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Facilitation of performance in a working memory task with rTMS stimulation of the precuneus: Frequency- and time-dependent effects

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ABSTRACT

Although improvements in performance due to TMS have been demonstrated with some cognitive tasks, performance improvement has not previously been demonstrated with working memory tasks. In the present study, a delayed match-to-sample task was used in which repetitive TMS (rTMS) at 1, 5, or 20 Hz was applied to either left dorsolateral prefrontal or midline parietal cortex during the retention (delay) phase of the task. Only 5 Hz stimulation to the parietal site resulted in a significant decrease in reaction time (RT) without a corresponding decrease in accuracy. This finding was replicated in a second experiment, in which 5 Hz rTMS at the parietal site was applied during the retention phase or during presentation of the recognition probe. Significant speeding of RT occurred in the retention phase but not the probe phase. This finding suggests that TMS may improve working memory performance, in a manner that is specific to the timing of stimulation relative to performance of the task, and to stimulation frequency.

1. Introduction

Transcranial magnetic stimulation (TMS) has long been used to disrupt cognitive, motor and perceptual functioning. For example, TMS to occipital cortex can mask a visual stimulus (Amassian et al., 1989) or increase memory scanning time (Beckers and Homberg, 1991), while TMS to left prefrontal cortex can arrest speech (Pascual-Leone et al., 1991) or cause word recall deficits (Grafman et al., 1994). Such TMS-induced disruption is typically attributed to temporary, virtual lesions in the cortical regions directly stimulated (e.g., Pascual-Leone et al., 2000). This method has been widely used to examine brain–behavior relationships.

More recently, however, TMS has been found to enhance performance in a number of tasks, including choice reaction time (Evers et al., 2001), picture naming (Topper et al., 1998), mental rotation of 3D objects (Klimesch et al., 2003), backward masking (Grosbras and Paus, 2003), Stroop (Hayward et al., 2004), recognition memory (Kohler et al., 2004), and analogical reasoning (Boroojerdi et al., 2001). For example, single-pulse

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TMS applied to the frontal eye fields (Brodmann area 8) just prior to a visual target increased discrimination of that target in a backward masking task (Grosbras and Paus, 2003). Performance enhancement has been seen in some studies in accuracy measures (Klimesch et al., 2003; Kohler et al., 2004), while other studies reported decreases in reaction time (RT) without change in accuracy (Boroojerdi et al., 2001; Evers et al., 2001; Sparing et al., 2001; Topper et al., 1998). Presumably, TMS-induced enhancements in these studies reflect facilitation of neural processing in localized cortical regions, rather than disruption, though this has not been definitively proven.

Working memory (WM), the cognitive mechanism that enables humans to keep a limited amount of information active for a brief period of time, has been studied extensively using TMS. Both single-pulse TMS (Desmond et al., 2005; Mottaghy et al., 2003; Mull and Seyal, 2001; Nyffeler et al., 2004; Oliveri et al., 2001) and repetitive TMS (rTMS; Herwig et al., 2003; Kessels et al., 2000; Mottaghy et al., 2000, Mottaghy et al., 2002; Pascual-Leone and Hallett, 1994) have been used to investigate WM. In all cases, TMS acted to disrupt performance, either by decreasing accuracy or slowing reaction time. However, there has been some indication of facilitatory effects using parietal rTMS stimulation during delayed memory tasks (Kessels et al., 2000; Oliveri et al., 2001). Kessels et al. (2000) found that RT was significantly faster with rTMS applied to a left parietal site compared to application at a homologous right site, although in neither case was RT significantly different from sham TMS, which had values intermediate between the two. In Oliveri et al. (2001), single-pulse TMS applied simultaneously to right and left parietal cortex resulted in a group mean RT 50 ms faster than in a no-TMS condition; however, this difference was not significant. Nonetheless, these hints of performance facilitation could be followed up by varying the timing of TMS application.

The timing of stimulation during performance of a psychological task is crucial to producing a TMS-related effect. Certainly disruptive effects of TMS depend not just on the region being stimulated, but on the times and durations of stimulation relative to the various phases of a task. Impaired performance often occurs when TMS is applied during processing of target information. A classic example is the masking of visually presented letters that occurs only when single-pulse occipital TMS is applied 70–100 ms after their presentation, about the time the stimulus information is first being processed in the striate cortex (Amassian et al., 1989). The production of facilitatory effects also appears to be time-dependent. In studies reporting facilitation, TMS is often applied immediately before a block of trials (Evers et al., 2001; Sparing et al., 2001) or, within each trial, immediately before a response is to be made (Grosbras and Paus, 2003; Klimesch et al., 2003; Topper et al., 1998). Indeed, in both Kessels et al. (2000) and Oliveri et al. (2001), parietal TMS was also applied prior to when the response was to be made, during the memory retention (or delay) intervals of the tasks. Thus, TMS applied to parietal cortex during the retention interval of a WM task appears to be a candidate for producing performance facilitation.

It should be noted that parietal TMS applied during the delay period has also impaired performance on a WM task (Mottaghy et al., 2003). However, Mottaghy et al. used an n-back WM task, in which the delay period is used not only for retention but also for responding. In this case the TMS may have disrupted response processing. In another WM study, 15 Hz parietal TMS applied during the last 3 s of a 6-s retention phase had no effect (Herwig et al., 2003). In both Kessels et al. (2000) and Oliveri et al. (2001), parietal TMS was applied in the first part of the retention interval, and it may be that facilitation is time sensitive. Additionally, it has been suggested that facilitatory effects depend on another timing parameter, the frequency of stimulation (Klimesch et al., 2003).

In the present study, TMS was applied during the performance of a delayed-match-to-sample task (DMS; Fig. 1), to test the hypothesis that task performance could be facilitated, depending on the stimulation frequency and time of occurrence, as well as on the location of stimulation. The DMS task is a variant of the Sternberg WM task (Sternberg, 1969). TMS has been used with Sternberg tasks twice before, using single pulses (Beckers and Homberg, 1991) and rapid trains of pulses (Herwig et al., 2003). In Beckers and Homberg (1991), occipital TMS during the probe phase increased the memory search time, the average time it took to scan for an item held in memory. In Herwig et al. (2003), 15 Hz stimulation to left premotor cortex during the retention period increased error rates yet had no effect on RT. While TMS thus impaired performance on the task in both WM studies, different choices of stimulation parameters might be expected to result in performance facilitation.

In a first experiment, active and sham TMS were applied at two different locations (left dorsolateral prefrontal cortex and a midline parietal site centered on the precuneus) over a range of stimulus frequencies (1, 5 and 20 Hz). In the case that some combination of frequency and site produced evidence of facilitation, a second experiment was performed to focus on that combination with a larger group of subjects, and to contrast it with TMS of the same frequency applied during a different phase of the task. In this way, we hoped to observe facilitation that was, in the first experiment, site and frequency specific, and in the second, time sensitive.

2. Results

2.1. *First experiment: facilitatory effects during the retention phase*

Percentage accuracy for all conditions is presented in Table 1. Accuracy was high for all participants, averaging 95.4% correct for trials with a set size of one and 89.5% for trials with a set size of six. In the ANOVA of accuracy for the frontal and parietal sites, only the main effect of Set Size was significant (Frontal: $F=28.0$, 1,22 *df*, $p<0.001$; Parietal: $F=17.8$, 1,18 *df*, $p<0.0005$). Such a set size effect is always expected in a DMS task. There were no significant effects of TMS at any frequency or set size at either site. It should be noted that the seemingly lower accuracy scores at the parietal site for active 5 Hz were strongly influenced by a single outlier. Without this subject, the mean scores for the active condition increase to 97.4 and 87.0 for set sizes one and six respectively, while remaining relatively unchanged in the sham condition at 95.3 and 91.7 (Table 2).

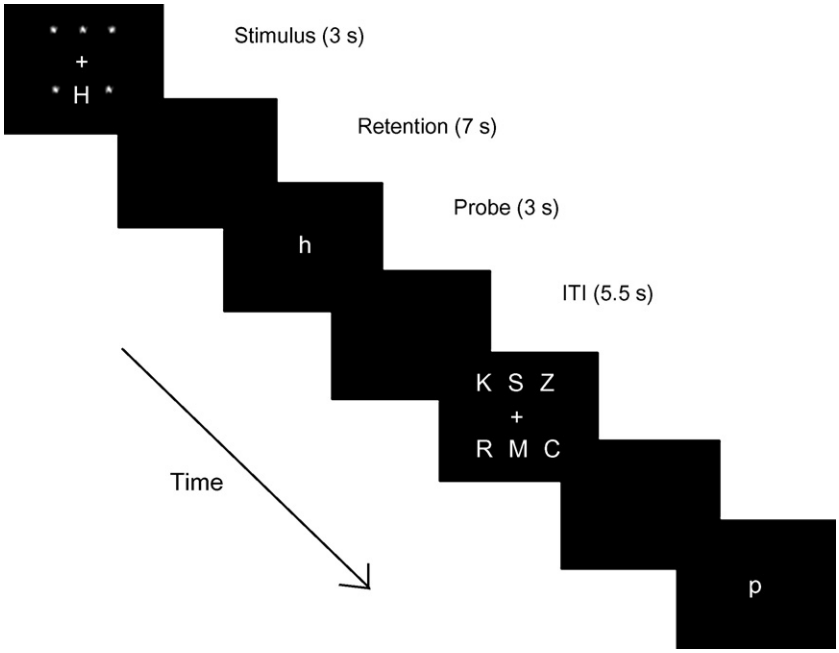

Fig. 1 – Schematic diagram of the delayed-match-to-sample paradigm. Two trials are shown, the first with a set size of one and requiring a "yes" response, and the second with a set size of six and requiring a "no" response. The trial phases and their durations are listed at the right (ITI = inter-trial interval).

In the ANOVA of the median reaction times for the frontal site, only the main effect of Set Size was significant ($F = 132.6$, 1,22 *df*, $p < 0.0001$). Again, such a set size effect is always expected in a DMS task. There were no significant effects of prefrontal TMS at any frequency or set size. Averages of the median reaction times for the parietal site at the three TMS frequencies during the retention phase are shown in Fig. 2. For the ANOVA at the parietal site, again Set Size was significant ($F = 122.5$, 1,18 *df*, $p < 0.0001$). In addition, there was a main effect of TMS ($F = 5.3$, 1,18 *df*, $p < 0.04$), and a TMS*Frequency interaction ($F = 4.7$, 1,18 *df*, $p < 0.025$). In post hoc testing, significance was achieved only in the case of 5 Hz TMS at the parietal site, where there was a mean decrease of 51 ms for memory set size of one ($t = 4.2$, 6 *df*, $p < 0.01$) and 76 ms for set size of six ($t = 2.9$, 6 *df*, $p < 0.015$). Five Hertz stimulation during the retention period was therefore chosen for the second experiment as a likely candidate for facilitation.

2.2. *Experiment 1A: lateral occipital comparison site*

An additional group of nine subjects performed the task while being stimulated by active and sham 5 Hz TMS applied over a third scalp location. This was done because active stimulation at the frontal site was uncomfortable for a number of subjects, which may have influenced task performance. The site chosen, the left middle occipital gyrus, was more comfortable for subjects than the frontal site. It was over a cortical location that was not part of the DMS task-related network found in Haybeck et al. (2004), although it was over extrastriate cortex

Table 1 – Percent accuracy (±SE) for active and sham TMS conditions at the parietal and frontal sites during the retention phase of the DMS task

Set size		1 Hz	5 Hz	20 Hz
Parietal site				
1	Active	91.1±4.0	90.2±7.3	98.2±0.9
	Sham	96.0±2.1	94.6±1.9	96.9±1.4
6	Active	88.4±2.1	82.6±4.9	87.9±2.8
	Sham	92.0±2.6	91.5±1.8	89.7±2.8
Frontal site				
1	Active	97.8±0.8	95.5±2.5	96.0±1.9
	Sham	98.2±0.9	95.2±2.2	94.6±3.0
6	Active	87.9±3.9	91.8±1.6	88.4±3.5
	Sham	91.1±2.1	90.9±1.9	91.5±3.0

Table 2 – Mean RT (±SE), in ms for active and sham TMS conditions at the parietal and frontal sites during the retention phase of the DMS task

Set size		1 Hz	5 Hz	20 Hz
Parietal site				
1	Active	522±40	491±26*	505±35
	Sham	527±34	542±37	488±28
6	Active	670±63	626±35*	652±46
	Sham	687±42	702±52	656±37
Frontal site				
1	Active	532±68	541±26	679±101
	Sham	500±43	557±33	605±98
6	Active	722±79	694±37	906±156
	Sham	670±69	759±48	798±107

* Significant difference between active and sham ($p < 0.02$).

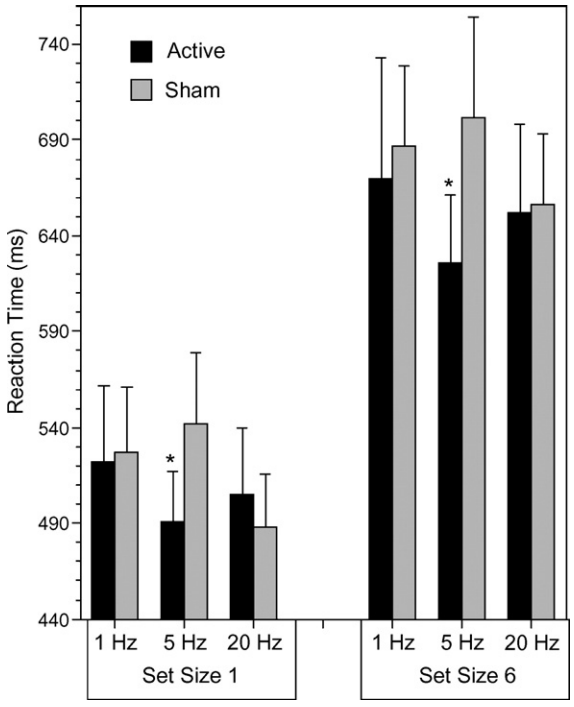

Fig. 2 – Mean reaction times in experiment 1 for active and sham TMS at the parietal site for the three stimulation frequencies.

responsible for visual processing. Performance results are shown in Table 3. At this site, subjects performed slightly worse with active TMS compared to sham at both set sizes. ANOVA results show an expected effect of set size for both accuracy ($F=12.5$, 1,8 *df*, $p<0.01$) and RT ($F=25.4$, 1,8 *df*, $p<0.001$). The difference between active and sham TMS was significant for accuracy ($F=6.8$, 1,8 *df*, $p<0.035$), although not quite so for RT ($F=4.8$, 1,8 *df*, $p<0.06$). At set size of one, subjects were significantly less accurate in the active condition ($t=3.0$, 8 *df*, $p<0.01$), and at set size of six, they were significantly slower with active TMS ($t=1.9$, 8 *df*, $p<0.05$).

2.3. *Second experiment: parietal 5 Hz stimulation during Retention and Probe phases*

Table 4 lists the group mean and SE accuracy scores for all conditions. Accuracy is quite similar between active and sham TMS for both set sizes in the retention phase, but is better with

Table 3 – Percent accuracy (±SE) and mean RT (±SE) in millisecond for active and sham 5 Hz TMS conditions at the occipital site

Set size		Accuracy	Reaction time
1	Active	91.8±1.6**	581±40
	Sham	95.2±1.2	554±31
6	Active	85.7±3.2	789±77*
	Sham	87.8±3.1	754±64

* Significant difference between active and sham ($p<0.05$).
** Significant difference between active and sham ($p<0.01$).

Table 4 – Percent accuracy (± SE) for active and sham 5 Hz TMS conditions at the parietal site during retention and probe phases

Set size		Retention	Probe
1	Active	95.1±0.9	96.8±0.7*
	Sham	95.2±1.2	90.1±2.5
6	Active	91.2±1.7	89.6±1.3
	Sham	89.8±1.7	86.6±2.4

* Significant difference between active and sham ($p<0.01$).

active TMS compared to sham in the probe phase. In the ANOVA, there is a main effect of set size ($F=25.6$, 1,19 *df*, $p<0.0001$), task phase ($F=5.5$, 1,19 *df*, $p<0.035$), and active or sham TMS ($F=6.2$, 1,19 *df*, $p<0.025$). In addition, there is an interaction of set size, phase and TMS condition ($F=6.0$, 1,19 *df*, $p<0.025$). In post hoc testing, the difference in accuracy in active compared to sham conditions at set size one in the probe phase ($t=-2.8$, 19 *df*, $p<0.01$) is primarily responsible for the effects.

Means and SEs of the median reaction times (RT) for 20 participants who had active and sham TMS at the parietal site during both Retention and Probe phases are shown in Fig. 3. The expected increase in RT between trials with memory set sizes of one and six was consistently present, averaging 186 ms. In only one case was there a noticeable effect of TMS: an 88 ms decrease in RT in the active condition compared to Sham for set size of six in the retention phase using 5 Hz TMS. A repeated-measures ANOVA on phase (Retention and Probe), Set Size (one and six), and TMS (Active and Sham) showed main effects of Set Size ($F=140.2$, 1,19 *df*, $p<0.0001$) and TMS

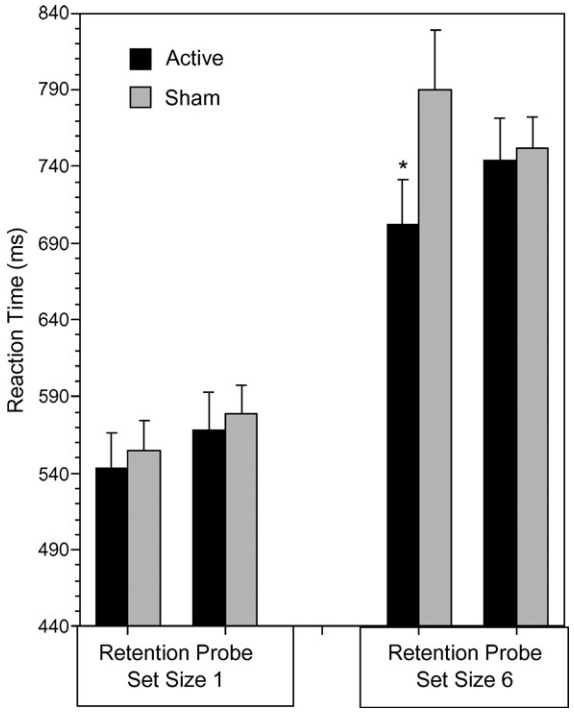

Fig. 3 – Mean reaction times in experiment 2 for active and sham TMS at the parietal site during Retention and Probe phases.

($F=6.15$, 1,19 *df*, $p<0.025$). Set Size*TMS ($F=8.0$, 1,19 *df*, $p<0.011$) and Phase*Set Size*TMS ($F=8.3$, 1,19 *df*, $p<0.01$) interactions were also significant. Post Hoc testing indicated that RT was shorter in Active than in Sham conditions for set size of six in the Retention phase ($t=-3.64$, 19 *df*, $p<0.001$). RT for the Active/Retention condition was also shorter than RT for Active/Probe ($t=-2.19$, 19 *df*, $p<0.02$) and Sham/Probe ($t=-2.47$, 19 *df*, $p<0.012$) conditions.

3. Discussion

Application of TMS to a midline parietal site resulted in facilitation of performance in a DMS task, while stimulation at a frontal site did not and occipital stimulation disrupted performance. Facilitation with parietal TMS was frequency dependent, occurring with 5 Hz stimulation, but not 1 or 20 Hz. TMS resulted in reduced RT with a memory set size of six when applied in the retention phase of the task, and increased accuracy with set size of one with TMS in the probe phase. This finding extends the list of cognitive processes for which TMS has been reported to improve performance to include working memory, which previously had only shown performance decrements with TMS.

In the classic Sternberg memory task, performance follows a very predictable pattern: as the set size is increased, RT increases while accuracy remains unchanged and at a high level. This task was chosen for this study in part because of the robustness of this pattern of performance, such that departures from it could most likely inferred to be effects of TMS. Over experiments 1 and 2, the RT and accuracy results did indeed consistently conform to the classic pattern, with a few departures from that pattern appearing as active/sham differences interpretable as effects of TMS. It should be noted that in the control experiment 1a, there was a departure from the classical result with a set size difference in accuracy as well as RT. In this case, all changes, including those between active and sham TMS, showed a worsening in performance, and helped to validate a site-specific effect on performance enhancement for parietal stimulation.

It might be argued that in the first experiment the effects of the different TMS frequencies are not comparable since there were not equal numbers of TMS pulses across conditions, and that the failure to induce a behavioral effect with 1 Hz TMS can be attributed to the low number of stimuli per train (eight stimuli for 1 Hz TMS vs. 36 stimuli for 5 Hz TMS). A dosage model is certainly the simplest approach to comparing frequencies. Unfortunately, this approach is not amenable to an examination of time-limited task phases. For example, to compare 1 and 5 Hz TMS in this way, 7-s 5 Hz trains must be compared to a 35-s 1 Hz trains. In this case, while the "doses" are equalized, either the durations of task phases must be unacceptably distorted to fit different train durations or stimulation is no longer time-locked to task phase. Nevertheless, even given the time constraints imposed by the task structure, the question can still be asked as to whether TMS of a certain frequency can affect task performance within a task phase. Results from this sort of probe provide useful information for modeling the neural dynamics involved in the task, with the caveat that for lower frequencies, a larger number of pulses may be needed to affect processing. In this regard, the period covered with 20 Hz TMS is problematic, since it could only be applied for 2 s for safety reasons. In another study stimulation in the second phase of a 6-s retention interval did not affect performance (Herwig et al., 2003), and so stimulation in the first phase was tried in the present study. However, 20 Hz TMS in the second part of the retention phase, or covering the complete period may also affect performance.

The same approach applies to the comparison across task phases. In experiment 2, 5 Hz TMS was applied during two task phases of unequal length. TMS was applied over the 7-s retention interval in one condition, but was applied for 4 s in the second condition (from 2 s before the onset of the probe to 2 s after). Probe processing was hypothesized to occur as preparation for the test letter, followed by encoding, memory search, decision, and response. As very few responses occurred beyond 2 s after probe onset, this was considered a boundary to this phase, and it made little sense to continue TMS after response generation simply to equalize pulses across conditions. The effects of TMS were thus compared within the time constraints of task phases, with the assumption that the minimum duration for the TMS to interact with the neural processing involved was not more than 4 s.

In this study, the implication is that the observed facilitation was due to TMS effects on the underlying cortical tissue; however, nonspecific effects can contribute to facilitation. For example, Pascual-Leone and Hallett (1994) reported that in a simple RT task, a single TMS pulse to motor cortex immediately before the cue to respond shortened response time. However, the same facilitation can be produced by sham stimulation in a non-site specific manner (Terao et al., 1997). Terao et al. suggested that what was actually being observed was intersensory facilitation (IF), an effect in which RT can be shortened if some stimulation, such as the auditory click of a TMS coil, occurs closely in time with the cue to respond. IF was also invoked to explain facilitation observed when rTMS was applied during a choice RT task similar to that used here, as RT decreases were seen whether TMS was active or sham (Nixon et al., 2004). It is unlikely that IF explains the facilitation found in the present study, since the same acoustic cues are present at 5 Hz in both active and sham conditions. In addition, the timing of TMS and probe onset met the requirements for IF in the 1 Hz conditions, and yet facilitation was not observed.

Nonspecific effects of TMS can also make comparisons across experimental conditions where TMS varies in frequency or duration problematic. In the present study, RT was higher with 5 Hz sham TMS than other sham conditions at the parietal site in both experiments (see Table 2 and Fig. 3). Distracting events such as the clicking noise of TMS pulses can alter subject set and strategies, and consequently, task performance, and these nonspecific effects may be frequency dependent. As a result, the best comparisons are those between active and sham conditions which share the same temporal or frequency characteristics. In this study, each active TMS condition was contrasted with a sham performed at the same frequency to mitigate this potential confound.

If the observed performance effects were indeed due to TMS acting on the underlying cortical tissue, the effects of TMS trains on the cortex might have come about through one of two different mechanisms. One possibility is that the TMS

train overlaps short but critical time windows during a task when cortical processing important to the task is occurring. The classic example is the 20–40 ms window centered around 80 ms post stimulus onset where single TMS pulses to occipital cortex masked visual stimuli (Amassian et al., 1989). Another possibility is that TMS trains can have effects that are not as time sensitive, but have a cumulative effect on cortical tissue. This cumulative effect could be frequency-dependent, perhaps an interaction of TMS frequency and natural cortical oscillatory activity. For example, in Klimesch et al. (2003), TMS trains at an individual's alpha brain wave frequency, but not same-duration trains of higher or lower frequencies, enhanced performance on a mental rotation task. A different sort of frequency dependency occurs with the first mechanism. In that case, increasing the frequency of the TMS train, from say 1 Hz to 5 Hz, increases the probability that a pulse or pulses may occur within a sensitive processing window. It should be noted that the frequency manipulation used in experiment 1 cannot be used as a direct test to distinguish these two mechanisms, because, due to safety restrictions, the 20 Hz trains could not cover the same periods as the 1 Hz and 5 Hz trains.

Given these two general mechanisms of TMS action, two explanations have been proposed for RT facilitation due to neural changes caused by TMS, one relying on the disruptive aspects of magnetic stimulation, and the other on neural modulation. In the present study, these two mechanisms may explain the two forms of performance facilitation observed: facilitation of performance accuracy for set size 1 trials with TMS during probe phase and RT enhancement seen with retention phase TMS. In the case of the first mechanism, neural processing which interferes with task performance is disturbed by TMS. For example, TMS applied to a superior occipital location which analyzes direction of motion resulted in an improvement in performance in a visual search task when stimuli were moving but direction of motion was irrelevant (Walsh et al., 1999). This sort of improvement through subtraction of irrelevant processing may also have occurred in a study of TMS effects on a Stroop task (Hayward et al., 2004). In that study, TMS applied to anterior cingulate cortex negated the addition to RT caused by Stroop interference. This suggested that this region is involved with evaluative processes that are not necessary in this task, such that their disruption allowed overall processing of the stimulus to be faster. Likewise, in the present study, perhaps midline parietal processes which normally interfere or compete with retention phase rehearsal of the memory items were disrupted. This might explain the improved accuracy with set size of one in the probe phase of the task, a case where processing is simpler and may not require parietal participation.

The second explanation posits that TMS delivered to cortex necessary for task performance just prior to its activation increases neural excitability in a way that can enhance performance under some conditions. For example, stimulation of neurons in the frontal eye fields during the 100 ms prior to a visual target improved target detectability (Grosbras and Paus, 2003; Moore and Fallah, 2001). Trains of TMS, especially at 5 Hz, have been shown to produce lasting effects on cortical excitability as measured by electrophysiological response (Barardelli et al., 1998; Peinemann et al., 2000) and with PET imaging (Siebner et al., 2000). In another study, 5 Hz TMS applied to somatosensory cortex immediately before a tactile discrimination task significantly improved performance (Ragert et al., 2003). In the present study, the application of 5 Hz TMS over the 7-s period just prior to probe presentation likewise may have increased the excitability of parietal neurons in a way that enhanced the comparison process between the probe and the memory items. The mechanism behind such enhancement is unknown. It has been suggested that a local increase in excitability, perhaps produced by a temporary increase in the amplitude of excitatory post-synaptic potentials (e.g., Iriki et al., 1989), may lead to a larger neural response. On the other hand, a general increase in neural activity might not explain enhancement of a more complex process of item comparison. Another possibility is that TMS affects the oscillatory dynamics of brain networks, perhaps by generating a resonance with local alpha activity (Klimesch et al., 2003). Studies have shown task performance to be positively correlated with the size of local alpha activity occurring prior to task processing and with the depth of alpha desynchronization after the onset of task-related stimuli (e.g., Neubauer et al., 1995). Klimesch et al. (2003) demonstrated that a train of parietal TMS applied at an individual's peak alpha frequency (about 10 Hz) immediately before a mental rotation task increased both performance accuracy and the depth of alpha desynchronization. In the present study, stimulation at 5 Hz, an approximate subharmonic to alpha frequency, may have generated a similar oscillatory effect, with concomitant enhancement of task performance.

If parietal cortex is involved with processing memory search, pre-conditioning with TMS during the retention phase prior to the search may have sped the process, possibly through local increases in excitation or resonant oscillatory activity. An imaging study using the DMS task (Haybeck et al., 2004) favors this mechanism. In that study, a brain network was found in the probe phase of the DMS task whose activation was related to performance, while activity during the retention phase was not. The regions of activation associated with the DMS task included a number of posterior regions, including midline parietal cortex. Previous TMS studies of parietal cortex in WM tasks have focused on more lateral sites (Herwig et al., 2003; Kessels et al., 2000; Mottaghy et al., 2003; Oliveri et al., 2001). This is in keeping with recent anatomical and neurological findings that lateral inferior parietal cortex plays an important role in the processing of verbal materials (Catani et al., 2005). However, more medial inferior parietal regions are also activated in verbal tasks (e.g., Bullmore et al., 2000), and in fact TMS at midline parietal sites has been found to alter RT in a verbal task (Lou et al., 2004).

A parietal role in verbal processing may also explain why facilitation with 5 Hz stimulation was seen for a set size of one in the first experiment but was not replicated in the larger sample of the second experiment. Most subjects reported using a mnemonic strategy that mixed rote rehearsal with semantic associations of the stimulus letters with words. The semantic strategy was quite useful in the case of six letters, but not necessarily with a set size of a single letter. Its application in the latter case would depend on an individual's cognitive style, and could be expected to vary considerably

between subjects. To the degree that the parietal cortex contributed to such verbal processing and TMS facilitated it, a small group of participants employing a similar cognitive strategy might demonstrate a benefit at a set size of one, while a larger, more varied group would not. Underlining this is the suggestion of a speed–accuracy trade-off between active and sham conditions at set size one seen in the parietal group from the first experiment, while the RT and accuracy at set size one for the larger group in the retention condition of the second experiment are roughly the same between active and sham.

While the facilitory effects of TMS suggest that parietal cortex may play a role in processing the DMS task, probe phase TMS did not appear to disrupt this processing, while disruption did occur with retention phase TMS at the occipital site. This was not entirely surprising, and points out that facilitation and disruption of performance by TMS may be caused by different mechanisms. Disruptive effects of TMS in WM tasks are often found to be dependent on the timing of pulses relative to stimulus presentation (Barardelli et al., 1998; Mottaghy et al., 2003). Parietal processing may not have been sensitive to disruption at the particular pulse times used with the 5 Hz stimulation, while processing at the occipital site may have been. On the other hand, while not dependent on exact timing of pulses, facilitory effects may be frequency dependent. In the present study, 1 and 20 Hz stimulation had little effect, while 5 Hz did. A number of other TMS studies have reported facilitory effects with 5 Hz stimulation (Barardelli et al., 1998; Peinemann et al., 2000; Ragert et al., 2003). In the present study, a strategy of applying TMS trains within the boundaries of overt task stages at three set frequencies was used. In future TMS experiments, train onset times and duration as well as frequency should be parametrically varied to further determine these time and frequency effects and to use them to understand the neural mechanisms underlying facilitory and disruptive effects. The techniques resulting from such studies could allow the use of TMS in exploring in a more sophisticated way the dynamics of functional networks illuminated through imaging, and the neuropsychological processes they support.

4. Experimental procedure

4.1. *Subjects*

Forty-four healthy male and female volunteers (14 female) with a mean age of 26.5 ± 3.2 years were recruited and signed written consent for the study. The study was approved by the Columbia University Investigational Review Board and the New York State Psychiatric Institute Investigational Review Board, and was performed under an approved FDA Investigational Device Exemption (IDE). Subjects were required to be right handed (as determined by using the modified Edinburgh Handedness Questionnaire), have normal or corrected-to-normal vision, and be native English speakers. Potential subjects were excluded if they had a history of current or past Axis I psychiatric disorder including substance abuse/dependence as determined by the Structured Clinical Interview for DSM-IV Axis I disorders (SCID–I/NP) or a history of neurological disease. All subjects were screened with physical and neurological examinations, blood and urine testing, urine drug screens, and pregnancy tests for women of childbearing capacity.

4.2. *DMS task*

Participants were trained on the delayed-match-to-sample (DMS) task0. Each trial was 20 s long, according to the following sequence of three task phases: first, an array of one or six upper case letters was presented on a computer screen for 3 s (the stimulus phase; see Fig. 1). Each letter subtended 1.1° of visual angle. Next, the screen was blank for 7 s (the retention phase), during which the subjects were asked to fixate on the center of the screen and keep the stimulus items in mind. Finally, a test stimulus, a single lower case letter, appeared for 3 s at the center of the screen (the probe period). At this time the subject was to indicate by a button press whether or not the probe letter matched a character in the stimulus array, using the right hand for matching probes and the left if it did not match. Subjects were instructed to respond as quickly and as accurately as possible. Following the probe phase was a 7-s inter-trial interval, during which the computer screen was again blank. Choice of set size and positive or negative probe for an individual trial was pseudo-random, with the restriction that there be 16 true positive and 16 true negative probes for each of the two set sizes over a block of 64 trials. TMS was applied every other trial in a block. This interleaving yielded an interval of 30 s between TMS trains, consistent with safety guidelines (Chen et al., 1997).

4.3. *TMS application*

Two types of stimulation were used: active and sham. Active TMS was applied using a vacuum-cooled figure 8 coil (5-cm diameter) powered by a Magstim Super-Rapid stimulator (Magstim Co., Whitland, South West Wales, UK). For sham TMS, a sham coil the same size and shape as the active coil was attached to the Super Rapid device, and was placed against the subject's head in the same way as for the active coil. This coil contained shielding to create the sound of a TMS train without actually delivering a magnetic stimulus. In addition, subjects were told that the coil would be placed at different sites, and that even very small differences in its exact location could result in very different sensations, depending on whether it was directly over a nerve or a muscle. The sham condition was reasonably convincing to the participants. When asked at the end of each session to make a best guess as to whether each condition was active or sham TMS, subjects were correct only 61% (±25%) of the time. When asked to rate their confidence in each guess on a scale of 0 (no confidence) to 3 (high confidence), they had a mean rating of 1.71 (±0.66) for those conditions they had correctly guessed, midway between low and moderate confidence.

TMS stimulus intensity was set at 100% of motor threshold of the left hemisphere, which was defined as the lowest intensity needed to evoke motor potentials of at least 50 μV recorded from the first dorsal interosseus in at least 5/10 stimulations.

Two sites were chosen for stimulation in the present study, based on cortical regions activated on preliminary analyses of fMRI recorded during performance of the DMS task in our

laboratories: left dorsolateral prefrontal cortex (DLPFC) and a midline parietal site centered on the precuneus (MPC) (Rakitin et al., 2004). Frontal and parietal sites are frequently activated in imaging studies of WM tasks (e.g., Petrides et al., 1993). For example, DLPFC shows increasing fMRI activation with increasing memory set size in the DMS task used here (Rypma et al., 2002). Prefrontal and parietal cortices have also been the most frequently targeted regions in TMS studies of WM. High-resolution structural MRI scans were obtained for each subject. The target sites were localized using Brainsight, a computerized frameless stereotaxy system (Rogue Research, Montreal, Canada). This system uses an infrared camera to monitor the positions of reflective markers attached to the participant's head. Head locations are correlated in real time with the participant's MRI data after the data are coregistered to a set of anatomical locations. Reflective markers are attached to the coil and the subject, so that relative positions of the coil to the head (and the MRI) can be tracked, allowing precise positioning of the coil with respect to previously chosen MRI locations. The parietal site was marked on the MRI as the point halfway between the occipital–parietal sulcus and the sulcus that defines the anterior portion of the precuneus in the mid-sagittal section. DLPFC was marked by starting from the most anterior point of corpus callosum in the mid-sagittal section, and then following a line along the coronal section in the plane of that point at a 45° angle from it to the exterior of brain in the left hemisphere.

TMS produced side effects in some subjects. Of the 44 subjects, 9 reported mild to moderate headaches or scalp pain at the end of at least one session. Over a total of 139 sessions, headache occurred 14 times. In addition, two of the 44 participants reported a moderate impairment in ability to concentrate (in one of six sessions for one and in two of five sessions for the other). No other side effects were reported and there were no seizures.

4.4. *Procedure for the first experiment*

The retention phase was chosen as the target for facilitation by TMS, following the timing of the other reported facilitatory effects (e.g., Grosbras and Paus, 2003; Klimesch et al., 2003; Topper et al., 1998), where TMS was applied immediately before a response was to be made. Three frequencies of TMS were used during the retention phase in alternate trials: 1, 5 and 20 Hz. Trains at 1 and 5 Hz could completely span the 7-s retention phase, but due to safety concerns, 20 Hz could only be applied for 2 s. For 20 Hz TMS, stimulation was applied in the first phase of the retention interval, since in previous work TMS given in the second half of a 6-s retention interval of a WM task did not result in facilitation (Herwig et al., 2003). It should be noted that in the present design, TMS frequencies were being compared by their effect within a defined task phase, rather than by equivalent numbers of stimuli across frequencies. Only one type of frequency was used in a block of trials. These stimulation parameters are well within safety guidelines (Wassermann, 1998). At a given site, two blocks of active and two blocks of sham TMS were run. Including Brainsight positioning, each set of two blocks lasted about 50 min. Due to the large number of conditions (two sites, three frequencies, active and sham TMS) and the amount of time required to complete a session, not every subject performed all conditions. For each site and frequency, seven participants completed four blocks of DMS trials with active and sham TMS, except in the case of DLPFC at 5 Hz. As a facilitation of RT with active (as compared to sham) TMS looked to be a possibility for that condition in pilot testing, 11 subjects were run. An ANOVA with a between groups factor of Frequency and repeated-measures factors of Set Size (one and six) and TMS (Active and Sham) was performed on the median RT data for each site. A facilitative effect would be indicated by a decrease in reaction time and/or an increase in accuracy when active TMS was used (as opposed to sham TMS). A facilitative effect would only be concluded if the decrease in RT was not accompanied by a decrease in accuracy. One condition, parietal 5 Hz, was found to have a significant facilitation of RT. This condition was chosen for the second experiment.

4.5. *Procedure for the second experiment*

Twenty-one subjects participated in the second experiment. None had received 5 Hz TMS in the first experiment. TMS at 5 Hz during the retention phase was compared with 5 Hz stimulation during the probe phase. The probe phase was chosen due to performance-related network activation during this period of the DMS task found using fMRI (Haybeck et al., 2004). As before, the 5 Hz retention phase train again ran through the entire 7-s retention interval. In addition, to cover the probe phase, a 5 Hz train began 2 s before the appearance of the probe and ended 2 s later (20 pulses). Again only one type of train was used in each block. All TMS was applied at the parietal site. Each participant received two blocks of trials in each of the two trial phases (Retention and Probe), in both active and sham conditions, for a total of eight blocks of trials. Participants returned to the laboratory over consecutive days until all conditions were performed. All conditions were counterbalanced across subjects. A repeated-measures ANOVA with factors of Phase (Retention and Probe), Set Size (one and six), and TMS (Active and Sham) was performed on the median RT data. One subject, whose RTs were greater than two standard deviations from the group mean, was excluded from the analysis as an outlier.

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