Protocol Title: Modulating the hippocampal and striatal memory networks with rTMS
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Précis:

Objective
The Behavioral Neurology Unit studies the human brain systems underlying learning and adaptation with the ultimate goal of finding interventions to make these processes more efficient. We are interested in whether repetitive transcranial magnetic stimulation (rTMS) can alter functional connectivity (FC) and behavioral efficiency in two memory networks in the brain: the hippocampal network, which supports the storage and retrieval of recallable facts, concepts, and events, and the striatal network, which supports the storage and retrieval of skills and habits. Additionally, because these networks interact behaviorally and can interfere with each other, an important question is whether neuromodulation of one network changes connectivity and efficiency in the other network. Pilot data from our group suggest that exogenous stimulation of one network causes it to expand its range of FC and co-opt resources from the other, which is a potential mechanism for the observed behavioral interaction. This study is designed to test a) whether rTMS- modulates within-network FC and memory supported by that network, and b) whether this also causes FC and behavioral changes in the other network.

Study population: Healthy Volunteers

Design
This study contains four between-subjects experiments and is a mixed inter-/intra subject design. Experiment 1 will use nominally excitatory stimulation targeting the hippocampal network to increase FC within the hippocampal network. We also expect to increase FC between the hippocampal and striatal networks, increased declarative memory, and a possible decrease in procedural, learning. Experiment 2 will use excitatory stimulation targeted to the striatal network. We expect this to cause stronger within-network FC in the striatal network, increased FC between the hippocampus and the striatal network and concomitant behavioral effects. Experiments 3 and 4 will be similar except that we will target nominally inhibitory stimulation to these networks and look for the inverse results. FC will be measured under resting and task-activated conditions and active rTMS will be compared to vertex sham.

Outcome measures

The primary outcome measure is the change in FC produced by rTMS within the targeted network. Between-network FC changes and corresponding memory changes will be secondary outcomes. Exploratory measures will include correlations between individual cognitive differences (questionnaires and NIH Toolbox scores), and our primary and secondary outcome measures.

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List of Abbreviations

rTMS	repetitive Transcranial Magnetic Stimulation
fMRI	functional Magnetic Resonance Imaging
BOLD	Blood-Oxygen Level Dependency
FC	Functional Connectivity
rsFC	resting-state Functional Connectivity
tbFC	task-based Functional Connectivity
WPT-F	Weather Prediction Task Feedback Version
WPT-O	Weather Prediction Task Observational Version
ARSQ	Amsterdam Resting-State Questionnaire

1. Introduction and Background

a. Background

Memory deficits are among the most debilitating problems in neurology (Nestor et al., 2005; Vakil, 2005), reduce quality of life for caregivers (Kreutzer et al., 1994), and tax the American public financially (e.g., $80-100 billion/yr for Alzheimer disease; CDC & NCCDPHP, 2000). Memory is affected disproportionately among cognitive functions in traumatic brain injury (TBI; Levin et al., 1988) and an estimated 3.2-5.3 million Americans are living with a TBI-related disability (Frieden et al., 2015). Because there are no accepted methods for improving memory in amnestic patients, new approaches are urgently needed. Additionally, the rise in popularity of "brain training" indicates a growing interest in improving memory amongst healthy adults (Rabipour & Raz, 2012). While behavioral approaches such as brain training have gained popularity, their utility to everyday human function is controversial (Simons et al., 2016).

Cognitive functions, such as memory, reside in brain networks (Power et al., 2011) and brain disorders disrupt these networks, causing effects on behavior (Gratton et al., 2012). The two characterized memory networks are the striatum-centered memory system (Keele et al., 2003; Knowlton et al., 1996), and 2) the hippocampal-centered memory system (Knowlton et al., 1996; Raichle et al., 2001). The striatal system supports the encoding and retrieval of skills and habits through repetition (e.g., riding a bike; procedural memories), whereas the hippocampal system supports the encoding and retrieval of recallable facts related to objects, events, or concepts (e.g., knowing a bike has two wheels; declarative memories). Although commonly discussed as discrete systems, they interact (Mattfeld & Stark, 2010, 2015; Poldrack et al., 2001), and learning typically involves both declarative and procedural acquisition. (Ghilardi et al., 2009). Evidence from neuroimaging (Mattfeld & Stark, 2010, 2015; Poldrack et al., 2001), behavioral studies in healthy subjects (Ashby & Crossley, 2010; Foerde et al., 2006, 2007) and patients (Foerde et al., 2013; Moody et al., 2004), and lesion experiments in rats (Lee et al., 2008; Packard & McGaugh, 1996), support a competitive relationship between systems. For example, engaging in learning of one memory type can interfere with learning of the other (Brown & Robertson, 2007; Cohen & Robertson, 2011).

rTMS has been shown to improve declarative memory (Wang et al., 2014; Figure 1), but it is unknown whether this enhancement is associated with behavioral effects on procedural memory or whether modulation of the striatal network affects declarative memory. Although TMS has been used to show a double dissociation between stimulation of different cortical sites and their effects on procedural and declarative memory (Cohen & Robertson, 2011; Galea et al., 2010), these studies did not include a measure of target engagement. Therefore, these studies cannot be considered mechanistically informative. To optimize the effect of rTMS on learning and memory, a mechanistic understanding of how these improvements occur is required. We will attempt to fill this gap by using functional connectivity (FC) as a marker of target engagement, and measuring changes in procedural and declarative learning. Because understanding mechanistic changes are necessary to optimize the effect on behavior, changes in FC will serve as our primary outcome measure.

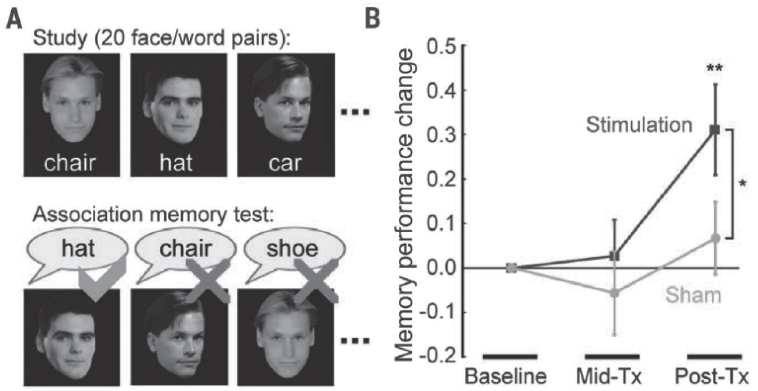

Figure 1. Results from Wang et al. (2014) demonstrating stimulus-dependent changes in associative memory. Panel A demonstrates the task used. Twenty face-name pairs were observed by participants during the study phase of the task. During the test phase, the faces were shown and the participant was asked to recall the corresponding word. Panel B indicates the results from baseline, after 3 days of stimulation, and 24 hours after the 5th day of stimulation for real and sham stimulation. Results are shown in proportion to baseline. * - $p < 0.05$, **-$p < 0.01$

FC 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 rsFC 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 rsFC across resting sessions with a success rate of at least 92.9% (see Figure 2). 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.

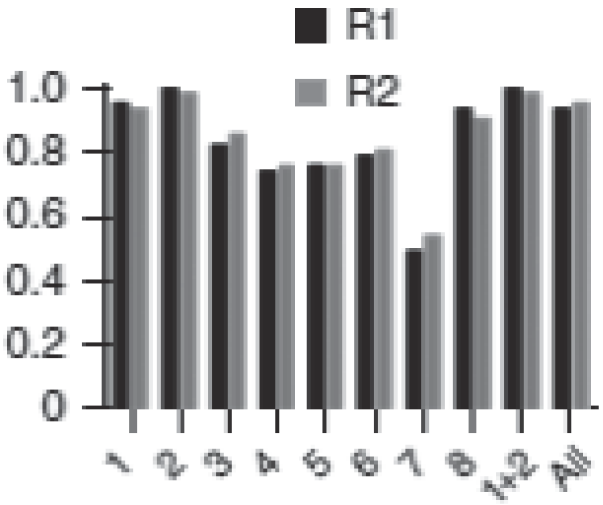

Figure 2. Results from Finn et al. (2015). Correct identification scores for machine classifier (y-axis) across eight different networks (x-axis) for FC patterns pulled from R1 and compared to the second session (R1; black bars) and for patterns pulled from the second session and compared to the first 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

TMS is a widely-used method to manipulate the "excitability" of brain pathways (Chen et al., 1997; Huang et al., 2005; Pascual-leone et al., 1994). Despite the limited radius of the effective electromagnetic

field, rTMS affects network-level activity beyond the site of stimulation (Fox et al., 2012). By applying rTMS to a cortical site known to be connected to a network of interest, it is possible to modulate the FC of that network (Eldaief et al., 2011; Rahnev et al., 2013; Steel et al., 2016; van der Werf et al., 2010; Vercammen et al., 2010). Wang, and colleagues (2014) delivered nominally excitatory (20 Hz) rTMS to the subject-specific posterior parietal cortex (PPC) site that was maximally connected to the anterior hippocampus and observed robust increases in hippocampal network FC. Specifically, this technique increased resting-state FC (rsFC) between the hippocampus and several cortical regions (Fig. 3 Image showing Stiumlation-dependent enhancement of hippocampal network). These changes were specific to the hippocampal network, and not the local site of stimulation, as the voxels throughout the brain with the highest baseline FC with the hippocampus showed the largest FC increase. Furthermore, these effects were present two-weeks later (Wang & Voss, 2015). The validity of using rsFC of a cortical area with a deep node (the hippocampus) as a subject-specific targeting guide and then using the change in FC as an outcome measure has been established by Wang et al (2014) and confirmed in our laboratory (see pilot data below).

Using this technique, we will attempt to increase and decrease FC within the hippocampal and striatal memory systems by selectively targeting each network with rTMS in separate experiments. We predict that rTMS will cause changes in FC and memory in the targeted network, which correspond with the stimulation protocol: Nominally excitatory stimulation will cause enhancement of the targeted network, and nominally inhibitory stimulation will cause FC to decrease. Additionally, because these networks are known to interact in behavioral and imaging experiments (Brown & Robertson, 2007; Cohen & Robertson, 2011; Mattfeld & Stark, 2010, 2015; Poldrack et al., 2001), we expect that altering the function of one network will cause changes in the pattern of FC in the other. Pilot data from our laboratory (Fig. 4 Connectivity changes with left Precuneus and medial Occipital Cortex) show that declarative network FC enhancement is accompanied by significantly increased rsFC between the striatum and the hippocampal network. "Capture" of one system by the other is a good explanation for why behavioral engagement in one learning type interferes with the other (Brown & Robertson, 2007; Cohen & Robertson, 2011).

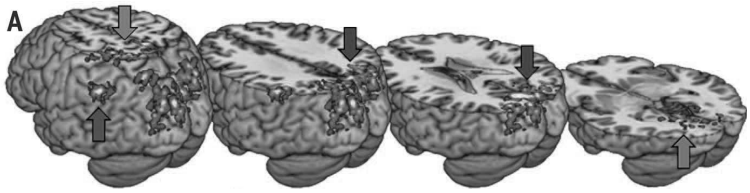

Figure 3. Hippocampal FC changes from Wang et al. (2014)
Stimulation-dependent enhancement of hippocampal network overlaid on a template brain from Wang et al. (2014). The highlighted regions represent the areas that increased in FC with the hippocampus caused by PPC stimulation.

b. Pilot Data

Pilot data from our laboratory show that enhancing the hippocampal network with rTMS via the PPC (Wang et al., 2014) is effective and replicable: Nine subjects tested showed significant FC increases between the hippocampus and regions that showed a group-level increase in FC with the hippocampus in Wang et al. (precuneus and medial occipital cortex; $t(8) = 2.73, p < 0.05, d = 0.75$; Fig. 4). To show that these effects were specific to the hippocampus, we calculated FC changes between the dorsolateral prefrontal cortex and the same regions (i.e. precuneus and medial occipital cortex), and found no change from pre- to post-stimulation ($p = 0.22$). Furthermore, the increase in hippocampus FC with the precuneus/medial occipital cortex were significantly greater than the change in global connectedness of the brain ($t(8) = 2.62, p < 0.05, d = 0.58$), showing that these effects were network-specific.

These pilot data, however, also suggest rTMS targeted to the hippocampus and connected areas also affects the striatal network. Figure 4 shows statistically significant FC increases between the *caudate* and the precuneus/medial occipital cortex. Thus, in addition to increasing FC in the hippocampal network, rTMS also caused the striatum to be pulled into tighter association with the hippocampal network.

Structural equation modeling, which can be used to determine the direction of influence between network nodes, indicated a robust increase in connectivity from the striatum to the precuneus and medial occipital cortex after stimulation. This may explain why behavioral engagement of the hippocampal network interferes with procedural learning (Brown & Robertson, 2007; Poldrack et al., 2001). Based on these preliminary findings, we will guide rTMS to the hippocampal network and examine the effects on both the hippocampal-dependent memory system and the striatal memory system. We will also target the striatal network, and look for similar effects on the hippocampal network.

Figure 4: Hippocampal FC changes from pilot data.

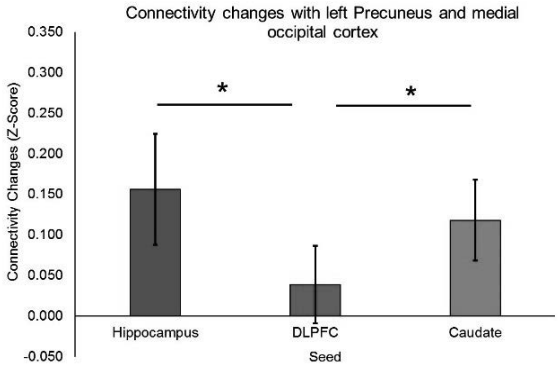

Figure 4. Pilot data (N=9) demonstrating increases in FC between precuneus and medial occipital cortex and three separate brain regions.
* - $p < 0.05$

c. Resting-State vs. Task-Based Connectivity

The results of Wang et al. (2014) and data from our laboratory show that rTMS can enhance rsFC within a targeted network. However, it is unknown whether the same is true of tbFC. Because tbFC may be a better measure of stimulation-induced changes, we will include this measurement in our experiments and compare the results to rsFC.

d. Measuring mentation during resting-state scans.

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

2. Study Objectives

The overall objective of this study is to determine whether the hippocampal and striatal memory networks can be modulated using rTMS. We will also examine cross network changes in FC by guiding nominally excitatory and inhibitory stimulation to the striatal and hippocampal memory networks and measuring the effects on both the targeted and non-targeted one. The results will be used to inform future clinical trials of rTMS in patients with disorders of learning and memory and possibly to enhance and accelerate learning in healthy individuals.

a. Primary aims

Primary Aim 1: Determine whether nominally facilitatory stimulation of the hippocampus increases rsFC or tbFC within the hippocampal memory network (Experiment 1; Replication of Wang et al., 2014).

Primary Aim 2: Determine whether nominally facilitatory stimulation of the striatum increases rsFC or tbFC within the striatal memory network (Experiment 2).

Primary Aim 3: Determine whether nominally inhibitory stimulation of the hippocampus decreases rsFC or tbFC within the hippocampal memory network (Experiment 3).

Primary Aim 4: Determine whether nominally inhibitory stimulation of the striatum decreases rsFC or tbFC within the striatal memory network (Experiment 4).

b. Secondary aims

Our secondary aims are to measure the effects of each type of stimulation at each site on declarative and procedural learning and memory and between network changes in task-based and resting-state FC.

c. Exploratory aim

We will search for predictors of the within and between network FC changes, including scores on the ARSQ, the NIH toolbox, and the procedural and observational versions of the Weather Prediction Task.

3. Subjects

a. Description of study population

We will study 104 healthy individuals (26 in each experiment). We are requesting up to 122 healthy volunteers as the accrual ceiling to account for dropouts and screening failures. Participants who are withdrawn or drop out from the study will be replaced. NIH employees who are not members of the BNU will be allowed to participate. Based on previous experience, our anticipated dropout rate is 15%.

b. Inclusion criteria

- Age 18-40 (inclusive)

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, such as those that lowers the seizure threshold such as neuroleptics, beta lactams, isoniazid, metronidazole; benzodiazepines, tricyclic or other antidepressants; or prescription stimulants.
- 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 2 hours
- Pregnancy, or plans to become pregnant during the study.
- Members of the NINDS BNU
- Subjects that received rTMS under protocol 17-N-0055 are excluded in order avoid learning effects from previously being exposed to the same behavioral tasks

- Subjects who have contraindications to MRI (we will follow the NMR Center guidelines for MR safety). Some of the exclusions are:
    1. Have non-MRI compatible metal in the body, such as a cardiac pacemaker, brain stimulator, shrapnel, surgical metal, clips in the brain or on blood vessels, cochlear implants, artificial heart valves or ferromagnetic fragments in the eye or oral cavity as these make having an MRI unsafe.
    2. Unable to lie flat on the back for the expected length of the experiment (2 hours).
    3. Have an abnormality on the brain imaging or neurologic examination not related to the diagnosis.
    4. Non-removable body piercing or tattoo posing MRI risk
    5. Pregnancy (urine pregnancy test)

An eligibility checklist is provided in Appendix A.

4. Study Design and Methods

a. Study overview

This protocol will include four experiments, each using rTMS and MRI, in a between-subjects design (Table 1). Experiments 1 and 3 will use PPC rTMS. Experiments 2 and 4 will use M1 stimulation.

	Active Stimulation Type	Active Stimulation Site	Targeted Network
Experiment 1	**Facilitatory (20-Hz)**	**PPC**	**Hippocampal**
Experiment 2	**Facilitatory (20-Hz)**	**M1**	**Striatal**
Experiment 3	**Inhibitory (cTBS or 1-Hz)**	**PPC**	**Hippocampal**
Experiment 4	**Inhibitory (cTBS or 1-Hz)**	**M1**	**Striatal**

Table 1. Experiment Details. PPC = Posterior Parietal Cortex, M1 = Motor Cortex.

Table 2 shows the timeline of each experiment. Each study will include FC and memory testing at baseline, 24-hours after, and two weeks after three days of rTMS. The number of days of rTMS is based on pilot data where three days of stimulation produces significant enhancement of rsFC in the hippocampal network in five out of six individuals tested. Each participant will make a maximum of 6 visits to the lab (not including screening), for a maximum time commitment of 14 hours. The 24-hour follow-up will occur between 12 and 48 hours after the last stimulation session, and the 2-week follow-up will occur 14-21 days after baseline measurements. Scanning will occur in the NIH NMRF. All visits will be outpatient.

	Baseline Day	rTMS Sessions	Post Stimulation Day	2-week follow-up
Group 1	**Baseline FC and Memory**	**Active Stimulation 3-sessions**	**FC and Memory Testing**	**FC and Memory Testing**
Group 2	**Baseline FC and Memory**	**Vertex Stimulation 3-sessions**	**FC and Memory Testing**	**FC and Memory Testing**

Table 2. Timeline of each experiment.

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 are listed in Appendix B.

Healthy participants will be recruited from the pool of individuals self-referring to the study directly and via the NIH Clinical Research Volunteer Program and via advertisements that will be pre-approved by the IRB. Although NIH employees will be allowed to participate, no direct solicitation of employees/staff by supervisors or co-workers will take place. Any recruitment material will be IRB approved. Participants who indicate interest will be pre-screened by phone. Pre-screening questions are listed in Appendix B.

IRB-approved ads will be posted on NIH listservs with the permission of the moderator and IRB required statement on how the receiver was identified. Listservs may include NIH sponsored recruitment list serves such as (NIH HV recruitment list serv). Listserv announcement will include:

"You are receiving this message because your email address is included in the above NIH Listserv/mailing list. The purpose of this message is to inform you of studies that are recruiting volunteers at NIH, Bethesda, Maryland. The moderator of the listserv/mailing list has permitted its use for this distribution".

i. Participant rescheduling

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 visit after baseline measures have been collected, besides the two week follow-up visit, baseline measurements (MRI and behavioral assessments) will be performed again. If a participant completes all visits, but is unable to attend the follow-up visit, their attendance in the study will be considered complete.

c. Screening

Participants who pass pre-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 A.

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

ii. Behavioral Tasks

Behavioral testing will occur at baseline, 24-hours, and two-weeks after stimulation.

Weather Prediction Task (feedback version; WPT-F) - 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 200 trials, with breaks after every 50 trials.

Weather Prediction Task (Observational version; WPT-O) – The observational version of the WPT resembles a paired associates test where the feedback and cards are displayed on the screen are shown on the screen together. After all pairs are shown the cards are again shown and the participant is asked to remember which feedback outcome (rain or fine) goes with that set of cards. Unlike the WPT-I, the WPT-E recruits brain regions that support declarative memory. Both version each take approximately 20 minutes. The order of the tasks will be counterbalanced across participants.

iii. MRI

We estimate a maximum of 2 hours for each scan in all experiments. 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.

1. MRI Anatomical scanning

All subjects will have anatomical (MPRAGE) scans at baseline, 24-hours, and 2-weeks after rTMS. 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 FC

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 10 min).

3. Task-Based FC

During scanning, participants will perform both versions of the WPT and tbFC will be measured. During the feedback version of the task, when a response is required, participants will respond using one of two buttons on a button box inside the scanner. Participants will respond verbally during the retrieval phase of the WPT-O.

iv. rTMS

The parietal target in Experiments 1 and 3 will be the region of the left PPC with the greatest connectivity with the left hippocampus derived from the baseline resting-state fMRI session, (similar to Wang et al. 2014). The left PPC was chosen because of its dense connections with the hippocampus (Cavada & Goldman-Racic, 1989; Mesulam et al., 1977).

The target in Experiments 2 and 4 will be the M1 area of maximal connectivity with the right striatum. M1 has extensive projections to the striatum (Parent & Hazrati, 1995), and these regions are functionally connected at rest (Di Martino et al., 2008).

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) or motor cortex will be used as our location of stimulation. 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.

20-Hz Stimulation (facilitation) 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).

Continuous Theta-Burst Stimulation (cTBS; inhibition) will be delivered at 80% of active motor threshold for 40 seconds (similar to Huang et al., 2005). cTBS consists of 50-Hz triplets separated by 200ms.

Control stimulation. As a negative control, rTMS will be delivered to the vertex with the same delivery parameter values and neuronavigation. Wang and colleagues (2014) used vertex stimulation as a control condition and found no significant effects on rsFC or behavior. In addition, there is no evidence that vertex stimulation causes other changes in rsFC (Jung et al., 2016).

Contingency for Experiments 3 and 4 if we are unable to inhibit each network. Because targeted inhibition of the hippocampal and striatal network with cTBS has not been demonstrated, it is possible that cTBS may not yield the expected inhibitory effects in Experiments 3 and 4. Thus, we will examine the results from the first three subjects receiving active stimulation in Experiments 3 and 4 and if there is no clear decrease of FC in the targeted network, we will run a cohort of 3 subjects using 1-Hz rTMS. We will proceed with the stimulation type that demonstrates the most reliable inhibitory effect.

1-Hz Stimulation. Stimulation will be delivered at 115% of the motor evoked potential threshold at 1-Hz continuously for 16 minutes (similar to Chen et al., 1997).

v. Cognitive Battery

The purpose of the battery is to detect effects on other memory processes other than those targeted in the study, characterize participants cognitive abilities at baseline, and to monitor for unexpected deleterious effects of stimulation. The data will be treated as exploratory. Some tasks will only be included at baseline (indicated below). These tests will not be given inside of the scanner. 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). This test will only be given at baseline.

2) 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). This test will only be given at baseline.

3) 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). This test will only be given at baseline.

4) 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) 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). This test will only be given at baseline.

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). This test will only be given at baseline.

7) 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). This test will only be given at baseline.

8) 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) 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) Verbal paired associates test (for verbal memory) – This test is similar to the WPT-O, 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) 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 – Participants report outcome measures through computer adaptive tests (CAT), short forms, or scales. (~ 20 minutes).

vi. 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 C). 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.

All clinical rating scales can be performed using an interpreter in any language. Interpreters will also be available for other protocol procedures as necessary.

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

a. 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.

b. Data

This protocol is not subject to the Genomic Data Sharing (GDS) policy. Data may also be shared with collaborating laboratories at NIH or outside of NIH and/or submitted to NIH-designated repositories and databases if consent for sharing was obtained

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 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

- Magnetic resonance imaging system operating in research mode
- Magstim Rapid^2 Therapy System and the
- Magstim Air Film Coil

According to 21 CFR 812.3 (m) and FDA "Information Sheet Guidance for IRBs, Clinical Investigators, and Sponsors: Significant Risk and Nonsignificant Risk and Nonsignificant Risk Medical Device Studies January 2006 (accessible at http://www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM126418.pdf), the use of these devices in the study are a Non-signficant Risk study. 21 CFR 812.3(m) enumerates four criteria for a Significant Risk Device Study; none of these apply to this study:

1. is intended as an implant and presents a potential for serious risk to the health, safety, or welfare of a subject;
   None of the three devices are implants.
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;
   None of the three devices are for use in supporting or sustaining human life.
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; or

The investigational use of the three devices is not of substantial importance in diagnosing, curing, mitigating, or treating disease, or otherwise preventing impairment of human health.

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

The use of the legally marketed MRI system in research mode will be performed under operating conditions below each of the limits (field strength, Specific Absorption Rate, time rate of change of gradient fields and sound pressure levels) deemed to be significant risk by the FDA as stated in the FDA guidance document, Criteria for Significant Risk Investigations of Magnetic Resonance Diagnostic Devices.

The Magstim Rapid2 Therapy System is legally marketed, indicated for "the treatment of Major Depressive Disorder in adult patients who have failed to achieve satisfactory improvement from prior antidepressant medication in the current episode." The Magstim Air Film Coil is legally marketed as "a stimulating coil solely intended for use with the Magstim® Rapid2 Stimulating Unit for the purposes of peripheral nerve stimulation. The Air Film Coil is an accessory of the Magstim® Rapid2 Unit." Both are used in combination in rTMS. Their use in this study is not within the labeled indications. The major risk of rTMS and cTMS is the risk of seizure. 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 50,000 sessions. For rTMS, safety guidelines have been developed (Wassermann, 1998) and updated (Rossi, Hallett, Rossini, & Pascual-Leone, 2009). These guidelines were incorporated into FDA's Class II Special Controls Guidance Document: Repetitive Transcranial Magnetic Stimulation (rTMS) Systems in Table 2. Maximum Safe Train Duration (seconds) Limits for Avoiding Seizures. The rTMS and and 1-Hz Stimulation in this study are below the limits defined in these documents. Furthermore, the study subjects are a lower risk since subjects with any neurological or psychiatric disorders, history of seizure or taking certain medications are excluded. cTBS was not included in the guidelines. However, there is only a single report of seizure with cTBS (Lerner, Wassermann, & Tamir, 2019; Oberman et al., 2011, Rossi et al., submitted manuscript). In that single patient, the study was conducted in at stimulation intensity levels higher than in this study. Over the past 20 years, the FDA has generally waived pre-IDE inquiries for TMS/rTMS studies on an NSR device basis.

All subjects will wear hearing protection.

The protocol will comply with the abbreviated IDE requirements under 21 CFR 812.2(b), available at:
https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfCFR/CFRSearch.cfm?fr=812.2

b. Gene therapy

- *N/A*

7. Risks and Discomforts

b. General

rTMS, as delivered under this protocol, involves more than minimal risk to participants. The behavioral tasks, neuroimaging procedures, and screening procedures are minimal risks to the participant.

c. 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. Participants will be fitted with ear plugs. 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 when hearing protection was used, 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 (Pascual-Leone et al., 1992) and more recent has found no increase in auditory threshold from TMS (Janicak et al., 2008; Levkovitz et al., 2007). 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).

d. 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 at subject's discretion.

ii. MRI

To mitigate the risk of damage 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, tattoos posing MRI risk, 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

Study staff will be trained in rTMS administration, rTMS safety, and the measurement of the TMS-evoked potentials by Dr. Eric Wassermann. Study staff will be trained to recognize and respond to signs of seizure and syncope. For each session, at least two rTMS-trained personnel will be in the laboratory. A licensed independent practitioner (LIP), a physician or nurse practitioner trained in rTMS, will be in house, specifically aware of the session, and reachable by phone for all TMS sessions. The TMS laboratory is in a clinic area with nursing and code team support. Each room has oxygen, suction, and an emergency call button. If a participant has a significant event, such as a seizure, the hospital emergency system (Code Blue) will be activated and treatment will be initiated as required.

Study staff will monitor individuals during participation and participants will be encouraged to tell experimenters of any discomfort. At each session, participants will be asked whether stimulation was (1) tolerable, (2) tolerable with some discomfort, or (3) intolerable. If the participant responds that stimulation is intolerable, the participant will be withdrawn from the experiment. Any participant exhibiting distress or who wishes to stop the experiment for any reason will be allowed to stop. Nonprescription drugs may be used to alleviate headache or pain discomfort.

Syncope will be treated safely by immediately placing the participant in a lying position with legs elevated. Should any participant become faint, the LIP covering the study will be called immediately.

Although no long-term deleterious effects of rTMS cognition have been reported, we will monitor cognition with the NIH Toolbox. This is a standardized assessment with age-adjusted normative values. An investigator will review NIH Toolbox results for each participant at the post-stimulation and 2-week follow-up assessments. If a participant experiences a significant drop from baseline, the IMM will be informed and the test(s) repeated in a few days. If the deficit persists, the participant will be withdrawn from the study and referred for clinical neuropsychological evaluation. If, in the judgment of Dr. Wassermann or IMM, a study procedure is causing frequent unexpected or adverse events of any kind, the study will be suspended until a review can be undertaken in consultation with the IRB. Depending on the outcome, the protocol may be amended, and/or specific language added to the protocol and documents to reflect the altered risk.

<u>TMS Training</u>
In accordance with safety guidelines, the TMS operator is a TMS-trained technician who operates under the supervision of the PI and/or Medically Responsible Investigator. Examples of TMS operators include medical assistants, technicians with relevant experience, psychologists, physicists, physiotherapists, engineers, physician assistants, nurses, nurse practitioners, and physicians.

Persons operating TMS equipment will have been certified by a NINDS laboratory in safe application of TMS.

Standard training procedures used include training in TMS device operation, supervised repeated practice in TMS procedures, and testing for inter-rater reliability in motor threshold determination. To be credentialed as a TMS technician, an individual must have passed the following criteria (as assessed by a designated BNU TMS instructor):

1) Training in TMS device operation
2) Supervised administration of at least 10 TMS procedures
3) Demonstration of inter-rater reliability on motor threshold determination
4) Current Basic Life Support certification
5) In-service training on basic TMS safety and risks by TMS-trained physician
6) In-service training on recognition and initial response to seizures

8. Subject Safety Monitoring

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 any experiment (i.e. both MRI scan and behavioral assessment) will be kept and analyzed. A researcher may end experimentation for the following reasons:

- Abnormal response to rTMS including the occurrence of a seizure, loss of consciousness, or the participant's unwillingness to continue or headache produced by rTMS.
- Withdrawal of consent and/or decision to terminate.
- The experiment will be ended if the participant is unwilling to continue due to pain, or frustration with the procedures

9. Outcome Measures

a. Primary outcome measures

- Pre-to-Post rTMS differences in rsFC and tbFC within the targeted memory network.

b. Secondary outcome measures

- Pre-to-Post rTMS differences in WPT-O scores
- Pre-to-Post rTMS differences in WPT-F scores
- Pre-to-Post rTMS differences in rsFC and tbFC between memory networks.

c. Exploratory outcome measures

- Correlations between network FC changes and:
  - ARSQ scores
  - NIH Toolbox Scores
  - WPT-F scores
  - WPT-O scores

10. Statistical Analysis

a. Analysis of data/ study outcomes

Our primary aim is to examine stimulation-dependent changes in FC within the targeted network in each study. Secondary aims for this protocol will include all behavioral change data from the WPT-F and WPT-O and changes in FC to the non-targeted network, and between networks.

To calculate FC from resting-state scans, the Pearson correlation coefficients (r) between time courses of BOLD activity in a seed region (i.e. hippocampus or striatum) will be calculated to determine the FC before and after rTMS stimulation. They will be Fisher-transformed to normalize r-values.

To calculate FC from task-based scans, the time series from a seed region will be inputted into a general linear model analysis as a regressor along with a regressor indicating when the task is occurring. For the WPT-F, this will include both the choice phase and the feedback phase during learning. For the WPT-O, this will include the observational trials and the retrieval trials. This analysis will be performed on each

individual to create a spatial map of beta values indicating the strength of the association between the seed and other regions during task performance.

Statistical analysis for Experiments 1 and 3 (targeting the hippocampal network)

iv. Preliminary Aims

Primary Aim 1
To confirm modulation of the hippocampal network, the average timeseries of the left hippocampal seed will be correlated with all other voxels with available data before and after stimulation. These data will be submitted to AFNI's 3dLME linear mixed effects function, which will be used to reveal potential areas of significant change in rsFC and tbFC.

v. Secondary Aims

Secondary Aims
Behavioral data will be submitted to a 3 x 2 x 2 ANOVA using timepoint (Pre-stimulation, post-stimulation, and follow-up), group (PPC vs. Vertex), and learning type (procedural and declarative) as factors. A significant interaction ($p < 0.05$) between time, group, and learning type will confirm network-dependent modulation of each memory type.

To confirm changes in FC, a similar analysis will be performed as Primary Aim 1, except that the timeseries of the right striatum seed will be used instead of the hippocampal seed.

Statistical Analysis for Experiments 2 and 4 (targeting the striatal network)

i. Preliminary Aims

Preliminary Aim 1
To confirm modulation of the striatal network, the average timeseries of the right striatum seed will be correlated with all other voxels with available data before and after stimulation. These data will be submitted to AFNI's 3dLME linear mixed effects function, which will be used reveal potential areas of significant change in rsFC and tbFC.

ii. Secondary Aims

Secondary Aims
Behavioral data will be submitted to a 3 x 2 x 2 ANOVA using timepoint (Pre-stimulation, post-stimulation, and follow-up), group (M1 vs. Vertex), and learning type (procedural and declarative) as factors. A significant interaction ($p < 0.05$) between time, group, and learning type will confirm network-dependent modulation of each memory type.

To confirm changes in FC, a similar analysis will be performed as Primary Aim 1, except that the timeseries of the left hippocampus seed will be used instead of the striatal seed.

Interim Analysis for Experiments 3 and 4. The data from 3 initial participants in Experiments 3 and 4 will be examined to confirm that cTBS is inhibiting our networks of interest. FC will be calculated within the targeted networks and analyzed by study investigators. If, in the opinion of the study team, inhibition of these networks is unreliable, a cohort of 3 subjects will be run and will receive 1-Hz stimulation instead. Following this cohort, the results will be compared between stimulation protocols and the study team will make an informed decision about whether to continue with cTBS or to switch to 1-Hz stimulation instead.

b. Power analysis (All experiments)

All experiments in this protocol are powered based on the primary outcome, which relates to modulating FC within the targeted networks.

A power analysis was conducted on our preliminary data using G*Power v3.1. We used a repeated-measures ANOVA model with FC changes between the hippocampus and the precuneus/medial occipital cortex as my dependent variable. The precuneus/medial occipital cortex was used because these regions showed the most robust response to stimulation in Wang et al., (2014). Based on these data (Mean Difference = 0.16, Pooled Standard Deviation = 0.21, Cohen's d = 0.75), and assuming power of 0.80 and $\alpha = 0.05$, 26 right-handed healthy participants between 18 and 40 years of age will be needed (13 per group) for each experiment (104 total). To account for attrition (estimated ~15%), we will request an accrual celling of 122.

There were no preliminary data to conduct a power analysis for stimulation involving the striatal network. Thus, we propose to have 13 analyzable subjects in the M1 group, the same number as in the parietal group from Experiments 1 and 3. We also plan to obtain 13 subjects in the control group, resulting in 26 analyzable healthy subjects (13 per group).

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 children

Children will not be included. The study is predicated on the work of Wang et al. (2014) and Poldrack et al. (2001; 2003), preliminary data from our laboratory for participants above the age of 18. Therefore, we will recruit a sample of the same age.

c. Justification for exclusion of subjects above the age of 40

Because neuroplasticity is reduced in older adults (Fathi, et al., 2010), including subjects above the age of 40 would reduce our power for detecting change in FC. A lower age of 40 years old for the upper limit, as older brains have less memory capability . Therefore, this group will be excluded.

d. Justification for the Exclusion of other Vulnerable Subjects

i. 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.

e. Justification of sensitive procedures

This study involves no sensitive procedures.

f. 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.

All investigators and staff authorized to consent subjects will complete consent training prior to obtaining consent.

12. 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.

13. 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 a study procedure is causing serious adverse events, or unanticipated problems it will be suspended. If there is a serious adverse event the study 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.

14. Quality Assurance (QA)

a. Quality assurance monitor

The NINDS Quality Assurance (QA) Audit Committee will periodically monitor the protocol.

b. Quality assurance plan

This protocol will undergo periodic review by the QA Audit Committee as outlined in the NINDS QA Standard Operating Procedure (SOP).

This protocol will undergo random review by the NINDS Quality Assurance (QA) Office as outlined in the NINDS QA Standard Operating Procedure. The purpose of the QA audit is to assess compliance with applicable regulatory requirements, good clinical practice guidelines, NINDS/NIH policies, as well as to provide recommendations for improving the management of clinical research data. The protocol will be audited according to the decision algorithm as described in the NINDS SOP. This protocol is classified as "more than minimal risk" and thus will be audited with a target frequency of once during the first year following IRB approval, and then approximately every three years thereafter.

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

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

16. 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.

17. Privacy

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

18. 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 in a locked office 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.

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).

19. 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.

20. Technology Transfer

N/A

21. Research and Travel Compensation

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

Compensation for time

First hour	$20
Additional hours	$10

Compensation for inconveniences

Subjects will be paid $10.00 per one Inconvenience Unit (IU). No payment will be offered for the screening visit.

fMRI testing (4 IU)	$40
TMS testing (4 IU)	$40
Behavioral Tasks (1 IU)	$20
Pregnancy Test (1 IU)	$10

Payment (check) will be mailed to participants after they complete the protocol, or by direct deposit if available. 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

22. References

Ashby, F. G., & Crossley, M. J. (2010). Interactions between declarative and procedural-learning categorization systems. *Neurobiology of Learning and Memory*, *94*(1), 1–12. https://doi.org/10.1016/j.nlm.2010.03.001

Brown, R., & Robertson, E. (2007). Off-Line processing: Reciprocal interactions between declarative and procedural memories. *Journal of Neuroscience*, *27*(39), 10468–10475. https://doi.org/10.1523/JNEUROSCI.2799-07.2007

Cavada, C., & Goldman-Racic, P. S. (1989). Posterior parietal cortex in rhesus monkey: I. Parcellation of areas based on distinctive limbic and sensory corticocortical connections. *The Journal of Comparative Neurology*, *287*, 393–421. https://doi.org/https://doi.org/10.1002/cne.902870402

CDC, & NCCDPHP. (2000). *Unrealized prevention opportunities: reducting the health and economic burden fo chronic illness*. Atlanta, GA, USA: US Department of Health and Human Services, Center for Disease Control and Prevention.

Chen, R., Classen, J., Gerloff, C., Celnik, P., Wassermann, E., Hallett, M., & Cohen, L. (1997). Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. *Neurology*, *48*(5), 1398–1403. https://doi.org/https://doi.org/10.1212/WNL.48.5.1398

Cohen, D. A., & Robertson, E. M. (2011). Preventing interference between different memory tasks. *Nature Neuroscience*, *14*(8), 953–955. https://doi.org/10.1038/nn.2840

Di Martino, A., Scheres, A., Margulies, D. S., Kelly, A. M. C., Uddin, L. Q., Shehzad, Z., Biswal, B., Walters, J. R., Castellanos, F. X., & Milham, M. P. (2008). Functional connectivity of human striatum: A resting state fMRI study. *Cerebral Cortex*, *18*(12), 2735–2747. https://doi.org/10.1093/cercor/bhn041

Eldaief, M. C., Halko, M. A., Buckner, R. L., & Pascual-leone, A. (2011). Transcranial magnetic stimulation modulates the brain's intrinsic activity in a frequency-dependent manner. *Proceedings from the National Academy of Sciences*, *108*(52), 21229–21234. https://doi.org/10.1073/pnas.1113103109

Foerde, K., Knowlton, B. J., & Poldrack, R. A. (2006). Modulation of competing memory systems by distraction. *Proceedings from the National Academy of Sciences*, *103*(31), 11778–11783. https://doi.org/10.1073/pnas.0602659103

Foerde, K., Poldrack, R., & Knowlton, B. (2007). Secondary-task effects on classification learning Secondary-task effects on classification learning. *Memory and Cognition*, *35*(5), 864–874. https://doi.org/10.3758/BF03193461

Foerde, K., Race, E., Verfaellie, M., & Shohamy, D. (2013). A role for the medial temporal lobe in feedback-driven learning: Evidence from amnesia. *The Journal of Neuroscience*, *33*(13), 5698–5704. https://doi.org/10.1523/JNEUROSCI.5217-12.2013

Fox, M. D., Halko, M. A., Eldaief, M. C., & Pascual-Leone, A. (2012). Measuring and manipulating brain connectivity with resting state functional connectivity magnetic resonance imaging (fcMRI) and transcranial magnetic stimulation (TMS). *NeuroImage*, *62*(4), 2232–2243. https://doi.org/10.1016/j.neuroimage.2012.03.035

Frieden, T., Houry, D., & Baldwin, G. (2015). *Traumatic Brain Injury in the United States: Epidemiology and Rehabilitation*.

Galea, J. M., Albert, N. B., Ditye, T., & Miall, R. C. (2010). Disruption of the dorsolateral prefrontal cortex facilitates the consolidation of procedural skills. *Journal of Cognitive Neuroscience*, *22*(6), 1158–1164. https://doi.org/10.1162/jocn.2009.21259

Ghilardi, M. F., Moisello, C., Silvestri, G., Ghez, C., & Krakauer, J. W. (2009). Learning of a sequential motor skill comprises explicit and implicit components that consolidate differently. *Journal of Neurophysiology*, *101*(5), 2218–2229. https://doi.org/10.1152/jn.01138.2007.

Gratton, C., Nomura, E. M., Pérez, F., & Esposito, M. D. (2012). Focal Brain Lesions to Critical Locations Cause Widespread Disruption of the Modular Organization of the Brain. *Journal of Cognitive Neuroscience*, *24*(6), 1275–1285. https://doi.org/https://doi.org/10.1162/jocn_a_00222

Gregory, M. D., Robertson, E. M., Manoach, D. S., & Stickgold, R. (2016). Thinking About a Task Is Associated with Increased Connectivity in Regions Activated by Task Performance. *Brain Connectivity*, *6*(2), 164–168. https://doi.org/10.1089/brain.2015.0386

Huang, Y. Z., Edwards, M. J., Rounis, E., Bhatia, K. P., & Rothwell, J. C. (2005). Theta burst stimulation of the human motor cortex. *Neuron*, *45*(2), 201–206. https://doi.org/10.1016/j.neuron.2004.12.033

Janicak, P. G., O'Reardon, J. P., Sampson, S. M., Husain, M. M., Lisanby, S. H., Rado, J. T., Heart, K. L., & Demitrack, M. A. (2008). Transcranial magnetic stimulation in the treatment of major depressive disorder: A comprehensive summary of safety experience from acute exposure, extended exposure, and during reintroduction treatment. *Journal of Clinical Psychiatry*, *69*(2), 222–232. https://doi.org/10.4088/JCP.v69n0208

Jung, J., Bungert, A., Bowtell, R., & Jackson, S. R. (2016). Vertex Stimulation as a Control Site for Transcranial Magnetic Stimulation : A Concurrent TMS / fMRI Study. *Brain Stimulation*, *9*(1), 58–64. https://doi.org/10.1016/j.brs.2015.09.008

Keele, S. W., Ivry, R., Mayr, U., Hazeltine, E., & Heuer, H. (2003). The Cognitive and Neural Architecture of Sequence Representation. *Psychological Review*, *110*(2), 316–339. https://doi.org/10.1037/0033-295X.110.2.316

Knowlton, B. J., Mangels, J. A., & Squire, L. R. (1996). A neostriatal habit learning system in humans. *Science*, *273*(5280), 1399–1402. https://doi.org/10.1126/science.273.5280.1399

Kreutzer, J. S., Gervasio, A. H., & Camplair, P. S. (1994). Primary caregivers' psychological status and family functioning after traumatic brain injury. *Brain Injury*, *8*(3), 197–210. https://doi.org/10.3109/02699059409150973

Lee, A. S., Duman, R. S., & Pittenger, C. (2008). A double dissociation revealing bidirectional competition between striatum and hippocampus during learning. *Proceedings of the National Academy of Sciences*, *105*(44), 17163–17168. https://doi.org/10.1073/pnas.0807749105

Levin, H. S., Goldstein, F. C., & Eisenberg, H. M. (1988). Disproportionately severe memory deficit in relation to normal intellectual functioning after closed head injury. *Journal of Neurology, Neurosurgery, & Psychiatry*, *51*(10), 1294–1301. https://doi.org/http://dx.doi.org/10.1136/jnnp.51.10.1294

Levkovitz, Y., Roth, Y., Harel, E. V., Braw, Y., Sheer, A., & Zangen, A. (2007). A randomized controlled feasibility and safety study of deep transcranial magnetic stimulation. *Clinical Neurophysiology*, *118*(12), 2730–2744. https://doi.org/10.1016/j.clinph.2007.09.061

Mattfeld, A. T., & Stark, C. E. L. (2010). Striatal and medial temporal lobe functional interactions during visuomotor associative learning. *Cerebral Cortex*, *21*(3), 647–658. https://doi.org/10.1093/cercor/bhq144

Mattfeld, A. T., & Stark, C. E. L. (2015). Functional contributions and interactions between the human hippocampus and subregions of the striatum during arbitrary associative learning and memory. *Hippocampus*, *25*(8), 900–911. https://doi.org/10.1002/hipo.22411

Mesulam, M. M., Van Hoesen, G. W., Pandya, D. N., & Geschwind, N. (1977). Limbic and sensory connections of the inferior parietal lobule (area PG) in the rhesus monkey: A study with a new method for horseradish peroxidase histochemistry. *Brain Research*, *136*(3), 393–414. https://doi.org/10.1016/0006-8993(77)90066-X

Moody, T. D., Bookheimer, S. Y., Vanek, Z., & Knowlton, B. J. (2004). An implicit learning task activates medial temporal lobe in patients with Parkinson's disease. *Behavioral Neuroscience*, *118*(2), 438–442. https://doi.org/10.1037/0735-7044.118.2.438

Nestor, P. J., Fryer, T., & Hodges, J. R. (2005). Declarative memory impairments in Alzheimer ' s disease and semantic dementia semantic dementia. *NeuoImage*, *30*, 1010–1020. https://doi.org/10.1016/j.neuroimage.2005.10.008

Oberman, L., Edwards, D., Eldaief, M., & Pascual-Leone, A. (2011). Safety of theta burst transcranial magnetic stimulation: a systematic review of the literature. *Journal of Clinical Neurophysiology*, *28*,

67-74. doi: 10.1097/WNP.0b013e318205135fPackard, M. G., & McGaugh, J. L. (1996). Inactivation of hippocampus or caudate nucleus with lidocaine differentially affects expression of place and response learning. *Neurobiology of Learning and Memory*, *72*(0007), 65–72. https://doi.org/https://doi.org/10.1006/nlme.1996.0007

Parent, A., & Hazrati, L. (1995). Functional anatomy of the basal ganglia. *Brain Research Reviews*, *20*(1), 91–127. https://doi.org/https://doi.org/10.1016/0165-0173(94)00007-C

Pascual-Leone, A., Cohen, L. G., Shotland, L. I., Dang, N., Pikus, A., Wassermann, E. M., Brasil-Neto, J. P., Valls-Solé, J., & Hallett, M. (1992). No evidence of hearing loss in humans due to transcranial magnetic stimulation. *Neurology*, *42*(3 Pt 1), 647–651. https://doi.org/10.1212/WNL.42.3.647

Pascual-leone, A., Valls-sole, J., Wassermann, E. M., & Hallett, M. (1994). Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex. *Brain*, *117*(4), 847–858. https://doi.org/https://doi.org/10.1093/brain/117.4.847

Poldrack, R. A., Clark, J., Pare-Blagoev, E. J., Shohamy, D., Moyano, J. C., Myers, C., & Gluck, M. A. (2001). Interactive memory systems in the human brain. *Nature*, *414*(6863), 546–550. https://doi.org/10.1038/35107080

Power, J. D., Cohen, A. L., Nelson, S. M., Wig, G. S., Barnes, K. A., Church, J. A., Vogel, A. C., Laumann, T. O., Miezin, F. M., Schlaggar, B. L., & Petersen, S. E. (2011). Functional Network Organization of the Human Brain. *Neuron*, *72*(4), 665–678. https://doi.org/10.1016/j.neuron.2011.09.006

Rabipour, S., & Raz, A. (2012). Training the brain: Fact and fad in cognitive and behavioral remediation. *Brain and Cognition*, *79*(2), 159–179. https://doi.org/10.1016/j.bandc.2012.02.006

Rahnev, D., Kok, P., Munneke, M., Bahdo, L., Lange, F. P. De, & Lau, H. (2013). Continuous theta burst transcranial magnetic stimulation reduces resting state connectivity between visual areas. *Journal of Neurophysiology*, *110*, 1811–1821. https://doi.org/10.1152/jn.00209.2013

Raichle, M. E., Macleod, A. M., Snyder, A. Z., Powers, W. J., Gusnard, D. A., & Shulman, G. L. (2001). A default mode of brain function. *Proceedings of the National Academy of Sciences*, *98*(2), 676–682. https://doi.org/https://doi.org/10.1073/pnas.98.2.676

Simons, D. J., Boot, W. R., Charness, N., Gathercole, S. E., Chabris, C. F., Hambrick, D. Z., & Stine-Morrow, E. A. L. (2016). Do "Brain-Training" Programs Work? *Psychological Science in the Public Interest, Supplement*, *17*(3), 103–186. https://doi.org/10.1177/1529100616661983

Steel, A., Song, S., Bageac, D., Knutson, K. M., Keisler, A., Saad, Z. S., Gotts, S. J., Wassermann, E. M., & Wilkinson, L. (2016). Shifts in connectivity during procedural learning after motor cortex stimulation: A combined transcranial magnetic stimulation/functional magnetic resonance imaging study. *Cortex*, *74*, 134–148. https://doi.org/10.1016/j.cortex.2015.10.004

Vakil, E. (2005). The Effect of Moderate to Severe Traumatic Brain Injury (TBI) on Different Aspects of Memory : A Selective Review. *Journal of Clinical and Experimental Neuropsychology*, *27*(8), 977–1021. https://doi.org/https://doi.org/10.1080/13803390490919245

van der Werf, Y., Sanz-arigita, E. J., Menning, S., & van den Heuvel, O. A. (2010). Modulating spontaneous brain activity using repetitive transcranial magnetic stimulation. *BMC Neuroscience*, *11*(1), 145. https://doi.org/https://doi.org/10.1186/1471-2202-11-145

Vercammen, A., Knegtering, H., Liemburg, E., den Boer, A., & Aleman, A. (2010). Functional connectivity of temporo-parietal region in schizophrenia: Effects of rTMS treatment of auditory hallucinations. *Journal of Psychiatric Research*, *44*, 725–731. https://doi.org/10.1016/j.jpsychires.2009.12.011

Wang, J., Rogers, L. M., Gross, E. Z., Ryals, A. J., Dokucu, M. E., Brandstatt, K. L., Hermiller, M. S., & Voss, J. L. (2014). Targeted enhancement of cortical-hippocampal brain networks and associative memory. *Science*, *345*(6200), 1054–1057. https://doi.org/10.1126/science.1252900

Wang, J. X., & Voss, J. L. (2015). Long-lasting enhancements of memory and hippocampal-cortical functional connectivity following multiple-day targeted noninvasive stimulation. *Hippocampus*, *25*(8), 877–883. https://doi.org/10.1002/hipo.22416

23. Attachments/ Appendices

a. Eligibility Checklist

Inclusion criteria

- ☐ 18 to 40 years of age

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 or have body tattoos.
- ☐ Claustrophobia or cannot lie supine for 2 hours.
- ☐ Taking medications acting on the CNS.
- ☐ NINDS Behavioral Neurology Unit Employee or fellow

b. Pre-Screening Checklist

Healthy Volunteers

ii. Healthy Volunteers

- ☐ Are you between 18 and 40 years of age?
- ☐ 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 two-hour MRI?
- ☐ Are you pregnant or plan to be pregnant?
- ☐ Do you have any tattoos?

c. Amsterdam Resting-State Questionnaire

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					
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					

24. Consent Forms

Healthy Volunteer Consent