PROTOCOL TITLE: Pairing Intermittent Hypoxia and Transcutaneous Electrical Spinal Cord Stimulation to Promote Arm Use After Cervical SCI

NCT#: NCT04854057

Date: 29 December 2022

I. BACKGROUND AND SIGNIFICANCE

Spinal cord injury (SCI) is damage to any part of the spinal cord that interrupts communication between the brain and the body. Depending on the level and the extent of the damage, individuals with SCI usually have permanent paralysis and disability. Consequently, SCI has a life-long, high socioeconomic impact on affected individuals, families, and health care systems. Estimated global SCI incidence is 40 to 80 new cases per million population per year (WHO, 2013). Each year about 17,700 new spinal cord injuries (SCI) occur in the United States (NSCISC, 2020). The estimated direct life-time cost of cervical SCI is between $2.3 and $5.0 million that varies depending on the level, severity, and age at injury (Cao, Chen, & DeVivo, 2011; NSCISC, 2020). Additionally, indirect costs such as work loss, reduced productivity, personal assistance expenses, and the burden on the family is estimated an average of $76,000 per year (NSCISC, 2020).

The majority of spinal cord injuries occur at the cervical level (NSCISC, 2020) that results in impairment in motor control and sensory function in the hand and arm. Restoration of upper extremity function is consistently rated as the highest treatment priority among people with cervical SCI (Anderson, 2004; French, Anderson-Erisman, & Sutter, 2010). The current standard of care to restore tetraplegic hand function is limited, and outcomes of these approaches are insufficient to improve the ability of self-care and independence. There is a need to for novel non-invasive treatment approaches to target plasticity that induce functional restoration in persons with chronic, cervical SCI. Brain-derived neurotropic factor (BDNF) expression plays a key-role in increasing neuroplasticity (Garraway & Huie, 2016). Daily acute intermittent hypoxia (AIH) and transcutaneous electrical spinal cord stimulation (TESS) are emerging therapies. Both of these interventions elicit an increase in BDNF. By combining these two approaches, augmentation of neuroplasticity could be achieved.

Daily AIH is a safe and highly promising non-invasive intervention as a plasticity primer for task-specific motor training in SCI rehabilitation (Trumbower, Hayes, Mitchell, Wolf, & Stahl, 2017; Trumbower, Jayaraman, Mitchell, & Rymer, 2012). Daily AIH upregulates BDNF and its high-affinity receptor (TrkB) that induce neuroplasticity (Baker-Herman et al., 2004). Human trials have demonstrated the efficacy of daily AIH in improving motor function both in upper (Trumbower et al., 2017) and lower extremities (Trumbower et al., 2012) and dynamic balance (Navarrete-Opazo, Alcayaga, Sepúlveda, & Varas, 2017). The results of a double blind sham controlled study showed that dAIH paired with task-specific training significantly improved hand dexterity, function, maximum hand aperture and muscle coordination in all participants compared to baseline ($p<0.05$) (Trumbower et al., 2017).

Over the past few years, a novel, non-invasive, transcutaneous electrical spinal cord stimulation (TESS) strategy adopting high carrier-frequency waveforms has emerged. This new strategy utilizes a unique waveform of biphasic or monophasic, rectangular, 1 ms pulses that are delivered at a frequency of 30 Hz. Each pulse is filled with an overlapping frequency of 10 kHz, which permits high stimulation intensities to pass through the skin and reach the spinal cord without causing discomfort (Gerasimenko et al., 2015). In patients with complete SCI, TESS improved lower extremity function in a gravity neutral position and facilitated stepping assisted by an exoskeleton (Gad et al., 2017; Gad et al., 2015). TESS is showing an exciting potential to restore upper extremity function effectively and non-invasively. Gad and

co-workers studied handgrip force improvements in 6 AIS B and AIS C chronic cervical SCI subjects (Gad et al., 2018). They reported that eight sessions of transcutaneous stimulation increased maximum voluntary handgrip forces by ~3-fold in the presence of stimulation and ~2-fold without simultaneous stimulation. In another case study, a subject who had C3 level chronic central cord injury improved substantially in terms of motor and sensory function (Inanici et al., 2018). Following four weeks of combined stimulation and sensorimotor training, the Graded Redefined Assessment of Strength, Sensation, and Prehension test score increased 52 points, and the upper extremity motor score improved 10 points. Pinch strength increased 2- to 7-fold in the left and right hands, respectively. Sensation recovered on trunk dermatomes, and the overall neurologic level of injury improved from C3 to C4. The subject began partial self-feeding at home in the second week of the intervention for the first time since his injury and continued this activity even after the intervention. Most notably, functional gains persisted for over three months of follow-up without further treatment. These data suggest that non-invasive electrical stimulation of spinal networks can promote neuroplasticity and long-term recovery following spinal cord injury. However, the exact mechanism of the electrical spinal cord stimulation remains to be determined. In animal studies, electrical stimulation of the nervous system increased BDNF and TrkB expression which promotes neuroplasticity (Al-Majed, Tam, & Gordon, 2004; Ghorbani et al., 2020). A possible mechanism that increases BDNF expression through electrical stimulation is via calcium influx through voltage-gated calcium channels leading to a chemical cascade with an increase of BDNF (Vermehren-Schmaedick, Khanjian, & Balkowiec, 2015; Wenjin et al., 2011).

II. SPECIFIC AIMS

A major goal of this proposal is to test the efficacy of daily AIH with TESS on restoring hand function in persons with chronic incomplete SCI.

Our fundamental hypothesis guiding this proposal is that daily AIH+TESS engage excitatory and inhibitory pathways, which converge on a common plasticity-promoting cascade that induces greater recovery of hand function than either one alone.

Both treatments appear to enhance motor function in persons with cervical SCI. Despite their independent effects on promoting functional benefits, we do not yet know if they may promote greater functional benefits when combined. To be effective as a long-term rehabilitation strategy, it is essential to determine the efficacy of combined protocols of recurring AIH+TESS.

Our specific aims are:

Aim 1. To investigate the therapeutic effect of combinatorial treatment of TESS and dAIH on motor performance.

We hypothesize that the combination of dAIH and TESS enhances recovery of fine motor skills of the hand, strength and muscle coordination that will be quantified using Graded Redefined Assessment of Strength, Sensibility, and Prehension (GRASSP), pinch-grip force measurements, and muscle coordination determined by agonist-antagonist coactivity ratio during grip and release movement, respectively.

Aim 2. To evaluate the effect of combinatorial treatment of TESS and dAIH on excitatory and inhibitory pathways of spinal networks and corticospinal tract.

We hypothesize that the combination of dAIH and TESS changes the excitability in neuronal pathways. To quantify neuronal pathway excitability, we will compare F-wave, H-reflex, somatosensory evoked potentials, motor evoked potentials and sympathetic skin responses before and after the treatment.

III. SUBJECT SELECTION

Subject Selection and Recruitment: Participants with cervical SCI will be recruited from Spaulding Rehabilitation Hospital and Massachusetts General Hospital. Potential subjects will be informed about the objectives of the study and must meet all inclusion and exclusion criteria. They will be required to read and sign approved consent and HIPAA forms prior to participation to allow access to medical information from their physicians. To determine the effects of the combinatorial treatment of acute intermittent hypoxia and transcutaneous electrical spinal cord stimulation, we propose to carry out a prospective, randomized, double blind, cross-over design study involving two alternating treatment arms. Intervention arms will consist of TESS and daily dAIH, paired with intensive upper extremity functional training. Based on prior recruitment experiences (Hayes et al., 2014), we anticipate a withdrawal rate of 20% in SCI. Our predicted sample size is total 10 persons with chronic cervical spinal cord injury. We incorporated the International Campaign for Cures of Spinal Cord Injury Paralysis (ICCP) inclusion/exclusion criteria recommendations (Tuszynski et al., 2007) to account for ethical considerations, safety, and potential confounds during subject recruitment.

ResearchMatch.org also will be utilized as a recruitment tool for this protocol. ResearchMatch.org is a national electronic, web-based recruitment tool that was created through the Clinical & Translational Science Awards Consortium in 2009 and is maintained at Vanderbilt University as an IRB-approved data repository (see IRB #090207).

Inclusion criteria: Participation requirements for individuals with SCI include: 1) 18 to 65 years old (the latter to reduce likelihood of significant comorbidities); 2) medically stable with medical clearance from physician to participate; 3) SCI at or below C3 and at or above C7; 4) non-progressive etiology of spinal injury; 5) American Spinal Injury Association Impairment Scale (AIS) C-D at initial screen; 6) at least 1 year post-injury (chronic). We plan to choose subjects greater that 1-year post-injury to ensure minimal confounding effects of natural spontaneous neurological recovery during the experiments. This will mean that changes in sensorimotor performance are more likely due to the interventions associated with the research study; 7) difficulty independently performing hand functions in activities of daily living; 8) able to attend 2 rounds of study sessions for 3 consecutive weeks and 2 follow-up visits for up to 16 weeks of study involvement; 9) stable medical condition without cardiopulmonary disease or frequent autonomic dysreflexia that would contraindicate participation in upper extremity rehabilitation or testing activities; 10) adequate social support to be able to participate in daily training for 6 weeks and a total of 16 weeks for all assessment sessions within the study period; 11) ability to read and speak English or communicate via medical interpreter.

Exclusion criteria: We will exclude individuals who have: **1)** dependence on ventilation support **2)** implanted stimulator (e.g. diaphragm pacing by phrenic nerve stimulation, vagus nerve stimulator, pacemaker, cochlear implant, epidural stimulator, baclofen pump, etc.); **3)** spinal cord injury related complications including unhealed pressure sore, severe neuropathic or chronic pain syndrome, history of frequent autonomic dysreflexia, infection (e.g. urinary tract), cardiovascular disease (e.g. deep vein thrombosis), pulmonary disease, heterotopic ossification in the upper extremities, severe osteoporosis, unhealed fracture, contracture of the upper extremity joints **4)** received botulinum toxin injections in upper extremity muscles in the prior 6 months; **5)** history of tendon or nerve transfer surgery in the upper extremity; **6)** history of additional neurologic disease, such as stroke, multiple sclerosis, traumatic brain injury, peripheral neuropathy (diabetic polyneuropathy, entrapment neuropathy, etc.) or peripheral nerve injury in the upper extremity **7)** history of concomitant diseases that would prevent full participation in intensive exercise therapy, such as uncontrolled hypertension, rheumatic diseases (rheumatoid arthritis, systemic lupus erythematosus, etc.), pulmonary disease, active cancer, chronic contagious disease, etc.; **8)** anticoagulation medication **9)** pregnancy because of unknown effects of TESS and dAIH, although women of childbearing potential will not otherwise be excluded; **10)** history of conditions that contraindicate transcranial magnetic stimulation. These include history of seizures or epilepsy, unexplained, recurring or violent headaches, nausea associated with medical procedures (needles, injections etc.), increased pressure inside the skull or significant heart disease, medication that may influence chances of having a seizure , and depression or bipolar disorder; **11)** history of allergic reaction or any skin reaction to use of adhesive electrodes **12)** < 24 on Mini-Mental Exam (Folstein, Folstein, & McHugh, 1975).

Inclusion of women and minorities: Of SCI cases, about 78% are male and 59% are non-Hispanic whites. SCI may affect individuals at any age, and average age at injury is 43 years (NSCISC, 2020). Our recruitment of subjects with cervical spinal cord injury and disability in their hand and arm function will include women and persons of any ethnicity and race. Since we do not have data that show any differences in responsiveness to TESS and daily AIH according to the age, gender or race of persons with SCI, we will balance based on age, gender, and race. However, women who are pregnant will not take part in this study as the effects of TESS and daily AIH on the developing fetus have not been studied. If a woman is of childbearing ability, birth control will be recommended for use throughout the study.

Based on these criteria, the team already has identified ~30 potential subjects eligible to participate in the experiments outlined in this proposal (see Human Subjects section for details).

Clinical Ratings: Potential subjects will undergo a screen by one of the licensed study clinicians. Physical evaluations will be completed by the study's licensed physical therapists, or occupational therapist, or trained research study staff.

IV. SUBJECT ENROLLMENT

a) ***Methods of enrollment, including procedures for patient registration and/or randomization:*** Our study coordinator, study physicians, physical therapists, PI (Trumbower),

and research collaborators will recruit subjects using a variety of strategies shown effective in the past. First, the team will identify potential subjects using Institutional Review Board (IRB)-approved resources to inform persons with SCI that are currently admitted to inpatient rehabilitation/outpatient units of Spaulding Rehabilitation Hospital and Massachusetts General Hospital. Subjects also will be recruited by word of mouth and flyers at these facilities.

Potential individuals with SCI will be identified by the following sources:

1. Attending physicians or therapists may refer their subjects to the study. We will provide physicians, therapists, and clinics with study information sheets, letters, and flyers. Prospective subjects will be encouraged to contact the study co-investigators.
2. Flyers posted in public areas across the Boston-land region, in the outpatient specialist clinics, or other private locations with given permission.
3. Possible subjects might also be identified through their medical records and their physicians might be asked to inform the subjects about the study.
4. Presentations at local, state hospitals, and rehabilitation facilities that include public and VA medical centers in the U.S. Northeast.
5. Attending public forums, conferences, or events at which the co-investigator will distribute IRB approved recruitment materials.
6. Partners Rally http://rally.partners.org
7. We also will provide a description of the study on http://www.ClinicalTrials.gov,
8. We will contact patients who have consented to be added to the Spaulding Rehabilitation Hospital's Spinal Cord Injury Model Systems (SCIMS) Database who have identified that they wished to be contacted for additional research studies. Research staff recruiting from the SCIMS database are also added as study staff on the SCIMS protocol.
9. ResearchMatch.org also will be utilized as a recruitment tool for this protocol. ResearchMatch.org is a national electronic, web-based recruitment tool that was created through the Clinical & Translational Science Awards Consortium in 2009 and is maintained at Vanderbilt University as an IRB-approved data repository (*see IRB #090207*).

b) Procedures for obtaining informed consent: Prospective subjects also will be informed of the methods, inclusion/exclusion criteria, and purpose of the study. We will provide a description of this study on ClinicalTrials.gov, as required by U.S. law. In all cases, recruitment will not be coercive, will not involve undue inducements, and will accurately reflect the study.

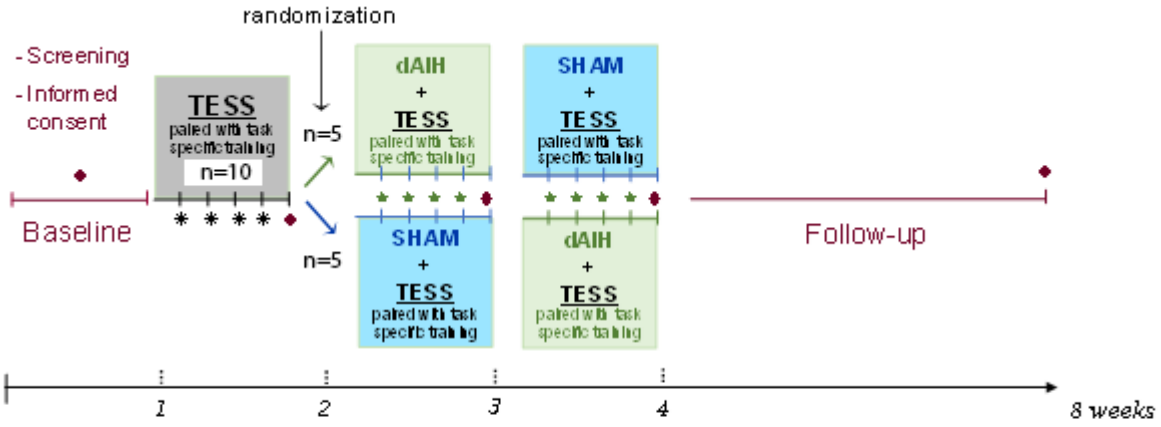
Eligible subjects will be screened at the designated site facilities (i.e., INSPIRE Laboratory, Spaulding Rehabilitation Hospital). At the screening visit, subjects will be informed of this project, their potential involvement, the possible benefits and risks, and their right to terminate participation at any time without penalty. Qualifying subjects in agreement with the study details will be asked to sign the informed consent and HIPAA form approved by Partner's IRB. All potential subjects will enter the study according to the inclusion/exclusion criteria above and following medical clearance. Experiments will be conducted at the *Spaulding Rehabilitation Hospitals (Boston, Cambridge).*

c) Treatment assignment, and randomization: Simple randomization of the participants will be used to assign for the cross-over order of the dAIH and sham treatments.

V. STUDY PROCEDURES

a) Study timeline:

The length of each arm of the study for each participant will last 8 weeks (up to 16 weeks total for both arms.) Figure 1 illustrates the stages of study timeline:



♦ – *Outcome measurements – 5 times*
[once at baseline, 3 times throughout intervention phase (at the end of each week), once at final follow-up]

* – ***45 min. cervical TESS paired with task-specific training, 4 sessions/week***

⋆ – ***37.5 min. AIH** (or **37.5 min. SHAM**) + 45 min. cervical TESS paired with task-specific training, 4 sessions/week*

Figure 1. Study design and timeline

b) Repeated measurements:

Measurements will be conducted at baseline, once at the end of each intervention week and at the end of one-month follow-up period.

c) Outcome measures:

1. GRASSP test. This test is validated for the tetraplegic population to quantify hand and arm function (Kalsi-Ryan et al., 2016; Kalsi-Ryan, Curt, Verrier, & Fehlings, 2012). Sub-scores of the GRASSP test include: a) Strength: manual muscle testing of 10 upper extremity muscles (i.e. deltoid, triceps, biceps, wrist extensors, finger flexors, finger abductors, extensor digitorum, opponens pollicis, flexor pollicis longus, and first dorsal interossei) on each side using Medical Research Council Motor Strength Scale; b) Sensation: Semmens-Weinstein monofilament testing of dorsal and palmar sensation of the fingers and thumb; c) Qualitative prehension: movement and active/passive positioning of finger-hand-forearm to form three prehension patterns, i.e. cylindrical grasp, lateral key pinch and tip to tip pinch; d) Quantitative prehension: scoring the performance during 6 pre-defined tasks, i.e. 9-hole peg test, pouring water, opening a jar, placing and turning a key in a lock, putting coins in a slot, and separating nuts from bolts.
2. Pinch and grip forces will be measured for both the right and left hands using standard

clinical dynamometer to quantify strength (Angst et al., 2010). To avoid tenodesis grip and pinch movement, tests will be performed in a standardized way with subjects seated upright against the back of a chair, shoulder adducted and neutrally rotated, elbow flexed 90 degrees, forearm in neutral position, when possible. Verbal encouragement will be provided to the subjects to exert maximum force. The average of three measurements per test session will be recorded.

d) Treatment Protocols:

1) Participants will receive **intensive upper extremity functional training** paired with TESS. Intensive training will consist of progressive functional task practice for upper extremity motor training. A total of 24 treatment sessions, which will last 45 minutes per session, will be completed in 3 weeks (4 days/week) per crossover arm. The functional task practice protocol is described previously and shown to be effective for enhancing functional performance and strength in chronic SCI (Beekhuizen, 2005; Beekhuizen & Field-Fote, 2005; Gomes-Osman, Tibbett, Poe, & Field-Fote, 2017; L. Hoffman & Field-Fote, 2013; L. R. Hoffman & Field-Fote, 2010). Functional task practice training will include repetitive unimanual (15 minutes each side) and bimanual (15 minutes) activities of gross upper extremity movement, isolated finger movements, simple and complex pinch and grip performance. For each category, 8-10 activities with various difficulty levels are designated and the subject will perform 1-2 activities within the same category in each treatment session. Activities in each category will be chosen according to the subject's ability and will be changed/modified as function progresses over time. For instance, the size of the coins will be reduced for simple pinch task or resistance level will be increased for TheraPutty exercises. Typical movement patterns will be encouraged by guidance and giving feedback (Vergara, Sancho-Bru, Gracia-Ibanez, & Perez-Gonzalez, 2014). When subjects have little to no voluntary movement, active assistance will be provided. 2-3 minute rest periods will be allowed between activities and when needed.

2) **Transcutaneous electrical spinal cord stimulation (TESS):** We will deliver TESS to the cervical spinal cord utilizing the experimental device developed by SpineX, Inc (Transcutaneous Electrical Spinal Cord Neuromodulator - TESCoN). The stimulator delivers programmable electrical current waveforms that are comprised of two modulated frequencies: (1) base frequency and (2) overlapping frequency, on up to four independent channels.

   This current waveform is adapted from kilohertz-frequency muscle stimulation, and permits high amplitude stimulation without discomfort (Ward, 2009; Ward & Shkuratova, 2002). Thus, stimulation over the skin can reach the spinal cord to activate spinal networks (Hofstoetter, Freundl, Binder, & Minassian, 2018). The rationale for the high overlapping frequency is that unmyelinated C-fibers in the skin can be selectively blocked by using high-frequency waveforms (Joseph & Butera, 2011; Joseph, Haeffele, & Butera, 2007; Ward & Robertson, 1998), and stimulation may penetrate more deeply due to lowering of the tissue impedance (Medina & Grill, 2014).

   We will use an electrical current waveform for transcutaneous spinal cord stimulation that is biphasic, 1 millisecond pulse width, 30 Hz base frequency, with a 10 kHz overlapping frequency (Fig 2).

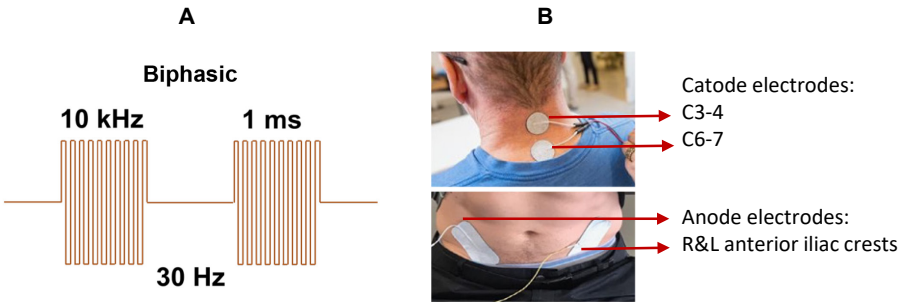


Figure 2. (A) Current waveform, (B) Cathode and anode electrode placement sites that will be used for TESS

Stimulation intensity will be adjusted between 0 and 130 milliamperes (mA) due to dose response curves obtained during the first stimulation session. We will use two 2.5 cm round self-adhesive hydrogel surface electrodes as cathodes and two 5 x 10 cm rectangular self-adhesive hydrogel electrodes as anodes (Axelgaard Manufacturing Co., Ltd., USA). Cathode electrodes will be placed midline on the skin of the neck, one above and one below the injury level with the guidance of the occipital inion and spinous processes as landmarks. Anode electrodes will be placed symmetrically over the iliac crests of the pelvis.

We will determine the optimal stimulation parameters for each participant based on the motor responses that will be tested using surface electromyography (EMG) electrodes (multichannel EMG system, Bortec AMT-8) placed on eight upper extremity muscles (deltoid, triceps, biceps, extensor digitorum, flexor digitorum, first dorsal interosseous, abductor pollicis brevis, and abductor digiti minimi) on each side. Subthreshold stimulation intensity will be used for therapeutic stimulation. Monophasic and biphasic stimulation waveforms activate neural circuits differently (Wang, Millard, Zheng, & Stanley, 2012), and both waveforms will be tested for their ability to enable functional movements. We will increase the stimulation intensity in increments of 5 mA until we reach direct muscle activation detected by surface EMG recordings.

Stimulation will be delivered for up to 45 minutes during each session of stimulation paired with training (6 weeks, 4 days/week). During the first week of the intervention period, participants will get only TESS paired with training. During the second and third weeks of the intervention period, TESS paired with training will be applied after daily AIH or SHAM (Figure 1). For safety precautions, we will closely monitor heart rate and blood pressure throughout each session along with blood oxygen saturation throughout AIH and SHAM sessions. Stimulation parameters will be re-adjusted as needed throughout the intervention phase of the study. For example, if stimulation interferes with the coordination of fine motor skills, the intensity will be lowered, or it will be increased for strengthening exercises if needed.

3) **<u>Daily Acute Intermittent Hypoxia (dAIH) and normoxia (SHAM):</u>** Each participant will be exposed to 4 sessions (1-week) of daily AIH and 4 sessions (1 week) of SHAM with a randomized alternating order. The order will be reversed for the second arm of the study. Daily AIH and SHAM treatment will precede the delivery of TESS paired with training on the second and third weeks of the intervention period (Figure 1). Each session

will consist of 15 episodes of 1.5 min hypoxia (fraction of inspired oxygen – [FIO_2] = 0.09 ± 0.02, i.e. 9% O2) for AIH or normoxia (FIO_2 = 0.21 ± 0.02, i.e. 21% O_2 or room air) for SHAM with 1 min intervals of room air.

Gas Delivery Methods: Intermittent gas mixtures will be delivered via manual adjustment of one- way valves attached to a hypoxia generator (HYP123, Hypoxico Inc., USA). The generator will fill reservoir bags attached to a non-rebreathing facemask worn by subjects (Hayes et al., 2014; Trumbower et al., 2012). We will deliver preset air mixtures of FIO_2 = 0.09 ± 0.02 (hypoxia) or FIO_2 = 0.21 ± 0.02 (normoxia). Oxygen concentration will be continuously monitored (OM-25RME; Maxtec Inc).

Participants will receive 4 treatment sessions of daily AIH and 4 daily SHAM per week throughout two consecutive weeks with an alternative order. A single AIH session consists of 15, 90 seconds episodes of breathing at a fraction of inspired oxygen (FIO_2) of 0.09 ± 0.02 with 60 seconds intervals of 0.21 ± 0.02 FIO_2 (room air) (Figure 3). While a single daily SHAM session consists of 15, 90 seconds episodes of 0.21 ± 0.02 FIO_2 with 60 seconds intervals of 0.21 ± 0.02 FIO_2 (room air). We provide the treatments via a custom air delivery system; see (Hayes et al., 2014; Trumbower et al., 2017; Trumbower et al., 2012) [10, 23, 32–34] for details. In brief, the delivery system directs a known air mixture from either a pressure-swing adsorption (PSA) system [HYP-123; Hypoxico Inc., USA] or a blower source to the non-rebreather facemask. PSA is a method that concentrates nitrogen from room air. For safety, oxygen concentration within the breathing circuit is continuously monitored [OM-25RME; Maxtec Inc]. Additionally, we continuously monitor blood oxygen saturation (SpO_2) and heart rate (HR) at 1-s intervals, and blood pressure (BP) every 5th breathing interval using a MASIMO system [MASIMO rainbow SET, Irvine, CA].

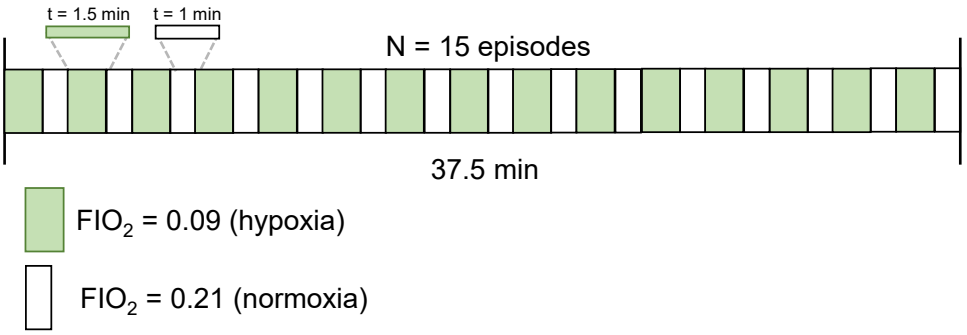


Figure 3. Acute Intermittent Hypoxia (AIH) protocol (FIO_2 - fraction of inspired oxygen)

e) Devices to be used

1. Transcutaneous Electrical Spinal Cord Stimulator
    - Transcutaneous Electrical Spinal Cord Neuromodulator (TESCoN) - Clinical Study Device, SpineX, Inc. Northridge, CA
2. Daily Acute Intermittent Hypoxia
    - Pressure-swing adsorption (PSA) system - HYP123, Hypoxico Inc.

- Oxygen concentration monitor - OM-25RME; Maxtec Inc.
- Blood oxygen saturation (SpO2), heart rate (HR) and blood pressure (BP) monitor - MASIMO rainbow SET, Irvine, CA.

VI. BIOSTATISTICAL ANALYSIS

Mixed-effects regression will be used to estimate and compare the rates of improvement in each performance measure and neurophysiologic parameter throughout the duration of each treatment phase.

Fixed effects will include time and treatment group, with the interaction of these two effects forming the primary indicator of a statistically significant treatment effect. Demographic and other patient characteristics will be assessed for a potential confounding relationship with treatment effect and outcome and will be added as fixed effects to each model as necessary.

Since mixed-effects regression can accommodate and compensate for missed assessments and subject drop-out, no cases will be excluded from the analysis for this reason. A two-sided alpha of 0.05 will be used to determine statistical significance, and all p-values will be adjusted for the experiment-wise error rate due to multiple correlations per Holm-Bonferroni.

Although the daily sessions throughout both 3-week study arms require substantial commitment, we do not expect more than 20% drop-out rate, based on our team's prior recruitment experiences with the spinal cord injured population (Hayes et al., 2014).

Given the early stage of research on combinatorial treatment of transcutaneous spinal cord stimulation and daily acute intermittent hypoxia for restoring upper limb function, power analysis and sample size are not computed.

VII. REFERENCES

The 2019 revision of the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI)-What's new? (2019). *Spinal Cord, 57*(10), 815-817. doi:10.1038/s41393-019-0350-9

Aboubakr, S. E., Taylor, A., Ford, R., Siddiqi, S., & Badr, M. S. (2001). Long-term facilitation in obstructive sleep apnea patients during NREM sleep. *J Appl Physiol (1985), 91*(6), 2751-2757. doi:10.1152/jappl.2001.91.6.2751

ACNS.org. (2006). Guideline 9A: Guidelines on evoked potentials. *J Clin Neurophysiol, 23*(2), 125-137. doi:10.1097/00004691-200604000-00010

Adams, M. M., Ginis, K. A., & Hicks, A. L. (2007). The spinal cord injury spasticity evaluation tool: development and evaluation. *Arch Phys Med Rehabil, 88*(9), 1185-1192. doi:10.1016/j.apmr.2007.06.012

Ainslie, P. N., & Poulin, M. J. (2004). Respiratory, cerebrovascular and pressor responses to acute hypoxia: dependency on PET(CO2). *Adv Exp Med Biol, 551*, 243-249. doi:10.1007/0-387-27023-x_37

Al-Majed, A. A., Tam, S. L., & Gordon, T. (2004). Electrical stimulation accelerates and enhances expression of regeneration-associated genes in regenerating rat femoral motoneurons. *Cell Mol Neurobiol, 24*(3), 379-402. doi:10.1023/b:cemn.0000022770.66463.f7

Anderson, K. D. (2004). Targeting recovery: priorities of the spinal cord-injured population. *J Neurotrauma, 21*(10), 1371-1383. doi:10.1089/neu.2004.21.1371

Angst, F., Drerup, S., Werle, S., Herren, D. B., Simmen, B. R., & Goldhahn, J. (2010). Prediction of grip and key pinch strength in 978 healthy subjects. *BMC Musculoskelet Disord, 11*, 94. doi:10.1186/1471-2474-11-94

Baker-Herman, T. L., Fuller, D. D., Bavis, R. W., Zabka, A. G., Golder, F. J., Doperalski, N. J., . . . Mitchell, G. S. (2004). BDNF is necessary and sufficient for spinal respiratory plasticity following intermittent hypoxia. *Nat Neurosci, 7*(1), 48-55. doi:10.1038/nn1166

Banister, E. W., & Woo, W. (1976). Training in hypoxia: effects on exercise tolerance in normoxia and hypoxia [proceedings]. *J Physiol, 263*(1), 228p-229p.

Baunsgaard, C. B., Nissen, U. V., Christensen, K. B., & Biering-Sorensen, F. (2016). Modified Ashworth scale and spasm frequency score in spinal cord injury: reliability and correlation. *Spinal Cord*. doi:10.1038/sc.2015.230

Beekhuizen, K. S. (2005). New perspectives on improving upper extremity function after spinal cord injury. *J Neurol Phys Ther, 29*(3), 157-162.

Beekhuizen, K. S., & Field-Fote, E. C. (2005). Massed practice versus massed practice with stimulation: effects on upper extremity function and cortical plasticity in individuals with incomplete cervical spinal cord injury. *Neurorehabil Neural Repair, 19*(1), 33-45. doi:10.1177/1545968305274517

Benavides, F. D., Jo, H. J., Lundell, H., Edgerton, V. R., Gerasimenko, Y., & Perez, M. A. (2020). Cortical and Subcortical Effects of Transcutaneous Spinal Cord Stimulation in Humans with Tetraplegia. *J Neurosci, 40*(13), 2633-2643. doi:10.1523/jneurosci.2374-19.2020

Benz, E. N., Hornby, T. G., Bode, R. K., Scheidt, R. A., & Schmit, B. D. (2005). A physiologically based clinical measure for spastic reflexes in spinal cord injury. *Arch Phys Med Rehabil, 86*(1), 52-59. doi:10.1016/j.apmr.2004.01.033

Berger, M. J., Hubli, M., & Krassioukov, A. V. (2014). Sympathetic skin responses and autonomic dysfunction in spinal cord injury. *J Neurotrauma, 31*(18), 1531-1539. doi:10.1089/neu.2014.3373

Bohannon, R. W., & Smith, M. B. (1987). Interrater reliability of a modified Ashworth scale of muscle spasticity. *Phys Ther, 67*(2), 206-207.

Burtscher, M., Haider, T., Domej, W., Linser, T., Gatterer, H., Faulhaber, M., . . . Bernardi, L. (2009). Intermittent hypoxia increases exercise tolerance in patients at risk for or with mild COPD. *Respir Physiol Neurobiol, 165*(1), 97-103. doi:10.1016/j.resp.2008.10.012

Cao, Y., Chen, Y., & DeVivo, M. J. (2011). Lifetime Direct Costs After Spinal Cord Injury. *Topics in Spinal Cord Injury Rehabilitation, 16*(4), 10-16. doi:10.1310/sci1604-10

Curt, A., & Dietz, V. (1999). Electrophysiological recordings in patients with spinal cord injury: significance for predicting outcome. *Spinal Cord, 37*(3), 157-165.

Curt, A., & Ellaway, P. H. (2012). Clinical neurophysiology in the prognosis and monitoring of traumatic spinal cord injury. *Handb Clin Neurol, 109*, 63-75. doi:10.1016/b978-0-444-52137-8.00004-8

Cutler, M. J., Swift, N. M., Keller, D. M., Wasmund, W. L., & Smith, M. L. (2004). Hypoxia-mediated prolonged elevation of sympathetic nerve activity after periods of intermittent hypoxic apnea. *J Appl Physiol (1985), 96*(2), 754-761. doi:10.1152/japplphysiol.00506.2003

Emad, R., Zafarghasempour, M., & Roshanzamir, S. (2013). Sympathetic skin response in incomplete spinal cord injury with urinary incontinence. *Ann Indian Acad Neurol, 16*(2), 234-238. doi:10.4103/0972-2327.112479

Fekete, C., Eriks-Hoogland, I., Baumberger, M., Catz, A., Itzkovich, M., Lüthi, H., . . . Brinkhof, M. W. (2013). Development and validation of a self-report version of the Spinal Cord Independence Measure (SCIM III). *Spinal Cord, 51*(1), 40-47. doi:10.1038/sc.2012.87

Folstein, M. F., Folstein, S. E., & McHugh, P. R. (1975). "Mini-mental state". A practical method for grading the cognitive state of patients for the clinician. *J Psychiatr Res, 12*(3), 189-198. doi:10.1016/0022-3956(75)90026-6

French, J. S., Anderson-Erisman, K. D., & Sutter, M. (2010). What do spinal cord injury consumers want? A review of spinal cord injury consumer priorities and neuroprosthesis from the 2008 neural interfaces conference. *Neuromodulation, 13*(3), 229-231. doi:10.1111/j.1525-1403.2009.00252.x

Freyvert, Y., Yong, N. A., Morikawa, E., Zdunowski, S., Sarino, M. E., Gerasimenko, Y., . . . Lu, D. C. (2018). Engaging cervical spinal circuitry with non-invasive spinal stimulation and buspirone to restore hand function in chronic motor complete patients. *Sci Rep, 8*(1), 15546. doi:10.1038/s41598-018-33123-5

Gad, P. N., Gerasimenko, Y., Zdunowski, S., Turner, A., Sayenko, D., Lu, D. C., & Edgerton, V. R. (2017). Weight Bearing Over-ground Stepping in an Exoskeleton with Non-invasive Spinal Cord

Neuromodulation after Motor Complete Paraplegia. *Front Neurosci, 11*, 333. doi:10.3389/fnins.2017.00333

Gad, P. N., Gerasimenko, Y. P., Zdunowski, S., Sayenko, D., Haakana, P., Turner, A., . . . Edgerton, V. R. (2015). Iron 'ElectriRx' man: Overground stepping in an exoskeleton combined with noninvasive spinal cord stimulation after paralysis. *Conf Proc IEEE Eng Med Biol Soc, 2015*, 1124-1127. doi:10.1109/embc.2015.7318563

Gad, P. N., Kreydin, E., Zhong, H., & Edgerton, V. R. (2020). Enabling respiratory control after severe chronic tetraplegia: An exploratory case study. *J Neurophysiol*. doi:10.1152/jn.00320.2020

Gad, P. N., Lee, S., Terrafranca, N., Zhong, H., Turner, A., Gerasimenko, Y., & Edgerton, V. R. (2018). Non-Invasive Activation of Cervical Spinal Networks after Severe Paralysis. *J Neurotrauma, 35*(18), 2145-2158. doi:10.1089/neu.2017.5461

Garraway, S. M., & Huie, J. R. (2016). Spinal Plasticity and Behavior: BDNF-Induced Neuromodulation in Uninjured and Injured Spinal Cord. *Neural Plast, 2016*, 9857201. doi:10.1155/2016/9857201

Gerasimenko, Y. P., Gorodnichev, R., Moshonkina, T., Sayenko, D., Gad, P., & Reggie Edgerton, V. (2015). Transcutaneous electrical spinal-cord stimulation in humans. *Ann Phys Rehabil Med, 58*(4), 225-231. doi:10.1016/j.rehab.2015.05.003

Ghorbani, M., Shahabi, P., Karimi, P., Soltani-Zangbar, H., Morshedi, M., Bani, S., . . . Ahmadalipour, A. (2020). Impacts of epidural electrical stimulation on Wnt signaling, FAAH, and BDNF following thoracic spinal cord injury in rat. *J Cell Physiol*. doi:10.1002/jcp.29793

Gomes-Osman, J., Tibbett, J. A., Poe, B. P., & Field-Fote, E. C. (2017). Priming for Improved Hand Strength in Persons with Chronic Tetraplegia: A Comparison of Priming-Augmented Functional Task Practice, Priming Alone, and Conventional Exercise Training. *Front Neurol, 7*, 242. doi:10.3389/fneur.2016.00242

Gozal, D., Row, B. W., Gozal, E., Kheirandish, L., Neville, J. J., Brittian, K. R., . . . Guo, S. Z. (2003). Temporal aspects of spatial task performance during intermittent hypoxia in the rat: evidence for neurogenesis. *Eur J Neurosci, 18*(8), 2335-2342. doi:10.1046/j.1460-9568.2003.02947.x

Hayes, H. B., Jayaraman, A., Herrmann, M., Mitchell, G. S., Rymer, W. Z., & Trumbower, R. D. (2014). Daily intermittent hypoxia enhances walking after chronic spinal cord injury: a randomized trial. *Neurology, 82*(2), 104-113. doi:10.1212/01.Wnl.0000437416.34298.43

Hoffman, L., & Field-Fote, E. (2013). Effects of practice combined with somatosensory or motor stimulation on hand function in persons with spinal cord injury. *Top Spinal Cord Inj Rehabil, 19*(4), 288-299. doi:10.1310/sci1904-288

Hoffman, L. R., & Field-Fote, E. C. (2010). Functional and corticomotor changes in individuals with tetraplegia following unimanual or bimanual massed practice training with somatosensory stimulation: a pilot study. *J Neurol Phys Ther, 34*(4), 193-201. doi:10.1097/NPT.0b013e3181fbe692

Hofstoetter, U. S., Freundl, B., Binder, H., & Minassian, K. (2018). Common neural structures activated by epidural and transcutaneous lumbar spinal cord stimulation: Elicitation of posterior root-muscle reflexes. *PLoS One, 13*(1), e0192013. doi:10.1371/journal.pone.0192013

Hubli, M., & Krassioukov, A. V. (2015). How reliable are sympathetic skin responses in subjects with spinal cord injury? *Clin Auton Res, 25*(2), 117-124. doi:10.1007/s10286-015-0276-z

Inanici, F., Samejima, S., Gad, P., Edgerton, V. R., Hofstetter, C. P., & Moritz, C. T. (2018). Transcutaneous Electrical Spinal Stimulation Promotes Long-Term Recovery of Upper Extremity Function in Chronic Tetraplegia. *IEEE Trans Neural Syst Rehabil Eng, 26*(6), 1272-1278. doi:10.1109/tnsre.2018.2834339

Joseph, L., & Butera, R. J. (2011). High-frequency stimulation selectively blocks different types of fibers in frog sciatic nerve. *IEEE Trans Neural Syst Rehabil Eng, 19*(5), 550-557. doi:10.1109/tnsre.2011.2163082

Joseph, L., Haeffele, B. D., & Butera, R. J. (2007). Conduction block induced by high frequency AC stimulation in unmyelinated nerves. *Conf Proc IEEE Eng Med Biol Soc, 2007*, 1719-1722. doi:10.1109/iembs.2007.4352641

Kalsi-Ryan, S., Beaton, D., Ahn, H., Askes, H., Drew, B., Curt, A., . . . Fehlings, M. G. (2016). Responsiveness, Sensitivity, and Minimally Detectable Difference of the Graded and Redefined Assessment of Strength, Sensibility, and Prehension, Version 1.0. *J Neurotrauma, 33*(3), 307-314. doi:10.1089/neu.2015.4217

Kalsi-Ryan, S., Curt, A., Verrier, M. C., & Fehlings, M. G. (2012). Development of the Graded Redefined Assessment of Strength, Sensibility and Prehension (GRASSP): reviewing measurement specific to the upper limb in tetraplegia. *J Neurosurg Spine, 17*(1 Suppl), 65-76. doi:10.3171/2012.6.aospine1258

Kumru, H., Vidal, J., Perez, M., Schestatsky, P., & Valls-Sole, J. (2009). Sympathetic skin responses evoked by different stimuli modalities in spinal cord injury patients. *Neurorehabil Neural Repair, 23*(6), 553-558. doi:10.1177/1545968308328721

Lu, P., Jones, L. L., & Tuszynski, M. H. (2005). BDNF-expressing marrow stromal cells support extensive axonal growth at sites of spinal cord injury. *Exp Neurol, 191*(2), 344-360. doi:10.1016/j.expneurol.2004.09.018

Lusina, S. J., Kennedy, P. M., Inglis, J. T., McKenzie, D. C., Ayas, N. T., & Sheel, A. W. (2006). Long-term intermittent hypoxia increases sympathetic activity and chemosensitivity during acute hypoxia in humans. *J Physiol, 575*(Pt 3), 961-970. doi:10.1113/jphysiol.2006.114660

McNeil, C. J., Butler, J. E., Taylor, J. L., & Gandevia, S. C. (2013). Testing the excitability of human motoneurons. *Front Hum Neurosci, 7*, 152. doi:10.3389/fnhum.2013.00152

Medina, L. E., & Grill, W. M. (2014). Volume conductor model of transcutaneous electrical stimulation with kilohertz signals. *J Neural Eng, 11*(6), 066012. doi:10.1088/1741-2560/11/6/066012

Navarrete-Opazo, A., Alcayaga, J. J., Sepúlveda, O., & Varas, G. (2017). Intermittent Hypoxia and Locomotor Training Enhances Dynamic but Not Standing Balance in Patients With Incomplete Spinal Cord Injury. *Arch Phys Med Rehabil, 98*(3), 415-424. doi:10.1016/j.apmr.2016.09.114

NSCISC. (2020). National Spinal Cord Injury Statistical Center, Facts and Figures at a Glance. *https://www.nscisc.uab.edu/Public/Facts%20and%20Figures%202020.pdf University of Alabama at Birmingham.*

Rossi, S., Hallett, M., Rossini, P. M., & Pascual-Leone, A. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin Neurophysiol, 120*(12), 2008-2039. doi:10.1016/j.clinph.2009.08.016

Rossini, P. M., Burke, D., Chen, R., Cohen, L. G., Daskalakis, Z., Di Iorio, R., . . . Ziemann, U. (2015). Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application. An updated report from an I.F.C.N. Committee. *Clin Neurophysiol, 126*(6), 1071-1107. doi:10.1016/j.clinph.2015.02.001

Row, B. W., Liu, R., Xu, W., Kheirandish, L., & Gozal, D. (2003). Intermittent hypoxia is associated with oxidative stress and spatial learning deficits in the rat. *Am J Respir Crit Care Med, 167*(11), 1548-1553. doi:10.1164/rccm.200209-1050OC

Sasaki, M., Radtke, C., Tan, A. M., Zhao, P., Hamada, H., Houkin, K., . . . Kocsis, J. D. (2009). BDNF-hypersecreting human mesenchymal stem cells promote functional recovery, axonal sprouting, and protection of corticospinal neurons after spinal cord injury. *J Neurosci, 29*(47), 14932-14941. doi:10.1523/jneurosci.2769-09.2009

Shatilo, V. B., Korkushko, O. V., Ischuk, V. A., Downey, H. F., & Serebrovskaya, T. V. (2008). Effects of intermittent hypoxia training on exercise performance, hemodynamics, and ventilation in healthy senior men. *High Alt Med Biol, 9*(1), 43-52. doi:10.1089/ham.2007.1053

Shimoyama, I., Ninchoji, T., & Uemura, K. (1990). The finger-tapping test. A quantitative analysis. *Arch Neurol, 47*(6), 681-684. doi:10.1001/archneur.1990.00530060095025

Srikrishna, S., Robinson, D., & Cardozo, L. (2010). Validation of the Patient Global Impression of Improvement (PGI-I) for urogenital prolapse. *Int Urogynecol J, 21*(5), 523-528. doi:10.1007/s00192-009-1069-5

Stinson, J. N., Kavanagh, T., Yamada, J., Gill, N., & Stevens, B. (2006). Systematic review of the psychometric properties, interpretability and feasibility of self-report pain intensity measures for use in clinical trials in children and adolescents. *Pain, 125*(1-2), 143-157. doi:10.1016/j.pain.2006.05.006

Trumbower, R. D., Hayes, H. B., Mitchell, G. S., Wolf, S. L., & Stahl, V. A. (2017). Effects of acute intermittent hypoxia on hand use after spinal cord trauma: A preliminary study. *Neurology, 89*(18), 1904-1907. doi:10.1212/wnl.0000000000004596

Trumbower, R. D., Jayaraman, A., Mitchell, G. S., & Rymer, W. Z. (2012). Exposure to acute intermittent hypoxia augments somatic motor function in humans with incomplete spinal cord injury. *Neurorehabil Neural Repair, 26*(2), 163-172. doi:10.1177/1545968311412055

Tuszynski, M. H., Steeves, J. D., Fawcett, J. W., Lammertse, D., Kalichman, M., Rask, C., . . . Privat, A. (2007). Guidelines for the conduct of clinical trials for spinal cord injury as developed by the ICCP Panel: clinical trial inclusion/exclusion criteria and ethics. *Spinal Cord, 45*(3), 222-231. doi:10.1038/sj.sc.3102009

Vergara, M., Sancho-Bru, J. L., Gracia-Ibanez, V., & Perez-Gonzalez, A. (2014). An introductory study of common grasps used by adults during performance of activities of daily living. *J Hand Ther, 27*(3), 225-233; quiz 234. doi:10.1016/j.jht.2014.04.002

Vermehren-Schmaedick, A., Khanjian, R. A., & Balkowiec, A. (2015). Cellular mechanisms of activity-dependent BDNF expression in primary sensory neurons. *Neuroscience, 310*, 665-673. doi:10.1016/j.neuroscience.2015.10.007

Wang, Q., Millard, D. C., Zheng, H. J., & Stanley, G. B. (2012). Voltage-sensitive dye imaging reveals improved topographic activation of cortex in response to manipulation of thalamic microstimulation parameters. *J Neural Eng, 9*(2), 026008. doi:10.1088/1741-2560/9/2/026008

Ward, A. R. (2009). Electrical stimulation using kilohertz-frequency alternating current. *Phys Ther, 89*(2), 181-190. doi:10.2522/ptj.20080060

Ward, A. R., & Robertson, V. J. (1998). Variation in torque production with frequency using medium frequency alternating current. *Arch Phys Med Rehabil, 79*(11), 1399-1404. doi:10.1016/s0003-9993(98)90234-9

Ward, A. R., & Shkuratova, N. (2002). Russian electrical stimulation: the early experiments. *Phys Ther, 82*(10), 1019-1030.

Wassermann, E. M. (1998). Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5-7, 1996. *Electroencephalogr Clin Neurophysiol, 108*(1), 1-16. doi:10.1016/s0168-5597(97)00096-8

Wenjin, W., Wenchao, L., Hao, Z., Feng, L., Yan, W., Wodong, S., . . . Wenlong, D. (2011). Electrical stimulation promotes BDNF expression in spinal cord neurons through Ca(2+)- and Erk-dependent signaling pathways. *Cell Mol Neurobiol, 31*(3), 459-467. doi:10.1007/s10571-010-9639-0

WHO. (2013). World Health Organization. International Perspectives on Spinal Cord Injury. *http://apps.who.int/iris/bitstream/10665/94190/1/9789241564663_eng.pdf?ua=1 Publications of the World Health Organization*.

Wong, D. L., & Baker, C. M. (2001). Smiling faces as anchor for pain intensity scales. *Pain, 89*(2-3), 295-300. doi:10.1016/s0304-3959(00)00375-4

Wuolle, K. S., Van Doren, C. L., Thrope, G. B., Keith, M. W., & Peckham, P. H. (1994). Development of a quantitative hand grasp and release test for patients with tetraplegia using a hand neuroprosthesis. *J Hand Surg Am, 19*(2), 209-218. doi:10.1016/0363-5023(94)90008-6

Xie, A., Skatrud, J. B., Puleo, D. S., & Morgan, B. J. (2001). Exposure to hypoxia produces long-lasting sympathetic activation in humans. *J Appl Physiol (1985), 91*(4), 1555-1562. doi:10.1152/jappl.2001.91.4.1555