Protocol Title: Functional connectivity as a biomarker of rTMS

Abbreviated Title: rTMS / functional connectivity

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Human Research Protections Program Investigator and Staff Training:

For this protocol, the following "Just in time" human subjects protection training courses are required for investigators and staff:

- CITI GCP modules
- Unanticipated Problems and Reporting Requirements in Biomedical Research

Total requested accrual: 101
101 Healthy Volunteers

Project Uses Ionizing Radiation: **x** No □ Yes

IND/IDE **x** No □Yes

Durable Power of Attorney **x** No □ Yes

Multi-institutional Project **x** No □ Yes

Data and Safety Monitoring Board **x** No □Yes

Technology Transfer Agreement **x** No □ Yes

Samples are being stored **x** No □ Yes

Flesch-Kincaid reading level of consent form: 8 (for HVs)

PRÉCIS

Objective: To use resting state functional connectivity (FC) as a biomarker of synaptic modulation by repetitive transcranial magnetic stimulation (rTMS) in paradigms intended to improve memory and learning. Ancillary outcomes include the effects of rTMS on the interaction between the explicit implicit memory systems.

Study population: Healthy adult volunteers

Design: The study contains two experiments. Experiment 1 is designed to establish the number of rTMS sessions required to produce a meaningful change in resting parieto-hippocampal FC in healthy subjects. Experiment 2 will replicate a prior experiment which used rTMS to enhance the explicit memory system in healthy subjects, and look for potential effects on the implicit system. This intervention will be contrasted with a negative control condition (vertex stimulation) in a between-groups design.

Outcome measures: The primary outcome measures are the change in FC produced by serially applied rTMS and improvement in explicit memory. We will explore whether enhancement of the explicit system has effects on resting state connectivity in the implicit system and whether white matter integrity predicts changes in FC in healthy subjects.

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List of Abbreviations

rTMS	Repetitive Transcranial Magnetic Stimulation
fMRI	functional Magnetic Resonance Imaging
FC	Functional Connectivity
WPT-I	Weather Prediction Task Implicit Version
AMT	Associative Memory Task
DTI	Diffusion Tensor Imaging
FA	Fractional Anisotropy
ARSQ	Amsterdam Resting-State Questionnaire

1. Introduction and Background

Justification for non-significant risk (NSR) designation for conventional TMS studies within published guidelines and theta-burst rTMS at up to MEP threshold intensity
21CFR812.2 SR states that a significant device

1. *is intended as an implant and presents a potential for serious risk to the health, safety, or welfare of a subject*

   TMS is not an implantable device.

2. *is purported or represented to be for a use in supporting or sustaining human life and presents a potential for serious risk to the health, safety, or welfare of a subject*

   TMS is not for use in supporting or sustaining human life. It does not present a potential for serious risk to the health, safety, or welfare of participants when used as described in this protocol.

3. *is for a use of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and presents a potential for serious risk to the health, safety, or welfare of a subject*

   TMS, as used under this protocol is not of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health and does not present a potential for serious risk to the health, safety or welfare of a subject.

4. *otherwise presents a potential for serious risk to the health, safety or welfare of a subject*

   TMS and its repetitive form, rTMS have been in use for over three decades and have been cleared by the FDA for treatment of several disorders. Safety guidelines have been developed (Wassermann, 1998) and updated (Rossi, Hallett, Rossini, & Pascual-Leone, 2009) allowing its dissemination to a wide range of clinical and non-clinical settings. For example, brief, self-limited, seizures were seen in early studies, before limits were established for combinations of delivery parameters. However, this risk has been reduced to the order of one in every 40,000 sessions, including for paradigms (theta-burst stimulation) not included in the guidelines (Lerner, Wassermann, & Tamir, 2019). In the past 20 years, the FDA has generally waived pre-IDE inquiries for TMS/rTMS studies on an NSR device basis. Hence, the CNS IRB, like most US IRBs, has accepted NSR designation for TMS/rTMS studies within these limitations.

Description of device

No device is specified in this protocol. TMS is delivered using standard, commercially available, equipment and guided with a frameless stereotaxic system similar to that used for neurosurgical procedures. TMS devices are cleared by the FDA for a range of applications including deep nerve stimulation and the treatment of psychiatric disorders.

Reports of prior investigations with device
The use of TMS/rTMS for investigational and therapeutic purposes was pioneered in NINDS laboratories, work which has resulted in over 1,000 publications. Worldwide, it is in use in hundreds of laboratories. See references for relevant safety literature.

Investigational plan
The objective of this study is to use resting state functional connectivity (FC) as a biomarker of synaptic modulation by repetitive transcranial magnetic stimulation (rTMS) in paradigms intended to improve memory and learning. Ancillary outcomes include the effects of rTMS on the interaction between the explicit implicit memory systems.

The study contains two experiments. Experiment 1 is designed to establish the number of rTMS sessions required to produce a meaningful change in resting parieto-hippocampal FC in healthy subjects. Experiment 2 will replicate a prior experiment which used rTMS to enhance the explicit memory system in healthy subjects, and look for potential effects on the implicit system. This intervention will be contrasted with a negative control condition (vertex stimulation) in a between-groups design.

a. Background

Repetitive transcranial magnetic stimulation (rTMS) has been attempted as a treatment for cognitive deficits in a variety of disorders (Bonnì, Mastropasqua, Bozzali, Caltagirone, & Koch, 2013; Pachalska, Lukowicz, Kropotov, Herman-Sucharsaka, & Talar, 2011; Pape, et al., 2009). Two assumptions underlie nearly all such studies. The first of these is the notion that rTMS exerts its effects by increasing the excitability of local neurons. This came from classical observations on the effects of rTMS on MEP amplitude (the motor evoked potential), which increased after high frequency (Pascual-Leone, Valls-Solé, Wassermann, & Hallett, 1994) and decreased after low frequency (Chen, et al., 1997) stimulation. This phenomenon was likely mediated by changes in synapses on neurons located in the motor cortex (M1) at or near the stimulated site. Later attempts to influence other brain areas have generally assumed and relied on local effects and it is frequently observed that rTMS is only effective at modulating activity in superficially located areas within reach of the stimulating current. The other, related, assumption is that stimulation has been actually delivered effectively to the target region or network simply because the stimulating coil was placed in its vicinity. This premise often appears tautologically in post-hoc, mechanistic explanations of behavioral outcomes.

If rTMS were a drug, preclinical evidence of "target engagement" would be expected before interventional trials. For better or worse, however, the field of noninvasive brain stimulation has generally skipped this step. While jumping directly to clinical outcomes

may have saved time, reliable biomarkers could have been helpful by providing surrogate endpoints and better mechanistic understanding. The absence of biomarkers or similar surrogate endpoints has also made the dosing parameter space difficult to explore efficiently, since this would have to rely on behavioral endpoints in most cases. In this context, it is notable that, despite intensive research, the field has delivered only one widely adopted use whose several delivery parameters have never been optimized. Biomarkers of target engagement or surrogate endpoints with less variability than behavioral outcomes might have avoided some of the blind alleys.

Wang et al. (2014) have provided a paradigm, which may prove useful in solving these and other problems for noninvasive brain stimulation in the clinical domain. As a way to target the hippocampus, a deep structure beyond the reach of TMS, they used a conventional functional magnetic resonance imaging (fMRI) measurement, functional connectivity (FC), to find an individually customized, upstream cortical target, connected by a known monosynaptic pathway from the parietal cortex to the hippocampus. By individualizing the stimulation site using fMRI, Wang and colleagues eliminated the effect of inter-individual differences in anatomy, which can impact the results of a stimulation intervention (Karabanov, Chao, Paine, & Hallett, 2012). By applying rTMS repeatedly to this target, they caused an increase in memory performance and a correlated change in FC between the stimulated area and the area of interest deep in the brain. Incidentally, rTMS also caused dramatically increased connectivity in a network of sites related to visual memory and outside the stimulated pathway. Their work suggested that FC could be used as a marker of target engagement for noninvasive brain stimulation studies and, possibly, as an endpoint in clinical trials.

This protocol is an attempt to use FC as an endpoint, first in a dose finding study aimed at determining the number of rTMS sessions required to produce a meaningful degree of modulation in the pathway studied by Wang et al., then in an experiment involving a different pathway, and finally in a clinical trial in amnestic TBI patients.

This study is sponsored by the Center for Neuroscience and Regenerative Medicine (CNRM). To address the profound issues related to the diagnosis and treatment of TBI, the United States Congress, through Public Law 110–252, established the CNRM as a collaborative intramural program in May 2008. The CNRM is a collaborative intramural federal program involving the United States Department of Defense (DoD), the Uniformed Services University of Health Sciences (USUHS), and the National Institutes of Health (NIH) joining clinicians and scientists across disciplines to catalyze innovative approaches to TBI research.

b. Functional Connectivity as a stable biomarker

Functional connectivity is stable across scanning sessions within individuals (Barch, et al., 2013; Finn, et al., 2015). Data from 126 participants collected from the Human Connectome Project (Van Essen, et al., 2013) were used to demonstrate that participants' pattern of resting state functional activity can be reliably identified amongst sets of participants and across days (Finn, et al., 2015). For example, Finn and colleagues (2015) demonstrated that a machine classifier can identify whole brain patterns of resting

state functional connectivity across resting sessions with a success rate of at least 92.9% (see Figure 1). High test-retest reliability has been found in several studies (Biswal, et al., 2010; Shehzad, et al., 2009; Zuo, et al., 2010; Zuo, et al., 2010) and the temporal and spatial measures of resting-state networks are stable across years (Choe, et al., 2015). Thus, we are confident that FC is stable enough to use as a biomarker for the effects of rTMS in serial test sessions.

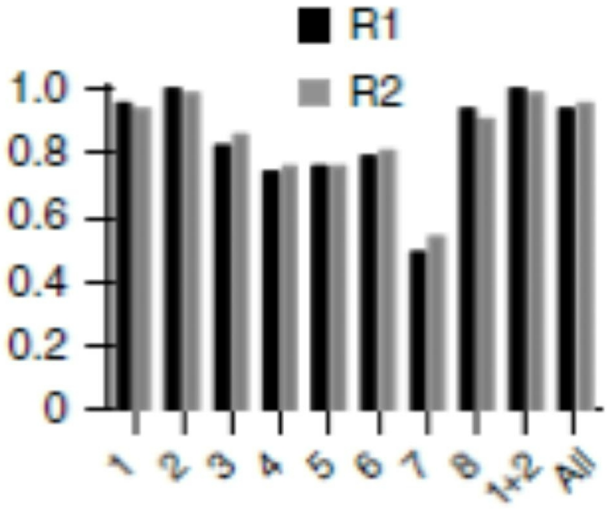

Figure 1. Results from Finn et al. (2015). Correct identification scores for machine classifier (y-axis) across eight different networks (x-axis) for FC patterns pulled from R1 and compared to the second session (R1; black bars) and for patterns pulled from the second session and compared to the first session (R2; gray bars). 1 = Medial Frontal, 2 = Frontoparietal, 3 = Default Mode, 4 = Subcortical-Cerebellum, 5 = Motor, 6 = Visual 1, 7 = Visual 2, 8 = Visual Association

c. Dose finding using continual reassessment method (CRM)

Wang and colleagues arbitrarily chose to deliver five sessions of rTMS. However, there are no empirical data on the duration of rTMS required to induce meaningful synaptic changes. Therefore, we will focus on the number of sessions as our rTMS parameter of interest while using the same stimulation parameters (e.g. frequency, intensity, duration) as Wang et al. (2014). We are choosing to focus on optimizing the number of sessions, rather than the other delivery parameters, because only one parameter can be explored at a time and reducing the number of sessions will have the greatest effect on the feasibility of this and future studies.

To find the minimum number of rTMS sessions required to produce a change in FC equivalent to that found by Wang et al., we will employ the continual reassessment method (CRM), a Bayesian, adaptive design, used to determine the dosing in Phase I drug trials. It is felt to be more efficient and accurate than simple dose escalation studies at estimating target drug doses (Garrett-Mayer, 2006). The parameter usually determined by this method is the minimum dosage expected to produce toxicity, or maximum tolerated dose (MTD). In this case, the MTD corresponds to the minimum number of sessions expected to produce the criterion change in FC. The logistical

function relating dose and effect and the MTD—the level where some predetermined proportion of subjects' experience "toxicity"— is initially assumed from prior knowledge and then adjusted dynamically, based on response data from small cohorts of subjects dosed at successively higher or lower levels as the MTD is approximated. Cohorts are run iteratively until either a pre-specified number of participants is run, or another stopping rule has been reached (see statistical analysis section).

d. Examining the effect of rTMS stimulation on implicit and explicit memory systems

Learning and memory rely on two primary systems, an "implicit" system, which supports learning by practice and experience under feedback conditions and an "explicit" system, which supports the storage of conscious knowledge. Implicit memory relies on parallel cortical-basal ganglia loops, which operate under the modulatory influence of catecholaminergic projections from the midbrain. The explicit system resides in the hippocampus, amygdala, and related areas. These systems may be in some degree of competition (Ashby & Maddox, 2011; Ashby & Maddox, 2005; Packard & Knowlton, 2002; Poldrack & Packard, 2003). As noted above, Wang et al. (2014) targeted the hippocampus and used a test of the explicit memory system as a behavioral outcome.

In our second experiment, we will use rTMS to increase connectivity in pathways an explicit memory task. We will target the parieto-hippocampal pathway, as Wang et al. (2014) did. We hypothesize that FC will increase only in the targeted pathways and that there will be a parallel dissociation in the effects on implicit and explicit memory. This will confirm our overall hypothesis that pathways involved in specific cognitive processes can be targeted selectively. We will also include a negative control condition (vertex stimulation) to control for sensory and other nonspecific effects of rTMS.

e. Measuring mentation during resting-state scans.

Recent work has revealed that mentation patterns during resting state scanning correlates with functional connectivity measurements (Gregory et al., 2016). Because differences in mentation between participants are difficult to control and could potentially explain our results, we have decided to include a measure of mentation (the Amsterdam Resting-State Questionnaire) as an exploratory measure to our protocol (see Appendix F).

f. Targeting the implicit learning system

The original version of this protocol included a dorsolateral prefrontal stimulation arm, the purpose of which was to attempt to enhance connectivity in the implicit learning system via fronto-striatal pathways. However, this proved infeasible, due to subject discomfort. This protocol now concentrates on the effects of parietal stimulation on FC and implicit and explicit learning.

g. Enhancing hippocampal network FC in TBI patients

We originally planned to pilot the paradigm for explicit memory system enhancement in amnestic TBI patients. This plan has been dropped because of time and resource constraints.

2. Study Objectives and hypotheses

The overall objective of this study is to determine whether functional connectivity can be used as a biomarker of rTMS synaptic modulation. The experiments outlined in this protocol will not be used to demonstrate the clinical effects of rTMS.

a. Aims

Experiment 1

Specific Aim 1: To determine the number of rTMS sessions required to produce a substantial change in FC between the parietal cortex and hippocampus in healthy subjects.

Experiment 2

Specific Aim 2: To determine whether rTMS delivered to the parietal cortex increases parietal-hippocampal FC in healthy subjects.

Specific Aim 3: To measure the effect of parietal rTMS on AMT and WPT-I, performance.

Specific Aim 4: To evaluate the relationship between fMRI connectivity and AMT and WPT-I outcomes after parietal rTMS in healthy subjects.

b. Hypotheses

i. Primary Hypotheses

Experiment 2: rTMS of the left parietal cortex will increase FC between the left parietal cortex and the hippocampus compared to rTMS of the vertex. rTMS of the left parietal cortex will significantly increase explicit memory compared to rTMS of the vertex.

ii. Secondary hypotheses:

Experiment 2: rTMS of the left parietal cortex will decrease FC between the left caudate (head) and parietal cortex compared to rTMS of the vertex. rTMS of the left parietal cortex will significantly decrease implicit memory compared to rTMS of the vertex.

3. Subjects

a. Description of study populations

We will study 50 healthy individuals (Experiment 1: 18; Experiment 2: 32). We are requesting up to 101 healthy volunteers as the accrual ceiling to account for dropouts and screening failures.

b. Inclusion criteria

Healthy individuals in Experiments 1 and 2.

- Age 18-50 (inclusive)
- English speaking and writing

c. Exclusion criteria

- Any current major neurological or psychiatric disorder such as (but not limited to) stroke, Parkinson disease, Alzheimer disease, schizophrenia or major depression
- History of seizure
- Medications acting on the central nervous system
- Ferromagnetic metal in the cranial cavity or eye, implanted neural stimulator, cochlear implant, or ocular foreign body
- Implanted cardiac pacemaker or auto-defibrillator or pump
- Non-removable body piercing
- Claustrophobia
- Inability to lie supine for 1 hour
- Pregnancy, nursing, or plans to become pregnant during the study.
- Members of the NINDS Behavioral Neurology Unit (BNU)
- For Experiment 2: Participation in Experiment 1

Eligibility Checklist: See Appendix B.

4. Study Design and Methods

a. Study overview

This study will include 2 experiments, each using rTMS and MRI. Experiment 1 will use parietal rTMS. Experiment 2 will involve parietal and vertex rTMS. Experiments 2 will have the same learning and memory testing.

Experiment 1 will require a maximum of 8 visits (contingent on the results of Experiment 1) and a maximum time commitment of about 13 hours.

Experiment 2 will require a maximum of 8 visits (contingent on the results of Experiment 1) and a maximum time commitment of about 15 hours.

b. Recruitment

Healthy participants will be recruited from the pool of individuals self-referring to the study directly and via the NIH Clinical Research Volunteer Program. Although NIH employees will be allowed to participate, no direct solicitation of employees/staff by supervisors or co-workers will take place. All recruitment material will be IRB approved. Participants who indicate interest will be pre-screened over the phone. Pre-screening questions will include the following questions (Appendix A):

Are you between 18 and 50 years of age?

Are you in good health?

Are you taking any medications?

Are you prone to seizures, stroke, or migraines?

Are you free of a history of significant neurological or psychiatric conditions?

Are you free of any metal in your body?

Are you comfortable doing a one-hour MRI?

Are you able to visit the lab for up to 8 visits in a two-week period, and up to 7 visits in a week?

Participants will not be invited to participate in more than one Experiment for this protocol. Thus, participants in Experiment 1 will not be invited to participate in Experiment 2.

It is likely that some participants will miss some sessions. If this occurs, and the participant is still willing to participate, we will reschedule. If the subject has already received rTMS, we will wait at least 30 days to reschedule the session so that the effects of stimulation wash out. If a participant misses a session after baseline measures have been collected, baseline measurements will be performed again (e.g. MRI, behavioral assessments).

The study is requesting a waiver of consent documentation to conduct phone pre-screening on potential subjects prior signing main research consent to verify eligibility.

c. Screening

Participants who pass initial screening will be invited to participate in the study and scheduled for consent and formal screening.

Upon arrival to the screening appointment, written, informed consent will be obtained by an investigator and formal screening will be done according to Appendix B.

Volunteers who have not had a neurological exam from an NINDS provider within the past two years will receive a neurological examination from an NINDS physician or nurse practitioner. This will not replace any exam the participant will receive for purposes of medical care; the exam will be for research purposes only. All women of child-bearing potential will have a urine pregnancy test (not earlier than 24 hours) before each MRI scan.

d. Study procedures

i. Behavioral tasks

Weather Prediction Task (Implicit version; WPT-I) - This is a test of the ability to learn an implicit, stochastic, association by trial and error and recruits a brain network including the dorsolateral prefrontal cortex and the head of the caudate nucleus, primarily in the right hemisphere. In the WPT, participants learn to predict a binary outcome, based on arbitrary stimuli with a hidden statistical link to that outcome. One, two, or three-card combinations of four possible cards are presented on a computer and the subject is asked to predict the "weather;" i.e. whether it will be rainy or fine. After each prediction, the subject receives corrective feedback. Each card is independently associated with one outcome with a fixed probability. For example, the probability of rainy might be 0.2 for squares, 0.4 for diamonds, 0.6 for circles, and 0.8 for triangles. We will use three different versions of the task at the three test sessions (see below). The task has 150 trials, with breaks after every 50 trials and takes approximately 17 minutes.

Associative Memory Task (AMT) – This is a test of the ability to learn and remember explicit associations between unrelated stimuli and involves the hippocampus and related visual processing areas. Participants are shown 20 face-name combinations (study phase) and are instructed to encode as many of these combinations as possible. Memory of the combinations is immediately assessed afterwards (test phase) by showing participants each individual face and asking them to recall the associated word. As with the WPT, we will use three different versions of the task at the three test sessions. The task takes approximately 10 minutes.

ii. MRI

1. MRI Anatomical scanning

All subjects will have anatomical (MPRAGE) scans before and after rTMS and diffusion tensor imaging at the beginning of the study. Participants who have not had one in the past year will receive a standard clinical MRI scan of the head, which will be submitted to the Diagnostic Radiology Department CC for interpretation. Depending on the requirement for a clinical scan, this phase will take 10-30 min.

2. Resting State Functional Connectivity

During scanning, participants will be instructed to lie motionless with open eyes fixated on a cross that is presented on a screen visible through a mirror attached to the MR head coil (approximately 20 min).

We estimate a maximum of 3 hours for the first scan in all experiments, and one hour for subsequent scans. Because scanner malfunctions and subsequent loss of data are common we will not report these as unexpected problems at the time of occurrence, but only at the time of continuing review. Subjects whose data are lost due to scanner malfunctions will be rescheduled, if possible.

MRI and its use in this research will be used per its FDA approved indication and specified parameters.

iii. rTMS

In all experiments, rTMS will be delivered at 100% of the motor evoked potential threshold and 20 Hz in trains lasting 2 s for 20 min with 28 second rests in-between trains (similar to Wang et al., 2014). If subjects are uncomfortable at 100% stimulation, the investigators will lower the stimulation by 5% increments up to 10% total decrease of stimulation. If subject is not comfortable at a 10% decrease, they will be withdrawn from the study.

The parietal target in all experiments will be the region of the left, lateral parietal area with the greatest connectivity with the left hippocampus derived from the baseline resting state fMRI session, as located using the targeting coordinates of Wang et al.

As a negative control, identical rTMS will be delivered at the vertex in Experiments 2 and 3. We justify the use of vertex stimulation as a control over sham stimulation as follows. A major criticism of sham procedures in rTMS neuromodulation studies is that conventional sham techniques do not reproduce the sensation of real rTMS on the scalp. This confound is so significant that basic validity of crossover designs has fallen into serious question. It is the consensus in the community that a more rigorous and scientifically valid procedure is to use real rTMS over a scalp site that is not thought to be part of the targeted network. This also serves as a control for nonspecific neural effects of the stimulation on the brain and further restricts the possibility of spurious or misinterpreted positive results (Sandrini et al., 2011; Ziemann et al., 2008).

Importantly, our paradigm is based on that of Wang and colleagues (2014) who used off-target stimulation as a control condition and found no significant effects on connectivity of behavior. Using an inactive sham that failed to replicate the sensory effects of rTMS would invalidate our study as a replication and lead to other questions about the source of any effects of active stimulation on connectivity or behavior.

rTMS targets will be marked in the participant's anatomical MRI volume and located with a frameless stereotaxic system. If any experiment, for any reason, fails to produce useful individual targets, a literature-based location for the parietal cortex (Wang, et al., 2014) will be used as our location of stimulation. In each experiment, rTMS will only delivered to the target in one hemisphere. To reduce the influence of diurnal variations in the responsiveness to neuroplasticity protocols (Sale, Ridding, & Nordstrom, 2008), we will make every effort to test subjects during the same time of day.

To allow participants the opportunity to experience rTMS at the parietal site and decide about continued participation, we will give them a "sample" before the first rTMS session. We will then ask the participant if they wish to continue the study.

For all experiments, FC and explicit/implicit memory will be measured within a week before the first rTMS session and immediately after the last rTMS session. Memory testing will be performed again 7-10 days after the last rTMS session. Experiment 1, however, will include no memory testing.

For all experiments rTMS and its use in this research will be used per its FDA approved indication and specified parameters.

iv. Cognitive Battery

All behavioral testing including the memory tests described above will take approximately three hours. Experiment 2 will include a 75-minute computerized battery that will be administered in parallel with pre- and post-stimulation assessments. The purpose of the battery is to detect effects on cognitive processes other than those targeted in the study. The data will be treated as exploratory. The battery includes the following tests:

1) Flanker Inhibitory Control and Attention Test (executive function and attention) - Participants respond to the direction of a target arrow while inhibiting attention to arrows flanking the target arrow (~3 minutes).

2) the Dimensional Change Card Sort Test (executive function and set-shifting) – Participants must match pictures based on one of two rules. These rules are transposed and participants' set-shifting abilities are measured by their accuracy and reaction time (~4 minutes).

3) the List Sorting Working Memory Test (for working memory) – Participants are given two lists of items (e.g. animals and foods) and are asked to list the items in size order. The task requires the participant to concurrently remember the items while sorting (< 10 minutes).

4) the Picture Sequence Memory Test (for story sequence memory) – Participants are given a list of objects and must remember the order of these objects in relation to each other. For example, if object A is listed in position X, the participant must recall what object is in position X+1 (~7 minutes).

5) the Oral Reading Recognition Test (for language) – The participant is asked to read individual words and the researcher records whether the word is read correctly (~3 minutes).

6) the Picture Vocabulary Test (for language) – Participants are aurally presented with a word and must match that word with one of four pictures that best represents the meaning of the word. The number of correct responses is recorded (~4 minutes).

7) the Pattern Comparison Processing Speed Test (for processing speed) – Participants are presented with two pictures and must determine whether they are the same or different. The number of correct responses in 90 seconds is recorded (~3 minutes).

8) the Georgia Complex Figures task (for visual memory) – Participants are asked to copy a complex drawing, and reproduce it from memory after a 20-minute delay. The number of successful elements of the complex figure are recorded (~ 5 minutes without delay).

9) the Category Fluency test (for category fluency) – Participants are given a category (e.g. animals, tools) and are asked to name as many category members in 60 seconds as possible (~ 2 minutes).

10) a verbal paired associates test (for verbal memory) – This test is similar to the AMT, but instead of new items used at each time point, the same pairs are retested at the end of the week of stimulation (~ 5 minutes).

11) the Everyday Memory Questionnaire (EMQ) (a subjective memory assessment) – Participants are asked 13 questions to assess their impression of their memory function. For each question, participants rate how often a particular memory problem has occurred, e.g. not being able to recall a word, having to check something that has already been done (~ 3 minutes).

12) Neuro-QOL forms: Depression, Fatigue, Sleep Disturbance, Cognition-General, Cognition-Executive (a subjective mental health assessment)– Participants report outcome measures through computer adaptive tests (CAT), short forms, or scales. (~ 20 minutes).

v. Amsterdam Resting-State Questionnaire

The ARSQ will be administered immediately after each resting-state scan and includes 50 questions related to patterns of mentation that could occur during resting-state scanning (see Appendix F). For each question, the participant is asked whether they agree or disagree with a statement related to their thoughts in the scanner. This questionnaire generally takes < 8 minutes to complete.

vii. Experiment 1 (Completed)

Experiment 1 will study only healthy volunteers. During the first day of experimentation, all participants will have an initial scanning session where anatomical, resting-state, and DTI images will be collected. This will be followed by 1-5 days of rTMS (20-minutes per day). Participants may schedule the first MRI session, and the first session of rTMS anytime within two days. On the day after the last rTMS session (within 12-36 hours), a final anatomical and resting-state-scan will be collected. Immediately following each

resting-state scan, we will administer the ASRQ. In this experiment, all participants will experience the same procedures, with the exception of the number of days where rTMS is administered (see Figure 2).

The search for the optimal number (1-5) of rTMS sessions will be performed using a continual reassessment method (CRM) design (O'Quigley, Pepe, & Fisher, 1990) (see Statistical Analysis section, below).

Participants will be run in cohorts of 3 (5 cohorts, for a total of 15 participants). Each cohort's FC results will be submitted to the CRM analysis, which will indicate how many rTMS sessions the next group will receive. For example, if Cohort 1 experiences a large increase in FC after 3 rTMS sessions (e.g. if the change is above the optimal cutoff point), the CRM will likely recommend a drop in the number of sessions for the next cohort. In this case, the CRM may recommend reducing the next cohort's sessions by 1 or 2. Alternatively, if a slight or no increase in FC is observed, the CRM will likely increase the number of sessions by 0, 1 or 2.

This procedure will be repeated until one of two stopping rules is achieved: 1) 15 participants have been tested, or 2) The CRM has recommended the same dose as the previous cohort 3 times in a row (see Figure 3). The first cohort will receive 3 sessions of rTMS. If the CRM does not reveal clear results by the end of data collection (15 subjects), we will default to 5 sessions of rTMS for Experiment 2.

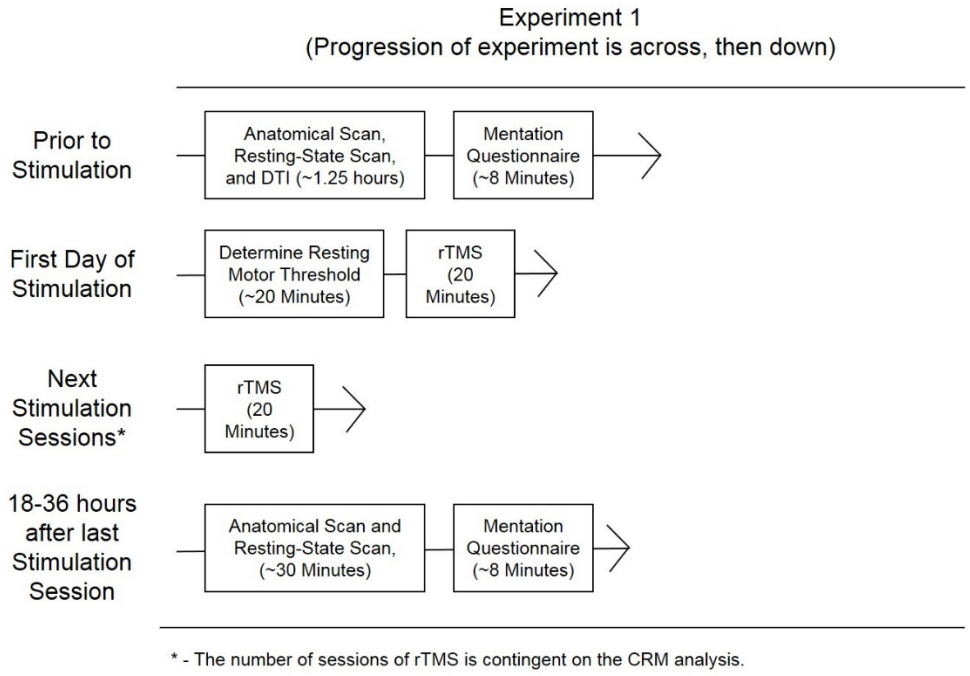

Figure 2. Procedures for Experiment 1. Time are estimates.

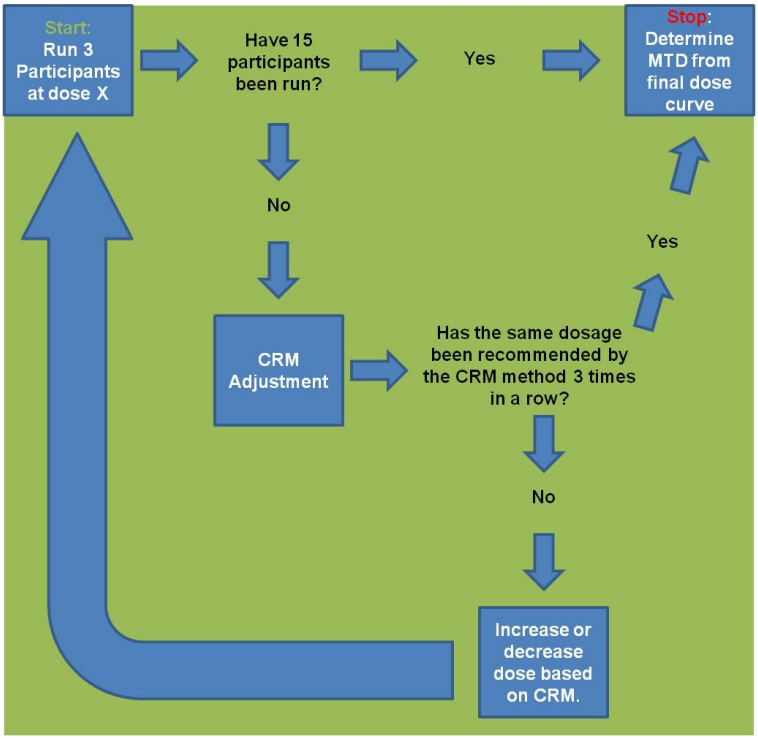

Figure 3. Flowchart of cohorts in Experiment 1. The experiment begins with an initial cohort at dose level 3 (3 sessions of rTMS). When a stopping rule has been met, the final MTD is determined from the CRM.

FC will be measured before and after each course of rTMS and we will obtain an anatomical scan in the first session. A timeline of each experiment is provided in Appendix C.

viii. Experiment 2

Experiment 2 will use a between-subjects design (see Figure 4) to study healthy volunteers. All participants will have an initial scanning session on the first day of the study where anatomical, resting-state, and DTI images will be collected. This will be followed by 1-5 days of rTMS (20-minutes per day). The number of days of rTMS will be derived from the results of Experiment 1. On the day after the last rTMS session (within 12-36 hours), a final anatomical and resting-state-scan will be collected. Immediately following each resting-state scan, we will administer the ASRQ. All participants will experience the same procedures, with the exception of the location of stimulation. Depending on group assignment, the participant will either receive stimulation to the parietal cortex, or vertex.

Contrary to Experiment 1, Experiment 2 will include behavioral testing at 3 different time points: 1) after the initial scanning session, 2) after the final scanning session, and 3) about a week after the last scanning session. This will include memory testing using the WPT-I and AMT, as well as the cognitive battery described above.

Subjects will be assigned to two groups (parietal and vertex), which will be balanced by age and sex. We will blind volunteers to stimulation type (group assignment) by recruiting participant who are naïve to the study hypothesis. Investigators scoring FC and behavioral data will be kept blind to rTMS assignment.

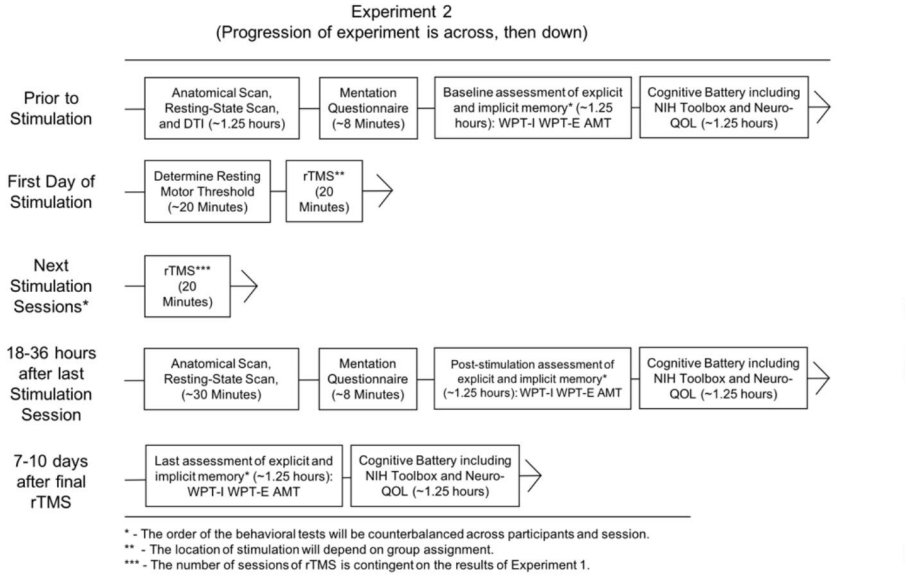

Figure 4. Procedures for Experiment 2. Times are estimates.

viiii. Duration of Study

Participation for healthy participants in Experiments 1 and 2 will be no more than 8 visits.

e. End of Participation

Volunteers will remain under the care of their own providers. No care will be offered to those participating in this protocol, except for any acute care required for adverse events. Findings of clinical significance, e.g., significant pathology on MRI will be shared with participants and any provider whom they designate.

5. Management of Data and Samples

i. Storage

The results of testing will be stored on password-protected computers or backed up on media stored in locked cabinets. Keys to participant identity will be stored in lab notebooks, available only to study investigators. Samples will not be stored under this protocol.

ii. Data and Sample Sharing Plan

This protocol is not subject to the Genomic Data Sharing (GDS) policy. The data will be shared at the time of publication, in response to a specific request to the PI. All data will be shared with the CNRM data repository and the Federal Interagency Traumatic Brain Injury Research (FITBIR) Informatics System upon study completion. Also, data may also be shared with collaborating laboratories at NIH or submitted to designated repositories and databases if consent for sharing was obtained.

All images will be transferred to a research *picture archiving and communication system* (PACS) located at the NIH Clinical Center. PHI will be removed, and a study ID identical to the CNRM Global Unique Identifier (GUID) described below. Imaging data will be processed and stored by the CNRM at the NIH Clinical Center.

The CNRM GUID is a number assigned by the CNRM Informatics Core. The Informatics Core has established an encrypted system and will provide access to the site for generation of a GUID), developed locally at each site, from personal health identifiers (PHI) data. Only the local site will have access to PHI. Local sites will maintain Master Keys matching GUIDs to PHI. Electronic/computer Master Keys will be kept on password protected terminal(s) in locked rooms with access limited to designated study personnel.

Electronic Master Key records will be backed up electronically at each site at least monthly. Physical print outs/copies of Master Keys will be kept in a locked cabinet in the office of a designated study investigator, and will be updated monthly or at more frequent intervals. The mapping from PHI to GUID will not be stored by or known to the

CNRM Informatics Core or NIH CIT personnel, but the central registration of issued GUIDs will help ensure uniformity of identifiers across sites and the ability to identify the enrolling site.

Subjects receiving follow up visits will retain initially-assigned GUIDs throughout their participation and all data will be stored linked to this GUID. Requirements or requests for subject future contact (re-identification) must pass through the enrolling site for GUID-PHI Master Key deciphering. CNRM Master Keys will contain the following information: GUID, last name, last 4 digits SSN, date of birth, and/or medical record number.

De-identified data will be stored in the CNRM Data Repository housed at NIH.

Data will be stripped of identifiers and may be coded ("de-identified") or unlinked from an identifying code ("anonymized"). When coded data is shared, the key to the code will not be provided to collaborators, but will remain at NIH. Data and samples may be shared with investigators and institutions with an FWA or operating under the Declaration of Helsinki (DoH) and reported at the time of continuing review. Sharing with investigators without an FWA or not operating under the DoH will be submitted for prospective IRB approval. Submissions to NIH-sponsored or supported databases and repositories will be reported at the time of Continuing Review. Submission to non-NIH sponsored or supported databases and repositories will be submitted for prospective IRB approval.

6. Additional Considerations

a. Research with investigational drugs or devices

- *N/A*

b. Gene therapy

- *N/A*

7. Risks and Discomforts

a. General

The behavioral tasks, neuroimaging procedures, and screening procedures are minimal risks to the participant.

b. Study Procedures

i. Behavioral measures

There are no major risks associated with these memory tests other than frustration or embarrassment associated with the participants' performance.

ii. MRI

People are at risk for injury from the MRI magnet if they have pacemakers or other implanted electrical devices, brain stimulators, some types of dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, implanted delivery pump, or shrapnel fragments. Welders and metal workers are also at risk for injury because of possible small metal fragments in the eye of which they may be unaware. People with fear of confined spaces may become anxious during an MRI. Those with back problems may have back pain or discomfort from lying in the scanner. The noise from the scanner is loud enough to damage hearing, especially in people who already have hearing loss. There are no known long-term risks of MRI scanning.

iii. TMS

There is a small possibility of a seizure during TMS. TMS has been found to produce hearing loss in experimental animals, caused by the click produced by the stimulating coil. However, no evidence of chronic hearing loss in several normal participants who had been extensively studied with TMS was found, nor transient changes in several participants tested before, and immediately after stimulation (Brasil-Neto, et al., 1992). All of our participants will wear earplugs to reduce the risk of cochlear damage. Other than this, TMS does not appear to pose any hazard to the brain beyond that of electric stimulation, which has been in clinical use for decades. The World Health Organization task group and the Food and Drug Administration concluded that brief exposure to static magnetic fields up to 2 Tesla have no adverse effects on human health).

c. Procedures to Minimize Risk

i. Behavioral measures

To minimize the risk associated with frustration or embarrassment, the researcher will maintain a positive attitude and observe the participants' behavior to determine if they are overly frustrated. Breaks will be encouraged if deemed necessary.

ii. MRI

To mitigate the risk of damage from associated with exposure to a powerful magnet, all magnetic objects (for example, watches, coins, jewelry, and credit cards) must be removed before entering the MRI scan room. In addition, participants will be screened for metal implants such as pacemakers or other implanted electrical devices, brain stimulators, some types of dental implants, aneurysm clips (metal clips on the wall of a large artery), metallic prostheses (including metal pins and rods, heart valves, and cochlear implants), permanent eyeliner, implanted delivery pump, or shrapnel fragments.

To minimize the risk of hearing damage, participants will be given earplugs or noise reducing headphones. To confirm that our female participants are not pregnant, thus removing any unknown risks of MRI on a fetus, women of childbearing potential will have a urine pregnancy test within 24 hours of participation in the fMRI experiment. Female participants will not be allowed to participate if the test is positive. There are no risks of pregnancy testing.

iii. TMS

To reduce the risk of a headache, migraine, or pain associated with rTMS, participants will be asked periodically if they are experiencing any of these symptoms, although typically these symptoms tend to subside after a short time away from rTMS.

To reduce the risks of hearing loss associated with rTMS, all subjects will be fitted with hearing protection.

8. Subject Safety Monitoring

For healthy subjects, a credentialed physician or nurse practitioner will be on site for all rTMS administration and immediately available. This is the standard of care in community and academic clinical and experimental rTMS centers, nationwide. Study staff will be trained in rTMS administration, rTMS safety, and the measurement of the motor evoked potential threshold and will have performed 10 measurements under supervision. Study staff will monitor individuals during participation and participants will be encouraged to tell experimenters of any discomfort. Any subject exhibiting distress or who wishes to stop the experiment for any reason will be allowed to stop. Participants may withdraw at any time. Data of those participants that have completed at least the first session of either Experiment 1-2 (i.e. both MRI scan and behavioral assessment) will be kept and analyzed. A researcher may end experimentation for the following reasons:

1) Abnormal response to rTMS including the occurrence of a seizure, loss of consciousness, or excessive pain or headache produced by rTMS.
2) Withdrawal of consent and/or decision to terminate.
3) Excessive frustration exhibited by participants on behavioral tasks.
4) Decision by legal proxy or patient guardian to terminate participation.
5) Subjects withdrawn due to high motor thresholds above the maximum capacity of the stimulator
6) Subjects withdrawn due to discomfort of TMS

9. Outcome Measures

a. Primary outcome measures

- Pre-to-post rTMS difference in FC between the left parietal cortex and the left hippocampus (Experiments 1-2) in healthy subjects (Experiments 1 and 2).
- Pre-to-post rTMS difference in AMT scores in healthy subjects (Experiment 2).

b. Secondary outcome measures

- Pre-to-post rTMS difference in WPT-I scores after rTMS in healthy subjects (Experiment 2).

c. Exploratory outcome measures

- Correlations between memory performance and white matter integrity (Experiment 2).

10. Statistical Analysis

a. Analysis of data/study outcomes

i. Experiment 1

Specific Aim 1: To determine the number of rTMS sessions required to produce a substantial change in FC between the parietal cortex and hippocampus in healthy subjects.

We will use the continual reassessment method (CRM) design (O'Quigley et al., 1990) to find the optimal number of sessions to produce a clinically meaningful change in fMRI connectivity between the parietal cortex and hippocampus in healthy subjects. The CRM is an adaptive design for dose finding studies, in which a "dose-toxicity" curve is fitted to the data and each patient will be assigned the dose most likely to be associated with the target toxicity level, designated as maximum tolerated dose (MTD) (Garrett-Mayer, 2006). It is important to note that while we retain the toxicity terminology from the CRM literature in the following description, we have adapted the technique to finding the threshold for efficacy, not toxicity.

In our study, the escalation decisions will be based on the efficacy of the rTMS. For the purpose of this study, the term dose will be used to mean the number of rTMS sessions. Similarly, the term toxicity will correspond to efficacy of the rTMS.

First, we used the data by Wang et al. (2014) to determine the threshold value for a clinically meaningful change in connectivity. The optimal cut-point for dichotomizing FC change was 0.028, which was determined by maximizing the Youden's index (sensitivity+specificity1) in the ROC analysis.

The details and assumptions to implement CRM are as follows.

- ***Dose limiting toxicity (DLT):*** We assume that a FC change equal to or greater than the threshold value 0.028 is an effective change. DLT will be defined as the FC change from baseline to post-stimulation ≥ 0.028

- ***Target toxicity level (TTL):*** The sensitivity for the dichotomized FC change (at 0.028) in the parietal simulation group was 87.50% in Wang et el. (2014). TTL is set to *12.5%,* which is equal to (1-sensitivity). *The MTD is then defined as the number of rTMS sessions that has a risk of DLT equal to the chosen TTL value of 12.5%.*

- ***Dose levels****:* The dose level refers to the number of consecutive rTMS sessions.

The range of sessions is 1 to 5.

- ***Number of healthy volunteers per dose level:*** 3 healthy volunteers per dose level

- ***Dose-toxicity model:*** We propose to use a one-parameter hyperbolic-tangent dose-response model by O'Quigley et al. (1990), where a Gamma (1, 1) prior will be used.

- ***Starting dose:*** 3 sessions

- ***Stopping rule****:* Healthy volunteers will continue to be recruited to the trial until a fixed sample size of 15 is achieved.

The following aims will be investigated using the optimal sessions determined in Specific Aim1.

ii. Experiment 2

Specific Aim 2 (Primary Aim 1): To determine whether rTMS delivered to the parietal cortex increases parietal-hippocampal FC in healthy subjects.

For each healthy subject, the Pearson correlation coefficients (r) between time courses of BOLD activity in the parietal and hippocampal areas will be calculated to determine the functional connectivity before and after rTMS stimulation. They will be Fisher-transformed to normalize r values. *We will call this the z scores.* The change in the z scores from pre- to post- stimulation will be assessed using the one-tailed, paired *t* test.

Specific Aim 3: To measure the effects of parietal rTMS on AMT and WPT-I.

First, the change in the AMT scores from pre- to post- stimulation will be calculated. Then, the two sample t-tests will be used to compare the changes in the AMT scores between the parietal and the sham groups. Additionally, we will consider using the Wilcoxon rank sum tests when the changes in the AMT scores are not normally distributed in each group.

Next, we will perform a similar analysis for WPT-I scores.

Specific Aim 4: To evaluate the relationship between fMRI connectivity and AMT and WPT-I outcomes in the parietal group in healthy subjects.

For each subject, the Pearson correlation coefficients (r) between time courses of the parietal and hippocampal regions will be calculated to determine the functional connectivity before and after rTMS stimulation. Then, the changes of the z scores (Fisher transformed r values) from pre- to post- simulation will be calculated. Similarly, the changes in AMT scores from pre- to post-simulation will be computed. The linear

regression models will be used to examine the relationship between the change in functional connectivity and the change in AMT scores.

Next, we will repeat a similar analysis using WPT-I scores.

b. Power analysis

Experiment 1: The maximum number of healthy subjects for Experiment 1 is set at 15.

Experiment 2: We propose to recruit 32 healthy participants (16 subjects in each of the parietal and control groups) to participate in Experiment 2.

We based our sample size estimate on the behavioral results from the study we are replicating (Wang et al., 2014). In Wang et al. (2014), the effect size of the memory increases caused by PPC stimulation, compared to sham stimulation, was 0.75 (Cohen's d). This was achieved using a within-subjects design. Using this effect size, to achieve a ß of 0.70 using the between subjects design described in this protocol (Experiment 2), 16 participants are required.

Sixteen subjects per group also achieves sufficient power to to detect FC changes. Wang et al. indicate that the mean difference in FC from post to pre stimulation in retrosplenial region was 0.1018 and its standard deviation was 0.09296. Based on their data, we assume that the mean change will be between 0.1008 and 0.1028. We also assumed its standard deviation (SD) will be between 0.085 and 0.10. A one-tailed, paired t-test was used at a significance level of 0.05. Computations were carried out using PASS (Hinze J. [2008] PASS, NCSS, LLC, Kaysville, Utah).

The following table shows estimates of power to address Primary Aim 1 given ten subjects per treatment group.

Mean of paired difference	Estimated standard deviation of the difference	Power
0.1008	0.085	0.96440
0.1018	0.085	0.96698
0.1028	0.085	0.96940
0.1008	0.093	0.93446
0.1018	0.093	*0.93833*

0.1028	0.093	0.94202
0.1008	0.100	0.90159
0.1018	0.100	0.90651
0.1028	0.100	0.91125

Table 1. Estimated power for primary aim 1 (N=10 in parietal group)

A sample size of ten subjects per group achieves 93.8% power to detect a mean of paired differences of 0.1018 with an estimated standard deviation of differences of 0.093 and with a significance level of 5% using a one-sided paired t-test. Under other scenarios, the estimated powers were greater than 90%.

Assuming a 50% dropout rate, we anticipate enrolling up to 101 healthy subjects (33 for Experiment 1; 64 for Experiment 2).

11. Human Subjects Protection

a. Subject selection

We will recruit healthy participants through referrals from the NIH Clinical Research Volunteer program or through self-referrals to the protocol. We will work to ensure equitable selection.

b. Justification for exclusion of subjects

i. Justification for exclusion of children

Children will not be included. The study is predicated on the work of Wang et al. (2014) who studied participants over the age of 18. Therefore, in order to replicate this result, we will recruit a sample of the same age.

Because we intend to compare the results of Experiments 1 and 2 and because we will be attempting to replicate Wang et al. (2014), we will focus our analysis on subjects between the ages of 18-50.

ii. Justification for exclusion of subjects above the age of 50

Because neuroplasticity is reduced in older adults (Fathi, et al., 2010) including subjects above the age of 50 would reduce our power for detecting change in FC. Therefore, this group will be excluded.

iii. Justification for the exclusion of non-English speakers/readers

We are excluding non-English speakers because differences in the ability to understand the task instructions might not be apparent to us when communicating via an interpreter,

but could affect performance significantly, introducing unpredictable differences between groups of subjects.

iv. Justification for participants who have already participated in Experiment 1.

Because the persistence of the rTMS effect is unknown, participants from Experiment 1 will not be invited to participate in Experiment 2.

c. Justification for the Exclusion of other Vulnerable Subjects

i. Persons without consent capacity

Because there is a minor increment above minimal risk and no expectation of benefit, we do not feel it would be appropriate to include participants without the capacity to give informed consent.

ii. Women who are Pregnant, Plan to Become Pregnant, or are Breast-feeding

The effects of MRI on fetal development and the health of pregnant women is unknown. Therefore, women who are pregnant will be excluded and women who can become pregnant will be excluded following a positive pregnancy test.

d. Justification of sensitive procedures

This study involves no sensitive procedures.

e. Safeguards for vulnerable populations

Since the effects of rTMS and MRI on fetal development are unknown, women of childbearing potential will have a pregnancy test before each rTMS and MRI session.

i. Safeguard of vulnerable subjects (NIH employees)

Protections for employees and staff participating in this study include 1) assuring that the participation or refusal to participate will have no effect, either beneficial or adverse, on the subject's employment or position at the NIH, 2) giving employees and staff who are interested in participating the "NIH Information Sheet on Employee Research Participation" prior to obtaining consent, and 3) assuring that there will be no direct solicitation of employees or staff.

Consent will not be obtained by a co-worker. We will only enroll NIH employees and staff when they are not members of the Behavioral Neurology Unit in the National Institute of Neurological Disorders and Stroke. This is the laboratory in which the research described in this protocol will occur. NIH employees and staff who participate during work hours must have permission from their supervisor. NIH employees and staff must either participate outside of work hours or take leave in order to receive compensation. The last stipulation does not apply to the home-monitoring period. Women of childbearing potential will have a pregnancy test, which must be negative before proceeding

12. Anticipated Benefit

This study does not offer direct benefit to participants but is likely to yield generalizable knowledge to the understanding of the effect of rTMS on FC in healthy adults.

13. Consent Documents and Process

a. Designation of Those Obtaining Consent

Study investigators designated as able to obtain consent are noted in the Study Personnel Document. All study investigators obtaining informed consent have or will complete the 'Elements of Successful Informed Consent' training prior to experimentation.

b. Consent Procedures

All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures and potential risks of the study and of their rights as research participants. Participants will have the opportunity to review the written consent form carefully and ask questions regarding this study before signing.

Consent will not be obtained by a co-worker.

c. Consent Documents

The consent form contains all required elements. Healthy Volunteer consent forms are submitted with this protocol.

14. Data and Safety Monitoring

a. Data and safety monitor

Data and safety will be regularly monitored by the Principal investigator. Dr Leonardo Cohen, a neurologist and an intramural NINDS investigator, will serve as the Independent Medical Monitor for this protocol.

b. Data and safety monitoring plan

Protocol investigators will evaluate the safety of study subjects throughout the conduct of the study and respond to adverse events (AEs) in a timely manner. The IMM will be informed of serious adverse events within 7 days and sent a summary of adverse events at the time of each annual review

If no interval data were collected, the monitor will be informed and a report will not be required. The IMM will also be sent protocol updates and other pertinent documents relating to the study on an as-needed basis. The IMM may also be consulted in person and as needed to discuss clinical issues. In person consultations with the IMM will be documented.

c. Criteria for stopping the study or suspending enrollment or procedures

If, in the judgment of the PI or IMM, a study procedure is causing frequent unexpected or adverse outcomes, that procedure will be suspended until a review can be undertaken in consultation with the IRB. Depending on that consultation, the procedure may be dropped from the protocol via an

amendment, or specific language may be added to the protocol and consent forms to reflect the changing risk level.

15. Quality Assurance (QA)

a. Quality assurance monitor

The CNRM will monitor the protocol:

1) After 5 subjects have completed participation in the study.
2) Every 6 months after the first five subjects have completed participation in the study
3) At the close of the protocol.

b. Quality assurance plan

The protocol will be monitored by CNRM for regulatory compliance and data quality in accordance with the established monitoring plan. The NINDS QA office will review the CNRM monitoring reports, but will not provide additional audits, unless deemed indicated by the findings of the CNRM monitor.

16. Reporting of Unanticipated Problems, Adverse Events and Protocol Deviations

Reportable events will be tracked and submitted to the IRB as outlined in Policy 801.

17. Alternatives to Participation

Participants do not receive rTMS in this study or forego any treatment in order to participate in this study. The alternative, therefore, is not to participate.

18. Privacy

All research activities will be conducted in as private a setting as possible.

19. Confidentiality

a. For research data and investigator medical records

All study investigators will have access to research records and data. Hard copy research data/records will be coded, no individual will be identified by name, and the data will be stored in a locked filing cabinet locked to protect subject anonymity. Electronic data with identifiers (including neuroimaging) will be saved password-protected NIH-issued computers on secured servers. Neuroimaging data will be maintained on a secure internet-based server. Only study investigators will have access to the data. De-identified results from clinical trials will be posted on cctrials.gov.

Clinical data will be managed according to NIH Clinical Center's standard policies (http://www.cc.nih.gov/participate/patientinfo/legal.shtml). Sensitive, private information (such as a urine drug test) will not be collected in this study, so no special protections for NIH employees and staff are necessary. However, confidentiality protections for them will be the same as those for all subjects. The PI will instruct all study personnel in the relevant SOPS and procedures to ensure the privacy of NIH employees and staff who participate in our study. All investigators will be required to read the SOP on participation of NIH personnel.

Participant research data will be de-identified and stored on secure computer systems. The only entries in the medical record will be to document participation in the research study. In laboratory records, all personally-identifying information will be removed. Participants will be identified by a number code, the key to which will be accessible only to the investigators. The information gathered during this study will be kept confidential to the extent that the law allows. The lab results will be kept safe in a locked room. The subjects will be informed that these results may be published for scientific purposes, provided their identity is not revealed.

Members of the CNRM, Uniformed Services University, Henry M Jackson Foundation, US Department of Defense and NIH, may have access to the study data for auditing purposes.

b. For medical records

Clinical data will be managed according to NIH's Clinical Center's policy (http://www.cc.nih.gov/participate/patientinfo/legal.shtml).

20. Conflict of Interest

a. Distribution of NIH Guidelines

NIH guidelines on conflict of interest have been distributed to all investigators.

b. Conflict of Interests

There are no conflicts-of-interest to report.

c. Role of a Commercial Company or Sponsor

There is no commercial company or sponsor.

21. Technology Transfer

N/A

22. Research and Travel Compensation

All participants will be compensated for time and research-related inconveniences in accord with NIH guidelines as follows:

Experiment 1	**Number**	**Pay per Procedure**	**Total**
Time for first hour	2-7	$20	40-160
Time for each additional hour	1-3	$10	30
rTMS	1-5	$60	60-300
MRI	2	$60	120
Urine pregnancy test	2-6	$10	20-60
Experiments 1 Total			**$250-650**
Experiment 2			
Time for first hour	3-8	$20	80-160
Time for each additional hour	6-11	$10	60
rTMS	1-5	$60	60-300
MRI	2	$60	120
Behavioral Tasks	3	$30	90
Urine pregnancy test	2-6	$10	20
Experiment 2 Total			**$250-840**

Payment (check) will be mailed to participants after they complete the protocol. If participants are unable to finish the study, they will be paid for the portion of the study completed. No reimbursement for travel or escort fee will be provided.

Employees and staff who participate during work hours must have permission from their supervisor. NIH employees must either participate outside of work hours or take leave in order to receive compensation

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25. Attachments/ Appendices

a. Appendix A: Pre-screening Questions

i. Healthy Volunteers

☐ Are you between 18 and 50 years of age?

☐ Are you in good health?

☐ Are you taking any medications?

☐ Are you prone to seizures, stroke, headaches, or migraines?

☐ Are you free of a history of significant neurological or psychiatric conditions?

☐ Are you free of any metal in your body?

☐ Are you comfortable doing a one-hour MRI?

b. Appendix B: Inclusion/Exclusion Checklist

Healthy Volunteers

Inclusion criteria

☐ 18 to 50 years of age

☐ Able to speak and write in English

Exclusion Criteria

☐ Pregnant/plans to be become pregnant during the study.

☐ Major neurological or psychiatric disorder

☐ History of seizure

☐ Ferromagnetic metal or implanted device

☐ Non-removable body piercing.

☐ Claustrophobia or cannot lie supine for 1 hour.

☐ Taking medications acting on the CNS.

☐ NINDS Behavioral Neurology Unit Employee or fellow

☐ For Experiment 2: participation in Experiment 1

c. Appendix C: Timeline of Study

The first rTMS session and consenting procedures may occur on the same day as the initial scanning session)

TIME	PROCEDURE
Experiments 1: Healthy Volunteers	
Day -30 to 1	Screening Procedures: Demographics, Neurological exam (if HV has not had one in past 2 years). Informed Consent
Day 1	Resting-state fMRI: 20 minutes Anatomical Scan: 10-30 minutes DTI: 20-30 minutes ARSQ: 8 minutes Urine pregnancy test
Stimulation Days 1-5* These procedures may occur on the same day as the baseline MRI or up to 4 days afterwards..	Resting Motor Threshold Measurement (30 minutes)** rTMS: (20 minutes)
Day 6	Anatomical Scan: 10 minutes Resting-state fMRI: 20 minutes ARSQ: 8 minutes Urine pregnancy test
Experiment 2: HVS	
Day -30 to 1	Screening Procedures: Demographics, Neurological exam (if HV has not had one in past 2 years). Informed Consent
Day 1	Resting-state fMRI: 20 minutes Anatomical Scan: 10-30 minutes DTI: 20-30 minutes Behavioral Tests: AMT, WPT-I Cognitive Battery (2.5 hours) ARSQ: 8 minutes Urine pregnancy test

TIME	PROCEDURE
Stimulation Days 1-5* **These procedures may occur on the same day as the baseline MRI or up to 4 days afterwards..**	Resting Motor Threshold Measurement (30 minutes)** rTMS: (20 minutes)
Day 6	Anatomical Scan: 10 minutes Resting-state fMRI: 20 minutes Behavioral Tests: AMT, WPT-I Cognitive Battery (2.5 hours) ARSQ: 8 minutes Urine pregnancy test
7 to 14 days after final scan	Behavioral Tests: AMT, WPT-I,Cognitive Battery (2 hours)

*The number of days of rTMS for Experiment 1 depends on the cohort of participants; HVs will receive between 1-5 sessions of rTMS. For Experiment 2, this number will depend on the results of Experiment 1.
** This measurement will be taken once during the first rTMS session.

d. Appendix D: Amsterdam Resting-State Questionnaire (ARSQ)

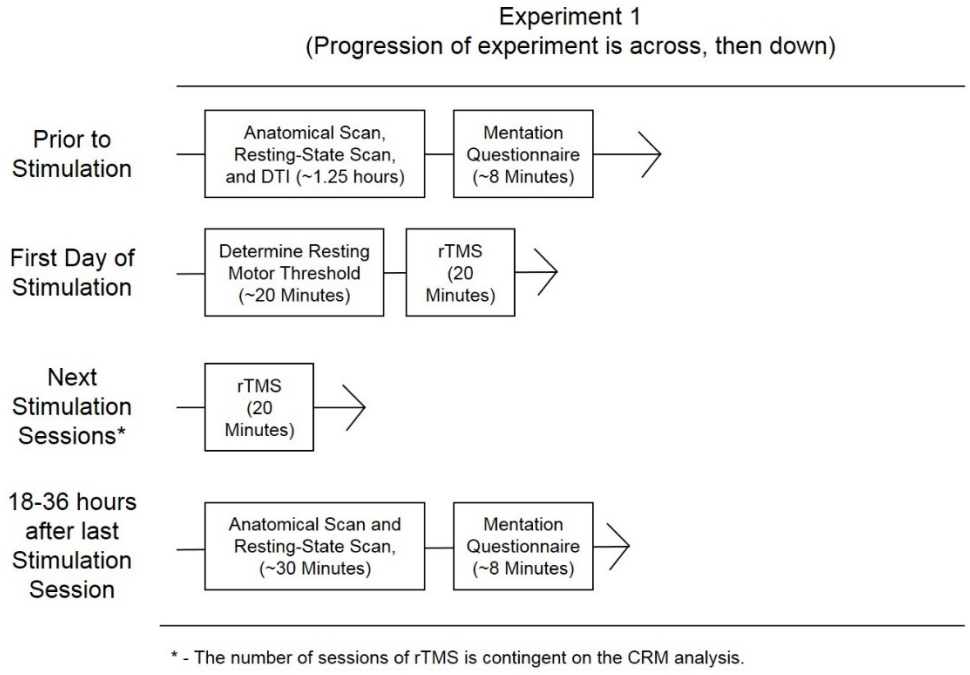
Here below are several statements regarding potential feelings and thoughts you may have experienced during the resting period in the scanner (when you were looking at the cross on the screen trying to think about nothing). Please indicate the extent to which you agree with each statement.

Questions	Completely Disagree	Disagree	Neither Agree nor Disagree	Agree	Completely Agree
I thought about my feelings					
I felt restless					
I felt tired					
I felt sleepy					
I felt comfortable					
I felt relaxed					
I felt happy					
I felt ill					
I enjoyed the session					
I had negative feelings					
I felt bored					
I felt nothing					
I felt the same throughout the session					
I thought about my health					
I thought about my work/study					
I thought about my behavior					
I had thoughts that I would not readily share with others					
I had busy thoughts					
I had similar thoughts throughout the session					
I thought about others					
I thought about myself					
I thought about pleasant things					
I had my thoughts under control					
I thought about solving problems					
I thought about the aim of the experiment					
I had difficulty staying awake					
I had rapidly switching thoughts					
I had superficial thoughts					

Questions	Completely Disagree	Disagree	Neither Agree nor Disagree	Agree	Completely Agree
I thought about the past.					
I thought about the present					
I thought about the future					
I had deep thoughts					
I thought about nothing					
I had difficulty holding on to my thoughts					
I thought about people I like					
I thought in images					
I thought in words					
I thought about things I need to do					
I was conscious of my body					
I thought about the sounds around me					
I thought about the odors around me					
I thought about my heartbeat					
I thought about my breathing					
I felt pain					
I placed myself in other peoples' shoes					
I felt motivated to participate					
I have difficulty remembering my thoughts					
I have difficulty remembering my feelings					
I had my eyes closed					
I was able to rate the statements					

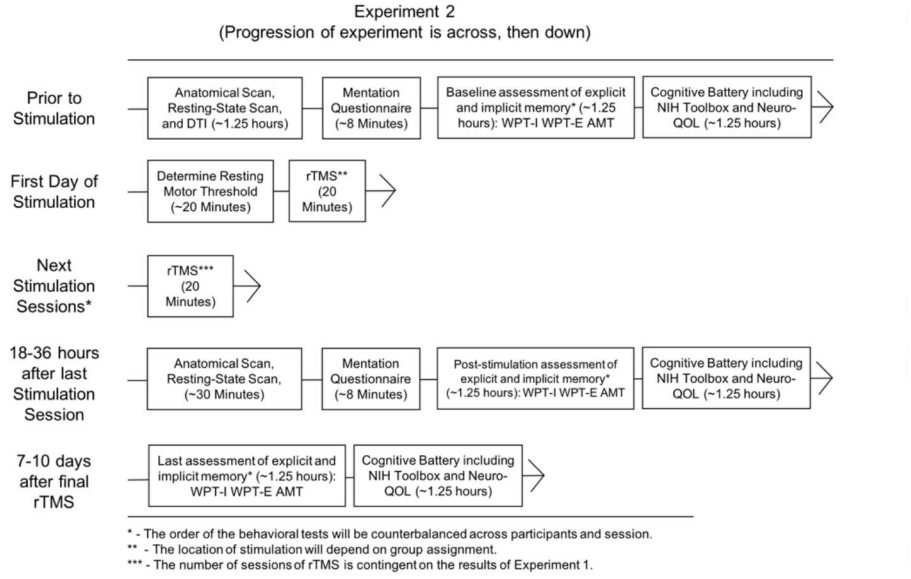
e. Appendix G : Timeline figures

Experiment 1 (from Section 4.d.vii)



* - The number of sessions of rTMS is contingent on the CRM analysis.

Experiment 2 (from Section 4.d.viii)



* - The order of the behavioral tests will be counterbalanced across participants and session.
** - The location of stimulation will depend on group assignment.
*** - The number of sessions of rTMS is contingent on the results of Experiment 1.

26. Consent Forms

Consent documents uploaded in iRIS:

- Standard Consent Form