Study Title:
Brain State-dependent Stimulation to Improve Movement (BrainSTIM)

NCT Number:
NCT05103176

Document:
Study Protocol and Statistical Analysis Plan

Document Date:
2/1/22

BRAIN BEHAVIOR LAB
UNIVERSITY OF MICHIGAN

IRB STUDY TITLE:
Multi-Session investigations of State-dependent brain network manipulation.

ClinicalTrials.gov BRIEF TITLE:
Brain State-dependent Stimulation to Improve Movement (BrainSTIM)

GRANT/ClinicalTrials.gov OFFICIAL STUDY TITLE:
Leveraging behavioral state to enhance specificity of non-invasive brain stimulation on motor circuits

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Who is eligible: Healthy, right-handed, English-speaking adults between the ages of 18 and 50 years with no history of neurological disorder

This study involves: Basic neurological assessments during a maximum of 9 visits

Compensation: up to $270

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SUBJECTS and SCREENING:

- To ensure experimental rigor, all research staff will be trained in Good Laboratory Practices, including standardized operating procedures and record keeping.
- We will recruit ~65 subjects (50% women), ages 18-50, to allow for drop-outs and subjects who cannot tolerate NIBS.
- All subjects should be in good health with normal or corrected-to-normal visual acuity, without any known contraindications to NIBS ("pre-screening" and "enrollment screening").
- Subjects will be screened prior to enrollment, at time of enrollment and prior to all subsequent sessions with questions that include the Transcranial Magnetic Stimulation Adult Safety Screen form in order to exclude subjects with contraindications for TMS and MRI (Keel et al., 2001; Rossi et al., 2011) and adhere to published safety guidelines and recommendations (e.g. Rossini et al., 2015)
- Subjects will be screened for handedness with the Edinburgh handedness inventory (Oldfield, 1971).
- Prior to the start of the study, all participants will provide informed written consent in accordance with the Declaration of Helsinki.
- After consenting and prior to the start of the study, all participants will provide written attestation that they do not believe they are pregnant. Participants will be offered a self-administered urine pregnancy test, free of charge, upon request.

- For subsequent study sessions, all participants will complete a screening ("Daily screening") to confirm there no changes in their eligibility.

Inclusion criteria:

1. Age 18-50
2. Right handed
3. English speaker
4. No history of neurological disorder
5. Able to provide written consent prior to admission

Exclusion criteria:

1. are left-handed
2. are younger than 18 or older than 50 years old
3. are pregnant, suspect you might be pregnant or are attempting to become pregnant
4. have a pacemaker, intracardiac lines or any other medically implanted device or medicine pump
5. have cochlear hearing implants
6. are taking GABAergic, NDMA-receptor antagonist, or other drug known to influence neural receptors that facilitate neuroplasticity
7. have non removable body piercings or have foreign objects in body
8. have metal anywhere in the head that could increase your risk of serious injury (not including braces, dental fillings, etc.):
    a. deep brain or vagus nerve stimulator
    b. aneurysm clips or coils
    c. stents in neck or brain
    d. implanted stimulators
    e. electrodes to monitor brain activity
    f. metallic implants in eyes or ears
    g. shrapnel or bullet fragments in or near the head
    h. facial tattoos with metallic or magnetic-sensitive ink
    i. other metal devices or objects implanted in or near the head,
9. have any of the below conditions that would put you at increased risk of having a seizure:
    a. a personal or family history of seizure/epilepsy
    b. taking prescription drugs that lower the threshold for seizures
    c. recent history of excessive alcohol consumption
    d. history of alcohol addiction/dependence
    e. recent history of recreational drug use
    f. history of drug addiction/dependence
10. have been diagnosed with any of the following:
    a. a stroke, brain hemorrhage, brain tumor, encephalitis, multiple sclerosis,
    b. Parkinson's disease or Alzheimer's disease
    c. depression in the past 6 months
    d. attention deficit disorder, schizophrenia, manic depressive (bipolar) disorder,

e. normal pressure hydrocephalus or increased intra-cranial pressure
f. diabetes requiring insulin treatment
g. any serious heart disorder or liver disease

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PROJECT SUMMARY:

Fundamental processes of the brain like learning and acquisition of new motor skills depend on neuronal plasticity in a number of spatially distributed but interconnected brain regions. Non-invasive brain stimulation (NIBS) is utilized in neuroscience research to investigate the causal relations between brain function and behavior in humans. It also is used to understand brain pathophysiology in neuropsychiatric diseases. Clinically, NIBS protocols have diagnostic and therapeutic utility across a wide spectrum of disease states. The last two decades have seen an exponential growth in the use of NIBS techniques in both basic neuroscience and clinical practice. NIBS is rapidly developing as a powerful technique for probing and modulating brain function. NIBS holds promise for the study and treatment of neurological disorders. However, the effects of NIBS on neuronal activity are highly variable and poorly understood. That is, there is a limited understanding of the effects of NIBS on brain and behavior.

The major challenge facing the therapeutic use of NIBS is the difficulty in predicting how underlying neural circuits will be altered by the application of electrical fields and concomitantly improve behavior. The effect of NIBS depends on numerous biological factors [e.g., the structure of the targeted neural circuit, the profile of neural activity during application, the responses of different cell classes (e.g., excitatory versus inhibitory; projecting versus local neurons), the resulting biochemical or structural modifications of synaptic connections, and the possible alterations of neuromodulatory inputs] and stimulation parameters [e.g., duration, frequency, intensity, and electric field orientation].

As a result, the past few years have been directed at developing protocols that systematically control for these numerous biological factors and stimulation parameters during the application of NIBS so that the stimulation-induced effects are more specific. Even so, a mechanistic framework to guide and optimize future applications of therapeutic NIBS is still lacking. There is a critical need for a basic understanding of how NIBS interacts with dynamic patterns of neural activity to induce persistent changes in neural circuits, and how it can be used to improve a person's ability to make hand movements necessary for daily life activities such as eating, dressing, tool-use and writing. This gap in knowledge precludes the development of biologically-based NIBS protocols in basic neuroscience and clinical practice.

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BACKGROUND:

The last two decades have seen an exponential growth in the use of noninvasive brain stimulation techniques in both basic neuroscience and clinical practice. Repetitive transcranial magnetic stimulation (rTMS) is rapidly developing as a powerful, noninvasive brain stimulation technique that uses magnetic fields to induce electrical activity in specific brain areas for probing and modulating brain function. rTMS

holds promise for the study and treatment of neurological disorders. Yet, there is a limited understanding of the effects of rTMS on brain and behavior. The efficacy of present rTMS therapy, in which the brain state is uncontrolled during stimulation, is highly variable. This is not surprising given that neural activity patterns at the time of stimulation can impact the effects of rTMS on brain and behavior outcomes. Leaving the brain state unconstrained during stimulation likely leads to aftereffects that can spread to remote (unintended) brain areas rather than targeting specific neuronal subpopulations and functional networks underlying the behavior of interest. The past few years have been directed at developing protocols that systematically control behavior to manipulate dynamic patterns of neural activity during stimulation so that the stimulation-induced effects are more specific. Even so, a mechanistic framework to guide and optimize future applications of therapeutic rTMS is still lacking. There is a critical need for a basic understanding of how rTMS interacts with dynamic patterns of neural activity to induce persistent changes in neural circuits underlying motor control. This gap in knowledge precludes the development of biologically-based rTMS protocols in basic neuroscience and clinical practice.

Significance:
The rationale for this project is that it will allow us to identify the mechanisms associated with improvements in motor function to target for future clinical applications of rTMS.

Our overall **objectives** in the current study are to:

1. Elucidate the neural mechanism by which rTMS paired with a motor task leads to improvements in motor function.
2. Develop more targeted network modulatory rTMS interventions to enhance motor function.

*Aim-specific objectives described in detail below under “research design”

The **specific aims / hypotheses** in the current study are:

1. Demonstrate improvement in action performance by manipulating the behavioral state during PPC stimulation. Based on preliminary data, our working hypothesis is that action performance will improve after PPC stimulation and will improve even more after stimulation during grasp performance.
2. Demonstrate modulation of neurophysiological aftereffects of PPC stimulation by manipulating behavioral state. Based on preliminary data, our working hypothesis is that motor excitability will increase after PPC stimulation and will increase even more after stimulation during grasp performance.
3. Assess the relationship between brain connectivity, plasticity and behavior in response to the behavioral state during brain stimulation. Our working hypothesis is that constraining the behavioral state during PPC stimulation will induce greater changes in functionally specific parietal-motor pathways within the targeted cortical grasping network, as compared to PPC stimulation alone. Exploratory analyses will examine correlations between changes in action performance, plasticity, and functional connectivity.

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RESEARCH DESIGN:

Participants will be asked to participate in up to nine sessions – the sessions will take place in either the TMS laboratory in the Kinesiology Building (830 N University Ave, Ann Arbor, MI 48109) or the Functional MRI Laboratory located in the Bonisteel building (2360 Bonisteel Blvd., Ann Arbor, MI 48109). The visit schedule is as follows:

Session 1: Screening + behavioral & neurophysiological measures (~2 hours)
Session 2: fMRI (~1 hour)
Session 3: TMS (~30 min)
Session 4: TMS (~30 min)
Session 5: TMS (~30 min)
Session 6: TMS + behavioral & neurophysiological measures (~3 hours)
Session 7: TMS + fMRI (~1 hour)
Session 8*: fMRI (~1 hour)
Session 9*: behavioral & neurophysiological measures (~1 hours)
*Option to complete visits 8 and 9 on the same day

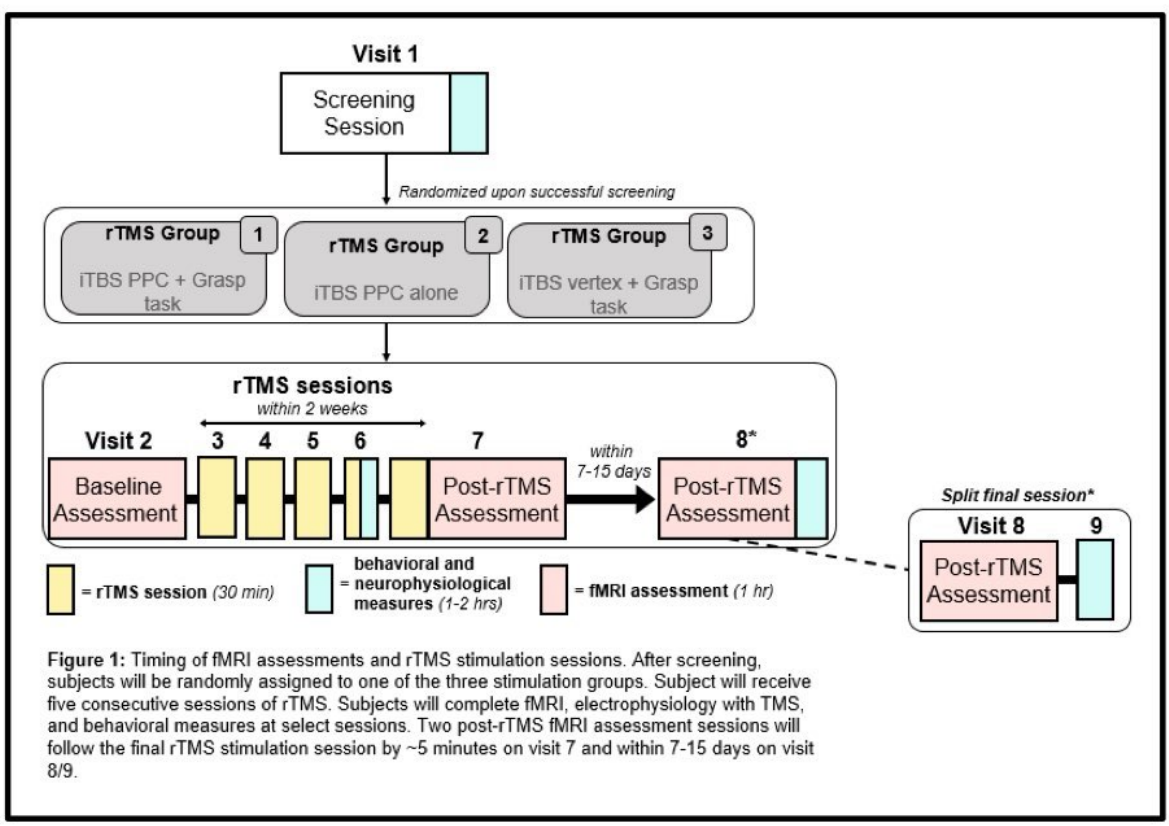

Figure 1: Timing of fMRI assessments and rTMS stimulation sessions. After screening, subjects will be randomly assigned to one of the three stimulation groups. Subject will receive five consecutive sessions of rTMS. Subjects will complete fMRI, electrophysiology with TMS, and behavioral measures at select sessions. Two post-rTMS fMRI assessment sessions will follow the final rTMS stimulation session by ~5 minutes on visit 7 and within 7-15 days on visit 8/9.

Subjects will be randomly assigned to one of three rTMS intervention groups in a 1:1:1 ratio. As depicted in **Figure 1,** subjects will complete fMRI scans, electrophysiological measures with TMS, and behavioral measures before and after five consecutive rTMS sessions. The first fMRI assessment session on visit 2, without rTMS, will obtain baseline measurements of hand motor function, electrophysiology with TMS, and fMRI-derived connectivity (providing the PPC target

for iTBS – see below). The five consecutive rTMS sessions will provide the experimental manipulation. One rTMS intervention will apply iTBS to the PPC, while subjects concurrently perform a grasp task (iTBS PPC + Grasp). Another rTMS intervention will apply iTBS to the PPC, while subjects are in an unconstrained, resting state (iTBS PPC alone). This will allow us to elucidate the effects of targeted rTMS enhancement of the parietal-frontal grasping network and motor function, and the interaction between parietal iTBS and behavioral state. To test the functional specificity of stimulation to the PPC, a third rTMS intervention will apply iTBS to a cortical region outside of the cortical grasping network (vertex)[1] while subjects concurrently perform the grasp task (iTBS Vertex + Grasp). This condition can control for the strong somatic sensations of receiving the iTBS, which neither sham nor 45-degree tilt of the rTMS coil protocols can achieve.[2] There is also evidence that the 45-degree tilt excites neural tissue.[3] This multimodal approach will allow us to visualize circuit-level modulation of the cortical grasping network related to targeted brain stimulation and motor behavior. Importantly, our pilot experiment shows that the average time from the end of rTMS to the beginning of the first post-rTMS fMRI is ~5 min, and that the fMRI assessment on visit 7 will be completed within 60 min. Findings from other groups[4], as well as our own data, demonstrate that the effects of iTBS last for 60 min, data acquisition will occur in the appropriate time frame to capture the immediate iTBS-induced effects on the proposed brain and behavior outcomes. We also will perform a second post-rTMS assessment approximately 7-15 days after stimulation to estimate more lasting effects of stimulation on brain and behavior.

Subjects and Screening: To ensure experimental rigor, all research staff will be trained in Good Laboratory Practices, including standardized operating procedures and record keeping. We will recruit ~65 healthy subjects (50% women), ages 18-50, to allow for drop-outs and subjects who cannot tolerate iTBS. Subjects will be screened with the Transcranial Magnetic Stimulation Adult Safety Screen[5,6,7] form in order to exclude subjects with contraindications for TMS and MRI, such as: a previous adverse reaction to TMS, a history of seizures or a condition that would increase the likelihood for seizures, a family history of epilepsy, taking medications that would increase the risk of seizure, pregnant or trying to become pregnant, presence of metal in the head (other than in the mouth), claustrophobia, presence of an implanted device like a pacemaker, metal in the brain, or other brain injuries or brain-related conditions.

Initial Assessment Session (screening): Subjects will watch a demo video of the 9-hole pegboard dexterity test (9-HPT, described below) from the NIH Toolbox for Assessment of Neurological and Behavioral function[8] (www.nihtoolbox.org) in order to standardize instructions and familiarize subjects with the task. Subjects then will practice on the 9-HPT to reduce subsequent practice effects across sessions. Subjects will also practice Choice Reaction Task and Precision Force Task, both tasks are described below. The last step in the initial assessment session will be exposing subjects to TMS, beginning with motor threshold determination, followed by an assessment of the targeted functional parietal-motor pathway for the grasp task using a dual-site TMS method described below, and then a 3-min trial run of iTBS to the PPC target. In our experience, ~10-15% of subjects cannot tolerate iTBS, so this will eliminate those subjects without having wasted an expensive fMRI session.

rTMS Sessions: Because multiple consecutive days of stimulation have been shown to produce longer-lasting effects on brain and behavior, 68,76-81 subjects will receive five consecutive rTMS sessions within a two-week period. A MagPro X100 magnetic stimulator with a 90mm figure-8 coil (MC-B70, MagVenture Inc.) will be used to apply rTMS to targeted locations marked on the structural MRI using a frameless infrared stereotactic neuronavigation system (Brainsight, Rogue Research,Montreal CA). Active motor threshold (AMT) will be obtained as the percentage of stimulator output that elicits a motor-evoked potential (MEP) of ≥ 200 μV peak-to-peak amplitude on 5 out of 10 trials while the subject maintains a 20% maximum contraction of the first dorsal interosseous (FDI) muscle of the right hand.[9,10] We then will deliver iTBS to PPC or vertex, [4,11] using 3 pulses of stimulation at 50 Hz, repeated every 200 ms, for 2s trains, repeated every 10s, for a total of 600 pulses in 190-s. Stimulation will be delivered at 80% of AMT, within consensus recommendations for safety.[12]

We will determine an individualized left PPC stimulation location based on peak functional connectivity with the left motor cortex seed obtained at the baseline assessment on visit 1 using functional neuroimaging. This will account for individual variability in recruitment of the cortical grasping network and maximize the reliability of the rTMS-induced modulation. We will use those coordinates as the site of stimulation for the PPC target. The stimulation targets will be transformed from MNI space into each subject's original MRI space for anatomically guided TMS. We then will use a robust dual-site TMS 'hunting' procedure, recently developed by my laboratory, to determine the optimal PPC scalp location where TMS can effectively exert a grasp-specific facilitation of motor cortex on an object-driven grasp task inspired by previous work from both our group[13,14,15] and others.[16,17,18,19,20] For the vertex (control) target, we will locate the vertex according to the standard 10-20 system. We then will confirm using individualized fMRI from the baseline assessment session that the underlying cortical site is outside the functionally defined regions of interest for the targeted cortical grasp network, and adjust the coil on the scalp accordingly. We will use neuronavigation to enable reproducible placement of the TMS coil over the cortical targets for the multiple rTMS sessions. For the PPC stimulation alone intervention group, subjects will be instructed to maintain gaze on a fixation LED during stimulation. For vertex and PPC stimulation augmented with a task sessions, subjects will perform an object-driven grasping task.[13,14,16,,21-26] Subjects will maintain fixation on a central LED in the midline (preview phase) for ~2 s. The illumination of an LED (green or red) then will instruct the subject to plan a precision grip towards either a small or large target object positioned in front of them. After 1 s, the LED will extinguish and cue subjects to execute the intended object-directed hand action. The presentation of the visual stimuli will be synchronized with the iTBS stimulation, which will occur 800 ms before the onset of every 'GO' cue in order to modulate cortical activity during both the planning and execution phase of the action.

Detailed objectives:
The objective of Aim 1 is to show improvement of manual dexterity after strengthening functional specific neural pathways with iTBS PPC + Grasp. To examine the effects of five-day stimulation and behavioral state on action performance, we will use a repeated measures model to test the hypothesis that the time to complete the 9-HPT will be reduced (i.e.,

performance improvement) after TBS PPC alone relative to iTBS Vertex + Grasp, and that the performance improvement will be greater for iTBS PPC + Grasp compared to iTBS PPC alone. We will use two sided tests, because it also is possible that performance may worsen, either with stimulation alone, or when combined with subjects performing the grasp task. It would be critical to know for future therapeutic purposes, for example, that manual dexterity was degraded by combining rTMS with a motor task. We also will perform an exploratory assessment on mean force and rectified EMG for a finger abduction task inspired by Bunday and Perez[27] to estimate persistent effects of stimulation on voluntary motor output. In addition to testing questions about performance, we also will examine relationships between action performance and the electrophysiology and fMRI measures from Aims 2 and 3, testing important questions around this mechanism.

The objective of Aim 2 is to determine inter-cortical interactions from PPC to motor plasticity. Descriptive statistics will be used to explore the distribution, central tendency, and variation of each measurement. Normality for each variable will be checked using a Shapiro-Wilks test, and appropriate transformations (i.e., log) will be used when necessary. To examine state-dependency modulation of rTMS-induced effects on motor plasticity, we will contrast the size of MEPs elicited in the resting hand muscle by TMS over M1 following iTBS PPC + Grasp with those following iTBS PPC alone. We will test the following: the size of MEPs evoked by TMS will be greater for iTBS PPC alone relative to iTBS Vertex + Grasp, and whether the MEPs will be greater for iTBS PPC + Grasp, compared to iTBS PPC alone. Because the MEP amplitude can be highly variable from trial-to-trial within the same subject,[28] we will collect 24 MEPs, the optimal number of single-pulse TMS trials required for the reliable assessment of motor cortical excitability.[29,30] In addition, to avoid the confounding influence of diurnal variations in levels of cortisol on the induction of cortical plasticity,[31] all experiments will occur at the same time of day. Based on prior work,[32] it is possible that subjects with more excitable motor systems will have greater behavioral improvements after rTMS. We, will therefore perform additional analyses examining the relations of inter-individual differences in MEP amplitude, magnitude of the plasticity effects in the motor system,[33] and the rTMS-induced behavioral effects.[34] Knowledge of this variability will provide insight into how interventions can be personalized and optimized for stroke recovery.

The third aim of this study is to assess the relationship between brain connectivity, plasticity and behavior in response to the behavioral state during brain stimulation. Exploratory analyses will test for correlations between the three measures, to provide a richer picture of iTBS effects. In order to assess functional connectivity between disparate brain regions, we will calculate correlations between the time series of disparate brain regions. For each seeded region of interest, we will produce a whole brain map of Pearson's r values that can subsequently be transformed using Fisher's r-to-z transform to allow for standard statistical testing. Regions that display a high degree of correlation ($p < 0.05$, corrected for multiple comparisons) are assumed to be "functionally connected" with that node. To examine the effect of iTBS on functional connectivity, we will directly contrast the MRI sessions following iTBS PPC + Grasp with iTBS PPC alone and iTBS Vertex + Grasp using our previous methods.[35] Exploratory analyses will search for regions outside the a priori defined network that change

with stimulation to develop a comprehensive picture of how iTBS interacts with brain networks. All data from our fMRI scans also will be publicly posted at Open fMRI (http://openfmri.org/) to allow other researchers to validate our results and perform supplemental analyses.

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MEASUREMENTS

The neurophysiological measurements for the current study are:

- **Motor threshold** in hand muscles as defined as the lowest TMS intensity needed to generate MEPs of >50 uV in at least 5 of 10 trials when the muscle is completely relaxed (resting MT; RMT) and the minimum intensity required to produce MEPs of $\geqq 200\mu V$ in at least 5 of 10 trials while the participant maintains 20% maximum contraction in the targeted hand muscle (active MT; AMT).

- **MEP amplitude** will be measured with 1 mV TMS intensity. A fixed stimulation intensity to elicit a ~1 mV MEP will be determined for each participant before testing to examine the changes in MEP after the different plasticity induction protocols. Twenty-four MEPs will be acquired at each time point. Stimuli will be applied every 5 s.

- **Dual-site TMS** will be used to measure functional connectivity in the motor system. The test pulse intensity over the primary motor cortex will be set to produce a MEP of ~1 mV at rest. The conditioning pulse intensity will be set between 80% - 120% resting MT. The interstimulus internal will range between 2 – 40 ms. These parameters will be determined based on our previous published work and work by others for each brain site. For PPC brain region, fifteen test pulses and conditioning pulses will be presented randomly at each time interval both at rest or during a task at each time points. Stimuli will be applied every 5 s.

  **See below for primary outcome 2, secondary outcomes 2, 11 & 12.*

  **(*see Appendix for details*)

General assessment procedures with TMS:

- Participants will be required to place their chin on a padded support during the trials to minimize head movement. The support/apparatus level will be placed so that the head is in a natural, upright, comfortable position. To avoid mental and physical fatigue, participants will be allowed breaks between blocks of trials (approximately every 10-15 minutes), where they can lift their head off the chin support or from the apparatus and rest if desired
- Motor-evoked potentials (MEPs) will be elicited by single-pulse TMS stimuli delivered from a MagPro X100 with MagOption Magnetic Stimulator and a 90 mm figure-of-

eight static-cooled coil (MagVenture Inc, Atlanta, GA) and two small figure-of-eight branding-iron coils (inner diameter: 50-70 mm) connected to a Magstim 200^2 stimulator (Magstim, Whitland, UK).

- MEPs will be recorded from abductor pollicis brevis (APB), first dorsal interosseous (FDI), and abductor digiti minimi (ADM) muscles in both hands with 9 mm diameter Ag-AgCl surface electrodes. The active electrode will be placed over the muscle belly, and the reference electrode over the metacarpophalangeal joint of the finger. The signal will be amplified (1000x), bandpass filtered (20 Hz– 2.5 kHz), digitized at 5 kHz using an amplifier and analog-to-digital interface (Intronix Technologies Model 2024F/ Digitimer D440-4 Low Noise 4-Channel Isolated Amplifier with Micro1401; Cambridge Electronics Design, Cambridge, UK) and stored in a computer for off-line analysis.
- Prior to stimulation the TMS coil and participants' physical locations will be co-registered using the Montreal Neurological Institute (MNI) template MRI image imported into the BrainSight™ TMS neuronavigation software (BrainSight 2.0, Rogue Research Inc., Montreal, QC). This system allows for precise and consistent stimulation of a desired target by tracking the position/orientation of the coil relative to the participant.

The fMRI measurements for the current study are:

- **MRI acquisition:** All neuroimaging data will be collected using a 3T GE MR 750 scanner at the University of Michigan's Functional Magnetic Resonance Imaging Laboratory. A standard 32-channel head coil will be used, and participant movement will be minimized by stabilizing the head with cushions and Velcro straps. Each imaging session will include acquisition of T1-weighted anatomical images, high-resolution anatomical images using an MP-RAGE sequence, and T2*-weighted functional images. Functional images will be acquired using a multi-band EPI sequence (acceleration factor of 3) that will allow us to collect whole brain data at a voxel size of 2 mm isotropic at a repetition time (TR) of 1.2 s. We will collect six functional runs of 5 minutes each.

- ***MRI preprocessing**:* Anatomical, functional and resting-state data will be preprocessed using fMRIPrep 20.1.1. The anatomical preprocessing pipeline performs brain-tissue segmentation and reconstruction of cortical surfaces. The functional preprocessing pipeline comprises susceptibility distortion correction, slice-timing correction, motion correction, coregistration with the subject's T1-weighted image, and nonlinear transformation to MNI152 space. Resting-state data will undergo additional preprocessing steps including interpolation across high-motion time-points (>0.5 mm FD), application of a bandpass filter to extract frequencies between 0.009 and 0.08 Hz, mean 'grayordinate' signal regression (MGSR), and censoring of high-motion time-points

- **Localization:** The high-resolution T1-weighted scan for each participant will be imported into BrainSight Frameless Neuronavigation system (Brainsight; Rogue Research, Montreal, Canada) and co-registered to digitized anatomical landmarks for TMS coil

placement during the experiment. Individualized TMS targets are to be mapped to Montreal Neurological Institute (MNI) space.

**See below for primary outcomes 3 & 4, secondary outcomes 3, 4, 5 & 6.*

The behavioral measurements for the current study are:

- **9 Hole Peg Test (9-HPT):** a brief, standardized, quantitative test of upper extremity function. Participants will take pegs from a container, one by one, and place them into the holes on the board as quickly as possible. Participants must then remove the pegs from the holes, one by one, and place them back into the container.

- **Precision force-tracking task:** To measure dynamic motor function in the scanner, we will use a precision force-tracking task (FTT). In this task (**Fig. 3**), participants must use a scanner-compatible force transducer (Current Designs, Inc., Philadelphia, PA) to continuously modulate their grip force to match a target force output. This task has already been implemented in Dr. Lee's lab. Each trial consists of a 15 second pattern of grip modulation during which participants attempt to keep the cursor (white circle) inside the bounds of a moving target (blue circle). The target will follow a trajectory generated by the linear combination of multiple different waveforms. At each frame refresh, we will calculate squared distance (error) from the cursor to the target which will allow us to calculate a root mean squared error (RMSE) for each trial as a dependent variable. During dual-task trials, participants will be required to simultaneously count the number of times the target circle flashes. This requires both tonic attentional vigilance as well as working memory maintenance, two executive functions previously implicated as important in the AMI. At the end of the trial, participants will report the number of target flashes they observed by responding to an on-screen prompt using the same grip force device.

- **Choice Reaction Time:** The choice reaction time (cRT) is a simple visuomotor task that will be used to control for visuomotor reaction times. We will use a 2-choice version of the cRT to assess the effect of simple visuomotor learned associations on visuomotor reaction time. For the task, a "1" or a "2" will appear on a computer screen ~80 cm in front of the participant and the participant will have to press the corresponding key on the keyboard as fast as they can. The probability of appearance of each number is set to 50%. Each task consists of 40 trials. Performance time will be calculated by the mean reaction time on correct trials at each time point. Participants will complete this task following each 9-HPT session. The participant will complete the cRT three times during each session.

**See below for primary outcome 1, secondary outcomes 1, 7, 8, 9 & 10.*

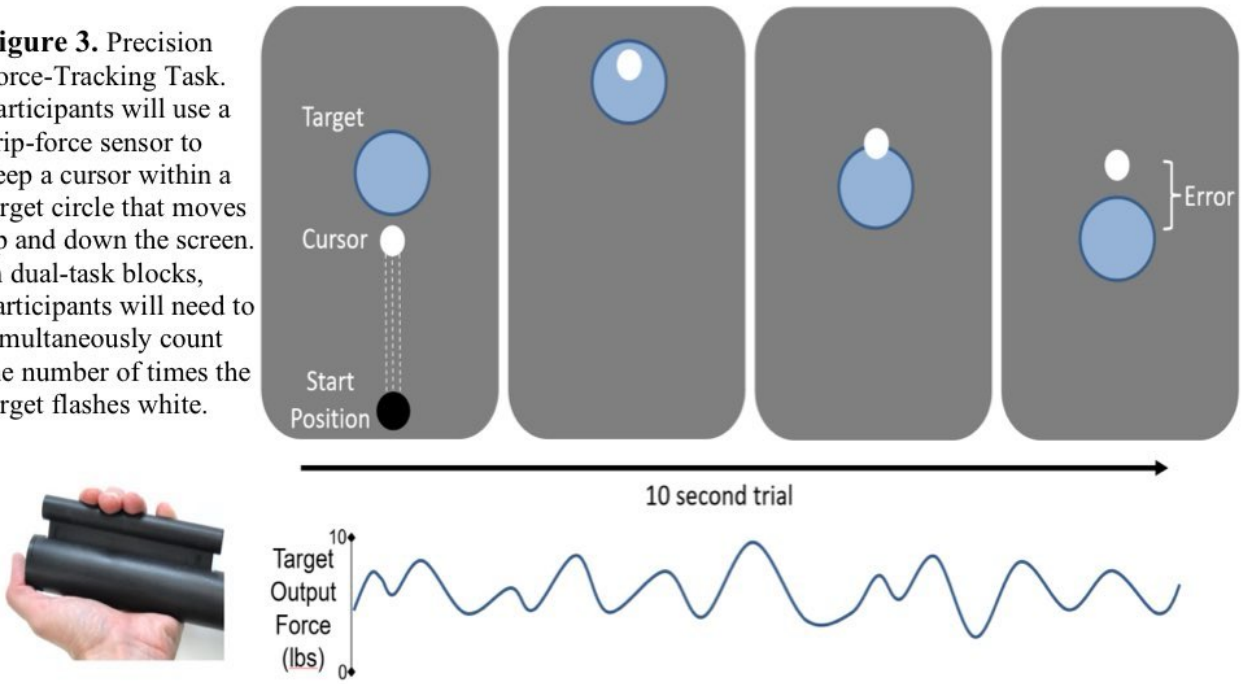

Figure 3. Precision Force-Tracking Task. Participants will use a grip-force sensor to keep a cursor within a target circle that moves up and down the screen. In dual-task blocks, participants will need to simultaneously count the number of times the target flashes white.

Surveys for the current study are:

- **Pre-screening:** Participants will be screened for preliminary eligibility prior to scheduling their first study session.
- **Enrollment screening:** Participants will again be screened to confirm eligibility prior to enrollment.
- **Daily screening:** Participants will be screened for eligibility prior to any NIBS administration to confirm enrollment.
- **Exit survey:** Participants will be asked to rate their comfort and experience upon completion of each study session.

NIBS interventions for the current study are:

- **Intermittent Theta Burst Stimulation (iTBS):** A MagPro X100 magnetic stimulator with a 90mm figure-8 coil (MC-B70, MagVenture Inc.) will be utilized to deliver brain stimulation. All participants will receive five consecutive days of stimulation within a two-week period. The 3-minute session of intermittent Theta Burst Stimulation (iTBS) will consist of 10 bursts of high-frequency stimulation (a 2 s train of 3 biphasic waveform pulses at 50 Hz repeated every 200 ms at 80% AMT) repeated every 10 s for a total of 190 s (600 pulses) to the target area. The target area will be located using BrainSight2 neuronavigation system. The baseline structural scan obtained during the scan 1 will be utilized for this localization process.

- **Object directed grasping:** Subjects will perform a precision grip with the right hand towards either a small or large target object positioned in front of them. The illumination of an LED (green or red) will instruct the subject to plan a precision grip towards either a small or large target object positioned in front of them. After ~1 second, the LED will extinguish and cue subjects to execute the intended object-directed hand action. The presentation of the visual stimuli will be synchronized with the iTBS stimulation, which will occur 800ms before the onset of every "GO" cue in order to modulate cortical activity during both the planning and execution phase of the action.

Outcome measures for the current study are:

Primary Outcome Measures:

1. Percentage change in the time to complete the nine-hole peg test (9-HPT) to immediate post-intervention. 9-hole peg test is a manual dexterity measure, estimated as the time required to complete the task (seconds). Time Frame: Baseline and immediate post-intervention.

2. Percentage change in amplitude of motor evoked potential (MEP) to immediate post-intervention. Motor cortical excitability is measured by electromyography using MEPs elicited by TMS Time Frame: Baseline and immediate post-intervention.

3. Change from baseline functional connectivity to PPC stimulation target within the cortical grasping network to immediate post-intervention. Resting-state connectivity of low frequency BOLD fluctuations for a seed at the PPC. Time Frame: Baseline and immediate post-intervention.

4. Change from baseline Blood Oxygen Level-Dependent (BOLD) activation, voxelwise in the cortical grasp network to immediate post-intervention. Parietal-frontal cortical grasping network defined by BOLD change while subject performs the precision force-tracking task. Time Frame: Baseline and immediate post-intervention.

Secondary Outcome Measures:

1. Percentage change in the time to complete the nine-hole peg test (9-HPT) to 1-week post-intervention. 9-hole peg test is a manual dexterity measure, estimated as the time required to complete the task (seconds). Time Frame: Baseline and 7-15 days post-intervention.

2. Percentage change in amplitude of motor evoked potential (MEP) to 1-week post-intervention. Motor cortical excitability is measured by electromyography using MEPs elicited by TMS. Time Frame: Baseline and 7-15 days post-intervention.

3. Change from baseline functional connectivity to PPC stimulation target within the cortical grasping network to 1-week post-intervention. Resting-state connectivity of low frequency BOLD fluctuations for a seed at the PPC. Time Frame: Baseline and 7-15 days post-intervention.).

4. Change from baseline Blood Oxygen Level-Dependent (BOLD) activation, voxelwise in the cortical grasp network to 1-week post-intervention. Parietal-frontal cortical grasping network defined by BOLD change while subject performs the precision force-tracking task. Time Frame: Baseline and 7-15 days post-intervention.

5. Change from baseline Blood Oxygen Level-Dependent (BOLD) activation, voxelwise in whole brain to immediate post-intervention. Parietal-frontal cortical grasping network defined by BOLD change while subject performs the precision force-tracking task. Time Frame: Baseline and immediate post-intervention.

6. Change from baseline Blood Oxygen Level-Dependent (BOLD) activation, voxelwise in whole brain to 1-week post-intervention. Parietal-frontal cortical grasping network defined by BOLD change while subject performs the precision force-tracking task. Time Frame: Baseline and 7-15 days post-intervention.

7. Percentage change in accuracy to precision force-tracking task to immediate post-intervention. Squared distance (error) from the cursor to the target in precision force-tracking task, estimated as the root mean squared error (RMSE). Time Frame: Baseline and immediate post-intervention.

8. Percentage change in accuracy to precision force-tracking task to 1-week post-intervention. Squared distance (error) from the cursor to the target in precision force-tracking task, estimated as the root mean squared error (RMSE). Time Frame: Baseline and 7-15 days post-intervention.

9. Percentage change in the mean choice reaction time to immediate post-intervention. Mean reaction time for subjects responding in the 2-choice reaction time control task, for correct responses. Time Frame: Baseline and immediate post-intervention.

10. Percentage change in the mean choice reaction time to 1-week post-intervention. Mean reaction time for subjects responding in the 2-choice reaction time control task, for correct responses. Time Frame: Baseline and 7-15 days post-intervention.

11. Percentage change in the normalized motor evoked potential (MEP) size to immediate post-intervention. Parietal-motor functional connectivity is measured by electromyography using MEPs elicited by dual-site TMS, while subjects

perform an object-directed grasp/subjects are at rest. Time Frame: Baseline and immediate post-intervention.

12. Percentage change in the normalized motor evoked potential (MEP) size to 1-week post-intervention. Parietal-motor functional connectivity is measured by electromyography using MEPs elicited by dual-site TMS, while subjects perform an object-directed grasp/subjects are at rest. Time Frame: Baseline and 7-15 days post-intervention.

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STATISTICAL DESIGN:

Because the effects of motor behavior are likely to be the most subtle, our main power analysis to determine sample size was based on our estimates of effect size for the stimulation aftereffects on action performance. We chose to calculate power based on a prior study that used TMS manipulation of associative plasticity in a premotor-motor circuit to enhance action performance.[32] In this study, the interaction between stimulation and experimental session was ηp2 = 0.11. Using G*Power 3.1.9.4 (www.gpower.hhu.de), we calculated the a priori required sample size to detect a significant effect at α < 0.05 with a power of 0.8. This resulted in a required sample size of 54 participants. In order to account for subject attrition and dropout, we will aim to recruit 65 participants. This sample size is larger than every combined rTMS-fMRI study in the field.[22,35,36]

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APPENDIX

METHODS BACKGROUND

Cortical output depends on the balance between excitatory and inhibitory systems. Transcranial magnetic stimulation (TMS) is a widely used technique to examine motor cortical physiology in intact human subjects. Depending on the stimulus parameters, TMS can be used to test different excitatory and inhibitory circuits in the brain. Changes in cortical excitatory and inhibitory circuits occur over the lifespan in healthy individuals and in many neurological and psychiatric disorders, and may mediate cortical plasticity. The aim of this research is to better understand the relationship and interactions between different inhibitory and excitatory systems in the human motor cortex.

Stimulation of the human motor cortex by TMS

TMS is a painless, non-invasive way to stimulate the human brain. TMS works by passing a large, brief current through a wire coil placed on the scalp. The transient current produces a large and changing magnetic field, which induces electric current in the underlying brain. When applied to the motor cortex, TMS produces muscle contraction in the contralateral muscles which can be recorded as a motor-evoked potentials (MEPs). Co-registration of scalp positions with

magnetic resonance images showed that the coil position that produces the largest MEP overlies the primary motor cortex (M1).

Different TMS measures of the motor cortex can evaluate different aspects of cortical excitability. Such measures are useful in understanding changes in brain physiology seen, for example, in the setting of cortical plasticity and brain disorders. Some of the common measures are listed here.

Measurement of corticospinal excitability by single-pulse TMS

Motor threshold (MT) and MEP amplitude

Motor threshold (MT) refers to the lowest TMS intensity capable of eliciting a small MEP based on predetermined criteria (usually 50 μV at rest and 100 μV with muscle contraction). MT reflects the excitability of a central core of neurons and is related to the excitability of individual neurons and their local density. MT also varies with current direction and is lowest with anteriorly directed current. MT likely reflects neuronal membrane excitability. MEP amplitude increases with stimulus intensities up to a maximum (plateau) which varies from subject to subject. Compared to neurons activated near threshold intensities, neurons activated at high TMS intensities either have higher threshold or are located further away from the center of activation by TMS. It should be noted that MT and MEP amplitude are influenced by cortical, subcortical as well as spinal excitability.

Measurement of cortical inhibition and facilitation by paired-pulse TMS

Paired-pulse TMS in a conditioning and test paradigm is a powerful way to examine cortical inhibition and facilitation. Motor cortex inhibition and facilitation due to stimulation of the motor cortex itself (intracortical inhibition or facilitation) will be described first followed by changes in motor cortex excitability induced by stimulation of other areas.

Measures of intracortical inhibition and cortico-cortical facilitation

At least two types of intracortical inhibition can be studied by applying paired-pulse TMS with one coil over the M1. These will be referred to as short interval intracortical inhibition (SICI) and long interval intracortical inhibition (LICI). SICI is a widely used protocol and involves a weak subthreshold conditioning stimulus followed by suprathreshold test stimulus. The test responses are inhibited at inter-stimulus intervals (ISIs) of 1 – 6 ms. LICI is elicited by suprathreshold conditioning and test pulses applied at ISIs of 50 – 200 ms. Intracortical facilitation (ICF) is tested using protocols similar to SICI and involves a weak subthreshold conditioning stimulus followed by suprathreshold test stimulus. Facilitation is observed at ISIs of 8 - 30 ms.

Inhibition/facilitation of the motor cortex induced by stimulation of other areas

Functional and effective connectivity studies can be conducted using TMS at two sites, often called the ‘‘dual-site” TMS approach (dsTMS). Many of these studies look at the influences on the M1 (M1) coming from various brain regions at rest, before and during movement. These paradigms involve stimulating other brain regions prior to M1

using two separate coils. MEP amplitudes are compared between baseline single-pulse MEP amplitudes and conditioned MEP amplitudes. Based on these findings, functional and effective connectivity are considered to be facilitatory or inhibitory, or to have no effect. Influences on the M1 come from other brain regions within the motor system such as bilateral posterior parietal cortices (PPC), ventral (PMv) and dorsal (PMd) premotor cortices, dorsolateral prefrontal cortex (DLPFC), supplementary motor area (SMA), pre-SMA and cerebellum. Using the dsTMS approach, interhemispheric inhibition (IHI) can also be demonstrated by applying a conditioning stimulus to the motor cortex, which reduces the size of the MEP produced by test stimulation of the opposite motor cortex at ISIs between 6 and 50 ms.

Inhibition of the motor cortex by cerebellar stimulation (cerebellar inhibition, CBI)

Cerebellar stimulation likely activates Purkinje cells in the cerebellar cortex, leading to inhibition of deep brain cerebellar nuclei such as the dentate nucleus which have a disynaptic excitatory pathway to the motor cortex through the ventral thalamus. We will use magnetic stimulation of the cerebellum with a TMS coil to inhibit the MEPs produced by stimulation of the contralateral motor cortex 5 to 7 ms later.

Effects of peripheral sensory stimulation on the motor cortex

The effects of peripheral sensory stimulation on motor cortex excitability can be assessed by applying a sensory stimulus, such as median nerve stimulation, followed by a test stimulus over the contralateral motor cortex. Inhibition of the test MEP has been reported at ISIs between 20 and 600 ms and is most consistent at two distinct ISIs at around 20 ms and 200 ms. At both ISIs inhibition occurs predominately at the cortical rather than spinal levels and abnormalities have been reported in neurological diseases. These will be termed short and long latency afferent inhibition and will be discussed separately.

Short latency afferent inhibition (SAI) refers to inhibition of test MEP evoked by stimulation of the motor cortex following contralateral median nerve stimulation at ISIs of about 20 ms. In normal subjects the inhibition begins between ISI of 19 to 21 ms and peaks at ISI of 21 ms on the average. Long latency afferent inhibition (LAI) is most consistent at ISI of about 200 ms following median nerve stimulation. SAI/LAI will be used to index sensory projections to motor cortex.

Summary of measures of cortical inhibition and facilitation by single- / paired-pulse TMS

Table 1 provides a summary of the different excitatory and inhibitory TMS assessment methods.

	SICI	LICI	ICF	dsTMS	IHI	CBI	SAI/LAI
Method							
Conditioning/ first stimulus (CS)	80% to 120% RMT	Supra-threshold TMS	80% to 120% RMT	Supra-threshold TMS	Supra-threshold TMS	Sub-threshold TMS-	Median nerve stim.

	Site: M1	Site: M1	Site: M1	Sites: Parietal-frontal network and cerebellum	Sites: Parietal-frontal network from opposite hemisphere	contra cerebellum	
Test stimulus/second stimulus to M1 (TS)	Supra-threshold TMS	Supra-threshold TMS	Supra-threshold TMS	Sub-threshold TMS	Supra-threshold TMS	Supra-threshold TMS	Supra-threshold TMS
Inter-stimulus interval, ISI (ms)	1-6	50-200	8-30	2-40	8-50	5-8	~20-25 200

Plasticity-inducing non-invasive transcranial brain stimulation (NTBS) protocols:

TMS can be used in a variety of ways to induce plastic changes in the brain, and this can be utilized to assess the capability for plasticity (**Table 2**). An effective way to modulate synaptic efficacy is to activate a cell with two or more inputs at close to the same time. If the stimuli come on the same synaptic pathway, this is called homosynaptic, and, if on different synaptic pathways, this is called heterosynaptic. Increased synaptic strength is called long-term potentiation (LTP); decreased synaptic strength is called long-term depression (LTD). TMS can also be used repetitively in a mode where very short, very high frequency trains of stimuli are delivered at theta frequency, about 5 Hz. This is called theta- burst stimulation (TBS). A typical paradigm would be three stimuli at 50 Hz, repeated at 5 Hz. If given intermittently, say 2 s of stimulation every 10 s, this leads to increased excitability. If given continuously over 40 s, this leads to decreased excitability. These approaches are described in detail below.

Repetitive paired-pulse stimulation

Paired Associative Stimulation (PAS): Heterosynaptic plasticity can be realized in humans with a peripheral stimulus paired with a TMS brain stimulus. A nice set of experimental paradigms has been developed by Classen and collaborators and called paired associative stimulation (PAS). If a median nerve stimulus at the wrist is paired with a TMS to the sensorimotor cortex at 25 ms, then the two stimuli arrive at about the same time, and the MEPs will be facilitated. If the interval is about 10 ms, the TMS comes about 15 ms before the median nerve volley arrives, and the MEP will be depressed. The former behaves like LTP and the latter like LTD.

Cortico-cortical Paired Associative Stimulation (ccPAS) is another way of looking at cortico-cortical relationships with TMS. Stimulation is delivered over a target site and

a conditioning stimulus is delivered over another site that has input to the target site. The two sites are stimulated repetitively at intervals where the timing of the arrival of the two stimuli at the target site might give rise to a long-term potentiation (LTP)-like effect or a long-term depression (LTD)-like effect. These effects have been successfully demonstrated, for example, between the M1s in the two hemispheres from SMA to M1 and from parietal areas to M1. Production of these effects not only shows the effective connectivity, but might be useful for producing plastic changes that could have functional benefits.

Repetitive transcranial magnetic stimulation (rTMS): Patterned rTMS refers to repetitive application of short rTMS bursts at a high inner frequency interleaved by short pauses of no stimulation. Most used to date are the different theta burst (TBS) protocols in which short bursts of 50 Hz rTMS are repeated at a rate in the theta range (5 Hz) as a continuous (cTBS), or intermittent (iTBS) train. For cTBS 3 pulses at 50 Hz are applied at 5 Hz for 40 s (600 stimuli) at an intensity of 80% active MT. For iTBS, we will deliver 3 pulses at a frequency of 50 Hz every 200 ms during 2 s (10 bursts) and repeated every 10 s for a total duration of 191 s (600 pulses).

Transcranial direct current stimulation (tDCS) is another method for influencing brain excitability using a low-level continuous electric current. Anodal stimulation will facilitate the motor cortex, and cathodal stimulation will inhibit it. It is thought that changes in cortical excitability is related to modulation in resting membrane potential which in turn alters spontaneous neuronal discharge rates. We will use one of the two tDCS units described below. The first unit uses a pair of saline-soaked surface sponge electrodes (7 x 5 cm, 35 cm^2) to the scalp, with one electrode over a specific node within the motor system and the other electrode placed over a different brain region. tDCs is performed using a battery-driven DC stimulator (Neuroconn DC-STIMULATOR PLUS, neuroConn GmbH, Illmenau, Germany) with a constant current flow of 1-2 mA for 15 to 30 minutes. The second Starstim tDCS system (Neuroelectrics, Barcelona, Spain), uses 3.14 cm^2 Ag/AgCl gelled electrodes placed in holes of a neoprene cap corresponding to the international 10/10 EEG system. The total injected current will not exceed 4 mA, and no more than 2 mA per electrode (montages with up to 8-electrodes) for 15 to 30 minutes, consistent with safety limits of the Starstim device. At these intensities and durations the subjects experience a slight itch under the electrodes but the procedure is not painful.

Summary of plasticity-inducing NTBS

Table 2 provides a summary of NTDS methods for excitation and inhibition.

Method	Excitatory Mode	Inhibitory Mode
rTMS/TBS	Intermittent 3 pulses, 50 Hz at 5 Hz for 40 s (600 stimuli) 80% active MT	Continuous 3 pulses, 50 Hz every 200 ms during 2 s (10 bursts) repeated every 10 s for a

		total duration of 191s (600 pulses), 80% active MT
PAS	synchronous heterosynaptic stimulation (PAS25)	asynchronous heterosynaptic stimulation (PAS10)
ccPAS	CS -->TS (ISI: 5-20 ms; 90-120% RMT) 5 s (0.2 Hz),15 minutes,180 pairs	TS -->CS (ISI: 5-20 ms; 90-120% RMT) 5 s (0.2 Hz),15 minutes,180 pairs
tDCS	Anodal (1-2 mA; 15-30 min)	Cathodal (1-2 mA; 15-30 min)

RISKS:

Risks associated with TMS:

1. Local scalp pain near stimulation site (common, not serious): Activation of muscles and nerves near the site of stimulation can cause substantial pain and discomfort, depending on the intensity and frequency of stimulation.

To minimize risk: The first step will be slight coil rotations (< 10 degrees), which often reduce stimulation-related pain. If this does not work, the coil will be moved slightly or the stimulation magnitude will be turned down.

2. Headache or neck pain (common, not serious): Headache and neck pain, typically lasting up to a few hours on the day of stimulation, are the most frequent side effects of TMS.

To minimize risk: These side effects are usually managed well with standard analgesics (e.g., single doses of aspirin, acetaminophen, or ibuprofen). Subjects will be instructed to contact the investigators if the headache persists on the following day.

3. Sound exposure (rare, serious): When producing a magnetic pulse train, the stimulating coil produces a series of brief clicks. No evidence of hearing loss has been found in humans exposed to TMS, despite extensive exposure to repeated stimulations over several years.

To minimize risk: Earplugs will be used in this study in all subjects as a precautionary measure.

4. Risk of Seizure Induction (rare, serious): The major safety concern about TMS is the possibility of eliciting a seizure, although TMS has rarely been associated with the induction of seizure, even in patients with epilepsy. For example, a recent international consensus conference rated the risk of seizure inductions during low-frequency rTMS as “rare, and usually protective.” In theory, medications that lower seizure threshold could present an added risk, but we believe this risk is no more than a minimal one in our protocol.

To minimize risk: For all studies, the parameters used will fall within the safety parameters established at the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation (Wassermann EM; Electroencephalogr Clin Neurophysiol 1998;108:1-16) and updated in 2009 (Rossi S, Hallett M, Rossini PM, Pascual-Leone A. Clin Neurophysiol 2009;120:2008-2039), as well as the safety guidelines for TMS protocols that are not repetitive in nature (e.g., single pulse studies). In the event of a seizure, subjects would receive counseling about the extremely low likelihood of a recurrent seizure. Mindful of the potential consequences of a report of a seizure in a medical record for a subject's insurability, driving, etc, medical documentation will include full details about the provoked nature of the seizure in an experimental protocol. Subjects will be informed of this possibility in the consent process, in addition to the possibility that a subsequent workup could reveal an increased risk of seizures.

In addition the following steps will be taken:

Subject Screening: Rigorous screening of subject participants will be done to exclude individuals with an increased risk of developing a seizure.

Training of all individuals who administer TMS: All personnel involved in TMS sessions will be familiar with the safety guidelines and with the seizure protocol.

Seizure protocol: A seizure protocol will be posted in a visible location in all rooms where TMS will be delivered. The seizure protocol is as follows:

In the event of a seizure

Protect from injury: Pull back coil apparatus, tilt chair back to flatten – move to the floor if possible to prevent participant from falling onto the floor from the chair. Protect head and body from injury using padding. Move any objects out of range that could potentially cause injury. Place a small folded blanket or other cushioning under the head if it is moving violently. Loosen any zips/ buttons close to the neck (e.g., jackets). If possible, turn subject on his/her side to prevent aspiration.

ABC's: (airway, breathing, circulation): Maintain a clear airway (head tilt, chin lift). Assess cardiorespiratory function (breathing and pulse).

Activate EMS (Emergency Medical Services): Call 911. If breathing and pulse are present, state, "Medical emergency at [location, building name and number]." If breathing and/or pulse are absent, state, "Cardiac arrest at [location, building name and number]".

Record time and duration of seizure.

Do not: restrict movement, insert of force objects into the mouth.

After the seizure: Maintain a clear airway: Subject to rest on his/her side to prevent aspiration. Help to reorient the subject: provide reassurance. Remain with the subject 1:1 until fully oriented and stable. Check vital signs. Have the subject transported to the Emergency Department for assessment.

Documentation: When the subject is stabilized, document the following: Incident preceding the seizure (stimulation parameters, duration of stimulation). Description of seizure (parts of the body involved in the seizure, types of movement, time and duration of seizure). Medical personnel notified and time. Treatment given or emergency measures taken. Any injury incurred during the seizure. Subject's clinical status post-seizure.

5. Light headedness and/or Syncope (rare, not serious): In the setting of altered sensory stimulation, subjects occasionally experience brief loss of consciousness, usually attributable to vasovagal syncopy.

To minimize this risk: Participants are encouraged to be well-hydrated in advance of the sessions. During breaks and upon completion of the protocol, we will ask that subjects rise slowly from the chair and we will monitor them for any signs of fading consciousness.

6. Risks from magnetic or induced stimulation (very rare, serious).

To minimize risk: Although the risk of adverse events due to magnetic fields or induced electrical currents acting on tissue at the parameters proposed in this experiment is virtually non-existent, there is some risk due to these currents interacting with metals or implanted devices in a potential subject. Therefore, rigorous screening procedures will occur to minimize this risk to acceptable levels.

7. For women of child-bearing potential: It is unknown if TMS can pose a risk to fetuses.

To minimize this risk: Participants are asked during their screening whether they are pregnant or are trying to become pregnant, and are not enrolled in the study if they are. Sexually-active women of child-bearing potential will be asked to use a reliable birth control method for the duration of this study.

Participants are asked about their experience at the conclusion of each visit; if they report symptoms, they are asked to rate them (see TMS exit survey in section 29)

Adverse events and withdrawals from the study will be documented for reporting during dissemination of research activities. Adverse events will be reviewed by Dr. Vesia to

determine their association with study protocol and assess any changes to the protocol that may be required. Study coordinator and/or PI will submit all reportable events to the IRB according to the reporting timetable.

Risks associated with fMRI:

1. Risk of hearing damage due to noise from the scanner (common).

   To minimize this risk, participants will be given foam earplugs to prevent against hearing damage and reduce discomfort due to the loud noises of the MRI scanner.

2. There is a minor risk of discomfort or anxiety from being in the confined space of the MRI scanner (infrequent).

   To minimize this risk we will provide the participant with pads and blankets for comfort as well as a two way intercom and an emergency squeeze ball so the participant can inform the fMRI technician they want to end the scan.

3. Sometimes participants report a temporary feeling of lightheadedness, slight dizziness, or nausea after the scanning session. (infrequent)

   To minimize this risk, participants will be told to inform the scanning technician as soon as this occurs so they can be helped up from the scanner.

4. Certain scanning protocols have the potential to cause peripheral nerve stimulation which is a light tingling sensation on the skin for a few seconds. It may cause minor discomfort but is not harmful. (Rare)

   To minimize this risk, the MRI machine is operated within FDA guidelines so the potential for inducing PNS is low.

5. Risk that the magnetic resonance image will reveal a minor or significant lesion in the brain (e. g. a tumor), previously unknown to the subject, and requiring additional follow-up. (Rare)

   In the event of anomalous finding on MRI, the PI would contact the participant and advise them to speak to their healthcare provider about obtaining a clinical MRI scan. Many such abnormalities are not clinically significant, but they may need or want to investigate them further. Participants are advised that scan images will not be routinely examined by a specialist trained to make medical diagnoses. Any abnormalities that a participant may currently have may not be noticed in the images obtained in this experiment and that if they have any current health concerns, they should consult their doctor.

6. Risk of injury from objects accelerated by the strong magnetic field of the magnet, striking the subject; or metallic substances on the skin or foreign bodies implanted deliberately or accidentally in the subject that acquire kinetic or thermal energy from the magnetic or radiofrequency emissions of the MRI, causing tissue injury. (Rare)

   To minimize risk, the MRI suite is kept clear of all objects that could be picked up by the magnetic field. MRI personnel are trained in safety procedures, which include training on what materials cannot be brought into the scanner room. Before the scan all participants are asked to fill out a safety form to assess their suitability to enter the MR environment. The MR technologist will review this form with the participant, and the participant will be asked multiple times to ensure there are no metallic objects on their person. Lockers are available for participants to store personal items during the scan.

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