Priming With High-Frequency Trans-spinal Stimulation to Augment Locomotor Benefits in Spinal Cord Injury

NCT04807764

Study Protocol and Statistical Analysis Plan

31 May 2022

SPECIFIC AIMS

The seemingly simple tasks of standing and walking require continuous integration of complex spinal reflex circuits between descending motor commands and ascending sensory inputs. This integration is markedly impaired by spinal cord injury (SCI), which severely compromises daily living activities. While these deficits are partially improved by locomotor training, even after multiple training sessions, abnormal muscle activity and coordination still persist. Thus, locomotor training alone cannot fully optimize the neuronal plasticity required to strengthen the synapses connecting the brain, spinal cord, and local circuits. As such, treatment interventions that *effectively promote neuromodulation of spinal locomotor networks and strengthen neural connectivity of the injured human spinal cord* are greatly needed. Our *overall hypothesis* is that transcutaneous spinal cord (transspinal) stimulation over the thoracolumbar enlargement can strengthen neural connectivity. Specifically, we expect that this stimulation will bring motoneurons closer to threshold, which is a pre-requisite for functioning descending and local inputs. Importantly, whether concurrent treatment with transspinal stimulation and locomotor training maximizes motor recovery after SCI is unknown. This question drives our current proposal to attempt to prime locomotor training in SCI using transspinal stimulation. With this approach, we will synergistically engage multiple activity-based plasticity mechanisms. **The primary objective of this clinical trial is to use transspinal stimulation to prime locomotor training and ultimately improve standing and walking ability in individuals with chronic incomplete SCI (iSCI).** We hypothesize that noninvasive high-frequency thoracolumbar transspinal stimulation administered before locomotor training will increase neuron responsiveness over multiple spinal segments, ultimately facilitating standing and walking. Because transspinal stimulation facilitates anti-gravity neuronal circuits, we further hypothesize that transspinal stimulation delivered during standing will augment gains compared to delivery in the supine position. To test these hypotheses, 45 individuals with iSCI will undergo 40 sessions of body weight-supported (BWS) step training primed with high-frequency (30 Hz) transspinal stimulation. Participants will be randomized to receive transspinal stimulation during standing (real or sham) or while supine (real). We will address our hypotheses with the following three specific aims:

Aim 1: Determine if priming locomotor training with transspinal stimulation increases motor evoked potential (MEP) amplitudes, and promotes appropriate MEP amplitude modulation during stepping. We *hypothesize* that real but not sham transspinal stimulation delivered during standing will increase MEP amplitudes and promote physiological MEP amplitude modulation during stepping. Alternatively, transspinal stimulation delivered while supine will increase MEP amplitudes but not induce comprehensive circuit reorganization. These results will support the notion that tonic high-frequency transspinal stimulation strengthens corticomotoneuronal connectivity through posture-dependent corticospinal neuroplasticity.

Aim 2: Determine if priming locomotor training with transspinal stimulation restores spinal inhibition, reduces hyperreflexia, and re-establishes physiological H-reflex modulation during stepping. We *hypothesize* that real but not sham transspinal stimulation delivered during standing will 1) restore soleus H-reflex rate-dependent depression, 2) increase the depth of reciprocal inhibition, and 3) improve soleus H-reflex modulation towards a more physiological pattern during stepping. We further *hypothesize* that rate-dependent reflex depression and H-reflex modulation during walking will recover more in the transspinal-standing group than in the transspinal-supine. These results will indicate that priming locomotor training with high-frequency transspinal stimulation evokes appropriate neuromodulation of spinal locomotor networks.

Aim 3: Determine if priming locomotor training with high-frequency transspinal stimulation improves limb coordination and the ability to stand and walk. Comparing locomotor electromyography (EMG) activity before and after the interventions will clearly demonstrate reorganized motoneuron activity. Alternatively, assessments of balance and walking with standard clinical tests will indicate functional gains. We *hypothesize* that the number of motor modules will increase in the transspinal-standing group, and that antagonistic muscles will be reciprocally active during stepping. These results will indicate that priming locomotor training with high-frequency transspinal stimulation improves the ability to stand and walk by facilitation of spinal locomotor neuronal networks.

We anticipate that the information gained from this mechanistic clinical trial will *greatly impact clinical practice.* This is because in real-world clinical settings, noninvasive transspinal stimulation can be more easily and widely implemented than invasive epidural stimulation. Additionally, by applying multiple interventions to accelerate motor recovery, we are employing a treatment regimen that represents a true clinical approach. Indeed, this multi-faceted approach meets the priorities of the National Institutes of Health for rehabilitation.

SIGNIFICANCE

The seemingly simple tasks of standing and walking require continuous integration of complex spinal reflex circuits involving descending motor commands from the brain and ascending sensory input from the legs.[1] Importantly, these circuits are disrupted by upper motoneuron lesions such as spinal cord injury (SCI). SCI, in addition to the clinical sequelae of paresis and ambulatory dysfunction, manifests as defects in multiple neurophysiological biomarkers, including motor evoked potentials (MEPs). MEPs provide information on the integrity of synaptic connections between the brain and spinal cord. After SCI, MEPs have significantly delayed onsets, decreased amplitudes, and increased thresholds.[2,3] SCI also hinders the ability of descending volleys to summate at the spinal cord and limits the electrophysiological transmission of uninjured axons.[4,5] Furthermore, damage to spinothalamic and dorsal column circuits disrupts transmission from receptors registering muscle stretch and cutaneous sensation.[6] These disruptions in the function and neurotransmission of uninjured fibers clearly demonstrate that SCI induces pathophysiology in the central nervous system.[7-13] Consequently, *interventions that effectively promote appropriate neuromodulation of spinal locomotor networks and strengthen neural connectivity of the injured human spinal cord* are greatly needed.

In individuals with incomplete SCI (iSCI), locomotor training in known to promote reorganization of corticospinal and spinal neuronal circuits.[15-20] However, even multiple sessions of locomotor training do not fully prevent pathological muscle activity during stepping.[21,22] Thus, locomotor training alone cannot fully optimize the neuronal plasticity required to strengthen synapses connecting the brain, spinal cord, and the local circuits they form.

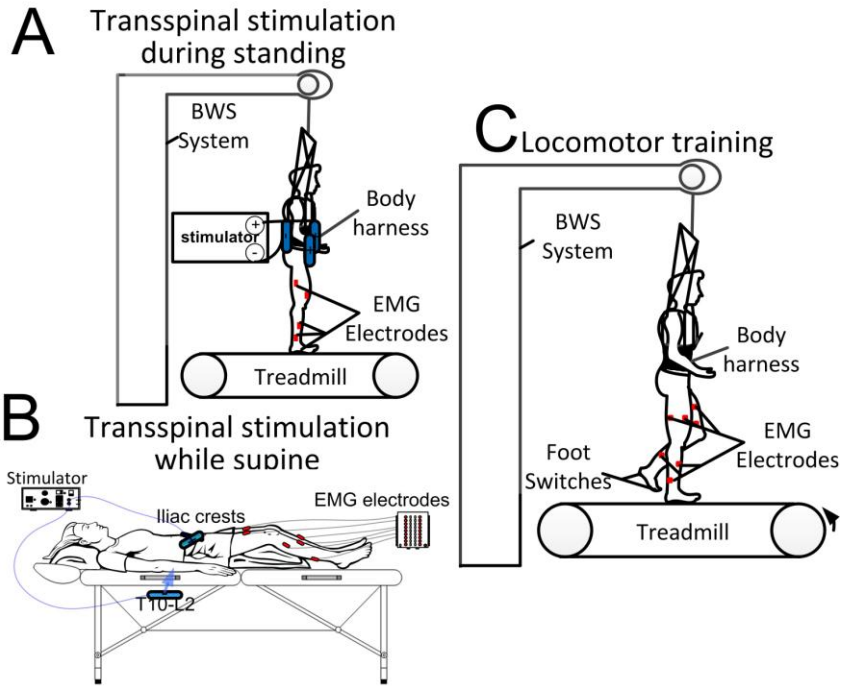


Fig. 1. Transspinal stimulation delivered during standing (A) and/or while supine (B) followed by locomotor training (C) within the same training session. EMG electrodes and foot switches are shown when recordings are taken at rest or during stepping.

One potential supplementary intervention is electrical stimulation of the nervous system. This approach has gained pronounced attention by the scientific community in the last two decades. Importantly, in individuals with complete SCI, epidural delivery of electrical current to the spinal cord promotes some recovery of standing and walking during or after treatment.[23-30] However, epidural stimulation is invasive and requires highly specialized teams, thereby limiting the number of patients that register for research trials and making translation to clinical rehabilitation centers difficult. Alternatively, noninvasive transspinal stimulation over the thoracolumbar enlargement can provide tonic excitatory inputs to spinal locomotor circuits. Such stimulation strengthens neural connectivity in a manner similar to that of epidural stimulation[31], and does not require complicated hardware or software arrangements. Thus, transspinal stimulation can be readily adapted in clinical rehabilitation settings.

The goal of this mechanistic clinical trial is to use noninvasive transspinal stimulation over the thoracolumbar enlargement, the spinal location of leg motor circuits, to prime locomotor training and improve recovery of standing and walking ability in individuals with chronic iSCI. To achieve this, we will randomly assign individuals with iSCI to receive a 40-session protocol involving high-frequency (30 Hz) transspinal stimulation for 30 min during standing (Fig. 1A) or while lying supine (Fig. 1B). This will be followed by followed by 30 min of locomotor training (Fig. 1C) within the same training session. In rehabilitation research, understanding the underlying **neuronal mechanisms of action of therapeutic interventions** is essential. This mechanistic clinical trial will provide a deeper understanding of how locomotor training primed with transspinal stimulation induces physiological and behavioral changes in neuronal circuits. Such understanding will enable the development of tailored neuromodulatory interventions, which are ideal to meet the specific needs of individuals with SCI.[32,33]

Potential targets of transspinal stimulation include proprioceptive afferents and spinal and brain neural circuits via dorsal column axons (Fig. 2). Our previous work confirms that transspinal stimulation-induced muscle responses (transspinal evoked potentials; TEPs) use the neuronal pathways that convey descending drive and sensory inputs from the periphery onto spinal neuronal circuits. Specifically, cortically-induced MEPs and spinally-induced TEPs, when evoked at specific times, summate at the muscle surface EMG.[34] This is a neural interaction that we recently demonstrated for the soleus H-reflex[35], providing strong evidence that transspinal stimulation induces neuromodulation. Indeed, transspinal stimulation clearly promotes neuroplasticity and motor function recovery, likely by activating spinal locomotor centers. This is supported by the step-like motor activity observed in individuals with SCI following transspinal stimulation when gravity is eliminated.[36-39] For example, in several cases of complete or iSCI, a single session of transspinal stimulation decreased spasticity, hyperreflexia, and ankle clonus, and increased leg muscle activity during stepping (see also preliminary studies).[40-42] While these studies did not examine reflex modulation, this proposed study will, specifically examining how transspinal stimulation confers benefits after SCI. We will further examine the effects of transspinal stimulation prior to locomotor training.

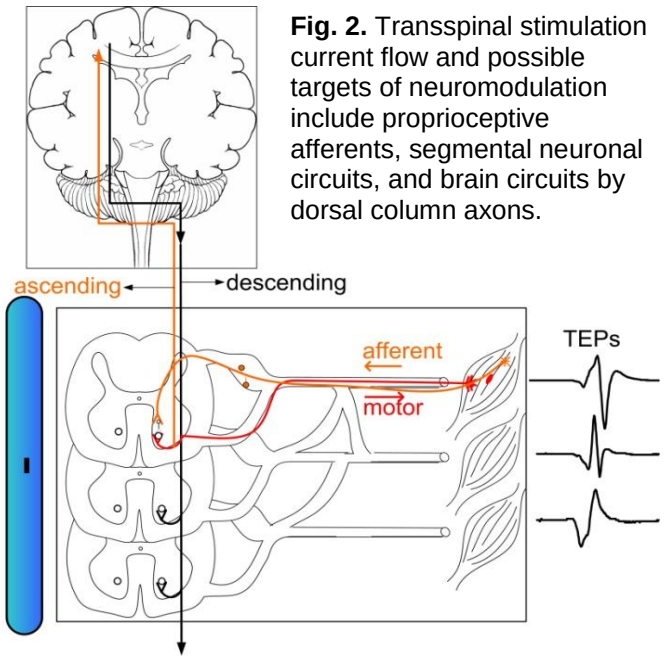


Fig. 2. Transspinal stimulation current flow and possible targets of neuromodulation include proprioceptive afferents, segmental neuronal circuits, and brain circuits by dorsal column axons.

During walking, appropriate engagement of corticospinal and spinal neuronal circuits at each step cycle phase is reflected by the phase-dependent amplitude modulation of the soleus H-reflex and MEPs (Fig. 3).[9,43-46] SCI disrupts soleus H-reflex modulation during walking (Fig. 3; orange line), varying from relatively normal in some patients to completely absent in others.[9,17] The most common change observed with locomotor training is partial return of soleus H-reflex depression during the swing phase; however, extensor motoneuron excitability during the stance phase remains unstable.[17] In addition, several MEP characteristics have been linked to walking disability[3,11,47] and locomotor recovery after SCI.[19,48-50] Consequently, the amplitude modulation of MEPs and the H-reflex during walking depict changes in both the pathological and reorganized behaviors of corticospinal and spinal neuronal circuits in individuals with SCI after therapeutic interventions.

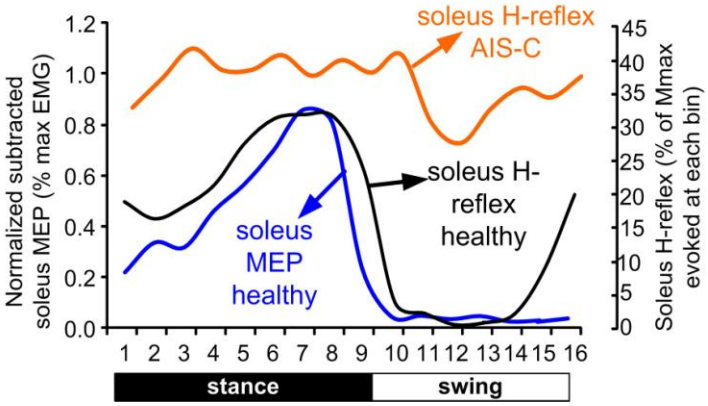


Fig. 3. Soleus H-reflex and MEP modulation during walking in individuals without SCI and in a group of AIS C. The soleus H-reflex and MEP are clearly modulated in a similar manner during walking, while the H-reflex is significantly impaired in AIS C SCI. Data adopted from PI's published works[9,17,43,44]

The primary goal of this proposal is to clearly demonstrate the neurophysiological changes in standing and walking ability in individuals with iSCI following high-frequency stimulation coupled with locomotor training. Over the course of 40 sessions, we will deliver high-frequency transspinal stimulation during standing to capitalize on the ability of injured spinal cord to process loading information.[51-53] To establish body-posture-dependent effects, we will compare the efficacy of this approach to high-frequency transspinal stimulation delivery with subjects lying supine. **Information gained from these experiments could greatly impact clinical practice,** mainly because noninvasive transspinal stimulation can be quickly implemented in real-world clinical settings to reduce motor dysfunction after SCI and other upper motoneuron lesions. Finally, this protocol represents approaches in **true clinical practice** by utilizing multiple interventions to accelerate functional recovery, which is consistent with the National Institutes of Health (NIH) priorities for rehabilitation.

INNOVATION

The use of noninvasive transspinal stimulation to prime locomotor training is a novel rehabilitation approach with significant clinical importance. Indeed, understanding how transspinal stimulation augments activity-based plasticity from neurophysiological and clinical perspectives is extremely important for the development of targeted and tailored neuromodulation interventions for SCI.

Neural circuitries and rhythmic motor behavior after SCI – Gait requires intrinsic spinal reflex circuits that control the rhythmic, alternating flexion and extension of each leg. These reflexes play critical roles in normal and pathological locomotor function and in recovery of walking ability after training in SCI.[54-56] SCI disrupts inhibitory spinal modulation following muscle stretch and cutaneous stimulation, leading to hyperreflexia. Furthermore, increased tone of anti-gravity muscles makes standing and walking difficult or even impossible. Persistent hyperreflexia also limits the benefits of rehabilitation and thus, motor recovery. For example, reduced H-reflex rate-dependent depression accounts for poor locomotor performance,[57,58] similar to disrupted inhibition between antagonistic muscles.[59,60] Alternatively, rehabilitation benefits are reflected as better transmission in spinal reflex pathways and central pattern generator circuits[56] and increases in corticospinal drive to spinal neurons.[61] *One notable innovation* of the proposed research is to examine how activity-based therapies affect descending and local circuitry changes in the same patients, directly addressing the lack of a single recovery outcome measure in individuals with SCI.[62]

Neural circuitries and dynamic balance – One of the key elements of locomotor control, which is greatly affected by SCI, is upright posture regulation.[63-68] During standing, local spinal inhibitory circuits continuously adjust the soleus H-reflex amplitude based on body sway amplitude.[69] H-reflex amplitude is directly related with postural instability and dynamic balance[70] and is susceptible to cortical control.[71,72] Specifically, upright balance control requires increased soleus MEPs and decreased soleus H-reflexes.[73,74] Recent data show that in persons with SCI, stand training with transspinal stimulation at varying frequencies (0.2 to 30 Hz) promotes self-assisted standing and upright trunk posture with minimal or no external assistance.[75,76] *One innovation* of the proposed research is that the effects of transspinal stimulation will be compared based on the degree of neural activity adjustment (standing vs. supine). Further, if we observe significant improvements in the supine position, this would indicate that clinical settings can utilize both body positions based on the availability of staff and recourses.

Transspinal stimulation and motor activity after SCI – After SCI, noninvasive transspinal stimulation over the thoracolumbar enlargement produces bilateral leg extension of paralyzed or paretic muscles similar to that observed in healthy humans (PI's personal observations). Multisite transspinal stimulation also produces step-like leg movements in non-injured subjects at 5-40 Hz when gravity is eliminated.[36,37,77] Alternatively, 30 Hz transspinal stimulation improves gait kinematics and locomotor EMG activity in subjects with SCI during weight-supported walking.[40] Thus, transspinal stimulation activates the neural circuits for standing and walking; however, the exact mechanism by which such stimulation affects these circuits when paired with step training is currently unknown and will be determined in this grant.

Multimodal rehabilitation strategies – Despite the realities of clinical practice and the NIH priorities, studies incorporating combined interventions are scare. Locomotor training has been coupled with transcranial or transspinal direct current stimulation, which improves walking speed and reduces spasticity[78,79] in manner similar to that after one session of transspinal stimulation, transcranial direct current stimulation, stretching, and cyclic movements.[80] After SCI, restoration of appropriate spinal reflex modulation and strengthening of corticomotoneuron synapses is critical for locomotor recovery. The endogenous activity-dependent plasticity of the neural circuits for standing and walking and their activation by transspinal stimulation invite the development of targeted therapeutic strategies. In this research project, we will evaluate how 40 sessions of transspinal stimulation coupled with locomotor training affect corticospinal and spinal reflex circuits. **We will determine the position-dependent effects of transspinal stimulation priming locomotor training. We will also evaluate the effects of this combined targeted therapeutic strategy from both neurophysiological and clinical perspectives**. Monitoring the impairment and recovery of reflex excitability and locomotor muscle activity in individuals with iSCI will reveal the potential to enhance the effects of locomotor training by tonic high-frequency transspinal stimulation. Ultimately, the novelty of this proposal lies in its unique, multidisciplinary approach involving several noninvasive therapeutic modalities that will hopefully restore standing and walking after paralysis.

APPROACH

PRELIMINARY STUDIES

Sustained transspinal stimulation alone restores spinal inhibition and decreases hyperreflexia in SCI – The soleus H-reflex is a neurophysiological biomarker for hyperreflexia and spasticity. After SCI, rehabilitative interventions seek to reduce excess H-reflex excitability and restore normal levels of H-reflex rate-dependent depression. Using our previously published protocol[81] we stimulated 6 people with iSCI using 10 sessions of low-frequency (0.2 Hz) transspinal stimulation delivered in the supine position. This regimen significantly increased soleus H-reflex rate-dependent depression in the less impaired left leg (Fig. 4A). In this leg, stimulation at 1.0 Hz promoted H-reflexes with amplitudes similar to those reported in healthy subjects (Fig. 4A). We also determined the H-reflex excitability in these patients using estimates from the recruitment curve. This revealed that stimulation significantly lowered H-reflex excitability in both legs (Fig. 4B). The decreased H-reflex amplitude coincided with decreased ankle clonus and reduced leg spasm frequency and severity. Polarizing current passing across the spinal cord produces membrane potential changes of muscle spindle afferents[82] and attenuates hypersensitivity by activating the dorsal columns.[83] Thus, the observed changes in spinal circuits may originate from modifications in the intrinsic properties and excitability of alpha motoneurons and muscle spindle afferents.

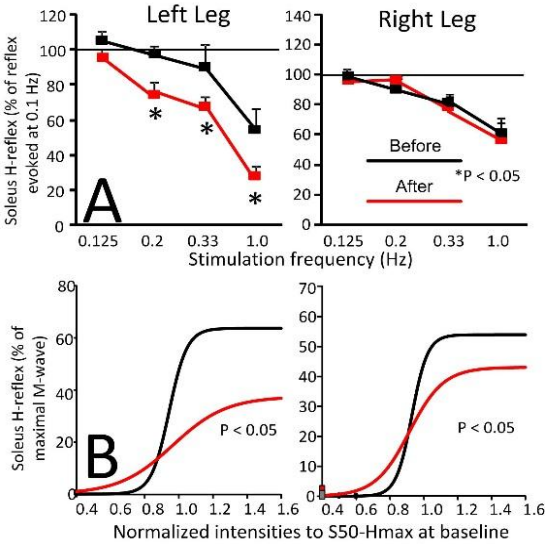


Fig. 4. Soleus H-reflex rate-dependent depression (A) and recruitment curves (B) before and after 10 sessions of transspinal stimulation in 6 persons with iSCI. Unpublished data.

Sustained transspinal stimulation alone increases motoneuron output of multiple segments in SCI – In SCI, a notable contradiction is that although hyperreflexia can produce significant muscle force output, it coincides with decreased motoneuronal excitability and volitional control.[84] The lack of neuromodulation continuously prevents motoneurons from firing at a frequency sufficient to generate volitional muscle contractions with sufficient force.

TEPs are easily recorded from all leg muscles in individuals with and without SCI. An example of TEPs recorded from an individual with iSCI (American Spinal Injury Association Impairment Scale [AIS] D) is indicated in Fig. 5A. Note that despite shape differences across muscles, TEPs recorded from knee muscles have shorter latencies than the more distal ankle muscles, consistent with our observations in individuals without SCI.[85,86] Our data indicate that TEP recruitment curves (common shape of TEP curves: gray box in Fig. 5) increase after 15 sessions of transspinal stimulation delivered supine to 6 people with iSCI for ankle and knee extensors (Fig. 5B).[87] These results strongly support our overall hypothesis that transspinal stimulation alters motoneuron excitability over multiple segments by bringing motoneurons closer to threshold, which is a pre-requisite for the integration of descending and local inputs to become functional.

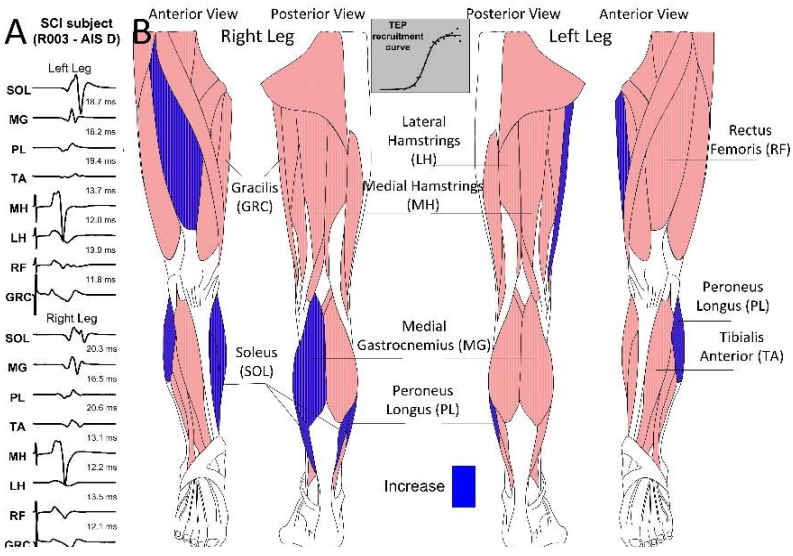


Fig. 5. (A). Representative TEPs recorded in one person with AIS-D SCI. **(B).** Increased motoneuron output is indicated as blue shaded area based on the TEP recruitment curves (example of TEP recruitment curve shown in grey box), assembled before and after 15 sessions of transspinal stimulation in 6 persons with AIS C-D SCI. Data submitted for publication, under review[87]

Transspinal stimulation alone strengthens corticospinal excitability – We previously showed that sustained transspinal stimulation over the cervicothoracic enlargement increases the corticospinal excitability of arm muscles after cervical SCI.[81] Such stimulation over the thoracolumbar enlargement also increases the corticospinal excitability of leg muscles after 1 session in 10 healthy control subjects.[86] We next wanted to confirm that sustained transspinal stimulation over the thoracolumbar enlargement strengthens corticomotoneuronal connections in leg muscles. To test this, we assembled the MEP recruitment curves recorded from the tibialis anterior (TA) muscle in 10 healthy control subjects while at rest before and 1 day after 10 sessions of transspinal stimulation. This demonstrated that transspinal stimulation significantly increased the TA MEP amplitude at intermediate and maximal intensities (Fig. 6). Increasing MEP amplitudes with increasing stimulation intensities (recruitment curve) are related to the strength of corticospinal projections[88] and are affected by the excitability of both cortical and spinal neurons.[89] Because MEPs and TEPs share common pathways,[34] it is possible that the observed changes result from non-Hebbian long-term potentiation of synaptic transmission.[90]

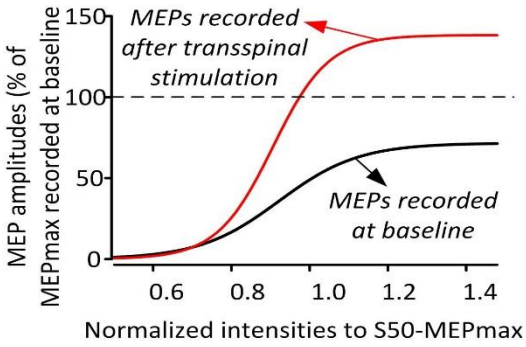


Fig. 6. TA MEP recruitment curves before and after 10 sessions of low-frequency transspinal stimulation in 10 healthy control subjects. Unpublished data.

Locomotor training alone restores spinal inhibition and decreases hyperreflexia in SCI – We previously showed that in 14 individuals with iSCI, locomotor training restored soleus H-reflex rate-dependent depression (Fig. 7A).[21] In 12 individuals with iSCI, locomotor training also restored soleus H-reflex modulation during body weight-supported (BWS)-assisted stepping (Fig. 7B).[17] Specifically, the soleus H-reflex was more depressed at late stance, allowing for easier transition from stance to swing. This improved H-reflex depression at the swing phase clearly resulted from reorganized reciprocal inhibition of ankle flexors onto ankle extensors. We wanted to confirm that locomotor training-mediated reflex recovery during stepping depended on the return of spinal inhibition. To test this, we designed experiments to assess changes of how reciprocal inhibition and Ib inhibition affected postsynaptic signaling and presynaptic inhibition on soleus Ia afferents.[15,21,22] These experiments confirmed that locomotor training in SCI promoted both spinal inhibitory circuit and spinal reflex excitability recovery. Together, this promoted more physiological intralimb muscle coordination as indicated by the L-shape of locomotor EMGs observed after training (Fig. 8).[21] We attributed these changes to plasticity of the glycinergic spinal inhibitory system that occurs independent of supraspinal influences.[91] This is supported by animal studies strongly indicating that locomotor training normalizes the proportion of excitatory and inhibitory synaptic inputs to spinal motoneurons,[92,93] improves synaptic inputs from Ia afferents,[94] and alters neurotransmitter concentration.[95]

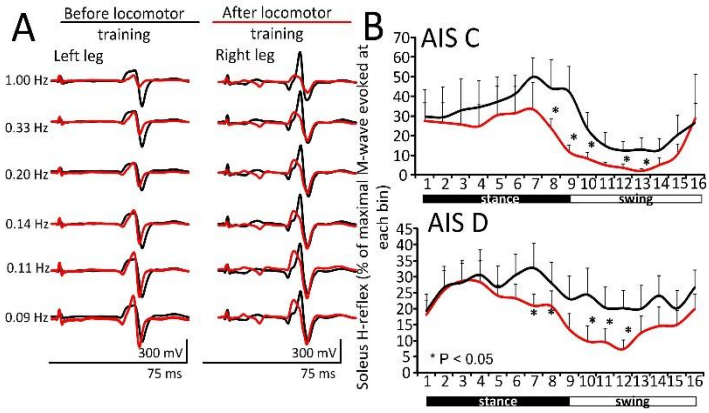


Fig. 7. Soleus H-reflex rate-dependent depression (A) and amplitude modulation during stepping (B) after locomotor training in iSCI. Data adopted from[17, 21]

Thus, we have comprehensive evidence from ongoing research studies that transspinal stimulation and locomotor training *alone* strengthen corticospinal and spinal neural connectivity, reduce hyperreflexia, and promote locomotor recovery. Based on these findings, we propose that priming locomotor training with transspinal stimulation will maximize improvements in standing and walking ability.

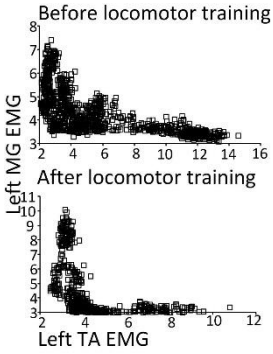


Fig. 8. EMG coordination between an ankle flexor and extensor muscle before and after locomotor training. Data adopted from[21]

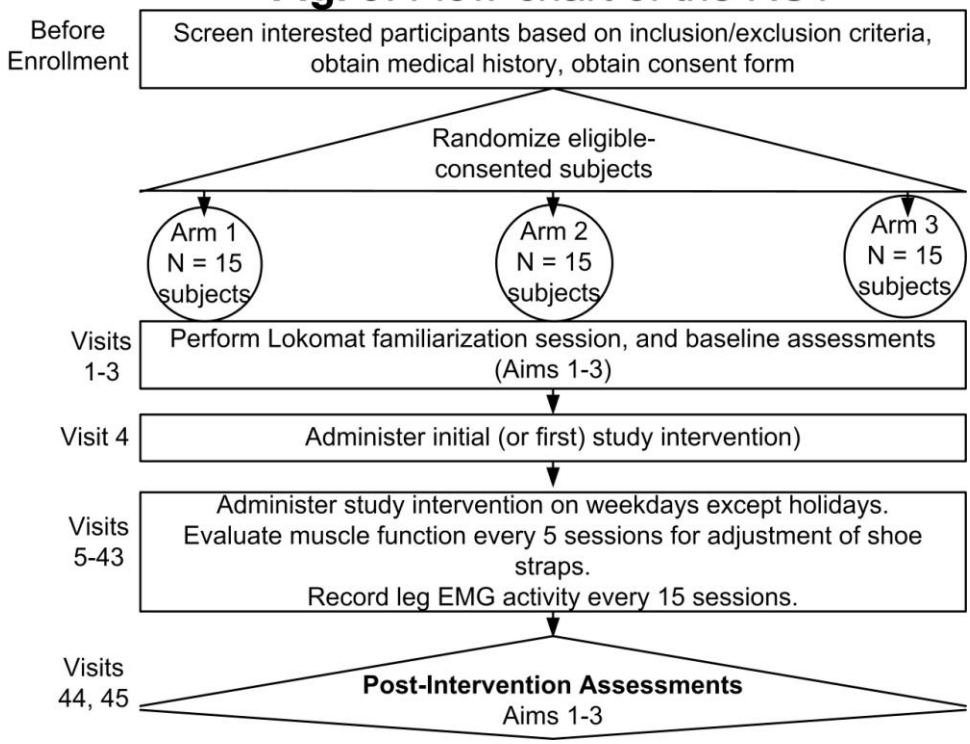
Based on the 20 years of the PI's and Co-PI's experimental work on human electrophysiology, SCI, human locomotion, management of clinical trials (3 completed, 2 currently active), the excellent

collaboration with the Co-PI, Noam Harel, MD, PhD, on a currently active randomized clinical trial (NCT03669302), we are highly capable of executing this research project.

SPECIFIC AIMS

Approach – This proposal is a mechanistic, sham-controlled, randomized, parallel design, multi-site, clinical trial comprised of three study groups/arms. 1: Real transspinal stimulation delivered during standing followed by locomotor training; 2: Real transspinal stimulation delivered while lying supine followed by locomotor training; and 3: Sham transspinal stimulation delivered during standing followed by locomotor training. The primary endpoints, which are linked to the specific aims, will independently evaluate corticomotoneuronal connectivity, spinal neuronal excitability and inhibition, and recovery of standing and walking ability. Together, these endpoints will indicate whether the reorganization of corticospinal and spinal neural activity depend on the patient's body posture during transspinal stimulation. Primary and secondary outcomes will not be analyzed based on ethnicity or SCI severity because AIS scale does not correlate with improvements in walking ability.[96] Gender, age, and baseline walking ability will be used separately as covariates.

Fig. 9. Flow-chart of the RCT



Participants – We anticipate that a total of 150 people with AIS C - D SCI will need to be screened to reach the target of 45 eligible participants of both genders and ages ranging 18-70 years. Eligible participants will be randomly assigned to one of the study groups, with 15 study members assigned to each group (Fig. 9). These group sizes were based on the assumption that 13 subjects are needed to establish significant differences before and after locomotor training in 1 out of the 3 primary outcomes. The basic inclusion/exclusion criteria are: ability to provide informed consent, chronic SCI, absent multiple injuries, absent traumatic brain injury, absent cauda equina lesion, injury level above T11, absent permanent ankle contractures, and absent pressure sores. We acknowledge the need for a control SCI group. We will draw data for the effects of locomotor training-only from our completed studies and our currently active randomized clinical trial (NCT03669302). Based on several discussions with current participants, it is likely that we will be unable to enroll persons with SCI for the stimulation-only intervention, as they are less likely to receive the same benefits as those receiving both transspinal stimulation and locomotor training. Thus, we will include only one sham group receiving transspinal stimulation during standing at an intensity that sensation is absent because cutaneous afferents affect spinal circuits in SCI.[7]

Therapeutic Intervention: Coupling Transspinal Stimulation & Locomotor Training – While participants are seated, the T10 spinous process will be identified via palpation and anatomical landmarks. Then, a single cathode electrode (Uni-Patch™, 10.2 cm × 5.1 cm, Wabasha, MA) will be placed along the vertebrae equally between the left and right paravertebral sides. Two interconnected electrodes (anode; same as the cathode), will be placed on the abdominal muscles or iliac crests, depending on self-reported levels of comfort. These electrodes will be connected to a constant current stimulator that will be triggered by Spike 2 scripts. Transspinal stimulation will initially be delivered as a single monophasic pulse of 1 ms. The intensities corresponding to the soleus TEP threshold and maximal amplitudes will be noted on the subject's data sheet. Soleus TEP amplitude will be assessed upon paired transspinal stimuli at 60-ms inter-stimulus interval to establish susceptibility to depression (Fig. 10). If TEP depression is not present upon paired transspinal

stimuli, the cathodal electrode will be adjusted. Consistent position of the cathodal transspinal electrode during the course of the intervention will be ensured by marking the area with a non-allergenic skin pen.

Transspinal tonic stimulation will be delivered at a frequency of 30 Hz (charge-balanced, symmetric, biphasic rectangular pulses of a 1-ms width per phase) for 30 min at 3 times the soleus TEP threshold.[97] Stimulation intensity will be lowered, if necessary, based on the subject's reported comfort. Transspinal stimulation intensity adjustments will be made only if the TEP threshold changes over the course of treatment. For participants receiving transspinal stimulation while lying supine, hips and knees will be placed in slight flexion and stabilized by holsters and towels to avoid external limb rotations as needed. Real transspinal stimulation at 30 Hz delivered during standi ng will be administered under BWS in a standing frame or in the Lokomat to ensure safety. The initial BWS will be equivalent to the one that knee buckling during standing is absent and will decrease over the training course to achieve full loading. Because prolonged standing may be demanding for individuals with SCI, a 2-min break will be given every 10 min of standing. Locomotor training following transspinal stimulation within the same session will also depend on the ability of each participant to step without foot dragging. Over the training course, we will use a clinical algorithm to adjust the BWS, leg guidance force, and treadmill speed.[17] Further, the positions of the ankle straps will be adjusted based on the right and left TA muscle strength as evaluated every five sessions.

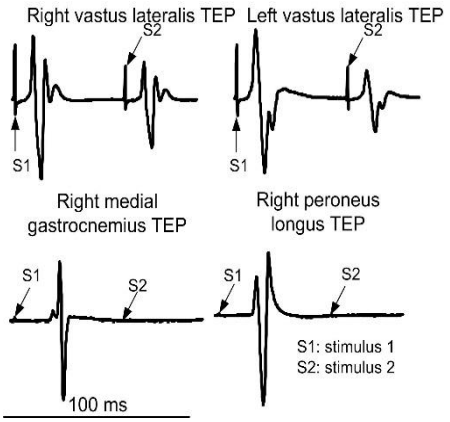


Fig. 10. Representative TEPs recorded from vastus lateralis muscles bilateral and from ankle muscles in one person with AIS D SCI upon paired transspinal stimuli at 60 ms inter-stimulus interval. Note that depression is larger in TEPs recorded from ankle muscles. Unpublished data.

Criteria for Selection of Therapeutic Intervention Parameters – In the literature, a consensus regarding the 'dosages' of locomotor training (intensity, volume, duration) has not been reached, with 20 - 120 sessions at varying intensities being reported.[98,99] Here, all participants will receive 40 sessions (weekdays, except holidays), because an average of 40 sessions results in significant neuromodulation of flexor and extensor reflex pathways.[14,15]

We will compare the delivery of transspinal stimulation during standing and while lying supine to evaluate body-posture dependent effects on therapeutic efficacy. The standing posture was selected because this position is actively maintained by the nervous system with small adjustments in the centers of pressure and mass[100] with tonic contractions of the calf muscles.[101] The injured spinal cord retains its ability to process loading information,[51,52,102] while standing provides sensory feedback that promotes coordinated hindlimb locomotion in the absence of training, drug application, or supraspinal influence.[53] SCI greatly affects trunk stability,[65-67,103] which is invaluable for locomotion and many daily living activities that are considered priorities by the SCI population.[104] Recent studies have shown that transspinal stimulation improves trunk control.[28,75,76] This motivated us to compare the effects of transspinal stimulation delivery during standing vs. lying supine. We will not deliver high-frequency transspinal stimulation during walking due to the possibility of blocking walking-related proprioceptive signals from reaching the spinal cord.[105]

Lastly, transspinal stimulation will be delivered at 30 Hz because this frequency facilitates voluntary movement, modulates spinal circuits, and produces rhythmic motor activity in both legs.[36,37,97] High-frequencies are known to increase neural excitability compared to low-frequencies that produce the opposite effects.[106]

We will deliver high-frequency transspinal stimulation to provide tonic excitatory inputs to spinal locomotor circuits and physiological neural signals from the periphery with locomotor training.

Feasibility of the Mechanistic Clinical Trial – A potential concern of the proposed experiments is the prolonged duration of the study, as each participant will need to come to the lab 45 times. Prolonged study participation may yield a high attrition rate. To counteract a 20% dropout rate, we included an additional two subjects to the predicted sample sized based on the statistical analysis of our preliminary data. Based on the PI's and Co-PI's extensive experience on randomized clinical trials (3 completed and 2 currently active), and our current 100% attendance rate, we do not foresee problems regarding participant withdrawal due to the study duration. Each participant will require ~2 months to complete the study. Further, the experimental procedures will bear, at most, moderate discomfort. Even at high intensities, transspinal stimulation does not

induce pain, alter blood pressure, nor induce skin irritation.[81] To ensure successful completion of the project within five years, we will deposit the protocol in Clinicaltrials.gov and begin recruitment immediately following grant award. We will enroll 1-2 persons concomitantly with SCI at both sites (College of Staten Island/CUNY and Bronx Veterans Medical Research Foundation), and thus, complete 11 people in YR01, 12 in YR02, 11 in YR03, and 11 in YR04. Data analysis and manuscript preparation for publication will occur during YR05.

Aim 1: Determine if priming locomotor training with transspinal stimulation increases MEP amplitudes and promotes appropriate MEP amplitude modulation during stepping.

Rationale – We will evaluate how priming locomotor training with high-frequency transspinal stimulation in iSCI alters corticomotoneuronal connectivity strength, as indicated by MEPs recorded from the legs (No. 1 circuit in Fig. 11). Specifically, MEP amplitude reflects the integrity of the corticospinal tract and excitability of cortical and spinal motoneurons.[107] Transspinal stimulation can strengthen corticomotoneuron connectivity, as indicated by the marked increase in maximal MEP (MEP_{max}) amplitudes following sustained transspinal stimulation. Both an increased MEP_{max} amplitude at rest and more physiological MEP amplitude modulation during assisted stepping will clearly demonstrate improved synchronization of descending volleys and/or responsiveness of motoneurons to descending inputs, which are directly linked to physiological locomotor function.

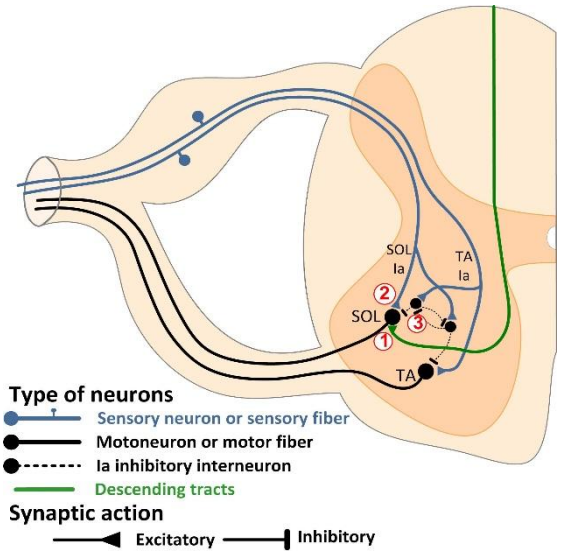


Fig. 11. The neuronal circuits investigated in this proposal are: **1**: corticomotoneuronal connections, **2**: soleus H-reflex rate-dependent depression exerted at Ia/motoneuron synapse, **3**: reciprocal inhibition exerted from ankle flexor group I afferents onto soleus motoneurons.

Power Analysis – We established the effect size[108] based on changes in the TA MEP_{max} amplitudes recorded in 10 healthy subjects while at rest before and after 10 sessions of transspinal stimulation delivered with subjects lying supine (Fig. 6). Using a paired t-test, we calculated that for an effect size of 0.8 (α = 0.05), 10 subjects are needed to provide a large effect size and detect a significant difference at baseline and post-intervention.

Experimental Methods and Data Reduction – Participants will have EMG electrodes placed on the TA, soleus, medial gastrocnemius (MG), and peroneus longus (PL) muscles. With participants in a seated position, single transcranial magnetic stimulation (TMS) pulses (0.1 Hz) will be delivered with a double-cone magnetic coil over the left primary motor cortex. This coil activates deep structures of leg cortical representation.[109] The magnetic coil will be placed 1 cm lateral and posterior from Cz, and the most optimal position will be established as the point that evokes selectively larger MEPs in the right soleus muscle. Then, the MEP threshold will be established and will correspond to the intensity that evokes 10 responses of at least 50 μV following 20 1-ms TMS pulses.

We will assemble the soleus MEP recruitment input-output curves with subjects seated while maintaining small-amplitude contractions. During assisted stepping, MEPs can be reliably recorded across different days.[44] Soleus MEPs will be recorded at 1.3 times the soleus MEP active threshold randomly at different phases of the step cycle (divided into 16 equal bins, or time windows) based on the signal from the right foot switch.[44] During stepping, MEPs will be collected prior to (baseline) and following (post) training using Lokomat settings matched to the baseline and those of the last training session. This will establish changes at matched settings and adaptations during more demanding stepping conditions.

MEPs recorded at different stimulation intensities (recruitment curve) will be measured as peak-to-peak amplitude and normalized to the soleus maximal M-wave (M_{max}) evoked by tibial nerve stimulation. This will counteract differences in muscle fiber composition across subjects. Stimulation intensities will be normalized to the intensity corresponding to the 50% of the MEP_{max} amplitude, as estimated based on the sigmoid function fit to the data.[81,110] Normalized MEPs will be plotted against the normalized intensities and grouped based on time of testing. During stepping, MEPs will be measured as linear EMG envelopes starting at 25 ms post-TMS for 60-ms intervals and subtracted by the control EMG. The resulting subtracted MEPs at each bin of the step cycle will be normalized to the maximal soleus locomotor EMG activity.[44] MEPs will be grouped based on bin number, time of testing, and subject group. The *main outcomes* from this experiment will be the MEP_{max} from the MEP recruitment curves and the MEP amplitudes at the mid-stance and mid-swing phases.

Statistical Analysis and Expected Outcomes – We will use a repeated measures analysis of covariance (ANCOVA) along with Bonferroni *post-hoc* t-tests (α = 0.05) at 2 x 3 levels (2: time, 3: groups) to test the main and interaction effects among subject groups with gender or age as covariates. We hypothesize that **H1.1**) transspinal stimulation delivered during standing will increase MEP_{max} amplitudes and promote appropriate MEP amplitude modulation during stepping. Furthermore, we hypothesize that **H1.2**) transspinal stimulation delivered in supine position will increase MEP_{max} amplitudes, but not induce circuit reorganization. We expect that in the transspinal-standing group, soleus MEPs at the mid-stance and mid-swing phases will show a 30% change from baseline. These findings will provide direct evidence that priming locomotor training with high-frequency transspinal stimulation strengthens and alters corticomotoneuronal connectivity.

Challenges and Alternative Strategies – It is possible that MEPs will only be evocable at stimulation intensities close to 100% of the maximum stimulation. MEP amplitudes may also be so low that they cannot be separated from background EMG activity. In this case, we will measure the soleus H-reflex amplitude following TMS at an intensity that does not directly activate the corticomotoneuron system (i.e. subthreshold to the MEP) according to methods we have previously utilized.[111] Descending subthreshold TMS volleys depolarize spinal motoneurons. This conditioning paradigm increases responsiveness of spinal motoneurons when afferent volleys arrive simultaneously or shortly after TMS-evoked descending motor volleys.[112] We will record TMS-dependent H-reflex modulation at rest at inter-stimulus intervals ranging from negative 5 to positive 20 ms to evaluate the time course of TMS conditioning on soleus H-reflex.[113,114] H-reflexes will be evoked according to the methods described in Aim 2.

Aim 2: Determine if priming locomotor training with transspinal stimulation restores spinal inhibition, reduces hyperreflexia, and re-establishes physiological H-reflex modulation during stepping.

Rationale – We will evaluate how priming locomotor training with high-frequency transspinal stimulation in iSCI affects reorganization and appropriate engagement of spinal neuronal circuits. SCI reduces spinal inhibition, alters the adjustment of ankle extensor activity during standing, and disrupts soleus H-reflex modulation during stepping.[8,9,53,115-117] Together, these changes result in poor standing and walking performance. Thus, the return of spinal inhibition and physiological soleus H-reflex amplitude modulation during stepping will clearly indicate reorganization of spinal neuronal circuits and the appropriate recruitment of motoneurons. These changes are directly linked to improved neural control of posture and locomotor function.[118-120] In this aim, we will perform three experiments on the same day to evaluate changes in the complex interconnected circuitry underlying neural control of standing and walking.[1] These experiments involve soleus H-reflex rate-dependent depression with changes occurring at Ia afferent/motoneuron synapses (No. 2 circuit in Fig. 11), motoneuron excitability via H-reflex recordings during stepping, and reciprocal inhibition exerted on soleus motoneurons (No. 3 circuit in Fig. 11) at rest and during stepping.

Power Analysis – We established the number of subjects based on a large Cohen's effect size[108] via a t-test comparing pre- and post-intervention values. Previously, we performed experiments evaluating the soleus H-reflex 1/10 s ratio in 6 persons with SCI recorded from the left leg before and after 15 sessions of transspinal stimulation delivered lying supine (preliminary data presented in Fig. 4). Using a paired t-test, we calculated that for an effect size of 0.8, 11 subjects are needed to obtain significant differences in the soleus H-reflex 1/10 s ratio between post-intervention and baseline values. We also performed a similar analysis for our previous experiments evaluating soleus H-reflex amplitudes at the mid-stance and mid-swing phases in 11 AIS C-D patients before and after locomotor training.[17] Using a paired t-test, we calculated that for an effect size of 0.8, 11 subjects are needed to establish a significant difference between pre- and post- intervention. Lastly, based on the amplitude of reciprocal inhibition before and after locomotor training in 13 persons with AIS C-D SCI,[22] 13 subjects provide a large effect size of 0.98.

Experimental Methods and Data Reduction – We are one of the few internationally recognized groups with extensive experience recording H-reflexes at rest and during walking. These experiments probe elements of spinal circuits in neurologically impaired patients.[8,9,17,21,121] We will adapt these previously published methods in this grant. Briefly, the soleus H-reflex that will be evoked on the ascending portion of the recruitment curve will be 20-30% of the soleus M_{max}. The soleus M-wave will also be monitored online to ensure that is similar under all recordings conditions. Control and conditioned soleus H-reflexes will be accepted when the M-wave ranges from 4-8% of the soleus M_{max}. We will use an ANCOVA along with Bonferroni *post-hoc* t-tests (α = 0.05) at 2 x 3 levels (2: time, 3: groups) to determine the locus of significant differences (α <0.05) for main and

interaction effects among subject groups. In addition to our main effects, we will include separately gender and baseline walking function as covariates.[50,122]

Experiment 1: *Soleus H-reflex rate-dependent depression:* To examine restoration of homosynaptic depression exerted at the Ia/motoneuron synapse, soleus H-reflexes following tibial nerve stimulation with a 1-ms pulse will be recorded from subjects seated at inter-pulse intervals of 1, 3, 5, 8, and 10 s. Homosynaptic depression is greatest at 1 s and fully recovers at inter-pulse intervals of 8 or 10 s.[123] Homosynaptic depression cannot be recorded during stepping because it is abolished by strong spinal inhibitory circuits and muscle contraction. The main outcome measure will be the H-reflex 1/10 s ratio.[124] Using a linear mixed model, we expect **H2.1)** a 30% decrease in the 1/10s ratio post-intervention compared to baseline in the transspinal-standing group, as indicated by a return of rate-dependent reflex depression.

Experiment 2: *Soleus H-reflex depression by antagonistic nerve stimulation:* To examine restoration of reciprocal inhibitory actions on antagonistic alpha motoneurons, the soleus H-reflex will be conditioned by common peroneal nerve (CPN) stimulation at conditioning-test (C-T) intervals of 0-4 ms, with subjects seated.[59,111,121,125] We will ensure constant CPN conditioning stimulation using a stable, small-amplitude TA M-wave. The C-T interval for which the reciprocal inhibition is present or the reciprocal facilitation is small will be further investigated during stepping. The main outcome will be the conditioned soleus H-reflex amplitude normalized to the control H-reflex. Using a linear mixed model, we predict that **H2.2**) the depth of reciprocal inhibition will increase by at least 50% post-intervention compared to baseline in the transspinal-standing group, as indicated by a return of reciprocal inhibition.

Experiment 3*: Control and conditioned soleus H-reflexes during stepping*: During the same session, each participant will be transferred to standing while wearing an upper body harness connected to overhead pulleys. During standing with BWS as necessary, the soleus H-reflex and M-wave recruitment curves will be assembled. Then, each participant will step with the assistance of the Lokomat, and we will record the soleus H-reflexes under control conditions and following CPN conditioning stimulation randomly across 16 equal time bins into which each step cycle is divided. The tibial nerve stimulation intensity will be adjusted in real-time online to evoke H-reflexes such that their corresponding M-waves are 2-8 % of the M_{max} evoked 80 ms after the H-reflex at each bin (Fig. 12). Control and conditioned soleus H-reflexes during stepping will be collected prior to (baseline) and following (post) training at Lokomat settings matched to baseline and the last training session. This will establish changes at matched settings and reflex adaptation at more demanding settings.[126]

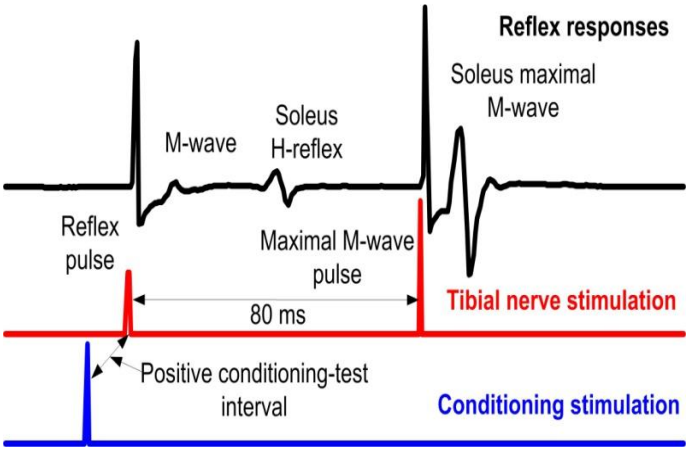


Fig. 12. Arrangement of stimuli during stepping. EMG channel, along with reflex and conditioning stimuli are shown as detected in Labview data acquisition software. Tibial nerve along with conditioning stimulation (that can be directed to M1, skin, or peripheral nerve as single pulses or pulse trains at variable frequencies) are indicated for an interval that can be adjusted based on the experimental protocol. Through foot switches (not shown) we determine the exact phase that stimulation occurs, i.e. the exact bin. Stimulation can be delivered randomly across the 16 bins that make up each step cycle.

During stepping, H-reflexes will be measured as peak-to-peak amplitude, accepted for M-waves ranging from 2-8% of the M_{max}, normalized to the M_{max}, and averaged for each bin of the step cycle. The main outcome will be the control and conditioned soleus H-reflex amplitude normalized to the M_{max} at mid-stance and mid-swing phases. We hypothesize that **H2.3**) the soleus H-reflex will be equal to 50% and 2% of M_{max} at the mid-stance and mid-swing phases, respectively. Furthermore, the depth of antagonistic inhibitory reflex actions will recover more in the transspinal-standing than in the transspinal-supine one.

Challenges and Alternative Strategies – Changes in synaptic transmission involving interneurons in polysynaptic pathways can affect the soleus H-reflex amplitude. These interneurons act on afferent axon terminals (i.e. presynaptic) or alpha motoneurons (i.e. postsynaptic). Our approach cannot depict the altered function of the numerous local spinal circuits that coordinate reflex responses linked directly to standing and walking.[1] Many local spinal circuits will likely undergo reorganization following locomotor training coupled with high-frequency transspinal stimulation. However, assessing the function of each element of these local circuits

requires that baseline and post-training experiments last two weeks each, which will be extremely tiring for the participants. These local spinal circuits, including loading pathways, ongoing presynaptic inhibition, and cross-inhibitory actions involving commissural interneurons, will all be established via conditioning H-reflex protocols.[1,54,127,128] They will be assessed in a subsequent mechanistic clinical trial focused on changes of pre- and postsynaptic motoneuronal control following locomotor training coupled with high-frequency transspinal stimulation.

Aim 3: Determine if priming locomotor training with high-frequency transspinal stimulation improves limb coordination and the ability to stand and walk.

LIMB COORDINATION

Rationale – Functional rhythmic alternating activity during human locomotion can be described in terms of a small set of motor modules.[129,130] Motor modules can be viewed as the temporal and spatial components of muscle synergies, represented as the EMG activities of many muscles simultaneously.[131,132] These modules correspond with the concepts of functional reflexes, while some elements of modularity conform to central pattern generator models.[129,133] Thus, these modules likely originate from the spinal cord. In upper motoneuron lesions, these modules change in organization and number,[134] which reflects abnormal coordination. Furthermore, the number of modules based on muscle activity decreases with an increasing degree of impairment.[135]

Power Analysis – This sub-aim is exploratory and thus the study is not powered based on these outcomes.

Methods – Activity during stepping will be collected with surface EMG electrodes from the left and right rectus femoris, vastus lateralis, gracilis magnus, medial hamstrings, TA, soleus, PL, and medial gastrocnemius muscles at 1 kHz along with signals from foot switches.

Data Analysis and Outcome Measures – Locomotor EMG activity will be collected to establish motor module rearrangement after training. EMG signals will be full-wave rectified, band-pass filtered, normalized to the homonymous maximal EMG, and plotted against the step cycle. Intralimb coordination will be determined by locomotor EMG distribution plots of agonist-antagonist muscles for each leg.[21] Co-contraction between the antagonistic muscles of the ankle and knee will be established via coactivation indices.[21] We will use a non-negative matrix factorization algorithm[136] and principal component analysis (Fig. 13) to extract patterns of activity or motor modules during different phases of a step cycle. A 90 % variance will be used for each muscle to quantify the number of modules needed to characterize an individual's gait.

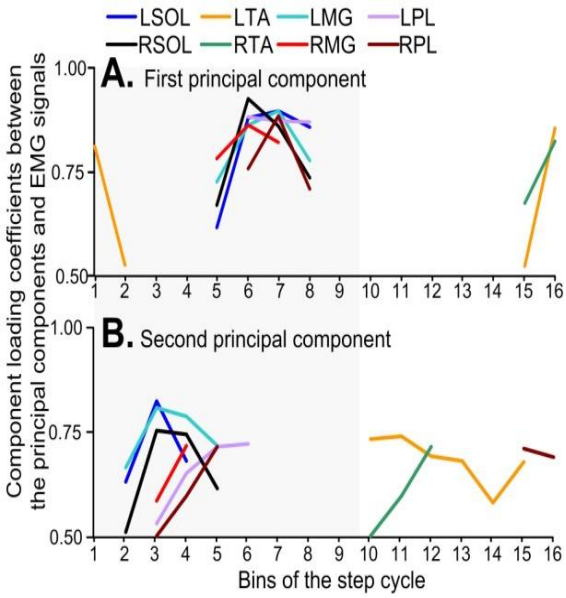


Fig. 13. Principal component analysis of EMGs recorded during walking in 13 healthy subjects. Note that motor modules during walking manifest in a similar pattern to the soleus H-reflex modulation. L: left, R: right, SOL: soleus, TA: tibialis anterior, MG: medial gastrocnemius, PL: peroneus longus Unpublished data.

Statistical Analyses and Hypotheses – We will use Pearson's correlation coefficient to evaluate the similarity of motor modules between groups. We hypothesize that ***H3.1)*** the number of motor modules will increase in the transspinal-standing group compared to transspinal-supine, indicating the engagement of spinal neuronal networks that were previously silent. We further hypothesize that ***H3.2)*** there will be significant changes in muscle activation patterns with flexor activity that coincides with extensor EMG suppression, improving intralimb coordination.

Expected Outcomes – We expect that improvements in motor coordination will be greater when transspinal stimulation is delivered during standing.

Challenges and Alternative Strategies – A common problem with measuring motor modules through non-negative matrix factorization or principal component analysis is that motor modules cannot precisely determine the altered neuronal mechanism. Thus, the recordings and analysis described in this section are independent form our experiments performed in Aim 2. Although unexpected, if Aim 2 shows persistent abnormal reciprocal

inhibition and H-reflex modulation during stepping in all subject groups, we will test for correlations between motor modules and spinal inhibition. Further, if the number of motor modules is consistent after training, correlations between the activation EMG profiles will be determined rather than the module composition. These changes will then be associated with walking ability at baseline and post-intervention.

ABILITY TO STAND AND WALK

Rationale – Our treatment intervention is based on fundamental principles underlying the neurophysiology of motor control and an in-depth understanding of effective neuromodulation strategies for rehabilitation. We will establish the benefits of this intervention through sophisticated neurophysiological and data analytical methods. However, we believe that in this mechanistic clinical trial, determining recovery of standing and walking is equally important. We will do so via well-established clinical tests using NIH common data elements. This will enable us to establish relationships between neurophysiological and clinical outcomes, thereby bridging the gap between scientists and therapists and making our intervention readily known to therapists who manage rehabilitation of this patient population.

Power Analysis – These are secondary endpoints; therefore the study is not powered based on these outcomes.

Methods – We will assess standing ability with the Berg Balance Scale (BBS) and static and dynamic stability with the BESTtest .[137,138] We will assess walking capacity with the 2-min and 10-meter walking tests.[139]

Data Analysis and Outcome Measures – The mean score of each test from each subject will be grouped based on time.

Statistical Analyses and Hypotheses – We will use linear mixed models followed by Bonferroni *post hoc* tests (α <0.05) for within-group and between-group comparisons for each score. We will also examine the relationship between neurophysiological changes and improvements in standing and walking ability. Rates of change in MEP_{max} and H-reflex 1/10 s will be correlated with the rate of change in 2-min and 10-meter walks. We *hypothesize* that ***H3.3***) both groups will show improved walking ability, but only the transspinal-standing one will have improved dynamic balance.

Expected Outcomes – We expect that both groups will have improved 2-min and 10-meter scores, while only the transspinal-standing group will have improved BBS and BESTtest scores.

Challenges and Alternative Strategies – Lack of improvements in standing and walking ability may not coincide with neuroplasticity and reflex reorganization. This would be completely unexpected[140]; however, if true, we will establish whether the lack of improvements correlates with lower extremity motor scores (LEMS). This is because LEMS is prognostic of walking ability.[141,142]

	AU 19	SP 20	SU 20	AU 20	SP 21	SU 21	AU 21	SP 22	SU 22	AU 22	SP 23	SU 23	AU 23	SP 24	SU 24
Aim 1: Corticospinal Circuits Plasticity															
Subject recruitment	■	■	■	■	■	■	■	■	■	■	■	■			
Experiments		■		■		■		■		■		■		■	
Training			■		■		■		■		■		■		
Analyze Data				■		■		■		■		■			
Writing												■	■	■	■
Aim 2: Spinal Circuits Plasticity															
Subject recruitment	■	■	■	■	■	■	■	■	■	■	■	■			
Experiments		■		■		■		■		■		■		■	
Training			■		■		■		■		■		■		
Analyze Data				■		■		■		■		■			
Writing												■	■	■	■
Aim 3: Locomotor EMG and standing-walking function															
Subject recruitment	■	■	■	■	■	■	■	■	■	■	■	■			
Experiments		■		■		■		■		■		■		■	
Training			■		■		■		■		■		■		
Analyze Data				■		■		■		■		■			
Writing												■	■	■	■

TIMELINE

The aims of this grant proposal are projected to be completed according to the timeline indicated in the Table. Four months are allocated for each AU: autumn, SP: spring and SU: summer. Subject recruitment, and experiments-training sessions will be continuous during the first 4 years of the grant.