A new definition of visual short-term memory
Sligte, I. G.

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4. Magnetic stimulation of the dorsolateral prefrontal cortex dissociates fragile visual short-term memory from visual working memory

To guide our behavior in successful ways, we often need to rely on information that is no longer in view, but maintained in visual short-term memory (VSTM). While VSTM is usually broken down into iconic memory (brief and high-capacity store) and visual working memory (sustained, yet limited-capacity store), recent studies have suggested the existence of an additional and intermediate form of VSTM that depends on activity in extrastriate cortex. In previous work, we have shown that this fragile form of VSTM can be dissociated from iconic memory. In the present study, we dissociate fragile VSTM from visual working memory by showing that magnetic stimulation of the right dorsolateral prefrontal cortex (DLPFC) disrupts visual working memory, while leaving fragile VSTM intact. Thus, this study shows that we can dissociate three stages in VSTM that have clearly different characteristics and rely on different neural structures. In addition, we observed that people with high DLPFC activity had superior working memory capacity compared to people with low DLPFC activity, and only people with high DLPFC activity really showed a reduction in working memory capacity in response to magnetic stimulation. Thus, it might be possible to predict whether a particular subject will respond to magnetic stimulation based on his/her functional MRI activity.

Introduction

Our brain essentially acts as a filter that reduces the amount of information at each subsequent step in the neural hierarchy. This mechanism is especially evident when looking at different stages in visual short-term memory (VSTM). Initially, people build up a very high capacity representation in iconic memory that lasts about half a second (Sperling, 1960), and this iconic image seems to be related to retinal firing beyond stimulus duration (Sligte, et al., 2008). At a slightly higher level in the neural hierarchy, in visual area V4 (Sligte, et al., 2009), a high-capacity representation (up to 15 objects) is maintained for up to four seconds after disappearance of a stimulus (Lepsien, et al., 2005; Sligte, et al., 2008). Whenever a new stimulus arrives, this high-capacity store is overwritten, but it is insensitive to meaningless flashes of light that do overwrite iconic memory traces (Sligte, et al., 2008). We will refer to this store as fragile VSTM. Finally, a maximum of four objects receives enough top-down amplification to be represented in parietal and frontal regions of the brain (Luck & Vogel, 1997; Pessoa, et al., 2002). This latter, capacity-limited store, operating at the top of the VSTM pyramid, is usually referred to as visual working memory.

This gradual account of VSTM composed of three stages adds fragile VSTM to the standard model of VSTM. However, it is important to rule out the possibility that fragile VSTM is a form of iconic memory or visual working memory. So far, we have dissociated fragile VSTM from iconic memory as 1) features are bound to form coherent objects in fragile VSTM (Landman, et al., 2003), 2) its traces last a factor 10 longer than iconic memory traces (Lepsien, et al., 2005; Sligte, et al., 2008), and 3) it is only overwritten by the presentation of new objects at the same location, not by homogenous patterns or flashes of light (Landman et al, 2003; 2004; Sligte et al., 2008). Nevertheless, the question remains whether fragile VSTM is some kind of high-capacity, but weakly represented form of visual working memory.

To answer this question, we aimed to show that maintenance of information in fragile VSTM does not depend on the neural substrate that is responsible for working memory maintenance. To be more specific, lesion studies (Goldman & Rosvold, 1970) and transcranial magnetic stimulation (TMS) studies (Koch, et al., 2005; Oliveri, et al., 2001; Turatto, Sandrini, & Miniussi, 2004) have shown that the right dorsolateral prefrontal cortex (DLPFC) is crucial for working memory maintenance. If fragile VSTM is a form of working memory, magnetic stimulation of the DLPFC during stimulus maintenance should reduce the capacity of both fragile VSTM and visual working memory.

To anticipate, we found that magnetic stimulation of the right DLPFC during stimulus maintenance reduced working memory capacity, but not fragile VSTM capacity. This implies that fragile VSTM is not a form of visual working memory. Moreover, we observed that only a
subset of our subjects displayed a reduction in working memory performance caused by TMS. Exploratory analyses showed that the amount of BOLD MRI activity in the targeted area and the size of the region of interest together explained 74% of the effectiveness of TMS in reducing working memory capacity. Future studies might further clarify this link between BOLD MRI measures and the effectiveness of TMS.

Methods

Subjects

13 right-handed adults (9 females) with normal or corrected-to-normal vision participated in this experiment for financial compensation. None of the subjects had a history of seizures, neurological diseases or other risk factors. All subjects gave their written informed consent to participate in the study, which was approved by the local ethics committee of the department of Psychology of the University of Amsterdam. One of the subjects could not tolerate transcranial magnetic stimulation (TMS) to the dorsolateral prefrontal cortex (DLPFC), so we decided to quit the session right at the beginning. In the end, 12 participants (8 females) finished the entire experiment and their data are reported in the paper. In total, each participant received 256 magnetic pulses at 110% of their resting motor threshold corresponding on average to 54 percent of the total machine output. In addition, eight of our subjects participated in a control experiment, in which repetitive TMS (rTMS) was delivered either at the left DLPFC or the right DLPFC in two different sessions. The order of stimulation (the left or the right DLPFC first) was counterbalanced across subjects. In total, participants received 1280 magnetic pulses in each rTMS session at 110% of their resting motor threshold. In two cases, subjects experienced a mild headache after an rTMS session that was alleviated by aspirin. In addition, in one case a subject reported being dizzy for 10 minutes after the rTMS session. Our subjects reported no other side effects.

Task design TMS experiment

We used a slightly modified version of the retro-cue/post-change cue change detection paradigm that was used in a previous experiment from our lab (Sligte, et al., 2008). In the present experiment, memory and test displays consisted of eight white (97.54 cd/m²) oriented rectangles on a black (1.09 cd/m²) background (see Fig. 4.1). Individual rectangles (2.22° × 0.55° in size) were presented at an eccentricity of 5° of visual angle and could be horizontal, vertical, 45° to the vertical or 135° to the vertical. Each orientation was presented at least once and at most three times. Throughout the entire experiment, a red fixation dot (0.66° × 0.66° in size) was present at the centre of the screen and it only turned green for
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100 ms to indicate the start of a trial. Spatial cues consisted of 2-pixel thick lines that were at one end touching the fixation dot and at the other end close (3.6°) to the centre of a single rectangle.

Figure 4.1 Subjects performed a visual short-term memory task, in which they had to detect changes that occurred between a memory and a test display. In addition, attention-directing cues were presented during the retention interval (Retro-cue trial) or thereafter during the test display (Post-change cue trial). Typically, people can report up to 15 items (out of 32) on retro-cue trials, but no more than 4 items on post-change cue trials; this reflects the capacity of fragile visual short-term memory representations and the capacity of visual working memory, respectively. On 50% of the trials, single-pulse transcranial magnetic stimulation (TMS) was applied to the right dorsolateral prefrontal cortex (DLPFC). 600 ms after offset of the memory display.

The trial design is shown in Figure 4.1. At the beginning of each trial, the fixation dot in the middle of the screen turned green for 100 ms. Then, a 250-ms memory display appeared and subjects were instructed to remember the orientation of each object in this display to their best ability. After offset of the memory display, a blank retention interval of 2000 ms was shown. Finally, a 2000-ms test display was shown in which the cued item was orthogonal compared to the memory display on 50 percent of the trials, and did not change on the other 50 percent. On retro-cue trials, a spatial cue was presented during the retention interval (between 1250 and 1750 ms after offset of the memory display) and this cue retrospectively singled out the item to potentially change (so-called retro-cue; Griffin et al., 2003). On post-
change cue trials, the spatial cue was shown on top of the test display (100 until 600 ms after onset of the test display). During the test display, subjects were required to respond by button press whether memory and test display were identical or whether the cued rectangle had changed. Furthermore, pseudo-randomly on 50% of the retro-cue trials and on 50% of the post-change cue trials, a transcranial magnetic stimulation (TMS) pulse was delivered at the right dorsolateral prefrontal cortex (DLPFC). This TMS pulse was presented during the retention interval, 600 ms after offset of the memory display, and had an intensity of 110% of the resting motor threshold. We chose to present the TMS pulse 600 ms after offset of the memory display because at that latency it caused a large reduction in working memory performance in a previous TMS study (Oliveri, et al., 2001). All trials were randomly intermixed within blocks of 128 trials. In total, subjects performed four blocks of 128 trials.

In addition, we performed a repetitive TMS control experiment. The trial design of this control experiment was almost identical to the above-described design, but altered in two ways: 1) the retention interval in the post-change cue condition was shortened to 1150 ms to keep encoding time identical for both conditions (time until retro-cue and post-change cue is now 1250 ms on both trials), and 2) we applied 5 TMS pulses at 200, 300, 400, 500, and 600 ms (5 pulses at 10 Hz) after memory display offset to rule out that performance on both retro-cue and post-change cue conditions depends on the DLPFC, albeit at different latencies.

**TMS specifications and stimulus presentation**

Transcranial magnetic stimulation (TMS) was delivered with the use of a 3.5T MagStim Rapid² Stimulator (Magstim Co., UK) and a figure-of-eight shaped coil (70-mm outer diameter). Before the experiment commenced, we determined the resting motor threshold of each individual. We followed the guidelines of the International Federation of Clinical Neurophysiology (Rossini, et al., 1994) to determine the minimum intensity that induced a visible movement to the contralateral first interosseus dorsalis muscle (read: a hand muscle).

Subsequently, we aimed the TMS coil at the right dorsolateral prefrontal cortex (DLPFC). The location of this area was determined, for each subject, with the use of BOLD-MRI (see below, and see Table 4.1 for details on the DLPFC for each subject). We aimed the TMS coil at the highest voxel value in the anterior part of the middle frontal gyrus with the use of the Visor system and dedicated ANT software (ANT – Visor system; ant-neuro.com). In our rTMS control study, we also aimed the TMS coil at the left DLPFC. The exact location of the left DLPFC mirrored the stereotactic location of the right DLPFC. The applied TMS intensity was 110% of the resting motor threshold, which corresponds on average to 54 percent of the total stimulator output.
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Pearson correlation with magnitude of TMS effect

|                  | -.13 | .60* | .67* | -.57* | .13 |

Table 4.1 Details on the right dorsolateral prefrontal cortex (DLPFC) of individual subjects

Talairach: The stereotactic location of the DLPFC is reported as the maximum activation in the anterior part of the middle frontal gyrus in standard BrainVoyager Talairach coordinates. Magnitude of TMS effect: the reduction in performance on the post-change cue condition caused by TMS, in %. Size: number of significantly activated and contiguous voxels, FDR-corrected for multiple comparisons. Max activity: highest activated voxel in the DLPFC (t-value based on contrast WM vs. Fixation-control). Mean activity: mean activation across voxels in the DLPFC, FDR-corrected for multiple comparisons (t-value based on contrast WM vs. Fixation-control). Depth: distance from the scalp to the nearest significantly activated voxel in the right DLPFC (FDR-corrected for multiple comparisons), going perpendicular from the scalp to the maximum activation in the right DLPFC. TMS intensity: 110% of the resting motor threshold reported as % output of the TMS apparatus.

Defining the DLPFC as region of interest

We used neuronavigation to aim the magnetic coil at the anterior part of the right dorsolateral prefrontal cortex (DLPFC). To derive the location of this region of interest (ROI), each subject
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performed a visual working memory task in the MR scanner that was similar to the task used by Pessoa and colleagues (Pessoa, et al., 2002); only the retention interval was two seconds instead of six seconds (see Fig. 4.2 for examples of the task).

**Figure 4.2** To derive the location of the right dorsolateral prefrontal cortex (DLPFC), each subject performed two blocks of a visual working memory task in the MR scanner (task adopted from Pessoa and colleagues, 2002; see Methods for details). By contrasting working memory trials with fixation control trials in which subjects do not have to maintain any information in working memory, we derived a widespread network of brain regions related to working memory maintenance. We used the single-subject activation map to navigate the TMS coil to the cluster showing the maximum activation in the anterior part of the right middle frontal gyrus. Note that activity in this figure is the mean activity over subjects (N = 12), Bonferroni-corrected for multiple comparisons. For details on the region of interest for each subject apart, see Table 4.1.

Details of the task are as follows: each trial started with the onset of a white fixation dot (46.4 cd/m²; 0.48° of visual angle) that was present for 1000 ms at the centre of a black display (0.2 cd/m²). Next, a 500-ms memory grey display (23.2 cd/m²) was shown that contained eight oriented rectangles (46.4 cd/m²; 0.39° by 1.56° of visual angle each) that were either horizontal, vertical, rotated 45° to the vertical or rotated 135° to the vertical. Subjects were instructed to remember the orientation of these rectangles to their best ability. After offset of the memory display, a 2000-ms blank display (0.2 cd/m²) was shown that acted as a retention interval. Next, a 2000-ms grey test display (23.2 cd/m²) was shown that was identical to the memory display in 50 percent of the trials (no change trials), and in the other 50 percent of the trials (change trials) one of the rectangles had an orthogonal orientation.
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cmpared to the memory display. Finally, the letter M was shown for 2,000 ms in the middle of a grey screen (23.2 cd/m²) indicating that subjects should decide whether the trial was a match trial (no change) or a non-match trial (change). Subjects responded by means of a button press. On 20 percent of the trials, no rectangles were presented in both memory and test display (so-called fixation control trials), but still the screen turned grey for 500 and 2,000 ms respectively. Subjects were instructed to maintain fixation on these fixation control trials and to press either one of the buttons when the “M” appeared on screen. A random inter-trial interval of 2000-6000 ms was used (steps of 1000 ms). Subjects performed two separate runs of this experiment in the MR scanner. Each run consisted of 40 working memory trials and 10 fixation control trials and started and ended with a 16 seconds lasting baseline in which no stimulation was delivered.

Scanner pulses triggered the start of each functional run. Stimuli were presented with Presentation (NeuroBehavioral Systems) and they were front-projected from a liquid crystal display (LCD) projector on to a screen at the feet of the supine subject. The supine subject viewed the screen through a mirror just above the eyes. We immobilized the subject’s head with foam pads (to reduce motion artifacts) and earplugs were used to moderate scanner noise. Total scanning time was approximately 30 minutes.

Magnetic resonance imaging (MRI) data were acquired from a Philips 3T scanner. For each individual, we first acquired an anatomical high-resolution image with conventional parameters [T1 turbo field echo; 182 coronal slices; flip angle (FA) of 8°; echo time (TE) of 4.6 ms; repetition time (TR) of 9.6 s; slice thickness of 1.2 mm; field of view (FOV), 250 × 250 mm; in-plane voxel resolution, 0.98 × 0.98 mm]. For our working memory experiment, blood-oxygenation level dependent (BOLD) MRI was measured using these parameter [T2*-weighted; 35 transversal slices; FA, 80°; TE, 30 ms; TR, 2.5 s; slice thickness, 3 mm; slice gap, 0.3 mm; FOV, 220 × 220 mm; 96 × 96 matrix; in-plane voxel resolution, 2.3 × 2.3 mm].

Image analysis was performed with the use of Brainvoyager QX (Brain Innovation, Inc.). Data preprocessing included image realignment, 3D motion correction, correction for slice scans acquisition order, temporal high-pass filtering (0.01 Hz) and linear detrending, and spatial smoothing with a kernel of 4 mm (FWHM). We then created statistical parameteric maps with the use of a multiple regression analysis convolved with a canonical hemodynamic function. By contrasting working memory trials with fixation-control trials (no items to remember), we derived a network of brain areas comprising bilateral LOC, bilateral posterior parietal cortex, bilateral frontal eye fields, and the right DLPFC (see Fig. 4.2, Bonferroni-corrected mean activation across subjects plotted). The right DLPFC was defined as the highest activated voxel in the anterior part of the middle frontal gyrus.
**Details on the right DLPFC**

All details on the right DLPFC can be found in Table 4.1, for each subject apart. In this section, we report how all details on the right DLPFC were calculated. **Size** of the right DLPFC was defined as the number of contiguous voxels that showed significant activation on the contrast working memory vs. fixation control, false discovery rate (FDR) corrected for multiple comparisons. **Mean activity** in the right DLPFC was defined as the mean activity (a t-value) across all contiguous voxels that showed significant activation on the contrast working memory vs. fixation control, FDR-corrected for multiple comparisons. **Max activity** in the right DLPFC was defined as the highest activated voxel (a t-value) on the contrast working memory vs. fixation control. **Depth** was defined as the distance from the scalp to the nearest significantly activated voxel in the right DLPFC (FDR-corrected for multiple comparisons), going perpendicular from the scalp to the maximum activation in the right DLPFC. **TMS intensity** is expressed as the percentage of total stimulator output and corresponds to 110 percent of the resting motor threshold of each individual. Finally, **Talairach** locations are reported in BrainVoyager format (x, y, and z separately).

**Procedure TMS study**

In the first session, we screened subjects on a history of seizures, neurological diseases and other factors that may pose a risk (Rossi, Hallett, Rossini, & Pascual-Leone, 2009). In addition, we tested their visual acuity. Thereafter, we trained them for one block (~10 min) on the fMRI task (see previous section). In the second session, we acquired a high-resolution anatomical image of each individual (~10 min). In addition, subjects performed two runs of the fMRI task (~20 min). In a two-week period following the MRI session, subjects were trained on the TMS experiment until they reached a performance of 75% (see Task design TMS experiment for task specifications). Most subjects (N = 9) were trained once for two hours, although three participants were trained twice for two hours to reach this performance criterion. Typically, subjects perform very well on retro-cue/post-change cue change detection experiments provided that they have trained the task for at least an hour. Finally, subjects performed the TMS experiment, while TMS was applied pseudo-randomly on 50% of the retro-cue and on 50% of the post-change cue trials. Subjects performed four blocks of 128 trials (32 retro-cue trials without TMS, 32 post-change cue trials without TMS, 32 retro-cue trials with TMS, 32 post-change cue trials with TMS). All trials were randomly intermixed within blocks. After the experiment, subjects were compensated financially for their participation.
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Procedure control study with rTMS

We invited all subjects who took part in the initial single-pulse TMS study to participate in the follow-up repetitive TMS control study, but only eight subjects were available for participation. In the first rTMS session, subjects were to perform one practice block without TMS (128 trials) to freshen up their memory about the task. Then, they performed four blocks of 128 trials (32 retro-cue trials without rTMS, 32 post-change cue trials without rTMS, 32 retro-cue trials with rTMS, 32 post-change cue trials with rTMS), while rTMS was applied either at the left DLPFC or the right DLPFC. The order of stimulating left or right DLPFC was counterbalanced between subjects. All trials were randomly intermixed within blocks. After the experiment, subjects were compensated financially for their participation.

Data analysis

We first converted the performance of individual subjects into an estimate of representational capacity, using Cowan’s K formula (Cowan, 2001). This formula is \( K = (\% \text{ hits on change trials} - \text{chance} + \% \text{ correct rejections on no-change trials} - \text{chance}) \times \text{set size}. \) A powerful aspect of this formula is that it corrects for guessing trials, but in essence it is a linear transformation of percentage correct. The basic statistical analyses were performed with repeated measures ANOVAs and paired t-tests. All tests reported in the paper are one-tailed.

In addition, we performed exploratory analyses to investigate why some subjects show a big reduction in working memory performance in reaction to TMS, while other subject do not show any effect at all (from now on called the “magnitude of the TMS effect”). As part of these analyses, we correlated mean activity in the right DLPFC and minimal depth to the right DLPFC with the magnitude of the TMS effect, using Pearson’s R. We also performed split-half analyses, where we divided the subjects in two groups of \( N = 6 \) with the highest mean activity in the DLPFC and the lowest mean activity in the DLPFC. Comparisons between groups were tested with one-tailed unpaired t-tests assuming unequal variance between groups. Comparisons within groups were tested with one-tailed paired t-tests. We also divided the subjects into two groups of \( N = 6 \) with DLPFC activation closest to the scalp and furthest away from the scalp. Tests for comparisons were similar as described above.

Both mean DLPFC activity and DLPFC depth were significantly correlated with the magnitude of the TMS effect on visual working memory, but DLPFC activity and DLPFC depth were also correlated with each other. Thus, it might be that only one functional measure is related to the between-subjects variations in TMS effects. To investigate this, we computed partial correlations between DLPFC activity and the magnitude of the TMS effect
controlled for DLPFC depth, and between DLPFC depth and the magnitude of the TMS effect controlled for DLPFC activity. These analyses showed that the correlation between DLPFC depth and the magnitude of the TMS effect was in fact mediated by DLPFC activity. We found confirmation of the partial correlation results in the results of a step-wise linear regression, where we entered the magnitude of the TMS effect on working memory as dependent variable and all details on the DLPFC as independent variables (size, mean activity, max activity, depth, TMS intensity, and Talairach locations x, y, and z separately). Again, this regression showed that DLPFC activity was the major factor in explaining the reduction in working memory performance by TMS, whereas DLPFC depth had little influence.

In a final analysis, we explored whether DLPFC depth could explain the magnitude of the TMS effect for people with high DLPFC activity. We sorted our subjects either according to mean activity or maximum activity in the DLPFC, and we calculated step-wise linear regressions (similar as explained above) for the subjects with highest activity (N=6) and lowest activity (N=6). Only, a sorting on maximum activity did reveal a significant effect of depth for the subjects with highest activity, whereas a sorting on mean activity did not.

Results

Dissociating fragile VSTM from visual working memory

The aim of the present study was to investigate whether fragile visual short-term memory (VSTM), a seconds-lasting and high-capacity form of VSTM, is a weak version of visual working memory, or a separate form of visual short-term memory. As visual working memory maintenance crucially depends on activity in the right dorsolateral prefrontal cortex (DLPFC) (Funahashi, et al., 1989; Fuster & Alexander, 1971; Goldman & Rosvold, 1970; Koch, et al., 2005; E. K. Miller, et al., 1996; Oliveri, et al., 2001; Turatto, et al., 2004), one would expect to see that transcranial magnetic stimulation (TMS) to this region will affect the capacity of fragile VSTM when it is a form of working memory, but not when it is a separate form of VSTM.

To test this hypothesis, we delivered TMS to the right DLPFC, while human volunteers performed a change detection task that measures fragile VSTM and visual working memory in a single experiment. The crucial aspect of this task is the delivery of a spatial cue that directs attention to a single item held in short-term memory. This cue is either presented during short-term memory maintenance (Fig. 4.1; retro-cue trial) or thereafter during the test display (Fig. 4.1; post-change cue trial). In effect, people can report almost all items on retro-cue trials reflecting the high capacity of fragile VSTM (Sligte, et al., 2008), but
no more than 4 items on post-change cue trials reflecting the limited capacity of visual working memory (Luck & Vogel, 1997). As retro-cues are presented more than one second after stimulus disappearance, the high capacity does not reflect iconic memory. In addition, features are bound in fragile VSTM (Landman, et al., 2003), which clearly sets it apart from iconic memory.

We used functional MRI data of a working memory task to navigate the TMS coil to the right DLPFC (see Fig. 4.2). The task and the analyses were almost identical to the already classic working memory study of Pessoa and colleagues (Pessoa, et al., 2002) (see Methods for details). For each participant, we defined the right DLPFC as the highest voxel value in the anterior part of the middle frontal gyrus. All details on the DLPFC are reported in the Table 4.1.

We observed that single-pulse magnetic stimulation of the right DLPFC reduced performance on post-change cue trials measuring visual working memory ($t(11) = 2.52, p = .015$), but not performance on retro-cue trials measuring fragile VSTM ($F(1,11) = 3.63, p = .042$). This suggests that fragile VSTM is not a form of visual working memory, but a separate form of VSTM that operates in between iconic memory and visual working memory. However, there are several alternative explanations that have to be ruled out before we can firmly draw this conclusion, namely; 1) fragile VSTM and visual working memory may both depend on activity in the DLPFC, but at different latencies after stimulus offset, 2) differences in encoding time between conditions (1250ms up to the cue in retro-cue condition; 2100 ms up to the cue in post-change cue condition) may cause differences in susceptibility to magnetic stimulation, and 3) difficult tasks may suffer more from distractions than easy tasks. As TMS produces large distractions, such as a loud click and muscle contractions, the selective decrement on the difficult, working memory task might be attributed to these nonspecific TMS effects. To rule out these alternative explanations, we performed a control experiment, where we applied repetitive TMS to the right DLPFC (addressing point 1), while people performed a retro-cue/post-change cue task that was matched in encoding time (addressing point 2). In addition, we applied rTMS to a control site in the left DLPFC that mirrored the location of the right DLPFC. We observed no activity related to visual working memory maintenance in the left DLPFC, but stimulation of this control site should evoke similar nonspecific TMS effects as right DLPFC stimulation. Thus, if stimulation of the left DLPFC evokes the same pattern of results as right DLPFC stimulation, our results can be attributed to nonspecific effects of TMS, but if stimulation of the left DLPFC has no effect on either condition, our prior results must be real (addressing point 3).
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Figure 4.3 Transcranial magnetic stimulation (TMS) of the right dorsolateral prefrontal cortex (DLPFC) caused a significant reduction in visual working memory capacity (in red), but had no effect on the capacity of fragile visual short-term memory (in blue). This was evident A when single-pulse TMS was delivered during retention, and B when repetitive TMS (rTMS) was delivered during retention, but rTMS of a mirrored location in the left DLPFC did not result in a performance decrement on either visual working memory or fragile VSTM. Altogether, this suggests that fragile VSTM, a long-lasting and high-capacity form of short-term memory, is not a form of visual working memory, but a separate form of VSTM in between iconic memory and visual working memory. Performance is depicted as mean Cowan’s K ± SEM; K is a common capacity measure that corrects for guessing. *p < .05, **p < .01, 2-way & 3-way: interaction tests.

Repetitive TMS of the right DLPFC produced a decrease in visual working memory capacity ($t(7) = 3.93, p = .003$), but no decrement in fragile VSTM capacity, just like the previous single-pulse TMS experiment (Fig. 4.3B; two-way interaction $F(1,7) = 6.05, p = .022$). The effect size was slightly bigger for repetitive TMS than for single-pulse TMS (partial $\eta^2 = .464$ vs. partial $\eta^2 = .248$ for the two-way interactions). Thus, we can rule out that fragile VSTM and visual working memory both depend on activity in the right DLPFC, albeit at different latencies, and that differences in encoding time between conditions result in different TMS effects between conditions. But most importantly, the selective decrease in working memory...
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performance when stimulating the right DLPFC was completely absent when the left DLPFC was stimulated (Fig. 4.3B; three-way interaction $F(1,7) = 4.00, p = .043$). Thus, we can rule out that the selective decrease in working memory performance is due to nonspecific effects of TMS (loud click, muscle contractions) that potentially have a larger disruptive effect on the difficult, working memory task than on the easier, fragile VSTM task. Note that Turatto and colleagues (Turatto, et al., 2004) already showed that rTMS only causes a decrease in change detection performance when aimed at the right DLPFC, but not when aimed at the left DLPFC or a control site. Our post-change cue condition is similar to the change detection task used by Turatto and colleagues, except for the cue that singles out the object to compare between displays. However, such a post-change cue does not alter performance on change detection tasks compared to when no cue shown (Vogel, et al., 2001). We observed no facilitation effects of rTMS, only a reduction in working memory capacity when the right DLPFC was stimulated.

Finally, it might be that fragile VSTM is sensitive to magnetic disruption of the right DLPFC, but that a ceiling effect in the data masks the TMS effect. To explore this, we plotted the distributions in performance (Fig. 4.4) for fragile VSTM without TMS (blue), fragile VSTM with TMS (light blue), working memory without TMS (red) and working memory with TMS (light red). The width of each distribution reflects the number of observations of a particular capacity and there are 80 data points in each distribution (single-pulse 12 subjects x 4 blocks; right-sided repetitive TMS 8 subjects x 4 blocks). It is evident that there is a minor ceiling effect in the fragile VSTM condition that is not evident in the working memory condition. Nevertheless, TMS causes a downward shift in the total distribution for the visual working memory condition, while this downward shift is not evident in the fragile VSTM condition. To express this more formally, the medians in total distribution were 6.5 for fragile VSTM without TMS, 6.5 for fragile VSTM with TMS, 4 for visual working memory without TMS and 3 for visual working memory with TMS.

When we split each distribution in quartiles and calculate the median for each quartile, the shift in distribution is even more evident. For the working memory condition, we observed that TMS caused a downward shift across the entire distribution (baseline vs. TMS in quartiles; 1st: 2-1.5; 2nd: 3-2.5; 3rd: 4.25-3.5; 4th: 5.5-5), whereas the shift in distribution seemed to be absent for fragile VSTM (baseline vs. TMS in quartiles; 1st: 4.5-4; 2nd: 6-6; 3rd: 7-7; 4th: 7.5-7.5). Even when there is a ceiling effect in the fragile VSTM condition, TMS should cause a change in distribution when fragile VSTM depends on the right DLPFC. However, this change in distribution is absent for fragile VSTM, while it is evident for visual
Magnetic stimulation of the DLPFC dissociates fragile VSTM from visual working memory. Therefore, we believe it is justified to conclude that fragile VSTM is a separate form of VSTM that operates in between iconic memory and visual working memory.

**Figure 4.4** Distributions in performance for fragile VSTM without TMS (blue), fragile VSTM with TMS (light blue), working memory without TMS (red) and working memory with TMS (light red). The width of each distribution reflects the number of observations of a particular capacity. Only half of each distribution is shown as the other half is its mirror image. It is evident that there is a minor ceiling effect in the fragile VSTM condition that is not evident in the working memory condition. Nevertheless, TMS causes a downward shift in the total distribution for the visual working memory condition, while this downward shift is not evident in the fragile VSTM condition (if anything, the opposite). Performance is depicted as mean Cowan’s K.

### Between-subjects variation in TMS effects explained by fMRI measures

One of the problematic aspects of TMS is that only a subset of the subjects really shows a decrement in performance when a particular brain site is stimulated. In our study for instance, we observed that about half of our subjects displayed a significant reduction in working memory capacity when their right DLPFC was stimulated, whereas the other subjects showed no effect at all. But why do some subjects show a big reduction in working memory performance in reaction to TMS, while other subject do not show any effect at all ("magnitude of the TMS effect")? We might be able to explain the magnitude of the TMS effect by looking at characteristics of the stimulated region of interest, such as the size of the DLPFC, max activity in the DLPFC, mean activity in the DLPFC, depth of the DLPFC, TMS intensity, and the exact location of the region of interest (in Talairach coordinates). For a description of all details on the region of interest, see Methods and the legend of Table 4.1.

In our initial analyses, we correlated the magnitude of the TMS effect with all DLPFC details described above and we found that both mean DLPFC activity in the right DLPFC ($r = .67$, $p = .016$) and the minimal distance from the scalp to this region of interest ($r = -.58$, $p =$
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.048) were highly correlated with the working memory performance decrement caused by single-pulse TMS. This suggests that functional MRI activity can in part explain why TMS is effective in some subjects, and not in other subjects.

The reduction in visual working memory capacity is largest in people...

Figure 4.5 Some people showed a large decrease in working memory capacity when TMS was applied to the right DLPFC, whereas others showed no decrease at all. Correlation analyses suggested that both the amount of DLPFC activity and the depth of the DLPFC from the scalp could explain the magnitude of the TMS effect on working memory capacity. A We divided our subjects into two groups of N = 6, those with the lowest and those with the highest DLPFC activity. People with high DLPFC activity had a significantly higher working memory capacity than people with low DLPFC activity. Moreover, only people with high DLPFC activity showed a reduction in working memory performance when TMS was applied. Performance is depicted as mean Cowan’s K ± SEM. B We divided our subjects into two groups of N = 6, those with shallow and those with deep DLPFC activity. It seemed that only people with shallow DLPFC activity really showed a decrease in working memory performance when TMS was applied. Performance is depicted as mean Cowan’s K ± SEM.

To further illustrate the relation between the amount of activity in the DLPFC and the magnitude of the TMS effect, we divided the subjects into two groups of N = 6 with the highest DLPFC activity and the lowest DLPFC activity (Fig. 4.5A). The group with high DLPFC activity had a significantly higher working memory capacity than the group with low DLPFC activity (t(8.62) = 1.88, p = .047; DOFs corrected for violations of homogeneity); on
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average, they maintained one additional object in working memory (3.2 vs 4.2 objects). Moreover, the group with high DLPFC activity did show a significant reduction in working memory performance when TMS was applied (t(5) = 3.31, \( p = .011 \)), whereas the group with low DLPFC activity did not respond to TMS (t(5) = .48, \( p = .375 \)). In another analysis, we divided the subjects into two groups of \( N = 6 \) with shallow DLPFC and deep DLPFC activity (Fig. 4.5B). Now, we did not observe a difference in working memory capacity between groups anymore (t(9.59) = .723, \( p = .361 \); DOFs corrected for violations of homogeneity). Still, the group with shallow DLPFC activity did show a significant reduction in working memory performance when TMS was applied (t(5) = 2.27, \( p = .036 \)), whereas the group with deep DLPFC activity did not (t(5) = 1.25, \( p = .134 \)).

While both DLPFC activity and depth are correlated with the magnitude of the TMS effect and while both seem to play different roles as suggested by our split-half analyses, DLPFC activity and depth are also highly correlated with each other (\( r = -.77, p = .003 \)). Therefore, it might be that only one functional measure is related to the between-subjects variations in TMS effects. Here, we report partial correlations and a linear regression analysis to get to the bottom of this. Partial correlations between the magnitude of the TMS effect and DLPFC activity were marginally significant when controlled for DLPFC depth (partial \( r = .45, p = .08 \)), while partial correlations between the magnitude of the TMS effect and DLPFC depth were far from significant when controlled for DLPFC activity (partial \( r = -.10, p = .386 \)).

Step-wise linear regression analyses showed that mean activity in the DLPFC could explain 45.3 percent of the variance in between-subjects TMS effects, and only the addition of the size of the DLPFC (in number of voxels) really made the model much better (\( R^2 = .739 \)). Again, the depth of the DLPFC did not play a role of significance in this linear regression analysis. Altogether, it seems that DLPFC activity is the major factor in explaining individual differences in susceptibility to TMS, and that correlations between depth of the region of interest and TMS effects are mediated by DLPFC activity.

No relation between TMS effectiveness and DLPFC depth?

It is somewhat strange that we do not observe a direct relation between DLPFC depth and the effectiveness of TMS as several studies have reported a link between scalp-cortex distance and the effectiveness of TMS before (Kozel, 2000; Knecht, 2005; Stokes, 2005, 2007). One major difference between the present study and these previous studies is the location of magnetic stimulation (DLPFC versus motor cortex). The location of the motor cortex varies little between subjects and the function of this area is clear; when stimulating the motor cortex above a certain threshold, a clearly visible motor response is evoked and
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this threshold can reliably be determined in each individual. However, working memory depends on complex interactions between multiple sites in the brain and it is still not well understood how each brain area contributes to working memory or how different working memory areas interact. Our study suggests that people with high DLPFC activity represent one additional object in working memory compared to people with low DLPFC activity. This additional object is zapped away by magnetic stimulation of the DLPFC. Therefore, it is likely that the amount of DLPFC activity indicates the extent to which people rely on this brain region for working memory maintenance. Depth would then only be able to explain effects in people with high DLPFC activity.

Figure 4.6 The relation between DLPFC depth and the magnitude of the TMS effect could be almost entirely explained by DLPFC activity. However, when we sort subjects based on their maximum activity in the DLPFC, we observe that DLPFC depth does influence the magnitude of the TMS effect for people with high DLPFC activity (in red), while DLPFC depth has no influence on the magnitude of the TMS effect for people with low DLPFC activity (in black).

To explore whether depth effects were only evident in people with high DLPFC activity, we sorted people according to mean activity in the DLPFC or according to max activity in the DLPFC as both activity measures significantly correlated with the magnitude of the TMS effect. We then selected the six subjects with the highest observed mean or max activity and we performed a step-wise linear regression with the magnitude of the TMS effect as dependent variable and all details on the DLPFC as independent variables (size, mean activity, max activity, depth, stimulator output in %, and Talairach locations). For the subjects with the highest mean activity, the regression analysis revealed only a good model fit for stimulator output ($R^2 = .663$), but not for depth. For the subjects with the highest max activity, however, the regression analysis did reveal a good model fit for minimal distance to the DLPFC ($R^2 = .714$) and we show these results in Figure 4.6. Power issues are probably the reason why we only observe this depth relation when sorting on max activity compared to a
Sorting on mean activity; if a single subject is swapped in an analysis with so little observations (N = 6), results may change dramatically. For now, we conclude that DLPFC depth seems to explain between-subjects variations in TMS effects, but only if the underlying brain area is active enough.

**Discussion**

Contrary to dichotomous models of visual short-term memory (VSTM) that distinguish between iconic memory (high-capacity and brief feature buffer) and visual working memory (limited-capacity and sustained object buffer), recent studies have provided evidence for an additional and intermediate VSTM stage (Griffin & Nobre, 2003; Landman, et al., 2003; R. Landman, et al., 2004; Makovski & Jiang, 2007; Makovski, et al., 2008; Matsukura, et al., 2007; Sligte, et al., 2008). Specifically, it seems that people retain a high-capacity representation of the outside world in extrastriate, visual cortex over the first couple of seconds after stimulus disappearance (Sligte, et al., 2009). This intermediate store is highly vulnerable to interference by subsequent visual stimulation (Matsukura, et al., 2007; Sligte, et al., 2008), yet features in this form of VSTM are bound to form coherent objects (Landman, et al., 2003), thereby setting it apart from iconic memory.

However, it is possible that this fragile form of VSTM actually is an expression of visual working memory, relying on the same neural substrate (prefrontal/parietal cortex) and the same psychological functions (attention and control), yet in a format that is too weak to enable report under normal circumstances. If fragile VSTM and working memory are indeed one and the same process instead of dissociable stages in VSTM, magnetic stimulation of the neural substrate supporting working memory (the present study) and manipulations of attention (Vandenbroucke, Sligte, and Lamme, this issue) should reduce the capacity of fragile VSTM and visual working to a similar extent.

Our results clearly suggest the opposite; in accordance with previous TMS studies (Koch, et al., 2005; Oliveri, et al., 2001; Turatto, et al., 2004), we found that disturbance of the right DLPFC caused a reduction in performance on post-change cue trials measuring the capacity of visual working memory, but performance on retro-cue trials was not affected by magnetic stimulation. In addition, manipulations of attention during encoding of the stimulus caused a large reduction in working memory capacity, but only a slight reduction in fragile VSTM capacity (Vandenbroucke et al., submitted). These combined results set fragile VSTM apart from the neural substrate and psychological functions underlying visual working memory, thus providing a solid framework for a three-stage model of VSTM consisting of iconic memory, fragile VSTM and visual working memory.
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The role of the DLPFC in visual working memory

Maintenance of information in visual working memory depends on activity in a widespread network of brain regions (Courtney, et al., 1997; Curtis, 2006; Curtis & D'Esposito, 2003; Pessoa, et al., 2002); besides activity in the dorsolateral prefrontal cortex, visual working memory maintenance is also related to activity in the anterior cingulate cortex, the frontal eye fields, the premotor cortex, the intraparietal sulcus, the superior parietal lobe, and many high-level visual areas in visual and temporal cortex. Note that this description matches the functional activity we found in relation to working memory maintenance (see Fig. 2).

Moreover, there are large individual differences in the capacity of visual working memory, ranging from two up to even five objects. Several groups have tried to relate these individual differences in working memory capacity to specific neural substrates. In the general approach of these studies, subjects perform a change detection task with varying set sizes (1, 2, 4, 8 objects to remember) while functional MRI or EEG is measured concurrently. Most notably, activity in the inferior intraparietal sulcus shows a pattern that mirrors behavioral performance when people have to memorize simple objects (Linden, et al., 2003; Todd & Marois, 2004). When more complex stimuli have to be memorized, behavior reaches plateau at much lower set sizes. Now, activity in lateral occipital complex and the superior intraparietal sulcus mirrors behavior (Xu & Chun, 2006), while activity in the inferior intraparietal sulcus is insensitive for stimulus complexity. This suggests that the superior intraparietal sulcus and lateral occipital complex keep track of the contents of visual working memory, while the inferior intraparietal lobe keeps track of a maximum of four spatial locations at the same time.

In the event-related potential (ERP) set-up (Magen, Emmanouil, McMains, Kastner, & Treisman, 2009; Vogel & Machizawa, 2004), subjects receive a cue before onset of the memory display that indicates to remember only the items that are on the left or right side of the memory display. This produces a laterization signal that scales in amplitude depending on the amount of objects that are maintained in visual working memory. Again, when behavior reaches plateau, so does this laterization component. The source of this laterization component seems to be driven by combined activity in parietal cortex and lateral occipital complex.

In the present study, we observed that people with high DLPFC activity have superior working memory capacity compared to people with low DLPFC activity. Nevertheless, the difference in capacity is just one object. When we relate this finding to previous findings, this might suggest that the bulk of individual differences in working memory capacity are determined by structures in the parietal lobe (and lateral occipital complex). However, high-
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capacity individuals effectively recruit additional resources in the dorsolateral prefrontal cortex.

A link between BOLD MRI and the effectiveness of TMS
It is quite logical that functional brain regions that lie relatively deep beneath the scalp are harder to disrupt, as the magnetic field falls off with increases in distance, and this has been documented before (Kozel, 2000; Knecht, 2005; Stokes, 2005, 2007). To our knowledge, however, a link between the height of BOLD activity in a targeted region of interest and the effectiveness of TMS – as we find here - has not been shown before. A lower BOLD response might indicate that some people rely less on the DLPFC for working memory maintenance and there are clues in this study substantiating this idea. It is then a logical consequence that TMS does not have a large effect in these people. However, for a solid conclusion that the effectiveness of TMS can be predicted from functional MRI activity, this finding should be replicated in different brain areas with entirely different experimental designs.