**Random Noise Stimulation to Enhance Corticomotor Drive for Improved Hand Function**

**Protocol # 762**

**Version 4. June 10th, 2021**

**Principal Investigator**

Anastasia Zarkou, PT, PhD

2020 Peachtree Rd NW Atlanta GA 30309

Phone: 404-603-4200

Email: Anastasia.zarkou@Shepherd.org

**Sub-Investigators:**

Edelle C. Field-Fote, PT, PhD, FAPTA

Jennifer Iddings PhD

Allison Ainsworth, MOT, OTR/L

Ashley Heleine, MS, OTR/L

Kyle Condon, PT, DPT

Cazmon Suri, MS

**Document History**

Original Protocol: 06/27/2018

Amendment: 05/22/2019

Amendment: 02/15/2021

Amendment: 06/10/2021

**This document contains confidential and proprietary information. Therefore, this document should be maintained in a secure location and should not be copied or made available for review by any unauthorized personnelStatement of Compliance**

This study will be carried out in accordance with Good Clinical Practice (GCP) as required by the United States Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, and 21 CFR Part 312).

\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ 06/10/2021\_\_\_\_\_\_\_\_

Principal Investigator Signature Date

Anastasia Zarkou\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Principal Investigator Printed Name

**Table of Contents**

##### 

1. Introduction
2. Study Aims
3. Study Design
4. Adverse Events
5. Data Management
6. Statistical Considerations
7. Ethical, Regulatory, and Administrative Considerations
8. Study Medication/Device/Intervention
9. References

### Introduction

Spinal cord injury (SCI) is a traumatic event for injured individuals and their loved ones as it affects every aspect of life. Cervical SCI results in upper extremity (UE) function impairments and reduced independence in performance of daily activities. UE dysfunction due to tetraplegia is attributable primarily to disruption of ascending and descending pathways, and secondarily to the maladaptive cortical reorganization following the initial injury [1]. Non-invasive brain stimulation (NIBS) approaches, even when applied for a short period of time [2], can modulate cortical excitability thereby enhancing transmission through the remaining corticospinal tract pathways to improve motor and functional recovery in individuals with SCI [3]. Specifically, transcranial direct current stimulation (tDCS) is a clinically accessible approach that has been associated with improved UE function in individuals with tetraplegia [4]. However, the literature indicates there is significant inter-individual variability in response to the application of tDCS [5], as well as inconsistency in temporal effects on cortical excitability [6]. In addition, current flow orientation varies depending on the polarity of the tDCS and the topography of the cortex [7], this variability influences the cortical response and clinical value of tDCS.

Transcranial random noise stimulation (tRNS) is a polarity-independent stimulation approach, which produces more consistent excitatory effects compared to tDCS [6]. In contrast to tDCS, tRNS is has excellent tolerability and no risk of burns. Importantly for research purposes, tRNS is imperceptible to most individuals, hence blinding is more effective [8]. A neurophysiological mechanism that forms the basis for the value of tRNS is stochastic resonance, a biological phenomenon in which a weak signal in the peripheral or central neuronal pathways is enhanced in the presence of low noise [9]. The literature has indicated that tRNS, when applied to the motor cortex, can induce facilitatory effects and enhance motor learning in healthy adults [8, 10]. Yet, there are no studies to date investigating the efficacy of tRNS on unimanual or bimanual UE functionality in individuals with tetraplegia.

Improving UE functional performance, even to a small extent, may have significant implications for the overall quality of life in individuals with SCI [11]. This study represents an important step in the development of clinically accessible rehabilitative interventions to prevent the downward spiral of UE motor dysfunction that occurs following cervical SCI. Specifically, we aim to investigate the efficacy of combined unihemispheric tRNS and FMT to enhance neuroplasticity for improved UE motor and sensory function in these persons. This project is innovative; it is the first time that the effects of tRNS on cortical excitability in individuals with SCI will be examined. This therapeutic intervention is significant as it has the potential to strengthen transmission through the residual pathways following SCI thereby promoting unimanual and bimanual motor and functional recovery. If tRNS can modulate corticospinal excitability, it may be a promising tool that could meaningfully advance neurorehabilitation research and therapeutic management in the field of SCI.

**2.0 Study Aims**

In individuals with tetraplegia (≥ 1 year post injury) who have at least trace extrinsic control of both hands, and one hand that has at least trace intrinsic control (thenar, first dorsal interosseous, hypothenar), we propose to investigate the efficacy of a 3-day combined tRNS and fine motor training (FMT) on descending cortical drive to enhance UE functional recovery. For the purposes of this study, regardless of the severity of the SCI injury, sufficient volitional control of intrinsic and extrinsic hand muscles suggests that there is potential for increased cortical drive through the spared connections for improving UE function.

**Specific Aim 1**: Compare effects on cortical excitability of a 3-day tRNS+FMT protocol to tDCS+FMT and sham stimulation+FMT in individuals with tetraplegia.

**Specific Aim 2:** Compare effects on motor and sensory function of a 3-day tRNS+FMT protocol to tDCS+FMT and sham stimulation+FMT in individuals with tetraplegia.

**3.0 Study Design**

**3.1 Definitions**

AMT: active motor threshold

EMG: electromyography

FMT: fine motor training

MSO: maximum stimulator output

MVC: maximum voluntary contraction

NIBS: non-invasive brain stimulation

RMT: resting motor threshold

tDCS: transcranial direct current stimulation

TMS: transcranial magnetic stimulation

tRNS: transcranial random noise stimulation

UE: upper extremity

**3.2 General Selection Criteria**

The following inclusion and exclusion criteria will be confirmed during the interview process with each potential participant.

**3.2.1. Inclusion Criteria:** Potential participants must meet all of the following criteria to be eligible for the study:

* Be 18-75 years of age
* Have cervical (neurological level C1-C8) SCI occurring more than a year ago
* Have a motor incomplete injury (ASIA/ISNCSCI C or D)
* Have self-reported functional limitation in at least one UE
* Ability to voluntarily move thumb or index finger in at least one UE
* Ability to voluntarily move their wrist in both UE
* Ability to follow multiple step commands
* Ability to communicate pain or discomfort
* Ability and willingness to consent to participate in the study and authorize use of protected health information.

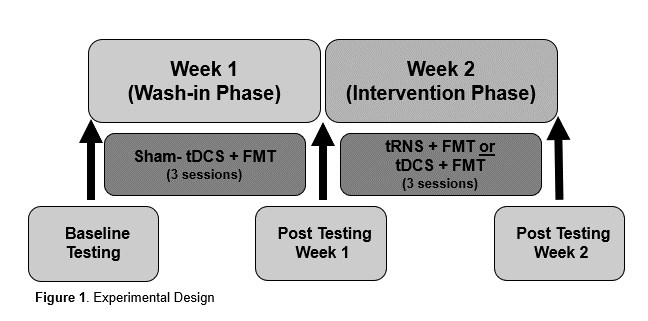
**3.2.2 Exclusion Criteria:** Potential participants will be excluded from the study if they meet any of the following criteria.

* Implanted metallic device in the head and/ or pacemaker
* History of seizures
* History of frequent and/ or severe headaches
* Received Botulinum toxin injection in the tested UE in the last 3 months
* Damage to the nerves of the arms/ hands (lower motor neuron damage) as documented in medical record, per participant report, or during in-person screening
* Prior tendon or nerve transfer surgery
* Severe pain or hypersensitivity of the arm/ hand that would limit participation in fine motor training
* Severe contractures of the arm/ hand that would limit participation in arm and hand training
* Current pregnancy

**3.3 Procedures**

**3.3.1 Study Design**

This is a 2-week wash-in control study. The primary objective is to compare the effects of combined tRNS+ FMT protocol to tDCS+ FMT and sham-stimulation+ FMT protocol on functional recovery in individuals with tetraplegia. Upon enrollment, participants will be randomized to receive tRNS or tDCS (12 per group). During the first week (wash-in phase), all participants will complete 3 sessions of sham-tDCS while practicing unimanual and bimanual functional tasks (FMT). In the second week (intervention phase), participants will receive either tRNS or tDCS during the FMT. All participants will complete three testing sessions: prior the wash-in phase (baseline testing), after the completion of the wash-in phase (post- testing week 1), and after the completion of the intervention phase (post- testing week 2) (Figure 1).



**3.3.2 Schedule of Events**

**Screening, consent and enrollment.** Study staff will meet with prospective participants to explain study details and screen participants to determine whether they meet eligibility criteria. Once eligibility and desire to participate in the study has been confirmed, informed consent will be obtained from the participants by a member of the investigative team authorized to consent for this study. The conversation is expected to include a thorough discussion of what is expected to happen during the study, risks and benefits of study participation, and any possible alternatives. Subjects will be given sufficient time to review the documents and ask questions. All participants will be informed that their involvement in the study is voluntary and that they may withdraw at any time while their medical care will not be affected by their decision on whether or not to participate. If they decide to participate, they will then sign the informed consent document and a copy will be given to them. Upon enrollment in the study, demographic characteristics and medical history of the participant will also be obtained.

All the participants will complete 3 testing sessions and 6 intervention sessions for the purposes of this study.

**Testing Procedures**

Each participant will undergo a neural excitability assessment and a comprehensive clinical evaluation, clinical accessors will be blinded to the subject’s group assignment. In particular, cortical excitability will be tested using corticomotor evoked potentials. Unimanual and bimanual performance will be assessed with the Grasp and Release Test (GRT) [12] and the Chedoke Arm and Hand Activity Inventory (CAHAI) [13], respectively. The revised Nottingham Sensory Assessment (rNSA) [14] and the tactile sensation subtest of the Graded Redefined Assessment of Strength Sensibility and Prehension (GRASSP) [15] will be administered to identify sensory impairments. Also, pinch and grasp strength will be quantified. The testing procedures will be repeated 3 times as described in the design section. The estimated time for each testing session will be approximately 3.5 hours.

***Cortical excitability*** will be assessed with transcranial magnetic stimulation (TMS). Motor evoked potentials (MEPs) of thenar, first dorsal interosseous, hypothenar, and wrist extensors muscles will be recorded. These muscles have been chosen because they play a key role in UE function for persons with tetraplegia. Specifically, thenar, first dorsal interosseous, and hypothenar are essential for functional hand activities, while wrist extensors allow for the formation of active tenodesis grasp in participants who have only trace amounts of intrinsic and/or extrinsic hand muscle control.

The MEPs will be induced using a Magstim 200 stimulator (The Magstim Company Ltd., Whitland, UK) with a figure-of-8 or round coil. The figure-of-8 coil will be positioned over C3 or C4 hand representation areas with the handle pointing 45 degrees posteriorly and laterally, as this position is known to most directly activate the corticospinal tract. The round coil will be used in the event of trace and/or absent muscle responses in the distal UE muscle groups (thenar, first dorsal interosseous, hypothenar) and will be positioned approximately over the Cz (vertex) area. Previous research has shown that using a round coil over Cz can induce responses from UE distal muscle groups in non-injured [16] and persons with SCI [17, 18]. In addition, observations from our lab suggest that in persons with SCI using the round coil over the vertex area (± 1 cm anterior, posterior, lateral) results in more robust MEP UE muscle responses compared to using the figure-of-8 coil over the hand representation areas (C3, C4) in individuals with chronic cervical SCI. A potential explanation is that the round coil stimulates a larger area of the brain than the more focal figure-of-eight coil, thus increasing the likelihood of obtaining distal upper extremity muscle responses during TMS. To ensure reliable positioning of the coil, a tight-fitting cap will be used for each participant. Markings of the inion, ears, a distance of 10 cm from the nasion, and vertex will be drawn on the cap during the first testing session and will be used as a reference in the subsequent sessions. Surface electromyography (EMG) signals will be recorded from the target muscles (Motion Laboratory Systems, Baton Rouge, LA). EMG sensor placement will be verified through observation of the raw EMG signal when manual resistance is applied to resist movement generated by the target muscle. The skin under each EMG sensor will be swabbed with alcohol and abraded with a mild abrasive cream (Nuprep; Weaver and Company, CO, USA) and the sensor will be secured in place by using a piece of hypoallergenic tape. Co-Flex, a latex free cohesive flexible bandage (Andover Healthcare, Inc.) can be used to further secure the EMG sensor placement. EMG signals will be amplified (x1k) and band pass filtered (10-20kHz). EMG will be digitized using a data acquisition interface (Power 1401, CED, Cambridge, England) controlled by Signal 2 software (CED) and stored on a computer for offline analysis. Finally, to ensure consistent positioning between testing sessions the EMG sensors will be traced onto a transparent sheet.

Initially, *resting and active motor thresholds* (RMT & AMT) will be recorded. RMT will be defined as the minimum output of the stimulator that will induce a reliable MEP (≥ 50μV) in 50% of the applied stimuli in resting muscles [19]. AMT will be defined as the minimum stimulus intensity that will elicit reliable MEP (higher responses compared to the muscular background activity [19] in 50% of the applied stimuli while participants maintain 10-20% of their maximum pinch strength as registered in a hand-held dynamometer. Visual feedback will be provided, to ensure that participants maintain the pinch in the target range (10-20% MVC) while TMS is applied. Participants will be given rest breaks as needed to prevent the influence of fatigue on the testing procedures. MEP peak-to-peak amplitude at 120%AMT and at a predefined percentage of the maximum stimulator output will be also obtained

***Unimanual Function***. The GRT is developed to assess unimanual performance in individuals with tetraplegia [12] and has good test-retest reliability [20]. For this test, participants will be required to grasp, move, and release six objects of different size and weight. Three of these objects (ie, peg, paperweight, and fork) will be manipulated with lateral prehension and 3 with palmar prehension (ie, block, can, videotape). For each task, the number of successful and unsuccessful attempts is 30 s for a total of 3 trials will be recorded for each UE.

***Bimanual Function*** will be assessed with the CAHAI-9 Version [13]. This test consists of 9 functional tasks that require bimanual coordination. The test items are: opening a jar of coffee, calling 911, drawing a line with a ruler, pouring a glass of water, wringing out a washcloth, doing up to 5 buttons, drying up with a towel, putting toothpaste on toothbrush, and cutting a medium resistance putty using a knife and a fork. Each item is graded on a 7-point activity scale (1: participant expends ≤25% of the effort for the task; 7: participant performs the task safely without modifications, assistive device, or aids, and within reasonable time) with higher scores suggesting better bilateral function. This test has good reliability and construct validity for individuals with stroke [13]. Our lab has established its validity in individuals with cervical SCI [21].

***Sensory Function*.** The sensation subtests of GRASSP will be used to quantify sensory function. GRASSP is a reliable and valid tool developed for individuals with tetraplegia [15]. The high responsiveness and sensitivity of GRASSP allows for identification of UE neurological and functional changes associated with the efficacy of therapeutic interventions [22]. During testing, Semmes and Weinstein Monofilaments will be applied on 3 dorsal and palmar sensory test locations in each hand. Each location is scored from 0 (no response) to 4 (participant is able to perceive the smallest diameter monofilament). For each hand, a total score will be computed by adding the individual scores for each site (range 0- 24).

The rNSA [14] is a standardized screening tool and a thorough sensory assessment approach utilizing simple and clinically available tools. It consists of 4 subscales: tactile sensation, proprioception, stereognosis, and two-point discrimination. All the subscales, except proprioception, will be assessed on a 3-point scale from 0 (the investigated sensory modality is absent) to 2 (the investigated sensory modality is normal). For the proprioception subscale, a 4-point scale will be used (0: no appreciation of movement taking place; 1: appreciation of movement taking place but direction is incorrect; 2: appreciation of the direction of movement taking place but inaccurate positioning; 3: appreciation of the direction of movement taking place and accurate positioning). For the purposes of this test, only the UE will be evaluated. The rNSA has acceptable inter-rater reliability for individuals with stroke [14]; nevertheless, it has never been applied to people with SCI. To examine its concurrent validity, we will validate it against the sensation domains of GRASSP. A score for each UE will be obtained.

***Strength.*** Changes in pinch and grasp strength will be assessed with a mechanical pinch gauge and hydraulic hand dynamometer (B&L Engineering, Santa Ana, CA). Research has shown that pinch and grasp strength in persons with SCI is improved following intervention comprised of neuromodulatory stimulation and training [2, 4, 23]. Participants will be in a seated position with their shoulder in neutral, elbow at 900 flexion, and forearm and wrist in neutral position. A total of 3 maximum voluntary contractions for each side will be collected with a 1-min resting period between trials. The average force in 3 trials will be recorded for each UE.

**Intervention procedures**

Combined stimulation and training protocols can lead to greater improvements in functional performance than stimulation or training alone [24]. Therefore, participants will receive a combined intervention for 6 days (3 days of sham-tDCS and 3 days of tRNS or tDCS during FMT). In each training session, a questionnaire prior and following the intervention will be administered. This questionnaire will include questions regarding factors that can potentially influence the effects of tRNS or tDCS and track sensations associated with its application. This questionnaire administration has been recommended for safety monitoring by recently published guidelines on low intensity transcranial electrical stimulation [25].

***tRNS.*** tRNS will be delivered by using a commercially available NIBS device (Starstim 8, Neuroelectrics, Cambridge, MA). The applied stimulation will be of random frequency (0- 500Hz) and intensity (gaussian white noise). The intensity of this noise signal follows the normal distribution with a mean value of 0μΑ, standard deviation of 334μΑ, and direct current offset of 0μΑ; thus, 99% of all randomly generated intensity values will range from -1002 to +1002μΑ. For NIBS approaches, this intensity level (ie, 2000μΑ) has been shown to be more effective in individuals with chronic SCI[26].

The stimulation will be delivered during the first 20 min of training using two rubber electrodes, placed in two saline-soaked sponges (35 cm2 per sponge). This stimulation duration has been previously used to increase behavioral performance in healthy individuals [27], and it is within the safety limits for applying NIBS approaches [25]. For the tRNS group the active electrode will be positioned over the primary motor cortex representation area (C3 or C4) of the more impaired hand- as long as it has intrinsic control and reliable MEP responses (active thenar and/ or first dorsal interosseous), and the reference electrode will be placed over the contralateral orbit. This stimulation set-up has been shown to be the optimal montage to enhance excitability of hand muscles [28].

***tDCS****.* tDCS will be delivered by using a commercially available NIBS device (Starstim 8, Neuroelectrics, Cambridge, MA). The stimulation will be delivered during the first 20 min of training using two rubber electrodes, placed in two saline-soaked sponges (35 cm2 per sponge). The active electrode (anode) will be positioned over the hand representation areas of the motor cortex (C3 or C4) whereas the reference electrode (cathode) will be positioned over the contralateral orbit as described above. The intensity of the stimulation will be at 2000μA as literature indicates that leads to increases in cortical excitability in individuals with chronic SCI [26].

***Sham-tDCS***. The sham-tDCS (control condition) will be delivered using the same commercially available NIBS device (Starstim 8, Neuroelectrics, Cambridge, MA). At the beginning and end of the sham-control intervention, a brief ramp-up and ramp-down of tDCS lasting 60 s will be applied, while no stimulation will be delivered in between. The sham-tDCS will be applied for a total of 20 min at the beginning of the training sessions. The electrode placement for sham tDCS will be identical to the process described above.

***FMT.*** Subjects in both stimulation groups will participate in repetitive task practice of unilateral and bilateral functional activities for a total of 45- 60 min. Unimanual practice will involve tasks primarily performed by the more impaired hand. The protocol will consist of a variety of repetitive functional tasks. Specifically, a variety of tasks will be used to promote subjects’ motivation and active participation, which are key components for a successful training protocol. Further, in light of evidence supporting that sensorimotor training can be beneficial for neurologic populations with motor and sensory impairments [29], we will include tasks focusing on sensory discrimination (see for example Borstad et al, 2013 [29]). Based on each participant’s performance, the training will be structured to progress in difficulty. Specifically, to ensure sensory demand, participants will be instructed to perform some of the tasks without visual feedback. We hypothesize that this protocol can facilitate both motor and sensory recovery and when combined with a NIBS approach its efficacious effect will be augmented.

**3.3.3 Laboratories**

The Hulse Spinal Cord Injury Research Laboratory will be used for all study sessions.

4.0 Adverse Events

**4.1 Definitions**

Adverse Event (AE) - any untoward physical or psychological occurrence or undesirable and unintended effect for a subject that may present itself during interventions and interactions used in the research or the collection of identifiable private information under the research, regardless of whether there may or may not be a relationship with the research intervention.

Unanticipated Adverse Event – any adverse event, the specificity, frequency or severity of which is not consistent with either:

* The known or foreseeable risk of adverse events associated with the procedures involved in the research that are described in the protocol related-documents, such as the IRB-approved research protocol, any applicable investigator brochure, and the current IRB-approved informed consent document, and other relevant sources of information, such as product labeling and package inserts; or
* The expected natural progression of any underlying disease or condition of the subject(s) experiencing the adverse event

Anticipated Adverse Event: - an adverse event that is not an unanticipated adverse event. The following adverse events are considered as anticipated:

Unanticipated Adverse Device Effect – any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

Related or possibly related to the research: - an event is related to the research if, in the opinion of the Principal Investigator, it could not have been produced by the subject’s clinical condition or environment, follows a known pattern of response to intervention, disappears or decreases with reduction in dose or cessation of intervention and/or recurs with re-exposure and/or it was more likely than not to be the result of the collection/disclosure of identifiable private information in the research and/or the interventions used in the research.

Unrelated to the research: - an adverse event is unrelated to the research if, in the opinion of the Principal Investigator, the adverse event is clearly due to extraneous causes (e.g., underlying disease or environment), does not follow a known pattern of response to intervention, and/or does not reappear or worsen with re-introduction of the intervention.

Serious Adverse Event: an event is considered serious if it results in death, is life-threatening, requires hospitalization or prolongation of hospitalization, causes persistent or significant disability or incapacity, is a birth defect or congenital malformation, represents, in the Principal Investigator’s judgment, other significant hazards or potentially serious harm to research subjects or others, or any other event as described in the research process.

**4.2 Reporting**

Each subject will be observed and queried in a nonspecific fashion at each contact during the study for any new or continuing symptoms since the last contact. All adverse events will be reported on the appropriate electronic Case Report Form (eCRF). Details will include the type of event, date of onset, duration, intensity, causality relationship to the study drug(s) (if applicable), and outcome. Wherever possible, a diagnosis rather than symptom(s) will be reported.

If an adverse event should occur, every attempt will be made to obtain as much information as possible about event evaluation and outcome. Documents of this follow-up will be maintained with the patient’s study records.

If a serious adverse event occurs, the treatment will be interrupted or discontinued at the physician investigator's discretion.

All protocol deviations will be reported to the investigator and the Institutional Review Board (IRB).

All adverse events will be reported to the IRB. All serious adverse events will be reported immediately to the IRB and the FDA (if applicable).

Endpoints will be adjudicated by the Principal Investigator. A written report detailing the endpoint adjudication will be provided by the Principal Investigator.

* 1. **Potential Side Effects**

TRNS has been shown to have minimal risk as it delivers a low alternating current and is a polarity-independent approach with excellent tolerability and no risk of burns [27, 30, 31]. Comparative studies on self-perceived stimulation-induced sensations indicate that tRNS is indistinguishable from sham stimulation and is imperceptible to most individuals, so blinding is more effective compared to other NIBS approaches [27, 30, 31]. TDCS has been shown to have minimal risk and has been associated with temporary itching, tingling, and burning sensations. It has also been associated with skin irritation, and superficial blistering, and occasionally headaches; however, the occurrences of these side effects is rare. TMS will be uncomfortable for some individuals, and does have the potential to cause headaches. In rare cases, repetitive TMS (a form of high frequency TMS that will not be used in this study) has been associated with seizure and hearing loss. However, these side effects have not been reported with the use of single pulse TMS for diagnostic purposes.

* 1. **Safety**

This study will be conducted in accordance with the principles of Good Clinical Practice as outlined in the Declaration of Helsinki and the International Conference on Harmonization.

All efforts will take place to ensure patient safety. Each subject will be monitored for safety throughout the trial utilizing clinical evaluations and laboratory markers.

Laboratory markers and/or clinical evaluations that are out of normal range will be recorded as adverse events and reviewed with the investigator.

SAE’s noted to be intervention related will be reported as appropriate, and interventions will be discontinued per the decision of the investigator.

All subjects will be triaged to the appropriate medical care based on investigators decision upon review of abnormal events.

.

**5.0 Data Management**

Following completion of the consenting process (including informed consent and Authorization for Use/Disclosure of PHI) and determination that the subject meets all of the inclusion and none of the exclusion criteria, enrollment will occur and data collection will commence.

**5.1 Case Report Forms**

Hospital, office, and research records for any admission or visit (including admission notes, discharge notes, operative reports, test results, and lab reports) are considered source documentation and will be collected and reviewed to confirm clinical events and may be utilized for data analysis. Data will be collected on all subjects via an eCRF. The CRF will contain no subject names. The subject code field will be a patient study number, numbered sequentially as entered into the electronic database. A separate master code list will be constructed by the investigator/study coordinator that will list the patient name with the designated subject code. This list will be maintained in a password protected file to which access is restricted.

Upon completion of data collection, each participant’s eCRFs will be signed by the Principal Investigator.

**5.2 Database**

The research database will be maintained in electronic form by Dr. Anastasia Zarkou. Statistical analyses will be the responsibility of Dr. Zarkou. The electronic database will be backed up per institutional guidelines.

**5.3 External Documentation:**

During administration of the patient questionnaire, if it is identified that a subject sought treatment from a source outside of the Hulse Spinal Cord Injury Research Laboratory after enrollment into the protocol, additional data will be obtained from external physician offices or hospitals to document and verify events. All data will be entered onto the follow-up eCRF.

**5.4 Quality Control**

Dr. Anastasia Zarkou will fulfill the responsibilities of the data manager including collecting and tracking data forms and instituting quality control measures for data entry verification and study compliance. She will request further documentation such as physician and/or procedure notes when complications are observed and reported. Dr. Zarkou will also be responsible for auditing the database and confirming the overall integrity of the data. She will ensure that all information pertaining to significant new developments and unanticipated adverse events are provided to the appropriate regulatory authorities, the investigators, and to the IRB.

Monitoring of the study will be conducted at regular intervals in order to ensure proper study operations and maintain Good Clinical Research Practice. These inspections are conducted in order to verify adherence to the protocol and the completeness and accuracy of the data being entered into the eCRF.

**6.0 Statistical Considerations**

**6.1 Statistical Analyses**

All data will be analyzed using SPSS (SPSS Inc., Chicago. IL, USA) with the level of significance set at p< 0.05. Pearson correlation coefficient will be computed to examine the relationship between rNSA and sensation subtests of GRASSP and establish the validity of the rNSA in individuals with SCI.

To investigate the efficacy of a combined NIBS and FMT protocol compared to sham-tDCS+FMT protocol on cortical excitability, motor and sensory function, all the outcome measures of interest will be examined separately by a two-way repeated measures ANOVA with 2 within factors (time: baseline testing, post testing week 1, post testing week 2; intervention: tRNS+FMT or tDCS+FMT, and sham-tDCS+FMT). Post-hoc analysis will include paired t-tests corrected for multiple comparisons. To investigate if tRNS results in greater improvements compared to tDCS when combined with FMT, separate 2 x 2 mixed model repeated measures ANOVA with time (baseline testing, post testing week 1, post testing week 2) as the within-subjects factor and group (tRNS+FMT, tDCS+FMT) as the between-subjects factor will be performed for the outcome measures of interest. Paired and unpaired t-tests will be performed for post-hoc analysis. We will also report effect sizes for all outcome measures.

**6.2 Sample Size**

Published recommendations in rehabilitation research suggest that a well-designed pilot study is important to produce preliminary data and provide an estimate of the variance that can be used in a formal sample size calculation when planning for a subsequent larger study [32–34].The literature suggests that a sample size of 12 individuals per group is appropriate since it allows for precise estimation of mean values and variability. Further increase in N does not make any profound difference in sample estimations while any precision gains are outweighed by practicalities regarding recruitment and funding limitations [32]. Therefore, in the proposed pilot study we plan to enroll a total of 24 individuals with tetraplegia (12 per stimulation group) to investigate the efficacious effect of a combined tRNS+ FMT protocol on UE function. Using a sample size of 24, an alpha of 0.05 and a power of 0.80, we anticipate an effect size of 0.59. This effect size is higher than 0.4 that has been recommended to be meaningful in clinical trials [34]. Finally, to account for 15% attrition we will recruit 28 subjects over 2 years to meet the target sample size.

**6.3 Estimated Duration of the Study**

This study will be completed during a 2-year period from the time of first enrollment. For all aims, subject recruitment, data collection and reduction will occur continuously during Years 1 & 2. Data analysis and manuscript preparation/submission will take place in Year 2. To monitor data quality, quality assurance checks will be performed throughout the duration of the study.

**7.0 Ethical, Regulatory, and Administrative Considerations**

**7.1 Informed Consent**

The principles of informed consent are described in the Code of Federal Regulations 21 CFR, part 50 and 45 CFR, part 46. Once the Investigator has determined the patient’s eligibility for the study, the background of the proposed study and the benefits and risks of the procedures and study must be explained to the subject. The subject must be able to comprehend the informed consent form and must sign it prior to performing any study specific procedures or prior to receiving medication. The subject will receive a copy of the informed consent. The original signed informed consent and Authorization for Use/Disclosure of PHI will be maintained in the subject’s research chart. Only those subjects who sign the IRB approved informed consent prior to participation are eligible to be in the study. Failure to provide written informed consent renders the patient ineligible for the study.

**7.2 Confidentiality**

All information and data collected and/or sent to study personnel concerning subjects or their participation in this study will be considered confidential. Only authorized personnel will have access to these confidential files. Authorized FDA personnel have the right to inspect and copy all records pertinent to this trial. All data used in the analysis and reporting of this evaluation will be without identifiable reference to any patient.

**7.3 Institutional Review**

The Principal Investigator will obtain approval for the study from IRB. All changes to the protocol must be reviewed and approved prior to implementation. The Principal Investigator will be responsible for obtaining annual IRB renewal through the duration of the study, or more frequently if required by the IRB. As Study Coordinator and Principal Investigator, Dr. Anastasia Zarkou will maintain all regulatory documents.

**7.4 Protocol Interpretation and Compliance**

The procedures defined in the protocol will be carefully reviewed by the Investigator and research staff prior to the time of study initiation to ensure appropriate interpretation and implementation. Any changes to the protocol in the form of an amendment must be submitted to the IRB.

**7.5 Completion of Case Report Forms**

The Principal Investigator or her designee will be responsible for completing, in a timely manner, an eCRF for each patient who is registered to participate in this study. The Principal Investigator will sign and date the indicated places on the eCRF. This signature will indicate that a thorough inspection of the data therein has been made and will thereby certify the contents of the form.

**7.6 Maintenance of Study Documentation**

It is the responsibility of the Principal Investigator to maintain a comprehensive and centralized filing system of all study-related documentation, which is suitable for inspection at any time by the FDA. Elements should include:

* Subject files – containing the completed CRFs, supporting source documentation, and the Informed Consent.
* Regulatory Files – containing the protocol with all amendments and accountability records.
  1. **Final Study Report**

Upon completion of the study, the Principal Investigator is required to submit a final study summary report for the patients enrolled in the study

* 1. **Record Retention**

All records, which are part of this study, will be retained for a period of two years following discontinuation/termination of the study.

**8.0 Study Medication/Device/Intervention/Other Procedure Details**

**Blinding:** Participants and assessors of the clinical measures will remain blinded to the randomized intervention order throughout the duration of the study.

**Assignment of Intervention:** Participants will be randomized to receive tRNS or tDCS. During the first week, all participants will complete 3 sessions of sham-tDCS while practicing unimanual and bimanual functional tasks (FMT). In the second week, participants will receive either tRNS or tDCS during the FMT.

**Dosing and Treatment:** Subjects will participate in a total of six 1-hour long FMT + stimulation (tRNS, tDCS, or sham-tDCS) sessions – 3 per week for 2 weeks.

**Identity of Medication/Device/Treatment:** TRNS and tDCS will be delivered using a commercially available NIBS device (Starstim 8, Neuroelectrics, Cambridge, MA).

1. **References**

1. Field-Fote EC. Spinal cord injury rehabilitation. F.A. Davis; 2009.

2. Gomes-Osman J, Field-Fote EC. Improvements in hand function in adults with chronic tetraplegia following a multiday 10-Hz repetitive transcranial magnetic stimulation intervention combined with repetitive task practice. J Neurol Phys Ther. 2015;39:23–30.

3. Gunduz A, Rothwell J, Vidal J, Kumru H. Non-invasive brain stimulation to promote motor and functional recovery following spinal cord injury. Neural Regen Res. 2017;12:1933.

4. Gomes-Osman J, Field-Fote EC. Cortical vs. afferent stimulation as an adjunct to functional task practice training: a randomized, comparative pilot study in people with cervical spinal cord injury. Clin Rehabil. 2015;29:771–82.

5. López-Alonso V, Cheeran B, Río-Rodríguez D, Fernández-Del-Olmo M. Inter-individual variability in response to non-invasive brain stimulation paradigms. Brain Stimul. 2014;7:372–80.

6. Inukai Y, Saito K, Sasaki R, Tsuiki S, Miyaguchi S, Kojima S, et al. Comparison of Three Non-Invasive Transcranial Electrical Stimulation Methods for Increasing Cortical Excitability. Front Hum Neurosci. 2016;10.

7. Rawji V, Ciocca M, Zacharia A, Soares D, Truong D, Bikson M, et al. tDCS changes in motor excitability are specific to orientation of current flow. Brain Stimul. 2018;11:289–98.

8. Terney D, Chaieb L, Moliadze V, Antal A, Paulus W. Increasing Human Brain Excitability by Transcranial High-Frequency Random Noise Stimulation. J Neurosci. 2008;28:14147–55.

9. Aihara T, Kitajo K, Nozaki D, Yamamoto Y. How does stochastic resonance work within the human brain? - Psychophysics of internal and external noise. Chem Phys. 2010;375:616–24.

10. Prichard G, Weiller C, Fritsch B, Reis J. Effects of different electrical brain stimulation protocols on subcomponents of motor skill learning. Brain Stimul. 2014;7:532–40.

11. Snoek GJ, IJzerman MJ, Hermens HJ, Maxwell D, Biering-Sorensen F. Survey of the needs of patients with spinal cord injury: impact and priority for improvement in hand function in tetraplegics. Spinal Cord. 2004;42:526–32.

12. Wuolle KS, Van Doren CL, Thrope GB, Keith MW, Peckham PH. Development of a quantitative hand grasp and release test for patients with tetraplegia using a hand neuroprosthesis. J Hand Surg Am. 1994;19:209–18.

13. Barreca SR, Stratford PW, Lambert CL, Masters LM, Streiner DL. Test-Retest Reliability, Validity, and Sensitivity of the Chedoke Arm and Hand Activity Inventory: A New Measure of Upper-Limb Function for Survivors of Stroke. Arch Phys Med Rehabil. 2005;86:1616–22.

14. Lincoln N, Jackson J, Adams S. Reliability and Revision of the Nottingham Sensory Assessment for Stroke Patients. Physiotherapy. 1998;84:358–65.

15. Kalsi-Ryan S, Curt A, Verrier MC, Fehlings MG. Development of the Graded Redefined Assessment of Strength, Sensibility and Prehension (GRASSP): reviewing measurement specific to the upper limb in tetraplegia. J Neurosurg Spine. 2012;17:65–76.

16. Groppa S, Oliviero A, Eisen A, Quartarone A, Cohen LG, Mall V, et al. A practical guide to diagnostic transcranial magnetic stimulation: Report of an IFCN committee. Clinical Neurophysiology. 2012;123:858–82.

17. Calancie B, Alexeeva N, Broton JG, Suys S, Hall A, Klose KJ. Distribution and latency of muscle responses to transcranial magnetic stimulation of motor cortex after spinal cord injury in humans. J Neurotrauma. 1999;16:49–67.

18. Smith H, Savic G, Frankel H, Ella way P, Maskill D, Jamous M, et al. Corticospinal function studied over time following incomplete spinal cord injury. Spinal Cord. 2000;38:292.

19. Nitsche MA, Seeber A, Frommann K, Klein CC, Rochford C, Nitsche MS, et al. Modulating parameters of excitability during and after transcranial direct current stimulation of the human motor cortex. J Physiol. 2005;568:291–303.

20. Mulcahey MJ, Smith BT, Betz RR. Psychometric rigor of the Grasp and Release Test for measuring functional limitation of persons with tetraplegia: a preliminary analysis. J Spinal Cord Med. 2004;27:41–6.

21. Hoffman LR, Field-Fote EC. Functional and Corticomotor Changes in Individuals With Tetraplegia Following Unimanual or Bimanual Massed Practice Training With Somatosensory Stimulation. J Neurol Phys Ther. 2010;34:193–201.

22. Kalsi-Ryan S, Beaton D, Ahn H, Askes H, Drew B, Curt A, et al. Responsiveness, Sensitivity, and Minimally Detectable Difference of the Graded and Redefined Assessment of Strength, Sensibility, and Prehension, Version 1.0. J Neurotrauma. 2016;33:307–14.

23. Gomes-Osman J, Field-Fote EC. Bihemispheric anodal corticomotor stimulation using transcranial direct current stimulation improves bimanual typing task performance. J Mot Behav. 2013;45:361–7.

24. Beekhuizen KS, Field-Fote EC. Sensory stimulation augments the effects of massed practice training in persons with tetraplegia. Arch Phys Med Rehabil. 2008;89:602–8.

25. Antal A, Alekseichuk I, Bikson M, Brockmöller J, Brunoni AR, Chen R, et al. Low intensity transcranial electric stimulation: Safety, ethical, legal regulatory and application guidelines. Clin Neurophysiol. 2017;128:1774–809.

26. Murray LM, Edwards DJ, Ruffini G, Labar D, Stampas A, Pascual-Leone A, et al. Intensity Dependent Effects of Transcranial Direct Current Stimulation on Corticospinal Excitability in Chronic Spinal Cord Injury. Arch Phys Med Rehabil. 2015;96:S114–21.

27. Fertonani A, Pirulli C, Miniussi C. Random noise stimulation improves neuroplasticity in perceptual learning. J Neurosci. 2011;31:15416–23.

28. Moliadze V, Antal A, Paulus W. Electrode-distance dependent after-effects of transcranial direct and random noise stimulation with extracephalic reference electrodes. Clin Neurophysiol. 2010;121:2165–71.

29. Borstad AL, Bird T, Choi S, Goodman L, Schmalbrock P, Nichols-Larsen DS. Sensorimotor Training and Neural Reorganization After Stroke. J Neurol Phys Ther. 2013;37:27–36.

30. Terney D, Chaieb L, Moliadze V, Antal A, Paulus W. Increasing Human Brain Excitability by Transcranial High-Frequency Random Noise Stimulation. J Neurosci. 2008;28:14147–55.

31. Pirulli C, Fertonani A, Miniussi C. The Role of Timing in the Induction of Neuromodulation in Perceptual Learning by Transcranial Electric Stimulation. Brain Stimul. 2013;6:683–9.

32. Moore CG, Carter RE, Nietert PJ, Stewart PW. Recommendations for planning pilot studies in clinical and translational research. Clin Transl Sci. 2011;4:332–7.

33. Julious SA. Sample size of 12 per group rule of thumb for a pilot study. Pharm Stat. 2005;4:287–91.

34. Dobkin BH. Progressive Staging of Pilot Studies to Improve Phase III Trials for Motor Interventions. Neurorehabil Neural Repair. 2009;23:197–206.