Protocol

1. Project Title

Cerebral networks of locomotor learning and retention in older adults

2. Investigator(s):

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3. Abstract:

Aging often leads to substantial declines in walking function, especially for walking tasks that are more complex such as obstacle crossing. This is due in part to a lack of continued practice of complex walking (sedentary lifestyle) combined with age-related deficits of brain structure and the integrity of brain networks. Neurorehabilitation can contribute to recovery of lost walking function in older adults, but major and persistent improvements are elusive. A cornerstone of neurorehabilitation is motor learning, defined as an enduring change in the ability to perform a motor task due to practice or experience. Unfortunately, in most clinical settings, the time and cost demands of delivering a sufficiently intensive motor learning intervention is not feasible. There is a need for research to develop strategies for enhancing motor learning of walking ("locomotor learning") in order to improve the effectiveness of neurorehabilitation.
The objective of this study is to use non-invasive brain stimulation to augment locomotor learning and to investigate brain networks that are responsible for locomotor learning in older adults. We have shown that frontal brain regions, particularly prefrontal cortex, are crucial to control of complex walking tasks. Our neuroimaging and neuromodulation studies also show that prefrontal cortex structure and network connectivity are important for acquisition and consolidation of new motor skills. However, a major gap exists regarding learning of walking tasks. This study is designed to address this gap. Our pilot data from older adults shows that prefrontal transcranial direct current stimulation (tDCS) administered during learning of a complex obstacle walking task contributes to multi-day retention of task performance. In this study we will build upon this pilot work by conducting a full scale trial that also investigates mechanisms related to brain structure, functional activity, and network connectivity. We will address the following specific aims:

Specific Aim 1: Determine the extent to which prefrontal tDCS augments the effect of task practice for retention of performance on a complex obstacle walking task.

Specific Aim 2: Determine the extent to which retention of performance is associated with individual differences in baseline and practice-induced changes in brain measures (working memory, gray matter volume, task-based prefrontal activity, and brain network segregation).

Specific Aim 3: Investigate the extent to which tDCS modifies resting state network segregation.
We anticipate that prefrontal tDCS will augment retention of locomotor learning, and that our data will provide the first evidence of specific brain mechanisms responsible for locomotor learning/retention in older adults. This new knowledge will provide a clinically feasible intervention approach as well as reveal mechanistic targets for future interventions to enhance locomotor learning and rehabilitation.

4. Background:

Aging often leads to substantial declines in walking function, especially for walking tasks that are more complex such as obstacle crossing. This is due in part to a lack of continued practice of complex walking (sedentary lifestyle) combined with age-related deficits of brain structure and the integrity of brain networks. Neurorehabilitation can contribute to recovery of lost walking function in older adults, but major and persistent improvements are elusive. A cornerstone of neurorehabilitation is motor learning, defined as an enduring change in the ability to perform a motor task due to practice or experience. Unfortunately, in most clinical settings, the time and cost demands of delivering a sufficiently intensive motor learning intervention is not feasible. There is a need for research to develop strategies for enhancing motor learning of walking ("locomotor learning") in order to improve the effectiveness of neurorehabilitation.

The objective of this study is to use non-invasive brain stimulation to augment locomotor learning and to investigate brain networks that are responsible for locomotor learning in older adult Veterans. We have shown that frontal brain regions, particularly prefrontal cortex, are crucial to control of complex walking tasks. Our neuroimaging and neuromodulation studies also show that prefrontal cortex structure and network connectivity are important for acquisition and consolidation of new motor skills. For instance, our published data shows that resting state connectivity of prefrontal networks predicts sensorimotor task performance and learning. Furthermore, prefrontal tDCS enhances multi-day retention of performance on a complex dart throwing task. However, a major gap exists regarding learning of locomotor (walking) tasks. This study is designed to address this gap. Our pilot data show that bihemispheric prefrontal tDCS administered during a single session of learning on a complex obstacle walking task contributes to multi-day retention of task performance. In this study we will build upon this pilot work by conducting a full-scale trial that also investigates mechanisms related to brain structure, functional activity, and network connectivity.

5. Specific Aims:

Specific Aim 1: Determine the extent to which prefrontal tDCS augments the effect of task practice for retention of performance on a complex obstacle walking task.

Hypothesis 1: Retention on the obstacle walking task at 1-week (primary outcome) and 1-month post-training will be significantly higher for an active prefrontal tDCS group versus a sham tDCS group.

Specific Aim 2: Determine the extent to which retention of performance is associated with individual differences in baseline and practice-induced changes in brain measures.

Hypothesis 2a: Better retention on the obstacle walking task will be associated with baseline greater working memory performance, greater prefrontal gray matter volume, and greater task-based prefrontal fNIRS activity.

Hypothesis 2b: Better retention on the obstacle walking task will be associated with training-induced increased prefrontal gray matter volume and reduction of task-based prefrontal fNIRS activity (indicating neural efficiency and/or reduced executive demands).

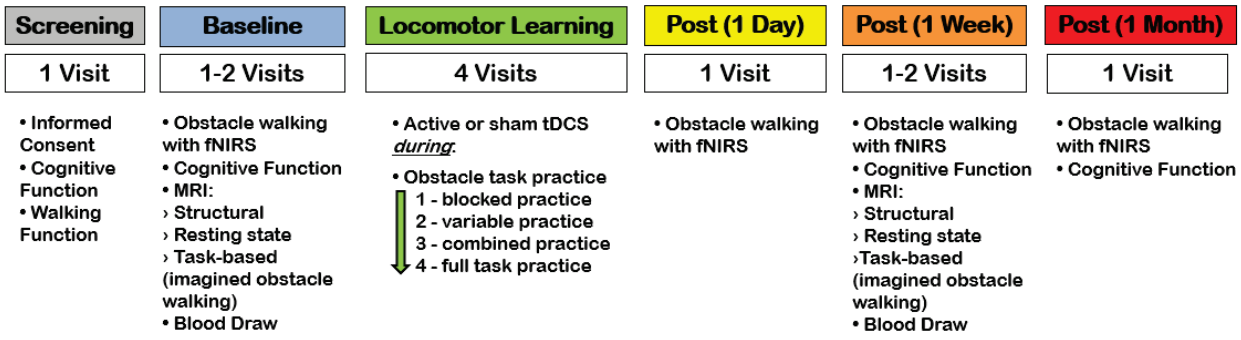
Hypothesis 2c: Better retention on the obstacle walking task will be associated with baseline network connectivity, including resting default mode network segregation.

Specific Aim 3: Investigate the extent to which tDCS modifies resting state network segregation.

Hypothesis 3: Compared to the sham tDCS group, the active tDCS group will demonstrate improved segregation of resting state networks at post-training relative to baseline.

6. Research Plan:

Study Overview



Up to one hundred older men and women will be enrolled in the intervention component of this study. An additional 15 participants (estimated) may enroll in the intervention but discontinue participation before completing the protocol. A larger number (up to 400) may participate in screening by telephone and/or onsite, but will fail the screening tests and not proceed to the study intervention. Participants will undergo the following sequence of events:

- *Telephone Screening*: Participants will be screened by telephone to determine if they meet basic enrollment criteria including age and general health status. If patients authorized us to view their medical records, those records may be examined to verify absence of exclusion diagnoses, to obtain complete/accurate medication lists, and to assess whether participants have successfully completed prior MRI scans.
- *Informed Consent and Onsite Screening Visit*: Participants who pass the phone screen will be invited to participate in on-site screening. Informed consent will be obtained. Mobility function, cognitive function, and health/safety screening will be conducted. This single visit will last approximately 3.5 hours. Qualifying participants will be invited to participate in the full study with or without MRI.
- *Baseline* Participants will undergo a test of walking over obstacles, turning while walking (90 degree turns and 180 degree turns), turning while standing in place (360 degree turn), cognitive function, blood draw, and MRI scans at the UF McKnight Brain Institute (structural, functional, and resting state MRI scans) if MRI screening passed. Baseline assessment will last about 4 hours and may be completed over 1 or 2 visits based on scheduling flexibility of the participant, study personnel, and equipment.
- *Study Intervention Visits:* Participants will attend four intervention sessions in which they will practice a “locomotor learning” task comprised of a 10m walking course with obstacles and transitions between hard and soft floor surfaces. During this task they will receive either active or sham transcranial

The obstacles walking task includes a series of foam obstacles and foam mats. This walking task is used for baseline visits, study intervention visits, and post visits.

direct current stimulation (tDCS) to the frontal portion of the brain. Each visit will last approximately one hour.

- *1-Day Post Assessment*: Participants will undergo a test of walking over obstacles.
- *1-Week Post Assessment*: Participants will undergo a test of walking over obstacles, cognitive function assessment, blood draw, and MRI scans (MRI eligible participants only) at the UF McKnight Brain Institute (structural, functional, and resting state MRI scans). Post assessments will last about 4 hours and may be completed over 1 or 2 visits based on scheduling flexibility of the participant, study personnel, and equipment.
- *Visit 9: 1-Month Post Assessment*: Participants will undergo a test of walking over obstacles, turning tasks, and cognitive function assessment. This visit will last approximately 2 hours.

Telephone Screening

Participants will be screened by telephone to determine if they meet the following criteria:

Inclusion criteria

- Age 65 - 95 years
- Willingness to be randomized to either intervention and to participate in all aspects of study assessment and intervention

Exclusion criteria

- Diagnosed neurological disorder or injury of the central nervous system, or observation of symptoms consistent with such a condition (spinal cord injury, Alzheimer's, Parkinson's, stroke, etc.)
- Contraindications to non-invasive brain stimulation (e.g., metal in head, wound on scalp)
- Contraindications to MRI (claustrophobia, metal anywhere in body, etc.).
- Use of medications affecting the central nervous system including, but not limited to, benzodiazepines, anti-cholinergic medication and GABAergic medication.
- severe arthritis, such as awaiting joint replacement
- current cardiovascular, lung or renal disease; diabetes; terminal illness
- myocardial infarction or major heart surgery in the previous year
- cancer treatment in the past year, except for nonmelanoma skin cancers and cancers having an excellent prognosis (e.g., early stage breast or prostate cancer)
- current diagnosis of schizophrenia, other psychotic disorders, or bipolar disorder
- difficulty communicating with study personnel (including people who cannot speak English)
- uncontrolled hypertension at rest (systolic > 180 mmHg and/or diastolic > 100 mmHg)
- bone fracture or joint replacement in the previous six months
- current participation in physical therapy for lower extremity function or cardiopulmonary rehabilitation
- current enrollment in any clinical trial
- clinical judgment of investigative team

Informed Consent and Onsite Screening Visit

Upon arriving to the research site, participants will undergo informed consent. We will explain the full study protocol including transcranial direct current stimulation and MRI.

All questions from the phone screening will be repeated, and additional health/medical screening criteria will be evaluated. This will include:

- Resting blood pressure
- Basic visual examination with Snellen eye chart
- Height, weight, age, sex, education level, socioeconomic status
- Medical history
- Obtain list of medications that the participant is currently using
- Various walking tasks including typical walking, fast walking, walking over obstacles, walking while carrying a tray, and combinations of these tasks.
- Somatosensory assessments of the foot based on tactile pressure sensation.
- Activities Specific Balance Confidence Scale: 16-item questionnaire that gauges confidence (on a scale of 0-100%) on various balance and walking tasks relevant to household and community ambulation.
- Berg Balance Scale: 14-item assessment of balance task performance to assess balance ability and fall risk.
- Movement-specific Reinvestment Scale: questionnaire that asked about the extent to which a person directs conscious attention to control of movement.
- Montreal Cognitive Assessment (MoCA)
- Trailmaking Test – paper based test where the person must “connect the dots” between numbers or between alternating letters and numbers.
- Sleep quality assessed by the Pittsburgh Sleep Quality Index, which is a questionnaire about sleep-related habits and experiences.

Following the screening visit, study staff will evaluate performance/responses relative to the study enrollment inclusion/exclusion criteria. BMI will be calculated from the height and weight collected. Medical records may be examined to verify absence of exclusion diagnoses, to obtain complete/accurate medication lists, and to assess whether participants have successfully completed prior MRI scans. Individuals who meet all criteria will be invited to enroll in the full study. Participants who screen out of MRI may also be invited to enroll for the study with the MRI portion excluded.

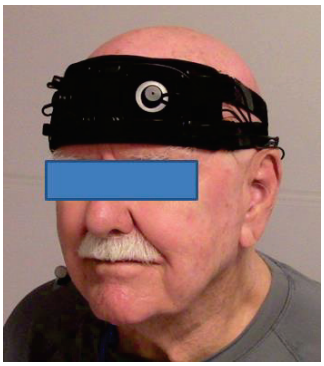
At the discretion of the Principal Investigator, any individual may be deemed ineligible for further participation in this study if there are concerns about the individual’s capability to perform study procedures or if it may be unsafe for the volunteer to participate in the study. Furthermore, minor exceptions to the inclusion/exclusion criteria may be permitted at the discretion of the Principal Investigator if those exceptions do not influence participant safety. For example, if a participant walks somewhat faster than the target inclusion criteria for walking speed. This is important to ensure that individuals are not excluded for insignificant reasons and to facilitate meeting enrollment benchmarks.

Baseline Visit(s)

Various assessments of walking performance and neural control will be made during the obstacle walking task or while at rest. Other walking tasks will also be assessed, including “complex” tasks such as walking while carrying a tray, walking backwards, walking while performing a cognitive task (such as naming words beginning with a certain letter), walking fast, and combinations of multiple tasks. Tests of gait will be performed on our CIRFace walkway, which measure parameters such as step length and swing time. Tasks will include straight walk, 90 degree turns, 180 degree turns and 360 degree turns. A computer-based cognitive function assessment will be administered.

Biomechanics and movement data

Performance on walking tests will be assessed using motion analysis instrumentation such as force plates, motion capture camera, and/or an electronic walkway. When motion capture cameras are used, we will tape small reflective markers to the participant's body which are visible to the camera and allow offline calculation of joint movements.



fNIRS headband

fNIRS prefrontal activation

Prefrontal brain activity may be measured with fNIRS during walking and cognitive assessments. fNIRS is a safe non-invasive technology for indirectly assessing cortical activation based on changes in blood flow and oxygenation. Sensors are held in place on the forehead by a headband, and produce infrared light that is able to pass through the skull and is absorbed or reflected by blood and other tissues in the head. Infrared light is considered safe and there is no sensation association with this procedure. Calculations made from the relative amount of light absorption/reflection provide an estimate of the metabolic activity level of underlying cortical tissue. We will use a commercially available fNIRS monitor (Octamon by Artinis Medical Systems).

Skin conductance

Skin conductance detects changes in activity of the sympathetic nervous system (fight or flight system) that occur when a person feels task-related acute stress. Skin conductance is increased with "nervous sweating" on the palmar surface of the hand. For example, walking over obstacles has been shown to elicit a larger skin conductance response compared to typical walking. Adhesive electrodes are placed on the palmar surface of two fingers on each hand. These electrodes are attached by a wire to small control unit worn on a belt.

Video, photos, and voice recordings

During this study, videos and/or photos may be taken during to document the functional abilities of participants, and for possible use in research presentations or education. We may also use voice recordings to capture performance on cognitive assessments such as a verbal fluency task performed during walking. At the time of consent, participants will choose what their videos/photos/recordings can be used for. We will avoid capturing images of the participants' faces, and will obscure or delete any such images.

Magnetic Resonance Imaging (MRI)

We will use multiple MRI to assess brain structure, resting state functional connectivity, and task-based connectivity during imagined walking. MRI testing on eligible participants will be performed at the McKnight Brain Institute on an MRI scanner with a 32 channel head coil. Participants will first be directed to a waiting room to complete an MRI safety screening. The participant's answers to this safety screening will be reviewed and approved by the MR technician. For all scans, participants will be instructed to remain awake and alert. For functional MRI during imagined walking, participants will be asked to imagine walking on the obstacle course shown in Figure 1 and over flat ground. Following the MRI we will use the "Vividness of Imagined Walking Questionnaire" to assess if the participant was able to imagine the task well.

Blood Draw

Blood draws will be conducted by a trained phlebotomist in the UF Center for Cognitive Aging and Memory (CAM) phlebotomy lab in the McKnight Brain Institute (MBI). Up to 45 milliliters of

blood will be drawn and processed by the phlebotomist in order to collect enough serum to be placed in 12 cryovials that will be stored in a -80 degree freezer also housed in the CAM lab at MBI (room LG-192). 2.5 milliliters of blood will be drawn directly into a PaxGene tube for genetic testing, with the primary goal of assessing polymorphisms in BDNF and COMT.

Cognitive Function Assessment

We will administer the Cognitive Assessment Battery from Creyos Research, formerly Cambridge Brain Sciences. The assessments currently remain named Cambridge Brain Science Assessment, which includes up to 12 cognitive tests. All are short, simple, computer-based assessment that assess domains of cognitive such as spatial working memory, response inhibition, and reasoning. Specific examples include:

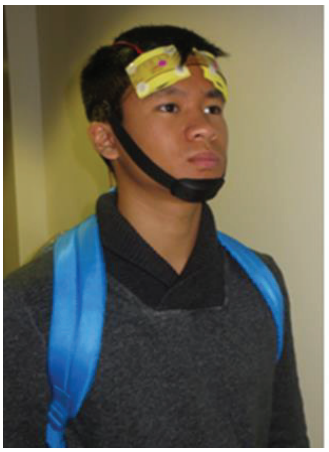
- Digit Span Test –determine the longest list of numbers that a person can remember.
- Visuospatial working memory –remember the sequence and location of items that appear and disappear from the screen.

Locomotor Learning Visits

Description of Locomotor Learning Task

Each of the four locomotor learning sessions will include 20 minutes of actual task practice. Short rest breaks are built in throughout this period, and participants will also be allowed to take additional rest breaks as often as they wish. Participants will wear a gait belt around their waist during this task, which allows research staff to more easily grip and assist a participant if he/she becomes unsteady. The sessions will be scheduled approximately equally over two weeks. To optimize learning and retention, we will use a tiered approach for task practice which includes blocked practice for Session 1, random practice for Session 2, combined practice for Session 3, and full course practice for Session 4. Blocked practice means that one isolated component of the task (e.g., transitioning between soft and hard surfaces) will be practiced before moving onto another isolated component (e.g., stepping over single obstacles), and then another (e.g., stepping over double-height obstacles). Random practice means that the aforementioned isolated components will each be practiced multiple times, but interspersed in a random order. Combined practice means that the components will no longer be practiced in isolation, but rather will be combined such as stepping over and obstacle during the transition between soft and hard surfaces. Full course practice means that the participant will practice on the actual course that is used for assessment, at both slower and fast speeds. This tiered approach to learning ensures that participants attain full understanding and mastery of each component early on. As mastery of the basic components is achieved, the complexity and attentional demands of the task are increased to promote robust learning and offline consolidation.

tDCS during Locomotor Learning



Participants will be randomized to receive either active or sham tDCS, which will be delivered simultaneously during the locomotor learning task. A commercially available tDCS unit (Soterix Medical Inc.) will be used for delivery of stimulation. The unit will be carried inside of a backpack so that stimulation can be delivered during walking. The tDCS unit is very lightweight, so wearing it in a backpack adds no appreciable effort to the walking tasks. Electrode preparation will involve saturating a sponge electrode with exactly 10cc of 0.9% saline solution using marked syringe (5cc/side). Electrodes will be placed using lightweight plastic "headgear" that is adjustable for head size and can hold the electrode montage in place during stimulation. We have considerable experience delivering this exact tDCS protocol during walking in our ongoing research studies.

Active tDCS: Twenty minutes of 2.0mA direct current through two biocarbon rubber electrodes encased in saline soaked 5cm2 sponges placed over the frontal cortices at F3 and F4 (based on the international "10-20 system" of standardized brain electrode placement). 2.0mA was chosen based on prior research.

Sham tDCS: Sham stimulation is performed with the same device and all procedures will be identical except for the duration of stimulation. Participants will receive 30 seconds of 2.0mA of direct current stimulation at the beginning of each rehabilitation session. Since participants habituate to the sensation of tDCS within 30-60 seconds of stimulation, this procedure provides the same sensation of active tDCS.

Post Visits

Participants will return to lab approximately 1 day, 1 week, and 1 month after completing the locomotor learning protocol. These visits will be very similar to the baseline visit, including each of the components listed above in that section. At each visit they will walk over the obstacle course again to assess changes in performance. At the 1 week and 1 month visits the cognitive function assessment will be repeated. At the 1 week post visit the MRI protocol and/or blood draw protocol will be repeated (except for the 2.5ml PaxGene, which is only needed at baseline) depending on MRI eligibility.

Possible Discomforts and Risks:

As with all physical activity, there is a risk of falling while we test or train walking ability. Falls can lead to injuries ranging from minor to serious. It is also possible that participants could experience musculoskeletal injury such as an ankle sprain. It is possible that the participant may experience fatigue, soreness, and discomfort due to physical activity associated with this study. These are unlikely to be worse than what he/she would experience due to increased physical activity outside of our study. These are normal responses to exercise and most discomfort would generally disappear within a matter of days. In general, walking exercise is strongly recommended for all adults, including elderly adults with medical conditions.

There is a risk that participants may find cognitive and functional tests challenging or uncomfortable if they have difficulty succeeding with the tasks. Participant may feel embarrassed if they perform poorly on cognitive tests. Participants may skip any question that they do not wish to respond do.

Transcranial direct current stimulation (tDCS) has been used by many prior studies and has an excellent record of safety and tolerability. The most often reported effect is tingling or itching sensations at the site of electrode placement, which may be accompanied by redness of the skin. Some people also report headache or a feeling of fatigue. All of these effects dissipate quickly, and tDCS has not been reported to have prolonged negative consequences. A recent literature review article compiled data from all tDCS clinical studies performed from 1998 to August 2010. Of 209 studies (172 articles, encompassing almost 4000 subjects), active tDCS and sham tDCS did not differ in the frequency of adverse effects that were observed. The most common adverse effects were headache, itching, burning sensation (without actual injury), discomfort and tingling, occurring in 10-40% of patients regardless of treatment group.

Magnetic resonance imaging is considered a safe, and there are no known long term effects from short term exposure to magnetic fields. The primary risk is to people with implanted metallic objects or medical devices, which could move or heat up as a result of the magnetic field. This could cause injury or discomfort. We will screen our participants carefully to ensure that no metal objects are present in or on their bodies. Another risk is that metal objects within the MRI testing area could become projectiles. We will be careful to ensure that no metal objects are present in the testing area. Some participants report claustrophobia while inside the scanner. Participants are screened for known claustrophobia before participating (and this is a contraindication for participation). If a participant begins to experience symptoms of claustrophobia during the scan, they will have the option of discontinuing the scan at any time. The scanner produces a loud noise, so participants will be provided with earplugs and/or MR-compatible headphones to alleviate any potential discomfort or possible hearing damage due to this loud noise.

fNIRS is considered safe. The infrared light used in assessment is not known to cause any harm or to alter the brain in any way. The sensors are secured to the participant's head using adhesive tape, which may cause minor skin irritation in some people, particularly those who are sensitive/allergic to adhesives. We will check with participants about any known sensitivities to adhesives.

Skin conductance involves placing adhesive electrodes on two fingers of each hand. It is sometimes slightly uncomfortable to remove the electrodes because they are very sticky.

The risks of drawing blood from a vein include discomfort at the site of puncture; possible bruising and swelling around the puncture site; rarely an infection; and, uncommonly, faintness from the procedure. There is also a risk of breach of confidentiality with regard to the data and samples collected. In order to minimize risks associated with the blood draw, samples will be obtained by a licensed phlebotomist using aseptic technique. A number of steps are taken to protect the participant's privacy. The collected information will be stored electronically with restricted access and password protection. Only approved research staff have password access to the database. Participant test results from the blood draws will be assigned a unique identification number, with the key linking sample numbers to study participant information kept in a separate location (i.e. on a restricted access server).

8. Possible Benefits:

There is no direct benefit to the participant.

9. Conflict of Interest:

None.

10. Statistical analysis and sample size calculations

For Specific Aim 1 we seek to determine whether active tDCS is superior to sham tDCS for enhancing retention of locomotor learning after one week. The primary outcome is the change in walking speed on the obstacle course between the end of the learning period to the one week post visit. We are powering the study to detect group differences in retention of walking performance. We will use an ANOVA model to test group differences, and will account for demographic covariates. To estimate sample size for this aim, we have conducted a pilot study with 12 older adult participants who were randomized to an active (n=5) or sham (n=7) tDCS group. To assess retention, the mean walking speed from the final three trials of the learning session were compared to the mean walking speed from the three retention trials measured one week later. The active tDCS group showed a between-session gain in walking speed of nearly 0.07 m/s. The sham tDCS group showed a drop in between-session speed of approximately -0.04 m/s. This between group-difference of 0.1 m/s is considered clinically meaningful and had a large effect size of 0.87. The whole-group standard deviation of response was 0.12. Based on these preliminary data, we will need 48 participants (n=24 per group) to detect a statistically significant group difference at alpha=0.05 and beta=0.20 (i.e., 80% power). To be conservative, we will enroll 60 participants to account for potential dropout and to ensure sufficient statistical power.

For Specific Aim 2 we seek to determine if several key measures of brain integrity/function help to explain individual differences in the retention of locomotor learning after one week. We will use multivariate regression models that account for demographic covariates. Based on the assumptions of univariate linear regression, a sample size of 60 with alpha=0.05 and beta=0.20 (i.e., 80% power) will allow us to significantly detect correlations coefficients as small as 0.355. Values below this level are generally considered very weak, and thus there would be little benefit to powering the study to detect smaller values. Based on our pilot data and findings from the literature, we are very likely to observe stronger associations that will achieve statistical significance. For instance, our pilot data show that working memory performance and prefrontal activation are associated with visuomotor learning with correlations of 0.57 and 0.48, respectively. Our pilot data also show that resting state default mode network connectivity is associated with retention of learning with a correlation of 0.46. Our statistical models will account for a number of demographic and other covariates, thereby accounting for some of the variability in the data set and improving our ability to detect these mechanism-related associations.

For Specific Aim 3 we seek to acquire evidence that tDCS modifies resting state network connectivity and segregation during motor learning. Prior studies have shown that even a single session of motor learning or a single session of tDCS can modify the connectivity of resting state brain networks. However, this remains an exploratory aim and we have not specifically powered the study for this purpose. Nevertheless, as with the other measures described above, if the effect size is moderate or large we are likely to detect it with statistical significance.

Data and Safety Monitoring Plan

Adverse Event Reporting

Adverse events will be reported according to the guidelines of the University of Florida Institutional Review.

Reporting within 5 days of the PI becoming aware will apply to adverse events that meet all of the following criteria:

- Serious
- Unexpected
- Related or the Relationship is “more likely than not”

Adverse events will be added to the cumulative event table and reported at continuing review when they meet either of the following criteria:

- Serious (but expected) and related or the relationship is “more likely than not”.
- Unexpected (but not serious) and related or the relationship is “more likely than not”.

A *serious adverse event* is any adverse event that results in any of the following outcomes:

- death,
- a life-threatening adverse event,
- inpatient hospitalization or prolonging existing hospitalization,
- a persistent or significant disability/incapacity,
- or a congenital anomaly/birth defect.

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse event when the event may jeopardize the patient or subject and/or may require medical or surgical intervention to prevent one of the outcomes listed in the definition above.

An *unexpected adverse event* is any adverse event that is not consistent with the current investigator brochure, protocol, consent form, or is not part of the normal disease progression. In addition, known adverse events may occur more frequently than expected. If so, then this meets the definition of “unexpected” and must be reported to the IRB.

Protection Against Risk

Staff training: All personnel will be thoroughly trained in the study procedures by the Principal Investigator or other appropriate member of the research team, and will complete all required trainings concerning human subjects research at the University of Florida.

Health monitoring and medical response: Volunteers at risk of health problems due to recent history of medical conditions (e.g., serious cardiac or pulmonary conditions) will be excluded, as noted above in the inclusion/exclusion criteria. Any adverse events will be recorded and monitored as required by our University of Florida Institutional Review Board. In the event of a medical emergency at the VA Hospital (our study site) we will call the hospital emergency response line, 6-9-1-1 and alert them to a code blue (medical emergency). Subjects will be able to terminate a study session at their request at any time without prejudice. Minimization of risk during neurorehabilitation and assessments will be accomplished by monitoring vital signs, with prescribed criteria for termination of the testing session. Vital signs will be monitored before, during and after assessment. Contraindications for participation will include resting heart rate >100 bpm or <50 bpm, resting systolic blood pressure >180 mmHg or <100 mm Hg or resting diastolic blood pressure >100 mmHg. Indications to terminate physical activity will include subject complaints of shortness of breath, light-headedness, dizziness, confusion, severe headache, dyspnea or onset of angina. If any of these conditions are greater than mild or persist after rest, the patient’s primary physician will be called and patient referred for evaluation. If the patient complains of angina at rest, loss of consciousness occurs, or cardiac arrest, emergency medical services through 911 will be called immediately. Portable defibrillators are available.

Confidentiality: Data will be used only in aggregate and no identifying characteristics of individuals will be published or presented. Confidentiality of data will be maintained by using research identification numbers that uniquely identify each individual. Safeguards will be established to ensure the security and privacy of participants' study records. Appropriate measures will be taken to prevent unauthorized use of study information. Data other than demographic information will not use names as an identifier. The research ID number will be used. The research records will be kept in a locked room in the study site. The files matching participants' names and demographic information with research ID numbers will be kept in a locked file that uses a different key from that of all other files. Only trained and certified study personnel will have access to these files, and they will be asked to sign a document that they agree to maintain the confidentiality of the information. Electronic records will be stored on password protected network server maintained by the university information technology department. In compliance with the Health Insurance Portability and Accountability Act (HIPAA) and the Standards for Privacy of Individually Identifiable Health Information of the Department of Health and Human Services, we access personal health information and medical records only after receiving signed informed consent.

REDCap: VA REDCap will be used to store de-identified data for increased efficiency and secure access by approved staff. This will eliminate the need for storage of written records, all data will be accessible in one secure location. REDCap is a secure database only accessible by authorized staff onsite from a VA connected computer password protected and maintained by VA OIT.

Safety during walking: The intensity and duration of walking activity will be carefully monitored to ensure that it is appropriate to the participant's capabilities. Participants will wear a gait belt during walking, which will better enable the therapist and/or assistants to provide support in the event of a loss of balance. Falls will be tracked and reported to the PI and IRB even if there is no injury associated.

tDCS Safety: Our protocol uses stimulation parameters that are considered standard practice, and have been used safely in prior research. The most common side effects of tDCS are slight itching, tingling, and reddening of the skin under the electrode. Participants typically habituate to itching or tingling sensations within 60 seconds of stimulation. To minimize risk associated with tDCS, participants will be monitored throughout stimulation sessions and asked to report any discomfort. If stimulation sensation is uncomfortable, the stimulation levels will be decreased to a comfortable level or will be stopped.

fNIRS Safety: fNIRS poses no safety risk. Infrared light from the fNIRS device is not harmful and elicits no sensation. Sensors may be secured to the skin using adhesive tape, which could lead to mild skin irritation in those who are sensitive to adhesives. If a person indicates or displays such a sensitivity, we will avoid using adhesive tape and instead use elastic fabric or straps to secure the sensors.

Questionnaire administration: Questionnaire data are collected in secure spaces where the interview cannot be overheard. Participants will be informed that they are not required to answer questions that they do not wish to answer.