**Full title:**

**Theta burst transcranial magnetic stimulation of fronto-parietal networks: Modulation by mental state**

**Principal Investigator:**

Stephan F. Taylor, M.D.,

Professor, Department of Psychiatry, University of Michigan

**Co-investigators:**

Taraz Lee, PhD

Assistant Professor of Psychiatry, University of Michigan

Luis Hernandez, PhD

Research Professor of Biomedical Engineering, University of Michigan

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**Table of Contents**

**Protocol Summary**

This proposal will examine a particular type of TMS, known as theta burst stimulation (TBS), which has been shown to induce longer lasting effects than other forms of TMS, making TBS an important tool for therapeutic applications. While TBS provides relatively focal stimulation, effects on the brain occur through interconnected networks in ways that are poorly understood. Moreover, stimulation is highly state-dependent, and the use of TMS in most therapeutic settings, such as the treatment of depression, leaves mental state uncontrolled. Augmenting TMS therapy by inducing specific mental states is an attractive idea for improving therapeutic TMS, but the relevant knowledge base is sparse. To address this critical gap, this exploratory R21 proposal will examine the effects of TBS on specific brain networks and the interaction between TBS and mental state. We will test the broad hypothesis that when TBS is applied during a controlled mental state, network changes will be facilitated, compared to stimulation when mental state is uncontrolled. We will focus on the dorsolateral prefrontal cortex (dlPFC) and the associated fronto-parietal network (FPN), which subserves cognitive control -- the ability to flexibly adapt and regulate behavior, an ability known to be impaired in neuropsychiatric conditions such as depression and dementia. We will use an 'n-back' task tapping cognitive control and the FPN. We will employ a within-subjects design with 40 healthy subjects in 4 MRI sessions. Each MRI session will consist of blood oxygenation level-dependent (BOLD) fMRI during an n-back task, resting state BOLD fMRI to measure connectivity and resting state arterial spin labeling (ASL) MRI to measure cerebral blood flow (rCBF) and examine effects on resting activity level. BOLD activation during the n-back will identify the FPN and the target site for dlPFC TBS. After a baseline fMRI session, subsequent sessions over different days will entail TBS, immediately followed by an MRI session to assess the effects of stimulation. TBS will involve: 1) dlPFC stimulation by active iTBS (600 pulses) alone or 2) while simultaneously performing an n-back cognitive task or 3) vertex (control) iTBS stimulation, alone. The following aims will be pursued:

**Population:** Healthy control subjects with no significant mental health problems.
**Site:** University of Michigan
**Study Duration:** 2 years
**Subject Participation Duration**: 3-4 weeks
**Study Design:** Basic experimental study in humans, single-blinded, cross-over
**Number of subjects:** up to 60 (to achieve 40 analyzable subjects)
**Description of Intervention**: Basic Experimental Studies with Humans (BESH)
**Estimated Time to Complete Enrollment:** 2 years

## 1.0 Introduction and Background

### *1.1 The need to improve our understanding of non-invasive brain stimulation*

Devices to deliver energy (magnetic, electrical, ultrasound) to nervous tissue have proliferated in the last decade, **but there remains a dearth of knowledge about how these devices affect brain networks.** This proposal will focus on TMS, which uses magnetic energy to induce electrical currents in nervous tissue, and even though several decades of work has detailed effects of TMS on cortical physiology,(*1, 2*) many questions remain unanswered about how TMS affects the brain, particularly at the meso-scale of brain networks which subserve complex behaviors. TMS has frequently been employed to disrupt cortical activity with focal stimulation, inducing a 'virtual lesion' and enabling inferences about the function of regions interrupted by stimulation,(*3, 4*) and it has also been used to enhance brain function.(*5, 6*) In the United States, it has been cleared for therapeutic use in the treatment of major depressive disorder,(*7*) obsessive-compulsive disorder(*8*) and migraine headache.(*9*) Numerous research studies have also reported benefits for many other neuropsychiatric conditions,(*10-15*) and more recent work has sought to combine TMS with behavioral interventions.(*8, 16-18*) While the combination of TMS with other interventions has intrinsic appeal, there is little scientific value in showing that two therapies, combined, work better than a single therapy, alone. If these efforts are to be more than therapeutic mashups, we will need a deeper understanding of the effects of TMS on brain networks, as this R21 project proposes.

Our approach will focus on cognitive control and the underlying FPN(s) which carry out this cognitive process. Cognitive control, also referred to as executive functioning, is the ability to flexibly adapt and regulate behavior in accord with goals and plans.(*19*) It is impaired in multiple neuropsychiatric conditions, such as depression (*20*), obsessive-compulsive disorder,(*21*) dementia(*22*) and schizophrenia.(*23*) Cognitive control is carried out in fronto-parietal networks,(*19, 24, 25*) including the dorsolateral prefrontal cortex, which is typically targeted by rTMS treatment for depression. As we review below, critical gaps in our knowledge exist about how TMS affects FPN and cognitive control, gaps which motivate the proposed work.

### *1.2 What is known about how TMS affects brain activity?*

Non-repetitive pulses of TMS stimulate neurons and affect local micro-circuitry in complex ways (see(*1, 2*)), but persisting, so-called 'plastic,' effects require repetitive TMS (rTMS). The exact mechanism(s) of enduring changes in neural activity remain unknown. Persistent rTMS effects are complex, involving direct and indirect effects on excitatory and inhibitory neurons at the focus of stimulation, as well as secondary and tertiary effects on connected regions.(*26, 27*) Timing, intensity, direction and frequency of stimulation all have differential effects.(*2, 27, 28*) In general, low frequency rTMS (≤1 Hz) reduces cortico-spinal excitability, measured as increasing levels of stimulation necessary to elicit a motor response ('motor evoked potential,' or MEP), whereas high frequency rTMS (generally ≥ 5 Hz) has an opposite, facilitatory effect on MEP, with effects generally lasting as long as the period of stimulation.(*2, 28, 29*) Longer lasting effects have been demonstrated by high frequency (50 Hz) bursts ('theta burst stimulation' or TBS), causing MEP facilitation for up to 15 minutes after a 190 sec period of intermittent stimulation (iTBS). On the other hand, continuous TBS (cTBS) for 40 sec causes MEP inhibition.(*30, 31*) The TBS protocols were designed to elicit long term potentiation and long term depression, the most widely studied mechanisms of neural plasticity,(*32*) although data suggests that responses measured in the MEP are more complex than LTP or LTD measured at the cellular level.(*33, 34*) A variety of cellular and molecular effects of rTMS/TBS have been described (see(*34*) for recent review), and meso-scale effects have also been studied with

electroencephalography,(*35-37*) but in order to best localize network effects, this proposal will focus on the persisting effects revealed through neuroimaging, including resting perfusion/metabolism, task-related activation and connectivity.

In general, the most consistent neuroimaging result observed in 'offline' (conducted after a period of rTMS stimulation) studies is that changes occur in regions beyond that stimulated, but anatomically and functionally connected.(*38-41*) Furthermore, it is not always possible to predict effects based on assumptions taken from MEP measurement. For example, 'inhibitory' cTBS increased cerebral blood flow in the motor cortex in one study,(*42*) and another study found that MEP measurement after 'excitatory' iTBS exhibited an inverse relationship between BOLD signal and the MEP increase induced by iTBS.(*43*) cTBS to the dlPFC increased connectivity amongst regions of the FPN,(*44*) and reduced the tuning of visual cortical activity and performance during a working memory task.(*45*) Overall, the effects of TMS on neuroimaging measures are difficult to predict and there are relatively few studies of offline effects of excitatory iTBS to the dlPFC. Therefore, **Aim 1 will address a question not sufficiently answered in the literature: What is the effect of iTBS to the FPN networks on resting perfusion, task-related activation and connectivity?**

*1.3 State-dependency of TMS stimulation*

A critical fact about TMS is that effects are highly state-dependent. The phenomenon known as 'metaplasticity' refers to neural plasticity modulated by prior activity in a neuron,(*46*) and an analogous process may occur with TMS.(*47, 48*) Extracellular recordings in animals have shown that increased visual cortical activity during excitatory TMS leads to greater post-TMS activity.(*49*) From the earliest days of TMS research in humans, it was noted that stimulation of motor cortex during active muscle contraction increased the size and number of descending volleys compared to stimulation when the hand was at rest.(*50, 51*) Since then, multiple examples of state-dependency have been described. For example, activating the ipsilateral hand during stimulation alters the response and coupling in the contralateral cortex during TMS to premotor cortex.(*52*) Directing attention to the contralateral hand during 5 Hz stimulation leads to larger MEP increases than when attention is directed to the ipsilateral hand.(*53*) Global changes in brain state, such as sleep, have demonstrated large effects, reducing the propagation of a single TMS pulse across the cortex when measured by surface electroencephalography.(*54*) In summary, multiple paradigms have demonstrated state-dependency of persisting TMS effects.

In spite of what is known about the state-dependency of TMS, most therapeutic uses of rTMS do not systematically control the mental state of subjects during stimulation, although this is starting to change. rTMS has been combined with psychotherapy for depression.(*16*) Cue exposure in addiction(*55*) and exposure therapy for post-traumatic stress disorder(*17*) and OCD(*8*) have been paired with TMS. A commercial system that combines rTMS with cognitive training in dementia patients has been cleared for marketing in the EU, even though differential effects in a sham-controlled study were not found (n=26).(*18*) In spite of this work, there are no neuroimaging studies identifying the neural effects of state-dependency in paradigms relevant to therapeutic TMS. Examining motor function, Narayana and colleagues showed that, in 4-week training paradigm, subjects who received 5 Hz rTMS to the motor cortex while they performed a digit sequence task showed improved motor performance and increased cerebral blood flow, relative to sham stimulation, in regions linked to skill learning.(*56*) For cognitive control, many studies have examined neural effects of TMS delivered 'offline'(prior to neuroimaging) to a brain at rest,(*39, 41, 57, 58*) but virtually no work has examined offline, persisting effects of TMS delivered while a person is engaged in a task. These persisting effects of TMS interacting with brain state are critical to understand how this interaction could be harnessed for improved therapeutic effect. Thus, in **Aim 2 we will address the question: How does mental state modulate the effect of rTMS on FPN networks?**

The phenomenon of task state interacting with TMS has been used to target specific neural populations within a stimulated region, a paradigm known as 'TMS adaptation.'(*59*) It has been used to augment brain mapping studies of language processing(*60*) and higher level perception.(*61*) For example, Silvanto and colleagues(*62*) showed that when subjects viewed visual motion while receive inhibitory cTBS to direction sensitive neurons in V1/V2, performance after stimulation was impaired for the direction-sensitive neurons that were not activated during the passive viewing task. In other words, neurons engaged by the task appeared to be 'protected' from inhibitory cTBS. This motivates our **Aim 3, asking whether excitatory iTBS stimulation while the FPN is engaged would improve subsequent performance**.

## 2.0 Study aims and outcome measures

*Specific Aim 1: Localize neural effects of dlPFC TBS* We will show that persisting neural changes induced by TBS to the dlPFC will affect the FPN. We predict that TBS alone (subjects not performing a cognitive task), compared to vertex stimulation, will increase fMRI activation in the FPN during the n-back and increase FPN connectivity during resting state BOLD. We also predict that TBS will increase resting perfusion in the FPN. Exploratory analyses will search for regions outside the FPN that change with stimulation to develop a comprehensive picture of how TBS to the dlPFC interacts with brain networks.

*Specific Aim 2: Demonstrate modulation of the effect of dlPFC TBS by a cognitive task* We predict that FPN changes during iTBS administered while subjects perform the n-back task will be greater than when they are not performing a task. We will also test the same hypothesis for BOLD resting state connectivity, and ASL-measured perfusion, in addition to exploratory hypotheses on brain networks outside the FPN.

Primary outcome measures for Aims 1 & 2:
1) 2-back minus 1-back BOLD activation, voxelwise in FPN
2) FPN connectivity to dlPFC TBS stimulation target
3) rCBF at stimulation target

Secondary outcome measures for Aims 1 & 2:
1) 2-back minus 1-back BOLD activation, voxelwise in whole brain
2) rCBF in FPN

*Specific Aim 3: Demonstrate improvement in cognitive control with TBS, modulated by cognitive task during stimulation* We will test predictions that n-back performance will improve following TBS to dlPFC, but not vertex, and will improve even more following TBS during n-back performance. Exploratory analyses will examine correlations between performance changes and network changes, suggesting mechanistic connections between TBS and performance changes.

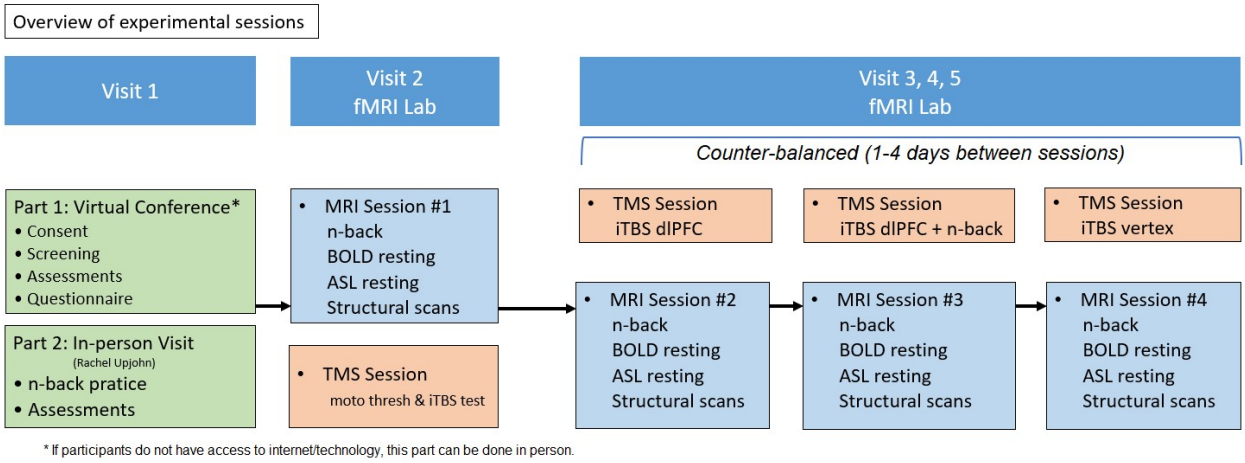
Primary outcome measure for Aim 3: accuracy to 2-back

Secondary outcome measures for Aim 3: d-prime, reaction time

## 3.0 Study design

The study is a within-subjects design that will expose subjects to three TMS/TBS sessions, with each followed by an MRI session. The first MRI session, without TMS, will obtain baseline

measurements of FPN activation (providing the target for iTBS stimulation – see below), resting state connectivity and resting state cerebral perfusion. The subsequent 3 MRI sessions will occur in a counterbalanced order, acquiring the same imaging sequence, and following one of three iTBS sessions: 1) To dlPFC, without a concurrent task; 2) to vertex, without any concurrent task, and 3) To dlPFC, while subjects perform an n-back task.




## 4.0 Study Participants

### *4.1 Participants inclusion/exclusion criteria*

Participants will be healthy individuals, ages 18-50. The upper limit of 50 will reduce variance from older subjects, who exhibit less specific activation patterns.(*63, 64*) We will recruit ~60 individuals (50% women) to allow for drop-outs and subjects who cannot tolerate TBS, aiming for 40 subjects in the final analysis.

*Inclusion/exclusion criteria:* (1) No history of past or current mental illness (except for simple phobias), but prior h/o substance dependence OK if in remission for greater than 5 years; (2) Not taking any medication, prescription or non-prescription, with psychotropic effects (birth control medications allowed); (3) No first-degree family members with a history of epilepsy; (4) Age 18 –50; (5) If a woman of child bearing age, not pregnant or trying to become pregnant; (6) No history of serious neurological illness (including, but not limited to, seizures/epilepsy) or current medical condition that could compromise brain function, such as liver failure; (7) No history of closed head injury, e. g. loss of consciousness > ~5 min, hospitalization, neurological sequela; (8) Ability to tolerate small, enclosed spaces without anxiety; (9) No family history of epilepsy in first degree relatives; (10) No metals, implants or metallic substances within or on the body that might cause adverse effects to the subject in a strong magnetic field, or interfere with image acquisition, e. g. aneurysm clips, retained particles (e.g. metal workers with exposures), neurostimulators, foil-backed transdermal patches, carotid or cerebral stents, CSF shunts; magnetic dental implants, ferromagnetic ocular implants, pacemakers, automatic implantable defibrillators; (11) Size compatible with scanner gantry, e. g. men over 6 feet tall that weigh more than 250 lbs, men under 6 feet tall that weigh over 220 lbs, women over 5'11" tall that weigh more than 220 lbs, or women under 5'10" tall that weigh more than 200 lbs. Subjects of these weights or greater typically have difficult fitting into the fMRI scanner properly; (12) Ability and willingness to give informed consent to participate.

### *4.2 Informed consent*

In most cases, potential subjects will learn basic information about the study during in-person or telephone screenings conducted by study personnel, and informed consent/assent forms will be given, mailed or emailed to those who are eligible for/interested in the study. A meeting with the

study coordinator will then be scheduled, at which time subjects will be given detailed information about the study and provided with an opportunity to enter our mock scanner to better understand how participation in an fMRI study "feels". During this meeting, the potential subject will be encouraged to ask questions about the study, after which the informed consent form will be reviewed and signed. A copy of the document will be given to the study participant and the original informed consent document will be kept in a locked cabinet in the principal investigator's laboratory, separate from all other study materials.

*4.3 Strategies for recruitment and retention*

These subjects will be recruited by community advertisements (flyers, social media campaigns) and word of mouth. A website, maintained by the University of Michigan provides a central recruitment portal for all subjects in this study (UMHealthResearch.org), although we expect that most of the subjects replying to this site will be healthy controls. Potential study participants go to the website and search for protocols in which they can participate. The website also features an email notification system that will let potential participants know when a new study is recruiting subjects.

When subjects contact the study team expressing an interest in participation, a pre-screening interview will occur. The interview will consist of a description of the study, and if the potential participant expresses interest, then pre-screening questions will be administered to determine initial eligibility and safety status. Participants will be asked questions from the fMRI Safety Screening form and the Transcranial Magnetic Stimulation Adult Safety Screen (TASS) (*71*) to ensure that there are no contraindications. Female subjects will be asked about pregnancy or the possibility becoming pregnant during this study. Part of this process may occur over email, with appropriate precautions taken to limit email exchange of sensitive information. For sensitive information, such as questions about history of mental illness or substance use, secure email messaging will be used. Subjects who pass pre-screening will be invited for a screening session. A waiver of HIPAA authorization for recruitment purposes will also be obtained so that medical records might be accessed in the UMHS system to address questions around eligibility. Subjects will be given detailed instructions, as well as reminder texts/emails/calls (their preference) about upcoming visits.

*4.4 Intervention assignment procedures*

All subjects will receive all interventions in a cross-over design. The three intervention sessions (visits 3, 4 & 5) will be given in counter-balanced order, stratified by gender. Subjects will be blind to the nature of the questions being asked; however, they will be told that different stimulation paradigms will be used, which will be evident to the subjects as they go through the procedures.

## 5.0 Description of intervention

This mechanistic intervention consists of rTMS, specifically iTBS, which will be delivered through a MagPro X100 with MagOption magnetic stimulator and a 90mm statically cooled figure-8 coil (MCF-B70, MagVenture Inc.). The MagVenture MagPro TMS system is a class II medical device, regulated by the FDA. Its intended use is for stimulation of peripheral nerves, and it has been cleared under FDA 510(K) regulations (K091940). Use in the submitted proposal constitutes off-label use of this device. It is not being used as a diagnostic device, and it is subject to Investigational Device Exemption regulations. In consideration of regulations 21 CFR § 812.3 (m) and 812.20 (a), the device is not a significant risk device, i. e. it is not intended as an implant, not for use in supporting or sustaining human life, not for substantial importance in diagnosing, curing, mitigating or treating disease and it does not present a potential for serious risk to health or safety of a subject. Therefore, the device qualifies as a non-significant

risk device, subject to the approval of the local Institutional Review Board, and the investigator will comply with the abbreviated IDE requirements, per 21 CFR § 812.2 (b).

**6.0 Study Schedule**

*Section 6.0 makes repeated references to remote visits, which will be conducted via an encrypted, HIPAA-compliant video conferencing platform (e.g., Blue Jeans, Zoom for Health, etc.) It will be standard practice to conduct said procedures remotely to better protect participants and staff against the risk of COVID-19. However, in cases where a remote visit is not possible, in-clinic visits will be allowed and the study will follow all UM safety guidelines for entering and being in UM buildings (e.g., COVID screenings, PPE, social distancing, etc.).*

*Pre-Screening assessment preparations:* Participants who screen-in from the phone screen will be scheduled for a remote video conference session. They will be emailed a link, given instructions about the visit (e.g., how to open the link on their phone or computer), and sent a blank copy of the consent to review prior to their session.

*6.1 Consenting procedures*

Informed consent will be obtained at the beginning of the remote visit. Electronic informed consent signatures will be collected from adult participants using SignNow. All procedures will adhere to the IRBMED Guidance for use of SignNow for Electronic Informed Consent Procedures. All woman of childbearing age will be given an option for a urine pregnancy test the day of the fMRI scan, or they may decline this option and sign a pregnancy attestation form via SignNow indicating that they do not believe they could be pregnant. Woman with a positive pregnancy test will not be permitted in the study.

> *If in-person visit required:* The day before their scheduled visit, study staff will contact participants to administer the COVID pre-screen questionnaire required for entry into the Rachel Upjohn Building. If cleared by the screener, participants will be scheduled to come to the Rachel Upjohn Building following current COVID protocols. Staff will escort them to a private interview room with a laptop or computer where a remote session will be established with the study team (in another room). Study staff will return to their office and provide all above-mentioned informed consent procedures remotely. All signatures will be collected using SignNow.

*6.2 Screening assessment visits*

The screening assessment visits will be separated into two parts: Part 1, which can be done remotely or as an in-person visit, and Part 2, which will be done in-clinic at the Rachel Upjohn Building.

*6.2.1 Part 1: Remote or in-person visit*

*Screening assessments:* The assessment session will include the following:

1) Acquisition of basic demographic data and medical history.
2) M.I.N.I. or SCID-IV (*65*) to exclude psychiatric illness and substance use disorders.
    - If suicide assessment in M.I.N.I uncovers suicidal plans, intentions, or recent behaviors, emergency evaluation will occur, and an appropriate plan of action will be followed, e. g. referral to psychiatric emergency room (see supplemental suicide protocol). The PI will be contacted and will join the decision-making process about how to manage the suicidal thoughts or behaviors.
3) The reading portion of the Wide-Range Achievement Test (*68*) to assess general educational attainment.
4) Digit span task (*70*) to assess executive function.
5) Beck Depression Inventory II(*66*) to assess sub-clinical mood symptoms.**

6) Short Form 12(*67*) to assess general health status.**

*Preparation for MRI scans, mock scanner and task practice*: The subject will enter a "mock scanner" to practice the task. The mock scanner has a moving table that takes the subject inside a large tube, similar to the real MRI scanner. Study personnel can stay in the room as the subject learns and practices the task. Subjects find the mock scanner less intimidating, although the experience familiarizes them with the scanner environment and helps to de-sensitize them to the actual scanner.

For subjects who complete the screening process, we will provide them with a schedule and instructions to set up expectations we have about being in a consistent frame of mind for each session. We will counsel participants to abstain from alcohol on the evenings prior to the TMS/MRI sessions and obtain a full night of sleep. We will ask them to maintain their usual caffeine habits, and not deviate from session-to-session.

This session should take approximately 1 hour.

*6.3 Visit 2: Baseline MRI session #1*
*Staff and participants will follow the fMRI Lab COVID-19 safety guidelines.*

This session will occur at the fMRI laboratory on North Campus. This visit will follow the screening session by up to ~2 weeks, depending upon availability of the fMRI and TMS suite. During the visit, we will acquire baseline MRI data, including localization data using the n-back task. We will also obtain motor threshold in the adjacent TMS suite and give subjects an initial exposure to iTBS stimulation to ensure tolerability.

This session should last approximately 2 – 3 hours.

*6.3.1 Safety assessment*: We will review the subject's safety screening forms for fMRI and TMS to make sure nothing has changed since the initial assessment. We will also record sleep and ingestions of psychoactive substances (caffeine, alcohol, nicotine). We will prepare them for the MRI scan, ensuring that they remove all metal from their person (lockers are provided at the fMRI laboratory to store personal items such as wallets, cell phones).

*6.2.2 MRI Image acquisition:* Scanning will be performed at the UM Functional Magnetic Resonance Imaging Laboratory (fMRI Lab) on a 3T MR 750 scanner (Waukesha, WI) using a 32-channel coil.

When we position a subject in the scanner, head movement will be minimized through: (a) instructions to the participant; and (b) packing the head inside the head coil with a system of foam padding and pillows that we have found is well-tolerated by the participants, yet limits movement. Extra care will be taken to ensure that the participant's head is positioned as straight as possible since that eases the task of identifying landmarks used in positioning slices to be acquired in the fMRI scans. A mirror or MR-compatible goggle system projects the computer display output into the bore of the scanner. Stimuli are controlled and responses recorded either by E-Prime (PST, Inc., Pittsburgh, PA) or Presentation (Neurobehavioral Systems, Albany CA) lab software. Participants will wear a response claw on their hands with buttons in order to make button-press responses during cognitive tasks. The stimulus-presentation and response-collection systems have been tested to ensure that no artifacts are introduced and that there has not been an increase in the image-level noise. The goggle system may contain an eye tracking camera, which can permit the monitoring and possible analysis of eye movements during the scan. In addition, MR-compatible earphones may be used and physiological sensors may be attached. These sensors consist of a strain gauge, or strap that

goes around the torso, to measure respiration, and plastic finger cuff, to measure heart rate by plethysmography and CO2 monitor (although latter may not be used if it is redundant with pulse oximeter). These non-invasive sensors are FDA-approved components (except for the CO2 monitor) of the scanner, and the fMRI Center has begun to incorporate the physiological information into the acquisition sequences in order to improve image reconstruction. Because this is an area of data analysis that remains under development, we may not employ these sensors in all scans. Once in the scanner, subjects can communicate via intercom with the technician running the scanner in the control room. A window separates the control room from scanner room, which is sealed off from the outside by a light vacuum seal. In addition to the intercom system, subjects are given a squeeze bulb. They are informed that should they need to exit the scanner immediately or gain the attention of the technicians, they can trigger an alarm in the control room by squeezing the bulb.

After localizer scans, subjects will undergo 3 scans: 1) BOLD-weighted fMRI (EPI, TR=800 ms, FOV=23, slice=2.4mm, 96x96 matrix) for the n-back task (8 minutes); 2) BOLD-weighted resting state scan for 10 minutes to obtain data for functional connectivity analyses (eyes open, looking at fixation cross); 3) pseudo-continuous ASL (pCASL) sequence following the recommendations of the ISMRM consensus(*75*). Our pCASL implementation will use a 3D stack of spirals acquisition(*76*) with 6 interleaves (TR=5000 ms, TE=4 ms, BW=125kHz, FOV=22 cm) preceded by a 1800 ms labeling period and an 1800 ms delay to allow the label spins to clear the arteries and enter the capillary/tissue compartment. Two hyperbolic secant inversion pulses at 600 and 1300 ms after labeling will suppress background signals, after first 2 frames to permit collection of spin density images for quantification. We will acquire 20 pCASL control-label image pairs, which will be reconstructed to a 128x128 matrix. Since the excitatory effects of iTBS have been demonstrated to last up to 60 minutes,(*30*) data acquisitions will occur in the appropriate time frame to capture iTBS effects. The last scan in the sequence will be a high-resolution structural scan (SPGR) for image normalization, as well as a T1 scan in the same prescription as the BOLD scans to facilitate normalization.

The scans and acquisition parameters described here are representative of what will be employed, although the actual acquisitions may differ slightly. Power-monitoring software on the scanner will ensure total energy delivered to the subject will remain within FDA guidelines. Specifically, the specific absorption rate (SAR) will be not greater than: 1) 4 W/kg averaged over the whole body for any period of 15 minutes; 2) 3 W/kg averaged over the head for any period of 10 minutes; 3) 8 W/kg in any gram of tissue in the head or torso; 4) 12 W/kg in any gram of tissue in the extremities, for any period of 5 minutes.

*6.2.3 Visit 2 TMS session*: This session will follow the MRI session for visit 2.

*6.2.3.1 Motor threshold*: Motor threshold will be obtained to calibrate the intensity of stimulation delivered for the TBS TMS sessions. Motor evoked potentials (MEPs) elicited using biphasic posterior-anterior stimulation will be recorded from right first dorsal interosseous (FDI) using surface electromyography (Rogue Research, Montreal, Canada). The motor cortical hotspot will be defined as the site of stimulation in left motor cortex that elicits the largest, consistent response in the FDI with the coil oriented at 45 degrees to the coronal plane. Active motor threshold (AMT) will be obtained as the percentage of stimulator output that elicits an MEP of ≥ 200 μV peak-to-peak on ten out of twenty trials while the subject is contracting the FDI muscle at approximately 20% of their maximum.(*77*)

*6.2.3.2 Trial run of iTBS:* In order to see if subjects can tolerate iTBS, we will do a 2-3 minute run of iTBS to the dorsolateral prefrontal cortex (approximate location). If a subject cannot tolerate iTBS, they will be withdrawn from the protocol (but receive payment for the 2 visits they completed).

*6.3 Visits 3,4,5, Combined TMS/MRI sessions*

These sessions involve the experimental manipulation, using iTBS TMS prior to the MRI session, in order to test hypotheses described in the Aims. These visits will also occur at the fMRI laboratory, which houses the TMS suite, across the hall from the MRI magnet. Each TMS session will differ, depending upon how iTBS is delivered and what the subject is doing while receiving stimulation. The MRI sessions will be identical.

We will seek to test participants at the same time of day to reduce circadian variability, separated by 1 to ~4 days. We will also work to schedule the 3 sessions in the same week, avoiding Monday. Each session will last approximately 2-3 hours. Below, we describe what happens for each of the three visits.

*6.3.1 Safety assessment:* We will review the subject's safety screening forms for fMRI and TMS to make sure nothing has changed since the prior session. We will also record sleep and ingestions of psychoactive substances (caffeine, alcohol, nicotine). We will prepare them for the MRI scan, ensuring that they remove all metal from their person (lockers are provided at the fMRI laboratory to store personal items such as wallets, cell phones).

*6.3.2 Neuronavigation for TBS sessions:* To determine the site for stimulation, we will use neuronavigation, with structural and functional information to account for individual variability in recruitment of the FPN. BOLD mages acquired from the first MRI session (Visit #2) will be analyzed with standard methods to determine the activation of 2-back minus 1-back, and aligned with the subject's high resolution SPGR structural scan. These data will be transferred to the Brainsight Frameless system (Rogue Research, Montreal CA). For the dlPFC target, we will locate the junction of the anterior and middle third of the left middle frontal gyrus (MFG), which corresponds to Brodman area 46,(*78, 79*), and within a zone that is 1/3 the length of the MFG, will search for the locus of greatest activation in BOLD activation (2-back minus 1-back, or 1-back minus 2-back) for each subject, using those coordinates as the site of stimulation. The Brainsight system will be used to identify the overlaying scalp position. For the vertex (control) target, we will locate the approximate vertex, according to standard 10-20 system, but in a region where no BOLD change is detected.

*6.3.3 TMS session:* We will deliver iTBS, which has been shown to increase cortical excitability,(*30*) using 3 pulses of stimulation at 50 Hz, repeated every 200 ms, for 2 sec trains, repeated every 10 sec, giving a total of 600 pulses in 190 sec. Stimulation will be delivered at 80% of AMT, which is within consensus recommendations for safety,(*80*) and used in published work.(*81*)

In counter-balanced order, subjects will receive one of three iTBS sessions on each of the 3 days: 1) Vertex stimulation and subjects will be instructed to maintain gaze on a fixation screen and rest; 2) dlPFC stimulation (from neuronavigated target described above, subjects will be instructed to maintain gaze on a fixation screen and rest; or 3) dlPFC stimulation augmented with an n-back task. A computer screen will display an n-back task, using the 2-back condition. Letters will appear for 0.5 sec every 2 seconds, and presentation will be synchronized with the iTBS stimulation, which will occur 0.5 sec before the onset of every 5$^{th}$ letter. Actual parameters may differ slightly when delivered in the experiment.

*6.3.4 MRI session:* MRI sessions will be identical to Visit #2. The MRI will follow immediately after the iTBS TMS session.

*6.4 Reimbursement of subjects*

In order to make participation worth the subjects' time, they will be reimbursed $20 for the assessment sessions and $50 for each MRI/TMS (total $220/subject). We have found that this amount provides a good incentive to encourage ongoing participation.

*6.5 Participant discontinuation/withdraw*

During the pre-screening process, subjects who are found to be non-eligible, before signing the consent form, will be considered *not enrolled*, and all data related to that subject will be destroyed. Subjects who consent, and are found not eligible during the assessment and screening will be considered *screen failures*, and all research and identifying data will be destroyed, except for the consent form and limited information to document the screen failure (age, gender, diagnosis, specific reason for exclusion). Subjects will receive a subject number at the time of consenting. Subjects who pass screening will be considered *enrolled*. Subjects who leave the study after signing the informed consent, and after being deemed eligible/enrolled/randomized, will be considered *withdrawn*. Subjects may withdraw from the study at any time and for any reason. Subjects may be withdrawn by the investigator when continued participation would present an unacceptable risk to the subject. Based on previous experience, we expect that most subjects will be compliant with the study protocol.

## 7.0 Assessment of Safety and Regulatory Reporting

*7.1 Data and Safety Monitoring Plan*

*7.1.1 Overall framework of monitoring:* Study monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonization Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s)

*7.1.2 Roles and responsibilities for regular operations*

*PD/PI:* The PI, a board-certified psychiatrist, will meet with study staff on a weekly basis to monitor the progress of the study. During phases when subject recruitment is occurring, the PI will review screenings and scheduling of assessments, as well as strategies to improve recruitment yield. Issues around maintaining confidentiality and privacy of participants during screening, assessment and data collection will be reviewed. Any protocol deviations and adverse events will be reviewed at this weekly meeting. For serious adverse events, the PI will be notified as soon as the SAE comes to the attention of study staff.

*Study coordinator/research assistant:* This bachelors level individual will carry out the screening and informed consent, and they will provide additional study information, all under the supervision of the PI. The individual will be trained in screening, administering consent and information to the participants. Questions about eligibility will be discussed with the PI. This individual will administer surveys and neuropsychological tests, monitor the participants during the procedure and administer the experimental intervention (TMS). They will receive training in the administration of TMS to ensure they can safely operate the device, are aware of potential side effects and can execute the seizure protocol if this is necessary. They will conduct any necessary follow-up interviews.

*Clinical Assessor:* Screenings will include thorough assessment of psychiatric health by a masters-level clinician with experience in administration of psychiatric interviews. They will ensure the absence of any neuropsychiatric conditions in the subjects, as well as health conditions that would be a contra-indication to TMS or MRI procedures.

*7.1.3 Frequency and type of other study monitoring:*

*Institutional Review Board monitoring:* Approval of all procedures, advertisements and materials given to subjects will be secured from the University of Michigan IRBMED. Annual reviews will be conducted by IRBMED, including the number of subjects screened, enrolled and withdrawn. The IRB will also review protocol deviations, adverse events (according to the reporting timetable of IRBMED) and complaints that arise in connection with the study.

*TMS Safety Committee monitoring:* The TMS Safety Committee (see eResearch application for composition of group and charter) will review the protocol prior to initiation of the first subject. The committee will receive an initial report after the first 3 subjects, and then semi-annual reports afterwards. The reports will include a log of all adverse events recorded during the study, number of subjects studied, and details of the specific TMS protocols employed. The committee will also review protocol deviations and any unanticipated events reported to the IRB. If the committee has concerns about subject safety, these concerns will be conveyed to IRBMED.

*Data monitoring:* Data entry will be audited by study staff, who were not involved in primary data entry.

*7.1.4 Adverse events arising in the course of participation:*

*7.1.4.1 Adverse Events Review:* Monitoring will occur by the clinical study coordinator. The coordinator will then bring all possible AEs to the attention of the PI for evaluation, gradation and appropriate reporting. AE's will be assessed for severity (mild, moderate, severe), expectedness (expected, unexpected) and relatedness (definitely related, possibly related, unlikely related, not related). A log of all AE's will be maintained by the study coordinator.

*7.1.4.2 Adverse Events Definitions and reporting timetable:* A Serious Adverse Event (SAE) in this study will be defined as an event that results in death, is life-threatening, requires hospitalization or prolongation of existing hospitalization, or results in persistent or significant disability or incapacity. The degree of probable relation between study procedures and the SAE will be carefully evaluated and documented.

Serious adverse events will be reported to the IRB within 7 days of occurrence (or notification) of the event if they are definitely or possibly related to the study interventions and unexpected. If the SAE is expected, it will be reported within 14 days. For SAEs that are not related to the study, reporting will occur at the time of scheduled continuation, or not at all if determined to be expected, per standard reporting timetable of the University of Michigan IRB.

Unexpected adverse events in this study will be defined as any adverse events for which the nature and severity are not consistent with expected or anticipated adverse events resulting from participation in the protocol.

Adverse events will be assessed as severe, moderate or mild. Moderate is defined as any non-serious event which causes discomfort and requires treatment, but does not pose any significant or permanent risk or harm to the subject or require in-patient hospitalization, including social or psychological trauma causing moderate or temporary distress, significant embarrassment, stigmatization of individual or community/ group, disruption of familial/social relationships or nontrivial emotional distress or upset. Moderate AEs will include evaluation in the emergency room not leading to hospitalization. Mild is defined as any non-serious event of less than moderate severity.

Moderate and mild AEs that are expected will not be reported to the IRB. Moderate/mild AEs that are unexpected and definitely or possibly related will be reported to the IRB at the time

of scheduled continuing review. Moderate AEs that are unexpected and definitely not related will not be reported to the IRB.

*7.1.5 Quality assurance and quality control for data acquisition*

*Clinical assessments:* Assessors will be trained on assessment instruments and established according to laboratory standards and good inter-rater reliability

*MRI data:* The fMRI lab maintains weekly schedule of calibration runs to assure consistent operation. Processing pipelines and standard operating procedures are in place ensure that data is processed in a uniform manner. Quality control at each step will be carried out by study staff to verify, through multiple metrics, image quality. Databases will track processing steps, in addition to automatically generated log files.

*7.1.6 Confidentiality and security of data*

*Confidentiality of data:* Multiple steps are taken to ensure the confidentiality of sensitive information. Subjects are reminded that they do not have to answer questions that make them feel uncomfortable, which reinforces the voluntary nature of their participation. The confidentiality of all information gathered directly from subjects or obtained from the patient's medical records is assured by assigning records a coded research number and identifying all computer and paper files only by this code. A single tracking file will link the codes with subject identifiers, such as name and hospital number. In addition, consent forms and payment forms will be kept in a separate, locked room, apart from research data. All files will be kept in locked file drawers in locked rooms, to which only authorized research personnel have access. Electronic records will be kept on secure servers, and records with identifying information will be kept separate from records used to collect research data. Only staff members who have a need to know personal identifying information will have access to this information. All research staff are trained in research ethics and HIPAA regulations pertaining to protected health information. Screening forms for subjects who do not qualify for the study will be destroyed, except for anonymous information unlinkable to the subjects (such as age, gender and education). After the completion of data analysis, the record linking subjects to the research codes will be destroyed, thereby anonymizing the data.

*7.2 Event Windows, Missed Assessments, Missed Sessions and Protocol Deviation Reporting*

Any safety-related assessments (TASS, fMRI screening) which are missed will be reported to the IRB as a protocol deviation. For the other assessments, missing assessments will not be reported as protocol deviations unless they constitute > 10% of the total assessments. In order to accommodate inevitable scheduling conflicts, holidays, etc., study team members will be allowed to schedule Motor Threshold (MT) and/or fMRI procedures with discretion as needed.

These deviations are expected to be minor and will not be reported, although they will be recorded. These allowances should affect neither the scientific integrity nor the safety monitoring provisions of the protocol.

## 8.0 Statistical analysis

Analyses described below represent planned analyses for primary and secondary measures. However, actual implementation of these analyses may differ slightly from what is described below, although the primary and secondary outcome measures will be as described.

**Specific Aim 1: Localize neural effects of dlPFC iTBS**

**Primary outcome measures:**

Primary outcome #1, BOLD activation: 2-back minus 1-back BOLD activation, voxelwise in FPN
Analysis Plan: MRI data analysis and strict quality control will use validated and established routines implemented in our laboratory. For processing BOLD images, we will use well-tested routines from FSL 5.0.1 and SPM12 for slice timing and realignment algorithms and spatial normalization (MNI-152) procedures implemented in VBM8. First-level analysis will use the general framework of the modified General Linear Model,(*82*) implemented in SPM12 with temporal convolution. For the *n-back data* the first-level design matrix will include regressors for 2-back and 1-back, iTBS/MRI session and 12 realignment parameters (including temporal derivatives of 6 realignment parameters). Second-level, between-subject analysis will consist of two-sided, one-sample t-tests for primary effects. For statistical inference across the FPN, and the entire brain, we will use topological false discovery rate (FDR)(*83*) providing corrected probabilities at $p<0.017$ (0.05/3, accounting for testing of 3 primary MRI measures). Given *a priori* hypotheses about FPN, a mask of activation (2-back minus 1-back, $p<0.001_{uncorr}$) at the group level, using all 4 MRI runs to provide the most representative mask, will be used as small volume correction.

(H1.1): iTBS to dlPFC, compared to vertex stimulation, will increase fMRI activation in the FPN during the n-back. To examine the effect of iTBS on FPN, we will directly contrast the MRI session following dlPFC stimulation with the session following vertex stimulation, with subjects in an unconstrained, resting state for both conditions. (2-back minus 1-back contrast, voxelwise analysis in SVC-corrected FPN mask). We are using two-sided tests, because unpredicted results have been described in the literature, where inhibitory cTBS has resulted in increased perfusion,(*42*) and increased connectivity,(*44*) and excitatory 5 Hz TMS to dlPFC demonstrated reduced BOLD activation.(*84*) For a secondary measure to test H1.1, we will examine whole brain activation, since change may occur outside this region of interest. For example, dlPFC stimulation has been observed to induce changes in a limbic anterior-cingulate network.(*85*)
Exploratory analysis: We will conduct exploratory analyses with a generalized psychophysical interaction (gPPI). To test for interaction between task and session, we will extract the time-course at the site of stimulation, deconvolve it with the canonical hemodynamic response function (HRF), and then generate an interaction regressor (task X time-course) which is tested (after-reconvolution with HRF) across the entire brain.(*86*) gPPI tests for changes in the slope of connectivity coefficients with task, so it reflects how task modulates connectivity. Testing for effects of iTBS will test for a modulation of this modulation. Statistical correction will use topological false discovery rate (FDR)(*83*), at $p<0.05$, corrected for whole brain search.

Primary outcome measure #2, BOLD connectivity: FPN connectivity to dlPFC iTBS stimulation target
Analysis Plan: Data processing of fMRI BOLD resting scans will begin with standard pre-processing steps. All scans will be slice-time corrected and realigned (FSL 5.0.9) to the 10th image acquired during a scanning session.(*87*) Subsequent processing will be performed with the Statistical Parametric Mapping SPM8 package (R4667; Wellcome Institute of Cognitive Neurology, London). Anatomic segmentation will be performed with the VBM8 toolbox in SPM8, using the high resolution SPGR T1 image, subsequently normalized to an MNI template using DARTEL. Normalizing warps to the MNI152 template are applied to the co-registered functional volumes, which are re-sliced and smoothed with an 8 mm isotropic Gaussian smoothing kernel. To assess and manage movement, we will calculate the frame-wise displacement (FD (*88*)) from the translation and rotation parameters from the realignment file, which is the sum (in millimeters) of the absolute values of frame displacement, i.e. successive 3D volumes, for all 6 parameters of rotation and translation. Rotational displacements are converted from degrees to millimeters moved on the surface of a 50 mm sphere. We use a 'scrubbing routine' to censor

any frame with FD > 0.5 mm from the regression analysis described below, yielding a scrub ratio for each subject. As it has been demonstrated that as many as 60% of frames can be removed and still yield analyzable results,(*88, 89*) we will use 60% deleted frames as a cutoff for analyzable subjects. Three-compartment segmentation of the high-resolution structural image from the VBM8 normalization will be applied to the functional time series to extract cerebral spinal volume (CSF) and white matter (WM) compartments, which will be subjected to a principal component analysis to identify the top 5 components in each,(*90*) which corresponds to heart rate and respiratory effects on the blood oxygenation level-dependent signal.(*91*) Adjusted time courses will be derived from sequential regressions of the time series with the following: Linear trend, 6 motion parameters, their temporal derivatives, the quadratics of these 12 parameters, 5 components from the PCA of CSF, 5 components of PCA of WM, followed by band pass filtering from 0.01 – 0.1 Hz, and then motion scrubbing. ICA-AROMA will be used to de-noise the data and remove additional, residual motion artifacts.(*92*) Global signal regression (GSR) will not done because of concerns about non-uniform transformation of correlations introduced by GSR, problematic for group comparisons.(*93*) For the primary analysis, the time-course from a 6 mm radius sphere at the site of stimulation will be entered into the multiple regression and correlated with every other gray-matter voxel time-course in the brain for each subject. Correlation coefficients are Z-transformed and then entered into the second level, between-subject analyses as two-sided, paired t-tests to compare between sessions. Statistical inference will be controlled as above.

(H1.2) iTBS to dlPFC, compared to vertex stimulation, will increase FPN connectivity during resting state BOLD. Because TMS effects are observed away from the site of stimulation,(*38-41*) we are testing functionally-defined FPN networks. We recognize that the FPN defined by n-back will not be identical to the FPN defined by a dlPFC seed-based connectivity, although the two significantly overlap.

Exploratory analysis, global brain connectivity: We will examine a graph-theoretic measures known as global brain connectivity (GBC).(*94*) GBC reflects the strength of local connectivity, similar to degree connectivity, except that no arbitrary thresholding of connections ('edges') is required; rather, the value of the connection strength (r-to-Z transformed) is summed. GBC in the dlPFC has been shown to decrease with electro-convulsive therapy, with the degree of change related to symptom improvement;(*95*) thus, we will test to see if iTBS to the dlPFC has an effect on GBC in the dlPFC. Two types of analyses will be conducted. In the first, we will average the connectivity for the 6 mm radius sphere at the site of stimulation and compare that for dlPFC stimulation and vertex stimulation. Alpha level will be $p<0.05$, two-sided. In the second analysis, we will search for network effects. Time courses will be extracted from 10 mm diameter spheres based on a set of 264 nodes distributed throughout the brain,(*96*) generating a cross-correlation matrix of Pearson r-values. Z-transformed r-values (connectivity strengths) for each of the 264 nodes with every other node (global brain connectivity) are summed, and connectivity strengths with the nodes within 13 specified networks are also summed. Connectivity measures (13 per subject) will then be entered into second level repeated measures ANCOVA, with gender as between factor, network and session (dlPFC, vertex) as within-factors, and meanFD and age as a co-variates, testing the hypothesis of change in GBC by network. We will also test exploratory effects across all 13 networks (with alpha level set to 0.05/13=0.0038), and we will test for effects on GBC (connectivity between all nodes).

Primary outcome measure #3, rCBF at stimulation target

Analysis plan: The *pCASL images* will be realigned using SPM12 realignment routines and denoised using compCor(*90*) and then used to calculate the perfusion rate at each voxel using a

two-compartment model, assuming gray matter T1 of 1400 ms. and 90% labeling efficiency. ROI analysis of the CBF images will occur in native space, using the FPN mask derived from n-back activation, warped to individual anatomy. A two-sided, paired t-test will test for effect of sessions, with $p<0.017$ as alpha. Secondary analyses will be performed on 6 cm radius sphere at the site of stimulation, in addition to a whole brain, voxel-wise search for changes in gray-matter tissue, using topological FDR to correct for multiple comparisons to $p<0.017_{corr}$.

(H1.3) iTBS to dlPFC, compared to vertex stimulation, will increase resting perfusion at the site of stimulation. PET studies of high frequency TMS have reported increased CBF at the dlPFC,(*97*) a finding which motivates our hypothesis here. However, a cTBS study by our co-investigator (T.L.) also found *increased* CBF after supposedly inhibitory stimulation of dlPFC,(*98*) so we will be performing a two-tailed t-test here. While the primary analysis will focus on the site of stimulation, a secondary analysis will focus on the FPN network, defined by the n-back task.

Exploratory analysis, amplitude of low frequency fluctuations (ALFF): ALFF, and fractional ALFF (fALFF), measures the magnitude of low frequency BOLD fluctuations, generally from 0.01 to 0.1 Hz.(*99*) fALFF measures the fractional strength (relative to the full spectrum) of BOLD fluctuations. ALFF is correlated with CBF,(*100, 101*) so this measure provides an alternative to supplement the pCASL analysis. This is a potentially important measure for brain stimulation, as fALFF/ALFF abnormalities have been reported in psychiatric disorders,(*102*-*104*) and fALFF in the subgenual cingulate gyrus has been shown to predict response to electroconvulsive therapy.(*105*) This analysis will use the BOLD resting data as above, except that frame censoring (scrubbing) will not be performed, since censoring can affect the frequencies in a run of BOLD-acquired MRI data. Each voxel's BOLD time series will be transformed into the frequency domain and the mean amplitude of the spectrum over the frequency range of 0.01–0.1 Hz will determine ALFF. fALFF for two bands (slow-4; 0.027-0.073 Hz; and slow-5, 0.01-0.027 Hz) will be calculated as the magnitude for that band over the magnitude of the full spectrum. This analysis will focus on the 10 mm diameter sphere under the site of stimulation. Alpha level for this analysis will be set at 0.017 (0.05/3), two-tailed.

Exploratory analyses, between measures: Exploratory analyses will also test for correlations between the three MRI measures, to provide a richer picture of iTBS effects. Lastly, we recognize that vertex stimulation, here being used as a control condition for dlPFC stimulation, may have some effects on brain networks, although it was selected to avoid the FPN. With some caveats (effect of order, since baseline is not counter-balanced), we can use baseline measures from the first MRI session (no preceding TMS session), to see if iTBS differences represent a change in the dlPFC-stimulated condition or the vertex-stimulated condition. An additional exploratory analysis will compare stimulation over the 'activated target' (2-back minus 1-back) versus the 'deactivated target' (1-back minus 2-back), based on how the target was selected.

**Specific Aim 2:** ***Demonstrate modulation of the effect of dlPFC iTBS by a cognitive task***
**Primary outcome measures:**

To examine state-dependency and modulation of iTBS effects by task, we will contrast MRI measures following iTBS applied to the dlPFC while subjects perform the n-back with MRI measures following iTBS compared to when they are in an unconstrained, resting state. The general analysis plan will use the same work-up of the imaging data as described above, the only difference being the MRI sessions that are compared with each other. Although directions of change are difficult to predict, we are predicting increasing activity as has been reported for iTBS applied during a deep encoding memory paradigm and not observed during shallow-encoding.(*106*) However, it is possible that cortex may become more efficient and less activation may be seen. The following primary hypotheses will be tested:

Primary outcome #1, BOLD activation: 2-back minus 1-back BOLD activation, voxelwise in FPN
(H2.1) For dlPFC stimulation during performance of a n-back task, compared to dlPFC stimulation in a resting, unconstrained mental state, FPN activation to the n-back task will be greater. For this analysis, the region of interest will be the FPN taken from the activation mask, as described above. For a secondary measure to test H2.1, we will examine whole brain activation, since change may occur outside this region of interest.

Primary outcome measure #2, BOLD connectivity: FPN connectivity to dlPFC iTBS stimulation target
(H2.2) For dlPFC stimulation during performance of a n-back task, compared to dlPFC stimulation in a resting, unconstrained mental state, connectivity with the dlPFC site of stimulation will increase. For this analysis, the region of primary interest will be connectivity to site of stimulation.

Primary outcome measure #3, rCBF at stimulation target
(H2.3) For dlPFC stimulation during performance of a n-back task, compared to dlPFC stimulation in a resting, unconstrained mental state, resting perfusion at stimulation site will increase. The primary region of interest will be the site of stimulation, and secondary analyses will focus on the FPN, defined by n-back activation.

**Specific Aim 3:** ***Demonstrate improvement in cognitive control with iTBS, modulated by cognitive task during stimulation***

Primary outcome measure: accuracy to 2-back
Analysis plan: For analysis of n-back performance, our primary performance measure will be accuracy to the 2-back condition. Secondary measures will be d-prime, to capture the sensitivity to the target and not bias to respond, and median reaction time. Although we found significant effects on d-prime for our list learning paradigm, that memory task was very different from the more demanding n-back, which requires updating and manipulation in short-term storage, while our list learning task involved only incidental memory. For each measure, we will conduct a repeated measures ANCOVA, with the performance measure across the three iTBS conditions as the repeated measure, and sex as a fixed, between-subject factor and age as a co-variate. Post-hoc, paired t-tests will used for specific contrasts.

(H3.1) 2-back accuracy will be greater for dlPFC stimulation (no task during iTBS) relative to vertex stimulation
(H3.2) 2-back accuracy will be greater for dlPFC stimulation applied while subjects carry out an n-back during iTBS stimulation during stimulation, compared to when they receive stimulation alone. The same hypotheses will be applied to d-prime, our secondary measure. Two-sided tests will be used, because it is also possible that performance may worsen, either with stimulation alone, or when combined with subjects performing the n-back. It would be critical to know, for example, that performance of a cognitive control task was degraded by combining TMS with a task. Although a commercial device is being tested on dementia patients, relying on the assumption that the combination is beneficial,(*18*) we are not aware of this assumption being tested as we propose to do here.
Exploratory analyses around performance correlations: In addition to testing questions about performance, we will also examine correlations between performance and the MRI measures from Aims 1 and 2, testing important questions around mechanism. Since we will systematically test for correlations between both performance measures and the three primary measures from each aim (18 correlations), alpha will be set at 0.05/18 = 0.0028.

## 9.0 Ethics and Protectionn of Human Subjects

*9.1 Ethical Standard and IRB Review*

The investigator will ensure that this study is conducted in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research, as drafted by the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and/or the ICH E6; 62 Federal Regulations 25691 (1997) and the Declaration of Helsinki and Good Clinical Practice (GCP).

All protocol amendments must be IRB approved prior to implementing, except when change is for patient safety.

*9.2 Subject Confidentiality*

Study staff will make every effort to limit identifiable information on potential subjects during recruitment. The minimum amount of information will be recorded, and staff are alerted to the dangers of printing, faxing and emailing sensitive information. Phone conversations with potential research subjects will occur behind closed doors, and staff will ask callers if they are in a location where sensitive information can be discussed without danger of revealing confidential information. Any information gathered on subjects who prove ineligible will be destroyed as soon as possible (a list of patients who have declined or screened out will be maintained through the recruitment phase in order to avoid contacting these subjects again).

*9.3 Potential Risks and Minimization of Risks*

9.3.1 Potential Risks

*9.3.1.1 Issues around confidentially and the disclosure of sensitive information:*

Subjects will grant the experimenters access to protected health information. They will also reveal sensitive information about themselves, which will include their psychiatric status and any history of substance abuse. Subjects may feel uncomfortable revealing this information, and there is a risk of losing confidentiality. Subjects will also be informed in the consent document that confidentiality will be limited in cases where the subject reveals intentions to harm themselves or others, and the investigator feels that the proper authorities may need to be notified in order to prevent the occurrence of harm to the subject, or others.

*9.3.1.2 The risks associated with the MRI study are:*

1) A minor risk of discomfort or anxiety from being in the confined space of the MRI scanner.

2) Fast imaging sequences, such as those employed in this study, have the potential to induce peripheral nerve stimulation (PNS). PNS can be described as a light touching sensation on the skin surface and may cause mild discomfort but is not harmful to the subject.

3) Risks of hearing damage due to loud noises produced by the scanner.

4) 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.

5) 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 to the subject.

6) Sometimes, subjects report a temporary, slight dizziness or light-headedness when they come out of the scanner.

7) Potential risk for pregnant women: According to the NIMH Council Workgroup on MRI research and Practices (September, 2005), "there is no known risk of MR brain scanning of a pregnant woman to the developing fetus for scanning at 4T or less, and no known mechanism of potential risks under normal operating procedures." Nevertheless, subjects should be warned about potential risks, not yet discovered.

*9.3.1.3 The 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.

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.

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.

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." Thus, seizure risk, while not zero, is so low as to be difficult to estimate. Even with rTMS therapy for depression, patients are routinely treated on psychotropic medications, and the risk of seizure induction is estimated at 1/30,000 treatments.(*7*)

5) Syncope (rare, not serious): In the setting of altered sensory stimulation, subjects occasionally experience brief loss of consciousness, usually attributable to vasovagal syncope.

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

*9.3.2 Protections Against Risk*

*9.3.2.1 Confidentiality of data:*

Multiple steps are taken to ensure the confidentiality of sensitive information. Subjects are reminded that they do not have to answer questions that make them feel uncomfortable, which reinforces the voluntary nature of their participation. The confidentiality of all information gathered directly from subjects or obtained from the patient's medical records is assured by assigning records a coded research number and identifying all computer and paper files only by this code. A single tracking file will link the codes with subject identifiers, such as name and hospital number. In addition, consent forms and payment forms will be kept in a separate, locked room, apart from research data. All files will be kept in locked file drawers in locked rooms, to which only authorized research personnel have access. Electronic records will be kept on secure servers, and records with identifying information will be kept separate from records used to collect research data. Only staff members who have a need to know personal identifying information will have access to this information. All research staff are trained in research ethics and HIPAA regulations pertaining to protected health information. Screening forms for subjects who do not qualify for the study will be destroyed, except

for anonymous information unlinkable to the subjects (such as age, gender and education). After the completion of data analysis, the record linking subjects to the research codes will be destroyed, thereby anonymizing the data.

*9.3.2.2 Risks from MRI scanning:*

1) The risk of discomfort and anxiety will be minimized by custom pads and pillows to make the subject as comfortable as possible. The subject is allowed to communicate with the machine operator via an intercom and may trigger an audible alarm at any time. Before the subject rolls into the bore of the magnetic, he or she is always reminded that they are free to stop the study at any time if they become uncomfortable. If they were to experience an anxiety reaction, the study would be halted, and the participant would receive immediate counseling from staff, with option to meet with the P.I. or another study psychiatrist. Participants find that once outside of the scanner, they experience immediate relief of any anxiety and discomfort. If the study team has any doubts about relief of anxiety, follow-up telephone calls would be made later that day or 1-3 days after the session to confirm the transient nature of their reaction.

2) The MRI machine is operated within FDA guidelines so the potential for inducing PNS is low.

3) All subjects are required to wear foam earplugs to reduce the risk of hearing damage.

4) In the event of anomalous finding on MRI, the PI would contact the subject and explain that the subject should contact their primary care provider to obtain a clinical MRI scan. A protocol for managing incidental findings has been developed, and it includes the following:

I. *Discovery of a finding:* Incidental findings that arise in the course of assessment, such as an abnormal finding in the MRI, will be brought to the immediate attention of the PI.
II. *Gathering additional information:* The PI will review the finding and seek consultation with a neuroradiologist who consults for the fMRI laboratory.
III. *Informing the subject:* If the PI is available while the subject is being scanned and can assess the finding and make a determination about informing the subject, that will be done immediately. If the PI is not available for a face-to-face meeting with the subject, study staff are instructed to complete as much of the protocol as is reasonable, without revealing the existence of an anomaly. The intention here is to control the circumstances by which the subject is informed of the anomaly, making sure that the PI (a board certified psychiatrist) is the person who talks to the subject, can answer questions and gauge the emotional reaction of the subject to the news. The subject will be informed by the PI, personally, either through a phone call or a face-to-face meeting. While a face-to-face meeting is preferred, this may not be immediately convenient for the subject, and the PI must weigh the relative benefits of the more personal setting versus anxiety engendered by anticipating a meeting to discuss something the subject did not expect to hear.
IV. *Informing the subject's health care provider:* Arrangements will be made to provide a summary of the finding to the subject's personal physician, with the patient's permission. The information conveyed will recommend that a follow-up, clinical MRI be obtained to evaluate the incidental finding.
V. *Informing the local IRB:* The incidental finding will be reported to the IRB.

5) 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 around the materials that cannot be brought into the scanner room. The technician administering the scan is also trained to review each subject's MRI safety form to assess their suitability to enter the MR environment. Subjects are screened at multiple points. An initial phone screening is done, in which the MRI is explained, and subjects are excluded from participation if they have contraindications to an MRI scan. On the day of the scan, the subject completes an MRI safety screening form, which is reviewed by study personnel and reviewed by the MRI technician. Subjects are prompted at two points to remove all metallic objects from their person, soon after the subject arrives at the MRI center. They are given instructions to empty their pockets, remove jewelry, watches, wallets, and shown a storage box where belongings can be safely stored. Immediately before entering the scanner room, they are prompted again, to ensure that they have removed any items that might interfere with the san or interact with the magnetic field.

6) To minimize risks from nausea, subjects are carefully eased out of the scanner, and a technician guides the subject from the scanner bed, counseling them to rise slowly, ensuring they have adequate balance.

7) Subjects are informed that pregnancy, or the intention to become pregnant, are contra-indications to receiving a research MRI scan. At the initial phone screening, female subjects are asked about pregnancy or the possibility of pregnancy. When the subject is consented for the study, language in the informed consent document mentions this exclusion, again. All woman of child bearing age will be given an option for a urine pregnancy test the day of the fMRI scan, or they may decline this option and sign a form indicating that they do not believe they could be pregnant. Woman with a positive pregnancy test will not be permitted to continue in the study.

*9.3.2.3 Risks associated with TMS*

1) Local scalp pain near stimulation site (common, not serious): 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): 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): Earplugs will be used in this study in all subjects as a precautionary measure.

4) Risk of Seizure Induction (rare, serious): 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 from 2009 and updated in 2009,(*107*) as well as the safety guidelines for TMS protocols that are not repetitive in nature (e.g., single pulse studies). Oberman et al(*80*) reviewed the available TBS literature to date. Out of over 1,000 participants, there was only 1 incidence of seizure reported. This incidence occurred after 50 trains (10 seconds) of TBS to the primary motor cortex at an intensity of 100% of resting motor threshold. Therefore, all TBS procedures used will stimulate at an intensity less than this level. While TBS does use a high frequency of stimulation, it is for a much shorter period of time than other types of TMS (typically 40 s for cTBS, 190 s for iTBS, versus 20 minutes of 1 Hz rTMS). In experimental protocols, stimulation will be delivered at no greater than 80% of active

motor threshold. Maximal frequency of stimulation will be the standard 3 pulses delivered at 50 Hz bursts. Inter-train interval will be no less than 10 sec.

In the event of a seizure, subjects would receive counseling about the 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 the room where TMS will be delivered. The seizure protocol is as follows:

Protect from injury: Pull back coil apparatus, tilt chair back to flatten. 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 Huron Valley Ambulance – 994-4111. 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 preceeding 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) Syncope: Subjects are encouraged to be well-hydrated in advance of the sessions. During breaks and upon completion of the protocol, they are advised to rise slowly from the chair and observed carefully for any signs of fading consciousness.

6) For women of child-bearing potential: 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.

*9.4 Potential Benefits and Justification of Risk*

There are no direct benefits for the participants in this research. However, the proposed experiments should reveal important information about how TMS interacts with the brain, and this information may be used to design new or improve existing TMS treatments, which may benefit individuals suffering from neuropsychiatric disorders. The proposal will test the broad hypothesis that when TMS is applied to a brain in a controlled mental state, network changes induced by TMS will be facilitated, compared to stimulation when mental state is uncontrolled. Results from this study will be used to optimize TMS therapy for depression and other neuropsychiatric disorders by controlling mental state to improve the efficacy of TMS treatment.

## 10.0 Data Handling and Record Keeping

*10.1 Data handling*

The investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All source documents will be completed in a neat, legible manner to ensure accurate interpretation of data. Dark ink is required to ensure clarity of reproduced copies. When making changes or corrections, the original entry will be crossed with a single line, and initial and date the change. For some assessments, subjects may enter information directly onto a computer, rather than a paper form. Only research ID numbers will link these data with the subjects, and data will be stored in encrypted form on password-protected, secure computers. Systems that are only used to collect data, such as laptop or tablet computers, will also have research files securely deleted after upload to the central database.

Research data will be maintained on password protected computers, on a secure linux cluster maintained in the Psychiatry Affective Neuroimaging Laboratory (PANLab) or on secure UMHS-administered servers. Backup procedures include RAID drives with mirroring, and off-site backup.

Recruitment tracking files are kept only for the duration of recruitment. Paper records are kept in locked file drawers in a locked room, to which only authorized research personnel have access. Paper records with identifying information (consent form, payment records) are kept in locked file cabinets, physically separate from the research records. Computer records with identifying information are kept on secure, password protected servers. Electronic databases will be used to store the coded data, and the key linking to identifiable subject information will be kept separate from the database with research data.

*10.2 Plans to destroy links between research data and subjects.*

At the point where additional data collection becomes unlikely, the identifying links between subject identity and the research data will be destroyed.

*10.3 Data Sharing*

The value of this research is significantly enhanced by making the data available for the widest possible use, and because of the rapidly changing world of data analysis, it is impossible to predict what future research will do with data collected for any particular project in the present. By keeping the data indefinitely, it will become available for projects, yet to be determined. These include projects run by the investigators, as well as those outside the institution through data sharing plans or central repositories. The NIMH, which funds this study, requires that data be uploaded into the NIMH Data Archive (NDA), which is a requirement of receiving funding. When data are transferred to co-investigators and data repositories, de-identification will be performed, removing all HIPAA identifiers from the files. To prevent reconstruction of facial likenesses from structural image datasets, we will blur/obscure/strip the information from the MRI image around the face.

Subjects will be informed in the consent document that the data will be stored and that it may be shared with other investigators, both at the University and outside the University, through central databanks. The risk posed to subjects will be very, very small. Storing the data for an indefinite amount of time will follow strict protocols, outlined above, that will ensure the safety of the data and minimize the risk of accidental disclosure. Data that is shared or uploaded to central databanks will be de-identified and will be effectively anonymized, since investigators using these central databases will not have access to the keys linking individual subjects to the data. Furthermore, no data will be shared without an agreement by stewards of the central databank to require data use agreements and prevent investigators from even attempting to identify subjects. It is extremely unlikely that an investigator would even be able identify subjects from shared data, without already having access to a subject's identifiers.

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