Official title:
Cervical and Lumbosacral Transspinal Stimulation to Reconnect the Injured Human Spinal Cord

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Study Protocol

Determine if cervical and lumbosacral transspinal stimulation reconnects the injured human spinal cord, strengthens the weak remnant neuronal pathways, and augments the benefits of locomotor training.

We will perform a pilot clinical trial on paired cervical and lumbar transspinal alone at rest or during locomotor training in people with SCI to establish safety based on adverse events, and efficacy based on clinical assessments and neurophysiological biomarkers. We *hypothesize* that cervical and lumbosacral transspinal stimulation administered alone at rest improves non-somatic functions and decreases spasticity, but interlimb coordination along with walking performance improves more in the group that receives cervical and lumbosacral transspinal stimulation during assisted stepping.

Participants

A total of 24 people with incomplete SCI (AIS B, C, D) will be enrolled to participate and will randomly be assigned to receive cervical and lumbosacral transspinal stimulation alone at rest or during assisted stepping.

Intervention

Cervical and lumbosacral transspinal tonic stimulation (Figure 1) will be delivered at a frequency of 30 Hz (charge-balanced, symmetric, biphasic rectangular pulses of a 1-ms width per phase; DS8R, Digitimer Ltd., UK) at paresthesia levels or higher depending on each subject's comfort while supine. This group will provide evidence on changes of neurophysiological and clinical outcomes attributed solely to cervical and lumbosacral transspinal stimulation.

Another group will receive cervical and lumbosacral transspinal tonic stimulation at 30 Hz during assisted stepping with the Lokomat 6 Pro ®. In both groups, the soleus TEP threshold will be tracked over the course of treatment, as this relates to responsiveness of spinal motoneurons.

Locomotor training will depend on the ability of each participant to

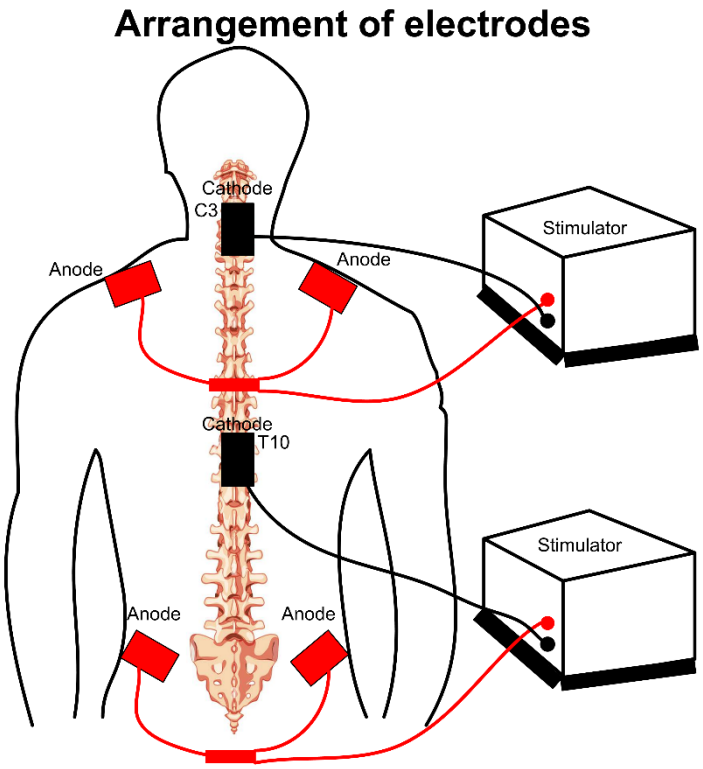

Figure 1. *Schematic illustration of the surface electrodes for cervical and thoracolumbar transspinal stimulation. Active electrodes are placed on the cervical and thoracolumbar spinal cord and reference electrodes on the clavicles and iliac crests.*

step without foot dragging. Over the training course, we will use the chart in Figure 2 to adjust body weight support, ankle straps position, and leg guidance force (Knikou 2013). Specifically, the tension of the ankle straps will be adjusted based on the right and left tibialis anterior muscle strength evaluated every 2 weeks. Body weight support and leg guidance force will be adjusted based on presence or absence of knee buckling during standing. Body weight support, ankle straps position, and leg guidance force may affect both neurophysiological biomarkers and clinical outcomes, but they will be individualized as is the case during outpatient rehabilitation.

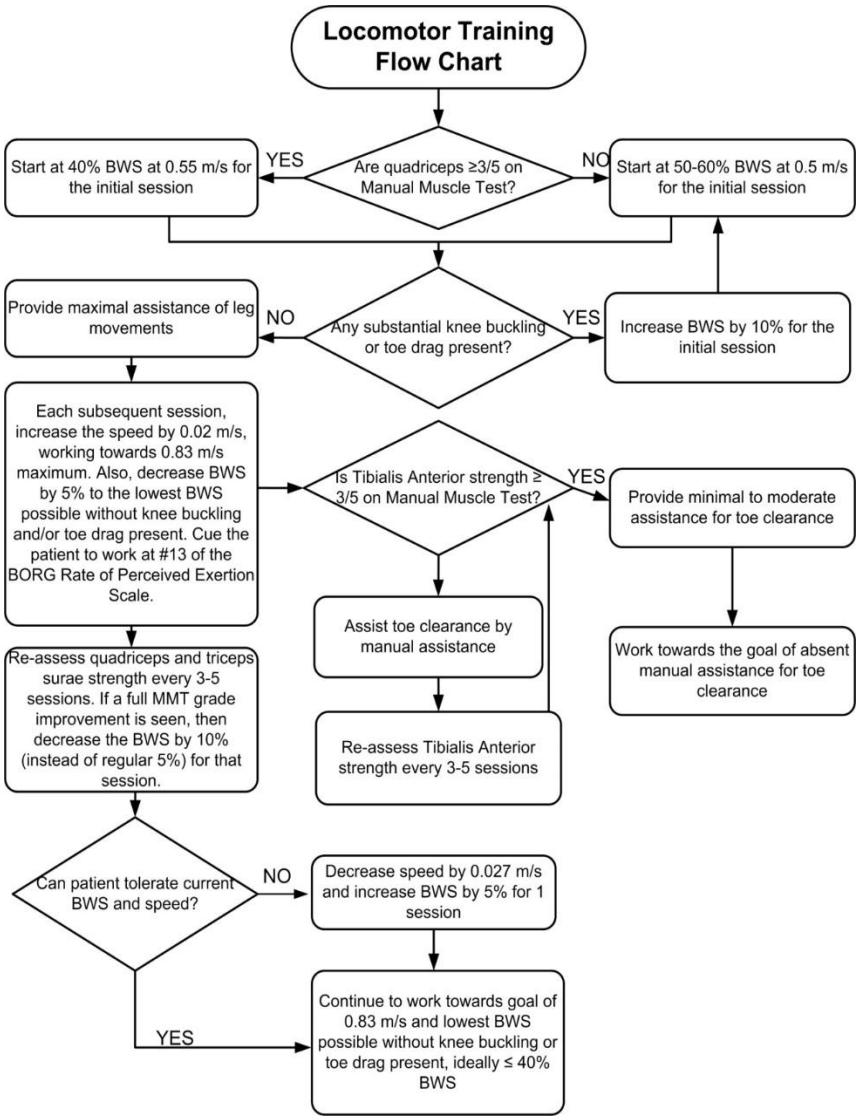

Figure 2. Locomotor training progression protocol*. The training protocol considers factors that are used to determine decreases in body weight support (BWS), increases in treadmill speed, and position of toe straps of the ankle braces over the course of the BWS robotic-assisted step training.*

Criteria for Selection of Therapeutic Intervention Parameters

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 (Gerasimenko et al. 2015; Danner et al. 2015). Medium frequencies increase neural excitability

compared to low frequencies that produce the opposite effects. Recent studies have shown that transspinal stimulation improves trunk control, likely because of the bilateral leg extension produced at higher intensities (Sayenko et al. 2018 and personal observations). This motivated us to use 30 Hz for transspinal stimulation. There is a possibility that transspinal stimulation delivered during assisted stepping blocks walking-related proprioceptive signals from reaching the spinal cord (Formento et al. 2018; Knikou and Murray 2018). However, only when cervical and lumbosacral transspinal stimulation is delivered during stepping we will be able to establish if this stimulation targets neurons connecting the enlargements of the spinal cord. A consensus regarding the 'dosage' of locomotor training (intensity, volume, duration) has not been reached, with 20 - 120 sessions being reported (Knikou 2013; Morrison et al. 2018). Each SCI participant enrolled in the pilot clinical trial will receive 5 sessions/week because it is superior to 2 or 3 sessions/week (Deng et al. 2019; Zhao et al. 2022).

Neurophysiological Biomarkers Before and After Intervention

For both groups we will perform state-of-the-art experiments to establish changes in neurophysiological biomarkers.

General Experimental Procedures

Electromyographic (EMG) recordings: Following standard skin preparation, single differential bipolar surface EMG electrodes (Motion Lab Systems Inc., Baton Rouge, LA, USA) will be placed bilaterally on the flexor carpi radialis (FCR), extensor carpi radials (ECR), biceps brachii (BIC), triceps brachii (TRIC), soleus (SOL), tibialis anterior (TA), rectus femoris (RF), vastus lateralis (VL), and semitendinosus (ST) muscles. EMG electrodes will be secured with 3M Tegaderm transparent film (3M, St. Paul, MN, USA). All EMG signals will be filtered with a cut-off frequency of 20 - 1000 Hz (1401 plus running Spike 2; Cambridge Electronic Design, Cambridge, UK) and sampled at 2000 Hz.

Transspinal stimulation: We will use our standard methods for transspinal stimulation (Einhorn et al. 2013; Knikou 2014; Murray and Knikou 2017, 2019; Knikou and Murray 2019; Pulverenti et al. 2022a, 2022b; Skiadopoulos et al. 2022). For cervical transspinal stimulation, a self-adhesive electrode (Uni-Patch^{TM}, 10.2 x 5.1 cm^2, Wabasha, MA, USA), will be placed over Cervical 3 - Thoracic 1 and two electrodes (anode; same type and size as the cathode) connected to act as one will be placed on each clavicle. For lumbosacral transspinal stimulation, a self-adhesive electrode (Uni-Patch^{TM}, 10.2 x 5.1 cm^2, Wabasha, MA, USA), will be placed over Thoracic 10 to Lumbar 1/2 and two electrodes connected to act as one (anode) will be placed on each iliac crest. For cervical or lumbosacral single pulse transspinal stimulation we will use a DS7A or DS7AH stimulator (Digitimer, Hertfordshire, UK).

Posterior tibial nerve stimulation for soleus H-reflex: With subjects seated and both feet supported by a footrest, a stainless-steel plate of 4 cm^2 in diameter (anode electrode) will be secured approximately 1 cm proximally to the patella. The optimal stimulation site of the right posterior tibial nerve will be probed with a 1-ms monophasic square-wave pulse via a hand-held monopolar stainless-steel head electrode (Knikou 2008) and will correspond to the site that the soleus H-reflex is elicited without a preceding soleus M-wave at low stimulation intensities and when stimulation intensity is increasing the soleus M-wave has a similar shape to that of the soleus H-reflex. When the optimal site is identified, the monopolar electrode will be replaced by a pre-gelled disposable electrode (SureTrace, Conmed, NY, USA) that will be maintained under constant pressure throughout the experiment with athletic foam pre-wrap.

Median nerve stimulation for FCR H-reflex: The FCR H-reflex will be evoked by percutaneous stimulation of the right median nerve with rectangular shocks of 1-ms duration every 5 s delivered by a constant current stimulator (DS7A, Digitimer, Hertfordshire, UK). A hand-held bipolar stainless-steel electrode will be placed medial to the brachial artery on the cubital fossa and used as a probe to determine the most optimal stimulation site (Knikou 2008). This site will correspond to the one during which at low stimulation intensities Ia afferents could selectively be excited with absent activation of motor axons (M-wave), and the shape of the FCR M-wave is similar to that of the H-reflex at both low and high stimulation intensities. When the optimal stimulation site is identified, the bipolar electrode will be maintained in place via an athletic wrap.

Before and one/two days after completion of the 20 sessions, we will assemble the 1) soleus and FCR H-reflex and arm-leg TEP recruitment input-output curves with subjects seated or supine while at rest, 2) the FCR and soleus H-reflex rate-dependent depression, and the soleus H-reflex and TEPs phase-dependent modulation during assisted stepping.

Recruitment input-output curves: For the recruitment curves, we will deliver single pulses of 1 ms duration at increasing intensities to the posterior or median nerves to evoke the soleus and FCR H-reflexes. Similarly, single 1 ms pulses at increasing intensities will be delivered to the cervical or low thoracic levels to assemble the arm and leg TEPs. We will analyze recruitment curves by fitting a sigmoid function and estimate predicted parameters of physiological importance like the threshold excitability and slope or gain of the system according to methods we have extensively utilized on the lab (Skiadopoulos et al. 2022).

Soleus/FCR 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 seated subjects seated at inter-pulse intervals of 1, 3, 5, 8, and 10 s. The same method will be used for FCR H-reflex following stimulation of the median nerve with a bipolar electrode. Homosynaptic depression is greatest at 1 s and fully recovers at inter-pulse intervals of 8 or 10 s (Crone and Nielsen 1989). The main outcome measure will be the H-reflex 1/10 s ratio (Aymard et al. 2000). Using a linear mixed model, we expect a 30% decrease in the 1/10s ratio of post-intervention compared to baseline in both groups, as indicated by a return of rate-dependent reflex depression.

Soleus H-reflex and TEPs phase-dependent modulation during stepping: Each participant will stand while wearing an upper body harness connected to overhead pulleys. While 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 soleus H-reflexes 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. Soleus H-reflexes during stepping will be collected before and after the intervention at Lokomat settings matched to before and the last training session. This will establish changes to matched settings and reflex adaptation at more demanding settings. 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 will record the leg TEPs during assisted stepping at 1.2 right soleus TEP threshold. Stimulations will be triggered based on the foot switch signal.

Clinical Measures Before and After Intervention

For each subject, at baseline and 1/2 days after completion of 20 intervention sessions, we will assess via questionnaires improvements in bladder and bowel function via the University of Michigan Spinal Cord Injury Model Systems Spinal Cord Injury Bowel and Bladder Treatment Index Short Form (SCI-BBTI-SF), sexual function via the PROMIS® V2.0 Brief Profile Sexual Function and Satisfaction (for male and female). Additionally, we will clinically evaluate spasticity via the Modified Ashworth Score and walking ability via the 2-minute and 10-meter walking tests.

Data Reduction and Statistical Analysis

All responses will be measured as peak-to-peak amplitude. H-reflexes recorded at different stimulation intensities (recruitment curve) will be 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 threshold of the H-reflex or TEP, as estimated based on the sigmoid function fit to the data. Normalized H-reflexes will be plotted against the normalized intensities and grouped based on time of testing. Similar analysis will be performed for the arm/leg TEPs with the only difference that they will be normalized to the homonymous TEP maximal amplitude.

For each dependent variable, we will perform descriptive statistics including frequencies and percentages for categorical variables and means and percentile distributions for continuous variables to examine the distribution of outcomes and describe the study population, followed by Shapiro-Wilk's test for normal distribution and Mauchly's test of sphericity for homogeneity of variances. If sphericity is not established, the Greenhouse-Geisser correction statistic will be used. We will group each dependent variable based on time of testing (before and after the intervention), and subject group (2 groups; 1st group cervical-lumbosacral transspinal stimulation at rest, 2^{nd} group cervical-lumbosacral transspinal stimulation during stepping). A repeated measures analysis of covariance (ANCOVA) one-way ANOVA along with Bonferroni post-hoc t-tests (α = 0.05) at 2 x 2 levels (2: time, 2: groups) to test the main and interaction effects among subject groups will be performed separately for each outcome. Further, repeated measures ANCOVA will be performed for each outcome separately with gender, age, level of SCI and clinically evaluated baseline walking function as separate covariates.

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