SCIMS PROJECT 1: ENHANCING CORTICOSPINAL ACTIVATION FOR IMPROVED WALKING FUNCTION.

ClinicalTrials.gov#: NCT03237234

Principal Investigator
Edelle C Field-Fote, PhD
2020 Peachtree Road, NW
404-603-4274
edee_field-fote@shepherd.org

Study Coordinator:
Nicholas H. Evans, MHS

Document History
Original Protocol: Version 2.0 11/15/2016

Statement 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).

_____ _____

Principal Investigator Signature Date

_____

Principal Investigator Printed Name

Table of Contents

1.0 Introduction

Spinal cord injury (SCI) occurs suddenly and unexpectedly to an estimated 12,500 people each year, and over 275,000 people are thought to be living with SCI in the United States alone.[1] Restoration of walking is cited as a priority among persons with SCI, regardless of severity, chronicity, or age at injury.[2] To address this priority, numerous approaches have been implemented to restore locomotor function either through physical training [3] and/or through the use of rehabilitation technology.[4] High-tech approaches to improving function after SCI are exciting and receive much media attention; however, a recent systematic review found that, of the many interventions aimed at improving motor function, the strongest evidence supports those strategies that include a physical training component.[5] Despite the potential benefits, current rehabilitation strategies aimed at the restoration of walking function often require access to intensive training programs conducted over extended periods of time and to facilities and equipment that may not be available in the homes or communities in which individuals with SCI live. Therefore, it would seem that the keys to optimizing long-term recovery of function are to identify those therapies that extract the greatest improvement over the shortest period of time and to identify training approaches that are readily accessible in the home environment. These considerations, along with three important findings in the research, form the basis of this project.

First, evidence from a large multi-million dollar locomotor training study involving persons with stroke found that a well-designed exercise program can be as effective for improving walking function as an intensive locomotor training program.[6] Second, a single session of moderate-intensity exercise has been found to promote motor skill acquisition, presumably through neurotrophic mechanisms, and to reduce spasticity.[7-9] In persons with SCI, 10 minutes of moderate-intensity activity (54% of maximal heart rate) was associated with a 1.5-fold increase in serum levels of neurotrophic factor.[7] Studies investigating locomotor training in persons with SCI [10] and in spinal rats suggests that exercise can restore the endogenous inhibition that is lost after SCI [11], which may affect the extent and severity of spasticity.[9] Third, supraspinal centers are known to be involved in locomotion, yet studies aimed at improving walking function have focused almost exclusively on activation of spinal circuits.[12] In rodent models with SCI, evidence indicates that recovery of locomotor function depends largely on activation of spared supraspinal pathways [13] and that there is an increased amplitude of lower limb motor evoked potentials following intensive locomotor training in humans with SCI.[14] These studies suggest a relationship between the strength of corticospinal drive and walking function. Therefore, it seems plausible that walking function can be improved by increasing the effectiveness and activation of spared descending pathways.

A preponderance of the evidence suggests that corticospinal drive is increased by ***combining*** moderately intense motor training with stimulation of supraspinal centers. This notion is supported by research demonstrating that intensive motor training aimed at increasing corticospinal drive improves muscle activation and walking function.[15] Furthermore, non-invasive brain stimulation has been shown to improve lower extremity strength, lower-limb control, gait, and balance.[16-19] Cortical stimulation with repetitive transcranial magnetic stimulation (rTMS) has been associated with improved walking function in persons with SCI [20], although this form of stimulation has inherent drawbacks that limit its use in clinical settings. An alternative to rTMS is transcranial direct current stimulation (tDCS), which is a low-risk, non-invasive brain stimulation technique involving low-intensity currents delivered to the motor cortex and aimed at modulating cortical excitability. tDCS is affordable and lacks many of the drawbacks associated with rTMS, making it a more clinically accessible technology. Emerging studies in persons with stroke have combined tDCS with moderately intense motor training and have demonstrated increased dorsiflexor control,[16] quadriceps strength,[17, 18] and balance.[17] tDCS may also have the capacity to modulate excitability of spinal networks and to decrease spasticity.[21, 22]

Based on the evidence described above, we put forward two primary goals of the proposed project: *1) determine whether a moderate-intensity training program designed to increase control of gait-related movements can improve functional and neurophysiologic measures,* and *2) determine whether training-related responses are larger when training is augmented with cortical stimulation to increase supraspinal drive.*

2.0 Study Aims and Hypotheses:

2.1 Aim 1. ***Quantify effects of moderate-intensity motor training alone (MT-only group) and moderate-intensity motor training + tDCS (MT+tDCS group) on walking function and balance.*** We hypothesize that training will be associated with improvements in locomotor function (walking speed and step height), balance (Berg Balance Test), and leg muscle strength (quadriceps and plantarflexor muscles). We also hypothesize that gains in the MT+tDCS group will be larger than those of the MT-only group.

2.2 Aim 2. ***Quantify effects of moderate-intensity motor training alone (MT-only group) and moderate-intensity motor training + tDCS (MT+tDCS group) on leg muscle strength and spasticity.*** We hypothesize that training will be associated with We also hypothesize that gains with MT+tDCS group will be larger than those with MT-only.

3.0 Study Design

3.1 Study Sample:

Thirty-five (35) participants with chronic (≥12 months post injury), motor-incomplete SCI (i.e. American Spinal Injury Association Impairment Scale (AIS) C or D)[23] will be recruited for the study. Upon enrollment, participants will be expected to have some, but limited, walking ability (detailed inclusion/exclusion criteria listed below).

3.1.1 Inclusion Criteria:

The research participants must meet all of the following criteria to be eligible for the study:

- Ability and willingness to consent and Authorization for use of PHI;
- Chronic SCI (≥12 months post-injury) at or above the neurological level of T10;
- Age 18-65;
- Able to stand (with or without the aid of an assistive device) for at least 5 minutes;
- Able to advance each leg independently for at least 3 steps. (For over ground locomotion, assistive devices including ankle-foot orthoses [unilateral or bilateral], knee-ankle-foot orthoses [unilateral only], walkers, crutches, or canes may be used);
- Able to rise from sit to stand requiring no more than moderate assistance from one person.

3.1.2 Exclusion Criteria:

The presence of any one of the following criteria would lead to exclusion:

- Inability or unwillingness to consent and Authorization for use of PHI;
- Progressive, or potentially progressive, spinal lesions including degenerative, or progressive vascular disorders of the spine and/or spinal cord;
- Injuries below the neurological spinal level T10;
- History of cardiovascular irregularities;
- Altered cognitive status;
- Presence of orthopedic pathology that would adversely influence participation in the protocol (e.g. knee or hip flexion contractures of greater than 10 degrees);
- Implanted metallic objects in the cranium (e.g. aneurysm clips);
- History of seizures;

NOTE: The use of prescription medications, including a baclofen pump for control of spasticity, will not be a basis for exclusion so long as the dosage has been stable for at least 90 days.

3.2 Procedures:

3.2.1 Research Design:

We propose a double-blinded, randomized, cross-over intervention study consisting of a moderate-intensity motor training circuit of gait-related upright motor control activities. We will also assess whether the addition of tDCS augments the training-related gains. Effects of training (and tDCS) on walking function, balance, strength, and spasticity will be assessed to determine whether there is preliminary evidence of efficacy of this approach.

Stratified randomization: To ensure equivalence of baseline data between groups, stratified randomization will be used. We have shown that persons with SCI who are ambulatory may be classified into one of two groups, with ***"less impaired"*** participants having lower limb motor scores of >15 for one leg and ≥10 for the other leg; participants not meeting this criteria are classified as ***"more impaired***".[3] To ensure equivalent distribution of more- and less-impaired participants between groups, individuals will be stratified into one of these two strata based on the above criteria, with randomization to groups within each stratum.

Schedule of Events:

Individuals will be asked to participate in the study over two, 5 consecutive day periods. Following completion of the fifth day, participants will be asked to take 2 weeks off before returning to the lab to repeat the intervention schedule but in the opposite group in which they originally participated (i.e. MT-only to MT+tDCS; MT+tDCS to MT-only). A detailed schedule of events including testing and intervention timing is provided in Figure 1.

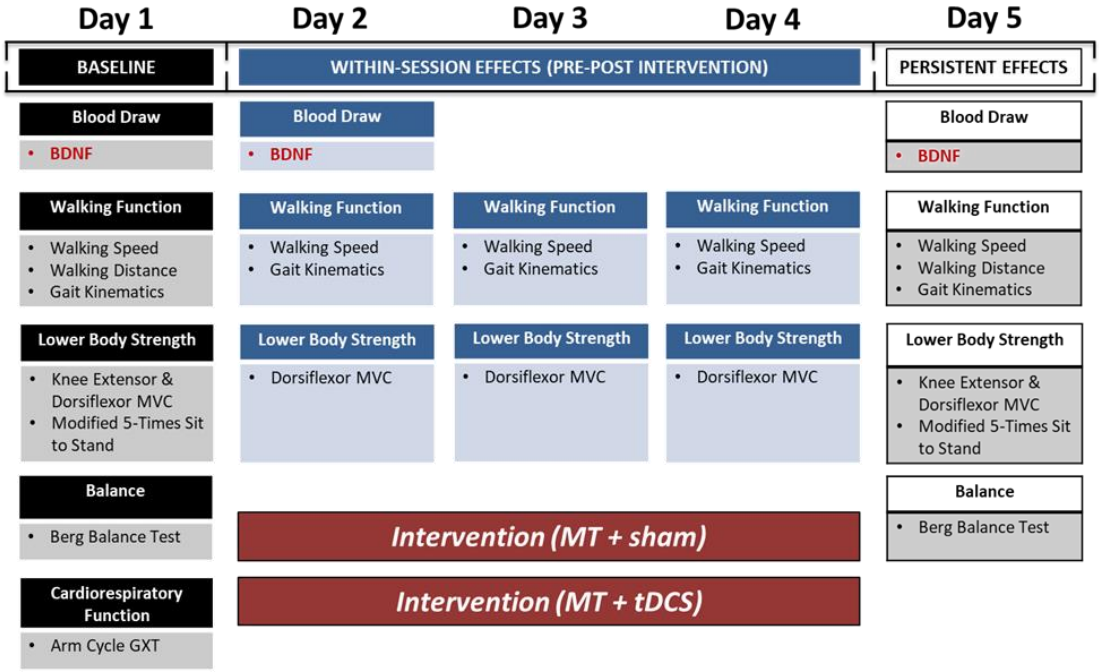


Figure 1. Timing of intervention and outcome measures.

3.2.2 Interventions:

Motor training (MT): The muscles targeted for motor training have been selected based on studies in persons with SCI that have identified the muscle groups that best predict walking ability.[24-27] Motor training (see Figure 2) will consist of a circuit of activities that, with the exception of the toe tap task, will be performed while standing to promote upright control (the toe-tapping activity will be performed in sitting as, in our experience, many people with SCI are unable to perform this activity when the hip and knee are in an extended position). Participants will perform each of the 6 different training activities for one minute each (*with modifications as necessary to allow successful execution*), alternating between legs, until 4 cycles of the circuit have been completed (approximately 25 minutes total). This exercise duration was selected based on prior studies showing that in persons with SCI, 10 minutes of moderate intensity activity (54% age-predicted maximum heart rate) increased serum levels of neurotrophic factor.[7] Furthermore, a single 15-minute session of gait and balance training in combination with tDCS improved walking speed and balance in persons with motor dysfunction.[19]

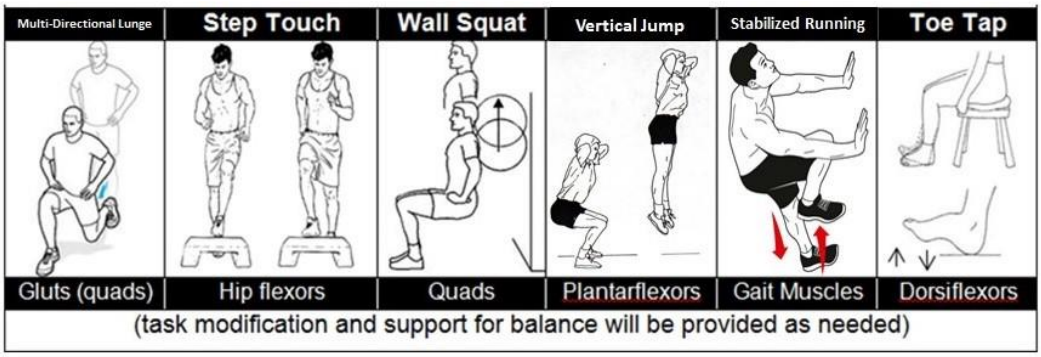


Figure 2. Moderate-intensity motor training activities. Motor training activities will be performed at an intensity intended to attain a target exercise intensity of 40–59% of heart rate reserve (HRR) for 5 of 6 activities; toe tapping (the exception) performed in sitting, will provide the opportunity for scheduled rest. During MT, all participants will wear a heart rate monitor chest strap synced to a wrist watch to ensure that the optimal HR range is achieved. HRR will be calculated from resting and peak heart rate measures obtained during baseline testing via administration of a graded-exercise test (GXT) (Appendix H). The GXT will consist of a maximal continuous upper-body exercise test performed on an arm crank cycle ergometer (Monark 881E Arm Ergometer). Cardiorespiratory data will be collected continuously from inspired and expired gases using a cardiometabolic testing device (COSMED Quark CPET, Chicago, IL). Heart rate will be assessed simultaneously using an integrated heart rate monitor (Polar WearLink® + transmitter, Polar Electro Inc., NY). Maximal GXT termination criteria and test procedures will be followed as per standards set forth by the American College of Sports Medicine.

Non-invasive brain stimulation (tDCS): The tDCS electrode placement is based on procedures shown to improve gait and balance in a single session when used in combination with gait training activities.[19] tDCS electrodes can simultaneously activate the bilateral leg motor areas when placed at the midline of the scalp slightly anterior to the vertex (anode) and at the inion (cathode), with a current intensity of 2mA. The tDCS device is lightweight, and can be worn in a backpack during the MT activities. As reported previously [19], participants in the MT-only group will receive sham tDCS to maintain analogous study procedures. *Appendix A* provides a detailed description of tDCS set-up and implementation.

3.2.3 Outcome Measures:

Outcome measures will be assessed at several time-points throughout the study (see Figure 1). We emphasize that both moderate-intensity motor training and tDCS have been reported to have significant single-session effects. Our testing schedule will allow us to capture both the immediate effects (also referred to as "online effects" [28]), and persistent effects (or "offline effects" [28]). ***Immediate Effects*** will be assessed within each intervention day, prior to and following the intervention. ***Persistent Effects*** will be assessed by comparing baseline measures to measures obtained following the 3rd intervention day. To minimize participant burden and because single-session effects may not last for prolonged periods, only a subset of outcome measures will be collected before and after each training session. Table 1 outlines the timing of specific outcome measures to be performed in order to determine the ***Immediate*** and ***Persistent Effects***.

Table 1. Outcome measures timing.

	Baseline Measures	**Immediate Effects**	**Persistent Effects**
Walking Speed	●	●	●
Walking Distance	●		●
Balance	●		●
Fear of Falling	●		●
Ankle dorsiflex strength	●	●	●
Knee extensor strength	●		●
Functional leg strength	●		●
Spasticity	●	●	●
BDNF	●	●	●

Walking Function: Walking speed will be the primary outcome measure for walking function, as speed has been the standard measure used in the literature and will allow us to assess outcomes relative to other published studies. As in our prior studies, functional walking capacity will be measured based on 2-minute walk test distance.[3] The use of the 2-minute rather than the 6-minute walk test allows us to include individuals whose impairments result in inability to walk for 6 minutes. Kinematic data related to walking performance will be obtained using an accelerometer-based motion capture system (Xsens MVN Biomech Awinda, Xsens, Enschede, Netherlands) as the participant walks along a 15-meter path, using only the passive assistive devices they typically use (e.g., lower limb orthotics, walker, forearm crutches). Secondarily, we will assess gait quality as reflected by step height, step length, and step symmetry. *Appendix B* provides a detailed description of walking function test set-up and implementation.

Balance*:* Balance will be measured using the Berg Balance Scale, which has been found to be valid for use in persons with SCI. *Appendix C* provides a detailed description of balance test set-up and implementation.

Fall Efficacy Scale-International (FES-I)

The fear of falling may be a major concern for persons with mobility impairments and may limit one's confidence or ability to perform activities of daily living [29, 30]. Fear of falling may also limit an individual's performance of specific overground motor tasks irrespective of functional ability to perform that task [31]. Therefore, the fear of falling will be an important factor to consider relative to the mobility interventions employed in the present study. The FES-I [32] will

be used to assess baseline and post-intervention perception of fear of falling. *Appendix D* provides a description of the FES-I test implementation.

Isometric muscle strength Ankle dorsiflexion (tibialis anterior) strength will be measured with the participant seated, with the test foot strapped onto a foot plate that is attached to a force transducer. An ankle dorsiflexion test was selected based on evidence indicating that the tibialis anterior is under the greatest corticospinal control.[33, 34] Maximum dorsiflexion torque will be calculated based on the maximum value obtained during three attempts. A similar approach will be used to assess knee extensor (quadriceps) strength, as prior studies have shown that a single session of tDCS improves quadriceps strength in persons with stroke.[18] *Appendix E* provides a detailed description of isometric strength test set-up and implementation.

Functional lower extremity strength*:* The Five Times Sit-to-Stand test has been validated for use in persons with motor-incomplete SCI and shown to be correlated with walking ability. Consistent with our previous publications, we will implement the Modified Five-Times Sit-to-Stand test as a measure of functional lower extremity strength.[35] In the modified version of this test, the participant will be seated on an adjustable mat table with height adjusted to 80% of lower extremity length (as measured in the standing position with shoes on from the greater trochanter to the floor). The participant's feet will be positioned shoulder width apart (as measured from the center of each foot equal to the distance between the acromion processes). The feet will be positioned either forward or backward until a position is reached in which the lower leg is perpendicular to the floor. Participants will be instructed to, "stand up, balance, and sit back down." The participant will begin in the seated position with back unsupported and will be given a "3-2-1-Go" command to initiate standing. Repetitions in which the participant fails to achieve full upright standing will not be counted (i.e., a standing attempt will not be counted if only a partial stand is completed). Participants will also be verbally encouraged to exert equal effort between legs when standing. The total time required to complete 5 repetitions of standing up and sitting down (without using the upper extremities for assistance) will be recorded. Participants unable to complete the task without upper extremity support will be given a maximum time of 180 seconds (3minutes) so that pre/post differences can be calculated. If participants are not able to obtain this position or cannot stand without modifying this position, they will also be given a score of 180 seconds. *Appendix F* provides a detailed description of functional lower extremity strength test set-up and implementation.

Spasticity: Both motor training [9, 11, 15] and the use of tDCS [21, 22] have been associated with decreased spasticity. We will use the Spinal Cord Assessment Tool for Spastic Reflexes to assess the impact of MT-only and MT+tDCS on spasticity. This measure is well correlated with electrophysiological measures of spasticity and is better correlated with self-reported measures of spasm frequency than the Ashworth test.[36] *Appendix G* provides a detailed description of spasticity test set-up and implementation.

Serum Brain-Derived Neurotrophic Factor (BDNF): Serum concentrations of BDNF will be measured in venous blood. Venous blood samples (10 mL) will be drawn by a Shepherd Center phlebotomist and stored in pre-chilled serum venipuncture tubes. Samples will be allowed to clot for 1 hour at room temperature and 1 hour at 4°C. Serum will be separated by centrifugation (1000 x g for 15 min) and stored in Eppendorf tubes at -70°C until analysis. At least once per month, blood samples will be transported by the study coordinator by car to the Georgia Institute of Technology, Department of Applied Physiology for analyses. Blood serum samples will be analyzed for BDNF by enzyme-linked immunosorbent assay (ELISA) using *Mature Rapid ELISA* kits from Biosensis (Cat #: BEK-2211-1P/2P, Biosensis Pty Ltd., SA, Australia) with a detection range from

7.8 pg/ml to 500 pg/ml. Preparation of mature BDNF standard and other assay procedures will be followed according to manufacturer's specifications (*Appendix I*). A total of 4 blood samples will be collected for each participant, and serum BDNF concentrations (ng/ml) will be compared across the following time points: baseline (Day 1), pre-exercise (Day 2), post-exercise (Day 2), and persistent test (Day 5).

3.2.4 Laboratories:

The following laboratory will be used as the primary site of this study:

Hulse Spinal Cord Injury Laboratory
Shepherd Center
2020 Peachtree Road, NW
Atlanta, GA 30309

The following laboratory will be used for blood sample analyses:

Ryanodine Receptor Laboratory
Georgia Institute of Technology
555 14th Street NW
Atlanta, GA 30332

4.0 Adverse Events

4.1 Definitions

Adverse Event (AE) - any untoward physical or psychological occurrence or undesirable and unintended effect for a participant 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 participant(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:

- Although tDCS has been shown to have minimal risk compared to other forms of non-invasive brain stimulation (i.e. TMS)[37], it has been associated with temporary itching/tingling at stimulation onset/offset and, occasionally, headaches. tDCS is associated with skin irritation and superficial blistering; however, the occurrence of these side effects is rare and, when they do occur, temporary.

- Motor training activities consist of dynamic exercises involving concentric and eccentric muscle contractions. This type of training may increase the mechanical forces placed on muscles and connective tissues of the lower extremities beyond that which participants may be accustomed. As a result, participants may experience signs and symptoms of delayed onset muscle soreness (DOMS) within the first 24-48 hours of training, the severity of which should lessen over the course of the training period. Participants' perceived severity of DOMS will be assessed prior to each session using a 1-10 verbal pain scale. Ratings of 8 or higher will result in a cancellation of that session. Participants will be instructed to take the day off, rest, and return for the next scheduled session. Participants will be assessed for pain/swelling of the lower limbs and joints prior to each training session and any abnormalities will be referred appropriately for medical management.
- There may be risks associated with blood tests. Taking blood may cause discomfort, bruising and, very rarely, infection where the needle goes into the skin. Participants may also experience dizziness, nausea, or fainting during blood draws. Participants will be instructed to inform the attending phlebotomist drawing the blood or other member of the study team if they are not feeling well while blood is being taken or at any time after blood has been taken.

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 to 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 participants.

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 participant'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 participants or others, or any other event as described in the research process.

4.2 Reporting

Each participant 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.

4.3 Potential Side Effects

Potential side effects have been listed under *Anticipated Adverse Events* in Section 4.1.

4.4 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 participant 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 study drug/device/research intervention related will be reported as appropriate and [study drug/device/ intervention is to be discontinued per the decision of the investigator].

All participants 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 it has been determined that the participant 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 participants via an eCRF. The CRF will contain no participant names. The participant 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 participant code. This list will be maintained in a password protected file to which only investigators involved in the project will have access.

Upon completion of data collection, an eCRF will be printed for the participant, signed by a Principal Investigator, and filed in the participant's research chart.

5.2 Database

Data will be collected and maintained in electronic form by Mr. Brandon Poe. Data entry, development of the primary database, and all statistical analyses will be the responsibility of Mr. Nicholas Evans. 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 participant sought treatment from a source outside of the Hulse Spinal Cord Injury 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

Mr. Brandon Poe will fulfill the responsibilities identified in their standard operating procedures. These responsibilities include collecting and tracking data forms and instituting quality control measures for data entry verification and study compliance. He will request further documentation such as physician and/or procedure notes when complications are observed and reported. Mr. Poe will also be responsible for auditing the database and confirming the overall integrity of the data. He 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 monitor study conduct 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

The study protocol must provide the research methodology with sufficient detail to answer the following questions:
1) Is the sample size and nature appropriate for the goals of the study?
2) For placebo-controlled studies, is there adequate justification for a placebo arm; is the duration of the intervention limited to that which is minimally necessary; is there adequate monitoring of participants receiving placebo?
3) Are there defined endpoints for discontinuing experimental treatment in the event of a worsening condition?
4) Is the proposed research design and statistical treatment of data appropriate and sufficient?
5) Does the proposed research carry enough likelihood of yielding valid information to warrant the participation of Shepherd Center patients?

6.1 Statistical Assumptions

All data will be managed using Microsoft Excel and analyzed using SPSS. Descriptive statistics, including means, standard deviations and 95% confidence intervals of the pre-post change for each condition will calculated for all measures. The baseline characteristics of the participants assigned to the 2 intervention groups will be compared using ANOVA and Chi-square analysis. The change from Baseline test (day 1) to Persistence test (day 5) for the two intervention groups will be compared using repeated measures ANOVA followed by pair-wise contrasts between the two groups. Pair-wise t-tests with Bonferoni correction for multiple comparisons will be used to evaluate within-day change for each of the 3 intervention days. We will identify the effect size and 95% confidence interval associated with effects to identify the outcome measures that are most sensitive to change following intervention with MT-only and MT+tDCS. We will also assess variables associated with responsiveness to intervention, using logistic linear regression to identify the variables that are most strongly associated with change in our primary outcome measure of walking function. Where significant pre/post differences exist among the primary outcome measures and blood serum concentrations of BDNF, Pearson correlation coefficients will be calculated to determine the strength of any relationship that might exist between measures of walking function and changes in BDNF concentration.

6.2 Sample Size

While our primary interest is in assessing the value of an intensive motor training program for improving measures of function, we are also interested in assessing between-groups differences in those who do versus do not receive tDCS to augment training. Our primary outcome measures are related to walking function. Sample size calculation is based on our published study from participants trained with intensive motor training alone, wherein the improvement in walking speed had an effect size of 0.69.[15] Based on this effect size, in order to identify pre-post differences due to training using the full study sample, a sample size of 15 participants is required to obtain a power of 0.8 with $\alpha = 0.05$. However, since we are also interested in between-group differences between the MT+tDCS and MT-only groups, if we assume an additive or larger effect of the tDCS, it will be necessary to double the sample size to 30, with 15 participants per group to identify significant between-groups differences at the same power and alpha levels. To accommodate an anticipated 15% attrition rate we will enroll 35 participants.

6.3 Project Implementation Plan

Table 3 provides a summary of the project implementation plan.

Table 3. Project Implementation Plan	**Timeline (Mths)**	**Investigator(s)**
Major Task 1: Management Activities		
Prepare regulatory documents and research protocol for the study. Modify the study according to Shepherd Center RRC recommendations.		
Refine eligibility criteria, exclusion criteria, screening protocol	1-3	EFF/NE
Finalize consent form & human participants protocol	1-3	EFF/NE
Submit RRC protocol for review	1-3	EFF/NE
Submit protocol for IRB approval	1-3	EFF/NE
Submit amendments, adverse events, and protocol deviations as needed	As Needed	NE
Annual IRB report for continuing review	Annually	EFF/NE
Major Task 2: Coordination Activities		
Coordinate training of project staff	1-6	EFF/NE
Coordinate supervision and fidelity checks as needed for attrition	Quarterly	EFF
Major Task 3: Research Activities		
Coordinate all study steps, data collection and database requirements	3-6	EFF/NE/BP
Finalize assessment measurements	3-6	EFF/NE
Begin participant recruitment	6	NE
Perform participant baseline evaluations/assessments	6-42	NE
Participants complete assigned intervention and "immediate effects" measured	6-42	NE
Participants complete "persistent effect" measurements	6-42	NE
Major Task 4: Data Analysis Activities		
Monitor data collection procedures and data quality	6-42	EFF/NE/BP
Perform all analyses according to specifications, share output and findings with study investigators	42	EFF/NE
Major Task 5: Dissemination Activities		
Prepare manuscripts, abstracts, and presentations	43-48	EFF/NE
Submit manuscripts, abstracts, and report findings at relevant national/international conferences	43-48	EFF/NE
Abbreviations: IRB (Institutional Review Board); EFF (Edelle Field-Fote); Nicholas Evans (NE); Brandon Poe (BP);		

6.4 Estimated Duration of the Study

It is estimated that this study will take approximately 48 months to complete. Accrual will require approximately 42 months for completion. Table 4 provides an estimated timeline of events for the study.

Table 4. Estimated timeline of study events.

Activity	Timeline (Months)
Enrollment	6-42
Treatment and Data Acquisition	3-42
Analysis of Primary Outcomes	42-48
Safety Monitoring	1-48

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 participant. The participant 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 participant 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 participant's research chart. Only those participants 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 participants 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. The study coordinator, Mr. Nicholas Evans, 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. No deviations from the protocol will be acceptable. 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 his 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, in coordination with Mr. Nicholas Evans and Mr. Brandon Poe, 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:

- Participant files – containing the completed CRFs, supporting source documentation, and the Informed Consent.
- Regulatory Files – containing the protocol with all amendments and accountability records.

7.7 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

7.8 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: Assessors will remain blinded to participant group allocation throughout the duration of the study.

Assignment of Study Intervention: To ensure equivalence of baseline data, stratified randomization will be used to ensure equal distribution of more- and less-impaired participants between groups.

Dosing and Treatment: Participants will participate in a total 1x2-hour baseline testing session, 3x1-hour MT or MT+tDCS sessions, and 1x1hour follow-up testing session over 5 consecutive days. MT activities will take approximately 18 minutes to complete.

Identity of Medication/Device/Treatment: tDCS will be delivered using a Soterix Medical, Inc. 1x1 tDCS device.

Unblinding Procedures: Data will remain blinded throughout participant enrollment and testing. Interim data analyses will not be performed for this study. Data will be unblinded by Mr. Brandon Poe upon completion of all evaluations for all study participants.

9.0 References

1. National Spinal Cord Injury Statistical Center, *Spinal Cord Injury (SCI): Facts and Figures at a Glance.* 2018, University of Alabama at Birmingham: Birmingham, AL.

2. Ditunno, P., et al., *Who wants to walk? Preferences for recovery after SCI: a longitudinal and cross-sectional study.* Spinal Cord, 2008. **46**: p. 500-506.

3. Field-Fote, E. and K. Raoch, *Influence of a locomotor training approach on walking speed and distance in people with chronic spinal cord injury: a randomized clinical trial.* . Physical Therapy, 2011. **91**: p. 48-60.

4. del-Ama, A., et al., *Review of hybrid exoskeletons to restore gait following spinal cord injury.* Journal of Rehabilitation Research, 2012. **49**: p. 497.

5. Gomes-Osman, J., et al., *A systematic review of experimental strategies aimed at improving motor function after acute and chronic spinal cord injury.* Journal of Neurotrauma, 2016. **33**: p. 425-438.

6. Duncan, P., K. Sullivan, and A. Behrman, *Body-weight-supported treadmill rehabilitation after stroke.* New England Journal of Medicine, 2011. **364**: p. 2026-2036.

7. Rojas Vega, S., et al., *Impact of exercise on neurplasticity-related proteins in spinal cord injured humans.* Neuroscience and Biobehavioral Reviews, 2008. **153**: p. 1064-1070.

8. Satton, M., et al., *A single bout of moderate aerobic exercise improves motor skill acquisition.* Plos One, 2015. **10**(10): p. e0141393.

9. Tillakaratne, N., et al., *Use-dependent modulation of inhibitory capacity in the feline lumbar spinal cord.* Jounral of Neuroscience, 2002. **22**(8): p. 3130-3143.

10. Manella, K. and E. Field-Fote, *Modulatory effect of locomotor training on extensor spasticity in individuals with motor-incomplete spinal cord injury.* Restorative Neurology & Neuroscience, 2013. **31**(5): p. 633-646.

11. Cote, M., et al., *Exercise modulates chloride homeostasis after spinal cord injury.* Journal of Neuroscience, 2014. **34**(27): p. 8976-8987.

12. Grillner, S. and E. Ekeberg, *Intrinsic function of a neuronal network - a vertebrate central pattern generator.* Brain Research Reviews, 1998. **26**(2-3): p. 184-197.

13. Singh, A., et al., *Role of spared pathways in locomotor recovery after body-weight-supported treadmill training in contused rats.* Journal Neurotrauma, 2011. **28**(12): p. 2405-2416.

14. Thomas, S., M. Gorassini, and L. Sarah, *Increases in corticospinal tract function by treadmill training after incomplete spinal cord injury.* Journal of Neurophysiology, 2005. **94**(4): p. 2844-2855.

15. Manella, K., K. Roach, and E. Field-Fote, *Operant conditioning to increase ankle control or decrease reflex excitability improves reflex modulation and walking function in chronic spinal cord injury.* Journal Neurophysiology, 2013. **109**(11): p. 2666-2679.

16. Madhaven, S., K. Weber, and J. Stinear, *Non-invasive brain stimulation enhances fine motor control of the hemiparetic ankle: implications for rehabilitation.* Experimental Brain Research, 2011. **209**(1): p. 9-17.

17. Sohn, M., S. Jee, and Y. Kim, *Effect of transcranial direct current stimulation on postural stability and lower extremity strength in hemiplegic stroke patients.* Annals of Rehabilitation Medicine, 2013. **37**(6): p. 759-765.

18. Tanaka, S., K. Takeda, and Y. Otaka, *Single session of transcranial direct current stimulation transiently increases knee extensor force in patients with hemiparetic stroke.* Neurorehabilitation and Neural Repair, 2011. **25**(6): p. 565-569.

19. Kaski, D., et al., *Improving gait and balance in patients with leukoaraiosis using transcranial direct current stimulation and physical training: an exploratory study.* Neurorehabilitation and Neural Repair, 2013. **27**(9): p. 864-871.

20. Benito, J., H. Kumru, and N. Murillo, *Motor and gait improvements in patients with incomplete spinal cord injury induced by high-frequency repetitive transcranial magnetic stimulation.* Topics in Spinal Cord Injury Rehabilitation, 2012. **18**(2): p. 106-112.

21. Roche, N., et al., *Effects of anodal tDCS on lumbar propriospinal system in healthy subjects.* Clinical Neurophysiology, 2012. **123**(5): p. 1027-1034.

22. Aree-uea, B., N. Auvichayapat, and T. Janyacharoen, *Reduction of spasticity in cerebral plasy by anodal transcranial direct current stimulation.* Journal of the Medical Assocaition of Thailand, 2014. **97**(9): p. 954-962.

23. American Spinal Injury Association, *International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI)*. 2015: Atlanta, GA.

24. van Middendorp, J., A. Hosman, and A. Donders, *A clinical prediction rule for ambulation outcomes after traumatic spinal cord injury: a longitudinal cohort study.* Lancet, 2011. **377**(9770): p. 1004-1010.

25. Kim, C., J. Eng, and M. Whittaker, *Level walking and ambulatory capacity in persons with incomplete spinal cord injury: relationships with muscle strength.* Spinal Cord, 2004. **42**(3): p. 156-162.

26. Wirth, B., H. van Hedel, and A. Curt, *Ankle paresis in incomplete spinal cord injury: relation to corticospinal conductivity and ambulatory capacity.* Journal of Clinical Neurophysiology, 2008. **25**(4): p. 210-217.

27. Crozier, K., et al., *Spinal cord injury: prognosis for ambulation based on quadriceps recovery.* Paraplegia, 1992. **30**(11): p. 762-767.

28. Reis, J., H. Schambra, and L. Cohen, *Noninvasive cortical stimulation enhances motor skill acquisition over multiple days through an effect on consolidation.* Proceedings of the National Academy of Sciences, 2009. **106**(5): p. 1590-1595.

29. Tinetti, M. and L. Powell, *Fear of falling and low self-efficacy: a case of independence in elderly persons.* Journal of Gerontology, 1993. **48**(Spec Issue): p. 35-38.

30. Goh, H., et al., *Falls and fear of falling after stroke: a case-control study.* Physical Medicine and Rehabilitation, 2016.

31. John, L., B. Cherian, and A. Babu, *Postural control and fear of falling in persons with low-level paraplegia.* Journal of Rehabilitation Research and Development, 2010. **47**(5): p. 497-502.

32. Dewan, N. and J. MacDermid, *Fall Efficacy Scale - International (FES-I).* Journal of Physiotherapy, 2014. **60**: p. 60.

33. Capaday, C., et al., *Studies on the corticospinal control of human walking: response to transcranila magnetic stimulation of the motor cortex.* Journal of Neurophysiology, 1999. **81**(1): p. 129-139.

34. Schubert, M., et al., *Corticospinal input in human gait: modulation of magnetically evoked motor responses.* Experimental Brain Research, 1997. **115**(2): p. 234-246.

35. Bosveld, R. and E. Field-Fote, *Single-dose effects of whole body vibration on quadriceps strength in individuals with motor-incomplete spinal cord injury.* Journal of Spinal Cord Medicine, 2015. **38**(6): p. 784-791.

36. Benz, E., et al., *A physiologically based clinical measure for spastic reflexes in spinal cord injury.* Archives of Physical Medicine and Rehabilitation, 2005. **86**(1): p. 52-59.

37. Nitsche, M., et al., *Transcranial direct current stimulation: state of the art 2008.* Brain Stimulation, 2008. **1**(3): p. 206-223.

10.0 Appendices – *Forms, Study Tools, Questionnaires, Device Manual, etc.*

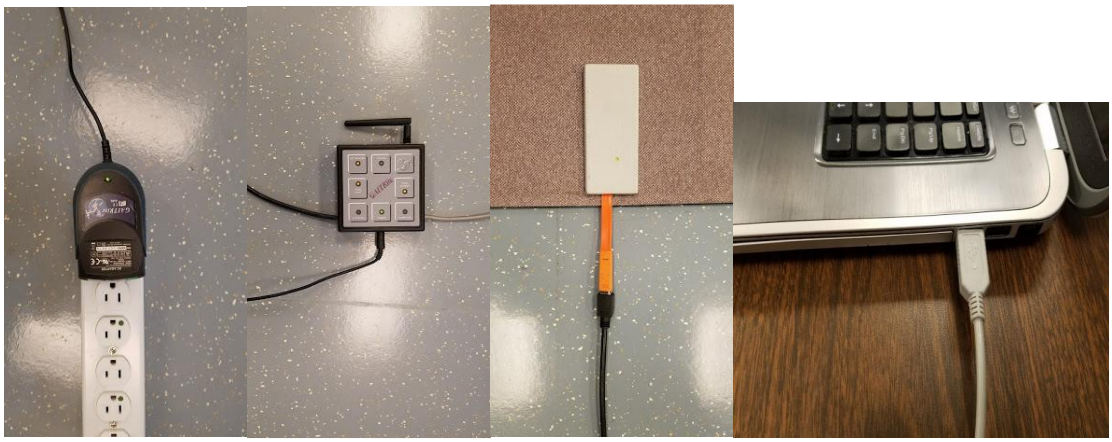
APPENDIX A. tDCS Set-up and implementation.

Skill
Note anode/cathode position per study protocol
Ensure tDCS kit is stocked: tDCS unit, cables, rubber electrodes, spare batteries, measuring tape, sponges, saline & syringe, coban, washcloths, alcohol swabs
Explain intervention to participant
Measure and record nasion-inion distance and distance between preauricular points
Calculate and record area for anode and cathode placement based on measurements and 10-20 EEG system
Inspect scalp for pre-existing irritation, cuts or lesions
Separate hair and clean underlying scalp with alcohol to reduce impedance
Connect tDCS unit, cables and rubber electrodes
Saturate sponges with saline (~6 ml/side) and insert rubber electrodes into sponges
Place anode in prepared location and secure with coban
Place cathode in prepared location and secure with coban
Turn on tDCS unit; confirm duration, intensity and sham settings
Start stim session; use relax button if stim is too intense for participant
Ensure participant is comfortable throughout stimulation
Turn off tDCS unit after session is complete (active button no longer lit)
Remove sponges from scalp
Inspect scalp for evidence of irritation and record observations
Ask participant how he/she is feeling and whether he/she has any questions
Wash saline from sponges and air dry if they are to be reused

APPENDIX B. Walking function test set-up and implementation.

A. Setup the Gaitrite hardware (10 min before subject arrives)

1) Take the gaitrite from its assigned space in the lab, and place it in the center of the gait test area.
2) This step requires two people, so ask for help
3) Connect the power extension cord to the power source
4) Connect the Gaitrite mat to the hub using the orange cable at the beginning of the mat (see picture)
5) Connect the USB cable from the hub to the recording PC with Gaitrite software installed



B. Setup the Gaitrite software and open subject session on PC

1) Open the Gaitrite application on the recording PC. You will see a User ID field. Click the left mouse button in the field three times to log in. Do not type any password.

Xsens Set-up:

Set-Up Checklist	
1.	Charge all MTw's using Awinda charging stations for at least 12 hours before use.
2.	Ensure that motion tracking is being set-up away from any strong magnetic fields.
3.	Turn on laptop and insert Sentinel flash drive into the USB port (this is necessary to run the *MVN Studio* software).
4.	Connect Awinda Wireless Station to the laptop using the Ethernet to USB cable (the Awinda Dongle can be used in place of wireless station but receiving range is limited).
5.	Connect power source to Allied Vision Technologies Camera and connect the camera to the laptop using the supplied Ethernet cable.
6.	From the laptop desktop, open the *MVN Studio* program.

1. Under File tab, select New Recording Session.
2. Current Configurations dialog box appears. In bottom right box next to the file directory name, enter the pre-determined Subject ID.
3. Accept Current Configurations by selecting Ok.
4. Turn on all MTw's by depressing the button located next to the charging port (the LED indicator on the top of each MTw should flash slowly when turned on).
5. Explain the set-up process and testing procedures to the participant.
6. Obtain "Offline Body Dimensions" (in standing position when possible) using the Segmometer and input the results (in cm) under the Body Dimensions tab to the left of the screen :
    - **Body Height**: Ground to top of head when standing upright.
    - **Foot Size**: Top of shoe nose to end of the heel.
    - **Arm Span**: Top of right fingers to top of left fingers in T-space.
    - **Hip Height**: Ground to most lateral bony prominence of greater trochanter.
    - **Knee Height**: Ground to lateral epicondyle on the femoral bone.
    - **Ankle Height**: Ground to distal tip of lateral malleolus.
    - **Hip Width**: Right to left anterior superior iliac spine.
    - **Shoulder Width**: Right to left distal tip of acromion (acromial angle).
    - **Shoe Sole Thickness**: Average thickness of the sole of the used shoes.
7. Once Body Dimensions have been entered, Save participant anthropometrics under pre-identified Subject ID (participant body dimensions can be re-loaded for future assessments).
8. For full-body motion tracking, use the headband, 9 FabriFoam Velcro straps, gloves, footpads, and Lycra shirt (or giger strap or adhesive athletic tape) to secure MTw's in place. Begin MTw placement for each body segment as indicated on the side of each MTw. Proceed from the top down as follows:
    - **HEAD**: Place the HEAD MTw in the headband pocket (adjust the headband to any comfortable position).
    - **SHOU**: Wearing the Lycra shirt, place left and right SHOU MTw's on Velcro tabs located on the bilateral scapulae.
    - **STERN**: Wearing the Lycra shirt, place the STERN MTw in the pocket located at the sternum. MTw should be placed flat, in the middle of the chest.
    - **UARM**: Using the upper arm FabriFoam straps, wrap the left and right UARM MTw's on the lateral side of the arm above the main thickness of the biceps.
    - **FARM**: Using the forearm FabriFoam straps, wrap the left and right FARM MTw's above the wrist on the lateral, flat portion across the distal radius and ulna.
    - **HAND:** Using the gloves, place the left and right HAND MTw's in the pockets located on the backside of the hands.
    - **PELV:** Using the pelvic FabriFoam strap, wrap the PELV MTw around the pelvis with the MTw positioned flat on the sacrum.
    - **ULEG:** Using the upper leg FabriFoam straps, wrap the left and right ULEG MTw's around the thigh with the MTw's placed on the lateral side at the midpoint between the hip and knee.
    - **LLEG:** Using the lower leg FabriFoam straps, wrap the left and right LLEG MTw's around the lower leg with the MTw's placed flat on the medial surface of the tibia at the midpoint between the knee and ankle.
    - **FOOT:** Using the Velcro foot pads, secure the left and right FOOT MTw's on the middle of the bridge of the foot between the shoe tongue and shoelaces. Tighten the shoestrings to secure the foot pad and MTw in place.
1. Under the MVN System tab, check the hardware status to ensure that all MTw's have been recognized by the *MVN Studio* software (a **green icon** indicates a given body segment is connected; a **black icon** indicates a given body segment is not detected).
2. Calibrate the system:
    - Have the participant stand and assume N-Pose (Neutral Pose) – standing upright on a horizontal surface with feet parallel (one foot width apart), face forward, and arms straight alongside the body with palms facing inwards and thumbs facing forwards. Physical assistance may be needed to assist the participant in this position.
    - When ready, under the "Calibration" tab to the left of the screen, select N-Pose icon and click the "Calibrate" and then "Start" buttons.
    - Hold the position for the time indicated on the computer, and if the result is "good" then click "accept". If the messages "poor" or "fail" appear, repeat the calibration before proceeding.
3. Position participant at the location where gait testing or other motor tasks will be performed (e.g. 10MWT, 6MWT, Agility T-test, etc.).
4. Record a motion tracking trial by clicking on the red record button located at the top of the *MVN Studio* screen toolbar.
5. When the participant completes the designated activity, click the same record button located at the top of the *MVN Studio* screen tollbar (during recording the button will be red and black).
6. After recording, a popup dialog box will appear so that relevant notes and comments regarding the motion tracking session can be entered and viewed when opening the file.
7. Save the current trial (the file name along with the date, time, and trial number will automatically be generated based on the Subject ID entered in Step 7).

	Skill
10-Meter Walk Test	
	Sets up 14 meter course with 2 meter marks at each end
	Gives appropriate instructions to subject
	Start timing when lead toes cross 2 meter mark
	Stops timing when lead toes cross 12 meter mark
	Records result in seconds
	Records assistive device used
2-Minute Walk Test	
	Ensure course is free of obstacles
	Gives appropriate instructions to subject
	Starts timing at “Go” command
	Marks distance at the end of 2 minutes
	Records distance in meters
	Records assistive device used

APPENDIX C. Berg Balance test implementation.

Berg Balance Scale

The Berg Balance Scale (BBS) was developed to measure balance among older people with impairment in balance function by assessing the performance of functional tasks. It is a valid instrument used for evaluation of the effectiveness of interventions and for quantitative descriptions of function in clinical practice and research. The BBS has been evaluated in several reliability studies. *A recent study of the BBS, which was completed in Finland, indicates that a change of eight (8) BBS points is required to reveal a genuine change in function between two assessments among older people who are dependent in ADL and living in residential care facilities.*

Description:
14-item scale designed to measure balance of the older adult in a clinical setting.

Equipment needed: Ruler, two standard chairs (one with arm rests, one without), footstool or step, stopwatch or wristwatch, 15 ft walkway

Completion:

Time: 15-20 minutes

Scoring: A five-point scale, ranging from 0-4. "0" indicates the lowest level of function and "4" the highest level of function. Total Score = 56

Interpretation:
41-56 = low fall risk
21-40 = medium fall risk
0 –20 = high fall risk

A change of 8 points is required to reveal a genuine change in function between 2 assessments.

Berg Balance Scale

Name: _____ Date: _____

Location: _____ Rater: _____

ITEM DESCRIPTION	SCORE (0-4)
Sitting to standing	_____
Standing unsupported	_____
Sitting unsupported	_____
Standing to sitting	_____
Transfers	_____
Standing with eyes closed	_____
Standing with feet together	_____
Reaching forward with outstretched arm	_____
Retrieving object from floor	_____
Turning to look behind	_____
Turning 360 degrees	_____
Placing alternate foot on stool	_____
Standing with one foot in front	_____
Standing on one foot	_____

Total _____

GENERAL INSTRUCTIONS

Please document each task and/or give instructions as written. When scoring, please record the lowest response category that applies for each item.

In most items, the subject is asked to maintain a given position for a specific time. Progressively more points are deducted if:

- the time or distance requirements are not met
- the subject's performance warrants supervision
- the subject touches an external support or receives assistance from the examiner

Subject should understand that they must maintain their balance while attempting the tasks. The choices of which leg to stand on or how far to reach are left to the subject. Poor judgment will adversely influence the performance and the scoring.

Equipment required for testing is a stopwatch or watch with a second hand, and a ruler or other indicator of 2, 5, and 10 inches. Chairs used during testing should be a reasonable height. Either a step or a stool of average step height may be used for item # 12.

Berg Balance Scale

SITTING TO STANDING
INSTRUCTIONS: Please stand up. Try not to use your hand for support.
( ) 4 able to stand without using hands and stabilize independently
( ) 3 able to stand independently using hands
( ) 2 able to stand using hands after several tries
( ) 1 needs minimal aid to stand or stabilize
( ) 0 needs moderate or maximal assist to stand

STANDING UNSUPPORTED
INSTRUCTIONS: Please stand for two minutes without holding on.
( ) 4 able to stand safely for 2 minutes
( ) 3 able to stand 2 minutes with supervision
( ) 2 able to stand 30 seconds unsupported
( ) 1 needs several tries to stand 30 seconds unsupported
( ) 0 unable to stand 30 seconds unsupported

If a subject is able to stand 2 minutes unsupported, score full points for sitting unsupported. Proceed to item #4.

SITTING WITH BACK UNSUPPORTED BUT FEET SUPPORTED ON FLOOR OR ON A STOOL
INSTRUCTIONS: Please sit with arms folded for 2 minutes.
( ) 4 able to sit safely and securely for 2 minutes
( ) 3 able to sit 2 minutes under supervision
( ) 2 able to able to sit 30 seconds
( ) 1 able to sit 10 seconds
( ) 0 unable to sit without support 10 seconds

STANDING TO SITTING
INSTRUCTIONS: Please sit down.
( ) 4 sits safely with minimal use of hands
( ) 3 controls descent by using hands
( ) 2 uses back of legs against chair to control descent
( ) 1 sits independently but has uncontrolled descent
( ) 0 needs assist to sit

TRANSFERS
INSTRUCTIONS: Arrange chair(s) for pivot transfer. Ask subject to transfer one way toward a seat with armrests and one way toward a seat without armrests. You may use two chairs (one with and one without armrests) or a bed and a chair.
( ) 4 able to transfer safely with minor use of hands
( ) 3 able to transfer safely definite need of hands
( ) 2 able to transfer with verbal cuing and/or supervision
( ) 1 needs one person to assist
( ) 0 needs two people to assist or supervise to be safe

STANDING UNSUPPORTED WITH EYES CLOSED
INSTRUCTIONS: Please close your eyes and stand still for 10 seconds.
( ) 4 able to stand 10 seconds safely
( ) 3 able to stand 10 seconds with supervision
( ) 2 able to stand 3 seconds
( ) 1 unable to keep eyes closed 3 seconds but stays safely
( ) 0 needs help to keep from falling

STANDING UNSUPPORTED WITH FEET TOGETHER
INSTRUCTIONS: Place your feet together and stand without holding on.
( ) 4 able to place feet together independently and stand 1 minute safely
( ) 3 able to place feet together independently and stand 1 minute with supervision
( ) 2 able to place feet together independently but unable to hold for 30 seconds
( ) 1 needs help to attain position but able to stand 15 seconds feet together
( ) 0 needs help to attain position and unable to hold for 15 seconds

Berg Balance Scale continued…

REACHING FORWARD WITH OUTSTRETCHED ARM WHILE STANDING
INSTRUCTIONS: Lift arm to 90 degrees. Stretch out your fingers and reach forward as far as you can. (Examiner places a ruler at the end of fingertips when arm is at 90 degrees. Fingers should not touch the ruler while reaching forward. The recorded measure is the distance forward that the fingers reach while the subject is in the most forward lean position. When possible, ask subject to use both arms when reaching to avoid rotation of the trunk.)
( ) 4 can reach forward confidently 25 cm (10 inches)
( ) 3 can reach forward 12 cm (5 inches)
( ) 2 can reach forward 5 cm (2 inches)
( ) 1 reaches forward but needs supervision
( ) 0 loses balance while trying/requires external support

PICK UP OBJECT FROM THE FLOOR FROM A STANDING POSITION
INSTRUCTIONS: Pick up the shoe/slipper, which is in front of your feet.
( ) 4 able to pick up slipper safely and easily
( ) 3 able to pick up slipper but needs supervision
( ) 2 unable to pick up but reaches 2-5 cm(1-2 inches) from slipper and keeps balance independently
( ) 1 unable to pick up and needs supervision while trying
( ) 0 unable to try/needs assist to keep from losing balance or falling

TURNING TO LOOK BEHIND OVER LEFT AND RIGHT SHOULDERS WHILE STANDING
INSTRUCTIONS: Turn to look directly behind you over toward the left shoulder. Repeat to the right. (Examiner may pick an object to look at directly behind the subject to encourage a better twist turn.)
( ) 4 looks behind from both sides and weight shifts well
( ) 3 looks behind one side only other side shows less weight shift
( ) 2 turns sideways only but maintains balance
( ) 1 needs supervision when turning
( ) 0 needs assist to keep from losing balance or falling

TURN 360 DEGREES
INSTRUCTIONS: Turn completely around in a full circle. Pause. Then turn a full circle in the other direction.
( ) 4 able to turn 360 degrees safely in 4 seconds or less
( ) 3 able to turn 360 degrees safely one side only 4 seconds or less
( ) 2 able to turn 360 degrees safely but slowly
( ) 1 needs close supervision or verbal cuing
( ) 0 needs assistance while turning

PLACE ALTERNATE FOOT ON STEP OR STOOL WHILE STANDING UNSUPPORTED
INSTRUCTIONS: Place each foot alternately on the step/stool. Continue until each foot has touched the step/stool four times.
( ) 4 able to stand independently and safely and complete 8 steps in 20 seconds
( ) 3 able to stand independently and complete 8 steps in > 20 seconds
( ) 2 able to complete 4 steps without aid with supervision
( ) 1 able to complete > 2 steps needs minimal assist
( ) 0 needs assistance to keep from falling/unable to try

STANDING UNSUPPORTED ONE FOOT IN FRONT
INSTRUCTIONS: (DEMONSTRATE TO SUBJECT) Place one foot directly in front of the other. If you feel that you cannot place your foot directly in front, try to step far enough ahead that the heel of your forward foot is ahead of the toes of the other foot. (To score 3 points, the length of the step should exceed the length of the other foot and the width of the stance should approximate the subject's normal stride width.)
( ) 4 able to place foot tandem independently and hold 30 seconds
( ) 3 able to place foot ahead independently and hold 30 seconds
( ) 2 able to take small step independently and hold 30 seconds
( ) 1 needs help to step but can hold 15 seconds
( ) 0 loses balance while stepping or standing

STANDING ON ONE LEG
INSTRUCTIONS: Stand on one leg as long as you can without holding on.
( ) 4 able to lift leg independently and hold > 10 seconds
( ) 3 able to lift leg independently and hold 5-10 seconds
( ) 2 able to lift leg independently and hold ≥ 3 seconds
( ) 1 tries to lift leg unable to hold 3 seconds but remains standing independently.
( ) 0 unable to try of needs assist to prevent fall

( ) TOTAL SCORE (Maximum = 56)

APPENDIX D. Falls Efficacy Scale-International test implementation.

FES-I

Now we would like to ask some questions about how concerned you are about the possibility of falling. Please reply thinking about how you usually do the activity. If you currently don't do the activity (e.g. if someone does your shopping for you), please answer to show whether you think you would be concerned about falling IF you did the activity. For each of the following activities, please tick the box which is closest to your own opinion to show how concerned you are that you might fall if you did this activity.

		Not at all concerned 1	*Somewhat concerned 2*	*Fairly concerned 3*	*Very concerned 4*
1	Cleaning the house (e.g. sweep, vacuum or dust)	*1* ☐	*2* ☐	*3* ☐	*4* ☐
2	Getting dressed or undressed	*1* ☐	*2* ☐	*3* ☐	*4* ☐
3	Preparing simple meals	*1* ☐	*2* ☐	*3* ☐	*4* ☐
4	Taking a bath or shower	*1* ☐	*2* ☐	*3* ☐	*4* ☐
5	Going to the shop	*1* ☐	*2* ☐	*3* ☐	*4* ☐
6	Getting in or out of a chair	*1* ☐	*2* ☐	*3* ☐	*4* ☐
7	Going up or down stairs	*1* ☐	*2* ☐	*3* ☐	*4* ☐
8	Walking around in the neighbourhood	*1* ☐	*2* ☐	*3* ☐	*4* ☐
9	Reaching for something above your head or on the ground	*1* ☐	*2* ☐	*3* ☐	*4* ☐
10	Going to answer the telephone before it stops ringing	*1* ☐	*2* ☐	*3* ☐	*4* ☐
11	Walking on a slippery surface (e.g. wet or icy)	*1* ☐	*2* ☐	*3* ☐	*4* ☐
12	Visiting a friend or relative	*1* ☐	*2* ☐	*3* ☐	*4* ☐
13	Walking in a place with crowds	*1* ☐	*2* ☐	*3* ☐	*4* ☐
14	Walking on an uneven surface (e.g. rocky ground, poorly maintained pavement)	*1* ☐	*2* ☐	*3* ☐	*4* ☐
15	Walking up or down a slope	*1* ☐	*2* ☐	*3* ☐	*4* ☐
16	Going out to a social event (e.g. religious service, family gathering or club meeting)	*1* ☐	*2* ☐	*3* ☐	*4* ☐

APPENDIX E. Isometric muscle strength test set-up and implementation.

	Skill
Isometric Quadriceps Strength	
	Explains procedure & models test for the subject
	Ensures dynamometer is secured to frame
	Ensures dynamometer output is in pounds
	Positions subject so that the hip is at 90 degrees and knee is flexed at 60 degrees.
	The lower leg is in contact with the dynamometer at a point just superior to the ankle joint articulation.
	Subject's legs are restrained to ensure that the subject is secure and hips are not used in the test.
	Once subject has been positioned, record seat height, bar height, and dynamometer angle.
	Give subject a "3-2-1" countdown and provide verbal encouragement to "Push!" and hold for 3 seconds.
	Provide 60secs rest between MVC efforts.
	Record results after each effort and repeat 3 times.
Isometric Dorsiflexor Strength	
	Explains procedure & models test for the subject
	Ensures dynamometer is secured to frame
	Ensures dynamometer output is in pounds
	Positions subject so that the hip, knee, and ankle are at 90 degrees with the foot fully on a step.
	The dynamometer should be in contact with the dorsum of the midfoot with a bias toward the first metatarsal.

APPENDIX F. Modified 5-Times Sit-to-Stand test set-up and implementation.

Modified 5 Times Sit-to-Stand Test	
	Adjusts mat table height to 80% of the subject's leg length
	Explains procedure & models test for the subject
	Ensures that subject's arms are not used
	Starts stopwatch on "Go" command
	Stops timing when subject sits the 5th time
	Records result as a time (seconds)

APPENDIX G. Spinal Cord Assessment Tool for SCI test implementation.

Spinal Cord Assessment Tool for Spastic Reflexes:

Adapted from Benz EN et al. A physiologically based clinical measure for spastic reflexes in spinal cord injury, Arch Phys Med Rehabil, 86: 52-9, 2005; paragraphs under "Instruments" - "SCATS: clonus" and "SCATS: flexor spasms" and "SCATS: extensor spasms". Used with permission from Elsevier Publishing.

R	L		
	SCATS: Clonus		Clonus of the plantarflexors was quantified in response to a rapid passive dorsiflexion of the ankle (A). The ankle was dorsiflexed at an angle that triggered clonus, and the duration of clonic bursts was timed. An ordinal rating from 0 to 3 was determined by the duration of clonic activity where 0 is no reaction; 1 is mild, clonus was maintained less than 3 seconds; 2 is moderate, clonus persisted between 3 and 10 seconds; and 3 is severe, clonus persisted for more than 10 seconds.
0	0	no reaction	
1	1	Mild <3 secs	
2	2	3< Moderate <10 secs	
3	3	Severe > 10 secs	

	SCATS: *flexor spasms.*		With the knee and hip extended to 0°, the clinician applied a pinprick stimulus for 1 second to the medial arch of the subject's foot (B). Excursion of the big toe into extension, ankle dorsiflexion, and knee and hip flexion were visually observed for severity. The rating scale consisted of a score from 0 to 3, where 0 is no reaction to stimulus; 1 is mild, less than 10° of excursion in flexion at the knee and hip or extension of the great toe; 2 is moderate, 10° to 30° of flexion at the knee nd hip; and 3 is severe, 30° or greater of knee and hip flexion.
0	0	no reaction	
1	1	less than 10° of excursion in flexion at the knee and hip or extension of the great toe	
2	2	moderate, 10° to 30° of flexion at the knee and hip	
3	3	severe, 30° or greater of knee and hip flexion.	

	SCATS: *extensor spasms*		With the contralateral limb extended, the tested knee and hip were positioned at angle of 90° to 110° of hip and knee flexion, and then both joints were simultaneously extended. One hand cupped the heel while the other was placed on the outside of the thigh (C). Once a reaction was elicited, the duration of visible muscle contraction in the quadriceps muscle was measured by observing superior displacement of the patella. The timed scale (0–3) that was used for clonus was also applied to the timed extensor spasms.
0	0	no reaction	
1	1	Mild <3 secs	
2	2	3secs < Moderate <10 secs	
3	3	Severe > 10 secs	

R	L	
	SCATS: Clonus	
0	0	no reaction
1	1	Mild <3 secs
2	2	3< Moderate <10 secs
3	3	Severe > 10 secs

	SCATS: *flexor spasms.*	
0	0	no reaction
1	1	less than 10° of excursion in flexion at the knee and hip or extension of the great toe
2	2	moderate, 10° to 30° of flexion at the knee and hip
3	3	severe, 30° or greater of knee and hip flexion.

	SCATS: *extensor spasms*	
0	0	no reaction
1	1	Mild <3 secs
2	2	3secs < Moderate <10 secs
3	3	Severe > 10 secs

APPENDIX H. Cardiopulmonary graded exercise test (GXT).

Cardiopulmonary Graded Exercise Test (GXT)

Subject Name: Date: Estimated HR Max 220 – Age = _____

85% Age Predicted Max HR = _____

Equipment Setup	
Machine Used	
Seat Setting	
Arm Length	
Back Tilt	
Miscellaneous	

Test Summary	
Time	
Peak VO2	
Peak Heart Rate	
Heart rate @ AT	
Other Notes	

Vitals

	Pre Test	Post Test	Recovery
Blood Pressure			
Heart Rate			
RPE			

Data Collection

	Primary		Secondary		
Time: (minutes)	**Heart Rate (bpm)**	**VO_2**	RPE	RER	BP
1:00					
2:00					
3:00					
4:00					
5:00					
6:00					
7:00					
8:00					
9:00					
10:00					
11:00					
12:00					
13:00					
14:00					
15:00					

APPENDIX I. Brain-Derived Neurotrophic Factor (BDNF) Protocol and Checklist

Equipment Checklist	Qty	Completed? (Y/ N)	Date	Employee's Initials	Supervisor's Initials
Serum separator vacutainer tubes					
PCR tube strips					
Transportation container (frozen shipper – no cost)					
Dry ice					
biosensis Mature BDNF Rapid ELISA Kit (96 well microplate)	12 x 8 wells				
Assay diluent A (1x)	2 x 25 mL				
Recombinant human mature BDNF standard	2 x 1000 pg				
Quality control sample (QC)	2 vials				
Mature BDNF detection antibody (100x)	1 x 110 µL				
Streptavidin-HRP (100x) – do not freeze	1 x 110 µL				
Wash buffer (10x)	1 x 33 mL				
TMB substrate (1x)	1 x 11 mL				
TMB stop solution (1x)	1 x 11 mL				
Plate sealer	1				
Pipettes (single channel)	10-1000 µL				
Plate shaker					
Microplate reader (absorbance at 450 nm)					

*Notes: To prevent sample variation, strict adherence to consistent sample preparation procedures among samples is necessary.

Serum Sample Collection & Preparation	Completed? (Y/ N)	Date	Employee's Initials	Supervisor's Initials
1. Serial venous blood draws will be collected on Day 1 (Baseline), before and after training on Day 2 (Intervention), and on Day 5 (Persistence) (i.e. 4 total samples per participant).				
2. Butterfly catheter placed in the participant's upper extremity.				
3. Samples collected in a 10mL serum separator vacutainer tube.				
4. Allow sample to clot for 1 hour at room temperature (research work room)				
5. Centrifuge at 1000g for 15 minutes.				
6. Prepare aliquots of 200µL in thin wall 0.2mL PCR tube strips.				

7. Store aliquots at -20°C until frozen samples are transported to Ga Tech within 24 hours.				
8. Store frozen samples at -80°C.				
9. Prior to beginning assay procedures, allow samples to slowly thaw. **(thaw time?)**				
10. Working pH of a sample should be near neutral (pH 6.8-7.5) – adjust with mild acid or base as needed.				
11. A minimum dilution of 1/50 to 1/1000 with Assay Diluent A is required.				

Preparation of Mature BDNF Standard	Completed? (Y/ N)	Date	Employee's Initials	Supervisor's Initials
12. See biosensis manufacturer specifications				

Other Reagents and Buffer Preparation	Completed? (Y/ N)	Date	Employee's Initials	Supervisor's Initials
13. Quality Control (QC) sample				

Assay Procedure	Completed? (Y/ N)	Date	Employee's Initials	Supervisor's Initials
14. All steps are performed at room temperature (20-25°C, 70-75°F)				
15. Add 100µL of diluted mature BDNF standards, QC sample, samples and blank (sample diluent only) to the pre-coated microplate wells				
16. Include sample-specific negative and positive control sample in the assay procedure (?).				
17. Seal the plate (plate sealer or parafilm).				
18. Incubate the plate on a shaker for 45 minutes (140rpm; 0.351 G*).				
19. Discard the solution inside the wells.				
20. Perform 5 washes with 1x wash buffer (200µL per well) – see technical hints for details				
21. Add 100µL of the detection antibody (1x) into each well.				
22. Seal the plate.				
23. Incubate the plate on a shaker for 30minutes (140rpm; 0.451 G*).				
24. Discard the solution inside the wells and wash as described in step 22.				
25. Add 100µL of the 1x streptavidin-HRP conjugate into each well.				
26. Seal the plate.				
27. Incubate the plate on a shaker for 30minutes (140rpm; 0.451 G*).				
28. Discard the solution inside the wells and wash as described in step 22.				
29. Add 100µL of TMB into each well.				

30. Incubate plate at room temperature for 4-8 minutes without shaking in the dark.				
31. Stop the reaction by adding 100µL of the stop solution into each well.				
32. Visible blue color will change to yellow.				
33. Read the absorbance at 450nm on a plate reader (read within 5minutes of adding stop solution and no longer than 30minutes).				