Non-invasive Cervical Electrical Stimulation for SCI

NCT03414424

PROTOCOL AND STATISTICAL ANALYSIS PLAN

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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 CES will potentiate spinal motor neuron responses to test TMS pulses. Depending on the timing of the effect, this would support the mechanism of heterosynaptic summation between segmental Ia input and descending corticospinal input[1], or sensory cortical facilitation of motor cortex excitability[2–4], or both.
Hypothesis 1.2: Conditioning CES will increase persistence of spinal motor neuron F-responses to retrograde stimulation over the median and ulnar nerves. This would further establish that CES could modulate motor neuron excitability at the segmental level.
Hypothesis 1.3: Conditioning subthreshold CES will reduce spinal motor neuron H-responses to afferent stimulation over the median nerve. This would indicate the potential for CES to reduce hyperactive muscle stretch responses (spasticity) via homosynaptic interactions.

Aim 2: Determine optimal CES parameters for acutely facilitating concurrent wrist and hand movements.
Volitional limb movements depend on the same corticospinal and motor neuron circuits as those activated by TMS and F-waves. Therefore, if CES facilitates TMS and/or F-responses, CES may also be able to facilitate volitional limb movements. The experiments in Aim 2 will shed light on this clinically relevant question.
Hypothesis 2.1: Subthreshold CES will *facilitate* concurrent wrist and hand muscle activation, supporting CES's ability to positively modulate corticospinal and/or motor neuron excitability. This would present an opportunity to directly translate this paradigm for clinical benefit by combining repetitive subthreshold CES with repetitive task-oriented physical exercise training.
Hypothesis 2.2: High-intensity suprathreshold CES will transiently *inhibit* concurrent wrist 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[5,6].

2. BRIEF REVIEW OF RESULTS AND CURRENT STATE OF KNOWLEDGE

Roughly 60% of spinal cord injuries occur at the cervical level[7]. Most injuries are anatomically incomplete. A large body of evidence in incomplete SCI as well as other forms of brain injury suggest that externally activating spared nerve circuits, whether by exercise, drugs, or electromagnetic stimulation, augments circuit and physiological function[8–16]. 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 SCI. 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[17,18]. Repetitive TMS (rTMS) at 1 Hz is generally inhibitory, whereas at 5 Hz or greater, rTMS is generally excitatory[11]. Higher-frequency (usually 50 Hz) 'theta burst' TMS may induce longer-lasting effects that are either inhibitory or excitatory based on burst patterns[18,19]. Seminal TMS mechanistic studies will serve as guides for some of the experiments in this proposal[17–21].

Direct current stimulation (DCS) delivers a low-power electric current that modulates neuronal excitability[22]. In distinction from TMS, DCS delivers exclusively *tonic, sub*threshold stimulation[23]. Various cranial and

spinal DCS configurations have shown promising effects in neurological and psychiatric disorders, as well as enhancement of normal function[24–35]. 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[36]. 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[37–39]. 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[8,13]. 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[40]. Adding simultaneous stimulation at the C5 and L1 levels increases motion coordination and range[41]. 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[42,43].

Transcutaneous biphasic stimulation can also elicit action potentials through afferent fiber pathways[44]. 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[45]. 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[46–49]. These paired stimulation techniques include paired associative stimulation (PAS), transspinal-transcortical stimulation, spinal associative stimulation, spike timing-dependent plasticity, and others[4,50–57]. 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[50]. 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[50]. 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[54,56,57].

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 an IRB-approved human pilot study 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[58].

While we are excited to test CES paired with either peripheral nerve stimulation or TMS in our ongoing pilot study (clinicaltrials.gov NCT02469675), 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[59]. 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.

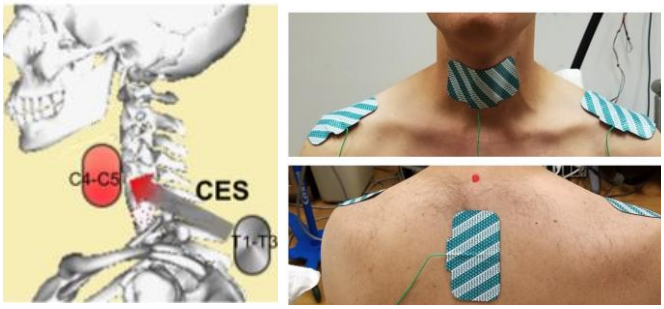

Figure 1. CES schematic. Biphasic electrical pulses are delivered noninvasively over the cervical spine. The cathode is placed posteriorly over T1-T3 levels, and the anode is placed anteriorly over C4-C5 levels. Ground electrodes are placed over the distal clavicles. Depicted schematically on left, photographically 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[1,44,45,60]. 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[18,21]. 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

Non-invasive, non-noxious cervical electrical stimulation (CES)

One 5x10 cm electrode is placed longitudinally over the midline with the top edge 2 cm caudal to the C7 spinous process (~T1-T3 vertebral levels). Another 5x10 cm electrode is placed horizontally centered over the thyroid cartilage, corresponding to the C4-C5 levels anteriorly (**Figure 1**). Two 5x10 cm common

ground electrodes are placed over the distal clavicles. Preliminary testing has shown that cathode-posterior biphasic (1 ms each phase) stimulation results in the best combination of subject tolerability, lowest motor threshold, and largest muscle response. We will use this configuration for all studies within the current proposal.

In preliminary studies, 20 subjects (6 with SCI) have undergone over 120 sessions of CES without procedure-related significant adverse events to date. Each session involves between 150-300 pulses of CES. All subjects have had easily obtainable CES responses. Across our studies involving TMS over hand motor cortex, 8 out of 9 subjects with cervical SCI have had readily elicited TMS responses in hand muscles (**Table 1**). This supports the physiological feasibility and safety of performing CES in individuals with and without SCI.

Age	Level	ASIA Grade	Duration	MEP Threshold (% MSO)	CES Threshold (mA)
64	C4	D	14yr	60	10
54	C4	D	4yr	neg	NT
57	C4	D	12yr	57	42
40	C4	D	15yr	65	24.5
36	C5	D	7yr	69	NT
42	C5	D	14yr	58	29
40	C5	D	5yr	67	NT
29	C8	C	3yr	47	32
51	C8	D	17yr	34	36

Table 1 – Demographics and average TMS and CES resting motor thresholds to hand muscles in subjects with SCI. **MEP**, motor-evoked potential; **MSO**, maximum stimulator output; **mA**, milliampere; **neg**, no response; **NT**, CES not tested in that subject.

CES activates multiple arm muscles simultaneously

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**).

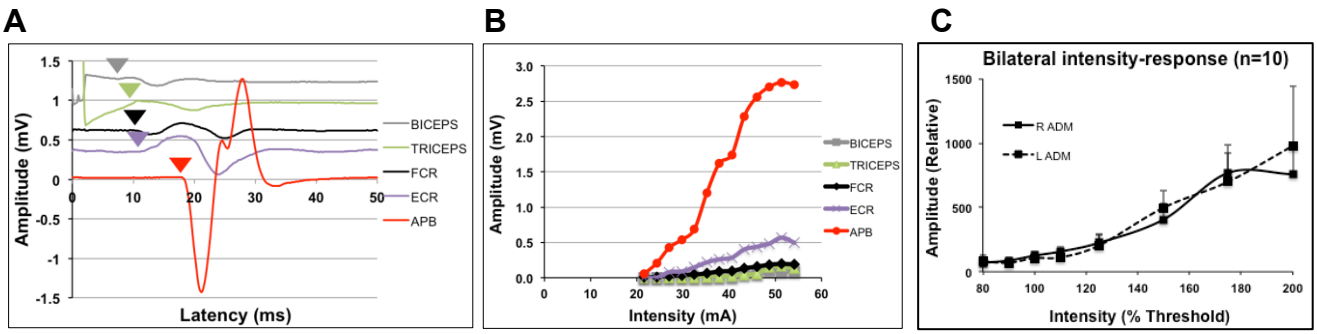

Figure 2. CES activates multiple muscles simultaneously. A) CES waveforms in one non-disabled volunteer depicted in raster format. CES stimulus at ~200% threshold. Note the proximal to distal gradient of latencies and amplitudes. **B)** Intensity-response curves of five different arm muscles, showing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

CES targets different circuits at different intensities

At peri-threshold intensity, latencies to the APB muscle are up to ~3-5 ms longer than the peripheral motor conduction time (not shown). At higher stimulation intensities, latency is equal to or shorter than the peripheral motor conduction time. Similar observations have been made in studies of other transcutaneous spinal stimulation paradigms[43,61,62]. These findings are consistent with lower intensity stimuli acting via

afferent sensory fibers that synapse onto lower motor neurons, and higher intensity stimuli acting via efferent motor fibers ~2-4 cm distal to the cell bodies. The longer route traversed by lower-intensity stimuli explains the longer latency.

These afferent versus efferent root transmission pathways are further demonstrated by the response to two sequential CES pulses given 40 ms apart (**Figure 3**). At low intensity, the amplitude of the second pulse is strongly reduced, suggesting post-activation synaptic depression as seen with H-reflexes and posterior root-muscle reflexes[1,44,45,60]. At high intensity, there is little to no decrement, suggesting direct efferent fiber axonal activation, which is not susceptible to post-activation depression.

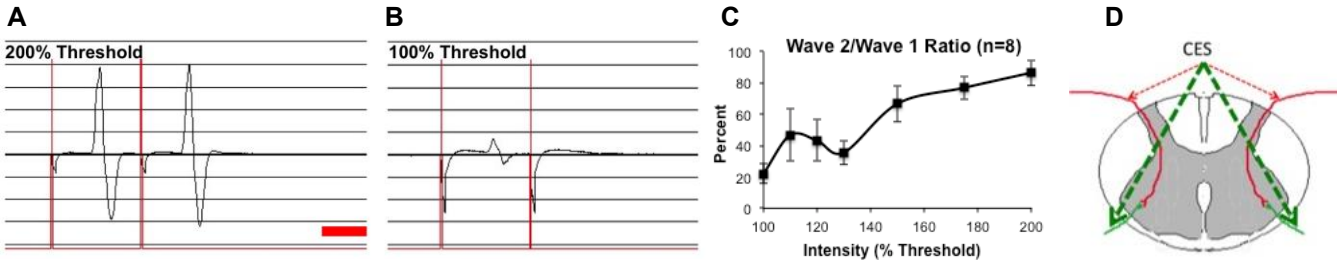

Figure 3. The CES circuit pathway is adjustable via stimulus intensity. APB recordings of double CES stimuli at 40 ms intervals. **A**, At high intensity (200% of resting motor threshold), repeat CES stimulus (red lines) shows no decrement. **B**, At peri-threshold intensity, repeat CES stimulus results 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>Subthreshold CES acutely facilitates muscle responses to cortical stimulation</u>

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 and SCI 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. <u>This is a finding with potential for direct clinical translation</u>. 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.

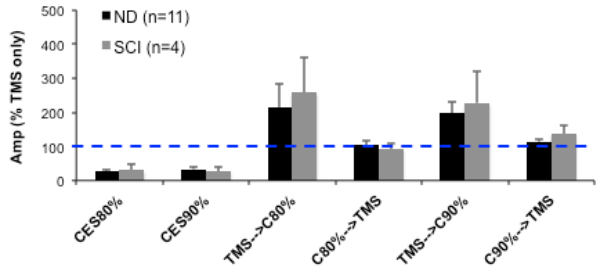

Figure 4. Subthreshold CES acutely facilitates TMS-evoked potentials. Suprathreshold (120%) TMS and subthreshold (80-90%) CES were given alone or in combination. Response amplitudes were normalized to the response to TMS alone. Subthreshold CES facilitated the response to TMS alone when the TMS pulse 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 <u>innovative</u>**:** the delivery patterns and configurations proposed here are novel. CES is <u>practical</u>**:** 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, amyotrophic lateral sclerosis, 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 and HAR-16-042. The HAR-16-042 protocol, “Acute interactions between electromagnetic stimulation and physical exercise”, was originally designed to obtain preliminary data to support several ongoing grant applications. Since that time, we have obtained funding for two new grants. Therefore, this and a concurrent new protocol being submitted by Dr. Yu-Kuang Wu are now more closely tailored to the respective grants we are being awarded.

3. PROCEDURES, METHODS AND EXPERIMENTAL DESIGN.

Participants: Ages between 18 and 75. n=15 subjects with SCI. n=15 able-bodied volunteers.

Inclusion criteria

Able-bodied participants

1. Age between 18 and 75 years; n=15 for both Aims;
2. No known central or peripheral neurological disease or injury.

SCI participants

1. Age between 18 and 75 years; n=15 for Aims 1 and 2;
2. Chronic (> 12 months) motor-incomplete SCI between neurological levels **{**C1-C8**}**;
3. 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;
4. Detectable F-wave responses of the left or right APB to median nerve stimulation and/or FDI to ulnar nerve stimulation;

Exclusion criteria

1. Multiple spinal cord lesions;
2. History of seizures;
3. Ventilator dependence or patent tracheostomy site;
4. Use of medications that significantly lower seizure threshold, such as tricyclic antidepressants, amphetamines, neuroleptics, dalfampridine, and bupropion;
5. History of stroke, brain tumor, brain abscess, or multiple sclerosis;
6. History of moderate or severe head trauma (loss of consciousness for greater than one hour or evidence of brain contusion or hemorrhage or depressed skull fracture on prior imaging);
7. History of implanted brain/spine/nerve stimulators, aneurysm clips, ferromagnetic metallic implants, or cardiac pacemaker/defibrillator;
8. Significant coronary artery or cardiac conduction disease;
9. Recent history (within past 6 months) of recurrent autonomic dysreflexia, defined as a syndrome of sudden rise in systolic pressure greater than 20 mm Hg or diastolic pressure greater than 10 mm Hg, without rise in heart rate, accompanied by symptoms such as headache, facial flushing, sweating, nasal congestion, and blurry vision (this will be closely monitored during all screening and testing procedures);
10. History of bipolar disorder;
11. History of suicide attempt;
12. Active psychosis;
13. Heavy alcohol consumption (greater than equivalent of 5 oz of liquor) within previous 48 hours;
14. Open skin lesions over the face, neck, shoulders, or arms;
15. Pregnancy
16. Unsuitable for study participation as determined by study physician.

Recruitment:

Recruitment will be accomplished by pre-existing relationships, physician referrals, and IRB-approved advertisements. Veterans with SCI who have an ongoing relationship with personnel of the Center of Excellence for the Medical Consequences of SCI, such as those who attend the pulmonary, endocrine, cardiovascular, gastroenterology, and SCI clinics, will be informed about the study. Physicians at the James J. Peters VA Medical Center will be informed of the goals and aims and the inclusion/exclusion criteria for this study. Interested non-veterans who contact us through word of mouth, public online sites such as clinicaltrials.gov, or through participation in other ongoing studies at the Center of Excellence will also be informed about the study. 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 a spinal cord injury, or are you an able-bodied volunteer?
- If you have spinal cord injury, did the injury occur greater than 12 months ago?
- Are you between the ages of 18 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 ≥ 50□V 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 a 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[63]. Pulse width is 0.2 ms for M/F wave stimulation and 1.0 ms for H-reflex stimulation.

Timing: Peripheral motor conduction time is calculated using F-wave and M-wave latencies using the formula ($Latency_M$+$Latency_F$ – 1) ÷ 2^{64}. 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.

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.

Aim 1: Map the cellular and synaptic targets of cervical electrical stimulation (CES).
This Aim investigates fundamental CES mechanisms. The experiments share a common structure comprising an electrical conditioning stimulus delivered 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 circuits activated by other exogenous neural stimuli.

Aim 1 Outcomes:
- Amplitudes: Peak-to-peak amplitudes from an average of 8-10 responses per condition.
- Persistence (F-waves): Persistence is defined as the percent of positive F-wave responses out of 25 pulses.

CES-TMS interactions:
- Goal: Measure interactions between afferent cervical nerve roots and descending corticospinal inputs into the cervical cord.
- Methods: Test TMS pulse intensity will be 120% of motor threshold. Conditioning CES pulse intensity will be between 30-95% of motor threshold, delivered within 300 ms of the test pulse. For conditioning CES, either single or "theta bursts" (3 pulses at 50 Hz)[19] will be delivered. All pulse combinations will be delivered at a rate of 0.1 Hz, in pseudorandom parameter order, until 8-10 responses per parameter have been recorded.
- Hypothesis 1.1: Conditioning subthreshold CES will potentiate motor neuron response to test TMS pulses.
- Interpretation: If subthreshold CES facilitation occurs at ISI between 2-20 ms, this would support the mechanism of heterosynaptic summation between segmental Ia input and descending corticospinal input[1]. If facilitation occurs at ISI between 20-60 ms, this would support the mechanism of sensory cortical facilitation of motor cortex excitability (based on analogous findings using peripheral nerve stimulation[4,2,3]). Because TMS travels along the same corticospinal and subcortical circuits used during volitional movement, this would also set the basis for using subthreshold CES to facilitate motor

responses during physical training programs (see Aim 2). Subjects with incomplete cervical SCI are expected to have reduced CES-TMS facilitation compared to uninjured subjects (see **Figure 4**).

CES-peripheral nerve interactions:

- Goal: Measure interactions between afferent and efferent cervical nerve roots, segmental interneuronal circuits, orthodromically conducting peripheral sensory fibers, and antidromically conducting peripheral motor fibers.
- Methods: F-wave pulse intensity will be ~110-120% of the intensity that results in maximal compound motor action potential (CMAP). H-reflex pulse intensity will be calibrated to result in H-reflex amplitude ~20-25% of maximal CMAP[63]. Conditioning CES pulse intensity will be set between 30-175% of motor threshold, delivered within 300 ms of test pulses. For subthreshold conditioning CES, either single or "theta bursts" (3 pulses at 50 Hz)[19] 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 25 (CES-F combinations) responses per parameter have been recorded.
- Hypothesis 1.2: Conditioning CES will increase persistence (number of responses per 25 stimuli) of median (APB) and ulnar (FDI) F-responses elicited up to 50 ms later.
- Hypothesis 1.3: Conditioning CES will reduce amplitude of median (FCR) H-responses elicited up to 50 ms later.
- Speculative hypothesis 1.2: Conditioning suprathreshold CES will block spinal motor neuron F-responses delivered up to 10 ms later.
- Interpretation: These experiments will lead to multiple insights. Through segmental interactions, confirmation of Hypothesis 1.2 would further demonstrate the ability of CES to positively modulate motor neuron excitability, and confirmation of hypothesis 1.3 would indicate the potential for CES to reduce spasticity[45]. Confirmation of speculative hypothesis 1.2 would demonstrate that at high intensity, CES pulses travel via efferent nerve roots and collide with retrogradely traveling F-waves.

Aim 1 data analysis: Peak-to-peak amplitude of all conditioned pulses will be normalized to the amplitude of unconditioned pulses. For each interaction paradigm, analysis of variance (ANOVA) with factors of subject group, stimulus intensity, interstimulus interval, and muscle will be performed. Post hoc pairwise comparisons will be made using Tukey's method. *Power*: Using a conservative predicted effect size of 0.4 for each interaction paradigm, >80% power will be achieved with a sample size of 10 for each analysis (repeated measure ANOVA) with four factors). 15 subjects in each group (SCI, able-bodied) will undergo testing.

Aim 1 Timeline (**Table 2**):
Each subject will undergo three 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 3 to 28 days. 30 subjects will be enrolled, resulting in 90 total testing sessions. A conservative projection of completing two testing sessions per week would result in a timeline of 45 weeks or 11-12 months for completing Aim 1.

Aim 2: Determine optimal CES parameters for acutely facilitating concurrent wrist 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 contraction (MVC) will be defined as the largest response from 3 attempts at maximal effort 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: opposition between the tips of the thumb and third finger (C8-T1 levels), or wrist extension 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: Subjects will be given a synchronized audio and visual cue to perform volitional motor tasks at 100%, 50%, or 10-20% of MVC. Volitional tasks will be performed for a maximum of 4 continuous seconds. At least 10 seconds will elapse between each CES/motor task combination. Conditioning CES pulses (or sham pulses at 0% intensity) will be delivered at intensity ranging between 30%-175% of CES resting motor threshold. For subthreshold conditioning CES, either single or "theta bursts" (3 pulses at 50 Hz)[19] will be delivered. Motor task, effort level, and CES parameters will be varied in pseudorandom order, until 8-10 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[68], 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 predicted findings of Hypotheses 1.1 and 1.2 and our early 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[69]. Furthermore, these experiments would shed light on whether CES modulates ongoing motor neuron activity differently at different spinal levels during level-specific motor tasks (thumb-finger opposition C8-T1, wrist extension C6). Most importantly, confirmation of Hypothesis 2.1 would represent an opportunity to directly translate and test this paradigm for clinical benefit by combining repetitive subthreshold CES with repetitive task-oriented physical exercise training.

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[5,6]. 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[5]. 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 as described above. Repeated-measure 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. Although we can only speculate on the predicted effect size, with use of a conservative effect size of 0.4 for each interaction paradigm, >80% power will be achieved with a sample size of 10 for each analysis (repeated measure ANOVA with five factors). 15 subjects in each group (SCI, able-bodied) will undergo testing.

Aim 2 Timeline:
Each subject will undergo two testing sessions on separate days, with a minimum of 48 hours and a maximum of three weeks between visits. Per subject, Aim 2 participation would therefore take 3 to 21 days. 30 subjects will be enrolled, resulting in 60 total testing sessions. A very conservative projection of completing 1.5 testing sessions per week would result in a timeline of 40 weeks or 10 months for completing Aim 2.

Overall timeline:

Stage	AB	SCI	Visits	Duration (wks)	Months			
					1-6	7-12	13-18	19-24
Startup					X			
Aim 1	15	15	3	1-4	XXXX	XXXXX		
Aim 2	15	15	2	1-3			XXXXX	XXX
Analysis/ Dissemination								XX

Table 2 – Study timeline. Extra time is allotted for study startup, data analysis, and dissemination of results. See also Timeline descriptions in proposal text.

4. Possible RISKS and protective actions

For subjects with SCI, 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 80 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, cardiac conduction disease, implanted pacemaker/defibrillators, or history of recurrent autonomic dysreflexia (defined in Exclusion Criteria) will be excluded from participation.

In subjects with SCI above the T6 vertebral level, there is a risk of autonomic dysreflexia – this is a potentially dangerous increase in blood pressure with simultaneous decrease in heart rate, usually accompanied by symptoms such as headache, facial flushing, sweating, nasal congestion, and blurry vision. There is a theoretical risk that it could occur in response to magnetic or electrical stimulation. Potential subjects who have a history of recurrent autonomic dysreflexia within the past 6 months will be excluded. If autonomic dysreflexia is suspected during a procedure, the procedure will be halted immediately. Head position, bowel, bladder, and other triggers will be addressed according to a standard algorithm[70]. Subjects who experience autonomic dysreflexia during any session will be withdrawn from further participation in the protocol.

To provide further caution against any adverse cardiac or autonomic event, the procedure will be closely monitored for cardiac or dysautonomic side effects with pulse oximetry, blood pressure, and spirometric measurements. Additionally, subjects will be monitored in real time for changes in cardiovascular and respiratory function using a three-lead electrocardiogram, 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 [71].

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 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 SCI 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 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.

Participants may achieve transient neurophysiological benefits. However, the study is not designed to provide long-lasting direct benefit. Rather, study participation may lead to new knowledge that could be applied toward the SCI population as a whole.

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