NCT03411863

Noninvasive Cervical Electrical Stimulation for ALS: Mechanistic and Safety Study

May 5, 2019

PART II
GUIDE TO RESEARCH PROPOSAL

1. SPECIFIC AIMS

Aim 1: Map the cellular and synaptic targets of cervical electrical stimulation (CES).
CES circuit interactions at both the spinal and supraspinal levels will be characterized using a common structure comprising an electrical or magnetic conditioning stimulus followed by a test stimulus delivered at a range of intensities, sites, and interstimulus intervals (ISI). This will shed insight into which circuits CES activates, and how CES circuits interact with circuits activated by other exogenous neural stimuli.

Hypothesis 1.1: Conditioning subthreshold CES will potentiate spinal motor neuron responses to test transcranial magnetic stimulation (TMS) pulses. Depending on the timing of the effect, this would support either the mechanism of heterosynaptic summation between segmental Ia input and descending corticospinal input[1], or sensory cortical facilitation of motor cortex excitability[2–4].

Hypothesis 1.2: Conditioning CES will increase persistence (number of responses per 20 stimuli) of spinal motor neuron F-responses to retrograde stimulation over the median and/or ulnar nerve. This would indicate CES's potential to modulate motor neuron excitability at the spinal level.

Hypothesis 1.3: Conditioning CES will reduce amplitude of median (flexor carpi radialis) H-responses, indicating that CES traveling via sensory afferents homosynaptically depresses ensuing H-reflexes[5,6]. This would demonstrate the potential for CES to reduce hyperactive muscle stretch responses (spasticity)[5] via segmental afferent interactions.

Aim 2: Determine optimal CES and volitional movement combination parameters for acutely facilitating concurrent arm and hand movements.
Volitional limb movements depend on the same corticospinal and motor neuron circuits as those activated by TMS and F-waves. Since preliminary data shows that subthreshold CES facilitates TMS responses, CES may also be able to facilitate volitional limb movements. The experiments in Aim 2 will shed light on this clinically applicable question.

Hypothesis 2.1: Subthreshold CES will *facilitate* concurrent arm and hand muscle activation, further indicating CES's ability to positively modulate corticospinal and/or motor neuron excitability. This would establish an opportunity to directly translate this paradigm for clinical benefit by combining repetitive subthreshold CES with repetitive task-oriented physical exercise training in a subsequent study.

Hypothesis 2.2: High-intensity suprathreshold CES will transiently *inhibit* concurrent arm and hand muscle activation. If observed, this 'spinal silent period' would shed insight into mechanisms underlying the 'cortical silent period' noted when cortical TMS is delivered during volitional contraction[7,8].

2. BRIEF REVIEW OF RESULTS AND CURRENT STATE OF KNOWLEDGE

Amyotrophic lateral sclerosis (ALS) is a spinal cord disease. Many of the most striking features of ALS – muscular atrophy, fasciculations, and findings of denervation on electromyography – demonstrate the consequences of damage to lower motor neurons within the spinal cord. The almost pathognomonic spasticity and hyperreflexia seen in atrophied limbs reflects degenerating corticospinal axons in the lateral spinal cord ("lateral sclerosis"). Remaining motor circuits are weak, uncoordinated, and spastic[9]. Therefore, ALS shares many key features with incomplete spinal cord injury (SCI). Thus, some of the therapies aimed at strengthening neural transmission in SCI may warrant testing in ALS as well.

A large body of evidence in brain and spinal cord injury suggests that activating spared nerve circuits augments circuit and physiological function[10–14]. Impressive gains have been made in the SCI field by applying electromagnetic stimulation, including transcranial magnetic stimulation (TMS), direct current stimulation (DCS), and invasive epidural electrical stimulation[10,15–21]. However, drawbacks exist, such as the unfeasibility of combining TMS with active exercises, the inability to directly map the timing or circuitry of DCS effects, and the risks of invasive surgery for epidural stimulation, especially in the ALS population.

Noninvasive electrical spinal stimulation lacks these drawbacks. We have developed a novel method of noninvasive cervical electrical stimulation (CES). Our technique draws on lessons learned from other forms of noninvasive spinal stimulation, mostly focused on the lumbar cord[5,22–26]. However, these studies need to be supplemented with experiments that further investigate CES circuit interactions, not only within the spinal cord, but also between the cord and other parts of the nervous system. This proposal will address gaps in mechanistic and therapeutic understanding in the field of noninvasive spinal cord stimulation, and will establish the rationale for applying our novel technique in individuals with ALS.

ALS has been neglected by the field of neural stimulation. To our knowledge, the small body of work using neural stimulation in ALS has centered on the brain **[[**or phrenic nerve**]]**, not the spine, and most studies have performed diagnostic rather than therapeutic stimulation[27–32]. A literature search revealed only seven primary studies of therapeutic repetitive TMS or transcranial DCS for ALS, the majority performed by the Di Lazzaro group in Italy, which have not shown a clear benefit in ALS progression[33–39]. A search of "amyotrophic stimulation" on the clinicaltrials.gov database showed one study with unknown active status recruiting ALS patients for therapeutic DCS in Italy, two studies recruiting ALS patients for diagnostic TMS in France, and our own unfunded pilot study of TMS paired with electrical stimulation at the James J. Peters VA Medical Center (see Preliminary Data). Both of these searches were last conducted on 21 February 2017. Thus, except for our own ongoing unfunded pilot study, there are no active clinical studies anywhere in the world of spinal stimulation for ALS. Given the limited therapeutic options available for ALS, this technology warrants testing even though electromagnetic stimulation may not halt or reverse underlying processes that contribute to ALS pathology such as inflammation, axonal transport defects, and protein aggregation[40].

Significance

ALS incidence is up to twice as high in Veterans from all eras of active duty[41]. Therefore, ALS is a 100% VA Service-Connected condition, and the experiments proposed here strongly align with RR&D's mission. Treatments are extremely limited, and there remains no cure. Individuals with ALS and their loved ones are generally eager to seek out new options – unfortunately, this is often exploited by unethical purveyors of ill-defined mixtures of stem cells or herbal remedies[42–44]. Therefore, rational, ethical, IRB-approved research studies are desperately needed. This pilot study, focused on mechanisms, safety, and short-term biomarkers of efficacy, represents an essential – and original – first step toward subsequent evidence-based studies of combined CES-exercise programs that will aim to improve clinical function in individuals with ALS.

This proposal focuses on non-invasive electrical stimulation over the spinal cord. Below, we will detail the state of this field, highlighting the gaps in mechanistic understanding and therapeutic application toward ALS and other conditions. We will thereby establish the rationale for testing and applying our novel technique of non-invasive cervical electrical stimulation.

Non-invasive *brain* stimulation

Transcranial magnetic stimulation (TMS) uses a transient focal magnetic field to induce action potentials in the underlying brain. TMS over the motor cortex transduces action potentials to descending corticospinal fibers[45,46]. Repetitive TMS (rTMS) at 1 Hz is generally inhibitory, whereas at 5 Hz or greater, rTMS is generally excitatory[47]. Higher-frequency (usually 50 Hz) 'theta burst' TMS may induce longer-lasting effects that are either inhibitory or excitatory based on burst patterns[46,48]. Seminal TMS mechanistic studies will serve as guides for some of the experiments in this proposal[45,46,48–50].

Direct current stimulation (DCS) delivers a low-power electric current that modulates neuronal excitability[51]. In distinction from TMS, DCS delivers exclusively *tonic, sub*threshold stimulation[52]. Various cranial and spinal DCS configurations have shown promising effects in neurological and psychiatric disorders, as well as enhancement of normal function[15,20,53–62]. However, there is no technique to directly map how the low-energy current distributes within the body, or to determine how individual variations in injury characteristics affect that distribution[63]. Furthermore, the continuous nature of DCS makes it difficult if not impossible to elucidate timing-dependent synaptic changes. Therefore, although DCS has therapeutic potential, its underlying mechanisms are quite likely to remain a black box.

Invasive *spinal* stimulation

Epidural electric stimulation through implanted lumbar electrodes delivers tonic, motor-subthreshold stimulation, usually targeted toward the locomotor central pattern generator. In rodent SCI models, epidural stimulation combined with physical training and monoaminergic drug stimulation has led to recovered ability to walk on previously paralyzed hindlimbs[19,64,65]. This work has been translated into humans with chronic motor-complete spinal cord injury as high as the C7 level, resulting in regaining some volitional leg movement while stimulation is turned on[10,17]. These incredibly encouraging results support the utility of subthreshold spinal stimulation to improve neural circuit function. However, stimulator implantation carries the risks of invasive surgery – these risks are significantly higher in the cervical than lumbar spine.

Non-invasive spinal stimulation

Multiple transcutaneous spinal stimulation approaches have been developed, largely targeted at thoracolumbar locomotor circuits. Tonic stimulation over the T11 level at 3 Hz induces coordinated walking movements in uninjured volunteers[23]. Adding simultaneous stimulation at the C5 and L1 levels increases motion coordination and range[22]. High-intensity phasic stimulation over the C7-T1 or T10-L1 levels activates efferent fibers in ventral motor roots to elicit action potentials in arm or leg muscles, respectively[24,25].

Transcutaneous biphasic stimulation can also elicit action potentials through afferent fiber pathways[6]. Subthreshold transcutaneous stimulation over T11-T12 for 30 minutes at 50 Hz led to reduced leg spasticity and evidence for improved motor function in three subjects with motor-incomplete SCI[5]. This effect was most likely mediated by activation of afferent spinal roots and their segmental interneuronal connections. We plan to exploit subthreshold cervical transcutaneous stimulation targeted at similar afferent pathways.

Temporally linked (paired) neuronal firing can lead to lasting effects on synaptic and neuronal excitability through Hebbian-like mechanisms such as long term potentiation and synaptic summation[66–69]. These paired stimulation techniques include paired associative stimulation (PAS), transspinal-transcortical stimulation, spinal associative stimulation, spike timing-dependent plasticity, and others[2,70–77]. In a demonstration of spike timing-dependent plasticity in the cervical cord, TMS over the hand motor cortex was paired with high-intensity electrical stimulation over the ulnar nerve at the wrist[70]. When repetitive (0.1 Hz, 100 repetitions) paired pulses were timed so the anterograde TMS signal arrived at cervical motor neurons 1-2 ms before the retrograde ulnar nerve signal, both able-bodied volunteers and subjects with SCI demonstrated increased TMS-evoked potentials in hand muscles and improved hand dexterity for at least 30 minutes after the end of paired stimulation[70]. This suggested that synapses between upper and lower motor neurons underwent Hebbian-like strengthening. Other studies have found more evidence for the highly timing-dependent nature of synaptic effects, both at cervical and lumbar levels[74,76,77].

The advantages of non-invasive cervical electrical stimulation (CES)

Our group recently developed a novel configuration of transcutaneous CES. Our technique draws on lessons learned from other forms of non-invasive spinal stimulation referenced above. We have already applied for and received FDA designation as non-significant risk, and we are conducting two other IRB-approved human pilot studies to establish basic CES mechanisms and safety. As presented in the preliminary data, CES (**Figure 1**) comfortably elicits action potentials over multiple spinal cord segments simultaneously in both arms. CES activates spinal motor neurons indirectly via nerve roots, targeting either afferent or efferent fibers depending on stimulus intensity. This afferent or efferent root-stimulation approach provides a flexible portal to access synapses between upper and lower motor neurons, even in contexts of damaged motor circuitry[78].

While we are excited to test CES paired with either peripheral nerve stimulation or TMS, TMS requires bulky, expensive equipment that makes it highly impractical to use in conjunction with concurrent physical exercise. In this proposal, we will take a more in-depth mechanistic approach to the CES technique itself, with the goal of establishing a single-modality stimulation paradigm that mediates beneficial synaptic plasticity in conjunction with physical exercise.

We emphasize that CES is inherently different from popularly used types of non-invasive electrical stimulation such as direct current stimulation (DCS, described above) and functional electrical stimulation (FES). FES targets peripheral nerves with suprathreshold pulses designed to directly stimulate motor units within one or more large muscles at a time. Separate FES electrodes are required for each targeted nerve/muscle. Critically, FES engages motor units in non-physiological order, from largest to smallest – this causes excessive muscle fatigue[79]. In contrast, CES targets multiple root levels and both sides simultaneously, with the goal of using subthreshold intensity to amplify endogenous volitional neural circuit signaling.

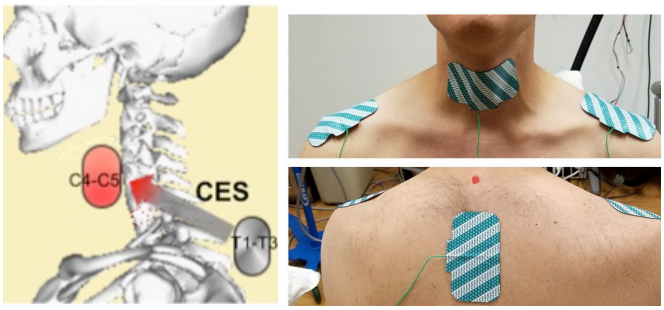

Figure 1. CES schematic. Biphasic electrical pulses are delivered noninvasively over the cervical spine. The cathode is placed posteriorly over T1-T3 levels, and the anode is placed anteriorly over C4-C5 levels. Ground electrodes are placed over the distal clavicles. Depicted schematically on left, photographically on right.

Gaps in mechanistic understanding of spinal stimulation

As opposed to stimulation configuration and pairing with other modalities, the effect of timing between successive spinal cord stimuli has received less attention in comparison to similar experiments in the field of TMS. Extensive studies have defined rTMS effects over a range of pulse frequencies and patterns, in a broad variety of injury and disease contexts. A similarly rigorous definition of spinal stimulation timing effects has not yet been achieved. One reproducible finding has shown that at low suprathreshold intensities, a conditioning spinal pulse inhibits a second spinal pulse given 20-50 ms later[1,5,6,80]. These findings, which we have confirmed in our own studies, indicate that at these intensities, spinal stimulation triggers afferent sensory fibers that activate motor neurons via synapses that are subject to homosynaptic depression. However, these studies need to be supplemented with experiments that more deeply investigate basic mechanisms of circuit interactions. In the TMS field for example, conditioning pulses inhibit test pulses given either 1-3 or 100-200 ms later, and facilitate test pulses given 8-15 ms later. The effects of these time intervals have revealed important brain circuit interactions termed short-interval cortical inhibition (SICI), long-interval cortical inhibition (LICI), and intracortical facilitation (ICF), respectively[46,50]. Using conditioning and test stimuli over a broad range of time intervals, site, and modality combinations, we now expect to define similar types of circuit interactions mediating the response to CES.

Our team is optimally positioned to address these gaps – we already conduct studies involving electrical and magnetic stimulation in a well-run clinical research center; and we have already obtained FDA and IRB clearance to conduct human studies using our novel CES technique.

PRELIMINARY DATA

This preliminary data derives from an ongoing study investigating basic parameters of CES and its interaction with TMS. That study does not involve volitional movement, and lacks many of the basic mechanistic experiments proposed in the current application.

Noninvasive, non-noxious cervical electrical stimulation (CES)

We have designed a surface electrode configuration that effectively stimulates motor responses from multiple cervical levels simultaneously at non-noxious current outputs. One 5x10 cm cathode is placed longitudinally over

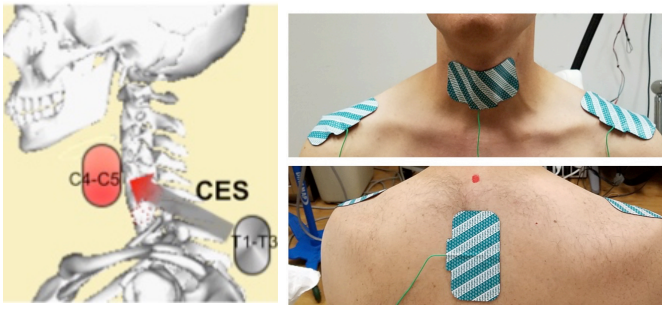

Figure 1. CES schematic. Biphasic electrical pulses are delivered noninvasively over the cervical spine. The cathode is placed posteriorly over T1-T3 levels; the anode is placed anteriorly over C4-C5 levels. Two ground electrodes are placed over the distal clavicles. Depicted schematically on left, photographically on right.

the midline with the top edge 2.5 cm caudal to the C7 spinous process (~T1-T3 vertebral levels posteriorly). Another 5x10 cm anode is placed horizontally centered over the thyroid cartilage, corresponding to the C4-C5 levels anteriorly (**Fig 1**). Two 5x10 cm ground electrodes are placed over the distal clavicles. Pulses are delivered via biphasic waveforms of 2-ms duration. In our ongoing pilot study comparing combinations of TMS with either CES or peripheral nerve stimulation, 19 subjects (5 with SCI, 3 with ALS) have undergone over 120 sessions of CES without procedure-related significant adverse events or intolerance. All subjects have had easily obtainable CES responses. Of 7 ALS subjects screened to date, 5 had hand motor responses to TMS, a requirement for inclusion in the ongoing study (**Table 1**). One subject did not wish to pursue further testing, and another subject presented with visual hallucinations before his first scheduled study visit, so we aborted his participation. Thus, 3 ALS subjects have undergone CES, without any significant adverse events.

Age	Gender	Duration	FRS	MEP	Resting Threshold (% MSO)
62	M	1.5 yr	37	+	84
60	M	4 yr	30	+	59
51	F	1 yr	42	-	n/a
48	M	4 yr	43	-	n/a
42	F	1.5 yr	42	+	83
54	M	3 yr	34	+	44
58	M	1.5 yr	35	+	50

Table 1 – Demographics and average TMS resting motor threshold to hand muscles in subjects with ALS. **FRS**, ALS Functional Rating Scale (out of 48); **MEP**, motor-evoked potential; **MSO**, maximum stimulator output.

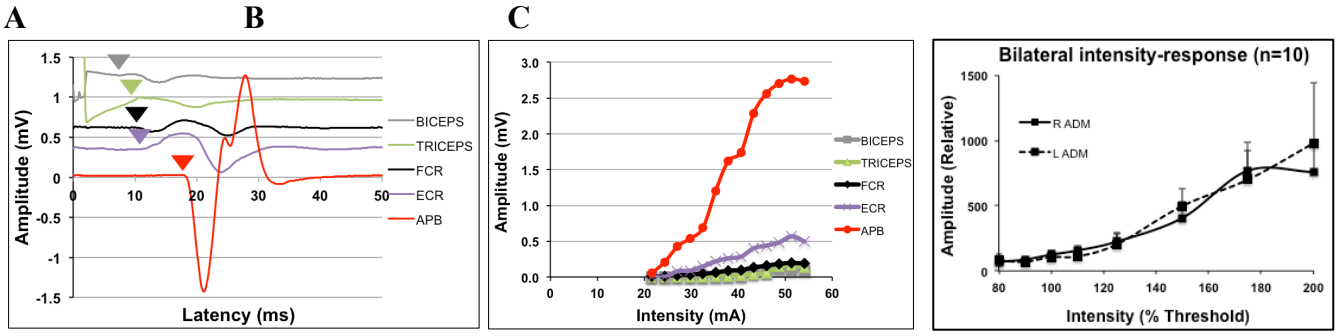

Figure 2. CES activates multiple muscles simultaneously. A) CES waveforms in one non-disabled volunteer depicted in raster format. CES stimulus at ~200% threshold. Note the proximal to distal gradient of latencies and amplitudes. **B)** Intensity-response curves of five different arm muscles, showing distal muscle selectivity. **C)** CES symmetrically activates homologous muscles on both sides **[[**updated, 10 non-disabled subjects**]]**. **FCR**, flexor carpi radialis; **ECR**, extensor carpi radialis; **ADM**, abductor digiti minimi; **APB**, abductor pollicis brevis.

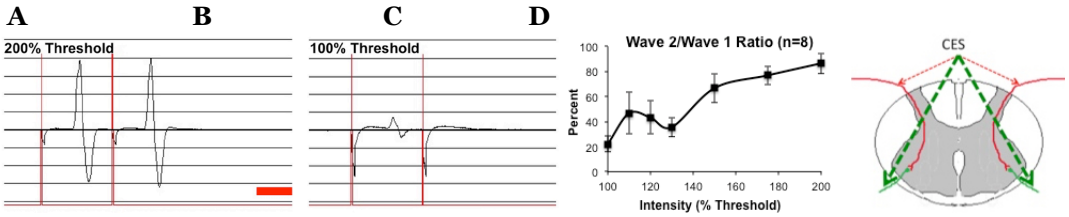

Figure 3. The CES circuit pathway is adjustable via stimulus intensity. APB recordings of double CES stimuli at 40 ms intervals. **A**, At high intensity (200% of resting motor threshold), repeat CES stimulus (red lines) shows no decrement. **B**, At peri-threshold intensity, repeat CES stimulus results in significant amplitude decrement. **C**, Second stimulus response increases as stimulus intensity increases, indicating direct axonal activation, not synaptic transmission. Average ± SEM in 8 non-disabled subjects. **D**, CES activates sensory afferent roots (red) at lower intensity, and motor efferent roots (green) at higher intensity. Red 20 ms-scale bar same for A and B.

<u>CES activates multiple arm muscles simultaneously</u>

Surface recording electrodes were placed in a non-disabled volunteer over the biceps (C5-C6), flexor carpi radialis (FCR, C6-C7), extensor carpi radialis (ECR, C6-C7), triceps (C7-C8), and abductor pollicis brevis (APB, C8-T1). At intensity levels sufficient to stimulate both proximal and distal arm muscles, relative latencies corresponded to the distance of each recording electrode from the cathode (**Fig 2A**). The hand myotomes more directly underlying the cathode

responded at lower thresholds than the arm myotomes (**Fig 2B**). Additionally, muscles in both arms simultaneously and symmetrically respond to CES in non-disabled subjects (**Fig 2C**).

CES targets different circuits at different intensities

When single CES pulses are delivered at peri-threshold intensity, latencies to the APB muscle are up to 3-5 ms longer than when delivered at higher stimulation intensity (not shown). When two CES pulses are given 40 ms apart at low intensity, the second pulse amplitude is strongly reduced, whereas at higher intensity, there is little to no decrement (**Fig 3**). These findings indicate that the CES circuit pathway can be selected by titrating intensity: at low intensity, CES travels a longer route from afferent to efferent neurons via synapses susceptible to post-activation depression[1,6]. At higher intensity, CES directly (non-synaptically) activates efferent fibers (**Fig 3D**).

Subthreshold CES acutely facilitates muscle responses to cortical stimulation

To test whether subthreshold CES (80-90% of motor threshold) could facilitate response to suprathreshold TMS (120% of motor threshold), CES was delivered either alone or paired such that the CES pulse arrived at cervical motor neurons 10 ms prior to TMS pulse arrival or 1.5-5.0 ms after TMS pulse arrival. Preliminary results show that subthreshold CES facilitates the response to TMS when the TMS pulse arrives first (**Fig 4**), with similar trends among non-disabled, SCI, and ALS subjects. Since TMS travels via the same corticospinal pathways that mediate volitional movement, the fact that subthreshold CES facilitates TMS suggests that it may also facilitate volitional movement. This is a finding with potential for direct clinical translation. Note, only three different interstimulus intervals (ISI) were tested in this experiment. A much wider range of intervals needs to be tested to better understand and optimize this phenomenon.

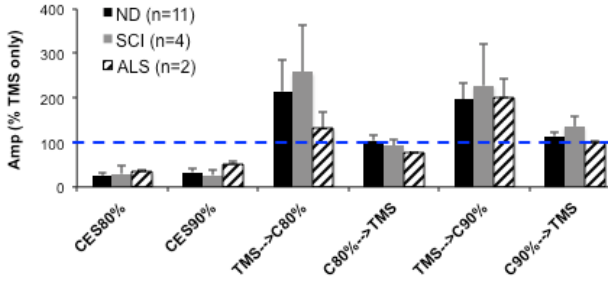

Figure 4. Subthreshold CES acutely facilitates TMS-evoked potentials. Suprathreshold (120%) TMS and subthreshold (80-90%) CES were given alone or in combination. Response amplitudes were normalized to the response to TMS alone. Subthreshold CES facilitated the response to TMS alone when the TMS pulse arrived prior to CES, but not when the timing was reversed. **Amp**, amplitude.

Rationale:

This proposal includes experiments designed to achieve both mechanistic insight and demonstration of therapeutic principle. CES is innovative**:** the delivery patterns, configurations, and subject population proposed here are novel. CES is practical**:** non-invasive stimulation carries significantly lower cost, lower risk, and greater ability to widely implement than surgically implanted stimulation, especially at the cervical level. Unlike TMS, surface electrical stimulation may be easily combined with simultaneous physical exercise. This approach is broadly applicable**:** it could be used for individuals with other neurological injuries such as stroke, spinal cord injury, multiple sclerosis, or traumatic brain injury. Finally, CES is compatible: it could be combined with drug or cell-based treatments to hopefully produce synergistic functional benefits in the future.

Please note that this IRB application involves similar neural stimulation procedures as already reviewed and approved in study HAR-15-001, HAR-16-042, and HAR-17-017. This application directly parallels the protocol of HAR-17-017, but in a different subject population and funded by a different agency, requiring separate IRB applications.

3. PROCEDURES, METHODS AND EXPERIMENTAL DESIGN.

Participants: Ages between 21 and 75. n=24 subjects with ALS. n=27 non-disabled volunteers.

Inclusion criteria

Non-disabled participants

1. Age between 21 and 75 years; n=15 for Aim 1, n=12 for Aim 2;
2. No known central or peripheral neurological disease or injury.

ALS participants

1. Age between 21 and 75 years; n=12 for Aims 1 and 2;
2. Diagnosis of definite or probable ALS by Escorial Criteria;
3. Weakness (not paralysis) or either hand: Score of 2, 3, or 4 (out of 5) on manual muscle testing of wrist extension, wrist flexion, finger extension, finger flexion, or finger abduction in left or right hand;

Exclusion criteria

1. History of other serious injury or disease of central or peripheral nervous system;
2. History of seizures;
3. Ventilator dependence or patent tracheostomy site;
4. Use of medications that significantly lower seizure threshold;
5. History of head trauma with evidence of brain contusion or hemorrhage or depressed skull fracture on prior imaging;
6. History of implanted brain/spine/nerve stimulators, aneurysm clips, ferromagnetic metallic implants, or cardiac pacemaker/defibrillator;
7. Significant coronary artery or cardiac conduction disease;
8. History of bipolar disorder or suicide attempt or active psychosis;
9. Heavy alcohol consumption (> equivalent of 5 oz of liquor) within previous 48 hours;
10. Open skin lesions over the face, neck, shoulders, or arms;
11. Pregnancy;
12. Unsuitable for study participation as determined by study physician

Recruitment:

Veterans with ALS will be recruited from the multidisciplinary ALS clinic at the James J. Peters VA Medical Center, Bronx, NY. After our ALS veteran population has been given first option to participate, eligible non-veterans with ALS will be permitted to participate. This will allow for a larger pool of subjects, and will help to ensure balanced allocation among treatment groups. Individuals who indicate desire to participate in clinical research will be referred from colleagues at ALS clinics at New York-Presbyterian Hospital and Mount Sinai Health System. We will not contact anyone unless referring physicians provide assurance that the individual would be interested in receiving further information about this study. Individuals who contact us through online postings at clinicaltrials.gov, which are distributed by various ALS online communities, will also have the opportunity to participate. Participants will be recruited without regard to gender, racial, or ethnic status. We will not contact any patients unless referring physicians provide us with assurance that the patient would be interested in receiving further information about this study.

Eligibility Screening:

Persons interested in participating will be assigned a number beginning at 1. To determine eligibility, interested participants will be asked the “yes” or “no” questions listed below. Persons who answer “no” to any of the following questions will not be eligible for the study:

- Do you have amyotrophic lateral sclerosis, or are you an able-bodied volunteer?
- Are you between the ages of 21 to 75 years?

If the potential participant answers “yes” to all questions, then the informed consent process will continue by inviting the participant for an in-person interview.

Enrollment:

At the in-person interview, the study will be explained by one of the study team members. All study team members will be trained to obtain consent. The study will be explained in its entirety. Along with explaining each of the testing and evaluation procedures, the study team member will explain every possible risk that the participant may encounter. The potential participant will be told that there is a possibility that he or she will not be eligible if any of the exclusion criteria are found to be true (i.e. a screening failure). This is an investigational, observational study. As such, no direct permanent benefits will be expected.

Potential participants will be encouraged to ask questions throughout the process. Potential participants will be informed of their right to withdraw at any time and that choosing to not participate will not infringe on any of their regular VA benefits or medical care. Once he/she has no further questions and the study team is confident that the potential participant fully understands the protocol and its risks, then the participant will be asked whether he or she is willing to sign the ICF.

Procedures:

General: All procedures are performed in a seated position with elbows at roughly 90 degrees and hands resting on a pillow in pronated position. Electrophysiological measurements are made using the dominant or stronger affected arm. Safety (blood pressure, heart rate, pulse oxygenation, peak expiratory flow rate, and structured symptom questionnaire) and tolerability are closely monitored throughout all experiments (see Human Subjects section).

Electromyography (EMG): EMG is recorded using surface sensors with 300x preamplification, 15-2,000 Hz bandwidth, and internal grounding (Motion Lab Systems). EMG input is collected at a sample rate of 5,000 Hz via digital acquisition board and customized LabVIEW software (National Instruments). Muscles recorded may include abductor pollicis brevis (APB), abductor digiti minimi (ADM), first dorsal interosseous (FDI), flexor carpi radialis (FCR), extensor carpi radialis (ECR), and/or biceps brachii.

TMS: TMS is performed using a MagPro X100 stimulator (MagVenture). The hand motor cortex 'hotspot' is found while monitoring EMG motor response. Location of the hotspot and all subsequent TMS stimuli are tracked using a neural navigation system (Brainsight). Resting motor threshold is determined by delivering pulses with increasing intensity until motor responses of ≥ 50μV are observed in the APB or FDI muscle in at least 5 out of 10 stimuli.

CES: CES is performed using two DS7A nerve stimulators or a dual DS8R stimulator (Digitimer) linked to deliver biphasic pulses (1 ms each phase; cathodal first). The cathode is a 5x10 cm surface electrode (Natus) placed longitudinally with the top edge ~2.5 cm caudal to the C7 spinous process (~T1-T3 vertebral levels posteriorly). The 5 x 10 cm anode is placed horizontally over the thyroid cartilage, corresponding to the C4-C5 levels anteriorly. Two 5x10 cm common ground electrodes are placed over the distal clavicles. Stimulus intensity ranges from 0-80 mA. Motor threshold is determined analogously to the method used in TMS. Blood pressure, heart rate, pulse oximetry, and peak expiratory flow rate are monitored every three minutes.

Peripheral nerve stimulation: Stimulation is delivered using a DS7A or DS8R nerve stimulator and dual surface electrodes (20 mm apart). M-wave and F-reflex responses are triggered over the median and ulnar nerves at the wrist, recording over the APB and FDI muscles, respectively. F-wave pulse intensity is ~110-120% of the intensity that results in maximal compound motor action potential (CMAP). H-reflex responses are triggered over the median nerve at the elbow, recording over the FCR muscle. H-reflex pulse intensity will be calibrated to result in H-reflex amplitude ~20-25% of maximal CMAP[81]. Pulse width is 0.2 ms for M/F wave stimulation and 1.0 ms for H-reflex stimulation.

Autonomic monitoring: In addition to standard vital sign monitoring of blood pressure, heart rate, pulse oxygenation, and forced vital capacity, real-time changes in cardiovascular and respiratory function will be monitored: A three lead electrocardiogram (ECG) (UFI; Morro Bay, CA. #Resp1EKG) will be used to determine HR and heart rate variability (HRV); recording electrode will be in the V6 position. These data will be intermittently monitored during study visits. The ECG data will be viewed in real-time and stored on

a secured desktop computer for future analysis using LabVIEW graphical software for instrumentation (National Instruments, Austin, TX, USA). Beat-to-beat finger BP will be monitored using photo-plethysmography for assessment of BP variability and baroreceptor reflex activity (CNSystems Medizintechnik; Graz, Austria; # CNAP Monitor 500). Impedance plethysmography will be used to monitor respiration rate (Biopac Systems, Inc.; Goleta, CA.; # RSP100C). These data will be viewed in real time and stored on a secured desktop computer for future analysis using LabVIEW.

Timing: Peripheral motor conduction time is calculated using F-wave and M-wave latencies using the formula ($Latency_M$+$Latency_F$ – 1) ÷ 2[82]. Central motor conduction time is calculated as the TMS-evoked potential latency minus the peripheral conduction time. These values are used to precisely synchronize arriving TMS, CES, and peripheral pulses at cervical motor neurons in the relevant experiments.

Replication: For Aim 1, testing will occur over three sessions. For Aim 2, testing will occur over two sessions. This will allow confirmation of key intensity and timing parameters defined for each subject over different sessions, improving reliability of the findings.

Aim 1: Map the circuit and synaptic targets of cervical electrical stimulation (CES).
This Aim investigates fundamental CES mechanisms using a classic conditioning-test stimulus paradigm. An electrical conditioning stimulus will be paired within 0-300 ms of a test stimulus delivered at a range of intensities and sites. This will shed insight into which circuits CES activates, and how CES circuits interact with inhibitory and facilitatory endogenous neural feedback pathways.

CES-TMS interactions:
- Hypothesis 1.1: Conditioning subthreshold CES will potentiate spinal motor neuron responses to test TMS pulses.
- Methods: Test TMS pulse intensity will be set at 120% of motor threshold (or 80-90% of maximal stimulator output if threshold not detectable). Conditioning CES pulse intensity will be set between 30-95% of CES motor threshold. For conditioning CES, either single or "theta bursts" (3 pulses at 50 Hz)[48] will be delivered. All pulse combinations will be delivered at a rate of 0.1 Hz, in pseudorandom parameter order, until 8 responses per parameter have been recorded.
- Interpretation: Depending on the timing of the observed effect, this would support either the mechanism of heterosynaptic summation between segmental Ia input and descending corticospinal input (facilitation expected to occur at ISI between 2-15 ms)[1], or sensory cortical facilitation of motor cortex excitability (facilitation expected to occur at ISI between 20-60 ms[2–4]). This would also set the basis for using subthreshold CES to facilitate spinal cord motor responses during physical training (see Aim 2).

CES-peripheral nerve interactions:
- Hypothesis 1.2: Conditioning CES will increase persistence of F-responses (per 20 stimuli) to retrograde stimulation over the median or ulnar nerve.
- Hypothesis 1.3: Conditioning CES will reduce amplitude of median (flexor carpi radialis) H-responses.
- Speculative hypothesis 1.2: Conditioning suprathreshold CES will block spinal motor neuron F-responses delivered up to 10 ms later.
- Methods: If obtainable, F-wave pulse intensity will be ~110% of the intensity that results in maximal compound motor action potential (CMAP). H-reflex pulse intensity will be calibrated for amplitude ~20-25% of maximal CMAP[81]. Conditioning CES pulse intensity will be set between 30-175% of motor threshold. For subthreshold conditioning CES, either single or "theta bursts" (3 pulses at 50 Hz)[48] will be delivered. All pulse combinations will be delivered at a rate of 0.1 Hz, in pseudorandom parameter order, until 8 (CES-H combinations) or 20 (CES-F combinations) responses per parameter have been recorded.
- Interpretation: Hypothesis 1.2 would indicate that CES positively modulates motor neuron excitability at the spinal level; Hypothesis 1.3 would indicate that CES traveling via sensory afferents homosynaptically depresses ensuing H-reflexes[6]. This would demonstrate the potential for CES to reduce hyperactive muscle stretch responses (spasticity) via segmental interactions[5,6]; ; and speculative hypothesis 1.2 would demonstrate that high-intensity CES pulses traveling anterogradely via efferent nerve roots collide with retrogradely traveling F-waves.

Aim 1 data analysis: Peak-to-peak amplitude (or F-wave persistence) of conditioned pulses will be normalized to that of unconditioned pulses. For each interaction paradigm, repeated-measure analysis of variance (ANOVA) with factors of subject group, conditioning stimulus intensity, interstimulus interval, and muscle will be performed. Post hoc pairwise comparisons will be made using Tukey's method.

Aim 1 Timeline:
Each subject will undergo two testing sessions on separate days, with a minimum of 24 hours and a maximum of two weeks between visits. Per subject, Aim 1 participation would therefore take 2 to 15 days. 27 subjects will be enrolled, resulting in 54 total testing sessions. A very conservative projection of completing 1.5 testing sessions per week would result in a timeline of 36 weeks or 9 months for completing Aim 1, not including recruitment.

Aim 2: Determine optimal CES parameters for acutely facilitating concurrent arm and hand movements.
Volitional limb movements depend on the same corticospinal and spinal motor circuits as those activated by TMS and F-waves. Therefore, since preliminary data shows that subthreshold CES facilitates TMS responses, CES may also be able to facilitate volitional limb movements. The experiments in Aim 2 will shed light on this clinically applicable question.

Volitional motor tasks: Maximal voluntary effort will be defined as the largest response from 3 attempts prior to testing. Effort will be measured using a customized dynamometer or the root mean square (RMS) of electromyographic activity in the target muscles. The tasks are: thumb-third finger opposition (C8-T1 levels), or wrist extension by extending the dorsal hand laterally against a mounted load cell (C6 level). A combined finger opposition/wrist extension task will also be tested. Subjects will be instructed to perform tasks at target effort levels based on real-time display of ongoing EMG or force output.

CES-motor task interaction: While performing volitional motor tasks at 100%, 50%, or 15-20% of maximal effort, conditioning CES (or sham) pulses will be delivered at a range of intensities between 30%-175% of resting motor threshold. For subthreshold CES, either single or "theta bursts" (3 pulses at 50 Hz)[48] will be delivered. The effect of CES pulses on RMS electromyographic activity over the subsequent 200 ms will be measured at several different arm and hand muscles and divided by the baseline RMS from the preceding 100 ms. At least 10 seconds will elapse between each CES/motor task combination. Effort level and CES pulse parameters will be varied in pseudorandom order, until 8 responses per parameter have been recorded.

Aim 2 Outcomes:
- Facilitation: At each muscle, the effect of CES pulses on RMS electromyographic activity over the subsequent 200 ms (in 25 ms bins) will be measured and normalized to the baseline RMS from the preceding 100 ms.
- Silent period: At each muscle, the duration of electromyographic silence is measured from the end of the CES-evoked potential until the resumption of volitional muscle activity (defined as mean rectified EMG amplitude less than or greater than mean baseline activity + 2 standard deviations[83], respectively).

Hypothesis 2.1: Subthreshold CES will *facilitate* concurrent wrist and hand muscle activation.

Hypothesis 2.2: High-intensity CES will transiently *inhibit* concurrent wrist and hand muscle activation.

Interpretation: Confirmation of Hypothesis 2.1 would substantiate the findings of Hypotheses 1.1 and 1.2 and our updated preliminary data that indicate CES's ability to positively modulate corticospinal and/or motor neuron excitability. We expect optimal facilitation when subthreshold CES is delivered at closer to motor threshold intensity. We do not know whether the effects will differ depending on intensity of volitional effort, but if so, further experiments could investigate the roles played by peripheral, spinal, and supraspinal components of effort intensity[84]. Most importantly, confirmation of Hypothesis 2.1 would represent an

opportunity to directly translate and test this paradigm for lasting clinical benefit by combining repetitive subthreshold CES with repetitive task-oriented physical exercise training in a subsequent study.

If high-intensity CES inhibits concurrent volitional activity as predicted by Hypothesis 2.2, this would be analogous to the 'cortical silent period' (CSP) noted when cortical TMS is delivered during volitional contraction[7,8]. The early phase of CSP (the first 50-75 ms after onset of the cortical motor-evoked potential) is mediated by poorly understood spinal mechanisms[7]. Our observations of the onset and duration of a 'spinal silent period' (SSP) in response to CES would shed mechanistic light on this phenomenon.

Aim 2 Data analysis: Rather than peak-to-peak amplitude, the effects of CES on concurrent volitional muscle contraction will be measured using RMS. RMS in 25 ms bins over the 200 ms following each CES pulse will be normalized to the baseline RMS during the 100 ms prior to each pulse. Repeated-measure analysis of variance (ANOVA) with factors of subject group, volitional effort intensity, CES intensity, post-stimulus timebin, and muscle will be performed. Post hoc pairwise comparisons will be made using Tukey's method.

Aim 2 Timeline:
Each subject will undergo two testing sessions on separate days, with a minimum of 24 hours and a maximum of two weeks between visits. Per subject, Aim 2 participation would therefore take 2 to 15 days. 24 subjects will be enrolled, resulting in 48 total testing sessions. A very conservative projection of completing 1.5 testing sessions per week would result in a timeline of 32 weeks or 8 months for completing Aim 2, not including results processing/dissemination.

4. Possible RISKS and protective actions

For subjects with ALS, there is a risk of falling during transfers between different testing positions. To minimize this risk, a clinician will be at the participant's side at all times to assess comfort and provide manual assistance/stabilization as necessary.

The surface electromyography recording and stimulating electrodes and tape have adhesive backing. Therefore, minimal risks such as transient skin irritation at the sites of surface electrode application may occur. Areas with excessive hair will be shaved prior to adhesive application.

Electrical stimulation involves currents up to 100 milliamperes. Electrical pulses may be transiently irritating or painful. Stimulation intensity will be reduced, or testing halted, if a subject is too uncomfortable. Electrical stimulation of the upper spinal cord may theoretically alter activity in vagal or other autonomic circuits. The most likely adverse risks of autonomic activation would be nausea, light-headedness, diaphoresis, or syncope. There is no risk of current crossing over cardiac muscle with the electrode configurations used in this protocol. Nevertheless, to provide further caution against cardiac damage or arrhythmia, subjects who have significant coronary artery disease or cardiac conduction disease, implanted pacemaker/defibrillators will be excluded from participation. To provide further caution against any adverse cardiac or autonomic event, the procedure will be closely monitored for cardiac or dysautonomic side effects with continuous pulse oximetry, blood pressure, and spirometric measurements. Additionally, to better understand potential autonomic effects of stimulation, subjects may be monitored in real time for changes in cardiovascular and respiratory function using three-lead electrocardiography, photo-plethysmography, and impedance-plethysmography. Any change in mean arterial pressure or pulse oximetry of greater than 15% from baseline, accompanied by symptoms such as sudden shortness of breath, chest pain, significant headache, or diaphoresis, will lead to immediate cessation of the protocol and further medical evaluation. Furthermore, if cardiac or dysautonomic side effects occur during the screening visit, the subject will be ineligible for further participation in the study. A medical doctor will be on the premises at all times during stimulation protocols. This technique has received formal designation by the FDA as Non-significant Risk (Q150053).

Transcranial magnetic stimulation (TMS) carries several potential risks. Most of these risks are much greater during application of *repetitive* TMS (defined as pulses given at a frequency of 1 per second or

more frequently), which will *not* be conducted in this study. We will be using a MagPro X100 device (MagVenture). This device has FDA 510(k) clearance (approval #K091940) for peripheral nerve stimulation, and has been cleared as a non-significant risk device by this and numerous other IRBs for research and clinical use. The most serious risk of TMS is induction of seizures. TMS-induced seizures are usually focal, but in some cases can become generalized. To minimize this risk, participants with underlying brain injury that increases the risk of TMS-induced seizures will be excluded from participation – this includes moderate or severe traumatic brain injury, stroke, tumor, multiple sclerosis, or abscess (see full list under Exclusion Criteria). Additionally, participants taking medications that significantly lower seizure thresholds, such as anti-psychotics, amphetamines, tricyclic antidepressants, bupropion, and dalfampridine will be excluded. Furthermore, the applied stimulus intensity will be kept below 200% of the motor threshold for each muscle. This intensity and single-pulse frequency fall far below the recommended safe guidelines delineated by an international workshop on TMS safety (Rossi et al. 2009). Furthermore, participants with implanted devices with electromagnetic properties, such as spine stimulators, deep brain stimulators, vagal nerve stimulators, cardiac pacemakers, cochlear implants, or aneurysm clips, will be excluded. The investigator who will be performing the TMS protocol (Noam Harel) is a neurologist experienced in treating seizures. There is no risk of seizure from electrical stimulation below the brain, as performed in this protocol.

There is a theoretical risk of *repetitive* TMS causing acute psychotic or manic symptoms in patients with depression. This risk is not clearly above the risk for sham-TMS, and it is not clearly above the natural rate of psychotic or manic symptoms that may arise in subjects with depression. Regardless, this protocol does *not* meet the definition of repetitive TMS, and any subject with history of bipolar disease, active psychotic symptoms, or history of suicide attempt will be excluded

TMS pulses generate loud auditory clicks. Hence, all participants will wear earplugs during the procedure. TMS may also cause scalp tingling sensations or pain that is almost always mild and transient. This occurs approximately half as frequently in participants exposed to sham-stimulation. This is much less common using single-pulse TMS than repetitive TMS. Our protocol will use only single-pulse TMS.

The research team has delivered TMS pulses at 5 to 10-second intervals extensively in prior and ongoing IRB-approved clinical studies. A standardized form to assess TMS side effects is used at the end of each TMS session. To repeat, the use of only single pulse TMS, as well as all the other precautions and exclusion criteria we will follow in our TMS protocol, far exceed the recommended guidelines established by an international group of TMS experts[85].

The Brainsight optical TMS tracking system (Rogue Research) uses passive reflectors placed on the subject's head and the TMS magnet to track the magnet's position and orientation relative to the subject's brain in real time. This is a passive detection device, with no penetrating electromagnetic stimulation. The infrared optical tracking portion of the system meets all applicable conformity standards (ANSI/AAMI ES60601-1:2005 +C1:2009 +A2:2010; see appendices), The infrared optical tracking portion of this system has been incorporated into numerous surgical tracking systems that have obtained FDA 510(k) approval in the setting of invasive brain and organ surgery. However, when used in combination with non-invasive TMS for research, it is not considered a medical device that requires FDA approval. There is no risk to the subject from using this tracking system.

Study coordinators and research assistants will be trained to conduct all procedures in a safe and effective manner that produces reproducible results. Study personnel with experience working with SCI and ALS participants will supervise all sessions. All staff will have undergone the appropriate training to use the equipment properly and safely. All subjects will be thoroughly questioned after each procedure to ensure that all possible adverse events, expected and unexpected, are ascertained. Study personnel will refer participants to the proper medical or psychological resources for any identified conditions or problems as a consequence of the research.

In the event of a serious adverse event, it will be reported to the IRB within 24 hours and study interventions will be discontinued until the study physician states that it is safe to resume the study. Adverse events will

be recorded in a data sheet and reported annually to the IRB. When appropriate, necessary medical or professional intervention will be provided for any serious or regular adverse event warranting treatment.

Any unexpected complications that may occur will be discussed with the study physician and/or the participant's personal physician. Dr. Miroslav Radulovic, a board-certified internist, will serve as the study physician.

Protection against Risk

Most of the information that participants provide will not be identifiable. Participant data results will be stored on the VA network in a password-protected file. No identifiable information will be linked to this file. The study team members will have a separate file of participant contact information, also stored on the VA network. Participants will be assured that any "hard copies" of their contact information or data will be kept in a securely locked cabinet in a locked private office. This data will not be destroyed. There is minimal risk of a breach of confidentiality or data security.

Study coordinators and research assistants will be trained to conduct all procedures in a safe and effective manner that produces reproducible results. Study personnel with experience working with SCI participants will supervise all sessions. All staff will have undergone the appropriate training to use the equipment properly and safely. All subjects will be thoroughly questioned after each procedure to ensure that all possible adverse events, expected and unexpected, are ascertained. Study personnel will refer participants to the proper medical or psychological resources for any identified conditions or problems as a consequence of the research.

In the event of a serious adverse event, it will be reported to the IRB within 24 hours and study interventions will be discontinued until the study physician states that it is safe to resume the study. Adverse events will be recorded in a data sheet and reported annually to the IRB. When appropriate, necessary medical or professional intervention will be provided for any serious or regular adverse event warranting treatment.

A formal Data Safety Monitoring Board will not be needed for this study.

Provisions for keeping data confidential are established. All electronic data will be kept on the secure VA network. All intake forms are de-identified according to HIPAA regulations. Consent forms, along with any other forms containing identifiable information, are kept in a locked filing cabinet. Only members of the investigative team will be able to use the information.

Any unexpected complications that may occur will be discussed with the study physician and/or the participant's SCI physician. Dr. Miroslav Radulovic, a board-certified internist, will serve as the study physician.

5. SIGNIFICANCE OF THIS RESEARCH.

The experiments proposed here address important questions in an entirely unexplored context that strongly align with RR&D's mission. ALS is a 100% VA Service-Connected condition due to its higher incidence and prevalence in Veterans[41]. There is no cure. There is no treatment that reliably reverses or halts progression. The rationally-based approach described in this Small Project In REhabilitation study will generate the data needed to advance this program to the next phase – the delivery of multiple sessions of facilitatory CES in conjunction with physical exercises to attempt to achieve lasting clinical benefit. The information obtained in this study may be useful scientifically to the subjects taking part in the study and to other researchers and patients. This could provide new information that leads to improved non-invasive techniques for strengthening nerve transmission after central nervous system injury and disease.

LIST OF KEY BIBLIOGRAPHIC REFERENCES.

1. Roy, F. D.; Bosgra, D.; Stein, R. B. Interaction of Transcutaneous Spinal Stimulation and Transcranial Magnetic Stimulation in Human Leg Muscles. *Experimental brain research* **2014**, *232*, 1717–28.

2. Stefan, K.; Kunesch, E.; Cohen, L. G.; Benecke, R.; Classen, J. Induction of Plasticity in the Human Motor Cortex by Paired Associative Stimulation. *Brain* **2000**, *123 Pt 3*, 572–584.

3. Aimonetti, J. M.; Nielsen, J. B. Changes in Intracortical Excitability Induced by Stimulation of Wrist Afferents in Man. *The Journal of physiology* **2001**, *534*, 891–902.

4. Devanne, H.; Degardin, A.; Tyvaert, L.; Bocquillon, P.; Houdayer, E.; Manceaux, A.; Derambure, P.; Cassim, F. Afferent-Induced Facilitation of Primary Motor Cortex Excitability in the Region Controlling Hand Muscles in Humans. *The European journal of neuroscience* **2009**, *30*, 439–48.

5. Hofstoetter, U. S.; McKay, W. B.; Tansey, K. E.; Mayr, W.; Kern, H.; Minassian, K. Modification of Spasticity by Transcutaneous Spinal Cord Stimulation in Individuals with Incomplete Spinal Cord Injury. *The journal of spinal cord medicine* **2014**, *37*, 202–11.

6. Minassian, K.; Persy, I.; Rattay, F.; Dimitrijevic, M. R.; Hofer, C.; Kern, H. Posterior Root-Muscle Reflexes Elicited by Transcutaneous Stimulation of the Human Lumbosacral Cord. *Muscle & nerve* **2007**, *35*, 327–36.

7. Farzan, F.; Barr, M. S.; Hoppenbrouwers, S. S.; Fitzgerald, P. B.; Chen, R.; Pascual-Leone, A.; Daskalakis, Z. J. The EEG Correlates of the TMS-Induced EMG Silent Period in Humans. *NeuroImage* **2013**, *83*, 120–34.

8. Potter-Baker, K. A.; Janini, D. P.; Frost, F. S.; Chabra, P.; Varnerin, N.; Cunningham, D. A.; Sankarasubramanian, V.; Plow, E. B. Reliability of TMS Metrics in Patients with Chronic Incomplete Spinal Cord Injury. *Spinal cord* **2016**.

9. Strong, M.; Rosenfeld, J. Amyotrophic Lateral Sclerosis: A Review of Current Concepts. *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders* **2009**.

10. Angeli, C. A.; Edgerton, V. R.; Gerasimenko, Y. P.; Harkema, S. J. Altering Spinal Cord Excitability Enables Voluntary Movements after Chronic Complete Paralysis in Humans. *Brain : a journal of neurology* **2014**, *137*, 1394–409.

11. Carmel, J. B.; Martin, J. H. Motor Cortex Electrical Stimulation Augments Sprouting of the Corticospinal Tract and Promotes Recovery of Motor Function. *Frontiers in integrative neuroscience* **2014**, *8*, 51.

12. Harel, N. Y.; Yigitkanli, K.; Fu, Y.; Cafferty, W. B. J.; Strittmatter, S. M. Multimodal Exercises Simultaneously Stimulating Cortical and Brainstem Pathways after Unilateral Corticospinal Lesion. *Brain research* **2013**, *1538*, 17–25, PMCID: PMC3873870.

13. McKay, W. B.; Ovechkin, a V; Vitaz, T. W.; Terson de Paleville, D. G. L.; Harkema, S. J. Long-Lasting Involuntary Motor Activity after Spinal Cord Injury. *Spinal cord* **2011**, *49*, 87–93.

14. Wolf, S. L.; Winstein, C. J.; Miller, J. P.; Taub, E.; Uswatte, G.; Morris, D.; Giuliani, C.; Light, K. E.; Nichols-Larsen, D. Effect of Constraint-Induced Movement Therapy on Upper Extremity Function 3 to 9 Months after Stroke: The EXCITE Randomized Clinical Trial. *Jama* **2006**, *296*, 2095–2104.

15. Fregni, F.; Boggio, P. S.; Lima, M. C.; Ferreira, M. J.; Wagner, T.; Rigonatti, S. P.; Castro, A. W.; Souza, D. R.; Riberto, M.; Freedman, S. D.; Nitsche, M. A.; Pascual-Leone, A. A Sham-Controlled, Phase II Trial of Transcranial Direct Current Stimulation for the Treatment of Central Pain in Traumatic Spinal Cord Injury. *Pain* **2006**, *122*, 197–209.

16. Gerasimenko, Y.; Lu, D.; Modaber, M.; Zdunowski, S.; Gad, P.; Sayenko, D.; Morikawa, E.; Haakana, P.; Ferguson, A. R.; Roy, R. R.; Edgerton, V. R. Noninvasive Reactivation of Motor Descending Control after Paralysis. *Journal of neurotrauma* **2015**.

17. Harkema, S.; Gerasimenko, Y.; Hodes, J.; Burdick, J.; Angeli, C.; Chen, Y.; Ferreira, C.; Willhite, A.; Rejc, E.; Grossman, R. G.; Edgerton, V. R. Effect of Epidural Stimulation of the Lumbosacral Spinal Cord on Voluntary Movement, Standing, and Assisted Stepping after Motor Complete Paraplegia: A Case Study. *Lancet* **2011**, *377*, 1938–47.

18. Carmel, J. B.; Kimura, H.; Martin, J. H. Electrical Stimulation of Motor Cortex in the Uninjured

Hemisphere after Chronic Unilateral Injury Promotes Recovery of Skilled Locomotion through Ipsilateral Control. *Journal of neuroscience* **2014**, *34*, 462–6.

19. Courtine, G.; Gerasimenko, Y.; Brand, R. van den; Yew, A.; Musienko, P.; Zhong, H.; Song, B.; Ao, Y.; Ichiyama, R. M.; Lavrov, I.; Roy, R. R.; Sofroniew, M. V; Edgerton, V. R. Transformation of Nonfunctional Spinal Circuits into Functional States after the Loss of Brain Input. *Nat Neurosci* **2009**, *12*, 1333–1342.
20. Hubli, M.; Dietz, V.; Schrafl-Altermatt, M.; Bolliger, M. Modulation of Spinal Neuronal Excitability by Spinal Direct Currents and Locomotion after Spinal Cord Injury. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology* **2013**, *124*, 1187–95.
21. Sayenko, D. G.; Atkinson, D. A.; Floyd, T. C.; Gorodnichev, R. M.; Moshonkina, T. R.; Harkema, S. J.; Edgerton, V. R.; Gerasimenko, Y. P. Effects of Paired Transcutaneous Electrical Stimulation Delivered at Single and Dual Sites over Lumbosacral Spinal Cord. *Neuroscience letters* **2015**.
22. Gerasimenko, Y.; Gorodnichev, R.; Puhov, A.; Moshonkina, T.; Savochin, A.; Selionov, V.; Roy, R. R.; Lu, D. C.; Edgerton, V. R. Initiation and Modulation of Locomotor Circuitry Output with Multisite Transcutaneous Electrical Stimulation of the Spinal Cord in Noninjured Humans. *Journal of neurophysiology* **2015**, *113*, 834–42.
23. Gerasimenko, Y.; Gorodnichev, R.; Machueva, E.; Pivovarova, E.; Semyenov, D.; Savochin, A.; Roy, R. R.; Edgerton, V. R. Novel and Direct Access to the Human Locomotor Spinal Circuitry. *J Neurosci* **2010**, *30*, 3700–3708.
24. Knikou, M. Neurophysiological Characterization of Transpinal Evoked Potentials in Human Leg Muscles. *Bioelectromagnetics* **2013**, *34*, 630–40.
25. Mills, K. R.; Murray, N. M. Electrical Stimulation over the Human Vertebral Column: Which Neural Elements Are Excited? *Electroencephalography and clinical neurophysiology* **1986**, *63*, 582–9.
26. Minassian, K.; Persy, I.; Rattay, F.; Pinter, M. M.; Kern, H.; Dimitrijevic, M. R. Human Lumbar Cord Circuitries Can Be Activated by Extrinsic Tonic Input to Generate Locomotor-like Activity. *Hum Mov Sci* **2007**, *26*, 275–295.
27. Carvalho, M. de; Miranda, P. C.; Luís, M. L.; Ducla-Soares, E. Cortical Muscle Representation in Amyotrophic Lateral Sclerosis Patients: Changes with Disease Evolution. *Muscle & nerve* **1999**, *22*, 1684–92.
28. Floyd, A. G.; Yu, Q. P.; Piboolnurak, P.; Tang, M. X.; Fang, Y.; Smith, W. A.; Yim, J.; Rowland, L. P.; Mitsumoto, H.; Pullman, S. L. Transcranial Magnetic Stimulation in ALS: Utility of Central Motor Conduction Tests. *Neurology* **2009**, *72*, 498–504, PMCID: PMC2677511.
29. Khedr, E. M.; Ahmed, M. A.; Hamdy, A.; Shawky, O. A. Cortical Excitability of Amyotrophic Lateral Sclerosis: Transcranial Magnetic Stimulation Study. *Neurophysiologie clinique = Clinical neurophysiology* **2011**, *41*, 73–9.
30. Mills, K. R.; Nithi, K. A. Peripheral and Central Motor Conduction in Amyotrophic Lateral Sclerosis. *Journal of the Neurological Sciences* **1998**, *159*, 82–87.
31. Vucic, S.; Kiernan, M. C. Utility of Transcranial Magnetic Stimulation in Delineating Amyotrophic Lateral Sclerosis Pathophysiology. *Handbook of clinical neurology* **2013**, *116*, 561–75.
32. Sangari, S.; Iglesias, C.; Mendili, M.-M. El; Benali, H.; Pradat, P.-F.; Marchand-Pauvert, V. Impairment of Sensory-Motor Integration at Spinal Level in Amyotrophic Lateral Sclerosis. *Clinical Neurophysiology* **2016**, *127*, 1968–1977.
33. Lazzaro, V. Di; Pilato, F.; Profice, P.; Ranieri, F.; Musumeci, G.; Florio, L.; Beghi, E.; Frisullo, G.; Capone, F.; Sabatelli, M.; Tonali, P. A.; Dileone, M. Motor Cortex Stimulation for ALS: A Double Blind Placebo-Controlled Study. *Neuroscience letters* **2009**, *464*, 18–21.
34. Lazzaro, V. Di; Dileone, M.; Pilato, F.; Profice, P.; Cioni, B.; Meglio, M.; Papacci, F.; Sabatelli, M.; Musumeci, G.; Ranieri, F.; Tonali, P. A. Long-Term Motor Cortex Stimulation for Amyotrophic

Lateral Sclerosis. *Brain stimulation* **2010**, *3*, 22–7.

35. Lazzaro, V. Di; Ranieri, F.; Capone, F.; Musumeci, G.; Dileone, M. Direct Current Motor Cortex Stimulation for Amyotrophic Lateral Sclerosis: A Proof of Principle Study. *Brain stimulation* **2013**, *6*, 969–70.
36. Lazzaro, V. Di; Rothwell, J. C. Corticospinal Activity Evoked and Modulated by Non-Invasive Stimulation of the Intact Human Motor Cortex. *The Journal of physiology* **2014**, *592*, 4115–28.
37. Munneke, M. A. M.; Stegeman, D. F.; Hengeveld, Y. A.; Rongen, J. J.; Schelhaas, H. J.; Zwarts, M. J. Transcranial Direct Current Stimulation Does Not Modulate Motor Cortex Excitability in Patients with Amyotrophic Lateral Sclerosis. *Muscle & nerve* **2011**, *44*, 109–14.
38. Munneke, M. A. M.; Rongen, J. J.; Overeem, S.; Schelhaas, H. J.; Zwarts, M. J.; Stegeman, D. F. Cumulative Effect of 5 Daily Sessions of Theta Burst Stimulation on Corticospinal Excitability in Amyotrophic Lateral Sclerosis. *Muscle & Nerve* **2013**, *48*, 733–738.
39. Zanette, G.; Forgione, A.; Manganotti, P.; Fiaschi, A.; Tamburin, S. The Effect of Repetitive Transcranial Magnetic Stimulation on Motor Performance, Fatigue and Quality of Life in Amyotrophic Lateral Sclerosis. *Journal of the Neurological Sciences* **2008**, *270*, 18–22.
40. Cleveland, D. W.; Rothstein, J. D. From Charcot to Lou Gehrig: Deciphering Selective Motor Neuron Death in ALS. *Nat Rev Neurosci* **2001**, *2*, 806–819.
41. Institute of Medicine Committee on the Review of the Scientific Literature on Amyotrophic Lateral Sclerosis in Veterans. *Amyotrophic Lateral Sclerosis in Veterans : Review of the Scientific Literature.*; National Academies Press: Washington, D.C., 2006.
42. Bedlack, R. S.; Joyce, N.; Carter, G. T.; Paganoni, S.; Karam, C. Complementary and Alternative Therapies in Amyotrophic Lateral Sclerosis. *Neurologic Clinics* **2015**, *33*, 909–936.
43. Bowman, M.; Racke, M.; Kissel, J.; Imitola, J. Responsibilities of Health Care Professionals in Counseling and Educating Patients With Incurable Neurological Diseases Regarding “Stem Cell Tourism”: Caveat Emptor. *JAMA neurology* **2015**, *72*, 1342–5.
44. Taylor-Weiner, H.; Graff Zivin, J. Medicine’s Wild West — Unlicensed Stem-Cell Clinics in the United States. *New England Journal of Medicine* **2015**, *373*, 985–987.
45. Chen, R.; Cros, D.; Curra, A.; Lazzaro, V. Di; Lefaucheur, J. P.; Magistris, M. R.; Mills, K.; Rosler, K. M.; Triggs, W. J.; Ugawa, Y.; Ziemann, U. The Clinical Diagnostic Utility of Transcranial Magnetic Stimulation: Report of an IFCN Committee. *Clin Neurophysiol* **2008**, *119*, 504–532.
46. Rossini, P. M.; Burke, D.; Chen, R.; Cohen, L. G.; Daskalakis, Z.; Iorio, R. Di; Lazzaro, V. Di; Ferreri, F.; Fitzgerald, P. B.; George, M. S.; Hallett, M.; Lefaucheur, J. P.; Langguth, B.; Matsumoto, H.; Miniussi, C.; Nitsche, M. A.; Pascual-Leone, A.; Paulus, W.; Rossi, S.; Rothwell, J. C.; Siebner, H. R.; Ugawa, Y.; Walsh, V.; Ziemann, U. 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. *Clinical Neurophysiology* **2015**, *126*, 1071–1107.
47. Ellaway, P. H.; Vásquez, N.; Craggs, M. Induction of Central Nervous System Plasticity by Repetitive Transcranial Magnetic Stimulation to Promote Sensorimotor Recovery in Incomplete Spinal Cord Injury. *Frontiers in integrative neuroscience* **2014**, *8*, 42.
48. Huang, Y.-Z.; Edwards, M. J.; Rounis, E.; Bhatia, K. P.; Rothwell, J. C. Theta Burst Stimulation of the Human Motor Cortex. *Neuron* **2005**, *45*, 201–6.
49. Chen, R.; Classen, J.; Gerloff, C.; Celnik, P.; Wassermann, E. M.; Hallett, M.; Cohen, L. G. Depression of Motor Cortex Excitability by Low-Frequency Transcranial Magnetic Stimulation. *Neurology* **1997**, *48*, 1398–403.
50. Nakamura, H.; Kitagawa, H.; Kawaguchi, Y.; Tsuji, H. Intracortical Facilitation and Inhibition after Transcranial Magnetic Stimulation in Conscious Humans. *The Journal of physiology* **1997**, *498 (*

Pt 3, 817–23.

51. Nitsche, M. A.; Paulus, W. Excitability Changes Induced in the Human Motor Cortex by Weak Transcranial Direct Current Stimulation. *J Physiol* **2000**, *527 Pt 3*, 633–639.
52. Brunoni, A. R.; Nitsche, M. A.; Bolognini, N.; Bikson, M.; Wagner, T.; Merabet, L.; Edwards, D. J.; Valero-Cabre, A.; Rotenberg, A.; Pascual-Leone, A.; Ferrucci, R.; Priori, A.; Boggio, P. S.; Fregni, F. Clinical Research with Transcranial Direct Current Stimulation (tDCS): Challenges and Future Directions. *Brain Stimul* **2012**, *5*, 175–195.
53. Boggio, P. S.; Nunes, A.; Rigonatti, S. P.; Nitsche, M. A.; Pascual-Leone, A.; Fregni, F. Repeated Sessions of Noninvasive Brain DC Stimulation Is Associated with Motor Function Improvement in Stroke Patients. *Restorative neurology and neuroscience* **2007**, *25*, 123–9.
54. Jeffery, D. T.; Norton, J. A.; Roy, F. D.; Gorassini, M. A. Effects of Transcranial Direct Current Stimulation on the Excitability of the Leg Motor Cortex. *Exp Brain Res* **2007**, *182*, 281–287.
55. Edwards, D. J.; Krebs, H. I.; Rykman, A.; Zipse, J.; Thickbroom, G. W.; Mastaglia, F. L.; Pascual-Leone, A.; Volpe, B. T. Raised Corticomotor Excitability of M1 Forearm Area Following Anodal tDCS Is Sustained during Robotic Wrist Therapy in Chronic Stroke. *Restor Neurol Neurosci* **2009**, *27*, 199–207.
56. Tanaka, S.; Takeda, K.; Otaka, Y.; Kita, K.; Osu, R.; Honda, M.; Sadato, N.; Hanakawa, T.; Watanabe, K. Single Session of Transcranial Direct Current Stimulation Transiently Increases Knee Extensor Force in Patients with Hemiparetic Stroke. *Neurorehabil Neural Repair* **2011**, *25*, 565–569.
57. Karok, S.; Witney, A. G. Enhanced Motor Learning Following Task-Concurrent Dual Transcranial Direct Current Stimulation. *PloS one* **2013**, *8*, e85693.
58. Lackmy-Vallée, A.; Klomjai, W.; Bussel, B.; Katz, R.; Roche, N. ANODAL TRANSCRANIAL DIRECT CURRENT STIMULATION OF THE MOTOR CORTEX INDUCES OPPOSITE MODULATION OF RECIPROCAL INHIBITION IN WRIST EXTENSOR AND FLEXOR. *Journal of neurophysiology* **2014**.
59. Winkler, T.; Hering, P.; Straube, A. Spinal DC Stimulation in Humans Modulates Post-Activation Depression of the H-Reflex Depending on Current Polarity. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology* **2010**, *121*, 957–61.
60. Lim, C.-Y.; Shin, H.-I. Noninvasive DC Stimulation on Neck Changes MEP. *Neuroreport* **2011**, *22*, 819–23.
61. Truini, a; Vergari, M.; Biasiotta, a; Cesa, S. La; Gabriele, M.; Stefano, G. Di; Cambieri, C.; Cruccu, G.; Inghilleri, M.; Priori, a Transcutaneous Spinal Direct Current Stimulation Inhibits Nociceptive Spinal Pathway Conduction and Increases Pain Tolerance in Humans. *European journal of pain (London, England)* **2011**, *15*, 1023–7.
62. Bocci, T.; Vannini, B.; Torzini, A.; Mazzatenta, A.; Vergari, M.; Cogiamanian, F.; Priori, A.; Sartucci, F. Cathodal Transcutaneous Spinal Direct Current Stimulation (tsDCS) Improves Motor Unit Recruitment in Healthy Subjects. *Neuroscience letters* **2014**, *578*, 75–9.
63. Edwards, D.; Cortes, M.; Datta, A.; Minhas, P.; Wassermann, E. M.; Bikson, M. Physiological and Modeling Evidence for Focal Transcranial Electrical Brain Stimulation in Humans: A Basis for High-Definition tDCS. *NeuroImage* **2013**, *74*, 266–75.
64. Brand, R. van den; Heutschi, J.; Barraud, Q.; DiGiovanna, J.; Bartholdi, K.; Huerlimann, M.; Friedli, L.; Vollenweider, I.; Moraud, E. M.; Duis, S.; Dominici, N.; Micera, S.; Musienko, P.; Courtine, G. Restoring Voluntary Control of Locomotion after Paralyzing Spinal Cord Injury. *Science* **2012**, *336*, 1182–1185.
65. Wenger, N.; Moraud, E. M.; Raspopovic, S.; Bonizzato, M.; DiGiovanna, J.; Musienko, P.; Morari, M.; Micera, S.; Courtine, G. Closed-Loop Neuromodulation of Spinal Sensorimotor Circuits

Controls Refined Locomotion after Complete Spinal Cord Injury. *Science Translational Medicine* **2014**, *6*, 255ra133-255ra133.

66. Hebb, D. O. *The Organization of Behavior; a Neuropsychological Theory*; Wiley: New York, 1949.
67. Rall, W.; Burke, R. E.; Smith, T. G.; Nelson, P. G.; Frank, K. Dendritic Location of Synapses and Possible Mechanisms for the Monosynaptic EPSP in Motoneurons. *J Neurophysiol* **1967**, *30*, 1169–1193.
68. Muller, D.; Nikonenko, I.; Jourdain, P.; Alberi, S. LTP, Memory and Structural Plasticity. *Curr Mol Med* **2002**, *2*, 605–611.
69. Caporale, N.; Dan, Y. Spike Timing-Dependent Plasticity: A Hebbian Learning Rule. *Annual review of neuroscience* **2008**, *31*, 25–46.
70. Bunday, K. L.; Perez, M. A. Motor Recovery after Spinal Cord Injury Enhanced by Strengthening Corticospinal Synaptic Transmission. *Current biology : CB* **2012**, *22*, 2355–61.
71. Cortes, M.; Thickbroom, G. W.; Valls-Sole, J.; Pascual-Leone, A.; Edwards, D. J. Spinal Associative Stimulation: A Non-Invasive Stimulation Paradigm to Modulate Spinal Excitability. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology* **2011**, *122*, 2254–9.
72. Einhorn, J.; Li, A.; Hazan, R.; Knikou, M. Cervicothoracic Multisegmental Transpinal Evoked Potentials in Humans. *PloS one* **2013**, *8*, e76940.
73. Knikou, M. Transpinal and Transcortical Stimulation Alter Corticospinal Excitability and Increase Spinal Output. *PloS one* **2014**, *9*, e102313.
74. McGie, S. C.; Masani, K.; Popovic, M. R. Failure of Spinal Paired Associative Stimulation to Induce Neuroplasticity in the Human Corticospinal Tract. *The journal of spinal cord medicine* **2014**, *37*, 565–74.
75. Mrachacz-Kersting, N.; Fong, M.; Murphy, B. A.; Sinkjaer, T. Changes in Excitability of the Cortical Projections to the Human Tibialis Anterior after Paired Associative Stimulation. *J Neurophysiol* **2007**, *97*, 1951–1958.
76. Shulga, A.; Lioumis, P.; Kirveskari, E.; Savolainen, S.; Mäkelä, J. P.; Ylinen, A. The Use of F-Response in Defining Interstimulus Intervals Appropriate for LTP-like Plasticity Induction in Lower Limb Spinal Paired Associative Stimulation. *Journal of neuroscience methods* **2015**, *242*, 112–7.
77. Taylor, J. L.; Martin, P. G. Voluntary Motor Output Is Altered by Spike-Timing-Dependent Changes in the Human Corticospinal Pathway. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **2009**, *29*, 11708–16.
78. Takeoka, A.; Vollenweider, I.; Courtine, G.; Arber, S. Muscle Spindle Feedback Directs Locomotor Recovery and Circuit Reorganization after Spinal Cord Injury. *Cell* **2014**, *159*, 1626–39.
79. Ibitoye, M. O.; Hamzaid, N. A.; Hasnan, N.; Abdul Wahab, A. K.; Davis, G. M. Strategies for Rapid Muscle Fatigue Reduction during FES Exercise in Individuals with Spinal Cord Injury: A Systematic Review. *PloS one* **2016**, *11*, e0149024.
80. Krenn, M.; Toth, A.; Danner, S. M.; Hofstoetter, U. S.; Minassian, K.; Mayr, W. Selectivity of Transcutaneous Stimulation of Lumbar Posterior Roots at Different Spinal Levels in Humans. *Biomedizinische Technik. Biomedical engineering* **2013**.
81. Serranova, T.; Valls-Sole, J.; Munoz, E.; Genis, D.; Jech, R.; Seeman, P. Abnormal Corticospinal Tract Modulation of the Soleus H Reflex in Patients with Pure Spastic Paraparesis. *Neurosci Lett* **2008**, *437*, 15–19.
82. Robinson, L. R.; Jantra, P.; MacLean, I. C. Central Motor Conduction Times Using Transcranial Stimulation and F Wave Latencies. *Muscle & nerve* **1988**, *11*, 174–80.
83. Barry, M. D.; Bunday, K. L.; Chen, R.; Perez, M. A. Selective Effects of Baclofen on Use-

Dependent Modulation of GABAB Inhibition after Tetraplegia. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **2013**, *33*, 12898–907.

84. Taylor, J. L.; Gandevia, S. C. A Comparison of Central Aspects of Fatigue in Submaximal and Maximal Voluntary Contractions. *Journal of applied physiology (Bethesda, Md. : 1985)* **2008**, *104*, 542–50.

85. Rossi, S.; Hallett, M.; Rossini, P. M.; Pascual-Leone, A. Safety, Ethical Considerations, and Application Guidelines for the Use of Transcranial Magnetic Stimulation in Clinical Practice and Research. *Clinical neurophysiology : official journal of the International Federation of Clinical Neurophysiology* **2009**, *120*, 2008–39.