Modulating interaction of motor learning networks in rehabilitation of stroke
NCT03086551
January 10th, 2018

SPECIFIC AIMS

Aim 1: Determine whether cTBS over contralesional dorsolateral prefrontal cortex prior to motor practice enhances motor learning post-stroke. Aim 1 will compare the efficacy of contralesional cTBS over dorsolateral prefrontal cortex paired with motor practice versus sham cTBS paired with practice for improving motor function.

Aim 2: Determine the effects of cTBS over dorsolateral prefrontal cortex upon motor networks. Our second aim is to determine the effect of our cTBS intervention upon local intracortical networks within primary motor cortex and across the motor network.

Aim 3: Provide preliminary evidence for individual predictors of the efficacy of dorsolateral prefrontal cortex cTBS post-stroke. Within our exploratory third aim, we will examine the relationship between pre-intervention behavioral and brain metrics and the responsiveness to dorsolateral prefrontal cortex cTBS.

RESEARCH STRATEGY

Design

Aims 1, 2 and 3 will be assessed in a single experiment. 16 individuals with first time middle cerebral artery (MCA) territory ischemic stroke will serve as their own matched control in a cross-over design. Each arm of the cross-over design will include the same 6 sessions (Figure 1). Each session within an arm of the cross-over design will be separated by at least 24 hours and the duration of each arm will not exceed three weeks. The only difference between the arms of the study will be the delivery active cTBS or sham cTBS over dorsolateral prefrontal cortex. The order of each arm will be counterbalanced. The four-session duration of the proposed intervention was informed by our past work pairing sensorimotor cortical stimulation with the same serial targeting task to be employed here. A three-week break between study arms will be employed to minimize carry over effects between each arm of the study. Therefore, total duration of the study from enrollment to completion

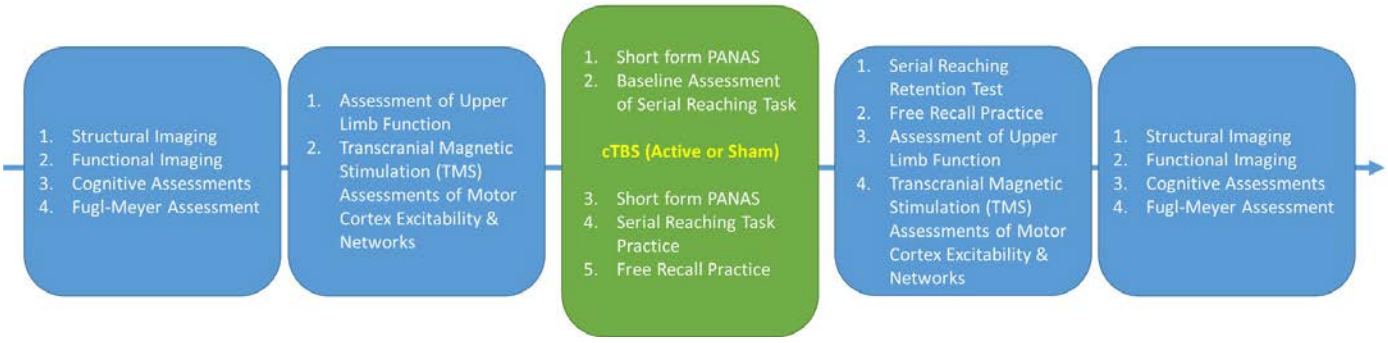

Figure 1 – Experimental procedure for one arm of the cross-over design. Both arms of the design will be similar. The only difference will be the NIBS intervention). A three-week break will separate Session 6 from Arm 1 to Session 1 of Arm 2. Sessions 1 and 6 are expected to take ~2.5 hours to complete. Sessions 2 thru 5 are expected to take ~1.25 hours to complete. SR task – serial reaching task. PANAS - Positive and Negative Affect Schedule

will be approximately 9 weeks (~3 weeks to complete arm 1, a 3 week break, ~3 weeks to complete arm 2).

***Inclusion Criteria*:** 1) Age between 40-75 years, 2) movement-related deficit associated with first time middle cerebral artery stroke, 3) greater than 3-months post-stroke, 4) Fugl-Meyer score between 15 and 66 and 5) ability to elicit a motor evoked potential from the ipsilesional cortex.

Exclusion Criteria: 1) a score <23 on the Mini-Mental Status Exam, 2) a history of seizure/epilepsy, head trauma, major psychiatric diagnosis, neurodegenerative disorder or substance abuse, 3) a history of congestive heart failure, 4) systolic blood pressure above 120 mmHg and/or diastolic pressure above 80 mmHg, 5) the taking of any GABAergic, NMDA-receptor antagonist or other drug known to influence the neural receptors that facilitate neural plasticity, 6) an infarct resulting from ischemic stroke of anterior or posterior cerebral artery OR an infarct that encroaches within 2cm of the site of cTBS stimulation, 7) absence of an MEP in response to single pulse transcranial magnetic stimulation over ipsilesional M1 and 8) any other contraindication to TMS.

Behavioral Test Battery: Serial reaching task**:** Motor performance will be indexed with a serial reaching task (Aim 1) (Figure 2). This task was chosen as: 1) it involves a reaching motion similar to that emphasized during rehabilitation, 2) we can quantify the effects of dorsolateral prefrontal cortex stimulation upon procedural knowledge tied to larger movement sequences in addition to sensorimotor control of simple reaching and 3) we can assess declarative knowledge about the practiced task. We developed and have previously published with this task confirming it can be performed and learned by our prospective stroke patient participants.

Patients will perform reaches with their paretic arm between sequentially presented targets using a touch screen. Each target will appear once patients have come to a complete stop within the target for 500ms.

Prior to each arm of the cross-over design patients will be informed of a repeated sequence of six targets that they will be required to reach to. Different sequences matched for difficulty will be used in each arm. Free recall will be performed prior to each session to assess any change in declarative knowledge.

During Sessions 2, 3, 4 and 5 of each cross-over arm participants will complete 30 reaches where the targets will occur in a random series. Participants will then complete a block of 60 reaches where the 6-element sequence will repeat 10 times. An additional block of 30 random reaches will then be completed. The pre-stimulation reaches will be used to assess sustained change in performance from session to session. This same set of 120 reaches will also be performed during Session 6 as a retention test.

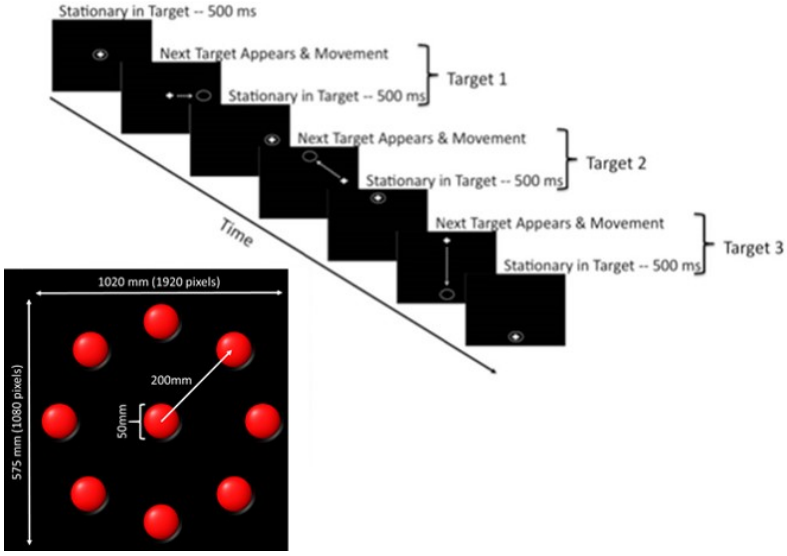

Figure 2 – Serial Targeting task. Participants will be seated in front of a touch screen angled on a desk in front of them and be asked to touch sequentially presented targets using their paretic limb. Targets will be presented sequentially at one of nine locations (inset bottom left).

For Sessions 2, 3, 4 & 5 participants will receive either active or sham cTBS over dorsolateral prefrontal cortex following the initial set of 120 reaches. Post-stimulation

participants will complete an additional 270 targeted reaches comprised solely of the 6-element repeating sequence.

The primary dependent measure for Aim 1 will be total response time to complete the sequence. As patients must stop within the target this metric captures both speed and accuracy. Comparison of response times during random reaches across session will be used to index sensorimotor procedural knowledge. Comparison of the difference between repeated and random reach response times will be used to index development of procedural knowledge relating to a larger sequence of movements.

Functional Assessment of the Paretic Limb: As secondary measures, the modified *Jebsen-Hand Function Test* and the *Box and Blocks* will be used to assess change in functional motor ability. The Jebsen-Hand Function Test is a valid and reliable test of hand function in healthy and individuals post-stroke and shows good responsiveness in chronic stroke to non-invasive brain stimulation interventions. We will employ a commonly used subset of the Jebsen-Hand Function Test: turning over cards, putting small objects in a can, mimicked spoon feeding, stacking checkers and moving weighted cans. To minimize learning effects each item will be tested 5 times but only the last two times will be used to quantify ability at each assessment. Scores for the paretic limb will be normalized to those of the non-paretic limb. The Box and Blocks test is a valid and reliable measure of gross manual dexterity. The Box and Blocks tests involves moving as many blocks from one side of a compartment over a partition to the other side in a 60-s window. The test is performed for both the paretic and non-paretic limb.

Attention, Spatial Working Memory, Verbal Working Memory: To comprehensively characterize our stroke groups we will use the Trail Making test to categorize visuomotor tracking and divided attention abilities. Where applicable, all participants will respond using their non-paretic limb.

TMS: TMS will be used to both quantify motor cortical excitability (Aim 2 - recruitment curves, short-latency afferent inhibition) and as an intervention (cTBS). TMS will be delivered using our own MagPro X100 with MagOption magnetic stimulator and a 90mm figure-8 coil (MCF-B70, MagVenture Inc.). All stimulation sites will be recorded using the BrainSight™ stereotactic guidance system to reduce variability within and across sessions.

TMS Assessments: Motor evoked potentials (MEPs) will be recorded from the paretic and non-paretic extensor carpi radialis using surface electromyography (PowerLab 8/30 and Bioamplifier, AD Instruments). The extensor carpi radialis was chosen because: 1) most patients with upper hemiparesis present with tonic wrist flexion and 2) the extensor carpi radialis is functionally relevant to the serial targeting task and chosen subtests of the Jebsen Hand Function Test. We have successfully stimulated the extensor carpi radialis in both healthy and clinical populations.

Single Pulse Recruitment Curves: Single-pulse recruitment curves will be used to assess motor cortical excitability. 70 monophasic TMS stimuli in posterior-anterior current direction ranging in intensity from 60% to 150% (15% increments) of resting motor threshold will be used to generate recruitment curves. Order of stimulation intensity will be randomized. Trials with pre-stimulus electromyography activity (100-ms prior to TMS) in excess of 15μV will be discarded and repeated at the end of the 70 trials.

Short Afferent Inhibition: Short-latency afferent inhibition will be used to index sensory projections to motor cortex. Short-latency afferent inhibition has been used to investigate the contributions of different intracortical M1 networks to motor learning . Pilot data from our group shows that directing attention to a high visual attention load task selectively alters short-latency afferent inhibition in the

motor network recruited by monophasic anterior-posterior current. This oligosynaptic motor network is the same network that has been linked to cerebellar mediated procedural learning and may be a prime substrate of declarative influence over procedural learning post-stroke.

Short-latency afferent inhibition within the ipsilesional motor cortex will be assessed using both anterior-posterior and posterior-anterior monophasic test stimuli over the extensor hotspot. 20 trials for each current direction will be preceded by median nerve stimulation (20ms interval, 3x sensory threshold) . Test stimulus intensity will be set to the intensity that elicits a motor evoked potential in the extensor carpi radialis of ~1mV for that current direction. An additional 20 trials for each current direction will be conducted in the absence of median nerve stimulation to serve as the unconditioned baseline. The same procedure will be repeated for contralesional motor cortex. In the event that sensory threshold cannot be established for the paretic limb median nerve stimulation intensity will be set to that of the non-paretic limb. In the event that a motor evoked potential of ~1mV cannot be evoked in the targeted limb stimulator output will be set to the minimal stimulator output at which extensor carpi radialis motor evoked potential amplitude stabilizes.

***Continuous Theta Burst Stimulation (cTBS)*:** cTBS (3 magnetic stimuli presented at 50 Hz every 200 milliseconds for 40 seconds) will be delivered over contralesional dorsolateral prefrontal cortex using the Brainsight™ stereotactic guidance system (Talairach coordinates 45 (or -45), 15, 38 (x,y,z)). The site of stimulation corresponds to the area of dorsolateral prefrontal cortex that we previously demonstrated to have greater blood oxygen level dependent response following implicit learning post-stroke. For active cTBS stimulation intensity will be set to 80% of active motor threshold for the non-paretic extensor carpi radialis. To monitor potential changes in affect associated with prefrontal stimulation the short form Positive and Negative Affect Schedule (PANAS) will be completed before and after cTBS during Sessions 2, 3 ,4 and 5.

Magnetic Resonance Imaging: Structural and resting state magnetic resonance imaging data will be acquired at the University of Michigan Functional Magnetic Resonance Imaging Laboratory on a 3.0 Tesla General Electric Discovery scanner using established sequences for anatomic and functional data acquisition.

Structural Magnetic Resonance Imaging: High resolution T1-weighted anatomical images (TR=8.9ms, TE=1.8ms, flip angle θ=15°, FOV=260, slice thickness=1.4mm, 124 slices) will be acquired during Session 1 and Session 6 of each study arm 1) for stereotactic guidance of the TMS coil, 2) to quantify lesion locations and volumes using the MRIcro region of interest tools, and 3) for co-registration of resting state data.

Resting State Functional Magnetic Resonance Imaging: T2*-weighted blood-oxygen level dependent scans (single-shot EPI, TR=2000ms, TE=30ms, flip angle θ = 90°, FOV=220x220mm, voxel dimension=3.4x3.4x3.2mm, 40 slices, 10 min/scan) will be acquired during Session 1 and Session 6 of each study arm. During these scans patients will lay supine with their eyes fixed on a white cross presented upon a black background. Temporal correlations in spontaneous metabolic demand between neuroanatomical regions in the resting state infer task-related functional connectivity in healthy individuals and those post-stroke . Reductions in resting state functional connectivity have been demonstrated in motor networks within and across hemisphere post-stroke (for a review see) and are predictive of rehabilitation outcomes . Therefore, we will use resting state functional connectivity to quantify changes neuronal networks underlying procedural learning (Aim 2) and whether pre-intervention resting state functional connectivity within the motor network may predict intervention outcomes (Aim 3).

Resting state data will be preprocessed as part of the standard processing stream at the University of Michigan that includes: removal of k-space outliers, field map corrections for inhomogeneity, removal of cardiac and respiratory rhythm, low pass filtering of data, slice time correction and motion correction (MCLIFRT, fMRIB software library) . Following pre-processing, the ARtifact detection tools (ART) toolbox will be used to identify global mean intensity outliers. An independent components analysis (Melodic, FSL) will be used to identify spatially distinct resting state networks including the motor, dorsal attention and default mode networks as well as any other networks that may be present.

Statistical Analyses and Power

The statistical approach and power analyses were developed in consultation with the Center for Statistical Consulting and Research at the University of Michigan. For Specific Aim 1 (**Determine whether cTBS over contralesional dorsolateral prefrontal cortex prior to motor practice enhances motor learning post-stroke**) the primary outcome metric will be response time during random reaches. The extent of sensorimotor procedural knowledge will be assessed using an Arm (active-cTBS, sham-cTBS) x Session (Session 2, Session 6) repeated measures ANOVA. The extent of procedural knowledge relating to the larger movement sequence will be assessed using a Arm (active-cTBS, sham-cTBS) x Sequence (Random, Repeated) x Session (Session 2, Session 6) repeated measures ANOVA. As a secondary assessment, the rate of procedural learning will be assessed for each measure by including Sessions 3, 4 and 5 as levels of the factor Session. An Arm (active-cTBS, sham-cTBS) x Session (Session 1, Session 6) repeated measures ANOVA will be used to assess time to complete the Jebsen-Hand Function Test

For Specific Aim 2 (**Determine the effects of cTBS over dorsolateral prefrontal cortex upon motor networks**) two separate sets of analyses will be performed. First, separate Arm (active-cTBS, sham-cTBS) x Hemisphere x Time (Session 1, Session 6) repeated measures ANOVA will be performed to assess change in intracortical motor networks (recruitment curves, short-latency afferent inhibition) across study arm. Motor evoked potential amplitude will be the dependent variable. Arm x Hemisphere x Time interactions will be decomposed using separate Arm x Time repeated measures ANOVAs for each Hemisphere.

Second, to assess changes in intercortical neural networks group level maps for each identified component in the independent component analysis will be assessed using separate Arm x Sequence random-effects analysis for Session 1 and Session 6. Statistical threshold will be set to $p<0.05$ and corrected for multiple comparisons using the false discovery rate.

Targeted enrollment for Aim 1 (Behavior) and Aim 2 (Motor networks) is 16 participants. This number was derived using effect sizes from our previous work. We observed effect sizes (partial η^2) for two-way repeated measures Stimulation Site x Session interactions of 0.135 (M1-cTBS vs. Control) and 0.107 (S1-cTBS vs. Control) when comparing response time from early practice to a delayed retention test. Brodie et al. observed effect sizes for motor and sensory improvements pre- to post an S1-5Hz repetitive TMS intervention that ranged between medium (partial $\eta^2 = 0.06$) to large (partial $\eta^2 > 0.16$). Based upon these effect sizes, the targeted enrollment that provides a power of 0.80 at an alpha level of 0.05 with a correlation of 0.7 among the repeated measures is between 5 and 12 participants (G*Power). These numbers are consistent with previously published work using similar cross-over designs to investigate the efficacy of M1 non-invasive brain stimulation interventions upon both behavior and M1 physiology post-stroke (i.e. recruitment curves and short-latency- afferent inhibition). In these studies sample sizes range from 6-16 stroke patients. To

account for effect sizes within the lower range of our estimates, overestimates of the correlations between the different levels in our repeated measures design as well as experimental mortality (see ***Potential Problems and Alternative Strategies***), we will target 16 individuals.

With respect to the measures of resting state functional connectivity (Aim 2), the majority of work post-stroke has been aimed at determining differences in network functional connectivity compared to non-stroke control individuals. To our knowledge, no studies have assessed changes in resting state connectivity across an intervention similar to that proposed here. The closest approximation is a longitudinal study looking at functional connectivity between ipsilesional and contralesional M1 post-stroke. This study (n=12 patients) demonstrated significant differences between 1, 3 and 6 months post-stroke. Therefore, 16 individuals should be sufficient to detect any changes in functional connectivity associated with our intervention.

To address our exploratory Aim 3 (**Provide preliminary evidence for individual predictors of the efficacy of dorsolateral prefrontal cortex cTBS post-stroke**), we will first perform separate Pearson Product Moment Correlations between change in response time from Session 1 to Session 6 and each of our predictive variables measured at Session 1 (Fugl-Meyer score, motor evoked potential laterality, ipsilesional short-latency afferent inhibition, resting state connectivity). As Aim 3 is exploratory we will use our predicted sample size of 16 from Aims 1 and 2 to identify predictors of interest for future work.

Potential Problems and Alternative Strategies

There are two potential methodological issues that have not been discussed thus far. The most prominent concern is that stimulation over contralesional dorsolateral prefrontal cortex prior to motor practice may compromise cognitive resources required to benefit from practice, regardless of declarative-procedural interactions. If cTBS over dorsolateral prefrontal cortex prior to practice compromises online learning we will move application of cTBS to the period of early consolidation immediately following all practice reaches. Consolidation of declarative and procedural knowledge is also competitive ; therefore suppression of dorsolateral prefrontal cortex during this early consolidation period has the potential to enhance procedural learning as well.

The second potential issue is experimental mortality. Of particular concern is the loss of patients who complete only one arm of our proposed cross-over design. Our choice of a three-week break in between study arms is a trade-off between Arm 1 effect dissipation and potential loss of contact with the patients. Our target enrollment of individuals will provide the option to adopt a partial cross-over design and a univariate mixed effect model statistical approach should experimental mortality between study arms occur.

Protection of Human Subjects

1. Human Subjects Involvement and Characteristics

Participants

16 individuals with chronic stroke will be recruited for the proposed studies. We will attempt to recruit equal numbers of men and women.

All potential participants will be screened with a health history questionnaire, neuropsychological assessments as well as transcranial magnetic stimulation (TMS) and magnetic resonance imaging (MRI) screening forms. The TMS safety screening questionnaire will be reviewed

by Dr. Stephan Taylor, Department of Psychiatry, University of Michigan Medical School, for contraindications. The MRI screening form will be reviewed by trained technicians at the University of Michigan functional MRI center.

Inclusion criteria

- Age between 40-75 years
- A score ≥23 on the Mini-Mental Status Exam
- Movement-related deficit associated with first time middle cerebral artery ischemic stroke
- >3-months post-stroke
- FM score between 15 and 66
- The presence of a motor evoked potential elicited by TMS over the ipsilesional cortex .

Exclusion Criteria:

- Age younger than 40 or older than 75 years
- A score <23 on the Mini-Mental Status Exam
- Primary infarct resulting from ischemic stroke of anterior or posterior cerebral artery OR infarct that encroaches within 2cm of the site of cTBS stimulation
- A history of seizure/epilepsy, head trauma, major psychiatric diagnosis, neurodegenerative disorder or substance abuse
- History of congestive heart failure
- Systolic blood pressure above 120 mmHg and/or diastolic pressure above 80 mmHg
- Taking of any GABAergic, NMDA-receptor antagonist or other drug known to influence the neural receptors that facilitate neural plasticity
- The absence of a motor evoked potential elicited from in the ipsilesional.
- Any other contraindication to TMS or MRI.

- Additional exclusion criteria related to anatomical and resting state MRI
  - Metallic medical implants (i.e. pacemaker)
  - Foreign objects in body
  - Non-removable body-piercings
  - Pregnancy
- Additional exclusion criteria related to TMS
  - Metal in the cranium (mouth excluded)
  - Cardiac pacemaker
  - Implanted medication pump
  - Implanted deep brain stimulator or vagus nerve stimulator
  - Intracardiac lines
  - Serious heart disease
  - Increased intracranial pressure
  - Pregnancy
  - History of seizures
  - Family history of seizures
  - Epileptogenic medication
  - Cochlear implants
  - Recent extended air travel resulting in jetlag or other sleep deprived state

Participants will be screened prior to each study session to determine any change in between sessions within a study arm and between study arms.

2. Potential Risks

In accordance with the classification of risk employed by the University of Michigan Medical School Institutional Review Board (IRBMED) the classification of risk is "***Minor increase over minimal risk***". This classification reflects both the probability and magnitude of harm associated with the theta burst stimulation protocol proposed. Dr. Meehan (former PI, current co-Investigator) and Dr. Taylor (Co-I) have successfully worked with IRBMED to develop procedures and accepted standards relating to theta burst stimulation since Dr. Meehan's arrival at the University of Michigan in 2011. Dr. Meehan has a history of IRBMED approved protocols using TMS and theta burst stimulation in healthy younger and older adults since 2011.

Below are the potential risks associated with theta burst stimulation. In brackets following each risk is the rate of occurrence and the designation of risk according to the standards used by IRBMED at the University of Michigan.

The risks associated with theta burst are:

Seizure (0.02%, rare): To date there has only been a single reported case of seizure during theta burst stimulation [82]. This case occurred in a healthy 33-year old male with no history of or risk factor for epilepsy. This individual had recently completed a transcontinental trip although they reported no signs of jetlag. The seizure occurred during the continuous variant of theta burst stimulation. Theta burst stimulation was delivered at 100% of resting motor threshold (or ~120% of active motor threshold). The convention is to deliver such protocols at 80% of active motor threshold.

Active motor threshold is defined as the percentage of stimulation output that elicits a motor evoked potential of 200 mV on 10 out of 20 trials while the participant maintains a contraction of 20% of maximum voluntary force . For the proposed work, active motor threshold will be defined according to this same standard. Theta burst stimulation will also be applied according to 80% of active motor threshold.

General pain, discomfort, (5% of participants, 1.1% of all sessions, infrequent)

- Mild headaches, infrequent (~2%)
- Non-specific discomfort (~0.5%)
- Mild discomfort caused by cutaneous sensation/neck muscle contraction (~0.5%)
- Worsening tinnitus in tinnitus patients (~0.3%)
- Light headedness or vagal responses (~1%)
- Nausea (~0.1%)
- Unilateral eye pain and excessive lacrimation during stimulation (~0.1%)

Finally, although there are no known risks associated with TMS to a developing baby, women who are pregnant will be excluded inline with precautions taken by other researchers. As stated there have been no reports of known risks however, given the risk benefit ratio women who are pregnant are generally excluded from participation. For the current study, if a woman answers "yes" to pregnancy during screening they will be excluded.

In addition to the risks associated with theta burst stimulation there are additional risks associated with Magnetic Resonance Imaging.

The risks associated with Magnetic Resonance Imaging are:

1. Discomfort: Participants, in particular older adults, may often experience discomfort as a result of having to lay in a fixed position for an extended period of time. Discomfort may be exacerbated by postural issues related to stroke.
2. Claustrophobia: Participants may experience a sense of panic associated with the confined space in the MRI scanner.
3. Hearing discomfort: The scanner is a loud environment. During data acquisition participants will hear loud knocking sounds that may cause discomfort.

3. Protection from Risks

Theta burst stimulation

The Brain Behavior Laboratory, where the TMS sessions will take place, has adopted the same procedures, formerly employed by the Human Sensorimotor Laboratory**,** to minimize the risks outlined above.

1. Screening procedures

First and foremost in reducing risk, is participant screening. The Brain Behavior Laboratory has adopted a TMS safety screening questionnaire modelled off of that proposed by Rossi et al. . This was developed in consultation with, and subsequently approved by, the University of Michigan's IRBMED.

The approved safety questionnaire is conducted by the participant during initial recruitment and prior to each study session. Our laboratory has traditionally been granted a waiver of informed consent by IRBMED to conduct this screening prior to the participant coming to the lab. This pre-screening is performed to minimize the probability of individuals travelling to the Brain Behavior Laboratory only to be excluded from participation. The pre-screening forms will be reviewed by Dr. Stephan Taylor (Co-Investigator) in the Department of Psychiatry, University of Michigan Medical School and provides the benchmark for all future safety screening. In the event of a change in screening testing will be delayed/re-scheduled in order to consult with Dr. Taylor to determine whether the participant would be allowed to continue participation in the study.

Of the risks associated with theta burst stimulation the risk with the most serious consequences is a seizure. With respect to seizures, the primary concerns with prospective participants are history of epilepsy, family history of epilepsy, neurological disorders and interactions between medication and TMS. History of epilepsy and family history of epilepsy are contraindications that result in exclusion of the participant. Neurological disorders, with the exception of stroke, are also an exclusionary criterion for the proposed work. Finally, drug interactions will not result in automatic exclusion. Dr. Taylor will assess the medications listed by the potential participant to determine if current or recent use of the indicated drugs (or interactions between drugs) may increase the risk of seizure.

Dr. Meehan, Dr. Taylor and IRBMED have worked together to establish a list of permissible medications. This list is reviewed regularly.

Medications that are permissible:

- Antihistamines

The most common medication reported in young individuals (as those that will be studied in the proposed activities) are anti-histamines. If an anti-histamine has been taken within 48-

hours of the scheduled testing session testing does not occur. The participant is not excluded from participation in the study but a minimum 48-hour delay in TMS testing is imposed from the time of screening provided the participant is willing to stay off anti-histamines for that duration.

- birth control pills
- Topical applications
- Low dose aspirin ('baby aspirin, or 81 mg dose)
- Fish oil (omega-3's)
- Coenzyme Q10
- Folate/folic acid
- Iron, vitamins, calcium supplements

Common medications that are permissible for older adults but will result in exclusion of younger adults

- Thyroid supplement (e. g. Synthyroid/levothyroxine)
- Statins (e.g. Lipitor/atorvastatin, Crestor/simvastatin, Pravachol/pravastatin)
- PPI/H2 blockers (e. g. Prilosec/omeprazole, Zantac/ranitidine)

Any other medications not on this list will be reviewed on a case by case basis.

2. All TMS protocols will follow established protocols where applicable.

All TMS protocols, including single pulse, short-latency afferent inhibition and theta burst stimulation will be performed according to accepted protocols and safety standards .

3. Training of Study Team Members

All study team members who administer TMS will be familiar with the International Society for Transcranial Stimulation (ISTS) "Consensus Statement on Managing the Risks of Transcranial Magnetic Stimulation". Specifically all participants will be familiar with the procedures for patient screening, the stimulation parameters for the proposed work, signs of potential adverse events during stimulation and first responder management in the event of a seizure.

4. Established and approved protocols in the event of an adverse event

The Brain Behavior Laboratory has a seizure protocol posted at multiple points in the laboratory. The adverse event protocol has been uploaded in Section 44 of the online form. All TMS testing is done with two laboratory members present.

Adverse events and withdrawals from study participation will be documented for reporting during dissemination of research activities. As part of the data safety monitoring plan, adverse events will be reviewed by Dr. Vesia, Dr. Meehan and Dr. Taylor to determine their association with the study protocol and assess any changes to the study protocol that may be required. Monitoring meetings will occur monthly even in the absence of an adverse event. An independent monitor will also be appointed to review study data and adverse events.

<u>Magnetic Resonance Imaging (MRI)</u>:

- Participants will be screened prior to entering the study. During screening participants will be informed that the presence of any metallic object in their body would prevent them from

participation (unless that objects can be removed, such as a piercing). The informed consent form repeats this inclusion criterion and an MRI screening form will be filled out immediately before entering the MRI scanner. This form is reviewed by a technician at the University of Michigan MRI center prior to the scanning session.

- Risk of discomfort associated with lying still during the short anatomical scans will be minimized by pads and pillows to make the subject as comfortable as possible. The participant is allowed to communicate with the machine operator via an intercom and will also be given a button to trigger an audible alarm at any time.
- Risk of claustrophobia will be minimized by screening and psychological preparation prior to scanning. These steps will help minimize the likelihood of distress.
- Hearing discomfort will be minimized by requiring participants to wear approved hearing protection supplied by the fMRI laboratory at the University of Michigan.

In addition to the steps taken above, participants will be informed of the risk of discovering something in their brain that would require an additional procedure/workup.

Confidentiality of Data

As outlined in Section 7 (**Sources of Research Materials**) subsection C (Access to Data and Data Sharing Practices and Policies) of the data management plan, no personal information or personal identifiers will be linked to any of the data. Personal information and identifiers will be stored in locked and secured facilities. Electronic copies of such information will be encrypted and stored on off-site servers. Access to these servers is restricted to approved laboratory personal through their unique username and password. Further, all University of Michigan researchers in the area of biomedical and health sciences are certified for research through the Program for Education and Evaluation in Responsible Research and Scholarship (PEERRS). Certification of completion of the PEERRS program is maintained in the laboratory to ensure all personal have completed the program. The Brain Behavior Laboratory has a full time research assistant who monitors all data collection, entry, and access to data collected in the laboratory.

4. Recruitment and Informed Consent

Participants will be recruited through the University of Michigan Health System, University of Michigan Honest Broker's Office, UMClinicalStudies.org (a web-based bulletin board used by the U of M Health Center to advertise for clinical studies) and local communities in southeastern Michigan and northwestern Ohio. All participants will be asked to read a comprehensive informed consent and given the opportunity to ask questions regarding the consent and the study. A waiver of consent will be required to perform initial screening prior to the arrival of the participant on the first day of the study. This initial pre-screening will be the only study activity conducted prior to obtaining informed consent. Prior to admittance into the study, participants must sign the informed consent document.

Participants will be paid a nominal sum for their participation.

5. Potential Benefits of the Proposed Research to Human Subjects and Others

It is possible that the individuals post-stroke who participate in this study may receive a direct benefit from participation. All participants will have the opportunity to participate in the "active" condition (cTBS over dorsolateral prefrontal cortex) as well as the sham control condition as part of the cross-over design. Participants may also receive indirect benefits from participation in the study in

general that includes an enhanced understanding of the neural basis of behavior, motor learning, neural plasticity and evaluations & strategies in human research. Further, participants may benefit indirectly from future application of our understanding of motor cortical neurophysiology and motor control post-stroke in the form of enhanced rehabilitative strategies for stroke-related movement disorders.

6. Importance of the knowledge to be gained

As outlined in the "Potential Risks" section the proposed work is designated as "*Minor increase over minimal risk"*. In contrast to the level of risk the benefits associated with understanding the role of dorsolateral prefrontal cortex to post-stroke recovery and the potential benefits of non-invasive brain stimulation to behavior and neurophysiology are great. This knowledge will provide a greater understanding of how to approach rehabilitation in general as well as adopt non-invasive brain stimulation as an effective adjunct to physical therapy. The proposed work is an important step towards developing evidence-based post-stroke interventions that promote fuller and quicker independence and re-establish quality of life.

7. Sources of Research Materials

The HSL has adopted a formal "Data Management Plan" outlined below to protect participant identities and ensure the prompt reporting of scientific results.

A. Types of Data, Samples and Other Materials to be Produced During the Course of the Project

Data collected will consist of 1) health history provided in response to the health history questionnaire, 2) scores from neuropsychological testing, 3) anatomical and resting state MRI scans, 4) electromyography data and TMS thresholds acquired during the TMS sessions, and 5) behavioral data stemming from measures of motor performance and learning. In addition to these data, data relating to participant screening and adverse events will also be recorded to ensure participant safety and accurate reporting of adverse events to promote enhancement of adopted scientific safety standards associated with the various techniques used as part of the research.

Our study team has considerable experience in using these techniques, processing acquired data using Labview and MATLAB, safe storage and dissemination of this data.

B. Data and Metadata Standards

The various data obtained during the course of the study will be stored in ascii and Excel formats. These formats have been chosen as they facilitate data sharing and offer a relatively simple method to store and retrieve data. Data dictionaries will also be generated to facilitate storage, retrieval, sharing and transfer of data management responsibilities that may occur during the course of the research. These data dictionaries will provide context and meaning to data elements, grouping and formats.

Metadata outlining experimental design, collection procedures, analysis methods and dissemination will also be stored. Metadata will be freely available through published articles, dissertation theses and web-based content.

C. Access to Data and Data Sharing Practices and Policies

All intellectual property and data generated through this project will be administered in accordance with University of Michigan procedures and NIH granting agency polices (http://grants.nih.gov/grants/policy/data_sharing). The PI does not anticipate any legal or ethical issues that may hinder or prevent the sharing of data.

Research outcomes will be made available through publication and presentation sources, subject to publisher subscription or printing charges. Experimental tasks, designs or methodological advancements will be made available for research use through publication and/or web-based material maintained by the Brain Behavior Laboratory (Director: Michael Vesia). Dissemination of research related materials will occur throughout the duration of the research plan as the information is validated internally and peer-reviewed. All data, experimental tasks, designs and methodological advancements for educational use will be made available through web-based material maintained by the Brain Behavior Laboratory.

To facilitate full access to all data generated as part of the proposed research a number of steps will be undertaken to protect participant anonymity. Data management will necessarily adhere to Federal Human Subjects Intuitional Review Board standards and the University of Michigan IRB. **All data will be encoded using participant codes. No personal information or personal identifiers will be linked to any of the data. Personal information and identifiers will be stored in locked and secured facilities.** Electronic copies of such information will be encrypted and stored on off-site servers. Access to these servers is restricted to approved laboratory personal through their unique username and password. Further, all University of Michigan researchers in the area of biomedical and health sciences are certified for research through the Program for Education and Evaluation in Responsible Research and Scholarship (PEERRS). **This includes any undergraduate students, graduate students, post-doctoral researchers and research assistants, who participate in research in the Brain Behavior Laboratory.** Certification of completion of the PEERRS program is maintained in the laboratory to ensure all personal have completed the program.

A full-time research assistant employed by the Brain Behavior Laboratory is currently responsible for monitoring all data collection, entry, and access to data collected in the laboratory.

D. Policies for Re-Use, Re-distribution and Production of Derivatives

The data generated from the current proposal will be of significant interest and benefit to a number of researchers and clinicians. Data will be made available only after data has been internally validated, relevant research hypotheses have been tested and study outcomes presented or published. In accordance with this policy it is likely that data will be made available no earlier than Year 2 of the proposed plan. All efforts will be made to accelerate this timeline provided scientific integrity is not compromised.

E. Data Archiving and Preservation

All raw and processed data, metadata, software (collection and analysis) will be archived locally on secured servers that are backed up at the University level on a daily basis. In addition to the dissemination strategies outlined above the Brain Behavior Laboratory will work with the Office of Research and Sponsored Projects (ORSP) at the University of Michigan to facilitate data archiving and preservation. ORSP is developing a Data Resource Sharing Center to support university researchers with data sharing and management (http://orsp.umich.edu/datasharing/).

Data and Safety Monitoring Plan

1. Plan for transcranial magnetic stimulation (including theta burst stimulation) monitoring

1.1 Subject Recruitment: All patients recruited will undergo repetitive rigorous screening to exclude individuals with an increased risk of developing a seizure or any other adverse reaction. The transcranial magnetic stimulation screening form to be used in the current study has been approved by the University of Michigan Medical School Institutional Review Board (IRBMED).

A waiver of informed consent will be used to perform screening over the phone prior to participant scheduling. The PI has successfully received a waiver of informed consent for other studies involving theta burst stimulation in younger and older healthy adults as well as stroke patients.

Screening results will be reviewed by Stephen Taylor, MD (Psychiatry) for suitability to participate. Stephen Taylor, MD is a Co-I on this application and has extensive experience using repetitive transcranial magnetic stimulation as a clinical intervention. Edward Claflin, MD (Consultant) will also be available to review screening forms if a second opinion is needed.

Participants will be re-screened for contraindications to transcranial magnetic stimulation prior to each session involving any transcranial magnetic stimulation activities to ensure no changes in status. Should any change be reported (i.e. a new medication) Dr. Taylor will re-evaluate the patient's risks and suitability for continued participation.

1.2 Training of all individuals who administer theta burst stimulation: All research personnel involved in the application of transcranial magnetic stimulation, including theta burst stimulation, will be familiar with published safety guidelines from the "Safety of TMS Consensus Group"[82]. Specifically, all personnel will be familiar with the procedures for patient screening for risk factors prior to participation as outlined in the informed consent document, the stimulation parameters to be used in this study, and first responder management in the event of a seizure. To facilitate first responder management our previously approved IRBMED approved adverse event protocols require two personnel be present at all times during stimulation regardless of study population.

1.3 Medical oversight of theta burst stimulation study protocol: In conjunction with the recommendations from the Safety of TMS Consensus Group[82] Michael Vesia, PhD is designated as the principal investigator for the proposed work. Co-Investigator Stephen Taylor, MD is designated as the medically responsible physician. Stephen Taylor, MD is a medically licensed physician in the School of Medicine at the University of Michigan. He has extensive experience in the use of transcranial magnetic stimulation as a clinical treatment for depression. As Co-Investigator he is intimately familiar with the study protocol and was involved in the protocol design. Dr. Taylor (or his designate) will review participant screening to determine suitability for participation.

1.4 Transcranial magnetic stimulation monitoring (including theta burst stimulation): All stimulation will be applied with at least two personnel present in the testing room at all times. Consistent with recommended guidelines[82] for studies applying theta burst stimulation over non-motor cortical areas electromyography of the first dorsal interosseous of both the paretic and non-paretic limbs will be monitored for spread of excitation from the stimulation site over dorsolateral prefrontal cortex. The first dorsal interosseous was chosen as this muscle has one of the lowest thresholds for production of motor evoked potentials. All participants will also be visually monitored throughout the application of continuous theta burst stimulation. Personnel will be trained to watch the participant for time-locked behavior time to stimuli. In addition to overt time-locked motor responses personnel will also be trained to identify the more subtle signs and symptoms of frontal lobe seizures.

1.5 Seizure protocol: The PI has an IRBMED approved seizure protocol in place that is universal for all transcranial magnetic stimulation studies. This protocol is posted in visible locations at various points in the room where transcranial magnetic stimulation will be delivered. The protocol entails

immediate activation of the Emergency Medical System (calling 911). Average EMS response times from the Ann Arbor Fire Department are 4.3 min. The site at which the transcranial magnetic stimulation protocol will be performed is less than 1 mile from the Emergency Department at the main University of Michigan Hospital and 1.1 miles from the nearest Ann Arbor Fire Department station. In the event that a seizure is not self-limited, the arrival of an EMS team should coincide with the appropriate time to administer therapy for status epilepticus, including anticonvulsant medications, EKG monitoring, and advanced life support protocols.

1.6 Adverse events arising in the course of participation: 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.

1.6.1 Serious adverse events will be reported to the IRBMED within 7 days of occurrence (or notification) of the event if they are definitely or possibly related to the study interventions. In the case of death occurring within 30 days of a study-related intervention, reporting to the IRBMED will occur within 7 days of the event (or notification of the event) regardless of relatedness to the study. For SAEs that are definitely not related to the study, reporting will occur at the time of scheduled continuation, per standard reporting timetable of the University of Michigan IRBMED.

1.6.2 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.

1.6.3 Adverse events not determined as serious will be graded as 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 adverse events will include evaluation in the emergency room not leading to hospitalization. Mild is defined as any non-serious event of less than moderate severity.

1.6.4 Moderate adverse events that are expected will be reported to the IRBMED at scheduled continuation review. Moderate adverse events that are unexpected and definitely or possibly related will be reported to the IRBMED within 14 days of occurrence. Moderate adverse events that are unexpected and definitely not related will be reported to the IRBMED at scheduled continuation review.

1.6.5 Expected, mild adverse events will not be reported to the IRBMED. Unexpected, mild AEs will be reported to the IRBMED at the scheduled continuation review.

1.7 Reporting responsibility: Michael Vesia, PhD (Primary Investigator) will have ultimate responsibility for event reporting. Michael Vesia, PhD will consult with Stephen Taylor, MD to determine the classification of the adverse event and draft the adverse event report. The study team will conduct regular meetings to discuss review the prevalence of expected mild adverse events. Moderate or severe adverse events, expected or not will trigger an immediate review of study procedures and adverse event response outside of these monthly meetings.

2. Incidental findings arising from assessment

The current protocol involves magnetic resonance imaging research scans. Incidental findings discovered in the course of a research scan have the potential to raise significant anxiety in subjects, while the nature of a research scan seriously limits the usefulness of the scan for resolving an incidental finding. At the first level, it is critical to make all participants aware of this risk. Another important consideration is to make subjects aware that they are receiving a research scan, which cannot be used to assess the clinical significance of any finding.

Standard operating procedure at the University of Michigan fMRI center does not entail reading of research MRI scans by neuroradiologists. Nevertheless, the experienced technicians and investigators collecting research data do encounter anomalous incidental findings, such as the presence of a large cyst or tumor. A protocol is in place to handle these events.

2.1: 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.

2.2 Gathering additional information: The PI will review the finding and seek consultation with Stephen Taylor, MD and others as appropriate.

2.3 Informing the subject: If the Stephen Taylor 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 Stephen Taylor, MD 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 Stephen Taylor, MD (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 Stephen Taylor, MD 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, Michael Vesia, PhD and Stephen Taylor, MD will 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.

2.4 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.

2.5 Informing the local IRB: The incidental finding will be reported to the IRB as an adverse event, with a gradation appropriate to the severity of the finding.

3. Procedures for dealing with suicidal thoughts, plans and intentions

Study personnel will be alert for the emergence of new suicidal thoughts amongst enrolled subjects during the course of participation. The PI will be promptly notified of any suspect comments or behaviors and consult with Stephen Taylor, MD (a board certified psychiatrist). Michael Vesia, PhD and Stephan Taylor, MD will formulate subsequent plans for additional emergency evaluation at the psychiatric emergency room (University of Michigan Psychiatric Emergency Services), if appropriate. Similarly, if the evaluation of a potential subject uncovers suicidal plans or intentions, the same procedures for emergency evaluation will be followed.