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Protocol Title: Assessing Cognitive Improvements, Brain Neuroplasticity and the Role of Genetic Factors After Aerobic Exercise in Sedentary Adults

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1) Protocol Title

Assessing Cognitive Improvements, Brain Neuroplasticity and the Role of Genetic Factors After Aerobic Exercise in Sedentary Adults

2) Objectives

The overall goal of the proposed study is to evaluate the effects of an 8-week aerobic exercise program on cognition and determine the relationship between cognitive improvements and neuroplasticity. Limited to Option A (See Study Options section), we will also explore the effect modification of BDNF levels and BDNF allelic status, and APOE4 status on cognitive response after exercise.

Aim 1: Quantify improvements in cognitive performance after 8 weeks (150 min per week) of moderate intensity aerobic exercise in sedentary adults ≥ 55 years of age. H1: We hypothesize that we will see improvements in overall cognitive performance after exercise, and greater improvements in some of the cognitive domains (e.g., in executive function and processing speed, as opposed to memory or language).

Aim 2: Determine an association between changes in plasticity, aerobic capacity, and cognitive performance overall and by cognitive domain, after 8 weeks of moderate intensity aerobic exercise in sedentary adults ≥ 55 years of age. H2: We hypothesize that a greater response in the brain plasticity will be associated with greater improvements in cognitive performance overall and in executive function after exercise.

Aim 3 (Option A): Explore the effect modification of BDNF (levels and Val66Met) and APOE e4 on cognitive response after exercise. H3: We hypothesize that individuals with greater levels of BDNF, being Val carriers or APOE e4carriers will have greater improvement in cognitive performance after exercise.

3) Study Plan/Options

Due to challenges posed by the COVID-19 pandemic, social distancing practices, and aiming to create a feasible scenario to continue the current study, we propose an additional option to safely adapt study methods to deliver the study in a home-based, fully remote manner. Importantly, this scenario also presents as an opportunity to collect meaningful data on our specific aims while translating this successful research program into a remote/home-based mode of delivery. A remote/home based option may also yield valuable preliminary data that will be relevant for planning future exercise studies in aging adults in the present "new normal".

Therefore, this research project will continue using a two-option design consisting of:

- Option A: Current, IRB-approved, in-person methods pending appropriateness based on current University of Miami recommendations and standard operating procedures as it relates to the COVID-19 pandemic.
- Option B: Remote option of current methods to maximize recruitment. This will be the only option until the study team obtains approval to resume Option A (as per University's safety guidelines, as mentioned above).

4) Background

Individuals 65 and older will reach 30% of the global population over the next 30 years, totaling approximately 2 billion people.[1] Many will develop age-related cognitive decline, a condition for which there is no effective prevention or treatment. Therefore, the development of successful

strategies to support cognitive health and remediate age-related cognitive decline is critically needed. Exercise is effective at improving cognitive performance,[2–4] but there is considerable inter-individual variability in cognitive outcomes after exercise. A greater understanding of this variability, the mechanisms, exercise dose and type is necessary to improve the effectiveness of exercise in prevention and remediation of age-related cognitive decline.

Improvements in cognition after exercise are attributed to neuroplasticity, which is generally defined as the reorganization of the functional connections and structure of the nervous system to adapt to changes in experience and environment. Neuroplastic changes after exercise include: neurogenesis,[5] angiogenesis,[6] synaptogenesis,[7] and production of neurotrophic molecules such as brain derived neurotrophic factor (BDNF).[8,9] All of these changes contribute to synaptic neuroplasticity that represents an increase in the efficacy of synaptic transmission in intracortical circuits related to cognitive function, learning and memory. Long-term potentiation (LTP)[10] is a form of synaptic neuroplasticity defined as an increased synaptic efficacy following a direct electrical current stimulus of hippocampal neurons. LTP may explain synaptic activity implicated in improved cognition after exercise.[11]

Transcranial magnetic stimulation (TMS) is a non-invasive device that applies a brief magnetic field close to the cranium to induce a very low amplitude current in the brain.[12–14] TMS is FDA approved for the treatment of drug-resistant depression, neurosurgery mapping, migraines and has been used in research settings since 1984.[13,14] TMS allows for an assessment of neuroplasticity that was modeled after the animal experiments of LTP synaptic plasticity in hippocampal neurons, but is performed through non-invasive stimulation of the motor cortex.[15–17] In our pilot work, we established the feasibility and safety of TMS neuroplasticity measures in exercise and cognition research in middle-aged adults,[18] and demonstrated that TMS plasticity assessment can identify individuals who derived greater cognitive benefits in executive function after 4 weeks of aerobic exercise with no adverse events.[18] However, TMS neuroplasticity has not been used to investigate exercise-mediated cognitive improvements in aging adults. Option A, we have been using TMS to gain insights into brain plasticity that may be relevant for exercise-induced cognitive improvements.

For Option B, we will use transcranial alternating current stimulation (tACS), which is one specific form of transcranial electrical stimulation (tES) combined with electroencephalography (EEG) to measure neuroplasticity that is similar to TMS neuroplasticity measurements. In both paradigms, the brain's response to a mild electrical stimulus is recorded. With TMS, a mild electrical stimulus is delivered with electromagnetic induction, and the response is measured with electromyography surface electrodes. With tACS, a mild electrical current is delivered with small electrodes placed on the scalp, and measured with electromyography surface electrodes. These two methods are under the same neurophysiological principles, and thus, yield comparable data.

The practical application of tACS is simple: a low intensity (1-2 mA) alternating electrical current is applied to the scalp to influence underlying cortical excitability, which is measured with non-invasive surface EEG electrodes. Direct current stimulation is presently FDA-approved for extracranial use, and FDA applications for cranial stimulation for management of mood disorder and chronic pain are in progress. The stimulator used in this study is the STARSTIM®-Home system (Neuroelectrics, Inc), a tES home-base system that recently received FDA approval for the Investigational Device Exemption supplemental protocol in a study for tES to be used to treat patients with Major Depression Disorder (MDD) due to COVID-19 related restrictions. STARSTIM®-Home system is capable of recording EEG before, during and after tACS stimulation.

Genetic factors may modify cognitive response to exercise. A common single nucleotide polymorphism of the BDNF gene (Val66Met) impairs activity-dependent release of BDNF.[19] We and others have demonstrated that the Val66Met allele attenuates improvements in cognitive performance after exercise, both in young[18,20] and old adults[21]. Furthermore, carriers of the apolipoprotein E (APOE) e4 alleles exhibit greater cognitive benefits after exercise through unknown mechanisms,[22,23] although

they have greater risk for AD.[24,25] The effects of genetic factors on response to exercise will only be evaluating in the Option A.

Our prior work has demonstrated that the link between exercise and cognitive improvements in older adults is largely based on cardiovascular fitness.[4] Therefore, we will quantify cognitive improvements following an aerobic exercise intervention in sedentary adults aged 55+ years, and determine the relationship between cognitive improvements and neuroplasticity. We will also explore the effect modification of BDNF levels and BDNF allelic status, and APOE4 status on cognitive response after exercise for Option A only.

This project is supported by a KL2 Career Development Grant from the Miami Clinical and Translational Research Institute at UM that is funded until December 2020. With the additional Option B of the study, our goal is to maximize the opportunities to gain valuable data that will be necessary for an R-type NIH submission in early 2021. Given the increasing use of ubiquitous mobile technologies in aging and older adults, especially in light of the COVID-19 pandemic, the addition of a remotely delivered clinical research program may facilitate recruitment, address barriers to participation, and accelerate the translation of our findings to a broader audience and clinical application.

5) Inclusion and Exclusion Criteria

Subjects will be screened by collecting information to ensure the participant's eligibility in the study. Screening will be considered visit/session #1.

Inclusion Criteria:

- age ≥ 55years
- no clinically detectable cognitive impairment (MoCA score ≥ 24)
- sedentary (defined as 'low' category using the International Physical Activity Questionnaire (IPAQ) short last 7 days).
- basic computer skills (accessing an e-mail or using the internet).

Exclusion Criteria:

- any unstable medical condition (i.e.: uncontrolled hypertension or uncontrolled diabetes)
- medical contraindication to physical exercise
- Contraindication to neuroplasticity assessment (TMS and tACS):
    a. Any current history of a psychiatric illness
    b. No medication is an absolute exclusion from TMS and tACS. Medications will be reviewed by the Principal Investigator and a decision about inclusion will be made based on the following:
        i. The subject's past medical history, drug dose, history of recent medication changes or duration of treatment, and combination with other CNS active drugs.
    c. Any metal in the brain, skull or elsewhere unless approved by the responsible MD
    d. Any medical devices (i.e. Cardiac pacemaker, deep brain stimulator, medication infusion pump, cochlear implant, vagal nerve stimulator)
    e. Intracranial lesion
    f. Substance abuse or dependence within the past six months
    g. Exclusively for Option A: TMS Neuroplasticity assessment, as per the TMS Safety Guidelines.[13]
        i. History of fainting spells of unknown or undetermined etiology that might constitute seizures
        ii. History of seizures, diagnosis of epilepsy, history of abnormal (epileptiform) EEG or family history of treatment resistant epilepsy

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- Pregnant women
- Vulnerable populations such as prisoners
- People unable to consent themselves
- Subjects who, in the Investigator's opinion might not be suitable for the study

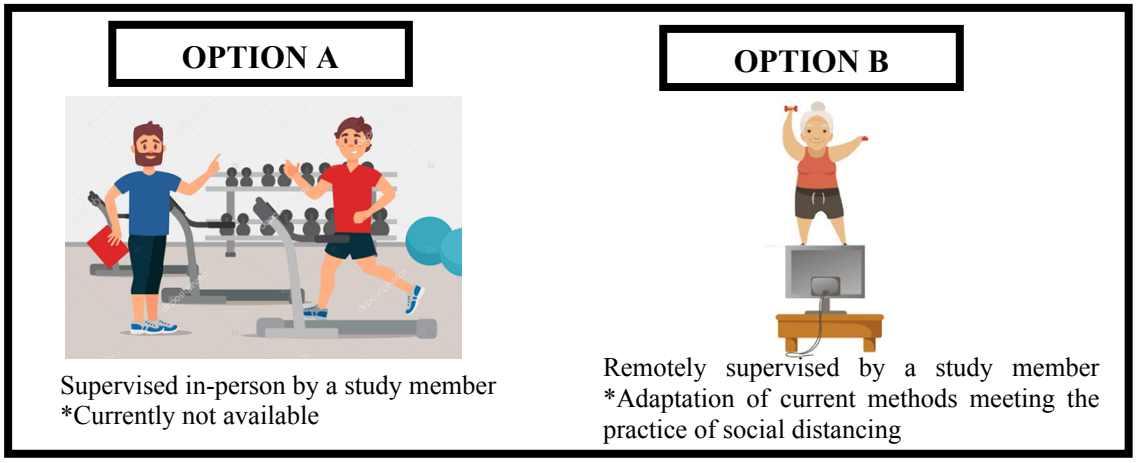
6) Number of Subjects

This is not a multi-center study. We will recruit a total of 80 adults. We will use a multifaceted approach by recruiting participants from the Evelyn F. McKnight Brain Institute community memory clinic, and by advertising the study using flyers throughout the University of Miami Miller School of Medicine (UMMSM) and Coral Gables Campuses, the Miami community and participant registries. We will also recruit via online research database tool including the ResearchMatch.org. In addition, we will access University Research Informatics Data Environment (URIDE), and potential subjects will be identified and contacted via the Consent to Contact Initiative.

With a significance level of 0.05, our sample of 80 individuals will provide 80% power to detect a Cohen effect size of 0.36 using a two-sided paired t-test for Aim 1, the effect size associated with improvements in executive function in a recent meta-analysis of healthy adults 55+,[26] and an attrition of 20%, not uncommon in exercise intervention trials. In addition, a sample of 80 participants will provide 80% power to detect a correlation of 0.34 between the change in neuroplasticity and the change in cognitive performance.

7) Study Timelines

The total time for a participant to complete this study will vary based on participant availability but is feasible to be completed within 10 weeks total (the intervention portion of the study is 8 weeks, with week 1 designated for pre-testing and week 10 for post-testing). Subjects will be recruited utilizing an open enrollment process over the course of 2 years. The following timeline will be utilized to guide and track intervention based on participant visit/session number and option selected as described in the following figure.



OPTION A: This option follows the current study timeline.

Visit #1: Screening process (may be partially done over a phone interview, but requires one visit in person to consent and collect physical measures, completed in week 1)

- Information will be collected to ensure that the participant is eligible to participate in the study. Estimated total time: 40 minutes [20 minutes (in person or phone interview, Attached) and 20 minutes in person]. These include:

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- Demographic information and medications/drugs in use
- Montreal Cognitive Assessment[27] (MoCA)
- International Physical Activity Questionnaire (IPAQ)[28] and self-report body mass index (BMI) measurement
- TMS screening and description of the study and overview of inclusion/exclusion criteria (may be done via phone with corresponding documentation)
- Medical History, Physical Ability Readiness questionnaire, Vital Signs Sheet. The decision to participate in the study will be made upon a review of the potential participant's medical history, vital signs sheet and, physical ability readiness questionnaire and a systems review performed by a licensed Physical Therapist who is a member of the Laboratory. Based on the results of this evaluation, if the subject is found to require further medical clearance, they will be referred to their physician for further evaluation and the assessment of potential eligibility in the present study.
    - Exercise Questionnaires (literacy, self-efficacy, barriers/motivators)

Visit #2 Pretest assessment- Part 1 (estimated time: 3 hours, completed in week 1, may be performed on same visit as Part 2)

- Blood draw
- Gait and postural control assessment
- Review of the adverse effects
- TMS plasticity measures
- Review adverse effects post TMS

Visit #3 Pretest assessment- Part 2 (estimated time: 1.5 hours, completed in week 1, may be performed at same visit as Part 1)

- Cognitive test battery
- Exercise testing
- Exercise Questionnaires (literacy, self-efficacy, barriers/motivators)

Visits #4-15: Physical exercise Intervention-Moderate Intensity (estimated time per visit: 60 minutes 3 times per week for weeks 2-5, total of 4 weeks of Moderate Intensity Exercise)

- Assessment of vitals (blood pressure, heart rate)
- 60 minutes of aerobic exercise (includes 5 minute warm-up/5 minute cool-down)
- Re-assessment of vitals

Visits #16-27: Physical exercise Intervention-High Intensity (estimated time per visit: 60 minutes for weeks 6-9, total of 4 weeks of High Intensity Exercise)

- Assessment of vitals (blood pressure, heart rate)
- 60 minutes of aerobic exercise (includes 5 minute warm-up/5 minute cool-down)
- Re-assessment of vitals

Visit #28 Posttest assessment- Part 1 (estimated time: 3 hours, maybe completed on same day as Part 2, completed in week 10)

- Blood draw
- Gait and postural control assessment
- Review of the adverse effects
- TMS plasticity

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- Review adverse effects post TMS

Visit #29 Posttest assessment- Part 2 (estimated time: 1.5 hours, may be completed on same day as Part 1, completed in week 10)

- Cognitive test battery
- Exercise Testing
- Exercise Questionnaires (literacy, self-efficacy, barriers/motivators)

Optional Test visits:

- An additional testing combining TMS and EEG during pre and post testing will be offered to participants. This testing is performed to gather information about direct measure of neuronal activity. This would add approximately two hours to each visit.

Visit #4: TMS/EEG pretesting measure (estimated time: 2 hours)

- Review of adverse events
- Single pulse TMS plasticity measures
- EEG recording
- Review adverse effects post TMS

Visit #31: TMS/EEG post testing measure (estimated time: 2 hours)

- Review of adverse events
- Single pulse TMS plasticity measures
- EEG recording
- Review adverse effects post TMS

OPTION B: This option is an adaptation of the current methods that includes updating testing timeline to include a familiarization session. We updated our assessment to include outcome measures that are appropriate for remote delivery. We eliminated our genetic testing as it not possible to be performed remotely. In this option, in-person **visits** will be replaced with remote **sessions**.

Adaptations of the current timeline include:
Session #1a: Screening process:

- A virtual consenting process will be completed using zoom for healthcare and REDCap e-consent as approved through the Clinical and Translational Science Institute (CTSI). The individual will be sent a REDCap link either through a secure email or through the chat feature of zoom. Once the individual opens the link, they will be asked to share their screen. At that time a trained study personnel will review the informed consent with the individual and answer any question the individual may have about the study. If the individual agrees to participate in the study, they will be asked to sign the e-consent form and provide the study personnel remote access of their web browser to also sign the consent form. The individual will then certify the information and submit the consent. At that time, they will have the option to download or print the completed consent form. A copy can be mailed at the individual's request. Participants will then be mailed a study kit.

Session #1b: Familiarization session- Review items delivered to place of residence using secured zoom (estimated time: 1.5 hours, completed in week one, follow up session may be scheduled as needed)

- Familiarize participant with all of the tools in the study kit.

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- The participant will learn to take their own blood pressure, oxygen saturation, put on the heart rate monitor, and proper safety and cleaning procedures.
- Participants will learn various zoom functions such as sharing screens which will be later utilized in the study.
- Participant will learn how to put on the EEG cap and how to operate the tablet.

Session #2 and #28 Pretest and Post assessment Part 1 (estimated time: 2 hours, completed in week 1)

- TMS plasticity assessment will be replaced with the tACS/EEG plasticity assessment.
- TMS-EEG will not be offered for Option B of the study.

Session #29 Posttest assessment- Part 2 (estimated time: 2 hours, completed in week 1).

- Return Study Kit.

8) Study Endpoints

Neuroplasticity Measures

- **TMS Plasticity Measures**
  - An index of the duration of the TBS-induced modulation of corticospinal excitability (the time-point at which the normalized mean MEP amplitude returns to baseline values) will be defined for each participant.
- **TMS-EEG**
  - The TMS-EEG integration provides real-time information on cortical reactivity and connectivity. EEG provides exquisite temporal resolution, a direct measure of neuronal activity, and is capable of differentiating inhibitory and facilitatory effects in response to TMS. Contrasting the results of TMS on EEG before and after TBS provides information about cortical plasticity (at the site of stimulation) and resulting network dynamic adaptations.
  - Brain functional connectivity and inter-regional coordination can be directly estimated from EEG's. Spontaneously occurring potentials across EEG channels will be detected prior and following stimulation using already developed event detection algorithms. Measurements following single-pulse TMS provide insights on cortical excitability. The EEG cap will be placed at the beginning of the session and will remain on for the duration of the visit.

- **tACS/EEG Plasticity**
  - An index of change in the tACS-induced modulation of cortical excitability will be defined for each participant. Brain functional connectivity and inter-regional coordination can be directly estimated from EEG's. Spontaneously occurring potentials across EEG channels will be detected prior to and following stimulation using already developed event detection algorithms.

Cognitive performance

- Cognitive performance will be assessed using a neuropsychological test battery. Executive function will be assessed using the Digit Span subtest of the Wechsler Adult Intelligence Scale – Fourth[30], processing speed will be assessed using the Trail-Making Test Part B[31]and the Stroop color-word test[29], global cognition will be assessed with the repeatable neuropsychological battery (RBANS)[30]; and the Delis-Kaplan Executive Function System (DKEFS)[31] will test for language.

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- Option B: the same cognitive battery, except for the Stroop color-word test, and with the addition of a TestMyBrain.org[32] battery of Digit Symbol Matching, Simple and Choice Reaction Time, and Gradual Onset Continuous Performance Test will be used via a secured Zoom Platform.

Gait and postural control

- The Timed Up-and-Go (TUG) test will be completed. Four, 90-second trials of walking will then be completed to assess gait under normal and cognitive dual task conditions. Standing postural control will be assessed by measuring postural sway with mobile sensors during 2 repetitions each of 30-second trials of standing—two with eyes open, two with eyes closed, and two while performing the serial subtraction cognitive task.
- Option B: The Timed Up-and-Go (TUG) test will be completed and supervised via a secured Zoom Platform.

Genetic Testing

- Blood samples will be collected for BDNF levels and DNA to assess brain-derived neurotrophic factor (BDNF) Val66Met polymorphism and the presence of apolipoprotein-E (APOE) e4 allele. We will store blood samples for future examination of other potential genetic, epigenetic, metabolic or pro-inflammatory markers.

Aerobic Capacity

- The Incremental Shuttle Walking Test (ISWT) will be performed to determine maximal walking velocity and walk distance. These values will be used to predict VO^2 peak changes over time as a measure of aerobic capacity. The test will be used to dose the aerobic exercise intensity. Additionally, changes in VO^2 peak will assessed for relationship with neuroplasticity and cognitive changes. The participants will be closely monitored (Heart rate, Blood pressure, rate of perceived effort) prior, during and after the test.
- Option B: We will adapt our exercise assessment to a submaximal aerobic capacity assessment using the 1-minute Sit-to-Stand Test (1-MSTST)[33] which can be completed in the participant's home. The 1-MSTST will be performed to determine maximal repetitions completed in one minute without using upper limbs. These values will be used to predict changes in aerobic capacity over time. The test is submaximal and will be used to dose the aerobic exercise intensity. Additionally, changes in aerobic capacity will be assessed for any relationship with neuroplasticity and cognitive changes. The participants will be closely monitored (heart rate, blood pressure, rate of perceived effort, and oxygen saturation) prior to, during and after the test.

9) Procedures Involved*

Study Design

This is an investigator-initiated open label study in neurologically healthy sedentary adults. Participants will undergo a total of 29 or 31 sessions, including the screening session, three to four pretest (baseline) assessments, 24 aerobic exercise sessions and three to four post-test assessments. For option A, assessments will take place at the Neuromotor Plasticity Lab located at Lois Pope Life Center located at 1095 14th Terrace, 1st Floor, Miami, FL 33136 and aerobic exercise visit will take place at either the UMMSM Medical Wellness Center or the UM Coral Gables Wellness Center. For option B, assessments and exercise sessions will take place in the participant home as they are monitored by a trained study member. An illustration of the experimental design is provided below:

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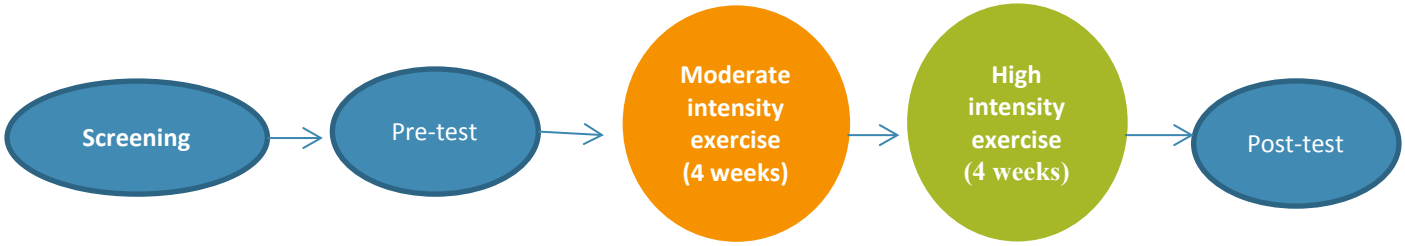

Methods:

Subjects will participate in the Option A pending appropriateness based on current University of Miami recommendations and standard operating procedures as it relates to the COVID-19 pandemic. Given the COVID-19 pandemic, it is worth of note that potential participants may remain less enthusiastic about in-person procedures for an undetermined length of time. Thus, even in the event that the study team obtains approval to resume activities as per Option A based on University guidelines, we would like to continue to offer Option B according to participant preference. All testing procedures described for the Option A will be adapted (as feasible) for the Option B. Testing procedures that differ for the Option B are described and noted in the Option B methods.

OPTION A METHODS

TMS Plasticity

Subjects will come in for a total of 2 plasticity visits (pre and post training). We will measure plasticity in the motor cortex of the dominant hand.

Subjects will be set up in a chair with EMG electrodes placed on the first dorsal interosseus (FDI) on the dominant hand to measure motor threshold over in the corresponding (contralateral) motor cortex (M1). The motor threshold serves as the basis for the following TMS-measures over the motor cortex. When administering TMS, the figure of eight coil will be placed tangentially to the scalp with the handle pointing posterior for all stimulations. We will proceed with determining motor threshold and conduct TMS (single pulse TMS and TBS) over the dominant motor cortex. After removing the EMG equipment from the subject, a post TMS safety evaluation will be conducted.

During each of the TMS/plasticity visits, the following procedures will take place:

Single Pulse TMS

TMS will be delivered using a biphasic figure of eight coil. This stimulation phase will consist of 3 batches of 30 TMS pulses delivered every 5-7 seconds at 120% of resting motor threshold (RMT) prior to TBS stimulation as a baseline. RMT will be defined as the minimum stimulus intensity that produced a small MEP (about 50 μV in 50% of 10 trials) during relaxation of the tested muscles. After TBS has been administered, batches of single pulse TMS will be administered at timed intervals for 40 minutes over the motor cortex.

TBS

For this study subjects will receive intermittent Theta Burst Stimulation (iTBS) over the motor cortex. iTBS consists of bursts of 3 pulses at 50 Hz repeated at intervals of 200ms for a total of 2 seconds (1 train) each train will be repeated every 10 seconds for 20 times for a total of 600 stimuli.

After TBS is applied to the motor cortex in an intermittent fashion (iTBS), TMS-induced potentials show increased amplitude for a period of 20-30 minutes.

Stimulation intensity will be delivered at 80% of active MT (AMT). AMT will be defined as the minimum stimulus intensity that produces a small MEP (about 200 μV in 50% of 10 trials) during isometric contraction of the tested muscles, at about 20% of maximum voluntary contraction.

Transcranial Magnetic Stimulation (TMS) and EEG

EEG provides exquisite temporal resolution, is a direct measure of neuronal activity, and can be correlated with cognitive performance, and is capable of differentiating inhibitory and faciliatory effects in response to TMS. Resting EEG will be compared with neuropsychological testing and imaging data. Event related potentials (ERPs) will be measured by EEG during TMS stimulation to assess for electrophysiologic response during testing. A TMS compatible EEG system would be set up and left in place for the duration of the visit. The TMS-EEG integration provides real-time information on central physiologic responses such as cortical reactivity and connectivity.

Subjects will be set up in a chair with EMG electrodes placed on the right hand for collection of motor evoked potentials (MEPs) during stimulation over the left primary motor cortex (M1). The EMG electrodes will remain in place throughout the TMS session. In order to keep the brain target constant throughout the stimulation session, we will use a frameless stereotactic neuronavigation system.

Baseline EEG Measures:

A 64 channel, TMS compatible EEG system will be set up at the beginning of the visit. Resting state EEG measures will be collected that includes a 3.5-min recording with eyes opened followed by a 3.5-min recording with eyes closed.

TMS with EEG measures:

Assessment of Motor Threshold:

Resting motor threshold (RMT) will be determined by applying single pulses of TMS to M1. RMT will be defined as the minimum stimulus intensity that produces a motor evoked potential (MEP) of at least 50 μV and up to 1 mv in the hand muscles in at least 5 of 10 trials. MEPs will be measured by electromyography (EMG) during relaxation of the tested muscles. Determination of RMT will be used to guide intensity to be used for single and paired pulses as described below. The optimal position for obtaining MEPs will be identified at the beginning of the assessment of RMT.

TMS Assessments of Intracortical Inhibition and Facilitation Using Single TMS Pulses:

Cortical reactivity will be assessed by applying paired and single pulses of TMS to the cortex and intracortical inhibition and facilitation will be assessed using a paired pulse technique. Paired pulse techniques measure the influence of a sub- or supra-threshold conditioning TMS pulse to M1 or DLPFC on a supra-threshold TMS pulse to M1 or DLPFC. TMS intensity will be set at 120% of each individual's resting motor threshold for reactivity measures with single pulses and sub- and supra-threshold TMS pulses will be set to 80% and 120%, respectively for paired pulse measures. Cortical reactivity will be assessed via EEG measures (TMS-evoked potentials - TEPs) and cortico-motor reactivity will be assessed at M1 by measuring peak-to-peak amplitude of MEPs (motor-evoked potentials as measured by EMG) induced in the hand muscles in response to the TMS.

Neuropsychological Test Battery

Participants will undergo 2 neuropsychological test visits (pre-test and post-test) to assess the influence of physical exercise on cognitive performance. This assessment will last approximately 1.5

hour.

- Visuomotor processing speed and cognitive flexibility/task switching: This will be assessed using the Trail Making Test (TMT),[34,35] is a two-part test (parts A and B). Part A consists of a series of circles with the numbers 1- 25 scattered on a sheet of paper. Individuals are asked to draw a line from one circle to the next following the sequential order of the numbers until all the encircled numbers are connected. They are asked to complete each of these tasks as quickly as they can without making mistakes. Part B consists of encircled numbers (1-13) and letters (A-L). Individuals are asked to connect the circles alternating between numbers and letters as quickly as they can without making mistakes. Each part is scored according to the number of errors made and the time (in seconds) to completion.
- Response inhibition, mental flexibility, and attentional control: This will be assessed using the **Stroop Color Word Test**[29]. The test consists of three components, each component consisting of 100 items, presented in five columns. On the first portion, participants are asked to read words printed in black ink (RED/GREEN/BLUE). On the second portion, participants are asked to identify the color of ink in which a series of Xs are printed. On the final section, participants are asked to identify the color of ink the color words are printed in. Participants are asked to complete each component as quickly and as accurately as possible. Each section is scored based on number of items completed accurately within a 45 second period.
- Attention and working memory: The **Digit Span subtest of the Wechsler Adult Intelligence Scale - Fourth edition**[36] will be used to asses this construct. This measure consists of three parts; namely, Digit Span Forward (DSF), Digit Span Backward (DSB), and Digit Span Sequencing (DSS). Participants are read increasingly longer lists of numbers (starting with 2 digits and increasing in difficulty up to 9 digits) and asked to repeat them verbatim, in backward order, and sequentially (i.e., from smallest to largest) on DSF, DSB and DSS respectively. Each portion is discontinued when participants cannot accurately repeat 2 consecutive trials of equal difficulty.
- Global Cognition: The **Repeatable Battery for the Assessment Neuropsychological Status (RBANS) Update**[30] is a brief battery used to measure an individual's cognitive state. The battery is composed of five cognitive domains including: Immediate memory, Visuospatial/Constructional, Language, Attention, and Delayed Memory. The whole assessment is completed in approximately 25 minutes.
    - Immediate memory is assessed through a list learning task of 10 words and a short story. Participants are asked to repeat a list of 10 words over a series of four learning trials. The score is dependent on the number of correctly recalled words over the four trials. Participants are then read a short story and asked to recall as many details from the story that they can remember over 2 trials. The score is based on the number of correct key details the participant can recall.
    - Visuospatial/Constructional is assessed using a figure copy and line orientation task. Participants are asked to copy a figure as accurately as they can while being timed. The score is based solely on the exactness of the copy of the figure. Participants are then shown 13 lines that are numbered 1 to 13 and are arranged in a fan shape that are 15 degrees apart from each other at the top of a page. Participants are shown a series of two lines, isolated from the fan shape, and asked to identify what numbers they correspond to on the fan shape. Participants are awarded one point for each time they correctly match the lines.
    - Language is assessed through picture naming and semantic fluency. Participants are asked to identify the name of ten different figures. The score is based on the number of figures the participant names correctly. Participants are then asked to name as many items from a specific category as they can for one minute. One point is awarded for each unique response.

- Attention is composed of a digit span and coding task. Participants are read a series of numbers increasing in length in which they are asked to repeat. Participants are awarded one point for each series of number they correctly recall. Coding is comprised of a key of nine symbols that are associated with a number one through nine. The participant is then asked to use the key to assign numbers to a list of matching symbols below, where the numbers are missing. One point is given for each symbol correctly assigned to a number based on the key.
- Delayed memory is measured by recalling the ten words and short story from the immediate memory task, recalling the figure copied during the visual spatial constructional task, and identifying the ten words from the list learning task among twenty other words.

- Verbal Fluency: The Verbal Fluency subtest of the **Delis-Kaplan Executive Function System (D-KEFS)**[31] will be used to assess verbal fluency. This subtest is comprised of letter fluency, category fluency, and category switching. During the letter fluency portion, participants are asked to name as many words as they can that begin with a certain letter of the alphabet within one minute. There are three trials with three different letters and the participant is awarded one point for each unique, appropriate response. For Category fluency, participants are asked to name as many items as they can that correspond to the category provided within one minute. Participants complete two trials with two different categories and are awarded one point for each unique, appropriate response. During the category switching the participant is asked to switch between naming items from two different categories within one minute. Participants are awarded one point for each unique appropriate response given for each category as well as each time they correctly switch between categories.

Postural Control assessment

Participants will undergo a functional evaluation of to assess the influence of physical exercise on postural control and gait. The duration of these assessments is approximately 0.5 hours.

- For the functional assessment, participants will be outfitted with wireless accelerometers (Mobility Lab, Seattle, WA) secured with Velcro straps to the wrists, ankles, sternum and lower back. The Timed Up-and-Go test will be completed, which is a valid and reliable test of mobility[37] that requires the subject to stand from a chair, walk 3 meters, turn and sit back down. Four, 90-second trials of walking will then be completed along a 35x4m indoor hallway to assess gait under normal and cognitive dual task conditions. The cognitive dual task will be verbalized serial subtractions of 7 from a random 3-digit number. Finally, standing postural control will be assessed by measuring postural sway (i.e., center-of-pressure fluctuations) during 6, 30-second trials of standing—two with eyes open, two with eyes closed, and two while performing the serial subtraction cognitive task.

Exercise Testing

A test of maximal aerobic capacity will be obtained using the Incremental Shuttle Walking Test[38] and prediction equations to determine VO^2 peak changes over time. Dr. Lawrence Cahalin, PT, PhD Professor of Physical Therapy and a leader in cardiorespiratory fitness testing and training, will serve as a consultant on this proposal and will oversee the development and implementation of the exercise testing and training, and guide the analysis and interpretation of the results.

Blood Collection

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Blood samples will be collected for BDNF levels and DNA to assess brain-derived neurotrophic factor (BDNF) Val66Met polymorphism and the presence of apolipoprotein-E (APOE) e4 allele. We will store blood samples for future examination of other potential genetic, epigenetic, metabolic or pro-inflammatory markers. DNA will be extracted by standard methodology and the prepIT L2P reagent.[39] The SNPs of the BDNF gene (rs6265, rs429358 and rs7412) and APOE4 will be analyzed using TaqMan genotyping assays. Blood samples will be delivered to the lab on ice within 2 hours of collection, centrifuged and stored at -80 °C until processing.

Physical exercise Intervention

The physical exercise intervention will be administered at the University of Miami Miller School of Medicine Wellness Center. Participants will be given a time slot, which will be preferred for the duration of the study but flexibility with the intervention time frame will be provided. Participants will be supervised by a member of the study team during all times and all sessions. Each participant will engage in 60 minute daily sessions delivered 3 times/week for 8 consecutive weeks (a total of 24 sessions).

Participants will be fitted with a heart rate monitor and will be instructed to walk (or trot) to maintain 55-64% of maximal heart rate (determined by exercise test) for the first 4-weeks of exercise or to maintain 65-90% of maximal heart rate (determined by exercise test) for the second 4-weeks of exercise during each session. Participants will be supervised at all times during the exercise sessions, and will be given the opportunity to rest if they indicate that they are uncomfortable. During all exercise sessions a research personnel who is trained in Basic Life Support or Cardiopulmonary Resuscitation (CPR) and First Aid certified will be present on site. During this session, heart rate and participant's exerted effort (measured with the Borg scale[40] will be monitored prior to the session, every 10 minutes of the 50 minute session and 5 minutes after the end of the session. The Borg scale is a numerical scale where 6 represents rest (no effort), and 20 represents maximal effort (see photo on the right).

A PT will be available by phone if subjects are reporting excessive discomfort in need of evaluation, and an appropriate referral will be made as necessary. In the event of an absence, participants will have the opportunity to make up the missed session.

20 Point Borg Scale

RPE
Rate of Perceived Exertion

POINT	EFFORT	DESCRIPTION	% OF MAXIMUM HEART RATE	√
6	No Exertion	Little to no movement, very relaxed	20%	
7	Extremely Light	Able to maintain pace	30%	
8			40%	
9	Very Light	Comfortable and breathing harder	50%	
10			55%	
11	Light	Minimal sweating, can talk easily	60%	
12			65%	
13	Somewhat Hard	Slight breathlessness, can talk	70%	
14		Increased sweating, still able to hold conversation but with difficulty	75%	
15	Hard	Sweating, able to push and still maintain proper form	80%	
16			85%	
17	Very Hard	Can keep a fast pace for a short time period	90%	
18			95%	
19	Extremely Hard	Difficulty breathing, near muscle exhaustion	100%	
20	Maximally Hard	STOP exercising, total exhaustion		

Source: Gunnar Borg, PhD, M.D.

Exercise Questionnaires:

We will perform the Lifetime Physical Activity Questionnaire, Exercise Self-Efficacy, and Barriers and Motivators to Exercise questionnaires.

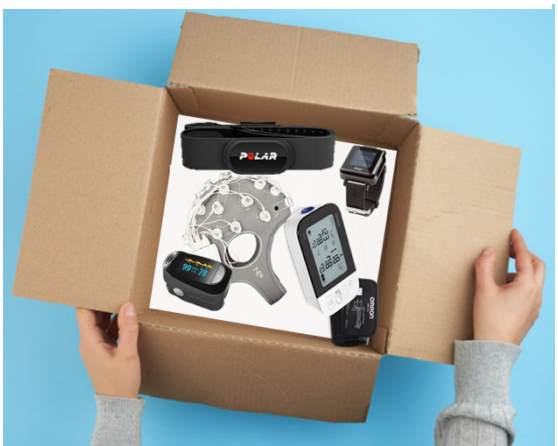
OPTION B METHODS

Study Kit

Participants will receive a study kit including items that have been previously sanitized using alcohol-based solution greater than 70% will be shipped to the participant's home. The study kit will include:

1. a sphygmomanometer (OMRON BP7350, Omron Healthcare, Inc.)
2. a heart rate monitor (Polar H10, Polar Electro Inc., Lake

Items included in the "Study Kit'



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Success, NY, USA)
3. a pulse oximeter (Diagnostix™ 2100 Fingertip Pulse Oximeter)
4. a physical activity monitor (Actigraph GT9X-BT Link, ActiGraph LLC, Pensacola, FL, USA)
5. a tape measure, masking tape, alcohol prep pads Isopropyl Alcohol 70%
6. a non-invasive brain stimulation/EEG hybrid STARSTIM®-Home (Neuroelectrics, USA) that includes a tablet.
7. a pre-paid return shipping label

When the study kit arrives, before beginning any assessments or intervention, we will schedule a remote meeting utilizing zoom for healthcare, to familiarize the participant with all of the tools in the study kit. The participant will learn to take their own blood pressure, oxygen saturation, put on the heart rate monitor, put on the EEG cap, and proper safety and cleaning procedures. If needed, we may schedule multiple meetings to ensure the participant is properly familiarized with all of the materials in the study kit.

Adaptations to Our Testing Procedures

tACS Plasticity

Subjects will come in for a total of 2 plasticity visits (pre and post training).

Subjects will be set up in a chair and will don the STARSTIM®-Home (Neuroelectrics, USA). The practical application of tACS is simple: a low intensity (1-2 mA) alternating electrical current is applied to the scalp to influence underlying cortical excitability, which is measured with non-invasive surface EEG electrodes. The study investigator will remotely supervise the session utilizing a tablet and zoom for healthcare that comprises the STARSTIM®-Home system (Neuroelectrics, Inc). The neuroplasticity assessment will follow the current guidelines for the safe application of neurophysiological measures recommended by the International Federation of Clinical Neurophysiology.[13,14]

Following the familiarization session, the study investigator will remotely supervise the session utilizing a tablet that comprises the STARSTIM®-Home system. TACS will be performed over two sites, approximately covering the scalp over the frontal lobe, and the test will last for approximately 30 minutes. STARSTIM®-Home is capable of recording EEG before, during and after tACS stimulation. The STARSTIM®-Home system can only be used at pre-specified times, determined and approved by the study investigator. In accordance with the safe application of neurophysiological measures recommended by the International Federation of Clinical Neurophysiology, we will collect and monitor adverse events.

During each of the tACS plasticity visits, the following procedures will take place:

Baseline Resting EEG

Resting EEG recordings will take place for 2 minutes with participant's eyes open and for 2 minutes with participant's eyes closed while the participant is seated.

tACS

A low intensity (1-2 mA) alternating current will be applied to the scalp approximately over the frontal lobe for 2 minutes via the STARTSTIM®-Home system (Neuroelectrics, Inc) as directed by the study investigator while the participant is seated.

tACS Plasticity EEG

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Resting EEG recordings will take place for 2 minutes with participant's eyes open and for 2 minutes with participant's eyes closed after receiving tACS stimulation. A change in baseline resting EEG potentials will be compared to post tACS-EEG potentials to determine neuroplasticity.

Neuropsychological Test Battery

This assessment will be performed in the same manner as previously described for Option A, except all testing will be delivered via a secured Zoom for Healthcare Platform. The Stroop Color-word test will be omitted from Option B. Additionally, Option B will use an additional battery from TestMyBrain.org.[32]

- TestMyBrain.org: As there are some tests that are not appropriately delivered via zoom, a few additional tests will be utilized for option. A TestMyBrain (TMB) link will be sent to participants during their assessment visit, and the investigator will guide them through the test. Overall this portion of the test will require 10 minutes.
    - Processing Speed is assessed using the TMB Digit Symbol Matching. Participants are shown symbols that match a corresponding number. Participants must quickly and accurately select the appropriate the number. They have 90 seconds to complete as many symbols as possible.
    - Basic psychomotor response speed will be measured with TMB Simple Reaction Time. Participants will be shown either "GO!" or "WAIT!". Participants must press the space bar on the computer as rapidly as possible, only when the word "GO!" is presented.
    - TMB Choice Reaction Time will be used to measure processing speed, response inhibition, and attention. Participants are shown 3 boxes with arrows inside of them. Two boxes have the same color, and one is different (odd color). Participants must rapidly select the direction of the arrow in the odd colored box.
    - TMB Gradual Onset Continuous Performance Test assesses sustained attention, response inhibition, and cognitive control. Participants asked to press a key when a city picture appears, and to not press when a mountain image appears. The images rapidly transition from one to the next with mountains appearing only 10-20% of the time.

Postural Control assessment

We will continue to use the Timed Up-and-Go as a measure of functional mobility in the same manner previously described. The participant will be instructed to set-up the chair and measure a 3-meter space with the provided tape measure and tape to complete this assessment in their home and the investigator will guide the timed up-and-go procedure and record their performance.

Exercise Testing

We will adapt our exercise assessment to a submaximal aerobic capacity assessment using the 1-min sit-to-stand test[41] which can be completed in the participant's home. Participants will be asked to join a zoom meeting where they will be asked to confirm their current geographical location and phone number. Participants will put on the heart rate monitor (Polar) and activity monitor (Actigraph). After this, vitals will be taken after 3-5 minutes of seated rest. Blood pressure, heart rate, and oxygen saturation will be documented by the investigator, and participants will complete a daily exercise questionnaire. The participant will then be asked to stand up and sit down from a sturdy chair for one-minute while being monitored by a trained study member. The number of sit-to-stands performed in 1 minute will be recorded. The participant's heart rate will be monitored continuously throughout the test and heart rate and blood pressure will be taken and documented immediately after the assessment. Heart rate and blood

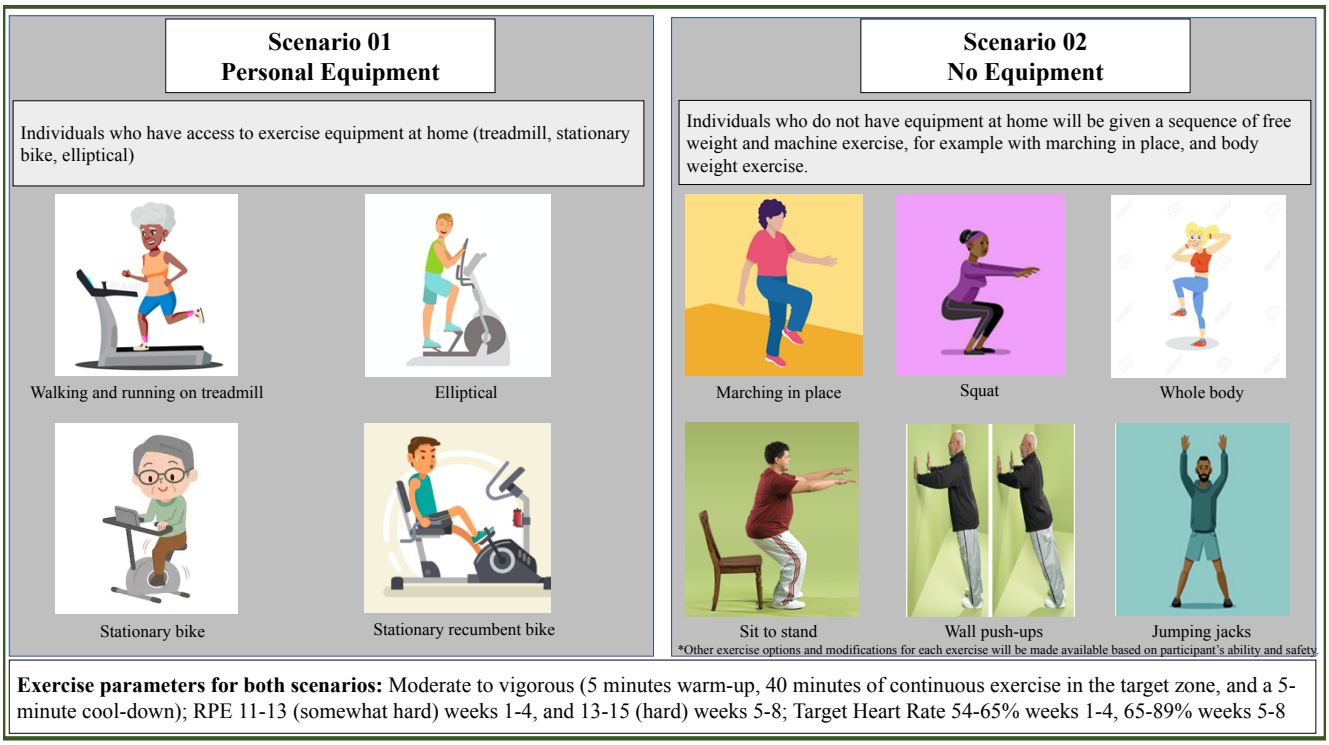
pressure will be taken again after 5 minutes of seated rest and will be continued to monitored until the participant has returned within 20% of their baseline measurement.

Exercise Questionnaires

Questionnaires and surveys will be completed by the participant using a REDCap link sent via a secured email. We are expanding our questionnaire to include a battery of structured surveys used to assess the 7 'pillars' of Brain Health (cognitive function, physical exercise, nutrition, comprehensive health, socialization, sleep, and vital plan [perceived meaning in life]), developed by the Barcelona Brain Health Initiative.[42]

Adaptation of Our Physical exercise Intervention

We have modified our 2-month, 3x/week in-person exercise intervention to be safely delivered



as a home-based exercise program. The study team will setup a zoom call to discuss the plan the exercise delivery modes, which will be individually planned, based on each participant's available resources and personal situations. For instance, participants whom have access to aerobic exercise equipment at home (treadmill, elliptical, or stationary bike) will be able to use them in the same manner as option A with a remote investigator monitoring and instructing. Individuals who do not have exercise equipment at home will be given a routine of exercises that do not require any equipment, and can be done safely in their homes. The body-weight exercise program was developed by a group of 4 physical therapists (one who is the Principal Investigator of the study) who are part of this study team. Modifications for each exercise will be tailored individually based on participant's ability levels and safety. Regardless of mode of exercise, the participant will warm up for 5 minutes, have 40 minutes of exercise, and 5 minutes to cool down. Mandatory rest and water breaks will be incorporated into the exercise session every 8 minutes for two minutes each, for a total of 10 minutes of rest breaks during the exercise session. The following figure shows a sample of exercise types for both scenarios.

Each session, participants will join a zoom meeting with a trained study personnel to ensure safety and guide the participant through the exercise session. The participant will be asked to put on the heart rate monitor and provide their geographical location and phone number as per our safety of exercise

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Time	Personal Equipment Group		Time	Body Weight (No Equipment) Group	
Minutes 0:00-4:59	**Warm-up 5 Minutes**		**Minutes 0:00-4:59**	**Warm-up 5 Minutes**	
Minutes 5:00-14:59	Block 1 Steady State Aerobic	Total Time=10 minutes Work= 8 minutes Rest and Water= 2 minutes	**Minutes 5:00-14:59**	Block 1 Body Weight A/B	Total Time=10 minutes Work= 8 minutes Rest and Water= 2 minutes
Minutes 15:00-24:59	Block 2 Steady State Aerobic	Total Time=10 minutes Work= 8 minutes Rest and Water= 2 minutes	**Minutes 15:00-24:59**	Block 2 Body Weight C/D	Total Time=10 minutes Work= 8 minutes Rest and Water= 2 minutes
Minutes 25:00-34:59	Block 3 Steady State Aerobic	Total Time=10 minutes Work= 8 minutes Rest and Water= 2 minutes	**Minutes 25:00-34:59**	Block 3 Body Weight E/F	Total Time=10 minutes Work= 8 minutes Rest and Water= 2 minutes
Minutes 35:00-44:59	Block 4 Steady State Aerobic	Total Time=10 minutes Work= 8 minutes Rest and Water= 2 minutes	**Minutes 35:00-44:59**	Block 4 Body Weight G/H	Total Time=10 minutes Work= 8 minutes Rest and Water= 2 minutes
Minutes 45:00-54:59	Block 5 Steady State Aerobic	Total Time=10 minutes Work= 8 minutes Rest and Water= 2 minutes	**Minutes 45:00-54:59**	Block 5 Body Weight I/J	Total Time=10 minutes Work= 8 minutes Rest and Water= 2 minutes
Minutes 55:00-60:00	**Cool-down 5 Minutes**		**Minutes 55:00-60:00**	**Cool-down 5 Minutes**	

plan. Heart rate, oxygen saturation, and blood pressure will be documented by study personnel after 5 minutes of seated rest. Participants will then complete the daily exercise questionnaire to ensure it is safe to begin exercising. The participant will have continuous heart rate monitoring throughout the intervention sessions using the heart rate monitor (Polar) and activity monitor CentrePoint Software (Actigraph, USA) permitting investigators the ability to monitor patients remotely and in near real-time. Participants heart rate and rate of perceived exertion at will be recorded at frequent intervals. Mandatory rest and water breaks will be implemented to ensure proper hydration.

Exercises will follow a standard format to ensure safety and adherence to study goals (See following table). All participants will complete a guided 5-minute warm-up. The intervention will be structured in five, 10-minute timed blocks consisting of eight minutes of exercise and two minutes of rest. For the body-weight group, participants will be shown two exercises. They will perform exercise "A" for one minute then exercise "B" for one minute. The participant will then repeat A-B sequence three more times for a total of 8 minutes of exercise. The participant will then take a rest and water break for two minutes to complete block one. The participant will complete five blocks using this format, completing a total of 10 unique exercises. The participant will then be guided through a cool down where they will slowly decrease exercise vigor to bring their heart rate down to baseline levels. Following exercise, the participant will be asked to take their blood pressure, heart rate and oxygen saturation and will monitored until the participant has returned within 20% of their baseline measurement.

10) Data and Specimen Banking*

Data will be stored indefinitely at the Lois Pope Life Center located at 1095 14th Terrace, 1st Floor, Miami, FL 33136. Data will be stored in a secured area within a locked cabinet accessed by only IRB-approved personnel.

For Option A, samples will be stored indefinitely at the John P. Hussman Institute for Human Genomics, University of Miami located at 1501 NW 10th Ave, Miami, FL 33136. Tubes will be labeled and managed according to the Laboratory Information System. Sample log-in and tracking will assign a unique sample ID to each specimen. The samples will be stored in a -80 freezer. Samples will be accessed only by IRB-approved personnel and PI designees. The release of data or samples will need prior authorization by the PI.

11) Data Management

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Data Monitoring

Data will be stored in accordance with University Policies. It will be kept in a locked cabinet, and the only people who will have access to this data are personnel approved by the IRB to work on this study. Data will be stored indefinitely at the Lois Pope Life Center located at 1095 14th Terrace, 1st Floor, Miami, FL 33136 and at 5915 Ponce de Leon Blvd, 5th Floor, Coral Gables, FL 33146. Data will also be stored using approved digital storage such as Box, Microsoft OneDrive, the data capturing system REDCap. The release of data or samples (only collected from in person) will need prior authorization by the PI. Data collection will be halted if the participant requests it. If this occurs, subject will be taken off the study and the reason of discontinuation will be documented.

Data Analysis:

With a significance level of 0.05, our sample of 80 individuals will provide 80% power to detect a Cohen effect size of 0.36 using a two-sided paired t-test for **Aim 1**, the effect size associated with improvements in executive function in a recent meta-analysis of healthy adults 55+,[42] and an attrition of 20%, not uncommon in exercise intervention trials. In addition, a sample of 80 participants will provide 80% power to detect a correlation of 0.34 between the change in TMS neuroplasticity and the change in cognitive performance for **Aim 2**, and 80% power to detect an R-Squared of 0.11 attributed to a genetic modification in the change in cognitive performance using an F-Test for **Aim 3 (Option A only)**. We expect an overall improvement in cognitive performance after aerobic exercise. Most robust improvements are expected in executive function as previously shown in elderly, but also in other cognitive domains such as processing speed and attention. [11]

Baseline characteristics will be summarized as means ± standard deviation or medians (interquartile ranges) for continuous variables and as frequency (%) for categorical variables. Normality for the distributions of continuous variables will be visually assessed and statistically tested. If the normality assumption is questionable, Box-Cox transformation will be used to reduce the skewness in the indicated analyses or non-parametric tests will be used. All statistical tests will be conducted against a 2-sided alternative hypothesis, employing a significance level of 0.05.

In the univariate analysis, we will compare the cognitive performance (overall and by domain) before and after exercise, using paired t-test to test for **Specific Aim 1**. For **Specific Aim 2**, we will examine the relationship between the change in TMS neuroplasticity and the change in cognitive performance using Pearson product-moment correlation coefficient. For **Specific Aim 3**, we will compare mean change in cognitive performance across subgroups by genotype using F- test. In the multivariable analyses, we will adjust for baseline characteristics (age, sex, race- ethnicity, education) using mixed effect modeling to quantify improvements in cognitive performance after exercise (**Specific Aim 1**), and to determine an association between changes in TMS plasticity, cognitive performance and changes in aerobic capacity (**Specific Aim 2**). We will use modification models by including interaction terms (time-by-genotype) to explore the relationship between change in cognitive performance after exercise and BDNF levels, Val66Met, and APOE e4 status (**Specific Aim 3-Option A only**).

12) Provisions to Monitor the Data to Ensure the Safety of Subjects*

Monitoring the safety of participants with respect to TMS neuroplasticity assessment

TMS has been used in a growing number of laboratories worldwide since 1984. A series of adverse events have been identified since then, and have been thoroughly reviewed for the development of recommended safety guidelines and precautions for the use of TMS, first at a consensus conference at the NIH in June, 1996 and, more recently, in 2008 in Siena (Italy), in a

meeting of an international panel of TMS experts (The Safety of TMS Consensus Group).

The principal investigator will ensure that these guidelines are well-known by all study investigators and will carefully follow these updated safety guidelines in the present study. Additionally, we will track and report any adverse events that may occur.

Monitoring the safety of participants with respect to tACS neuroplasticity assessment

Transcranial Alternating Current Stimulation (tACS) is a form of Transcranial Electrical Stimulation (tES). TES is uniquely suited for a home-based remote intervention as it can be delivered with a lightweight, portable and inexpensive device. Importantly, tES stimulation is painless and has a favorable safety profile. Two independent papers that represent broad consensus efforts between experts in the field, have concluded that tES is safe if appropriate guidelines are followed, that it lacks serious adverse effects and that it is well tolerated. These consensus efforts include the paper by Bikson et al. (2016)[43] that considered 33,200 tES sessions across 1000 subjects undergoing repeated sessions. This report included data from healthy individuals and diverse clinical populations. It also includes the paper by Antal et al. (2017)[44] reflecting the outcome of a consensus meeting, which took place in Göttingen, Germany, on September 6-7, 2016 and follow-up committee work by email correspondence. The committee reviewed safety, ethical, legal, regulatory considerations, and provided application guidelines for tES. The data considered included over 18,000 tES courses of multiple sessions administered to healthy subjects, neurological and psychiatric patients. The conclusion of both these large consensus efforts – and reflecting a true consensus between all participants in both groups – is that tES represents a minimal risk intervention.

As previously mentioned, participants will have a familiarization session where they will be instructed on how to appropriately place the EEG cap. The study investigator will remotely supervise the session utilizing a tablet that comprises the STARSTIM®-Home system. The STARSTIM®-Home system can only be used at pre-specified times, determined and approved by the study investigator. In accordance with the safe application of neurophysiological measures recommended by the International Federation of Clinical Neurophysiology, we will collect and monitor adverse events.

Monitoring the safety of participants with respect to Exercise

The decision regarding ability to perform exercise will be made upon a review of the potential participant's medical history, vital signs sheet, Physical Activity Readiness Questionnaire (PAR-Q) and a systems review performed by a licensed Physical Therapist. Based on the results of this evaluation, if the subject is found to require further medical clearance, they will be referred to a Medical Physician for further evaluation and the assessment of potential eligibility in the present study.

All exercise intervention will be performed by trained study personnel, under the guidance of a Physical Therapist to ensure participant safety during exercise and to respond appropriately in case of an adverse event. There are three physical therapists on our team. As participants will have been screened prior to study enrollment and only subjects appropriate for exercise will be included, no more than minimal risk is present in regards to exercise intervention.

13) Withdrawal of Subjects*

The principal investigator will withdraw participants who become ineligible to participate in the study (as per study inclusion and exclusion criteria), or if there is a safety concern for the participant or study investigators. When subjects withdraw from the study any data already collected will still be used, but further data will not be collected.

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14) Risks to Subjects*

All risks involved in study procedures are outlined below. As some testing is specific to either the Option A or the Option B only, these designations are stated. If no designation is present than these risks are inherent to both options. Each option will have a specific Informed Consent Form that will identify the unique risk based on their option participation.

Risks associated with TMS (Option A Only):

TMS has been used in a growing number of laboratories worldwide since 1984. A series of adverse events have been identified since then, and have been thoroughly reviewed for the development of recommended safety guidelines and precautions for the use of TMS, first at a consensus conference at the NIH in June, 1996 and, more recently, in 2008 in Siena (Italy), in a meeting of an international panel of TMS experts (The Safety of TMS Consensus Group). We will carefully follow these updated safety guidelines in the present study.

More Common

- Headaches and/or Neck Pain: Up to 20%-40% of subjects undergoing TMS experience headaches or neck pain, which are believed to be due to muscle tension. All prior cases of headaches induced by TMS have promptly resolved with a single dose of acetaminophen (Tylenol®) or aspirin. In some cases, TMS may cause facial discomfort on the same side of stimulation.

Rare

- Seizures: TMS can cause a seizure; however, this is an extremely rare problem. Repetitive TMS can induce a seizure even in the absence of pre-existing brain lesions, epilepsy, or other seizure risk factors, both in patients and healthy subjects. From the several thousands of studies that have used TMS to date, a total of 16 cases have been reported, of which 9 cases occurred after the 1998 safety guidelines. Based on the available data, the reported risk of seizures is less than 1 in 1000 for repetitive TMS. Additionally, TMS has been rarely associated with seizure-like events and syncope. Nevertheless, this is a very concerning complication and to make the subjects' risk as small as possible, the investigators will follow precautions that are recommended by the International Society for Transcranial Stimulation and mentioned in the 2008 updated safety guidelines of The Safety of TMS Consensus Group.

- Hearing Problems: TMS produces a loud clicking sound when the current is passed through the stimulation coil. This loud click can result in ringing in the ear and short-term decreased hearing if no protection is used. In order to prevent this potential adverse effect subjects will be given earplugs. Animal and human studies have demonstrated that earplugs can effectively prevent the risk of hearing disturbance due to TMS. In one case[45] a subject's hearing protection fell out and resulted in permanent hearing loss during a 1Hz rTMS session at 120% of the motor threshold. The authors were using a special TMS coil called the H-coil that is designed to reach deep into the subject's brain and is shaped like a helmet fitted onto the subject's head. However, the authors claimed that the loudness of the H-Coil they were using was not different from other coils. This type of coil will not be used in this study. In order to inform and protect the subjects, we will take the following precautions:
    - Inform subjects of the risk of permanent hearing loss, if an earplug should loosen, become detached, or fall out.

- Inform subjects that they should immediately report to the investigator any loosening or detachment of an earplug during TMS.
- Immediately stop TMS to replace earplugs if the subject reports or if an investigator observes that an earplug has loosened or has fallen out.
- Ask the subject if they are experiencing any hearing problems following every session of TMS
- Prompt referral for auditory assessment of all individuals who complain of hearing loss, tinnitus, or aural fullness following completion of TMS.

The risk of hearing loss with our TMS coil is very low with no reported incidence of transient or lasting problems using the methods outlined in this study.

- Syncope: Syncope can occur due to anxiety and psycho-physical discomfort during testing and treatment with TMS. This is reported less than seizure activity but the true number may be higher due to under-reporting. Subjects will be monitored for feeling any signs or symptoms of a pending syncopal event (i.e. feeling dizzy or lightheaded). TMS will immediately be stopped and the subject will be assisted.

- Memory: TMS could induce short-term changes in memory, attention and other cognitive and mental functions. This is a rare risk, as safety studies conducted found these events to be rare and transient.

- Mood: Acute psychiatric effects have been described in patients receiving rTMS. Although single cases suggest a causal relationship between rTMS and mania, the overall rate (13 cases) across 53 randomized controlled studies in depression appears to be low (0.84% mania for active rTMS vs. 0.73% for sham rTMS) and even below natural switch rates in patients with bipolar disorders receiving mood stabilizers (2.3–3.45%). Similarly, cases ofrTMS induced psychotic symptoms, anxiety, agitation, suicidal ideation and insomnia, have been reported, but it is unknown whether these occur at higher rates compared to the natural course of disease being treated or associated with other interventions. Psychotic symptoms and suicidal ideation have never been described in normal subjects during or after rTMS. Subjects with psychiatric problems will not be included in this study, so mood changes are not anticipated.

- Dental Pain: The possibility of dental pain during rTMS has been reported. This potential adverse effect of TMS would occur during the application of the stimulation itself. Should such discomfort occur, we encourage the participant to alert the study investigator. The stimulation session will be immediately terminated, and the participant will be encouraged to seek a dental evaluation. This is a very rare occurrence, but it may point to the presence of a cavity that may require care. This adverse effect should not lead to any lasting problems or complications.

- Finally, even though TMS has been used in several laboratories worldwide since 1984, there could be some unforeseen complications, and the patients will be informed about this possibility.

Risks associated with tACS (Option B Only):

As mentioned before in the 'Monitoring the safety of participants with respect to tACS

neuroplasticity assessment' section, there is a consensus that tACS as in the form of tES is painless and has a favorable safety profile. Also, two independent papers that represent broad consensus efforts between experts in the field, have concluded that tES is safe if appropriate guidelines are followed, that it lacks serious adverse effects and that it is well tolerated, representing a minimal risk intervention.

In accordance with the safe application of neurophysiological measures recommended by the International Federation of Clinical Neurophysiology, we will collect and monitor adverse events. Possible adverse events can include:

- Mild tingling sensation
- Slight Itch
- Temporary mild redness under cap

Electroencephalography (EEG): There are no risks associated with an EEG. The test is painless and safe.

Risks of Gait and Postural Testing

Potential risks of standing and walking include fall, fatigue, muscle strains, sprains, muscle soreness and light headedness. Participants will be supervised at all times during physical function testing, and will be given the opportunity to take breaks if they indicate not feeling well. Typically, muscle soreness is temporary and is resolved within a few days, but if muscle soreness persists, we will do our best to accommodate a "make up" session.

Risks of Exercise Testing and Physical Exercise Intervention

Potential risks of exercise and exercise testing include fatigue, muscle strains, sprains, muscle soreness and light-headedness. There are possible, but infrequent complications of the training exercises such as dizziness, fainting, fall, irregular heartbeat, and very rarely, heart attack, cardiac arrest, or death. Participants will be supervised at all times during the physical exercise intervention and physical exercise testing, and will be given the opportunity to take breaks if they indicate not feeling well. Typically, muscle soreness is temporary and is resolved within a few days, but if muscle soreness persists, we will do our best to accommodate a "make up" session.

The standard procedure for handling emergencies at the University of Miami Medical Wellness Center is to call 911 (emergency response system). Additionally, all study personnel who will be supervising exercise sessions will be trained in CPR.

We have developed a new safety plan specifically for option B to ensure participant wellbeing throughout the intervention which includes confirming the participant's geographical location and phone number at every session. In the event that the participant experiences any adverse event, the trained study personnel will follow the original study protocol, which states to call 9-1-1. The study team will use this geographical information collected prior to the session to assist emergency personnel. We will be enforcing mandatory rest and water breaks during exercise to promote proper hydration and rest. We have created a daily exercise questionnaire to provide strict guidelines for when someone should or should not engage in exercise. The daily exercise questionnaire and participant vitals will be used to determine if participants are appropriate and safe to proceed with the exercise intervention. We have also developed a strict guide to recognize when to stop an exercise.

Electromyography (EMG, Option A only):

The sticky pads used for the test may cause skin irritation or redness. Taking the sticky pads off causes discomfort similar to when taking off a band-aid.

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Neuropsychological (Cognitive) Testing:

Participants may feel tired or frustrated during the cognitive tests, and will be given breaks as needed.

Psychological Stress

Some of the questions we will ask you as part of this study may make participants feel uncomfortable. They have the option to refuse to answer any of the questions and take a break at any time during the study. In addition, participants will be made aware that they may stop participation in the study at any time.

Collection of Blood (Option A only)

There are minor risks associated with having blood drawn for laboratory testing (risks not higher than for any routine blood sampling). The risks of blood drawing include: fainting, the occurrence of temporary discomfort and/or bruise at the site of puncture; rarely, infection or the formation of a small clot or swelling to the vein and surrounding area may occur.

Unforeseeable Risks

There may be unforeseen risks that the investigators are not aware of. In the event this happens, these will be responded to appropriately for subject safety and reported to the IRB.

15) Potential Benefits to Subjects

It is not possible to predict whether subjects will benefit directly from participation in this study. However, participating in regular exercise has been demonstrated to improve overall feelings of well-being and fitness.

16) Vulnerable Populations

This study will not enroll pregnant women, prisoners or children. Subjects have to be able to sign and personally (Option A) or digitally (Option B) date the written/digital ICF as approved by the CTSI. Subjects should be able to follow instructions for exercise interventions and assessment battery.

17) Sharing of Results with Subjects*

Study information will be shared in the format of academic presentations, scientific manuscripts and poster presentations. As this study will involve community recruitment outside of the UHealth system, study results and information will not be uploaded into UChart.

18) Setting

Option A

- Screening and TMS procedures will take place at the Lois Pope Life Center located at 1095 14th Terrace, 1st Floor, Miami, FL 33136.
- Exercise interventions will take place at the University of Miami (Medical and Coral Gables Wellness Centers).

Option B

- Screening, assessments, and exercise sessions will take place at the participant's place of residence. A study kit including items needed to complete assessments will be mailed to the participant. Participants will be asked their geographical location and phone number at the beginning of every assessment and exercise session.

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19) Resources Available

All investigators, trainees, and staff will be required to be trained and up to date in the CITI course for the Responsible Conduct of Research, Conflict of Interest and other modules related to patient oriented research, as per University policy. Prior to any participant activities, the study will be reviewed and approved by the University of Miami Human Subjects Research Office, and all Key Personnel, including investigators, trainees, and staff will be listed in the protocol.

Contact information for the Principal Investigator and other designees will be provided to all participants and we will make sure they understand whom to contact in case of any emergencies, adverse events, or other questions. We will monitor adverse events in all participants throughout the interventions, and will review these regularly.

Dr. Joyce Gomes-Osman. PhD, PT is the principal investigator in this study. She is a research neuroscientist with extensive experience in clinical research and non-invasive brain stimulation approaches, which include transcranial magnetic stimulation (TMS) and electroencephalography (EEG). Dr. Gomes has a publication record that include studies utilizing non-invasive brain stimulation to characterize the neurophysiology and induce neurostimulation (as a potential therapeutic approach) in individuals with neurologic impairments from spinal cord injury. Her experience in clinical trials are a result of 6 years working at the Miami Project to Cure Paralysis, University of Miami, where she was a project coordinator for two R01 grants, while working on her doctoral studies. Dr. Gomes has expanded her knowledge in Neurology and non-invasive brain stimulation techniques during her postdoctoral fellowship with Dr. Alvaro Pascual-Leone, an internationally recognized leader in this field, at the Berenson-Allen Center for Non-Invasive Stimulation at Beth Israel Deaconess Medical Center at Harvard Medical School. She remains affiliated as a research scholar, and is currently conducting studies to investigate the effects of aerobic exercise on neuroplasticity and cognitive function in healthy individuals. In addition, she is a lecturer at the "Intensive Course in Transcranial Magnetic Stimulation", organized by this Center.

Dr. Lawrence Cahalin, PT, PhD, will CCS is Board Certified in Cardiopulmonary Physical Therapy. He has 33 years of clinical experience in physical therapy. His clinical practice has focused on independent exercise testing, cardiac and pulmonary rehabilitation, physical therapy for patients with end-stage heart and lung disease before and after heart or lung transplantation, and breathing retraining. Dr. Cahalin has an interest and expertise in exercise testing, exercise training, and assessment of functional capacity. His training in both physical therapy and gerontology has provided him with clinical and research skills to better appreciate and understand the effects of aging in older adults with and without heart and lung disease. Much of his work has examined the dynamic interaction between the cardiac and pulmonary systems at rest and during exercise with the goal of improving diagnoses, functional performance, and quality of life. He has made significant contributions to science and clinical research addressing (1) the safety of exercise testing, (2) the clinical utility of the 6-minute walk test in heart failure and end-stage lung disease, (3) inspiratory muscle testing and training in heart failure, (4) the clinical utility of heart rate recovery during maximal and submaximal exercise, and (5) promoting the development and implementation of worksite health and wellness programs as vehicles to prevent non- communicable disease and provide cardiac rehabilitation.

Dr. Jordyn Rice, PT, DPT is the lead physical therapist in this study. She is a research associate in the Neuromotor and Plasticity lab currently working to complete her PhD studies in Physical

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Therapy under the supervision of Dr. Joyce Gomes-Osman. She has a clinical background working in a variety of settings and has an active PT practice.

Trainees: Several trainees are planned to come in contact with participants. Our team has extensive experience with successful mentoring and we have designed an excellent educational program that runs throughout the fellowship.

20) Prior Approvals

No approvals need to be obtained prior to commencing the research Recruitment Methods.

21) Recruitment Methods

Participants for this study will be recruited via a recruitment flyer on the Miller School of Medicine Campus, University of Miami Coral Gables Campus, and greater Miami community. In addition, online research database tool (ResearchMatch.org) and the Consent to Contact Initiative will be used as recruitment methods. Individuals who are interested in participating in the study will be instructed to contact study staff via email or telephone to discuss the study details. At this time, the study will be explained to the potential participants. If subjects appear to fulfill eligibility criteria and are showing further interest in participating, a meeting will be scheduled to take place at the Lois Pope Life Center located at 1095 14th Terrace, 1st Floor, Miami, FL 33136 or virtually via Zoom. During this meeting, individuals will have the opportunity of choosing (or not) to enroll in the study.

Option A:

Participants will be compensated $60 at the beginning of the study and $60 upon completion of the study for a total of $120. Additionally, for the Option A only, participants will be compensated $2 for parking or transportation cost during their visits. Parking payments will be paid at the end of each week (ie: if participants attended 3 exercise sessions, they will receive $6 on their last visit of the week).

Option B:

Participants will be compensated $120 upon termination of all study visits via a gift card.

22) Local Number of Subjects

Approximately 80 participants will be enrolled in this study.

23) Confidentiality

Data will be stored indefinitely at the Lois Pope Life Center located at 1095 14th Terrace, 1st Floor, Miami, FL 33136 in a locked cabinet. For the Option A only, the BDNF and APOE4 samples will be analyzed and stored indefinitely at the Hussman Institute for Human Genomics (HIHG) Biorepository, 1501 NW 10th Avenue Biomedical Research Building(BRB) Rm 448 Miami, FL 33136. Identifiers will be removed from blood samples that are collected during this research and may be store and used for future research studies.

24) Provisions to Protect the Privacy Interests of Subjects

Information about study patients will be kept confidential and managed according to HIPAA requirements. Regulations require a signed patient HIPAA Authorization informing the participant of the following:

- What protected health information (PHI) will be collected
- Who will have access to that information and why

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- Who will use or disclose that information

If a participant withdraws consent or revokes authorization to collect or use PHI, following regulations, the investigator retains the ability to use all information collected prior to revocation of patient authorization.

All of the subjects PHI will be kept secure with paper information in a locked cabinet and all electronic data password protected, and only used as necessary for study purposes. This will be explained to subjects at the beginning of the study.

25) Compensation for Research-Related Injury

In the event of an injury, individuals will be instructed to follow-up with their medical insurance in the event of an injury.

26) Economic Burden to Subjects

- Option A: Participants are required to transport themselves to all study visits and pay for transportation and parking costs as necessary but will be reimbursed for $2 each study visit.
- Option B: Participants will not incur any economic burden and will be provided with all resources necessary to participate in this study.

27) Consent Process

Option A: The informed consent process will take place when the potential participant comes in for visit #1 at the Lois Pope Life Center located at 1095 14th Terrace, 1st Floor, Miami, FL 33136. The study team will thoroughly discuss the study aims, requirements, schedule, procedures, possible benefits, potential risks and alternatives. If subjects remain interested in participating, they will be given an informed consent document, and ample time to review it in privacy. After the potential participant has indicated that he/she has completely read the document, they will have the chance to ask any questions before deciding to give written consent.

Option B: A virtual consenting process will be completed using zoom for healthcare and REDCap e-consent as approved through the Clinical and Translational Science Institute (CTSI). The individual will be sent a REDCap link either through a secure email or through the chat feature of zoom. Once the individual opens the link, they will be asked to share their screen. At that time a trained study personnel will review the informed consent with the individual and answer any question the individual may have about the study. If the individual agrees to participate in the study, they will be asked to sign the e-consent form and provide the study personnel remote access of their web browser to also sign the consent form. The individual will then certify the information and submit the consent. At that time, they will have the option to download or print the completed consent form. A copy can be mailed at the individual's request.

_____The investigator will emphasize to each potential participant that his/her participation is voluntary and there is no consequence to refusing participation. Ample time will be taken to familiarize potential subjects with procedures.

Only when they declare that they are comfortable with all aspects of the study and its procedures, will they be allowed to offer consent. The subject will be interviewed for their introspections and comments about the experiment to confirm their understanding. At that point the potential participant will share their screen and electronically sign the informed consent document.

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They will then give the study member remote access to their browser and allow the study member to also sign the consent form. The potential participant will then verify the information and submit the consent. At that point they will be given the option to download a copy or print the signed informed consent. The participant may also request a mailed copy be sent to them.

The consent form document will not have the UM footer as the research team will not be inserting or extracting any data from the participant's medical record.

28) Process to Document Consent in Writing

Only the study personnel listed on this protocol and designated by the PI to consent patients are authorized to conduct the IC process.

Informed consent will be obtained in accordance with US 21 CFR 50.25 and ICH Good Clinical Practice. HSRO Investigator Manual HRP-103 guidance will be followed, as well as HSRO guidance for Informed Consent. COVID-19 mitigation HSRO consenting process will used to obtain remote consent.

29) Authorization for Use and Disclosure of Protected Health Information (HIPAA)

Type of Request:

☒ Waiver of Authorization for access to medical record for subject identification/recruitment.

☐ Waiver of Authorization for access to medical record to obtain data for the research.

Confirm that you will destroy or de-identify the information you collect at the earliest opportunity.

☒ ***I confirm***

Confirm that the information you collect will not be reused or disclosed to any other person or entity, except as required by law, for authorized oversight of the research study or for other research for which the use or disclosure of PHI is permissible.

☒ ***I confirm***

30) Drugs or Devices

Device: Magpro X100, MagVenture Corporation, Farum, Denmark

This device was purchased by the Department of Physical Therapy for the Neuromotor Plasticity Laboratory, directed by Dr. Gomes-Osman.

The Transcranial Magnetic Stimulator is the original device developed by the *Magstim Corporation* for the purpose of electromagnetic neural tissue stimulation in 1984. The devices consist of a bank of capacitors that can be charged up to a predetermined amount of energy and then quickly discharge via solid state switches into a coil of copper wire. This coil of copper wire is encased in plastic. As the current passes through the coil, a magnetic field is generated following Faraday Laws. This magnetic field will in turn induce a secondary current in any tissue with capabilities to conduct electricity that is placed in the proximity of the induced magnetic field. For the purpose of brain stimulation, the coil is held over the subject's head and as the current passes through it, i.e., a magnetic field is generated that penetrates the scalp and skull without being attenuated. This magnetic field

induces a flux of current in the brain. This secondary current induced in the brain influences neuronal activity by virtue of mostly transsynaptic effects. The effects induced by the stimulation of the brain depend on the site of stimulation, since different areas of the brain control different functions. In addition, stimulation parameters such as focality of the magnetic field, stimulus intensity and stimulus frequency also condition the induced effects in the brain. The Transcranial Magnetic Stimulator has no electric contact with the patient or subject. There is no failure mode of the device that could result in the delivery of an excessive amount of stimulation intensity or number of stimuli given a circuit breaking safety device. Therefore, the device allows noninvasive stimulation of the human brain without the use of direct electrical contact between the device and the subject. In principle, this device provides the means of an electrodeless, noninvasive, and painless stimulation of the human brain. It is used in cognitive research and treatment of some neurologic and psychiatric conditions.

Support for non-significant risk device

The non-invasive brain stimulation device (TMS) utilized in the present study has been cleared by the FDA for the treatment of depression. There have been nearly 2,000 research studies published utilizing this technique, and the safety of this device has been established. The First International Safety Conference on Transcranial Magnetic Stimulation took place at the National Institutes of Health in Bethesda, MD, in June 1996. Representatives of the Food and Drug Administration (FDA) were present at that meeting and they shared the consensus that the evidence accumulated over the previous 10 years supports the safety of TMS. More recently in 2008, an international panel of TMS experts (The Safety of TMS Consensus Group) met for a consensus conference to review and update safety guidelines in TMS based upon review of published data (Rossi et al., 2009). The evidence reviewed by this group of experts in the field continues to support the safety of TMS with the application of recommended guidelines. These TMS guidelines and recommended safety screening are incorporated into this study of physiologic measures. In the present study, we will strictly follow the Safety Guidelines to TMS.

Storage and usage of device

The Magpro X100 will be stored at the Lois Pope Life Center located at 1095 14th Terrace, 1st Floor, Miami, FL 33136. The device will be used by the principal investigator, and only be used by members of the Laboratory following adequate training.

	Applicable to:		
FDA Regulation	***IND Studies***	***IDE studies***	***Abbreviated IDE studies***
21 CFR 11	*X*	*X*	
21 CFR 54	*X*	*X*	
21 CFR 210	*X*		
21 CFR 211	*X*		
21 CFR 312	*X*		
21 CFR 812		*X*	*X*
21 CFR 820		*X*	

tACS Device Related Information

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This device will be purchased or rented by the Department of Physical Therapy for the Neuromotor Plasticity Laboratory, directed by Dr. Gomes-Osman. Transcranial Alternating Current Stimulation (tACS) is a form of Transcranial Electrical Stimulation (tES).

For over a decade tACS has been used in a growing number of studies due to its cost effectiveness and overall feasibility as a form of non-invasive brain stimulation. TACS is a brain stimulation technique that is designed to target and manipulate brain oscillations. An alternating sinusoidal current is applied between two electrodes which can vary on the strength, position, and frequency of the current. Only a fraction of the current enters the brain which in turns causes the membrane potential of a group of neurons to change which changes the probability of the generation of an action potential. In conjunction with EEG readings, tACS can provide us insights into the brain functioning.[46] TES is uniquely suited for a home-based remote intervention as it can be delivered with a lightweight, portable and inexpensive device. Importantly, tES stimulation is painless and has a favorable safety profile. Based on the conclusion of large consensus efforts – and reflecting a true consensus between all participants in both groups – is that tES represents a minimal risk intervention.

The practical application of tACS is simple: a low intensity (1-2 mA) alternating electrical current is applied to the scalp to influence underlying cortical excitability, which is measured with non-invasive surface EEG electrodes. The study investigator will remotely supervise the session utilizing a tablet and zoom for healthcare that comprises the STARSTIM®-Home system (Neuroelectrics, Inc). The neuroplasticity assessment will follow the current guidelines for the safe application of neurophysiological measures recommended by the International Federation of Clinical Neurophysiology.[13,14] STARSTIM®-Home is capable of recording EEG before, during and after tACS stimulation. The STARSTIM®-Home system can only be used at pre-specified times, determined and approved by the study investigator. In accordance with the safe application of neurophysiological measures recommended by the International Federation of Clinical Neurophysiology, we will collect and monitor adverse events.

Support for non-significant risk device

Transcranial Alternating Current Stimulation (tACS) is a form of Transcranial Electrical Stimulation (tES). TES is uniquely suited for a home-based remote intervention as it can be delivered with a lightweight, portable and inexpensive device. Importantly, tES stimulation is painless and has a favorable safety profile. Two independent papers that represent broad consensus efforts between experts in the field, have concluded that tES is safe if appropriate guidelines are followed, that it lacks serious adverse effects and that it is well tolerated. These consensus efforts include the paper by Bikson et al. (2016)[43] that considered 33,200 tES sessions across 1000 subjects undergoing repeated sessions. This report included data from healthy individuals and diverse clinical populations. It also includes the paper by Antal et al. (2017)[44] reflecting the outcome of a consensus meeting, which took place in Göttingen, Germany, on September 6-7, 2016 and follow-up committee work by email correspondence. The committee reviewed safety, ethical, legal, regulatory considerations, and provided application guidelines for tES. The data considered included over 18,000 tES courses of multiple sessions administered to healthy subjects, neurological and psychiatric patients. The conclusion of both these large consensus efforts – and reflecting a true consensus between all participants in both groups – is that tES represents a minimal risk intervention.

The stimulator used in this study is the STARTSTIM®-Home device (Neuroelectrics, Inc), a tACS home-base system that recently received FDA approval for the Investigational Device Exemption (IDE) supplemental protocol in a study for tACS to be used to treat patients with Major Depression Disorder (MDD) due to COVID-19 related restrictions. Several devices

that enable direct current stimulation are cleared by the FDA for extracranial use, particularly aiming for iontophoretic medication delivery. Pivotal studies aiming to gain FDA approval for different tCS devices for treatment of major depression, epilepsy, and chronic pain are in progress. It is important to note that the IDE in this instance was requested by the company because they are conducting a pivotal study for tCS in epilepsy and could not risk endangering their effect and thus wanted to ensure coordinated action with the FDA. Both the company and the FDA felt it valuable to issue the IDE as part of the concerted effort to highlight targeted actions to address the COVID-19 pandemic and its impact on mental health.

Storage and usage of device

The STARTSTIM®-Home device (Neuroelectrics, Inc) will be mailed to participants' homes for pre and post assessments. Participants will then mail the device back to the investigator using a pre-paid return label for sanitization and storage at the Lois Pope Life Center located at 1095 14th Terrace, 1st Floor, Miami, FL 33136. The device supervision and application will only be performed by the principal investigator, and only be used by members of the Laboratory following adequate training.

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