Transcranial Magnetic Stimulation enhances working memory

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A thesis submitted to the Department of Psychology for honors

Duke University

Durham, North Carolina

2016

Abstract

Cognitive decline associated with aging affects a large proportion of America's progressively older population. To remedy this decline, various working memory (WM) training protocols are emerging, the most novel of which utilize Transcranial Magnetic Stimulation (TMS) to excite neuronal activity, induce long-term potentiation, and enhance cognitive functioning. Ultimately aiming to remediate WM decline in aging adults by using TMS, this study first sought to establish ideal TMS parameters to induce WM improvements. Using a delayed match-to-sample (DMS) WM task with both maintenance and manipulation conditions, it was hypothesized that active TMS, relative to sham TMS, would differentially impact task performance depending on its timing of administration, either before encoding or at the end of the delay phases. Following screening and practice, subjects trained on the DMS task for 4 hours over 2 days, receiving 5s of either active 5Hz TMS at 100% of motor threshold to the dorsolateral prefrontal cortex (DLPFC) or sham TMS. The phase of active versus sham TMS stimulation was counterbalanced across participants. The results suggest that active TMS improved DMS reaction time and accuracy as compared to sham TMS. Specifically, maintenance task performance improved with TMS before encoding, while manipulation task performance was aided by TMS during the delay period. Although promising, these results should be bolstered by increased sample sizes and individualized fMRI-based DLPFC targeting before deciding on the optimal timing of TMS for each DMS task condition in aging adults.

We all have experienced the frustration of trying and failing to remember information, such as a telephone number, for short periods of time. However, imagine the feeling of debilitation involved in consistently loosing track of the topic during a conversation, forgetting where important objects like keys or medications were placed, or being unable to mentally compute basic calculations such as a tip in a restaurant. For a large proportion of America's progressively aging population, this form of cognitive decline that particularly afflicts executive processing and working memory (WM) becomes a serious setback (Fiore et al., 2012; Salthouse, 1994).

Working Memory

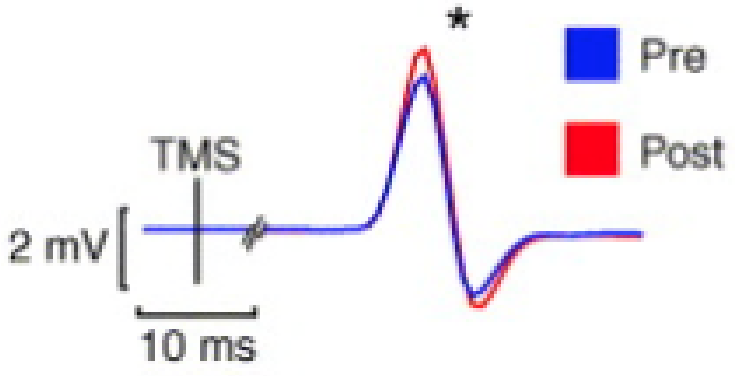
WM encompasses the transient memory for concepts currently being processed and plays a significant role in long term memory storage, retrieval from long term memory, social interactions, formulation of present and future goals, and problem solving ability (Kimberg et al., 1997). Lesion experiments in animals and functional imaging studies in humans have revealed various brain regions associated with WM, including the frontal cortex, parietal cortex, anterior cingulate cortex, and parts of the basal ganglia. There are divergent WM processes identified as maintaining or manipulating information in WM. To successfully maintain information in WM, one must simply recall information that was previously provided; however, to manipulate information in WM requires interacting with and modifying the information as it is stored in WM in some meaningful manner. While both processes tend to decline with age, manipulation's greater demand of executive processing, occurring primarily in frontal regions such as the dorsolateral prefrontal cortex (DLPFC), is suggested to disproportionally decline relative to maintenance in aging adults (Petrides, 2000).

One behavioral WM task that highlights differences between maintenance and manipulation functions is the delayed-match-to-sample (DMS) task. In this task, a set of letters is encoded and then, following a delay period, a probe letter is presented. Participants must respond by categorizing the probe as a member of the initial letter set or not (Sternberg, 1969). To recruit maintenance versus manipulation processes, participants are instructed to either recall whether the test letter was present in the initial set (maintenance trials) or to determine whether a presented letter is paired with the number correctly indicating its proper alphabetical order within the initial set (manipulation trials) (D'Esposito et al., 1999).

Transcranial Magnetic Stimulation

Correlational methods, such as fMRI or EEG, have traditionally been utilized to study the WM circuitry activated by cognitive tasks; however, the development of noninvasive brain stimulation has enabled the generation of stronger causal inferences. Transcranial Magnetic Stimulation (TMS), a type of noninvasive brain stimulation, was originally developed as a disruptive agent that interferes with neural activity to create a temporary virtual lesion. However, more recently, TMS has also been explored as a mechanism for enhancing cognitive functioning (Luber and Lisanby, 2013). TMS generates brief pulses of current through a stimulating coil placed tangentially to the head that produces a magnetic field around the coil. This, in turn, induces an electric field that stimulates neurons within the focal region of approximately two cm. This neural stimulation is thought to lead to long-term neuronal potentiation or depression, the classical models for learning and memory (Bliss and Lomo, 1973). Esser et al. (2006) demonstrated this potential for inducting long-term potentiation in humans by using high-density electroencephalography to measure potentiated neural responses following rTMS sessions **(Figure 1)**.

Figure 1. Potentiated EEG response following rTMS to the Motor Cortex **[Esser et al., 2006]**

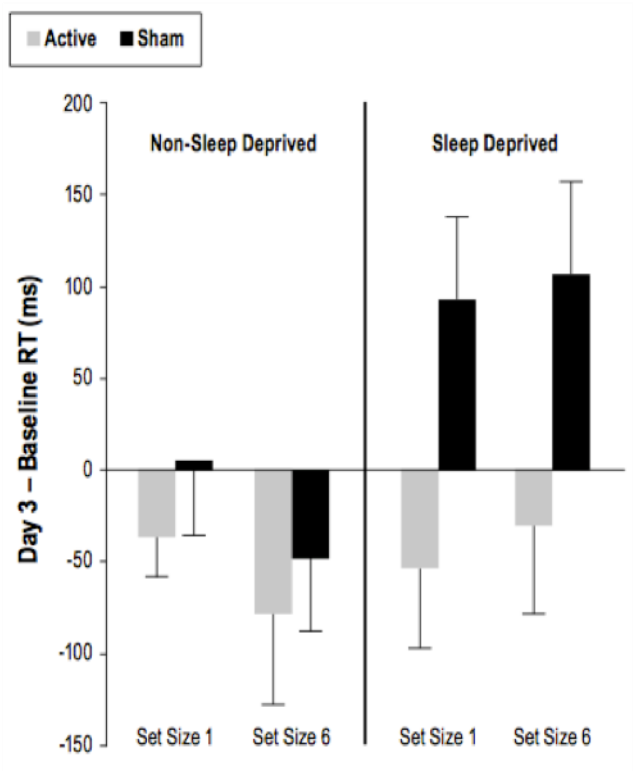


The effect of rTMS on neural activity varies as a result of its frequency, intensity, and timing parameters. If administered at a low frequency of approximately 1Hz, cortical activity tends to be suppressed. This method is often employed by studies intending to create virtual lesions, or a temporary and reversible disruption of neural activity in underlying cortical areas (Whitney et al., 2012). Higher frequency rTMS administered between 5-20Hz, however, generally increases cortical excitability and can facilitate cognitive enhancement (Hsu et al., 2015).

For the specific cognitive function of WM, rTMS has previously been utilized both to disrupt (Postle et al., 2006; Basso et al., 2010) and enhance (Yamanaka et al., 2010) WM. Significantly, previous research by our lab demonstrated that 5Hz rTMS can remediate sleep deprivation-induced WM deficits (Luber et al., 2013). 5Hz rTMS was administered during the delay period of a DMS task to the lateral occipital complex, a region associated with maintenance WM functions, or to the vertex, a sham region not involved in WM. Both immediately after and 18 hours after the last rTMS session, sleep deprived individuals who received active TMS performed the same as non-sleep deprived individuals, while the sleep deprived participants receiving sham rTMS performed significantly worse **(Figure 2)**. This WM

enhancement with 5Hz rTMS suggests that rTMS may augment WM performance by driving brain oscillations to theta rhythm (4-7Hz). As investigated by Roux and Uhlhaus (2014), various neural oscillations serve distinct functional roles during WM performance. Theta activity in particular has been observed in tasks involving sequential coding of items in the WM, as is involved in the DMS task (Roux and Uhlhaus, 2014).

Figure 2. Reaction time improves with TMS and remediates sleep deprivation **[Luber et al., 2013]**



Stemming from these investigations of brain oscillation frequencies, the timing of rTMS administration can also impact its result by dictating the WM state that the stimulation will interact with. Brain oscillations vary according to the stage in memory consolidation that is concurrently being performed. For example, although theta activity has been observed during the sequential encoding of items, alpha rhythm (8-13Hz) is commonly implicated during the initial orienting of attention and inhibition of task-irrelevant information (Palva et al., 2011). In order to

enhance cognitive functioning, it is important to deliver the rTMS frequency that specifically aligns with the neural activity occurring at the time of administration. If incorrectly aligned, rTMS might drive neural oscillations to a frequency serving an incorrect functional role. Thus, although previous research in our lab has administered rTMS during the end of the delay period in the DMS task (Luber et al., 2013), this timing decision needs to be based on direct experimental comparison. Furthermore, since maintenance and manipulation WM tasks involve qualitatively different executive functions, it is possible that the optimal timing for TMS during the DMS task is not equivalent for both.

Current Study

Since many of the questions addressed above remain unanswered, the current study intended to analyze several of these design considerations regarding TMS before delving into a more time- and resource-intensive WM training protocol. This study aimed to first establish the feasibility of incorporating time-synchronized rTMS into a two-hour DMS WM training session. Then, by designing a randomized controlled trial that directly compared performance following DMS training with TMS dispensed at two different phases of the WM cascade, we intended to determine which timing of TMS administration led to the greatest cognitive enhancement. Both maintenance (recall) and manipulation (alphabetize) tasks were included to analyze whether the optimal TMS settings are equivalent or distinct for WM tasks that recruit a different extent of higher-order processing.

To accomplish these goals, subjects were recruited to participate in a three-day, six-hour procedure. On the first day, participants underwent screening, motor threshold determination, and practice on the DMS WM task until performance was static. The next two days involved training on the DMS task with either active 5Hz TMS or sham stimulation applied to the left

DLPFC. To compare temporal effects, TMS was delivered either before stimulus presentation or during the delay period of each DMS task trial. In accordance with the paradigms indicating cognitive enhancement with 5Hz rTMS, we hypothesized that performance will improve on WM tasks when participants receive active rather than sham TMS. Additionally, we expect that there will be a difference in performance between groups receiving active TMS before stimulus presentation and during the delay period of each trial. By elucidating the optimal delivery period of TMS administration and ensuring the procedure's feasibility, the results can be applied to our ultimate goal of demonstrating generalized, long term cognitive enhancement in aging adults following WM training with TMS.

Methods

Participants

A total of 10 subjects were recruited and consented for participation. [mean age = 22.7y, SD = 4.52y; 7 female; 3 male; races represented = African American, Caucasian, Asian, and Hispanic; mean education = 15y, SD = 2y]. Of these participants, five completed all three visits, two completed visits one and two before cancelling, one completed only the screen before canceling, and two did not participate due to a screening fail. Participants were recruited from the Duke community, either by word of mouth or via the Center for Cognitive Neuroscience Research Participation Website (http://participate.mind.duke.edu/default.asp). All gender, race, and minority groups were accepted. Participants were compensated $20 per hour, or approximately $120.00 total for participation in the screening (2hrs) and two rTMS sessions (4 hours). Payment by check was mailed several weeks after the end of each participant's study involvement.

Following recruitment, subjects were consented to the risks and benefits of participating and were able to make an informed decision regarding their continued participation. Duke Medicine's Institutional Review Board approved all consenting and screening materials, equipment, and the procedural protocol (Pro00065334). Study exclusion criteria existed to minimize the risks associated with rTMS and included:

- Current or recent substance abuse or dependence (urine test)
- Current serious medical illness (self report)
- Failure of TMS Adult Safety Screening (TASS) form
- Unwillingness to provide informed consent
- Any Axis I DSM-IV disorder diagnosis (MINI, DSM-IV)
- Presence of a clinically defined neurological disorder
- Increased risk of seizure due to prior diagnosis of increased intracracranial pressure, history of seizures, or current medication that lowers the seizure threshold
- Presence of a cochlear implant
- Women who are pregnant or breast-feeding (urine test)

Materials

Screening. All participants supplied a urine sample that was used for a pregnancy test (women) and a drug panel. They then took the Edinburgh handedness inventory to ensure that the effects of TMS were matched with the neural configurations associated with right hand-dominant individuals. Next, the TMS Adult Safety Screening (TASS) form was completed to detect any individuals with an increased susceptibility to seizing, whether due to family history, implanted metal, or medication. The MINI (DSM-IV) was administered to screen for any Axis I DSM-IV disorder or clinically defined neurological disorder. A TMS side effect rating scale and mood ratings were administered after each TMS session to monitor the health of participants after TMS administration.

TMS. Subjects were seated comfortably in a chair, facing a computer screen for visual stimulus presentation. A head frame and chin rest held the participant's head steady throughout the procedure at a fixed distance of about 30 inches from the monitor. A coil holder frame locked

to maintain the TMS coil position throughout the procedure. Location of the DLPFC was standardized based on a coordinate selected in Blumenfeld and Ranganath's review of fMRI-based WM studies (2006). This coordinate was then logged into Brainsight neuronavigational software (Rogue Research Inc., Montreal, Canada), which uses infrared stereotactic navigation to align TMS coil placement with a preselected location and angle on a calibrated MRI.

Timing pulses controlled by the computer drove a MagPro X100 (MagVenture Inc, Denmark) TMS device that delivered focal magnetic pulses through a figure-eight coil. Five-second trains of 5 Hz rTMS (25 pulses) were administered at 100% of the individual's motor threshold (MT) with the TMS coil tangential to the scalp. Sham rTMS was administered with a sham coil equipped with shielding to block magnetic field output while retaining the auditory and tactile aspects of active TMS. A low amplitude current simulator was placed over the subject's forehead to supply a similar skin sensation. The TMS administration was time-locked relative to stimulus presentation on the computer monitor using MATLAB software (MathWorks, Natick, Massachusetts). All known TMS risk prevention strategies, including screening, exclusionary criteria, and the use of motor threshold for dosing were utilized by the lab. Administrators were also certified in TMS, BLS, and seizure prevention and management, and licensed clinicians were present in the immediate vicinity during all sessions that included TMS.

Procedure

Timeline. Participants came to the lab on three separate days for two hours each. In almost all cases, these three days were within the same week. The first visit included consent and screening processes, determination of the participant's motor threshold, and as many practice DMS runs as possible (range 3-8 runs). The second and third visits were identically structured,

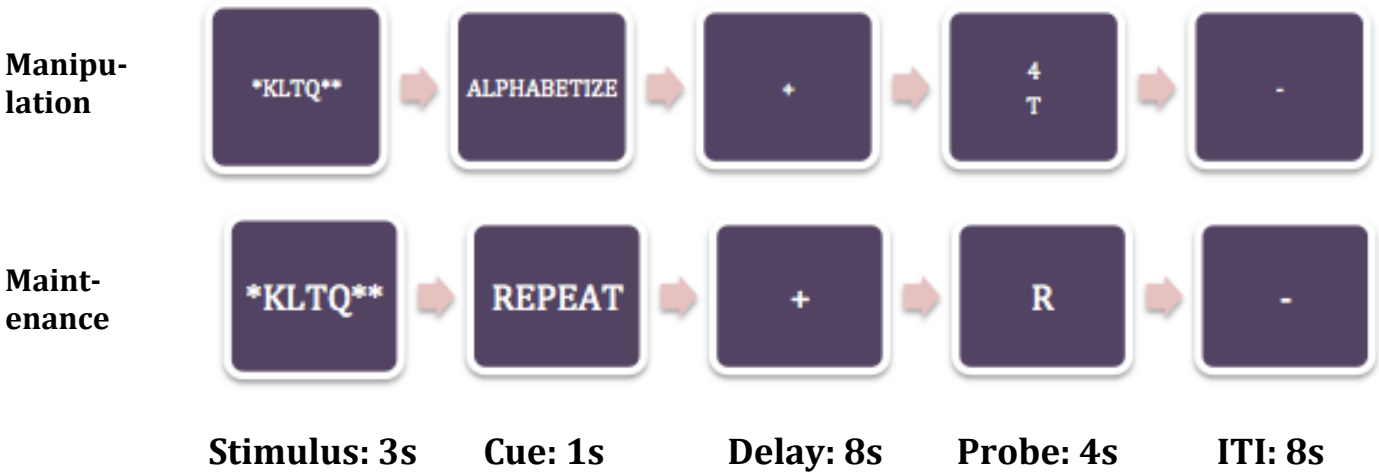
counterbalanced training sessions with rTMS applied to the left DLPFC. Both days consisted in 10 DMS runs total, with 2 practice no stimulation runs followed by 4 runs each of active, sham, pre-stimulus, and delay-period TMS delivery **(Table 2)**.

Table 1. Timeline of Study Procedures

Assessment	Screening Session	rTMS Session 1	rTMS Session 2
1. Subject consent & screening	X		
2. Practice with WM task	X		
3. Motor Threshold	X		
4. WM task + rTMS		X	X
5. Side Effect Checklist		X	X
6. Mood Ratings		X	X

Working memory tasks. The behavioral task employed modeled Sternberg's (1969) delayed match-to-sample (DMS) task, modified by D'Esposito, Postle, and Ballard in 1999. The procedure resembled **Figure 3**. Trials began with a pre-array period of 5s, then stimuli were presented for 3s, followed by a task cue for 1s, a delay or encoding period of 8s, a probe of 4s, and the inter-trial interval of 3s. Trials requiring participants to *maintain* letters in their working memory were prompted by a "REPEAT" command, and trials requiring participants to *manipulate* letters into alphabetical order were prompted by an "ALPHABETIZE" command. At the probe phase, subjects were instructed to respond to indicate a correct or incorrect match as quickly as possible, emphasizing a quick over an accurate response. Average performance accuracy and reaction times were collected across all training days as indicators of progress. The number of letters in the stimulus array was indicative of task difficulty. Following the results of our pilot study that determined optimal set sizes for eliminating ceiling and floor effects, set sizes were either 3 or 5 letters for *manipulation* trials, and were either 5 or 9 letters for *maintenance* trials.

Figure 3. Manipulation and Maintenance trials of the DMS task, respectively



Motor threshold determination. Motor threshold (MT) is defined as the minimum magnetic flux needed to elicit a threshold EMG response in a target muscle in 5 out of 10 trials. MT is the field's standard mechanism for establishing an individualized dosage of TMS intensity, applying the maximum intensity level that also minimizes the risk of seizures. EMG will be used to measure the motor evoked potentials (MEP) for the contralateral first dorsal interosseus. The lowest TMS intensity, applied to the motor cortex, that is able to evoke 5 MEP's of $\geq$ 50 µV in peak-to-peak amplitude out of 10 trials will be established as that individual's resting MT, defined using the "threshold hunting" method (Awiszus, 2003).

Analysis. The experiments utilized a factorial design with 16 levels [2 rTMS (active vs. sham) x 2 Timing (PRE vs POST) x 2 Task (Maintenance vs. Manipulation) x 2 Difficulty (Easy vs. Difficult set size)]. Each level serves as an independent variable over which the dependent variables, reaction time and accuracy, could depend. Subjects were randomly assigned to one of four groups (A, B, C, or D) that counterbalanced the order with which each received active versus sham TMS, and whether the stimulation was given PRE or POST stimulus presentation

(Table 2). Mixed model ANOVAs with repeated-measures factors of rTMS type and difficulty level were performed separately by task type and timing for median reaction time and average accuracy data.

Table 2. Group assignment for ordering of TMS conditions

Group A

SCREENING VISIT Screening, Consent, Practice (6 blocks), & MT = 90 mins	
Screening	5 mins
Consent	5 mins
Practice	30 mins
MT	20 mins
Practice	30 mins

VISIT 1A Randomized TMS Pre- vs. Post-encoding at DLPFC & WM task = 2 hrs.	
2 No Stim	20 mins
Active-Pre	10 mins
Active-Post	10 mins
Active-Pre	10 mins
Active-Post	10 mins
Sham-Pre	10 mins
Sham-Post	10 mins
Sham-Pre	10 mins
Sham-Post	10 mins

VISIT 2A Randomized TMS Pre- vs. Post-encoding at DLPFC & WM task = 2 hrs.	
2 No Stim	20 mins
Sham-Pre	10 mins
Sham-Post	10 mins
Sham-Pre	10 mins
Sham-Post	10 mins
Active-Pre	10 mins
Active-Post	10 mins
Active-Pre	10 mins
Active-Post	10 mins

Group B

SCREENING VISIT Screening, Consent, Practice (6 blocks), & MT = 90 mins	
Screening	5 mins
Consent	5 mins
Practice	30 mins
MT	20 mins
Practice	30 mins

VISIT 1B Randomized TMS Pre- vs. Post-encoding at DLPFC & WM task = 2 hrs.	
2 No Stim	20 mins
Sham-Pre	10 mins
Sham-Post	10 mins
Sham-Pre	10 mins
Sham-Post	10 mins
Active-Pre	10 mins
Active-Post	10 mins
Active-Pre	10 mins
Active-Post	10 mins

VISIT 2B Randomized TMS Pre- vs. Post-encoding at DLPFC & WM task = 2 hrs.	
2 No Stim	20 mins
Active-Pre	10 mins
Active-Post	10 mins
Active-Pre	10 mins
Active-Post	10 mins
Sham-Pre	10 mins
Sham-Post	10 mins
Sham-Pre	10 mins
Sham-Post	10 mins

Group C

SCREENING VISIT Screening, Consent, Practice (6 blocks), & MT = 90 mins	
Screening	5 mins
Consent	5 mins
Practice	30 mins
MT	20 mins
Practice	30 mins

VISIT 1C Randomized TMS Pre- vs. Post-encoding at DLPFC & WM task = 2 hrs.	
2 No Stim	20 mins
Active-Post	10 mins
Active-Pre	10 mins
Active-Post	10 mins
Active-Pre	10 mins
Sham-Post	10 mins
Sham-Pre	10 mins
Sham-Post	10 mins
Sham-Pre	10 mins

VISIT 2C Randomized TMS Pre- vs. Post-encoding at DLPFC & WM task = 2 hrs.	
2 No Stim	20 mins
Sham-Post	10 mins
Sham-Pre	10 mins
Sham-Post	10 mins
Sham-Pre	10 mins
Active-Post	10 mins
Active-Pre	10 mins
Active-Post	10 mins
Active-Pre	10 mins

Group D

SCREENING VISIT Screening, Consent, Practice (6 blocks), & MT = 90 mins	
Screening	5 mins
Consent	5 mins
Practice	30 mins
MT	20 mins
Practice	30 mins

VISIT 1D Randomized TMS Pre- vs. Post-encoding at DLPFC & WM task = 2 hrs.	
2 No Stim	20 mins
Sham-Post	10 mins
Sham-Pre	10 mins
Sham-Post	10 mins
Sham-Pre	10 mins
Active-Post	10 mins
Active-Pre	10 mins
Active-Post	10 mins
Active-Pre	10 mins

VISIT 2D Randomized TMS Pre- vs. Post-encoding at DLPFC & WM task = 2 hrs.	
2 No Stim	20 mins
Active-Post	10 mins
Active-Pre	10 mins
Active-Post	10 mins
Active-Pre	10 mins
Sham-Post	10 mins
Sham-Pre	10 mins
Sham-Post	10 mins
Sham-Pre	10 mins

Results

With the ultimate goal of creating a WM training paradigm to remediate WM and cognitive decline in aging adults, we first analyzed the feasibility of a two-hour DMS WM training session and the optimal characteristics of rTMS that lead to the greatest cognitive enhancement. Specifically, maintenance (recall) and manipulation (alphabetize) DMS tasks were

included to analyze whether the ideal rTMS settings are equivalent or distinct for WM tasks that recruit a different extent of higher-order processing. Participants were recruited for a three-day study, over which the first day included screening and DMS practice, and the second and third days consisted in 10 DMS blocks. Task conditions, such as set size and task type, were programmed to occur randomly for each trial. During the last two days, the order at which participants received a total of four blocks each of active TMS or sham stimulation, delivered either before stimulus presentation or during the delay period of each DMS trial, was counterbalanced **(Table 2)**. Although a total of ten participants were consented for the study, only data from the five subjects who completed all three training sessions was included in the graphical and statistical analysis.

First, the general efficacy of 5Hz rTMS was investigated by comparing the results of active versus sham stimulation from days 2 and 3. It was hypothesized that performance would improve on WM tasks when participants received active rather than sham rTMS. Additionally, we hypothesized that there would be a difference in performance when comparing the timing of rTMS administration, either before the stimulus presentation or during the delay period of each trial. For each set size and task type, the average percent correct and average response time (RT) (in ms), with outliers > 2 SD removed, were plotted **(Figure 4 and 5)**. The data obtained from aggregating trial-wise results across participants for each task condition were statistically analyzed using a repeated measure ANOVA with a post hoc univariate t-test of significance to discriminate among between-variable interactions.

Overall, the results suggested that active rTMS to the left DLPFC does improve DMS performance, as compared with sham stimulation in some conditions. For both task types, there

was a condition of rTMS administration that resulted in significant improvements in performance, as indicated by decreased RT and/or increased accuracy.

Figure 4. RTs for maintenance and manipulation tasks, averaged by groups of set size and stimulation type

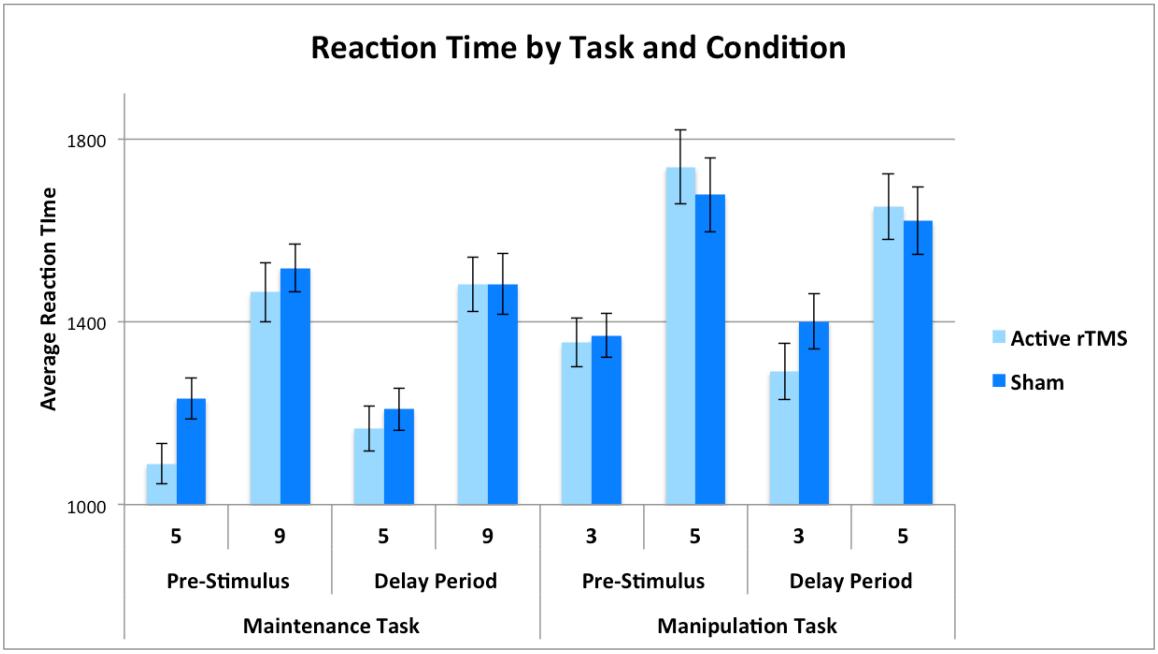
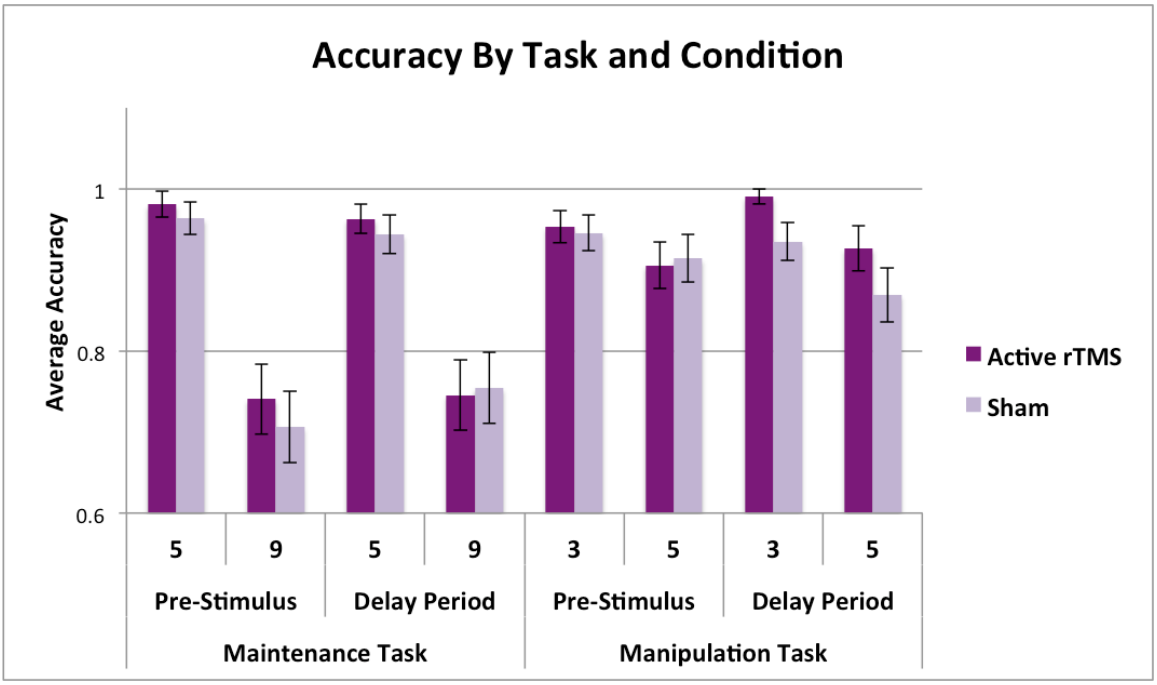


Figure 5. Accuracy on maintenance and manipulation tasks, averaged by groups of set size and stimulation type



Our analysis also suggested that the timing of TMS administration does impact DMS performance, and that these phase dependencies differ between task types. For the maintenance task, only trials in which active rTMS was received before stimulus presentation (1089 $\pm$ 44.4 ms and 1464 $\pm$ 65.1 ms) resulted in a reduced average RT than those receiving sham stimulation during the same phase (1231 $\pm$ 44.4 ms and 1517 $\pm$ 51.8 ms) [$F(1, 104) = 5.94$], $p = 0.016$. There was no set size by stimulation-type interaction, indicating that this reduction in RT was significant for set sizes of both 5 and 9 letters. Accuracy did not significantly increase or decrease between active TMS or sham stimulation for either delivery period.

For manipulation trials, however, only trials in which active rTMS was administered during the delay period of each trial resulted in improved performance as compared to sham stimulation. Using a repeated measures ANOVA for RT, the effect of stimulation type was not significant, but there was a trending set size by stimulation-type interaction [$F(1, 105) = 2.934$], $p = 0.089$. Post-hoc t-test analysis revealed that for a set size of 3, the RT decrease with active rTMS (1292 $\pm$ 62.0 ms) as compared to sham stimulation (1401 $\pm$ 60.3 ms) neared significance [$t(106) = -1.92$], $p = 0.057$. There was no significant difference in RT for trials with a set size of 5. Furthermore, accuracy for manipulation trials significantly increased when active rTMS was delivered during the delay period of each trial (0.991 $\pm$ 0.00914 % and 0.935 $\pm$ 0.0236 % correct), as compared to sham stimulation (0.927 $\pm$ 0.0277 % and 0.869 $\pm$ 0.0333 % correct) [$F(1, 105) = 4.118$], $p = 0.038$. For accuracy, no set size by stimulation-type interaction existed, signifying that the increase in performance accuracy was significant for set sizes of both 3 and 5 letters.

Although interested in the conditions for rTMS administration that most significantly enhanced DMS task performance, an imperative auxiliary goal was to ensure that general DMS

results mirror published expectations. As a whole, the results expected from a DMS task were apparent (Sternberg, 1969; D'Esposito, 1999). In the maintenance task, for all types of stimulation and timing of stimulation delivery, there was a significantly lower average RT and greater average accuracy for the lower set size (5 letters) as compared with the greater set size (9 letters), $p<0.001$ for all conditions. For the manipulation task, all but one condition resulted in a significantly lower average RT and greater average accuracy for lower set size (3 letters) as compared with the greater set size (5 letters), $p < 0.005$. Average accuracy for the manipulation task when stimulation was delivered before the stimulus presentation of each trial was the only condition that did not produce significant differences in performance depending on the set size (3 versus 5 letters) [$F(1, 104) = 2.645$], $p = 0.106$.

Discussion

The long-term goals of this study are to demonstrate that rTMS and WM training can remediate cognitive decline in aging adults (60-80 years old). Critical to this goal, we aim to validate that rTMS combined with WM training leads to greater cognitive enhancement than WM training alone, and that this improvement in performance applies to both the trained DMS task and a battery of untrained cognitive tasks. Before delving into this protocol, however, it is necessary to answer several design-related concerns for rTMS. Notably, does 5Hz active rTMS to the left DLPFC lead to better performance on our version of the DMS WM task than sham stimulation? And furthermore, is there a delivery period within the DMS task that interacts most favorably with this 5Hz rTMS, and is this period the same or different between maintenance and manipulation tasks?

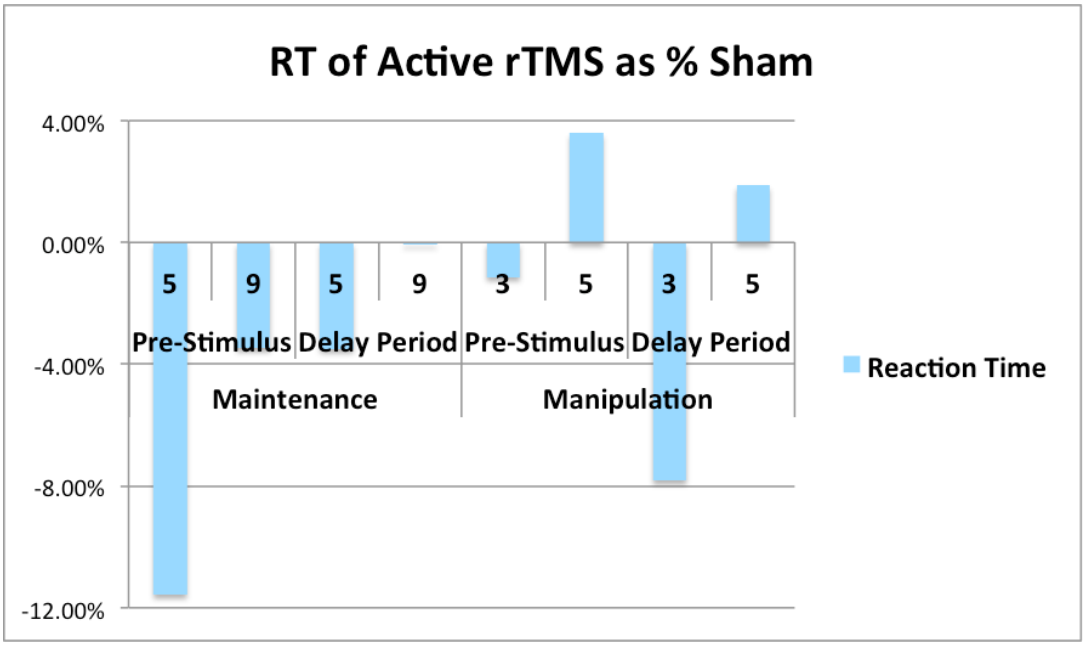
It was hypothesized that active rTMS would improve DMS task performance more than sham stimulation. Furthermore, we postulated that there would be a difference in performance

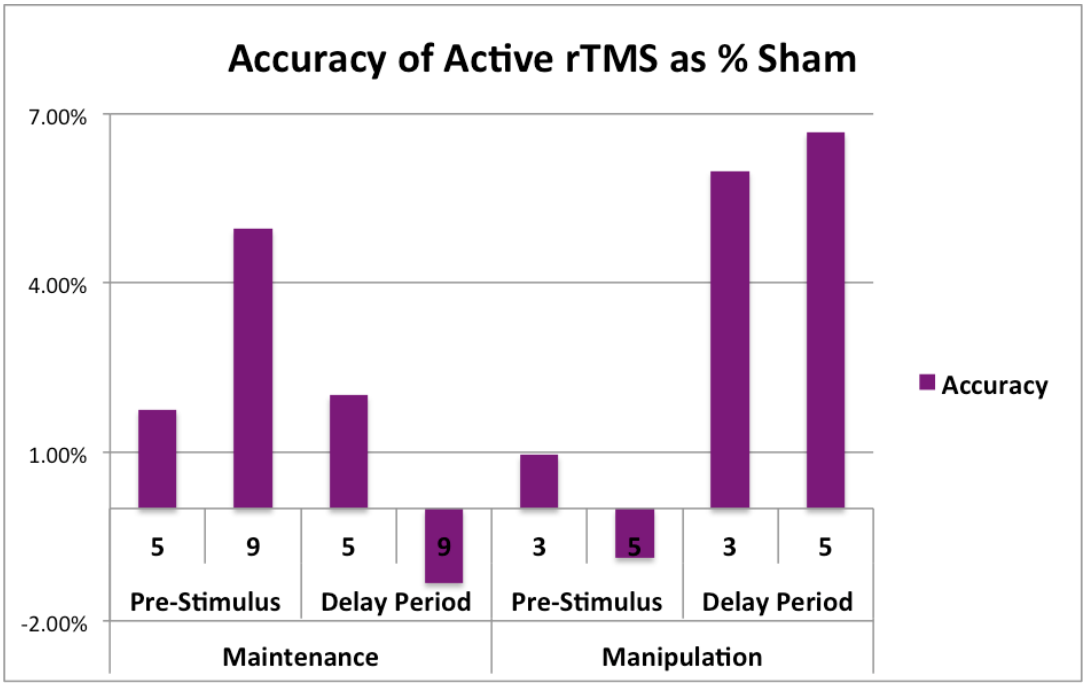
when active rTMS is applied before stimulus presentation versus during the delay period of each trial. Our results provided preliminary support for both of these hypotheses. For the maintenance task, RT decreased significantly when active rTMS was delivered before stimulus presentation of each trial. In contrast, for the manipulation task, RT decreased significantly (set size of 3 only) and accuracy increased significantly when active rTMS was administered during the delay phase of each trial. While these results stem from only 5 subjects and are underpowered for drawing any strong conclusions, **Figure 6** illustrates how, in general, rTMS effects trended in the hypothesized direction. For "Maintenance – Pre-stimulus," average RTs were all lower and average accuracies were all higher with active rTMS. For "Manipulation – Delay Period," all results except for the average RT with a set size of 5 letters mirror the same trends. For the less effective delivery period for each task type, the results for active rTMS as compared to sham TMS do not improve DMS performance in as many conditions and, even in the conditions where RT decreases or accuracy increases, this improvement is not as severe.

This study also sought to establish the feasibility of incorporating time-synchronized rTMS into a two-hour DMS WM training session. The results indicated, overall, that this protocol would be feasible. First, the robust differences in task performance based on set sizes, a measure of task difficulty, indicated that participants were able to engage with the DMS task for the entire two-hour session. Maintaining or manipulating tasks should become more difficult as more letters are added to the stimulus. Therefore, had the performance not differed by set size, this would have signified a lack of effort and overreliance on mental shortcuts for indicating correct or incorrect probes by participants. Additionally, as illustrated in **Figure 4**, average RTs were longer for the manipulation task as compared to the maintenance task, while accuracies were closer in value **(Figure 5)**. This result was also expected since the task of mentally

alphabetizing letters requires a greater extent of executive functioning (D'Esposito et al., 1999). Lastly, by administering mood ratings and talking with participants after each session, it was confirmed that the session's demands were perceived as manageable.

Figure 6. Average RT and accuracy for all task conditions reported as the percent by which active rTMS results are greater or less than sham stimulation results





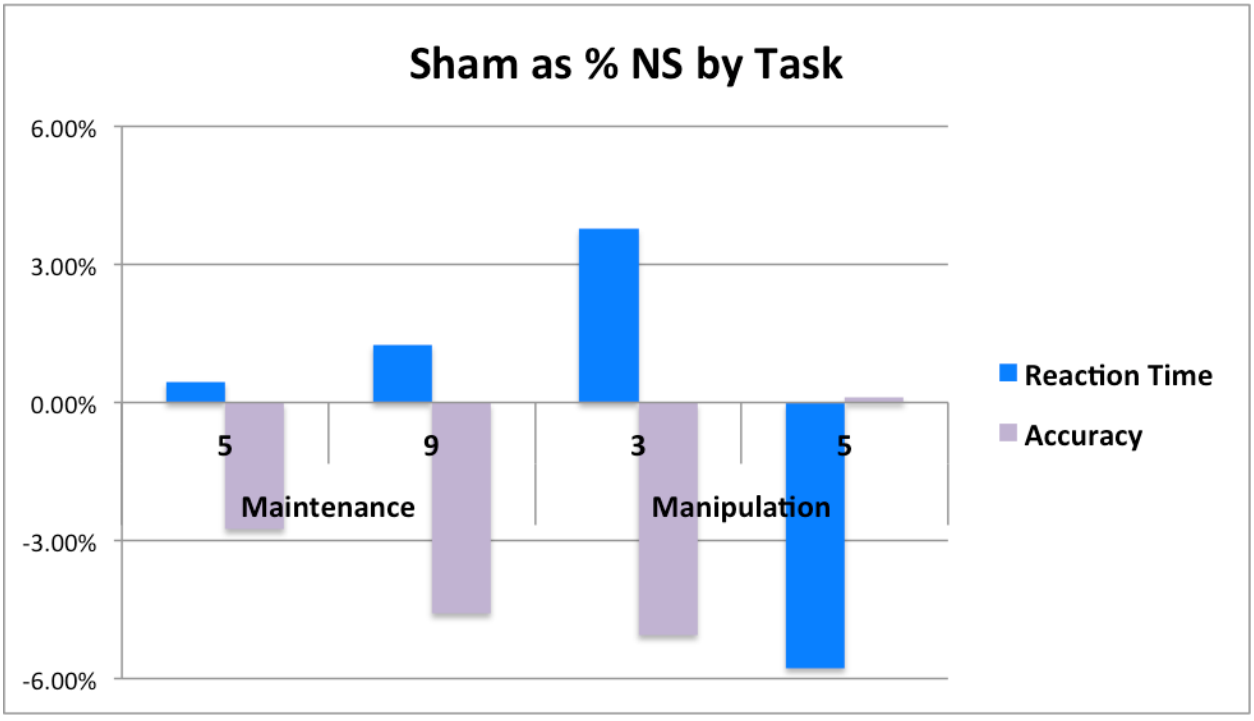
Overall, theses results are consistent with the literature. Activity in the DLPFC has been shown to be active in maintenance- and manipulation-like WM tasks and is predictive of subsequent memory particularly in "reorder" tasks (Blumenfeld and Ranganath, 2006; Petrides, 2000). Therefore, it is not surprising that rTMS at this location improved performance on similar WM tasks. The results also are consistent with meta-analyses claiming that high frequency rTMS tends to facilitate cortical excitability while low frequency rTMS generally suppresses cortical excitability (Hsu et al., 2015; Luber and Lisanby, 2013). However, several individual studies disagree, claiming that high frequency rTMS can have disruptive effects as well (Postle et al., 2006; Wagner et al., 2006). To continue validating or adjusting TMS guidelines, additional individual experiments and meta-analyses need to be published.

Of interest is the relatively surprising preliminary observation that the optimal timing of rTMS administration differed for maintenance and manipulation conditions. Although the DLPFC is thought to have a greater role in the executive functioning of manipulation tasks (D'Esposito et al., 1999), the DLPFC should be activated for both tasks (Petrides, 2000). If, after including the data of more subjects in the analysis, these results are confirmed, future studies could investigate the cause of this delivery period difference. Do the two tasks engage the DLPFC during different phases of memory consolidation? Do the two tasks initiate theta band cortical oscillations at different phases of the DMS task? By examining the mechanism by which rTMS could optimally enhance performance at different phases in the DMS for maintenance and manipulation conditions, significant discoveries in relation to the neural processes of memory consolidation could emerge.

Despite promising results, there are several methodological limitations worth noting. As discussed, participants generally performed better when receiving active rTMS as opposed to

sham stimulation. However, performance on the two control conditions, sham stimulation and no stimulation (NS), also differed from each other. The NS condition consisted of results from the first two practice blocks that were conducted on days 2 and 3. These two blocks were intended to prevent errors, perhaps related to readjusting to the experimental conditions or forgetting the task instructions, from impacting the rTMS results. Therefore, it was expected that performance on NS trials would be worse than on sham stimulation trials. However, average RT tended to be higher and average accuracy tended to be lower for sham trials as compared to NS **(Figure 7)**. This could signify that the noise and tactile sensations associated with sham stimulation acts as a distracting factor in DMS task completion. As we move forward with the study and collect data from more participants, it will be important to keep watch over the two control conditions to determine whether this performance difference continues and whether it reaches statistical significance, indicating that the sham stimulation might have inhibitory effects.

Figure 7. Average RT and accuracy for all task conditions reported as the percent by which sham stimulation results are greater or less than no stimulation results



Another important consideration regarding the data collected to this point concerns the TMS targeting approach. While the parent project includes fMRI and e-field modeled targeting, localization to this point has utilized a coordinate estimation of the DLPFC based on Blumenfeld and Ranganath's review of the brain regions that are activated as subjects complete various WM tasks, as visualized by fMRI (2006). A stereotactic neuronavigation software (Brainsight, Rogue Research Inc.) did enable us to calibrate this coordinate with the size of each individual's head and to ensure accurate TMS coil placement. While Blumenfeld and Ranganath's coordinate was founded on functional targeting, by selecting a hotspot that was most frequently active across studies, this coordinate represents an estimation. To improve DLPFC targeting, we now plan to obtain fMRIs for each participant, which will capture the regions that are activated while completing the DMS task. Since cortical structure varies between individuals, functional targeting will ensure that the proper coordinate is selected for all participants. This improved methodology, similarly to increasing sample size, will increase the statistical power of our results.

Since we eventually intend to apply the results of this study to a two-week WM training protocol aiming to remediate cognitive decline in aging adults, it is important to consider several aspects that will not align between the studies. First, the young adults participating in the current study (M = 22.7yo) may respond differently than older adults (60-80yo) to 5Hz rTMS. This concern was illuminated by Yamanaka et al. (2014), who reported that over-recruitment of oxygen-hemoglobin and alterations in frontal-parietal networks likely caused rTMS to the right parietal cortex to improve WM performance to a lesser extent for older adults as compared to younger adults. Moreover, the current and future studies emphasize training to a different degree. In the current study, all participants received both active and sham rTMS, and only the

instantaneous effects of rTMS on individual task performance over two training sessions were analyzed. The two-week procedure, however, will compare the improvement over time between two different groups, where participants are randomized to train on either active or on sham rTMS. To serve as a potential cognitive therapy for aging adults, the effects of rTMS must extend beyond several seconds. Although the claim that high-frequency rTMS can have cumulative effects has been scrutinized, if WM training with rTMS is indeed effective, then all sham conditions occurring after the first set of rTMS blocks might have been affected by this active stimulation, making differences between conditions less pronounced (Hamidi et al., 2011).

In conjunction, this study successfully analyzed many of the design considerations that emerged when attempting to construct a TMS protocol imbedded within a DMS WM task. Moving forward, we plan to recruit more participants for the current study and to validate the results by applying functional targeting to localization of the DLPFC. These methodological advances will improve the statistical power of the results and enable a more confident selection of the optimal delivery period of rTMS for both maintenance and manipulation conditions. While crucial to our project moving forward, elucidating timing parameters through direct experimentation will contribute to the rapidly growing literature on TMS parameters and WM neural mechanisms alike. Since the feasibility of the training sessions was confirmed by the current study, once the delivery periods are comprehensively supported, we can progress to study the impact of WM training combined with rTMS in aging populations. By merging both approaches we hope to generate long-term potentiation and neural plasticity, preventing the decline in WM and cognition that frequently impacts adults as they age. If successful in demonstrating that a TMS and WM training paradigm produces lasting and generalizable

cognitive enhancement in the elderly, TMS can be further investigated as a potential therapeutic tool for remediating cognitive decline and dementia in aging adults.

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