Study Title:	Neural Facilitation of Movements in People With SCI
NCT number:	NCT05354206
IRB ID #:	202111151 (The Washington University in St. Louis IRB)
PI:	Ismael Seáñez
Document Type:	Protocol
Document Date:	11/10/2022

Neural facilitation of stimulation-assisted movements in people with spinal cord injury

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Version: November 10, 2022

Synopsis

Study title	Neural facilitation of stimulation-assisted movements in people with spinal cord injury
Objective	To understand the contribution of individual neural tracts to movements facilitated by transcutaneous spinal cord stimulation using transcranial magnetic stimulation, loud auditory stimuli, wearable sensors, motion tracking, and electromyography.
Hypothesis	Previous studies supported a potential role of spinal cord stimulation on the excitability of corticospinal and reticulospinal tract. We aim to identify the specific contributions of cortico- and reticulospinal networks towards reinforcing different types of movements enabled by transcutaneous spinal cord stimulation in unimpaired volunteers and volunteers with spinal cord injury. The results from this study may serve as preliminary data for the development of targeted rehabilitation strategies that take advantage of residual connections to maximize recovery after spinal cord injury.
Study period	Planned enrollment duration: approximately 12 months Planned study duration: 1-2 hours per subject, 5 visits
Number of subjects	112 (100 unimpaired, and 12 volunteers with spinal cord injury)
Study design	This study will measure changes in corticospinal and reticulospinal excitability associated with spinal cord stimulation during different leg movements.
Inclusion and exclusion criteria	Inclusion Criteria: Healthy volunteers 1. Age between 18 and 65 years 2. Healthy people with no major comorbidities of any organ systems Exclusion Criteria: 1. Subjects younger than 18 or older than 65 years 2. Subjects not providing consent or not able to consent 3. Subjects with any acute or chronic pain condition 4. Subjects with any acute or chronic disease of a major organ system 5. Use of analgesics earlier that 5 half-lives after the latest intake prior to study period 6. The use of caffeinated products within 3 hours prior to study period 7. History of epilepsy

	8. Implanted metal 9. Active medical problems SCI volunteers 1. Age between 18 and 65 years 2. Traumatic injury at the C4-T9 level, complete (ASIA A), or incomplete (ASIA B, C, or D) 3. At least one-year post injury 4. Stable medical condition 5. Difficulty independently performing leg movements in routine activities of daily living 6. Able to follow simple commands 7. Able to speak and respond to questions Exclusion Criteria: 1. Subjects younger than 18 or older than 65 years 2. Subjects not providing consent or not able to consent 3. Subjects with any acute or chronic pain condition 4. Subjects with any acute or chronic disease of a major organ system 5. Use of analgesics earlier that 5 half-lives after the latest intake prior to study period 6. The use of caffeinated products within 3 hours prior to study period 7. Presence of tremors, spasms, and other significant involuntary movements 8. Etiology of SCI other than trauma 9. Concomitant neurologic disease, such as traumatic brain injury that will significantly impact the ability to follow through on study directions, multiple sclerosis, stroke, or peripheral neuropathy 10. History of significant medical illness including cardiovascular disease or insufficiency, uncontrolled diabetes, uncontrolled hypertension, osteoporosis, cancer, chronic obstructive pulmonary disease, severe asthma requiring hospitalization for treatment, renal insufficiency requiring dialysis, autonomic dysreflexia, etc. 11. Severe joint contractures disabling or restricting lower limb movements. 12. Unhealed fracture, contracture, pressure sore, urinary tract infection or other uncontrolled infections, other illnesses that

	might interfere with lower extremity exercises or testing activities 13. Depression, anxiety, or cognitive impairment 14. Deficit of visuo-spatial orientation 15. Sitting tolerance less than 1 hour 16. Severe hearing or visual deficiency 17. Miss more than 3 appointments without notification 18. Unable to comply with any of the procedures in the protocol 19. Botulinum toxin injection in lower extremity muscles in the prior six months 20. Any passive implants (osteosynthesis material, metallic plates or screws) below T9. 21. Any implanted stimulator in the body, e.g., pacemaker, vagus nerve stimulator, etc. 22. History of alcoholism or another drug abuse 23. Pregnancy (or possible pregnancy) 24. Having an Intrathecal Baclofen Therapy Pump (ITB pump) 25. History of epilepsy
Measurements	Movement kinematics with video, muscle activity, motor evoked potentials by transcranial magnetic stimulation, and muscle responses after a loud auditory stimulus, before, during, and after transcutaneous spinal cord stimulation; depression and anxiety evaluation using the Beck Depression Inventory and Beck Anxiety Inventory questionnaires.

TABLE OF CONTENTS

1. Research aims

Electrical spinal cord stimulation (SCS) combined with exercise training has been shown to restore posture, stepping, and voluntary walking in humans with spinal cord injury (SCI). However, the neural basis of SCS induced recovery remains poorly understood, presenting significant barriers towards maximizing its use in rehabilitation. A better understanding of the specific improvements in motor function and potential for cortical and spinal plasticity could allow clinicians and physical therapists to personalize SCS mediated therapy according to individual needs. Additionally, allowing the brain to play an active role in the delivery of therapy could further enhance and accelerate the potential for recovery. In this study, we combine body-machine interfaces based on wireless wearable sensors with spinal cord stimulation to determine which kinds of SCS-facilitated movements are mediated by the corticospinal and reticulospinal tracts. We will evaluate corticospinal and reticulospinal tract excitability via transcranial magnetic stimulation (TMS) and loud auditory stimuli respectively. If successful, this study will allow us to study whether different types of training used in rehabilitation can be used to enhance the excitability of these neural structures and evaluate their role in the learning of skilled motor tasks. An understanding of how we can accelerate plasticity through residual cortical and reticulospinal connections may enable the development of rehabilitation strategies that take advantage of patient-specific residual functions to further augment recovery.

2. Introduction

Spinal cord injury (SCI) is a life-altering event that leads to long-lasting motor impairment, and currently, there is no 'cure' for paralysis[1–3]. Through my work and that of others, electrical spinal cord stimulation (SCS) has been gaining momentum as a neuromodulation technique to re-enable movement of paralyzed areas[4–6]. Although regained movements below the injury have enabled activity-based interventions to induce unprecedented functional improvements in the chronic stage of paralysis[7–10], the mechanisms of neurorecovery induced by electrical neuromodulation of the spinal cord remain poorly understood[11,12].

To better understand SCS-mediated neurorecovery, studies in rodent models of SCI using viral tracing[13] and optogenetics[14] suggest that corticospinal and reticulospinal projections mediate the voluntary control of paralyzed areas enabled by SCS, and activity-dependent reorganization of these circuits may be a primary contribution towards the restoration of function that has been observed in humans[7–9]. While direct recordings of these neural structures through viral tracing and optogenetics are not currently possible in humans, experimental paradigms exist to evaluate their involvement and contribution during SCS and motor tasks[11,15]. Twenty minutes of SCS during rest has an excitatory effect at subcortical, but not cortical levels[11]. However, it remains unclear whether this excitatory effect can be further enhanced by combining SCS with active voluntary movement. Indeed, despite the motor-enabling effects of SCS having been first observed half a century ago[16], a major breakthrough in SCI research came from the combination of SCS with activity-based training[4,5,7–9,17]. Moreover, while SCS can immediately enable a broad range of behaviors in animal models of paralysis[13,18–20], independent stepping without stimulation in humans has only been observed after more than a year of intense rehabilitation with SCS[8,9,21]. In this work, we will evaluate corticospinal and reticulospinal tract excitability via transcranial magnetic stimulation (TMS) and reaction to a loud auditory stimuli (StartReact) respectively. Having a clear understanding of the neural mechanisms that are enhanced by SCS can allow the development of therapies that directly target the excitability and plasticity states of these structures towards improved and accelerated recovery.

3. Background and Significance

3.1 Rationale for this study

This 1-year study will provide the necessary data to prepare a clinical trial investigating whether activity-dependent reorganization of cortico- and reticulospinal circuits are primary contributors towards the long-lasting improvements in function that have been observed in humans. Moreover, we will study whether different types of training used in rehabilitation can be used to enhance the excitability of these neural structures and evaluate their role in the learning of skilled motor tasks[22–25]. An understanding of how we can accelerate plasticity through residual cortical and reticulospinal connections may enable the development of rehabilitation strategies that take advantage of patient-specific residual functions to further augment recovery.

4. Study Objectives

The goal of this project is to generate evidence-based knowledge of changes in the short-term excitability of corticospinal and reticulospinal neural structures that may mediate immediate improvements in motor function enabled by SCS.

Aim 1. Determine which kinds of SCS-facilitated movements are mediated by the corticospinal tract. I will use TMS to test whether SCS can enhance motor evoked potential (MEP) response time and amplitude as individuals use their legs to perform motor control tasks with different levels of dexterity.

Aim 2. Determine which kinds of SCS-facilitated movements are mediated by the reticulospinal tract. Using the same protocol, I will test whether SCS can enhance StartReact response time and amplitude.

5. Selection of Subjects – Inclusion/Exclusion Criteria

We plan to include in this study a total of 100 unimpaired and 12 SCI human volunteers over the course of one year.

Data collection will be performed at Washington University in St. Louis Danforth and Medical campus. Subjects will be referred by Drs. Eric Leuthardt, Brenton Pennicooke, Kerri Morgan, Theresa Notestine, and Neringa Jukins, who are co-investigators on this project.

Approximately equal numbers of men and women will be recruited from all ethnic and racial groups. The racial composition of the population in St. Louis is approximately 44% Caucasian, 46% Black or African American, 4% Asian, 4% Hispanic, 2% Other. This will be the expected composition of our volunteer pool, of which the minority portion exceeds the national norm.

5.1 Healthy volunteers

Inclusion criteria

1. Age between 18 and 65 years
2. Healthy people with no major comorbidities of any organ systems

Exclusion criteria

1. Subjects younger than 18 or older than 65 years
2. Subjects not providing consent or not able to consent
3. Subjects with any acute or chronic pain condition
4. Subjects with any acute or chronic disease of a major organ system
5. Use of analgesics earlier that 5 half-lives after the latest intake prior to study period
6. The use of caffeinated products within 3 hours prior to study period
7. History of epilepsy
8. Implanted metal

9. Active medical problems

5.2 SCI volunteers

Inclusion criteria

1. Age between 18 and 65 years
2. Traumatic injury at the C4-T9 level, complete (ASIA A), or incomplete (ASIA B, C, or D)
3. At least one-year post injury
4. Stable medical condition
5. Difficulty independently performing leg movements in routine activities of daily living
6. Able to follow simple commands
7. Able to speak and respond to questions

Exclusion criteria

1. Subjects younger than 18 or older than 65 years
2. Subjects not providing consent or not able to consent
3. Subjects with any acute or chronic pain condition
4. Subjects with any acute or chronic disease of a major organ system
5. Use of analgesics earlier that 5 half-lives after the latest intake prior to study period
6. The use of caffeinated products within 3 hours prior to study period
7. Presence of tremors, spasms, and other significant involuntary movements
8. Etiology of SCI other than trauma
9. Concomitant neurologic disease, such as traumatic brain injury that will significantly impact the ability to follow through on study directions, multiple sclerosis, stroke, or peripheral neuropathy
10. History of significant medical illness including cardiovascular disease or insufficiency, uncontrolled diabetes, uncontrolled hypertension, osteoporosis, cancer, chronic obstructive pulmonary disease, severe asthma requiring hospitalization for treatment, renal insufficiency requiring dialysis, autonomic dysreflexia, etc.
11. Severe joint contractures disabling or restricting lower limb movements.
12. Unhealed fracture, contracture, pressure sore, urinary tract infection or other uncontrolled infections, other illnesses that might interfere with lower extremity exercises or testing activities
13. Depression, anxiety, or cognitive impairment
14. Deficit of visuo-spatial orientation
15. Sitting tolerance less than 1 hour
16. Severe hearing or visual deficiency
17. Miss more than 3 appointments without notification
18. Unable to comply with any of the procedures in the protocol
19. Botulinum toxin injection in lower extremity muscles in the prior six months
20. Any passive implants (osteosynthesis material, metallic plates or screws) below T9.
21. Any implanted stimulator in the body, e.g., pacemaker, vagus nerve stimulator, etc.
22. History of alcoholism or other drug abuse
23. Pregnancy (or possible pregnancy)

24. Having an Intrathecal Baclofen Therapy Pump (ITB pump)
25. History of epilepsy

5.3 Withdrawal Criteria

1. Subjects will be allowed to withdraw from the study at any time upon request
2. Subjects' participation will be terminated if any of the inclusion criteria ceases to be valid or if any exclusion criteria will manifest after enrollment
3. Subjects' participation will be terminated if any significant depression is identified on the collected information on depression. Subjects will also be referred to a mental health professional.

Prior to scheduling, subjects will be screened over a phone interview to determine whether they meet the inclusion and exclusion criteria. All research personnel will have received training in the responsible conduct of human research as required by Washington University's Institutional Review Board (IRB).

6. Human Subjects Research

6.1 Protection of Human Subjects

The study will be performed after proper Washington University Institutional Review Board (IRB) approval of the protocol, consent forms, and recruitment materials. This study will include human subjects and thus will be conducted in conformity to the Helsinki declaration. The study will be conducted under the supervision of the Dr. Ismael Seáñez who is experienced in the conduct of human studies on SCS. The motion tracking, EMG, and TMS data will be analyzed qualitatively and quantitively in collaboration with team members with substantial expertise (laboratories of Dr. Ismael Seáñez in the Department of Biomedical Engineering and Dr. Rita Haddad in the Department of Psychiatry), to identify corticospinal responses associated with movement and transcutaneous SCS.

6.2 Recruitment and Informed Consent

Our recruitment procedure for SCI subjects uses word-of mouth, fliers (posters), and phone calls. Healthy subjects that are affiliated with Washington University will be recruited by flyers, and word of mouth. We will stress in recruitment and during the study that participation is completely voluntary and will not affect their benefits or employment status.

Subjects with SCI will be referred by Drs. Eric Leuthardt and Brenton Pennicooke (Washington University School of Medicine, Neurosurgery), Dr. Neringa Jukins (Barnes-Jewish Hospital, Physical Medicine and Rehabilitation, Spinal Cord Injury Medicine), Dr. Theresa Notestine (The Rehabilitation Institute of St. Louis), and Kerri Morgan (Paraquad, Stephen A. Orthwein Center), and identified through the EPIC system if they meet the recruitment criteria. They will be told about the study in person or contacted by phone and read the "Phone Script". We will make it clear that participation does not imply any certainty for favorable outcome or other benefits.

Recruitment from TRISL and Paraquad will be purely in the form of posting recruitment flyers and referrals. No patient database access will be requested or used.

Subjects will be given verbal (initially) and then written descriptions of the study aims, procedures, risks, and benefits, and will be required to give written informed consent. A member of the investigative team provides all study descriptions, informed consent, and answers all questions. Subjects are informed verbally and in writing that participation is voluntary, and they may refuse to participate and may withdraw from the study at any time without penalty.

6.3 Potential benefits of the proposed research to the subjects and others

There is no expected direct benefit to study subjects. If this study is able to identify specific neural tracts that are involved in movements enhanced by spinal cord stimulation, we may implement this knowledge for the development of patient-specific rehabilitation strategies towards improvement of movement after SCI. Society may benefit from a novel paradigm for restoration of function after SCI.

6.4 Compensation

There will be no costs to participants for being in this study. Participants with SCI will be paid $20 for every hour of participation during this research study. Unimpaired participants will be paid $10 for every hour of participation. A prorated rate of $5 dollars for every completed 15 and 30 minutes for SCI and Control participants respectively will be used to account for incomplete hours or participation. If they withdraw from the study, they will be paid for a percentage of the experiment that they participated. The maximum amount of money that they can receive for a visit to the lab is $60. Transportation expenses from home to the hospital will be reimbursed when needed. Transportation expenses from home to the hospital will be reimbursed upon receipt. These funds are provided to help support participants with time and travel associated with participation.

6.5 Inclusion of women

Studies actively encourage the participation of women in the research. Our studies routinely and deliberately attempt to include equivalent numbers of women and men. However, due to a small size of the study, we may not be able to enroll equal number of male and female subjects. Pregnant or possibly pregnant women are excluded from our research protocols.

6.6 Inclusion of minorities

All of our studies actively encourage the participation of minorities in the research. Our minority recruiting of healthy subjects typically matches the demographic composition of the Washington University community from which subjects will be recruited (78% white, 21% Black, <1 % Hispanic). The racial composition of the population in St. Louis is approximately 44% Caucasian, 46% Black or African American, 4% Asian, 4% Hispanic, 2% Other. This will be the expected composition of our SCI volunteer pool, of which the minority portion exceeds the national norm.

6.7 Inclusion of children

Due to the newly identified rare risk of autonomic dysreflexia, children will not be included in the study.

7. Study design and methods

7.1 Procedure Schedules – Full Protocol

SCI and Controls – Complete protocol

	7.2 INITIAL EVALUATION			7.3 AIM 1	7.4 AIM 2
PROCEDURE	Visit #1 Functional Mapping	Visit #2 Range of Motion	Visit #3 Familiarization	Visit #4 TMS	Visit #5 StartReact
Kinematic recordings					
EMG recordings					
Transcutaneous SCS					
BoMI					
Motor tasks					
TMS					
StartReact					

7.2 Initial evaluation: Determine the effect of SCS on motor responses and range of motion

To determine an initial baseline for each participant, we will evaluate motor responses elicited by transcutaneous SCS in the initial evaluation phase. Participants will visit the lab for 1) a functional mapping where we measure spinal responses elicited by SCS, 2) a range of motion evaluation where we measure the range of motion on different joints with and without SCS, and 3) a familiarization phase where participants learn to control the body-machine interface using their leg movements supported by SCS.

Participants: 100 unimpaired participants and 12 participants with spinal cord injury (spinal cord injury level C4-T9) with motor impairments in the legs (complete ASIA A, or incomplete ASIA B, C, or D) and in the chronic stage of SCI (>6 months postinjury) will participate in this experiment, which will be submitted for review and approval to Washington University School of Medicine Institutional Review Board. Unimpaired participants will be recruited to perform either one of the five potential visits (Visits# 1-5) or all five visits. Approximately 16 unimpaired participants are expected to complete each type of protocol. SCI participants will be recruited for the five visits in total.

Experiments with unimpaired, control participants will be used to refine collection procedures and instruments and to prepare an improved, more refined research design that will ensure effective and high-quality data collection with SCI participants. Data collected on control subjects will also be used to establish baseline behavior for comparison of SCI participant task performance and may be used to generate generalizable knowledge.

Kinematic recordings*:* Kinematic recordings will be obtained at a 100 Hz sampling rate using a 3D markerless motion capture system (Miqus-Hybrid, Qualisys, Sweden) consisting of 10 HD cameras covering the entire workspace. Head, trunk, and bilateral upper and lower extremity kinematics will be captured by these 2MP cameras or by infrared cameras and positioning virtual markers over standardized anatomical landmarks.

Muscle activity recordings (EMG)*:* Muscle activity (EMG) will be acquired bilaterally at 1 kHz

using a 16-channel wireless system (Trigno Avanti, Delsys, Natick, MA). The area around muscles will be shaved and cleaned with an abrasive cream and alcohol. Bipolar surface electrodes will be placed over the form of the iliopsoas, rectus femoris, vastus lateralis, semitendinosus, biceps femoris, tibialis anterior, medial gastrocnemius, and soleus muscles. Prior to electrode placement, the area around each muscle will be shaved and cleaned with an abrasive cream and alcohol. EMG signals will be processed according to the SENIAM (Surface Electromyography for the Non-Invasive Assessment of Muscles) standards for EMG recordings and band-passed filtered between 10 and 450 Hz using a 4^{th} order Butterworth filter. A moving average of the rectified EMG signal within a centered 250 ms time window will be used to generate normalized EMG envelopes for quantification.

Transcutaneous spinal cord stimulation (SCS): During the stimulation phases of the study, non-invasive transcutaneous electrical stimulation will be delivered to the lumbar segments of the spinal cord below the injury using a DSR8 biphasic constant-current stimulator (Digitimer, UK) with a range of 0-1,000 mA. Stimulation will be administered using two pairs of conductive self-adhesive electrodes with a diameter of 3.2 cm placed on the skin over the spinal midline between the spinous processes of the L4 and L5 vertebrae to act as cathodes. The center of the cathodes will be located ~2 cm left and right of the midline. Two additional 5x10 cm electrodes will be placed symmetrically on the skin over the abdomen to be used as anodes[56,57]. The stimulation waveform will be biphasic, rectangular, with 1 ms pulses at a frequency of 1-100 Hz. As an additional alternative, the stimulation will be filled with a carrier frequency of 5 KHz. It is thought that a 5-10 KHz carrier frequency is beneficial for improving muscle strength[58] and for suppressing the sensitivity of pain receptors[59], and is therefore more comfortable for human participants[11,60,61].

Functional mapping*:* Recordings will be performed with single-pulse, graded stimulation amplitudes as subjects lay supine on a table in order to compute the degree of recruitment of each muscle with increasing stimulation amplitudes as previously described in my work[7].

Range of motion: Kinematic recordings will be performed to quantify the range of motion at the different joints with and without transcutaneous SCS.

Body-machine interface*:* Participants will be fitted with a body-machine interface to control a computer cursor using the legs. Leg movements will be recorded using non-invasive, wireless inertial measurement unit (IMU) motion sensors (YostLabs, Portsmouth, OH) attached with Velcro® to adjustable straps around the legs or with double-sided skin tape. User-specific body motions will be decoded and converted into the two coordinates of the cursor in real-time as previously described in my work[62].

Familiarization: Participants will have the opportunity to familiarize themselves with the control of the BoMI using SCS and perform different types of game-like activities like center-out reaching, pong, solitaire, Tetris, and other flash games, as well as the motor tasks (described below).

7.1 Aim 1: Determine which kinds of SCS-enabled movements are mediated by the corticospinal tract

An increasing number of studies supports the view that SCS can restore posture, stepping, and voluntary walking in humans with SCI[4,7,17]. The prevailing view is that by elevating the functional state of spared spinal sensory-motor networks below the lesion, residual descending pathways can transmit activity that enables and amplifies voluntary motor control[15]. Although epidural SCS can enable force production in paralyzed muscles[7], whether motor improvements are mediated by the corticospinal tract or the reticulospinal remains unknown.

In a 45-min session, we will deliver non-invasive transcutaneous SCS to the lumbosacral enlargement as participants use their SCS-facilitated leg movements to control a non-invasive body-machine interface (BoMI) on a computer screen and perform game-like activities[62,63]. To determine which kinds of SCS-enabled movements are mediated by corticospinal networks, participants will perform two motor tasks before and after training requiring different degrees of dexterity: precision control vs. maximum range of motion; using two types of movements: hip vs. ankle flexion. To quantify changes in neural excitability of the corticospinal tract, we will compare motor-evoked potentials (MEPs) elicited by transcranial magnetic stimulation of the leg motor cortex[64] as participants perform the motor tasks. We postulate that MEP responses will be enhanced for the precision-control task in distal muscles (tibialis anterior), which are thought to require a high involvement from the corticospinal tract. *Our hypothes is that SCS will further enhance the MEP response for these movements, but not for range-of-motion movements, or movements with proximal muscles (rectus femoris).*

Transcranial magnetic stimulation (TMS): Motor evoked potentials (MEPs) in the tibialis anterior and rectus femoris muscles will be evoked by TMS of the contralateral motor cortical leg area using a Magstim 200^2 stimulator (Magstim, UK) through a double cone coil and a monophasic current waveform delivered at 4-s intervals. The motor threshold for both muscles will be identified[65] and that intensity will be used to elicit and average twenty MEPs at each timepoint[24]. Average MEP response amplitude will be used to quantify changes in corticospinal tract excitability[15]. If no motor threshold is detected at 100% device stimulation intensity due to SCI (and interrupted circuits), the 100% stimulation intensity or the maximum tolerable intensity will be used. MEP responses will be measured during rest and during an active contraction (Motor Tasks, see below) before and after BoMI training, as well as with and without SCS. To estimate changes in MEPs, response amplitudes under different conditions will be normalized to the baseline (before training, resting) response.

Motor tasks: Immediately before after the BoMI session, participants will wear a VR headset (Oculus Quest 2, Facebook, CA) to block their view. The IMUs on the legs will measure and digitize their leg joint angles. A real-time virtual representation of one of their joint angles will be presented through the VR. Participants will be asked to perform two tasks requiring different types of motor control. In the range-of-motion task, participants will be instructed to flex or extend their legs to surpass a virtual target of a specified amplitude. In the precision-control task, participants will have to move their joint and hold the position within a target range of a specified amplitude for 1 second. We will examine motor-evoked potentials elicited by TMS (*Aim 1*) and the StartReact response (*Aim 2*) as individuals perform the task under two conditions: with SCS and without SCS using the knee and ankle joints. As an alternative to the VR headset, a wall projector will be used to perform the motor tasks.

BoMI Training: Participants will perform visuo-spatial motor tasks over a 45min session with SCS. The tasks will consist of different types of game-like activities like center-out reaching, pong, solitaire, Tetris, and other flash games.

Expected outcomes, potential problems, and alternative strategies*:* Based on previous studies by other groups, it is our expectation that MEP responses will be enhanced for the precision-control task in distal muscles (tibialis anterior), which are thought to require a high involvement from the corticospinal tract. *We hypothesize that SCS will further enhance the MEP response for these movements, but not for range-of-motion movements, or movements with proximal muscles (rectus femoris*.

The main challenge in this study will be the recruitment of a sufficiently large group of volunteers with spinal cord injuries in the C4-T9 level. If this will not be feasible, we will extend the study to individuals with lesions at the C2-C3 level that have complete or partial paralysis in the legs. Furthermore, we can seek to further extend the study to other hospitals in the St. Louis region like the Barnes Jewish Hospital, the Rehabilitation Institute of St. Louis, SSM Rehabilitation Hospital, and the VA Medical Center Spinal Cord Injury Program. Another potential difficulty may arise because of the high expected attrition with severely disabled volunteers. As a consequence, some evaluations might be interrupted. To preserve uniformity of statistical power across the study, we will retain the results of participants with an incomplete number of sessions in separate data sets. The analysis of incomplete data sets will take explicitly into account the causes for abandoning the study (e.g. dissatisfaction with the methodology, change in medical condition, etc).

Our approach will have –by design an objective—a degree of customization based on each individual's specific mobility and potential for movement restoration with SCS. Therefore, there might be a small initial difference in performance. However, our previous studies have demonstrated an improvement in performance with training independent of differences in the BoMI map between subjects[22,62,66,67].

One possibility is that 45 minutes of BoMI training is not sufficient to observe changes in corticospinal and reticulospinal tract excitability within a single session. If this is the case, we will increase the duration of BoMI training to 2 hours. Twenty minutes to 2 hours of SCS and motor training have been shown to be sufficient to observe changes in motor function[61,68,69] as well as spinal[15], cortical[24], and subcortical[11] excitability in human subjects.

Support of limb weight may introduce a force production confound when participants have to generate torque to move their foot compared to their whole leg. If this is the case, we will perform the motor task evaluations as participants lay on their side as manual support is provided to counter the effects of gravity.

7.2 Aim 2: Determine which kinds of SCS-enabled movements are mediated by the reticulospinal tract

Using the same protocol as Aim 1, we will test whether SCS can enhance the StartReact response time and amplitude. To quantify changes in neural excitability of the reticulospinal tract, we will compare the StartReact response[70] (a shortening in response time after a startling auditory stimulus) as participants perform the motor tasks before and after BoMI training.

StartReact response: Participants will be instructed to reach the target as fast as possible after it is presented. Response time will be identified from EMG activity[71] and measured when the target is presented alone (visual reaction time), when it is paired with a soft auditory stimulus (80 dB, 500 Hz, 50 ms, visual-auditory reaction time), or with a startling acoustic stimulus (120 dB, 500Hz, 50ms, visual-startle react time) presented through audio speakers (T-15, Polk Audio). To quantify changes in StartReact responses, response times under different conditions will be normalized to the baseline response.

Expected outcomes*:* Based on previous studies by other groups[47,71,72], I expect the StartReact response time to be the shortest for the startle response, where the reticulospinal tract is engaged. Furthermore, I expect that SCS will have an additional enhancement of this response, but only for tasks that require a high involvement from the reticulospinal tract. *We hypothesize that SCS will enhance the StartReact response for the range-of-motion task in proximal muscles (rectus femoris), but not for precision-control movements, or movements with distal muscles (tibialis anterior).*

8. Data Analysis and Statistical Plan

Average MEP and StartReact responses (of 20 responses) will be used to quantify changes in corticospinal and reticulospinal tract excitability[15]. A paired sample t-test of response amplitude and latency (time from stimulation to muscle response) will be used to test the hypothesis that BoMI training with SCS results in enhanced corticospinal tract excitability during precision-control movements and reticulospinal tract excitability during range-of-motion movements. In addition, a Student's *t*-test with a Holm-Sidak adjustment for multiple comparisons will be performed to compare the difference between changes in corticospinal excitability induced by the type of motor task (precision control vs. range-of-motion), joint (ankle vs. hip), and group (control vs. SCI).

8.4 Sample Size

We plan to include in this study a total of 100 unimpaired and 12 SCI human volunteers over the course of two years. We expect that only 90% of unimpaired and 70% of SCI subjects initially recruited will conclude the experiment. Therefore, we will need to recruit 110 unimpaired and 16 SCI volunteers in order to reach the recruitment targets.

The number of subjects has been powered from the results of previous studies using similar methods to investigate structural changes in the brain after BoMI practice[22,73]. Although the power is adequate, it might be possible that we are unable to detect significant changes in motor capacity. If no within-subject significant changes are found in preliminary experiments, we can extend the duration of training within one session, or extend the protocol to several sessions. If no significant group changes are found, we can recruit six more SCI participants for the study (a 50% increase).

9. Management of Intercurrent Events

9.1 Adverse Experiences

The investigators will monitor the subjects closely for any local or systemic adverse events from the study. If any such events are noted, they will be managed appropriately and reported. Subjects will be followed until satisfactory resolution. The description of the adverse event will include time of onset, duration, severity, etiology, relationship to the study (none, unlikely, probably, highly probable) and treatment that was required.

9.2 Premature Discontinuation

Subjects may be discontinued from the study if the investigators decide that it is in the best interest of the subject, or the subject requests to be withdrawn. Subjects' participation will be terminated if any significant depression is identified on the collected information on depression. Subjects will also be referred to a mental health professional.

9.3 Potential Risks

Use of motion sensors. There are no known risks associated with the use of the instrumented motion sensors. The trunk and leg motions made in the proposed research are well within the normal range of motion of adult subjects, and there are no physical injuries expected from the use of the motion sensors. Fatigue from the repeated movement of legs, or dizziness from the computer screen are a possibility.

Surface electrodes. Surface electrodes for recording of muscle activity via electromyography (EMG) and transcutaneous spinal cord stimulation are widely used and present a low-risk to the subject. Skin redness, dryness, or irritation is possible from the shaving and skin cleaning required for the application of the surface EMGs. Skin lacerations from shaving due to dry contact or repeated shaves are possible. Subjects are instructed that they may stop any procedure at any time with no adverse consequences.

Transcutaneous spinal cord and peripheral nerve stimulation. Unpleasant or even painful paresthesia could be experienced during stimulation. In most cases, stimulation parameters can be adapted to be tolerated by the subject. However, there is a likelihood that stimulation cannot be tolerated by a subject. Subjects are instructed that they may stop any procedure at any time with no adverse consequences.

Autonomic dysreflexia (AD) is an acute episodic hypertension resulting from sympathetic hyperactivity[46] and is typically defined as increased systolic blood pressure greater than 20 to 30 mmHg[47]. AD may be experienced by individuals with injuries above the 6th thoracic level (T6), most commonly triggered by noxious irritation of urinary bladder or bolon[48], although it can also be triggered by nonnoxious or noxious stimuli[47,49]. A recent study testing the use of tSCS for upright posture in children with SCI detected an episode of AD in one participant (5% occurrence), rating it as "*very unlikely to occur*"[50]. The stimulation was immediately stopped, and, within 3 min, the episode resolved, and blood pressure returned to baseline levels. Although a study specifically investigating the incidence of AD by spinal cord stimulation in people with SCI failed to observe an adverse event[51], and numerous other studies have not reported incidences of AD[10,11,32,52–55],

we found two other studies reporting signs of AD in SCI while using SCS[56,57]. In one study, one out of nine participants was observed to experience symptoms of AD, but was later found to have an injury to the left calf, likely due to the sit-to-stand training paradigm[57]. Although the other study mentions that there were no confirmed incidents of AD, one out of fifteen participants demonstrated an elevation of systolic blood pressure greater than 60 mmHg, and the stimulation was stopped.

History of AD is already an exclusion criteria of our study, and participants with SCI typically have experience in identifying the onset of AD symptoms. However, we believe that although the risk of inducing AD through tSCS may be rare, it is important to monitor blood pressure and other signs of AD (decreased heart rate, face flushing, headache, and sweating) to manage it in time should it occur.

Transcranial magnetic stimulation. The TMS device to be used is the MagStim 200^2 with a 110mm double cone coil and an EMG interface module. The TMS devices are non-significant risk devices. The principal component of the TMS is a "figure-8" coil made of insulated copper bus bar and capacitor-containing booster modules. The flow of electricity through the coil is regulated by a control module and a computer. When electricity flows through the coil, a magnetic field pulse is generated according to Faraday's law. This magnetic pulse in turn causes nearby neurons to discharge in a physiological variant of the Hall effect. No electrical current is ever passed between the machine and the participant. Currently, this MagStim 200^2 machine has FDA approval for peripheral nerve stimulation, but we will use it for cortical stimulation to evoke motor evoked potentials from the motor cortex[15,24,71,72].

Generally, TMS is considered safe and well-tolerated. Patients receiving repetitive TMS, which has increased risks compared to single-pulse TMS[74] (Table 1), are able to drive home or return to work immediately after a repetitive TMS session. In this study, we will only use single-pulse TMS. The single-pulse TMS techniques performed in this study have been used extensively in thousands of research studies and on tens of thousands of participants in the United States and around the world. Single-pulse TMS techniques are considered remarkably safe[74] if the appropriate guidelines are followed [75].

Table 1

Potential side effects of TMS. Consensus has been reached for this table.

Side effect	Single-pulse TMS	Paired-pulse TMS	Low frequency rTMS	High frequency rTMS	Theta burst
Seizure induction	Rare	Not reported	Rare (usually protective effect)	Possible (1.4% crude risk estimate in epileptic patients; less than 1% in normals)	Possible (one seizure in a normal subject during cTBS) (see para 3.3.3)
Transient acute hypomania induction	No	No	Rare	Possible following left prefrontal stimulation	Not reported
Syncope	Possible as epiphenomenon (i.e., not related to direct brain effect)				Possible
Transient headache, local pain, neck pain, toothache, paresthesia	Possible	Likely possible, but not reported/ addressed	Frequent (see para. 3.3)	Frequent (see para. 3.3)	Possible
Transient hearing changes	Possible	Likely possible, but not reported	Possible	Possible	Not reported
Transient cognitive/ neuropsychologial changes	Not reported	No reported	Overall negligible (see Section 4.6)	Overall negligible (see Section 4.6)	Transient impairment of working memory
Burns from scalp electrodes	No	No	Not reported	Occasionally reported	Not reported, but likely possible
Induced currents in electrical circuits	Theoretically possible, but described malfunction only if TMS is delivered in close proximity with the electric device (pace-makers, brain stimulators, pumps, intracardiac lines, cochlear implants)				
Structural brain changes	Not reported	Nor reported	Inconsistent	Inconsistent	Not reported
Histotoxicity	No	No	Inconsistent	Inconsistent	Not reported
Other biological transient effects	Not reported	Not reported	Not reported	Transient hormone (TSH), and blood lactate levels changes	Not reported

Adapted from [76].

In general, the risks associated with single-pulse TMS are very minimal. Some of the risks that may be associated with TMS include unpleasant temporary side effects including headache, scalp discomfort at the site of stimulation, paresthesia, toothache, hearing changes, spasms, or twitching of facial muscles, and lightheadedness.

Studies suggest that the loud clicking noise produced by the TMS discharge may exceed 140 dB of sounds pressure level, and can cause hearing loss [74]. However, a majority of studies report no change in hearing after TMS when hearing protection is used [74].

There is a very small risks of seizures in repetitive TMS done with very intense, high-frequency stimulation or stimulations separated by less than 1 second. Such intensities and frequencies will not be used in this study, as only single-pulse TMS will be employed. A questionnaire[77] sent to 2510 authors in 174 groups using a variety of coils and protocols in TMS reported over 16 seizures apparently caused by TMS in over 200,000 sessions for a standardized risk of 8/100,000. However, 13 of these occurred in high-risk subjects. The other 3 had no known risk factors [74].

Loud auditory stimulus. Loud noises can cause temporary side effects including headache, and discomfort in the ear.

Loss of confidentiality. There is a risk of loss of confidentiality with participation in this study. Investigators will aim to keep the identity of the subjects confidential to the extent permitted by law. However, it is possible that others may become aware of the subject's participation in this study and may inspect and copy records pertaining to this research. Some of these records could contain information that personally identifies subjects.

This is an observational study, and no treatments will be tested in this study. Treatment for SCI will be directed by the participant's physician.

9.4 Procedures to minimize potential risks

This study will be conducted at Washington University under the supervision of the PI. The PI is trained and well-experienced in performing human subject research. Study subjects will be monitored throughout the experiment by a co-investigator. Inclusion and exclusion criteria and monitoring are designed to ensure that the risks are minimal. Subjects are informed that participation is voluntary, and they may refuse to participate and may withdraw from the study at any time without penalty.

The responses to the Beck Depression Inventory questionnaire will be reviewed in real time by team members. If, based on the questionnaires, a participant is suspected of having suicidal intent, the PI will be notified immediately and referrals will be made as needed for further evaluation.

Study staff complete training in HIPPA regulations and do not divulge confidential information regarding participants. Participant records are kept confidential with paper records in a secure location and computer records password-protected, available only to the study staff, and with subject identifiers removed.

Use of motion sensors. To avoid fatigue, experiments will be self-paced by the subject. Subjects will be instructed that they may ask to have the procedure stopped if they have any discomfort or other concerns.

Muscle activity sensors (EMG). Washing the electrode gel from the skin surface and applying unscented skin lotion after the experiments will be applied to minimize skin irritation. Shaving cream will be used to reduce the likelihood of lacerations. In case of lacerations, first aid will be applied as necessary and the EMG application will be discontinued in that area.

Transcutaneous spinal cord and peripheral nerve stimulation. Stimulation will always start at a minimum amplitude and slowly increased as subjects are instructed to give feedback on their comfort levels. Amplitudes will be chosen as the level at which a subject feels paresthesia (tingling sensation) or a motor response is elicited. These amplitudes are normally way below pain thresholds. If a subject feels discomfort at a certain stimulation amplitude, the amplitude will be lowered to a comfortable level, or stopped.

To identify potential incidences of autonomic dysreflexia, blood pressure and heart rate will be monitored before, during, and after the use of continuous SCS. A baseline measurement will be recorded at the beginning of the experiment. A second recording will be performed as soon as the continuous stimulation has reached the amplitude necessary for the experiment. A final recording will be performed at the end of the experiment. Signs of autonomic dysreflexia will include an increase in systolic blood pressure by more than 20 mmHg, absolute pressure greater than 140 mmHg systolic or 100 mmHg diastolic, a decrease in heart rate by more than 40 bpm, face flushing, sweating, and headache. If a sign of autonomic dysreflexia is detected, stimulation

will be immediately stopped, participants will be moved to an upright position to induce an orthostatic hypotension response, and blood pressure and heart rate will be monitored once every 5 minutes until they return to baseline levels.

Transcranial magnetic stimulation. Stimulation will always start at a minimum device amplitude and slowly increase as subjects are instructed to give feedback on their comfort levels. Stimulation amplitudes will be chosen as the level at which muscle responses are elicited. These amplitudes are normally below pain thresholds. If a subject feels discomfort at a certain stimulation amplitude, the amplitude will be lowered to a comfortable level, or stopped.

All participants in our study will be required to wear approved hearing protection (earplugs or earmuffs) during TMS evaluations as recommended by TMS safety guidelines.

Loud auditory stimulus. When delivered continuously for prolonged times (longer than 2 minutes), noises above 110dB can result in temporary or permanent hearing loss. Noises in our experiment will have a short 1/5 second duration to prevent adverse consequences. Subjects will be instructed that they may stop any procedure at any time with no adverse consequences.

Loss of confidentiality. Upon enrollment in this study, 1) all subjects will be assigned a study ID number, 2) The link to identifiers will be destroyed at the end of the study, 3) Data will be stored under lock and key (office, file cabinet) and only the investigators and research team will have access. If data are published, there will be no link to identifiers. Study data will not be revealed to any organization or individuals other than the subjects themselves, 4) Study data will not be entered in subjects' medical records.

Adverse experiences. The investigators will monitor the subjects closely for any local or systemic adverse events from the study. If any such events are noted, they will be managed appropriately and reported to the HRPO and QASMC according to institutional guidelines. Subjects will be followed until satisfactory resolution. The description of the adverse event will include time of onset, duration, severity, etiology, relationship to the study (none, unlikely, probably, highly probable) and treatment that was required.

Premature discontinuation. Subjects may be discontinued from the study if the investigators decide that it is in the best interest of the subject, or the subject requests to be withdrawn. Subjects' participation will be terminated if any significant depression is identified on the collected information on depression. Subjects will also be referred to a mental health professional.

10. Data Safety and Monitoring Plan

The specific monitoring plan for this investigation is commensurate with the risks and the size and complexity of the investigations planned. This study involves a single site (Washington University in St. Louis) and a single intervention (behavioral tasks), with minimal risks. Therefore, we have selected the monitoring authority of: ***Dr. Ismael Seáñez and Washington University***

Institutional Review Board.

Dr. Ismael Seáñez, the Principal Investigator, will take primary responsibility for monitoring participant safety. He will review all serious adverse events (SAEs), adverse events (AEs), and unanticipated problems (UPs) on an ongoing basis, and ensure that that the Washington University Institutional Review Board (IRB) receives reports via its online reporting system following the IRB's required timeframes. Specifically:

- All SAEs, regardless of relatedness or expectedness, will be reported to the safety officer within two working days of becoming aware of the event.
- AEs and SAEs that meet the definition of a UP, including breach of confidentiality, will be reported to the safety officer within two working days and to the IRB within 10 working days of becoming aware of the event.
- All other AEs and SAEs that do not meet criteria as a UP will be reported to the IRB at the time of continuing review in accordance with its policy.
- Any major deviations from protocol will be reported within 10 working days, and changes to protocol initiated without IRB approval to alleviate immediate hazards will be reported within 24 hours.
- Participant complaints (which do not rise to the level of UP) and minor deviations from protocol will be reported annually, but any pattern/series of minor deviations will be reported within 10 working days.

In addition, the following records will be added to the study regulatory binder:

- For all reports to the IRB, the Coordinator will retain a copy of the notification to the IRB
- Each SAE and UP will be recorded individually in an Adverse Event Form
- AEs will be recorded as part of the AE Tracking Log.

Dr. Seáñez will ensure that all study personnel are adequately trained and provisioned to work with participants to resolve complaints, trained to identify and respond to expected AEs (e.g. intolerability of spinal cord stimulation or headaches or seizures after transcranial magnetic stimulation), and otherwise trained to ensure compliance with the protocol.

During quarterly whole-study meetings, Dr. Seáñez and the clinical research coordinator will lead a review of any issues related study data and safety with all members of the team.

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