic trigeminal sensory axons are sensitive to ephrin-A5-fc: implications for lobe specific axon guidance. *Neuroscience*. 2005;135(1):97-109. doi:10.1016/j.neuroscience.2005.05.052
116. Van Exan RJ, Hardy MH. A spatial relationship between innervation and the early differentiation of vibrissa follicles in the embryonic mouse. *J Anat*. 1980;131(Pt 4):643-656.
117. North H, Karim A, Jacquin M, Donoghue M. EphA4 is necessary for spatially selective peripheral somatosensory topography. *Dev Dyn*. 2009;239(2):630-638. doi:10.1002/dvdy.22185
118. Yaron A, Huang P, Cheng H, Tessier-Lavigne M. Differential requirement for Plexin-A3 and -A4 in mediating responses of sensory and sympathetic neurons to distinct class 3 Semaphorins. *Neuron*. 2005;45(4):513-523. doi:10.1016/j.neuron.2005.01.013
119. Kettunen P, Løes S, Furmanek T, et al. Coordination of trigeminal axon navigation and patterning with tooth organ formation: epithelial-mesenchymal interactions, and epithelial Wnt4 and Tgfβ1 regulate semaphorin 3a expression in the dental mesenchyme. *Development*. 2005;132(2):323-334. doi:10.1242/dev.01541
120. Butler S, Dodd J. A role for BMP heterodimers in roof plate-mediated repulsion of commissural axons. *Neuron*. 2003;38(3):389-401. doi:10.1016/s0896-6273(03)00254-x
121. Guha U, Gomes W, Samanta J, Gupta M, Rice F, Kessler J. Target-derived BMP signaling limits sensory neuron number and the extent of peripheral innervation in vivo. *Development*. 2004;131(5):1175-1186. doi:10.1242/dev.01013
122. Engelhard C, Sarsfield S, Merte J, et al. MEGF8 is a modifier of BMP signaling in trigeminal sensory neurons. *Elife*. 2013;2:e01160. doi:10.7554/elife.01160
123. Miyashita T, Yeo S, Hirate Y, et al. PlexinA4 is necessary as a downstream target of Islet2 to mediate slit signaling for promotion of sensory axon branching. *Development*. 2004;131(15):3705-3715. doi:10.1242/dev.01228
124. Ma L, Tessier-Lavigne M. Dual branch-promoting and branch-repelling actions of slit/Robo signaling on peripheral and central branches of developing sensory axons. *J Neurosci*. 2007;27(25):6843-6851. doi:10.1523/jneurosci.1479-07.2007
125. Rochlin M, O'Connor R, Giger R, Verhaagen J, Farbman A. Comparison of neurotrophin and repellent sensitivities of early embryonic geniculate and trigeminal axons. *J Comp Neurol*. 2000;422(4):579-593. doi:10.1002/1096-9861(20000710)422:4<579::aid-cne7>3.0.co;2-g
126. Lopez G, Krimm R. Epithelial overexpression of BDNF and NT4 produces distinct gustatory axon morphologies that disrupt initial targeting. *Dev Biol*. 2006;292(2):457-468. doi:10.1016/j.ydbio.2006.01.021
127. Huang T, Krimm R. BDNF and NT4 play interchangeable roles in gustatory development. *Dev Biol*. 2014;386(2):308-320. doi:10.1016/j.ydbio.2013.12.031
128. Vilbig R, Cosmano J, Giger R, Rochlin M. Distinct roles for Sema3A, Sema3F, and an unidentified trophic factor in controlling the advance of geniculate axons to gustatory lingual epithelium. *J Neurocytol*. 2004;33(6):591-606. doi:10.1007/s11068-005-3329-8
129. Rios-Pilier J, Krimm R. TrkB expression and dependence divides gustatory neurons into three subpopulations. *Neural Dev*. 2019;14(1):3. doi:10.1186/s13064-019-0127-z
130. Fei D, Huang T, Krimm R. The neurotrophin receptor p75 regulates gustatory axon branching and promotes innervation of the tongue during development. *Neural Dev*. 2014;9(1):15. doi:10.1186/1749-8104-9-15
131. Tang T, Donnelly C, Shah A, Bradley R, Mistretta C, Pierchala B. Cell non-autonomous requirement of p75 in the development of geniculate oral sensory neurons. *Sci Rep*. 2020;10(1):22117. doi:10.1038/s41598-020-78816-y
132. Patel A, Krimm R. Neurotrophin-4 regulates the survival of gustatory neurons earlier in development using a different mechanism than brain-derived neurotrophic factor. *Dev Biol*. 2012;365(1):50-60. doi:10.1016/j.ydbio.2012.02.008
133. Rochlin M, Farbman A. Trigeminal ganglion axons are repelled by their presumptive targets. *J Neurosci*. 1998;18(17):6840-6852. doi:10.1523/jneurosci.18-17-06840.1998
134. Mbiene J, Mistretta C. Initial innervation of embryonic rat tongue and developing taste papillae: nerves follow distinctive and spatially restricted pathways. *Cells Tissues Organs*. 1997;160(3):139-158. doi:10.1159/000148006
135. Huang T, Krimm R. Developmental expression of Bdnf, Ntf4/5, and TrkB in the mouse peripheral taste system. *Dev Dyn*. 2010;239(10):2637-2646. doi:10.1002/dvdy.22412
136. Liebl D, Mbiene J, Parada L. NT4/5 mutant mice have deficiency in gustatory papillae and taste bud formation. *Dev Biol*. 1999;213(2):378-389. doi:10.1006/dbio.1999.9385
137. Patel A, Huang T, Krimm R. Lingual and palatal gustatory afferents each depend on both BDNF and NT-4, but the dependence is greater for lingual than palatal afferents. *J Comp Neurol*. 2010;518(16):3290-3301. doi:10.1002/cne.22400
138. Ma L, Lopez G, Krimm R. Epithelial-derived brain-derived neurotrophic factor is required for gustatory neuron targeting during a critical developmental period. *J Neurosci*. 2009;29(11):3354-3364. doi:10.1523/jneurosci.3970-08.2009
139. Runge E, Hoshino N, Biehl M, Ton S, Rochlin M. Neurotrophin-4 is more potent than brain-derived neurotrophic factor in promoting, attracting and suppressing geniculate ganglion neurite outgrowth. *Dev Neurosci*. 2012;34(5):389-401. doi:10.1159/000342996
140. Lopez G, Krimm R. Epithelial overexpression of BDNF and NT4 produces distinct gustatory axon morphologies that disrupt initial targeting. *Dev Biol*. 2006;292(2):457-468. doi:10.1016/j.ydbio.2006.01.021
141. Nosrat C, Ebendal T, Olson L. Differential expression of brain-derived neurotrophic factor and neurotrophin 3 mRNA in lingual papillae and taste buds indicates roles in gustatory and somatosensory innervation. *J Comp Neurol*. 1996;376(4):587-602. doi:10.1002/(sici)1096-9861(19961223)376:4<587::aid-cne7>3.0.co;2-y
142. Nosrat C, MacCallum D, Mistretta C. Distinctive spatiotemporal expression patterns for neurotrophins develop in gustatory papillae and lingual tissues in embryonic tongue organ cultures. *Cell Tissue Res*. 2001;303(1):35-45. doi:10.1007/s004410000271
143. Nosrat C, Olson L. Brain-derived neurotrophic factor mRNA is expressed in the developing taste bud-bearing tongue papillae of rat. *J Comp Neurol*. 1995;360(4):698-704. doi:10.1002/cne.903600413

144. Hoshino N, Vatterott P, Egwiekhor A, Rochlin M. Brain-derived neurotrophic factor attracts geniculate ganglion neurites during embryonic targeting. *Dev Neurosci*. 2010;32(3):184-196. doi:10.1159/000313902
145. Mistretta C, Goosens K, Farinas I, Reichardt L. Alterations in size, number, and morphology of gustatory papillae and taste buds in BDNF null mutant mice demonstrate neural dependence of developing taste organs. *J Comp Neurol*. 1999;409(1):13-24. doi:10.1002/(sici)1096-9861(19990621)409:1<13::aid-cne2>3.0.co;2-o
146. Giger R, Wolfer D, De Wit G, Verhaagen J. Anatomy of rat semaphorin III collapsin-1 mRNA expression and relationship to developing nerve tracts during neuroembryogenesis. *J Comp Neurol*. 1996;375(3):378-392. doi:10.1002/(sici)1096-9861(19961118)375:3<378::aid-cne3>3.0.co;2-#
147. Dillon T, Saldanha J, Giger R, Verhaagen J, Rochlin M. Sema3A regulates the timing of target contact by cranial sensory axons. *J Comp Neurol*. 2004;470(1):13-24. doi:10.1002/cne.11029
148. Treffy R, Collins D, Hoshino N, et al. Ephrin-B/EphB signaling is required for Normal innervation of lingual gustatory papillae. *Dev Neurosci*. 2016;38(2):124-138. doi:10.1159/000444748
149. Yarmolinsky D, Zuker C, Ryba N. Common sense about taste: from mammals to insects. *Cell*. 2009;139(2):234-244. doi:10.1016/j.cell.2009.10.001
150. Barretto R, Gillis-Smith S, Chandrashekar J, et al. The neural representation of taste quality at the periphery. *Nature*. 2014;517(7534):373-376. doi:10.1038/nature13873
151. Zhao G, Zhang Y, Hoon M, et al. The receptors for mammalian sweet and umami taste. *Cell*. 2003;115(3):255-266. doi:10.1016/s0092-8674(03)00844-4
152. Mueller K, Hoon M, Erlenbach I, Chandrashekar J, Zuker C, Ryba N. The receptors and coding logic for bitter taste. *Nature*. 2005;434(7030):225-229. doi:10.1038/nature03352
153. Lee H, Macpherson L, Parada C, Zuker C, Ryba N. Rewiring the taste system. *Nature*. 2017;548(7667):330-333. doi:10.1038/nature23299
154. Ramón Y, Cajal S. *Notas Anatomicas. I. Sobre La Aparición De Las Expansiones Celulares En La Médula Embrionaria. II. Sobre Las Terminaciones Nerviosas Del Corazón De Los Batracios Y Reptiles, Barcelona*. San Diego, CA: Brain Corporation; 1890.
155. Koehler K, Nie J, Longworth-Mills E, et al. Generation of inner ear organoids containing functional hair cells from human pluripotent stem cells. *Nat Biotechnol*. 2017;35(6):583-589. doi:10.1038/nbt.3840
156. Lee J, Rabbani C, Gao H, et al. Hair-bearing human skin generated entirely from pluripotent stem cells. *Nature*. 2020;582(7812):399-404. doi:10.1038/s41586-020-2352-3
157. Hisha H, Tanaka T, Ueno H. Lingual epithelial stem cells and organoid culture of them. *Int J Mol Sci*. 2016;17(2):168. doi:10.3390/ijms17020168

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