Protocol Title: Task-dependent effects of TMS on the neural biomarkers of episodic memory
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Abbreviated Title: ***Task-dependent TMS and memory***

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Title: Task-dependent effects of TMS on the neural biomarkers of episodic memory

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TABLE OF CONTENTS

STATEMENT OF COMPLIANCE

The protocol will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and trial site staff who are responsible for the conduct, management, or oversight of NIH-funded trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1 SYNOPSIS

Title: Task-dependent effects of TMS on the neural biomarkers of episodic memory

Study Description: The Behavioral Neurology Unit studies the human brain systems underlying learning and adaptation with the goal of finding interventions to make these processes more efficient. In this study, we are interested in examining how the task state of the episodic memory network influences the effect of faciliatory TMS on memory and its EEG neural correlates. It is hypothesized that Network-targeted parietal-TMS will improve memory performance and enhance EEG biomarkers of successful memory performance, but that these changes will be modulated by the ongoing task activity during stimulation.

Objectives:

Primary Objectives:

- Investigate how TMS modulates EEG neural measures of successful memory and the association of this modulation with behavioral performance
- Examine how memory task state influences susceptibility to plasticity via TMS and determine the optimal neural state for improving memory

Exploratory Objectives:

- Search for MRI predictors of the effects of TMS

Endpoints:

- Primary Endpoints: Memory performance, Late Positive Posterior ERP, evoked theta/alpha power (secondary), EEG functional connectivity (secondary)
- Exploratory Endpoints: fMRI resting state functional connectivity, fractional anisotropy

Study Population: 32 Healthy Volunteers ages 18-40. Referrals will occur from the NIH Clinical Research Volunteer program or through self-referrals to the protocol.

Description of Sites/Facilities: Protocol activities will occur at the OP5 clinic and 7SW clinic at the NIH clinical center.

Enrolling Participants: 50 Healthy Volunteers ages 18-40

Study Duration: 60 months

Participant Duration: 1 month

1.2 SCHEDULE OF ACTIVITIES (SOA)

Procedures	Enrollment/Screening Day -7 to -1	Baseline Visit 1, Day 1	Study Visit 2 Day 4 +/-3 day	Final Study Visit 3 Day 13 +/-8 day
Informed consent	X			
Demographics	X			
Medical history	X			
Concomitant medication review	X			
Physical exam	X			
Vital signs	X			
Height	X			
Weight	X			
3T MRI		X		
EEG			X	X
TMS			X	X
Cognitive Tasks			X	X
Pregnancy test [b]		X		
Complete Case Report Forms (CRFs)				X

a:
b: Urine pregnancy test (women of childbearing potential).

2 INTRODUCTION

2.1 STUDY RATIONALE

The Behavioral Neurology Unit studies the human brain systems underlying learning and adaptation with the goal of finding interventions to make these processes more efficient. This study will observe neural reorganization underlying episodic memory performance. The protocol will investigate how engaging in a memory task alters the activity and connectivity of the network and the relevance of those changes to successful task performance. Also, how this adaptation influences plasticity of the network will be examined. Using simultaneous TMS, EEG, and cognitive testing, this study will examine the network basis of memory processing, as well as elucidating potential mechanisms of TMS-induced memory improvement.

The overall objective of this study is to (1) examine the effects of TMS on EEG biomarkers of successful memory performance and (2) investigate how the task state of the episodic memory network influences the effect of faciliatory TMS on memory.

The results will answer basic science questions about the network basis of memory processing, with potential application to other cognitive networks and domains. Additionally, these data will provide a greater understanding of TMS as a tool for influencing the hippocampus, the network, and memory performance, allowing for optimization of stimulation for that purpose. Finally, this work will address clinical questions on how to improve memory and re-channel activity in networks via noninvasive stimulation for clinical purposes.

2.2 BACKGROUND

Episodic memory provides a means by which we reflect on the past, make decisions about the future, and form a learned identity. Deficits in this system occur in a variety of neurological and psychiatric disorders[1–5], making them one of the most debilitating problems in neurology[6,7]. However, there currently exist no effective treatments for memory impairment. A better understanding of how the brain adapts to perform a memory demand will not only expand our basic knowledge of memory processing but could inform novel treatments for individuals with memory impairments.

Episodic memory depends on the hippocampus[8,9] and a distributed set of regions which form the hippocampal-cortical network (HCN), including medial prefrontal, posterior cingulate, and medial and lateral parieto-occipital cortex[10–13]. This network shows changes in fMRI activity and connectivity in response to successful memory encoding and retrieval[14–18]. Likewise, abnormal function of this network has been associated with memory impairment in many disorders, including traumatic brain injury[19,20], epilepsy[21,22], and Alzheimer's disease[23,24]. Additionally, the HCN generates real-time EEG signatures of successful encoding and retrieval, most notably the Late Positive Posterior Event-Related Potential (ERP)[25] and oscillatory activity in the theta/alpha band[26].

There has been substantial recent interest in using brain stimulation to manipulate brain networks, both to test the role of particular regions and networks in memory processing causally and as a potential therapeutic tool in treating memory disorders[27–29]. Noninvasive transcranial magnetic stimulation (TMS) has been used to manipulate activity and connectivity in the HCN and improve memory performance in healthy adults[30–39]. Repeated treatment over days produces

lasting effects[30–36], but single sessions of cTBS have been effective[37,38] and time-locked cTBS can produce facilitation at the trial-by-trial level[39]. However, while enhancement of connectivity in the HCN is positively correlated with memory improvement, how TMS treatment increases the retention of information in the HCN and particularly the hippocampus remains unknown. Additionally, while the effects of time-locked cTBS have been examined in relation to fMRI activity in the hippocampus[39], the effect of HCN cTBS on EEG measures of successful memory has not been studied. Establishing these mechanisms will improve the basic understanding of how the brain successfully meets a memory demand and inform translational studies for the treatment of memory impairment. One goal of this project is to examine the effects of on-line TMS on the electrophysiological signatures of successful memory performance to provide more information on the mechanism by which TMS improves episodic memory to develop new and specific biomarkers of target engagement for future clinical studies.

The effects of brain stimulation have been shown previously to vary depending on neural state, such as the phase of oscillations relevant to the stimulated network[40,41] and network activation by simultaneous task performance[42–44]. However, the influence of brain state on HCN facilitation with TMS has not been investigated, despite its likely importance. A second goal of this project is to examine this question of task state-dependent modulation for the HCN and episodic memory.

Using simultaneous EEG, TMS, and memory testing, we will investigate the EEG activity related to successful memory performance and examine how TMS modulates that activity. In healthy volunteers, we will deliver brief, facilitatory TMS before and during the encoding trials of a memory task to either the HCN, via an inferior parietal cortex site with maximal hippocampal connectivity, or to the vertex as a control. We will additionally deliver stimulation during a control task with similar attentional demands and processing load, and there will be a no-stimulation condition for both tasks. We will record task performance and time-locked EEG activity, focusing on episodic memory performance and the late positive posterior ERP, theta/alpha power, and EEG functional connectivity measured during encoding and retrieval as our outcome measures.

2.3 RISK/BENEFIT ASSESSMENT

2.3.1 Known Potential Risks

TMS

Brief, self-limited, seizures were seen in early studies, before limits were established for combinations of delivery parameters and still occur occasionally. However, this risk has been reduced to the order of one in every 50,000 sessions in individuals without specific risk factors. For TMS, safety guidelines have been developed[45] and updated[46]. 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. Furthermore, the study participants are a lower risk since participants 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[47]. In that single patient, the study was conducted in at stimulation intensity levels higher than in this study.

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[48] and more recent has found no increase in auditory threshold from TMS[49,50]. All participants will wear earplugs to reduce the risk of cochlear damage.

Other than this, TMS does not appear to pose any hazard beyond that of electrical brain stimulation, which has been in clinical use for decades.

Behavioral measures

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

EEG

There are no major risks associated with EEG recording, however there is a possibility of discomfort from the electrode cap and abrading of the scalp during electrode application. Additionally, there may be electrode gel remaining in the hair following cap removal.

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.

Procedures to Minimize Risk

TMS

Study staff will be trained in TMS administration, TMS 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 TMS-trained personnel will be in the laboratory. A licensed independent practitioner (LIP), a physician or nurse practitioner trained in TMS, 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.

Persons operating TMS equipment will have been certified by a NINDS TMS laboratory or the NIMH Noninvasive Neuromodulation Unit (NNU) 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 user, an individual must have passed the following criteria, as assessed by senior BNU or NNU staff:

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) Training on basic TMS safety and risks by TMS-trained physician
6) Training on recognition and initial response to seizures

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 participant's request.

EEG

To reduce discomfort during electrode application, the researcher will use care in securing the cap for comfort and in moving hair to clear access to the scalp. Additionally, after cap removal, participants will be provided with shampoo and sink space to clean gel from their hair should they desire.

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.

2.3.2 Known Potential Benefits

This study does not offer direct benefit to participants but is likely to yield generalizable knowledge about TMS and the effects on memory.

2.3.3 Assessment of Potential Risks and Benefits

Brief, self-limited, seizures were seen in early studies, before limits were established for combinations of delivery parameters and still occur occasionally. However, this risk has been reduced to the order of one in every 50,000 sessions in individuals without specific risk factors. Extensive training will be administered by Dr. Eric Wassermann, a neurologist and an expert in TMS, so study staff can effectively recognize and respond to adverse effects. Additionally, a licensed independent practitioner (LIP), a physician or nurse practitioner trained in TMS, will be in house, specifically aware of the session, and reachable by phone for all TMS sessions.

Although this risk is more than minimal, the information to be gained is of great benefit. Episodic memory deficits occur in a variety of neurological and psychiatric disorders[1–5], making them one of the most debilitating problems in neurology[6,7]. However, there currently exist no effective treatments for memory impairment. TMS has shown promise as a method of improving memory in healthy adults[30–39], including older adults[51]. A better understanding of the effects of this tool on memory processing could inform novel treatments for individuals with memory impairments.

3 OBJECTIVES AND ENDPOINTS

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
Primary		
1. Investigate how TMS modulates EEG	1. Memory performance	1. Memory performance measures will allow us to

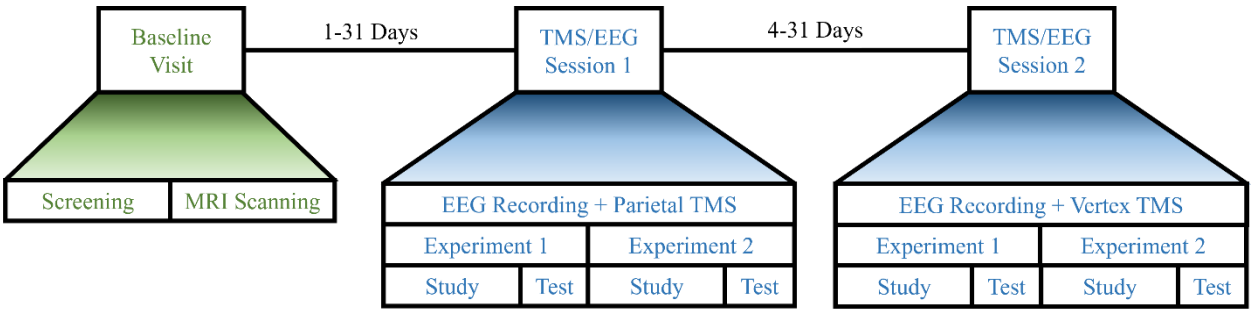
OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
neural measures of successful memory and the association of this modulation with behavioral performance 2. Examine how memory task state influences susceptibility to plasticity via TMS and determine the optimal neural state for improving memory	• Accuracy: Percentage of successful (later remembered hit and correct rejection) vs unsuccessful (forgotten miss and false alarm) trials for item recognition (remember/familiar/new) and context recollection (spatial location) • d': Analysis of hits (correctly remembered as encoded) relative to false alarms (new objects labelled as encoded) for item recognition 2. Late positive posterior ERP • ERP amplitude 500-700 ms after trial onset (encoding and retrieval) over parietal and occipital electrodes 3. Theta/alpha power (secondary) • Power in the 4-13 Hz band 0-1000 ms after trial onset (encoding and retrieval) 4. EEG functional connectivity (secondary) • Pearson correlation coefficients (r) between electrode time courses after trial onset (encoding and retrieval)	examine the effects of TMS on performance, as well as to sort successful vs unsuccessful trials for EEG analyses 2. The late positive posterior ERP has been previously shown to be a robust marker of successful memory performance[25] 3. Task-induced changes in this frequency band have been previously associated with successful memory performance[26] 4. fMRI functional connectivity modulation via network-targeted TMS has been previously associated with TMS-induced memory improvement. Whether EEG functional connectivity will be similarly altered, and the relevance to memory changes, remains unknown
Tertiary/Exploratory		
1. Search for MRI predictors of the	1. Baseline fMRI functional connectivity	It is hypothesized that TMS influences downstream targets

OBJECTIVES	ENDPOINTS	JUSTIFICATION FOR ENDPOINTS
effects of parietal TMS	• Resting state functional connectivity at baseline between the hippocampus and cortical regions 2. Baseline DTI structural connectivity • Fractional anisotropy (FA) at baseline between the hippocampus and cortical regions	(i.e., regions other than the stimulation site) via network connections. Associations between TMS outcomes and structural or functional connectivity would support this hypothesis, elucidating potential mechanisms of TMS effects

4 STUDY DESIGN

This protocol is a single site study. The study will include a baseline 3T MRI session, and two experiments conducted during two sessions of simultaneous TMS, EEG, and cognitive testing (Figure 1). Both experiments will use the same participants to allow for within-subjects comparisons and the order of experiments will be counterbalanced. Participants who miss a session will be rescheduled or replaced (see Recruitment). Participants will make three visits to the lab, a baseline assessment session and two TMS/EEG sessions, for a time commitment of approximately 8 hours. TMS/EEG Session 1 will occur 1-31 days following the baseline visit and TMS/EEG Session 2 will occur 4-31 days following TMS/EEG Session 1 (Figure 1).

Figure 1. Study Design



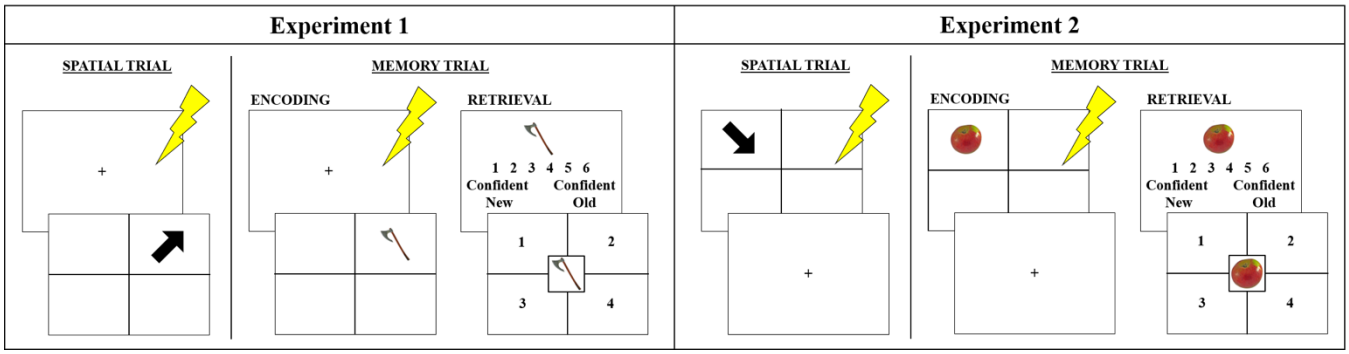
On each TMS/EEG day, participants will undergo EEG recording using a 64-channel elastic cap. During recording, they will complete two blocks of a task, one for each experiment, which differ only in their timing of TMS delivery. Each block consists of 90 study trials of episodic memory encoding (45 trials) and spatial processing (45 trials) for a total of 180 trials (90 memory and 90 spatial processing) per TMS/EEG session (Table 1).

Table 1. Conditions and trial counts.

	Parietal Stimulation				Vertex Stimulation				TOTAL	
	Stim On		Stim Off		Stim On		Stim Off			
	Memory	Spatial	Memory	Spatial	Memory	Spatial	Memory	Spatial		
Experiment 1	30	30	15	15	30	30	15	15	**180**	**360**
Experiment 2	30	30	15	15	30	30	15	15	**180**	

TMS will be paired with task trials and consist of 2-second trains of continuous theta burst stimulation (cTBS; see Study Procedures). In Experiment 1, TMS will be delivered during the rest phase immediately prior to trial onsets (Figure 2). In Experiment 2, TMS pulses will occur during the trial while participants are actively engaged in the task (Figure 2). The order of experiments will be counterbalanced across subjects within each session. TMS will be delivered to an episodic memory network-connected location in posterior parietal cortex (see 4.4.2. TMS, below) or to the vertex as an inactive control condition, and the order of stimulation location will be counterbalanced across TMS/EEG sessions. One-third of trials will contain no stimulation as an additional control to examine behavioral and neural effects in the absence of the TMS sensory artifact (Table 1). Participants will then be tested on encoded items, also with EEG recording. EEG data will be examined in an event-related design, with timeseries following trial onsets compared across task (memory encoding, spatial processing, and memory retrieval) and stimulation (parietal-TMS, vertex-TMS, and no-TMS) conditions.

Figure 2. Experiment Design



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 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".

It is possible that some participants will miss a session. If this occurs, and the participant is still willing to participate, we will reschedule. If a participant no longer wishes to continue with the study, a new participant will be recruited for the full study. If they have data from one experiment, those data will still be included in analysis for that experiment but excluded from any comparisons across experiments.

It is possible that technical problems may arise. If this occurs, and the participant is willing to continue with the study, we will reschedule.

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.

In some cases, consent, screening, and examination (when required) may be performed on the same day as the baseline MRI.

Study procedures:

Behavioral Tasks

Memory Task: This is a test of episodic memory, including item recognition and context recollection[30,52,53]. In the task, participants will be shown a series of objects in one of four quadrants on the screen. They will be instructed to remember the objects and where they were located, both of which will be tested later. To ensure attention and for consistency with the spatial task (see below), they will be asked to indicate by button press whether the object is primarily used outdoors (e.g., a baseball) or indoors (e.g., a television). During the encoding phase, each object will be presented for 4 seconds with 4 seconds between trials. In Experiment 1, TMS will occur during a 2 second cue before each trial and in Experiment 2, TMS will occur in the first 2 seconds of stimulus object presentation. There will be 180 encoding trials, interspersed with 180 trials of the spatial task (described below). Trial order will be randomized. The retrieval phase will occur following the end of encoding. Participants will be shown, sequentially and in randomized order, the 180 encoded objects and 180 new lure objects, for a total of 360 trials. For all trials, they will respond with whether the object was old (previously seen) or new (not previously seen) and their confidence on a scale of 1-6 (1=confident new,

6=confident old) as a measure of item recognition. If the object was previously seen, participants will then be asked which quadrant the item was presented in to provide a measure of context recollection. We will use two different versions of the task with different and non-overlapping sets of objects at the two test sessions and the order of versions will be counterbalanced across participants.

Spatial Task: This is a test of spatial processing, which will serve as a control task to isolate neural markers of memory engagement, versus engagement in any task with similar visuospatial qualities. Notably, spatial processing activates a network of frontoparietal including the posterior parietal cortex regions[54,55], allowing it to further serve as a control to isolate hippocampal network effects of TMS from the local effects of TMS on the stimulated region. In each trial of this task, participants will be shown an arrow pointing to one corner of the screen and displayed in one of four quadrants. They will be instructed to indicate by button press whether the arrow points to the corner in which it is displayed ("match," e.g. pointing to the upper right corner in the upper right quadrant) or a different corner ("no-match," e.g. pointing to the upper right corner in the upper left quadrant). Accuracy of match/no-match responses will be assessed. Each arrow will be presented for 4 seconds with 4 seconds between trials. In Experiment 1, TMS will occur during a 2 second cue before each trial and in Experiment 2, TMS will occur in the first 2 seconds of stimulus presentation. There will be 180 trials, interspersed with encoding trials of the memory task. We will use two different versions of the task at the two test sessions.

TMS

The parietal target will be the region of the left posterior parietal cortex with the greatest connectivity with the left hippocampus derived from the baseline resting-state fMRI session[31]. This region was chosen because of its dense connections with the hippocampus[56,57]. Thus, stimulation of this location can modulate function of the hippocampal-cortical network[30–36,38].

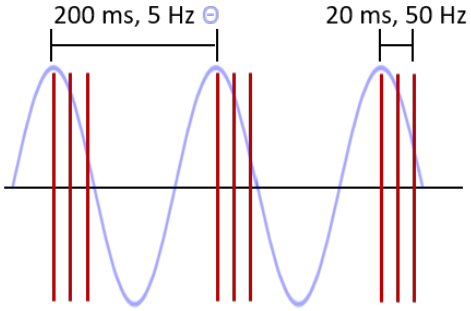
The control target will be vertex, a commonly used active control site which does not influence neural or behavioral activity[58].

TMS 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[31] will be used as our location of stimulation. To reduce the influence of diurnal variations in the responsiveness to neuroplasticity protocols[59], we will make every effort to test subjects during the same time of day.

Stimulation will be delivered during the encoding phase of the behavioral task, either prior to trial onset (Experiment 1) or simultaneously with trial onset (Experiment 2; see 4.4.1 Behavioral Tasks for timing) in the form of 2-seconds of theta-burst stimulation (50 Hz pulse triplets every 200 ms; Figure 3) at 80% of the motor evoked potential threshold of the right abductor pollicis brevis muscle or 44% of maximum stimulator output, whichever is lower. This protocol has been

shown previously to particularly influence the hippocampal-cortical network and memory performance[37–39].

Figure 3. Theta-burst stimulation.



EEG

Participants will be fitted with a 64-channel elastic EEG cap for the duration of each experiment session. Data will be recorded continuously during the study and test phases of the behavioral experiment. The EEG signal will be amplified online, filtered, and digitized continuously along with stimulus onset codes used for subsequent analysis. Independent component analysis and/or other processing techniques will be performed to isolate and remove recurring artifacts from the signal.

Single-trial waveforms will be screened for artifactual contamination. Trials will then be divided based on task type (spatial, memory encoding, accuracy of item recall (memory task) and spatial task performance. The timeseries from trials of the same type and/or accuracy will then be averaged, and the resulting waveforms will be compared across categories. For theta/alpha band analysis, we will additionally use time-frequency decomposition of the event-related averages.

MRI

We estimate a maximum of 1 hour for baseline scanning. Because scanner malfunctions and subsequent loss of data are common, we will not report these as unexpected problems. Participants whose data are lost due to scanner malfunctions will be rescheduled, if possible. Participants whose MRI sessions cannot be completed may enter the experiment if the MPRAGE (see below) data have been acquired. They will be rescheduled for the other scans when possible.

MRI anatomical scanning: All participants will have anatomical (MPRAGE) scans at baseline. 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.

Resting-state scanning: All participants will receive a resting-state fMRI scan at baseline for identification of a TMS target. 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).

Diffusion-weighted scanning: Participants will have a diffusion-weighted scan for diffusion tensor imaging (DTI) scanning at baseline to estimate white matter structural connectivity. This phase will take approximately 20 min. This may be omitted in some cases due time constraints.

Justifications for MRI and TMS non-significant risk (NSR) designation

Research Pulse Sequences:
a. These custom sequences all operate under the International Electrotechnical Commission (IEC) 60601-2-33, First Level-Controlled Operating Mode. The IEC/FDA set operating mode limits on the MRI for RF heating (Specific Absorption Rate) and Time-varying magnetic field gradients (dB/dt) that are fully operational for all research pulse sequences. This device has not received pre-market approval or 510 (K) clearance by the FDA.

MRI Research Analysis and Reconstruction Software:
a. This software conforms to operate under the International Electrotechnical Commission (IEC) 60601-2-33, First Level-Controlled Operating Mode. The IEC/FDA set operating mode limits on the MRI for RF heating (Specific Absorption Rate) and Time-varying magnetic field gradients (dB/dt) that are fully operational for all research pulse sequences. This device has not received pre-market approval or 510 (K) clearance by the FDA.

Magstim Rapid2 with Air Film coil TMS (Magstim):

a. This device is classified as an FDA cleared device with 510(K) clearance by the FDA #K143531, K162935. The indicated use according to the 510(K) is the treatment of Major Depressive Disorder in adult patients who have failed to achieve satisfactory improvement from prior antidepressant medication in the current episode. In this study, this device is intended to be used to investigate how TMS modulates EEG neural measures of successful memory and the association of this modulation with behavioral performance, and not for diagnostic purposes. Therefore, it does not conform to the 510(k) label.

BrainVision actiCHamp Plus EEG (Brain Vision):

a. The Sponsor has confirmed with the manufacturer that there is no market clearance or approval for this device in the US and that the device does not meet criteria for exemption under 21CFR812. This device has not received pre-market approval or 510 (K) clearance by the FDA.

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-significant 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;
   No. All devices listed in this section are not intended as implants and do not present a potential for serious risk to the health, safety, and welfare of a subject
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;

   No. All devices listed in this section are not purported or represented to be for
3. *a use in supporting or sustaining human life and presents no potential for serious risk to the health, safety, or welfare of a subject.*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

   No. All devices listed in this section are not intended for use of substantial importance in diagnosing, curing mitigating, or treating disease, or otherwise preventing impairment of human health and presents no 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

   No. All devices listed in this section do not present a potential for serious risk to the health, safety, or welfare of subjects.

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

Devices determined to be used on label or other exempted devices pursuant to 21CFR812.2(c):

GE Discovery MR 750 System–3T. 510k: K163331

a. This device and associated system carries an FDA 510(k) clearance . The Sponsor has determined that the device is used in the trial in accordance with this label.

Primary Objective

Primary Objective 1: Investigate how TMS modulates EEG neural measures of successful memory and the association of this modulation with behavioral performance

- Hypotheses

- Network-targeted parietal-TMS will improve memory performance (context recollection; Primary Outcome Measure 1) on parietal-TMS relative to vertex-TMS trials
- Relative to vertex-TMS, parietal-TMS will also increase the Late Positive Posterior ERP (Primary Outcome Measure 2) and theta power (Secondary Outcome Measure 1) during encoding on successful trials, relative to unsuccessful trials
- The EEG effects will correlate with memory improvement (Correlation, Primary Outcome Measure 1 and Primary Outcome Measure 2/Secondary Outcome Measure 1)
- Additionally, parietal-TMS will alter EEG functional connectivity patterns (Exploratory Outcome Measure 1) at encoding during successful trials relative to unsuccessful trials
- Parietal-TMS will not influence neural activity or accuracy for spatial trials, relative to vertex-TMS and no-TMS

Primary Objective 2: Examine how memory task state influences susceptibility to plasticity via TMS and determine the optimal neural state for improving memory

- Hypotheses
  - Network-targeted parietal-TMS during memory encoding will have a larger effect on source recollection (Primary Outcome Measure 1), relative to pre-trial parietal-TMS and vertex-TMS during encoding
  - Exploratory Analyses:
    - Parietal-TMS during memory encoding will increase the Late Positive Posterior ERP (Primary Outcome Measure 2) and theta-power (Secondary Outcome Measure 1), relative to pre-trial parietal-TMS and vertex-TMS during encoding
    - Additionally, parietal-TMS will alter EEG functional connectivity patterns (Exploratory Outcome Measure 1) during later-remembered trials, relative to pre-trial parietal-TMS and vertex-TMS during encoding
    - Network-targeted TMS will not influence neural activity during arithmetic trials, relative to vertex-TMS and no-TMS

Exploratory Objective:

Search for MRI predictors of the effects of parietal TMS

- Measurement of baseline resting-state functional connectivity (Exploratory Outcome Measure 3) and fractional anisotropy (Exploratory Outcome Measure 4) between the hippocampus and cortical regions of the episodic memory network, particularly the precuneus[60] , and look for correlations with the effects of TMS on memory and task-related EEG events across participants.

End of participation

Participants 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.

4.1 CONCOMITANT THERAPY

Not applicable

5 STUDY POPULATION

5.1 INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Stated willingness to comply with all study procedures and availability for the duration of the study
2. Ability of subject to understand and the willingness to sign a written informed consent document.
3. Age 18-40 (inclusive)

5.2 EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1.) Any current major neurological or psychiatric disorder such as (but not limited to) stroke, Parkinson disease, Alzheimer disease, schizophrenia or major depression
2.) History of seizure
3.) 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.
4.) Inability to provide informed consent
5.) Ferromagnetic metal in the cranial cavity or eye, implanted neural stimulator, cochlear implant, or ocular foreign body
6.) Implanted cardiac pacemaker or auto-defibrillator or pump
7.) Non-removable body piercing
8.) Claustrophobia
9.) Inability to lie supine for 1 hour
10.) Pregnancy, or plans to become pregnant during the study.
11.) Members of the NINDS BNU
12.) Subjects who have contraindications to MRI will follow the NMR Center guidelines for MR safety

An eligibility checklist is provided in Appendix A.

5.3 INCLUSION OF VULNERABLE PARTICIPANTS

5.3.1 Participation of Employees

NIH employees may be enrolled in this study as this population meets the study entry criteria. Neither participation nor refusal to participate as a subject in the research will have an effect, either beneficial or adverse, on the participant's employment or position at NIH.

Every effort will be made to protect participant information, but such information may be available in medical records and may be available to authorized users outside of the study team in both an identifiable an unidentifiable manner.

The NIH Information Sheet on Employee Research Participation will be made available. Please see section **9.1.5** for consent of employees.

5.3.2 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 of childbearing potential will have a pregnancy test before each MRI session. Women who are pregnant will be excluded and women who can become pregnant will be excluded following a positive pregnancy test.

5.3.3 Adults who lack capacity to consent

Adults who are unable to provide initial informed consent will be excluded from this study. Adults who permanently lose the capacity to provide on-going consent subsequent to giving initial consent will be removed from the study and compensated for what they have completed.

5.4 LIFESTYLE CONSIDERATIONS

Not applicable

5.5 SCREEN FAILURES

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently entered in the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this study (screen failure) because of a medication or pregnancy may be rescreened. Rescreened participants should be assigned the same participant number as for the initial screening.

5.6 STRATEGIES FOR RECRUITMENT AND RETENTION

The protocol will study 32 healthy individuals. 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 current experience, the anticipated non-completion rate is 22%, due to screen failures, individual characteristics making stimulation impossible, such as very high motor evoked potential threshold, non-removable hair braiding, and other issues discovered after consenting.

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. 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.

It is possible that some participants will miss a session. If this occurs, and the participant is still willing to participate, they will be rescheduled. If a participant no longer wishes to continue with the study, a new participant will be recruited for the full study. If they have data from one experiment, those data may be included in analysis for that experiment but excluded from any comparisons across experiments.

It is possible that technical problems may arise. If this occurs, and the participant is willing to continue with the study, they will be rescheduled.

When data from a subject are not usable due to noise or other technical problems, a new participant will be recruited for the full study to achieve a sample of 32 complete subjects.

5.6.1 Costs

N.A.

5.6.2 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

Participants will be paid $10.00 per one Inconvenience Unit (IU).

fMRI testing (4 IU)	$40
TMS testing (4 IU)	$40
EEG testing (2 IU)	$20

Behavioral Tasks (2 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.

6 PARTICIPANT DISCONTINUATION/WITHDRAWAL

6.1 PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Participants are free to withdraw from participation in the study at any time upon request.

An investigator may discontinue or withdraw a participant from the study for the following reasons:

- Significant non-compliance
- If the participant meets an exclusion criterion (either newly developed or not previously recognized) that precludes further study participation
- Subject has completed the study follow-up period
- Death
- Screen Failure

The reason for participant discontinuation or withdrawal from the study will be recorded on the Case Report Form (CRF).

6.2 LOST TO FOLLOW-UP

A participant will be considered lost to follow-up if he or she fails to return for two scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will make one attempt to contact the participant and reschedule the missed visit within one week and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make one attempt to regain contact with the participant either by email or telephone.
- Should the participant continue to be unreachable, despite this attempt, he or she will be considered lost to follow-up.

- It is possible that a participant deemed lost to follow-up will recontact the investigators to return to the study. In this case, if it is within six months of their consent date, they will be rescheduled. The subject will keep their previously assigned subject number.

7 STUDY ASSESSMENTS AND PROCEDURES

7.1 SCREENING PROCEDURES

7.1.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects.
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images.

7.1.2 Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the subject has signed the consent this study:

- Pregnancy test
- Clinical MRI scan, if none has been obtained within the last year

7.2 CLINICAL EVALUATIONS

MRI: 3T MRI will take an estimated 1 hour for baseline scanning.

MRI anatomical scanning: All participants will have anatomical (MPRAGE) scans at baseline. 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.

Resting-state scanning: All participants will receive a resting-state fMRI scan at baseline for identification of a TMS target. 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).

Pregnancy testing: All participants of childbearing potential will undergo pregnancy testing before each MRI scan.

Assessment of adverse events will be monitored, tracked, documented, and reported by study investigators and the PI

7.3 BIOSPECIMEN EVALUATIONS

Not applicable

7.3.1 Correlative Studies for Research

Not applicable

7.3.2 Samples for Genetic/Genomic Analysis

Not applicable

8 STATISTICAL CONSIDERATIONS

8.1 STATISTICAL HYPOTHESIS

The primary aims are to investigate how TMS alters the EEG biomarkers of successful memory performance and how memory state-related neural changes influence plasticity of the brain. Secondary aims for this protocol will include examining the MRI connectivity predictors of TMS effects.

- Primary Objective 1*:* Investigate how TMS modulates EEG neural measures of successful memory and the relevance of modulation to behavioral performance
  - Hypotheses
    a. Network-targeted parietal-TMS will improve memory performance (context recollection; Primary Endpoint 1) relative to vertex-TMS trials
    b. Relative to vertex-TMS, parietal-TMS will also increase the Late Positive Posterior ERP (Primary Endpoint 2) and theta power (Primary Endpoint 3) during encoding on successful trials, relative to unsuccessful trials
    c. Parietal-TMS will alter EEG functional connectivity patterns (Primary Endpoint 4) at encoding during successful trials relative to unsuccessful trials
    d. The EEG effects will correlate with memory improvement (Correlation, Primary Endpoint 1 and 2-4)
    e. Parietal-TMS will not influence neural activity or accuracy for spatial trials, relative to vertex-TMS and no-TMS

- Primary Objective 2: Examine how memory task state influences susceptibility to plasticity via TMS and determine the optimal neural state for improving memory
  - Hypotheses
    a. Network-targeted parietal-TMS during memory encoding will have a larger effect on memory performance (Primary Endpoint 1), relative to pre-trial parietal-TMS and vertex-TMS during encoding
    b. Exploratory Analyses:
       i. Parietal-TMS during memory encoding will increase the Late Positive Posterior ERP (Primary Endpoint 2) and theta-power (Primary Endpoint 3), relative to pre-trial parietal-TMS and vertex-TMS during encoding
       ii. Additionally, parietal-TMS will alter EEG functional connectivity patterns (Primary Endpoint 4) during later-remembered trials, relative to pre-trial parietal-TMS and vertex-TMS during encoding
       iii. Network-targeted TMS will not influence neural activity during arithmetic trials, relative to vertex-TMS and no-TMS

- *Exploratory Objective:* Determine MRI predictors of the effects of noninvasive stimulation
  We will examine the correlations between stimulation effects on our behavioral and neural outcome measures (Primary Endpoint 1 and Primary Endpoints 2-4) and MRI measures of structural and functional connectivity (Exploratory Endpoints 1-2). For each participant, the Pearson correlation between (1) the difference in outcome measures paired with parietal-TMS and vertex-TMS and (2) hippocampal FA or hippocampal functional connectivity will be calculated. Thresholds for significant effects will be set at $p<0.05$ following FDR correction for multiple comparisons.

8.2 SAMPLE SIZE DETERMINATION

All experiments in this protocol are powered based on the primary outcome, which relates to the effects of TMS on memory and its neural correlates.

A power analysis was conducted using G*Power v3.1.9.7. We used a repeated-measures, within-factors ANOVA based on a previous study with a similar design[39]. Like the proposed experiments, this study delivered 2-second trains of TMS prior to trials of memory encoding or a control task and examined the effects on later memory performance and on neural activity, in this case hippocampal fMRI activity, during encoding. They used a three-way repeated-measures ANOVA to examine the interactions between TMS presence (on vs off), frequency (theta-burst vs 12.5 Hz beta), and location (parietal cortex vs supplementary motor area) and found an effect size of theta burst parietal-TMS on memory performance of $\eta^2=0.44$ and fMRI hippocampal activity of $\eta^2=0.28$. As the proposed analyses will also use three-way repeated-measures ANOVAs (see Section 8.3.2), we calculated subject number based on these effect sizes, power of 0.80, and $\alpha = 0.05$ shared across two primary outcomes, memory performance and the late positive posterior ERP ($\alpha = 0.025$ for each outcome). Thirty-two participants with complete data will be needed. To achieve n=32, we will recruit up to 50 subjects to account for potential non-completion (e.g., screen failure, subject withdrawal) or unusable data (e.g., due to noise).

8.3 STATISTICAL ANALYSES

8.3.1 General Approach

The primary aims are to investigate how TMS alters the EEG biomarkers of successful memory performance and how memory state-related neural changes influence plasticity of the brain. Secondary aims for this protocol will include examining the MRI connectivity predictors of TMS effects.

8.3.2 Analysis of the Primary Endpoints

- Primary Endpoint 1*:* Investigate how TMS modulates EEG neural measures of successful memory and the relevance of modulation to behavioral performance
  This aim will examine behavioral and EEG neural outcome measures (Primary Endpoints 1-4). We will perform 3x3 ANOVAs on each outcome measure with the factors trial type (successful/ unsuccessful/ spatial), stimulation site (parietal/ vertex/ none), and their interaction. We will particularly focus on the main effect of stimulation site to determine

the influence of parietal-TMS on memory performance and its neural correlates, and the interaction effect to determine the task specificity of TMS effects. Thresholds for significant effects will be set at $p<0.025$ following FDR correction for multiple comparisons.

- Primary Endpoint 2: Examine how memory task state influences susceptibility to plasticity via TMS and determine the optimal neural state for improving memory
  This aim will examine behavioral and EEG neural outcome measures (Primary Endpoints 1-4). We will perform 3x3x2 ANOVAs on each outcome measure with the factors trial type (successful/ unsuccessful/ spatial), stimulation site (parietal/ vertex/ none), stimulation timing (pre-trial and mid-trial) and their interaction. We will particularly focus on the interaction effect of stimulation site and timing to determine the influence of memory task-state parietal-TMS on memory performance and its neural correlates, and the three-way interaction effect to determine the task specificity of state-dependent TMS effects. Thresholds for significant effects will be set at $p<0.025$ following FDR correction for multiple comparisons.

8.3.3 Tabulation of individual Participant Data

Not applicable

8.3.4 Exploratory Analyses

Determine MRI predictors of the effects of noninvasive stimulation: The correlations between stimulation effects on our behavioral and neural outcome measures (Primary Outcome Measures 1-2, Secondary Outcome Measure 1, Exploratory Outcome Measure 1) and MRI measures of structural and functional connectivity (Exploratory Outcome Measures 2-3) will be examined. For each participant, the Pearson correlation between (1) the difference in outcome measures paired with parietal-TMS and vertex-TMS and (2) hippocampal FA or hippocampal functional connectivity will be calculated. Thresholds for significant effects will be set at $p<0.05$ following FDR correction for multiple comparisons.

9 REGULATORY AND OPERATIONAL CONSIDERATIONS

9.1 INFORMED CONSENT PROCESS

9.1.1 Consent/Assent Procedures and Documentation

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.

The consent form contains all required elements. The consent form is submitted with this protocol.

9.1.2 Consent for minors when they reach the age of majority

Not applicable

9.1.3 Telephone consent

Not applicable

9.1.4 Telephone assent

Not applicable

9.1.5 Considerations for Consent of NIH employees

Consent for NIH employees will be obtained as detailed above with following additional protections:

Consent from employees will be obtained by an individual independent of the employee's team whenever possible. Otherwise, the consent procedure will be independently monitored by the CC Department of Bioethics Consultation Service in order to minimize the risk of undue pressure on the employee.

9.1.6 Consent of Subjects who are/become Decisionally Impaired

Not applicable

9.2 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, and the Institutional Review Board (IRB), and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the IRB.

9.3 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence.

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

The study monitor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator,

including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the NIH. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and by NIH research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

9.4 FUTURE USE OF STORED SPECIMENS AND DATA

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored on password-protected computers or backed up on media stored in locked cabinets within locked BNU offices. Keys to participant identity will be stored in lab notebooks, available only to study investigators. Samples will not be stored under this protocol. All data will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number.

Genetic testing will not be performed.

9.5 SAFETY OVERSIGHT

9.5.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis (approximately weekly) when subjects are being actively enrolled/evaluated on the study to discuss each subject.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in HRPP Policy 801 will be submitted within the required timelines.

The principal investigator will review all data on each subject to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

9.6 CLINICAL MONITORING

Not applicable

9.7 QUALITY ASSURANCE AND QUALITY CONTROL

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

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

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.

9.8 DATA HANDLING AND RECORD KEEPING

Data Collection and Management Responsibilities

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Hardcopies of the study visit worksheets will be provided for use as source document worksheets for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into CiSTAR, a 21 CFR Part 11-compliant data capture system provided by the NINDS. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

9.8.1 Study Records Retention

Study documents should be retained as per the NIH Intramural Records Retention Schedule. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the investigator when these documents no longer need to be retained.

9.9 UNANTICIPATED PROBLEMS

9.9.1 Definition of Unanticipated Problems (UP)

Any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review

Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied; and

- Related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places participants or others (which many include research staff, family members or other individuals not directly participating in the research) at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or expected.

9.9.2 Unanticipated Problem Reporting

The investigator will report unanticipated problems (UPs) to the NIH Institutional Review Board (IRB) as per Policy 801.

9.10 Protocol Deviations and Non-Compliance

It is the responsibility of the investigator to use continuous vigilance to identify and report deviations and/or non-compliance to the NIH Institutional Review Board as per Policy 801. The investigator is responsible for knowing and adhering to the reviewing IRB requirements.

9.10.1 NIH Definition of Protocol Deviation

A protocol deviation is any changed, divergence, or departure from the IRB-approved research protocol.

- Major deviations: Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.
- Minor deviations: Deviations that do not have the potential to negatively impact the rights, safety or welfare of subjects or others, or the scientific integrity or validity of the study.

9.11 Publication and Data Sharing Policy

9.11.1 Human Data Sharing Plan

This study will be conducted in accordance with the following publication and data sharing policies and regulations:

National Institutes of Health (NIH) Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

This study will comply with the NIH Data Sharing Policy and Policy on the Dissemination of NIH-Funded Clinical Trial Information and the Clinical Trials Registration and Results Information Submission rule. As such, this trial will be registered at ClinicalTrials.gov, and results information from this trial will be submitted to ClinicalTrials.gov. In addition, every attempt will be made to publish results in peer-reviewed journals. Data from this study may be

requested from other researchers after the completion of the primary endpoint by contacting Dr. Eric Wassermann.

9.11.2 Genomic Data Sharing Plan

Not applicable

9.12 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NINDS has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

10 ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of Covariance
CFR	Code of Federal Regulations
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
cTBS	Continuous Theta Burst Stimulation
DCC	Data Coordinating Center
DTI	Diffusion Tensor Imaging
EC	Ethics Committee
EEG	Electroencephalography
ERP	Event Related Potential
eCRF	Electronic Case Report Forms
FA	Fractional Anisotropy
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IRB	Institutional Review Board
MRI	Magnetic Resonance Imaging
NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan

SMC	Safety Monitoring Committee
SOA	Schedule of Activities
SOP	Standard Operating Procedure
TMS	Transcranial Magnetic Stimulation
UP	Unanticipated Problem
US	United States

11 REFERENCES

1. L. Beason-Held, L. Dementia and the Default Mode. *Curr. Alzheimer Res.* **8**, 361–365 (2011).

2. Henson, R. N. *et al.* The effects of hippocampal lesions on MRI measures of structural and functional connectivity: Connectivity Change after Hippocampal Lesion. *Hippocampus* **26**, 1447–1463 (2016).

3. Sheline, Y. I. *et al.* The default mode network and self-referential processes in depression. *Proc. Natl. Acad. Sci.* **106**, 1942–1947 (2009).

4. Whitfield-Gabrieli, S. & Ford, J. M. Default Mode Network Activity and Connectivity in Psychopathology. *Annu. Rev. Clin. Psychol.* **8**, 49–76 (2012).

5. Whitfield-Gabrieli, S. *et al.* Hyperactivity and hyperconnectivity of the default network in schizophrenia and in first-degree relatives of persons with schizophrenia. *Proc. Natl. Acad. Sci.* **106**, 1279–1284 (2009).

6. Nestor, P. J., Fryer, T. & Hodges, J. R. Declarative memory impairments in Alzheimer ' s disease and semantic dementia semantic dementia. *NeuoImage* **30**, 1010–1020 (2005).

7. Vakil, E. The Effect of Moderate to Severe Traumatic Brain Injury (TBI) on Different Aspects of Memory : A Selective Review. *J. Clin. Exp. Neuropsychol.* **27**, 977–1021 (2005).

8. Scoville, W. B. & Milner, B. LOSS OF RECENT MEMORY AFTER BILATERAL HIPPOCAMPAL LESIONS. *J. Neurol. Neurosurg. Psychiatry* **20**, 11–21 (1957).

9. Squire, L. R. Memory and the hippocampus: A synthesis from findings with rats, monkeys, and humans. *Psychol. Rev.* **99**, 195–231 (1992).

10. Buckner, R. L., Andrews-Hanna, J. R. & Schacter, D. L. *The Brain's Default Network*: *Anatomy, Function, and Relevance to Disease*. *Ann. N. Y. Acad. Sci.* **1124**, 1–38 (2008).

11. Rugg, M. D. & Vilberg, K. L. Brain networks underlying episodic memory retrieval. *Curr. Opin. Neurobiol.* **23**, 255–260 (2013).

12. Eichenbaum, H. A cortical–hippocampal system for declarative memory. *Nat. Rev. Neurosci.* **1**, 41–50 (2000).

13. Ranganath, C., Heller, A., Cohen, M. X., Brozinsky, C. J. & Rissman, J. Functional connectivity with the hippocampus during successful memory formation. *Hippocampus* **15**, 997–1005 (2005).

14. Paller, K. A. & Wagner, A. D. Observing the transformation of experience into memory. *Trends Cogn. Sci.* **6**, 93–102 (2002).

15. Buckner, R. L., Wheeler, M. E. & Sheridan, M. A. Encoding Processes during Retrieval Tasks. *J. Cogn. Neurosci.* **13**, 406–415 (2001).

16. Warren, K. N. *et al.* Increased fMRI activity correlations in autobiographical memory versus resting states. *Hum. Brain Mapp.* **39**, 4312–4321 (2018).

17. Gratton, C., Laumann, T. O., Gordon, E. M., Adeyemo, B. & Petersen, S. E. Evidence for Two Independent Factors that Modify Brain Networks to Meet Task Goals. *Cell Rep.* **17**, 1276–1288 (2016).

18. King, D. R., Chastelaine, M. de, Elward, R. L., Wang, T. H. & Rugg, M. D. Recollection-Related Increases in Functional Connectivity Predict Individual Differences in Memory Accuracy. *J. Neurosci.* **35**, 1763–1772 (2015).

19. Sharp, D. J. *et al.* Default mode network functional and structural connectivity after traumatic brain injury. *Brain* **134**, 2233–2247 (2011).

20. Mayer, A. R., Mannell, M. V., Ling, J., Gasparovic, C. & Yeo, R. A. Functional connectivity in mild traumatic brain injury. *Hum. Brain Mapp.* **32**, 1825–1835 (2011).

21. Pittau, F., Grova, C., Moeller, F., Dubeau, F. & Gotman, J. Patterns of altered functional connectivity in mesial temporal lobe epilepsy: Functional Connectivity in MTLE. *Epilepsia* **53**, 1013–1023 (2012).

22. Luo, C. *et al.* Altered functional connectivity in default mode network in absence epilepsy: A resting-state fMRI study. *Hum. Brain Mapp.* **32**, 438–449 (2011).

23. Damoiseaux, J. S., Prater, K. E., Miller, B. L. & Greicius, M. D. Functional connectivity tracks clinical deterioration in Alzheimer's disease. *Neurobiol. Aging* **33**, 828.e19-828.e30 (2012).

24. Sheline, Y. I. & Raichle, M. E. Resting State Functional Connectivity in Preclinical Alzheimer's Disease. *Biol. Psychiatry* **74**, 340–347 (2013).

25. Rugg, M. D. & Curran, T. Event-related potentials and recognition memory. *Trends Cogn. Sci.* **11**, 251–257 (2007).

26. Herweg, N. A. *et al.* Theta-Alpha Oscillations Bind the Hippocampus, Prefrontal Cortex, and Striatum during Recollection: Evidence from Simultaneous EEG–fMRI. *J. Neurosci.* **36**, 3579–3587 (2016).

27. Suthana, N. & Fried, I. Deep brain stimulation for enhancement of learning and memory. *NeuroImage* **85**, 996–1002 (2014).

28. Kim, K., Ekstrom, A. D. & Tandon, N. A network approach for modulating memory processes via direct and indirect brain stimulation: Toward a causal approach for the neural basis of memory. *Neurobiol. Learn. Mem.* **134**, 162–177 (2016).

29. Sreekumar, V., Wittig, J. H. J., Sheehan, T. C. & Zaghloul, K. A. Principled Approaches to Direct Brain Stimulation for Cognitive Enhancement. *Front. Neurosci.* **11**, (2017).

30. Kim, S. *et al.* Selective and coherent activity increases due to stimulation indicate functional distinctions between episodic memory networks. *Sci. Adv.* **4**, eaar2768 (2018).

31. Wang, J. X. *et al.* Targeted enhancement of cortical-hippocampal brain networks and associative memory. *Science* **345**, 1054–1057 (2014).

32. Warren, K. N., Hermiller, M. S., Nilakantan, A. S. & Voss, J. L. Stimulating the hippocampal posterior-medial network enhances task-dependent connectivity and memory. *eLife* **8**, e49458 (2019).

33. Hermiller, M. S., Karp, E., Nilakantan, A. S. & Voss, J. L. Episodic memory improvements due to noninvasive stimulation targeting the cortical–hippocampal network: A replication and extension experiment. *Brain Behav.* **9**, e01393 (2019).

34. Freedberg, M. *et al.* Persistent Enhancement of Hippocampal Network Connectivity by Parietal rTMS Is Reproducible. *eNeuro* **6**, (2019).

35. Nilakantan, A. S., Bridge, D. J., Gagnon, E. P., VanHaerents, S. A. & Voss, J. L. Stimulation of the Posterior Cortical-Hippocampal Network Enhances Precision of Memory Recollection. *Curr. Biol.* **27**, 465–470 (2017).

36. Tambini, A., Nee, D. E. & D'Esposito, M. Hippocampal-targeted Theta-burst Stimulation Enhances Associative Memory Formation. *J. Cogn. Neurosci.* **30**, 1452–1472 (2018).

37. Hermiller, M. S., Chen, Y. F., Parrish, T. B. & Voss, J. L. Evidence for immediate enhancement of hippocampal memory encoding by network-targeted theta-burst stimulation during concurrent fMRI. *J. Neurosci.* JN-RM-0486-20 (2020) doi:10.1523/JNEUROSCI.0486-20.2020.

38. Hermiller, M. S., VanHaerents, S., Raij, T. & Voss, J. L. Frequency-specific noninvasive modulation of memory retrieval and its relationship with hippocampal network connectivity. *Hippocampus* **29**, 595–609 (2019).

39. Dave, S., VanHaerents, S. & Voss, J. L. Cerebellar theta and beta noninvasive stimulation rhythms differentially influence episodic memory versus semantic prediction. *J. Neurosci.* JN-RM-0595-20 (2020) doi:10.1523/JNEUROSCI.0595-20.2020.

40. Kundu, B., Johnson, J. S. & Postle, B. R. Prestimulation phase predicts the TMS-evoked response. *J. Neurophysiol.* **112**, 1885–1893 (2014).

41. Hyman, J. M., Wyble, B. P., Goyal, V., Rossi, C. A. & Hasselmo, M. E. Stimulation in Hippocampal Region CA1 in Behaving Rats Yields Long-Term Potentiation when Delivered to the Peak of Theta and Long-Term Depression when Delivered to the Trough. *J. Neurosci.* **23**, 11725–11731 (2003).

42. Silvanto, J., Bona, S. & Cattaneo, Z. Initial activation state, stimulation intensity and timing of stimulation interact in producing behavioral effects of TMS. *Neuroscience* **363**, 134–141 (2017).

43. Silvanto, J. & Cattaneo, Z. Common framework for "virtual lesion" and state-dependent TMS: The facilitatory/suppressive range model of online TMS effects on behavior. *Brain Cogn.* **119**, 32–38 (2017).

44. Silvanto, J., Muggleton, N. & Walsh, V. State-dependency in brain stimulation studies of perception and cognition. *Trends Cogn. Sci.* **12**, 447–454 (2008).

45. Wassermann, E. M. Risk and safety of repetitive transcranial magnetic stimulation: report and suggested guidelines from the International Workshop on the Safety of Repetitive Transcranial Magnetic Stimulation, June 5–7, 1996. *Electroencephalogr. Clin. Neurophysiol. Potentials Sect.* **108**, 1–16 (1998).

46. Rossi, S., Hallett, M., Rossini, P. M. & Pascual-Leone, A. Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clin. Neurophysiol.* **120**, 2008–2039 (2009).

47. Lerner, A. J., Wassermann, E. M. & Tamir, D. I. Seizures from transcranial magnetic stimulation 2012–2016: Results of a survey of active laboratories and clinics. *Clin. Neurophysiol.* **130**, 1409–1416 (2019).

48. Pascual-Leone, A. *et al.* No evidence of hearing loss in humans due to transcranial magnetic stimulation. *Neurology* **42**, 647–51 (1992).

49. Levkovitz, Y. *et al.* A randomized controlled feasibility and safety study of deep transcranial magnetic stimulation. *Clin. Neurophysiol.* **118**, 2730–2744 (2007).

50. Janicak, P. G. *et al.* 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. *J. Clin. Psychiatry* **69**, 222–232 (2008).

51. Nilakantan, A. S. *et al.* Network-targeted stimulation engages neurobehavioral hallmarks of age-related memory decline. *Neurology* **92**, e2349–e2354 (2019).

52. Ranganath, C. & Ritchey, M. Two cortical systems for memory-guided behaviour. *Nat. Rev. Neurosci.* **13**, 713–726 (2012).

53. Staresina, B. P., Duncan, K. D. & Davachi, L. Perirhinal and Parahippocampal Cortices Differentially Contribute to Later Recollection of Object- and Scene-Related Event Details. *J. Neurosci.* **31**, 8739–8747 (2011).

54. Corbetta, M. & Shulman, G. L. Control of goal-directed and stimulus-driven attention in the brain. *Nat. Rev. Neurosci.* **3**, 201–215 (2002).

55. Wiesman, A. I., Heinrichs-Graham, E., Proskovec, A. L., McDermott, T. J. & Wilson, T. W. Oscillations during observations: Dynamic oscillatory networks serving visuospatial attention. *Hum. Brain Mapp.* **38**, 5128–5140 (2017).

56. Cavada, C. & Goldman-Racic, P. S. Posterior parietal cortex in rhesus monkey: I. Parcellation of areas based on distinctive limbic and sensory corticocortical connections. *J. Comp. Neurol.* **287**, 393–421 (1989).

57. Mesulam, M. M., Van Hoesen, G. W., Pandya, D. N. & Geschwind, N. 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 Res.* **136**, 393–414 (1977).

58. Jung, J., Bungert, A., Bowtell, R. & Jackson, S. R. Vertex Stimulation as a Control Site for Transcranial Magnetic Stimulation : A Concurrent TMS / fMRI Study. *Brain Stimulat.* **9**, 58–64 (2016).

59. Sale, M. V., Ridding, M. C. & Nordstrom, M. A. Cortisol Inhibits Neuroplasticity Induction in Human Motor Cortex. *J. Neurosci.* **28**, 8285–8293 (2008).

60. Freedberg, M. *et al.* Multiple parietal pathways are associated with rTMS-induced hippocampal network enhancement and episodic memory changes. *bioRxiv* 2020.07.09.195172 (2020) doi:10.1101/2020.07.09.195172.