

Parieto-frontal Interactions in Visual-object and Visual-spatial Working Memory: Evidence from Transcranial Magnetic Stimulation

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This study aimed to investigate whether transcranial magnetic stimulation (TMS) can induce selective working memory (WM) deficits of visual-object versus visual-spatial information in normal humans. Thirty-five healthy subjects performed two computerized visual *n*-back tasks, in which they were required to memorize spatial locations or abstract patterns. In a first series of experiments, unilateral or bilateral TMS was delivered on posterior parietal and middle temporal regions of both hemispheres after various delays during the WM task. Bilateral temporal TMS increased reaction times (RTs) in the visual-object, whereas bilateral parietal TMS selectively increased RTs in the visual-spatial WM task. These effects were evident at a delay of 300 ms. Response accuracy was not affected by bilateral or unilateral TMS of either cortical region. In a second group of experiments, bilateral TMS was applied over the superior frontal gyrus (SFG) or the dorsolateral prefrontal cortex (DLPFC). TMS of the SFG selectively increased RTs in the visual-spatial WM task, whereas TMS of the DLPFC interfered with both WM tasks, in terms of both accuracy and RTs. These effects were evident when TMS was applied after a delay of 600 ms, but not one of 300 ms. These findings confirm the segregation of WM buffers for object and spatial information in the posterior cortical regions. In the frontal cortex, the DLPFC appears to be necessary for WM computations regardless of the stimulus material.

Introduction

Neurophysiological studies in animals (Ungerleider and Mishkin, 1982; Goodale and Milner, 1992; Wilson *et al.*, 1993) and neuroimaging studies in humans (Haxby *et al.*, 1994; Grady *et al.*, 1994; Kohler *et al.*, 1995; Ungerleider, 1995; Owen *et al.*, 1996; Courtney *et al.*, 1996; Smith and Jonides, 1997, 1999) have suggested that the distinction between a ventral occipito-temporal pathway for object processing and a dorsal occipito-parietal pathway for spatial processing may also apply to working memory (WM), the process of actively maintaining a representation of information for a brief period of time so that it is available for use (Baddeley, 1992; Smith and Jonides, 1997). This suggests the existence of an anatomical segregation, in the posterior cortical regions of the brain, of the neural circuits involved in the short-term retention of the visual aspect and the spatial location of the objects: the posterior parietal cortex, involved in spatial perception within the dorsal stream, would also implement a buffer for the retention of spatial information; conversely, the infero-temporal cortex, involved in object features perception, would also play a role in storing object information.

In addition to posterior cortical regions, the frontal cortex plays a critical part in certain aspects of WM for both spatial and non-spatial material (Goldman-Rakic, 1987; McCarthy *et al.*, 1994, 1996; Petrides 1994, 1995; Courtney *et al.*, 1996; D'Esposito *et al.*, 1998).

A debated issue in the neuropsychological literature is whether the prefrontal cortex shows a 'domain-specific' organ-

ization similar to the one described in posterior brain regions, with functionally distinct subdivisions that subservise different aspects of WM. One prevalent view is that dorsolateral frontal regions are concerned primarily with memory for spatial material, while ventrolateral frontal regions subservise memory for non-spatial material (Goldman-Rakic 1987, 1990, 1995; Wilson *et al.*, 1993; Courtney *et al.*, 1996; McCarthy *et al.*, 1996; Levy and Goldman-Rakic, 1999). In this field, most evidence supporting the notion of domain specificity in human prefrontal cortex comes from studies indicating the middle frontal gyrus in the dorsolateral prefrontal cortex (DLPFC) (McCarthy *et al.*, 1994, 1996; Sweeney *et al.*, 1996; Carlson *et al.*, 1998) or the superior frontal gyrus/sulcus (SFG/SFS) (Courtney *et al.*, 1996, 1998; Carlson *et al.*, 1998; Ungerleider *et al.*, 1998; Haxby *et al.*, 2000) as the human frontal region specialized for spatial WM. In particular, studies showing an activation of the human SFG during spatial WM tasks have suggested that this area is the true functional homologue of the DLPFC in monkeys, or perhaps that the major site of spatial WM in monkeys is more posterior than was originally believed. This hypothesis was supported by the recent description of a patient showing selective impairment of visual-spatial WM after damage to the SFG and the precuneus region in the right hemisphere (Carlesimo *et al.*, 2001).

An alternative proposal is that a functional distinction can be drawn between the different frontal cortical regions, based on the type or nature of the processes that are carried out by those regions, rather than on the type of information being temporarily stored (Petrides 1994, 1995; Owen *et al.*, 1996, 1999; D'Esposito *et al.*, 1998; Postle and D'Esposito, 1999; Rypma and D'Esposito, 1999). According to this 'process-specific model', the mid-ventrolateral prefrontal cortex (BA 45/47) is concerned primarily with the passive maintenance and explicit retrieval of recently processed information; in contrast, the DLPFC (BA 9/46) is recruited only when active manipulation or 'monitoring' of such information within WM is required.

Another critical issue concerns the relation between the computations of the prefrontal regions and those of more posterior areas in WM functions (Carpenter *et al.*, 2000). It has been suggested (Chafee and Goldman-Rakic, 1998, 2000) that the reciprocal anatomical projections between parietal and prefrontal neurons may entrain their parallel activation as multi-modal domain-specific networks in WM tasks. Although the temporal relations among the various network components may be critically important (Fuster, 1995), they are only beginning to be addressed by neuroimaging studies (Cohen *et al.*, 1997).

Further findings on these issues could be provided by transcranial magnetic stimulation (TMS) as a technique able to produce focal, transient and fully reversible disruption of cortical network function during the performance of cognitive

tasks in normal humans (Walsh and Rushworth, 1999; Pascual-Leone *et al.*, 2000). This kind of approach can induce 'virtual lesions' useful for establishing the necessity of a cortical region for a given behaviour. Moreover, by disrupting activity for only a short time, TMS allows us to obtain information on precisely when activity contributes to task performance [i.e. the chronometry of cognition (Oliveri *et al.*, 2000; Pascual-Leone *et al.*, 2000)].

Following this theoretical framework, the aim of our study was to investigate whether single-pulse TMS of posterior (parietal and temporal) and frontal (SFG and DLPFC) cortical regions could induce focal, material-specific WM deficits of visual-object and visual-spatial information in normal humans. In addition, we wanted to examine the temporal dynamics of the TMS interference on frontal versus posterior cortical areas, for both visual-object and visual-spatial WM tasks.

Materials and Methods

Subjects

Thirty-five right-handed normal subjects (12 males, 23 females; mean age 25.2 ± 3.7 years), with normal, or corrected to normal, vision, participated in the experiments after having provided written informed consent. The experimental procedure was approved by the local ethical committee. All subjects but one were naive of the purposes of the study.

Experimental Procedure

The experiments were conducted in a soundproof, dimly lit room. Subjects sat comfortably in an armchair, at a distance of ~50 cm from the computer monitor, the centre of which was aligned with the subject's eyes.

We used two computerized tasks (a visual-spatial and a visual-object WM task), with an 'n-back' paradigm, already adopted in neuroimaging studies of WM functions (Smith *et al.*, 1996; Smith and Jonides, 1997; Owen *et al.*, 1998). In these tasks, the subjects were requested to code the stored positions (or visual patterns) with respect to their temporal position, and to constantly change the temporal codes as new stimuli were presented.

In particular, the subjects were required to continuously monitor a sequence of 22 visual stimuli (locations or abstract patterns) presented on a 14" computer screen, and to respond, after a delay period following each stimulus, by selecting the one that had been presented n steps earlier in the sequence. The subjects responded by pressing with the index, middle and ring finger of the right hand one of three buttons, which corresponded, respectively to left, middle and right locations on the screen.

A series of pilot studies conducted in other normal subjects in order to combine high memory load with good performance level, suggested to adopt a $n - 2$ procedure in both tasks: this meant that the subjects had to respond by selecting the stimulus that was presented two steps earlier in the sequence.

Before starting the experiment, all subjects received extensive training in the testing procedure, until they were sufficiently confident with it, and reached a high level of accuracy (>80% of correct responses) and a stable performance (no progressive decrease of response latencies in the course of the 22 trials).

Visual-spatial Working Memory Task

In this task, three different locations (represented by white squares) were highlighted in a continuous sequence on the screen. On each of 22 consecutive trials, one of the three white locations was randomly selected by the computer program and transiently (300 ms) changed from white to black and then back to white again (study phase), indicating the next position in the series to be remembered. Following a 3 s delay, during which the screen appeared blank, the three white squares appeared again on the screen for 2 s (response phase). After a delay of 1 s, the next trial was presented. The subjects responded by pressing the button

corresponding to the location highlighted two steps back in the sequence (i.e. $n - 2$).

Visual-object Working Memory Task

In this task, three abstract patterns were presented in the centre of the screen in a continuous sequence. In each trial of an ongoing sequence of 22, one of three possible patterns was selected randomly by the computer and appeared in the centre of the screen for 300 ms (study phase). Following a 3 s delay, the three patterns were presented simultaneously on the screen for 2 s (response phase). Each pattern was randomly positioned inside one of the three boxes. After a delay of 1 s, the next trial was presented. In each trial, the subjects had to respond by pressing the button corresponding to the visual pattern presented two steps back.

In all experiments, a complete set of 22 visual stimuli was presented for each stimulation site. Since the subjects had to respond to the stimulus presented two sequences back, they did not have to respond to the first two stimuli of the sequence; therefore a total of 20 responses for each block of trials was finally computed.

Figure 1a,b,c illustrates the experimental sequences schematically.

Transcranial Magnetic Stimulation

The computer was able to trigger one or two magnetic stimulators with adjustable stimulus onset asynchronies (SOA) between the visual stimulus and TMS. Single-pulse TMS was delivered with Novamatrix MagStim 200 magnetic stimulators (MagStim Co., Ltd, Withland, UK) using a figure of eight coil (double 70 mm coil, MagStim). With the adopted coil, the magnetic pulse has a rise time of ~200 μ s and a duration of 1 ms, but the effects in the underlying cortical region are reported to last for up to 10 ms (Jalinous, 1991; Ilmoniemi *et al.*, 1997).

The excitability threshold was determined separately for the two hemispheres and defined as the lowest stimulus intensity able to elicit a muscle twitch from thenar muscles. Stimulus intensity was fixed at 130% of motor threshold and kept constant throughout the experiments. This TMS intensity was determined in order to maximize the chance of local disruption of neural function without producing excessive discomfort.

In the various experiments, TMS was delivered with different SOAs from the visual stimulus in the study phase, over target scalp sites marked on a plastic swimmer's cap.

TMS was well tolerated by all subjects. Throughout the experiments, no significant eye movements or excessive blinking were observed.

No significant interhemispheric asymmetries of motor threshold were found in any subject; therefore, the intensity of stimulation was the same for the two hemispheres in all experiments.

The entire course of the study consisted of five experiments.

Experiment 1: Effects of Bilateral Parietal and Temporal TMS Delivered during the Memory Delay of Visual-object and Visual-spatial WM Tasks

Eight subjects (five males, three females; mean age 25.4 ± 3.5 years) participated in this experiment, performed in order to test the effects of synchronous stimulation of parietal or temporal sites of both hemispheres, delivered during the memory delay of visual-object and visual-spatial WM tasks.

Two identical magnetic stimulators, connected to two identical coils, simultaneously delivered single pulses to homologous areas of the right and left parietal or temporal scalp (corresponding to the P4, P5, T5, T6 labels of the 10–20 EEG system). These scalp positions are known to correspond to posterior parietal (Homan *et al.*, 1987; Elkington *et al.*, 1992; Pascual-Leone *et al.*, 1994; Oliveri *et al.*, 1999; Walsh *et al.*, 1999) and middle temporal regions. The coils were placed tangential to the skull, with the handle pointing backward parallel to the midline. This induced a current flowing in a posterior–anterior direction in the underlying brain areas. The mean TMS intensity was $65.6 \pm 4.2\%$ of the stimulator output.

In accord with the findings of previous event-related potential (ERP) studies addressing the issue of distinct memory buffers for object and spatial information (Ruchkin *et al.*, 1997), in both WM tasks we used an interval of 300 ms from the offset of the target visual stimulus to the application of TMS. This SOA was judged sufficiently outside the time window for perceptual operations. Therefore, we were confident of

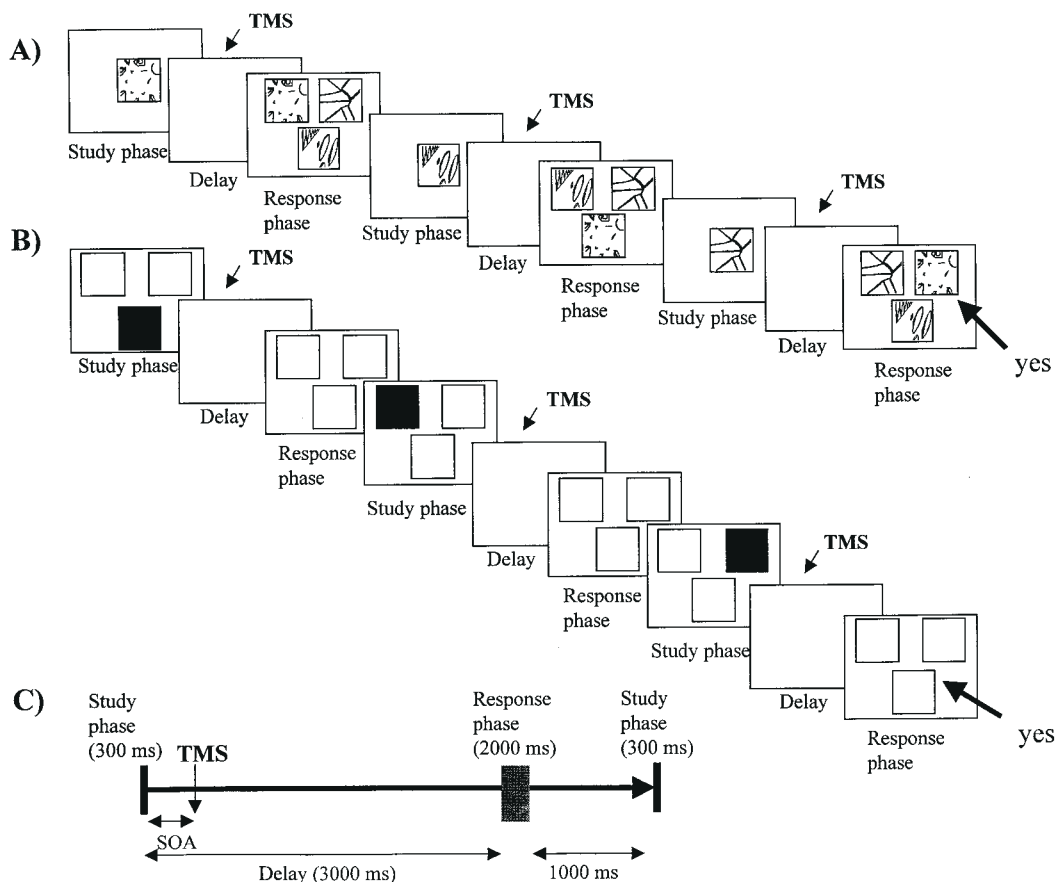


Figure 1. Experimental procedure: (a) visual-object WM task; (b) visual-spatial WM task. The diagrams represent three trials; the arrow indicates the pattern presented two steps earlier the subjects had to respond to (see text for details). (c) Time scale of visual stimuli in the two tasks. A single trial is shown. The study phase (in this phase there was a visual presentation of the target stimulus — abstract pattern or spatial location) lasted 300 ms; there was then a delay interval of 3 s, followed by the response phase (in this phase the three visual patterns or spatial locations were simultaneously presented and the subject had to respond by pressing the appropriate button), lasting 2 s. A 1 s time period separated the response phase from the following study phase. TMS was applied, in each trial, during the delay interval, after various SOAs from the offset of the study phase (see text for details).

applying the interfering stimulus at the time of the retention interval (see also control experiment 3 for a test of this assumption).

Baseline trials (visual stimulation without the interfering TMS) were randomly intermingled with test conditions. Therefore, the experiment consisted of three conditions (baseline, parietal TMS, temporal TMS) for each WM task. The order of the different experimental conditions was randomized across subjects.

Experiment 2: Effects of Unilateral TMS of Parietal and Temporal Scalp Sites Delivered during the Memory Delay of Visual-object and Visual-spatial WM Tasks

Six of the previously examined subjects (four males, two females; mean age 24.7 ± 3.9 years) participated in this experiment, performed in order to investigate the presence and extent of lateralization of visual WM buffers in posterior cortical areas.

TMS was applied unilaterally to the parietal or temporal scalp positions of each hemisphere at a SOA of 300 ms. Baseline trials were randomly intermingled with test conditions. Therefore, the experiment consisted of five conditions (baseline, right parietal TMS, left parietal TMS, right temporal TMS, left temporal TMS) for each WM task. The order of the experimental conditions was randomized across subjects.

The adopted TMS intensities were the same as the ones in the previous experiment.

Experiment 3: Effects of Parietal and Temporal TMS Delivered during the Encoding and Motor Response Phases of Visual-object and Visual-spatial WM Tasks

Six subjects (two males, four females, mean age 22.3 ± 0.8 years, three of

whom were examined in experiment 1) participated in this experiment, performed in order to detect a possible TMS interference with the encoding and motor processes during WM operations.

Given the observed lack of effects of unilateral TMS, for each WM task bilateral focal TMS was delivered over the selected parietal and temporal sites at two SOAs, in two separate blocks of trials: at 0 ms from the offset of the visual stimulus (i.e. at the end of the study phase) and at 3300 ms, during the response phase. Baseline trials were randomly intermingled with test conditions. Therefore, for each block (i.e. SOA = 0 and 3300 ms) there were three experimental conditions: baseline, parietal TMS and temporal TMS.

The mean TMS intensity in this experiment was $58.5 \pm 7\%$ of the stimulator output

Experiment 4: Effects of SFG and DLPFC TMS on Visual-object and Visual-spatial WM Tasks

A total of 25 subjects (seven males, 18 females; mean age 23.4 ± 2.7 years) participated in these experimental sessions. Two of them were examined in the previous experiments.

According to the guidelines of previous studies (Pascual-Leone and Hallett, 1994; Jahanshahi *et al.*, 1998; Mottaghy *et al.*, 2000), for stimulation of the left/right DLPFC the tip of the intersection of the two coil loops was lined up with the F3/F4 sites of the 10–20 EEG system. Localization of the SFG was made making reference to the hand area of the motor cortex (see Ro *et al.*, 1999). This area is easily identified with TMS since its stimulation induces visible contractions of the contralateral hand. Therefore, after localizing the area of motor cortex that produced the most reliable, visible contraction of the contralateral hand

at suprathreshold TMS intensity, a scalp marking was made on each subject over a site 2 cm rostral to this location. This scalp site was considered as overlying the SFG.

Despite the possibility of individual differences, the stimulated points were considered to be over the left/right DLPFC and SFG also on the basis of a MRI scan performed in a single representative subject after marking the target regions with capsules containing soya oil (Fig. 2). A T1-weighted image was acquired with a Siemens 1.5 T Vision Magnetom MR system (Erlangen, Germany; MPRAGE sequence, 1 mm isotropic voxel). The capsules of soya oil and the underlying brain cortex were identified with DISPLAY (Brain Imaging Center, Montreal Neurological Institute, McGill University), a program that permits labelling of a region of interest on each slice of the MRI volume and allows its simultaneous visualization within the sagittal, horizontal and coronal planes of MRI as well as on a three-dimensional reconstruction of the brain surface (Fig. 2, top). The three-dimensional brain surface was made by means of three-dimensional model-based surface deformation algorithm (MacDonald, 1998). The stimulated scalp sites were localized following the Economo and Koskinas map (Economo and Koskinas, 1925). As can be seen from Figure 2, a line tangential to the surface of the scalp and the related perpendicular line originating in the centre of each capsule were drawn on the coronal slices. These lines indicate, respectively, the coil orientation and the centre of the area where the induced magnetic field was at its maximum. The white filled circles (Fig. 2*a,b*) represent the centre of the circular area (corresponding to the 1 cm² dotted circle; Fig. 2 top) targeted by the magnetic field. Figure 2 (top) also shows that the dotted circle on the brain corresponding to the most caudal capsule (α) was over the gyrus frontalis primis, in proximity of the sulcus gyri frontalis primi (i.e. SFG: BA6); the dotted circle below the most rostral capsule (β) was over the dorsolateral prefrontal cortex, at the level of the gyrus frontalis secundus and of the caudal portion of sulcus frontalis medius (i.e. DLPFC: BA9/46).

Bilateral simultaneous stimulation was performed with two focal coils positioned over homologous scalp sites. For TMS of the DLPFC, the coils were held tangentially to the scalp, with the current flowing parallel to the sagittal axis. For TMS of the SFG, in order to prevent or minimize activation of the motor cortex (and so hand muscle twitches that could alter RTs in the response phase), the direction of the coils was such that current flow in the underlying cortex was anterior to posterior, which is opposite to the direction optimal for stimulation of the motor cortex. With the adopted coils and intensities of stimulation, the direction of the peak of the induced current flow is perpendicular to the coil position, and the spatial resolution of TMS is assumed to be in the order of 1 cm² (Maccabee *et al.*, 1990) (see also Fig. 2 for details).

For each WM task, TMS was applied at two different intervals after the offset of the visual stimulus: 300 ms (11 subjects) and 600 ms (14 subjects). The SOA of 600 ms was selected according to the findings of previous ERP (Ruchkin *et al.*, 1997) and TMS (Muri *et al.*, 1996) studies of WM processes, which showed a frontal lobe activation starting 400–700 ms after the visual stimulus.

Baseline trials were randomly intermingled with test conditions, and the order of the trials was randomized across subjects. Therefore, the experiments consisted of three conditions for each SOA: baseline, SFG TMS and DLPFC TMS.

The mean TMS intensity was 54.5 \pm 7.1% of the stimulator output. With the adopted intensities, no muscle twitches were induced in the contralateral arm by TMS of either scalp site (SFG or DLPFC).

Experiment 5: Effects of DLPFC and SFG TMS, Delivered during the Encoding and Motor Response Phases of Visual-object and Visual-spatial WM Tasks

Six subjects (two males, four females; mean age 25.3 \pm 4.1 years) participated in this experiment. Five of them were examined in experiment 4.

For each WM task, bilateral focal TMS was delivered over the selected frontal sites at two SOAs, in two separate blocks of trials: at 0 ms (i.e. at the end of the study phase) and at 3300 ms, during the response phase. Baseline trials were randomly intermingled with test conditions. Therefore, for each block (i.e. SOA = 0 and 3300 ms) there were three experimental conditions: baseline, SFG TMS and DLPFC TMS.

The mean TMS intensity was 55 \pm 6% of the stimulator output.

Data Analysis

Responses were measured in terms of accuracy (mean number of correct responses) and reaction times (RTs: interval of time from the onset of the test visual stimulus in the response phase to the subject's response). Responses were scored as errors in the case of a wrong response, no response or a response given outside the duration of the response phase.

Repeated measures ANOVAs, with Task (two levels: visual-object versus visual-spatial WM), Condition (three levels in experiments 1 and 3: baseline versus parietal versus temporal TMS; five levels in experiment 2: baseline versus right parietal versus left parietal versus right temporal versus left temporal; three levels in experiments 4 and 5: baseline versus DLPFC TMS versus SFG TMS) and Side (two levels in experiment 2: right versus left hemisphere TMS) were performed for RTs and accuracy level.

Since RTs in the visual-object WM task were considerably longer than those obtained in the visual-spatial task, in all experiments a separate ANOVA was performed on normalized RTs; they were computed by subtracting the values obtained during control trials from those obtained in TMS trials.

In all of these analyses, whenever an overall significant level was found, planned comparisons were made to test the significance between single factors. The threshold of significance was set at $P < 0.05$.

Results

TMS of Temporal and Parietal Areas

Experiment 1: Effects of Bilateral Parietal and Temporal TMS Delivered during the Memory Delay of Visual-object and Visual-spatial WM Tasks

Table 1 shows mean levels of accuracy and RTs in the two WM tasks across the various experimental conditions.

The ANOVA performed on the absolute RT data revealed significant Task [$F(1,7) = 226.8$; $P < 0.001$] and Condition [$F(2,14) = 6.3$; $P = 0.01$] main effects. In fact, the RTs were significantly shorter in the spatial than in the object visual WM task. This reflected a surplus (extra) time necessary for processing visual information (i.e. abstract patterns) in the visual task. The significance of the Condition effect reflected the finding that, when considering together the two tasks, temporal TMS elicited longer RTs (739 ms) than baseline trials (683 ms; $P = 0.01$); RTs after parietal TMS (708 ms) did not differ significantly from either temporal TMS or baseline conditions.

The Task \times Condition interaction was highly significant [$F(2,14) = 13.3$; $P = 0.0005$], reflecting the opposite effects produced by parietal and temporal TMS in the visual-spatial and visual-object tasks. In particular, in the visual-spatial task, parietal TMS elicited significantly longer RTs compared with both baseline [$F(1,7) = 13$; $P = 0.008$] and temporal TMS [$F(1,7) = 14.06$; $P = 0.007$] conditions, which, in turn, did not differ from one another. In contrast, in the visual-object task, temporal TMS induced a significant increase in RTs compared with both baseline [$F(1,7) = 15.9$; $P = 0.005$] and parietal TMS [$F(1,7) = 11.7$; $P = 0.01$] conditions, which did not differ from one another.

The overall pattern of results did not change when analysing normalized rather than absolute RTs (Fig. 3). In this case, due to normalization, the main effects of Task [$F(1,7) = 5.1$; $P = 0.06$] and Condition [$F(1,7) = 3.8$; $P = 0.09$] did not reach statistical significance. However, a clear dissociation of the TMS effects on parietal versus temporal sites depending on the WM task emerged from the significant Task \times Condition interaction [$F(1,7) = 16.2$; $P = 0.005$]. Planned comparisons showed that, in the visual-spatial WM task, parietal TMS induced a significant increase in RTs compared with temporal TMS [$F(1,7) = 14.06$; $P = 0.007$]. On the other hand, in the visual-object WM task,

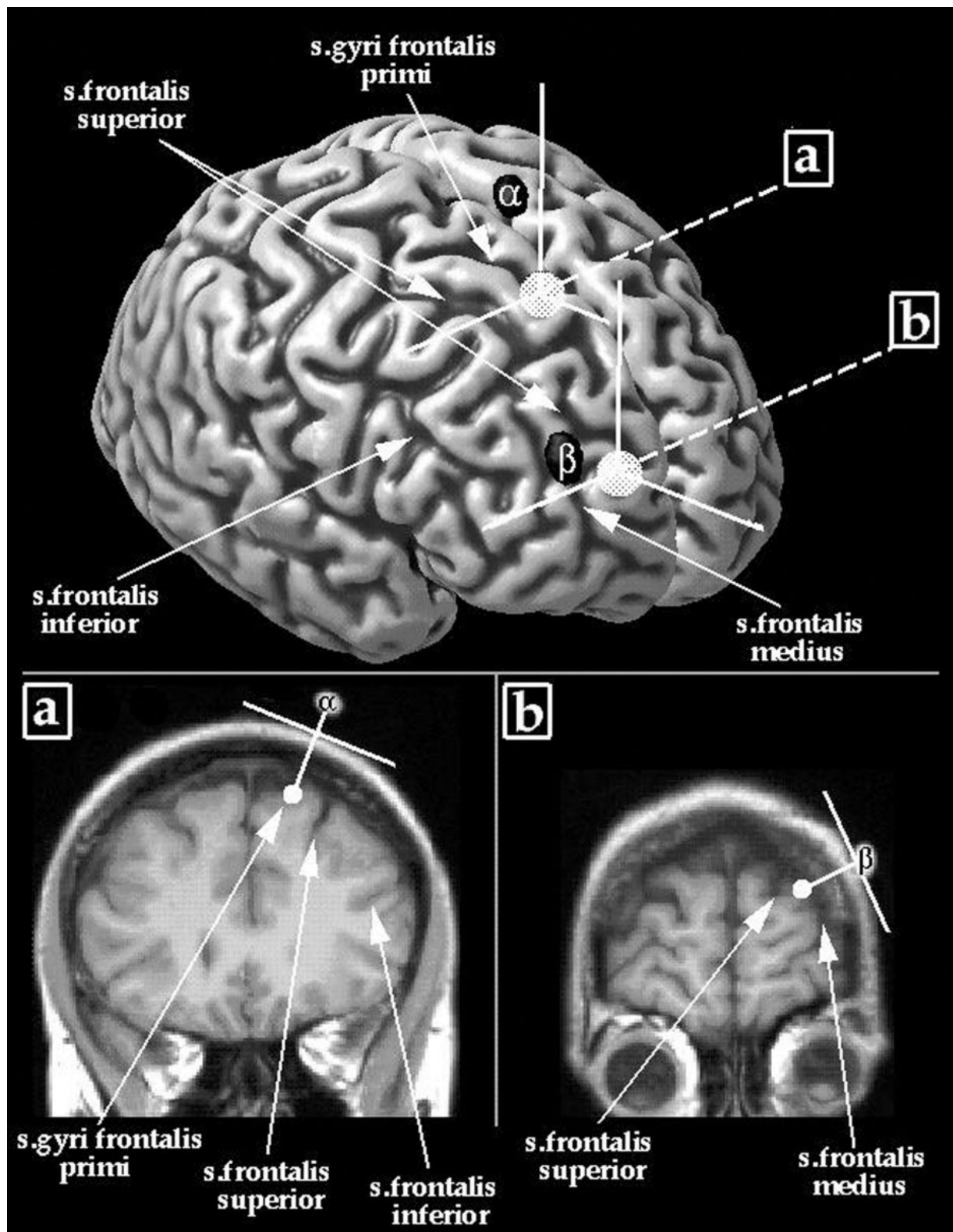
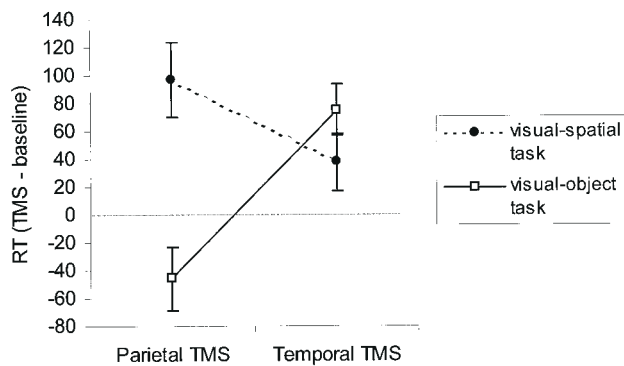


Figure 2. Top: three-dimensional surface brain reconstruction. The dotted lines *a* and *b* indicate the cutting plane corresponding to the coronal slices presented in the panels *a* and *b*. α and β represent the capsules containing soya oil positioned on the scalp for localizing the cortical regions targeted by TMS. The filled convergent lines indicate the cursor employed for anatomical localization and the orthogonal planes into the space. The cursor marks the circular area where the induced magnetic field was at its maximum. The dotted circles (1 cm²) indicate the supposed outer perimeter of the areas affected by TMS. Panels *a* and *b*: MRI coronal slices. The perpendicular lines indicate the coil orientation and the direction of the induced current flow in the brain; the white filled circles represent the centre of the circular area affected by TMS. (s.) = sulcus.

Table 1Mean level of accuracy (no. of correct responses \pm SD) and RTs in the two WM tasks during bilateral TMS of posterior cortical areas at SOA of 300 ms

	Baseline	Parietal TMS	Temporal TMS
Accuracy			
Visual-spatial WM	18.37 \pm 1.77	17.0 \pm 3.07	16.87 \pm 2.42
Visual-object WM	17.50 \pm 1.31	17.25 \pm 2.76	14.50 \pm 3.70
RT (ms)			
Visual-spatial WM	480.87 \pm 117.30	577.37 \pm 156.84	518.12 \pm 121.51
Visual-object WM	885.62 \pm 149.54	839.68 \pm 106.64	960.62 \pm 167.79

**Figure 3.** Normalized RTs (TMS – baseline) in visual-object versus visual-spatial WM tasks as a function of the stimulated scalp sites (parietal and temporal) in experiment 1. Error bars indicate 1 SEM.

temporal TMS yielded a significant increase in RTs compared with parietal TMS [$F(1,7) = 10.2$; $P = 0.005$].

When evaluating the performance as the number of correct responses, there was no significance of Task [$F(1,7) = 2.65$; $P = 0.14$] and Condition [$F(2,14) = 2.83$; $P = 0.09$] main effects, or of the Task \times Condition interaction [$F(2,14) = 2.23$; $P = 0.14$]. These results are indicative of the fact that the average level of accuracy was similar in both tasks and that it was unaffected by TMS of the various scalp sites (see Table 1).

In sum, this experiment showed a dissociation of the TMS effects on parietal versus temporal sites depending on the WM task: temporal TMS selectively increased RTs in the object WM task, whereas parietal TMS increased RTs only in the spatial WM task. The response accuracy was not affected by TMS in either WM task.

Experiment 2: Effects of Unilateral TMS of Parietal and Temporal Scalp Sites Delivered during the Memory Delay of Visual-object and Visual-spatial WM Tasks

Table 2 shows mean levels of accuracy and RTs in the two tasks and in the various experimental conditions.

Repeated measures ANOVA performed on absolute RTs showed a significance of the Task main effect [$F(1,5) = 213.83$; $P < 0.001$]. The Condition effect was not significant [$F(4,20) = 0.7$; $P = 0.6$]. The interaction Task \times Condition was significant [$F(4,20) = 3.15$; $P = 0.04$]. This reflected the fact that TMS of all scalp positions elicited opposite, albeit non-specific effects in the two tasks. In fact, while in the visual-spatial task RTs were slightly prolonged after TMS of all scalp positions compared with baseline, the opposite pattern was observed for the visual-object task: in this case, TMS of all scalp positions resulted in a consistent reduction of RTs compared with baseline.

When analysing normalized RTs, there was a significant main effect of Task [$F(1,5) = 7.0$; $P = 0.04$], reflecting the opposite

effects elicited by TMS in the two tasks. However, there was no significance of the Condition [$F(1,5) = 0.6$; $P = 0.45$] and Side [$F(1,5) = 0.09$; $P = 0.78$] effects, or of the interactions between the various factors.

Similarly, when evaluating accuracy level, ANOVA did not show any significance of Task [$F(1,5) = 2$; $P = 0.21$] and Condition [$F(4,20) = 2.01$; $P = 0.13$] effects, or of the Task \times Condition interaction [$F(4,20) = 2.63$; $P = 0.07$].

These findings suggest that unilateral parietal or temporal TMS was ineffective in disrupting performance in both WM tasks.

Experiment 3: Effects of Parietal and Temporal TMS Delivered during the Encoding and Motor Response Phases of Visual-object and Visual-spatial WM Tasks

The main results of this experiment are summarized in Table 3.

Two separate repeated measures ANOVAs, with Task (visual-spatial versus visual-object WM) and Condition (baseline, parietal TMS, temporal TMS) as within-subject factors, were performed on RTs and accuracy for each block (= SOA: 0 and 3300 ms) of trials.

As regards RTs, a significant main effect of Task was found, both for blocks with 0 ms [$F(1,5) = 150.87$; $P = 0.00006$] and for those with 3300 ms SOA [$F(1,5) = 338.45$; $P = 0.000009$]. On the other hand, no significant main effects of Condition or Task \times Condition interaction were found at either SOA.

As regards accuracy, in blocks with 0 ms SOA, no significant main effects of Task and Condition or significant interactions were found. In blocks with 3300 ms SOA, there was a significant main effect of Task [$F(1,5) = 7.95$; $P = 0.04$], reflecting a lower number of correct responses in the visual-object compared with visual-spatial WM task. On the other hand, no significant effects of Condition or significant interactions were found.

In sum, TMS of posterior cortical areas, when delivered in the study phase or in the response phase, had no perceptual or motor disruptive effects on visual-spatial and visual-object WM tasks.

TMS of frontal cortical areas

Experiment 4: Effects of SFG and DLPFC TMS Delivered during the Memory Delay of Visual-object and Visual-spatial WM Tasks

Table 4 shows mean levels of accuracy and RTs in the two tasks and in the various experimental conditions.

TMS trials with 300 ms SOA. For the RTs, only the main effect of Task was reliable [$F(1,10) = 239.2$; $P < 0.001$], again reflecting the longer RTs in the visual-object compared with the visual-spatial WM task. Conversely, the Condition effect was not significant [$F(2,20) = 0.05$; $P = 0.95$], nor was the Task \times Condition interaction [$F(2,20) = 1.10$; $P = 0.35$].

Table 2Mean level of accuracy (no. of correct responses \pm SD) and RTs in the two WM tasks during unilateral TMS of posterior cortical areas at SOA of 300 ms

	Baseline	Right parietal TMS	Left parietal TMS	Right temporal TMS	Left temporal TMS
Accuracy					
Visual-spatial WM	18.0 \pm 0.9	17.8 \pm 2.0	17.3 \pm 1.3	18.0 \pm 1.1	16.0 \pm 2.7
Visual-object WM	18.0 \pm 1.4	16.0 \pm 2.1	15.8 \pm 3.5	17.3 \pm 1.9	17.5 \pm 2.7
RT (ms)					
Visual-spatial WM	511.2 \pm 141.6	519.5 \pm 124.5	527.3 \pm 135.1	525.5 \pm 153.5	538.5 \pm 185.0
Visual-object WM	920.3 \pm 227.8	847.8 \pm 149.6	870.2 \pm 168.4	844.8 \pm 169.2	821.2 \pm 144.1

Table 3Mean level of accuracy (no. of correct responses \pm SD) and RTs in the two WM tasks during bilateral TMS of posterior cortical areas at SOAs of 0 and 3300 ms

	Baseline		Parietal TMS		Temporal TMS	
	SOA: 0	SOA: 3300	SOA: 0	SOA: 3300	SOA: 0	SOA: 3300
Accuracy						
Visual-spatial WM	17.8 \pm 1.5	19.2 \pm 1.2	18.7 \pm 1.8	19.7 \pm 0.5	17.8 \pm 1.7	17.8 \pm 1.7
Visual-object WM	17.0 \pm 1.3	18.5 \pm 1.9	18.0 \pm 2.4	15.3 \pm 4.8	18.0 \pm 2.5	16.5 \pm 2.5
RT (ms)						
Visual-spatial WM	526.8 \pm 143.8	478.7 \pm 113.5	523.2 \pm 157.8	447.5 \pm 100.5	536.3 \pm 136.5	463.0 \pm 102.3
Visual-object WM	861.2 \pm 145.7	820.2 \pm 136.8	852.2 \pm 158.0	748.3 \pm 69.2	838.0 \pm 147.8	785.0 \pm 77.9

Table 4Mean level of accuracy (no. of correct responses \pm SD) and RTs in the two WM tasks during bilateral TMS of frontal cortical areas at SOAs of 300 and 600 ms

	Baseline		SFG TMS		DLPFC TMS	
	SOA: 300	SOA: 600	SOA: 300	SOA: 600	SOA: 300	SOA: 600
Accuracy						
Visual-spatial WM	18.0 \pm 2.9	18.3 \pm 2.1	15.6 \pm 5.3	17.9 \pm 2.4	15.8 \pm 5.3	16.5 \pm 4.1
Visual-object WM	17.6 \pm 3.5	18.7 \pm 1.9	16.5 \pm 3.7	17.6 \pm 2.3	15.9 \pm 4.9	16.2 \pm 3.8
RT (ms)						
Visual-spatial WM	488.5 \pm 161.7	419.7 \pm 89.2	471.9 \pm 195.1	465.6 \pm 96.8	492.8 \pm 185.0	445.0 \pm 93.9
Visual-object WM	805.5 \pm 150.4	775.7 \pm 116.8	821.4 \pm 151.4	776.2 \pm 113.2	791.4 \pm 167.3	824.9 \pm 116.4

This pattern of results did not change when analysing the TMS–baseline difference in RTs.

Regarding accuracy, there were neither significant main effects nor interactions between the factors.

In sum, TMS delivered 300 ms after visual stimulus offset (as in the previous set of experiments conducted in posterior cortical areas) had no effects on either visual-spatial or visual-object WM. *TMS trials with 600 ms SOA.* Examination of the RTs when TMS was delivered 600 ms after the visual stimulus offset revealed main effects of Task [$F(1,13) = 190.1$; $P < 0.001$] and Condition [$F(2,26) = 10.4$; $P = 0.0004$]. This reflected the fact that, when considering the two tasks together, TMS of both the SFG [$F(1,13) = 20.4$; $P = 0.0005$] and the DLPFC [$F(1,13) = 19.6$; $P = 0.0006$] elicited longer RTs than did baseline trials. RTs did not significantly differ between the two TMS conditions [$F(1,13) = 1.8$; $P = 0.19$].

The Task \times Condition interaction was significant [$F(2,26) = 4.7$; $P = 0.01$], reflecting the different effects produced by SFG and DLPFC TMS in the visual-spatial and visual-object tasks. In particular, in the visual-spatial task, TMS of the SFG elicited significantly longer RTs than did the baseline [$F(1,13) = 24.8$; $P = 0.0002$], and a clear trend in this direction was observed also after TMS of the DLPFC [$F(1,13) = 3.9$; $P = 0.06$]. On the other hand, no significant differences in RTs were observed between the two TMS conditions [$F(1,13) = 2.75$; $P = 0.12$]. In contrast, in

the visual-object task, TMS of the SFG did not significantly alter RTs compared with baseline [$F(1,13) = 0.01$; $P = 0.96$]; after TMS of the DLPFC, instead, RTs were significantly slower compared with both baseline [$F(1,13) = 8.64$; $P = 0.01$] and SFG TMS [$F(1,13) = 6.62$; $P = 0.02$] conditions.

The reported pattern of results did not change when considering normalized, rather than absolute, RTs. In this case, the main effects of Task [$F(1,13) = 0.28$; $P = 0.60$] and Condition [$F(1,13) = 1.63$; $P = 0.22$] did not reach statistical significance. However, a clear dissociation of the TMS effects on the SFG versus the DLPFC depending on the WM task still emerged from the significant Task \times Condition interaction [$F(1,13) = 6.03$; $P = 0.02$] (see Fig. 4). Planned comparisons showed that, in the visual spatial WM task, RTs were similar after TMS of the SFG versus the DLPFC [$F(1,13) = 2.13$; $P = 0.16$]. In the visual-object WM task, instead, TMS of the DLPFC yielded a significant increase in RTs compared with TMS of the SFG [$F(1,13) = 5.28$; $P = 0.03$].

When evaluating the performance as the number of correct responses, there was a significant main effect of Condition [$F(2,26) = 9.31$; $P = 0.0009$], indicating an increase in error rates induced by TMS. In fact, considering the performance on the two WM tasks together, the accuracy was significantly reduced by TMS of the DLPFC compared with baseline [$F(1,13) = 16.8$; $P = 0.001$], whereas TMS of the SFG failed to significantly alter it

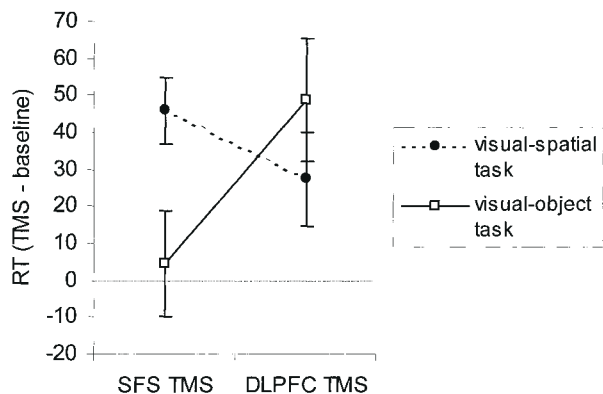


Figure 4. Normalized RTs (TMS – baseline) in visual-object versus visual-spatial WM tasks as a function of the stimulated frontal scalp sites in experiment 4. The data refer to SOA of 600 ms. Error bars indicate 1 SEM.

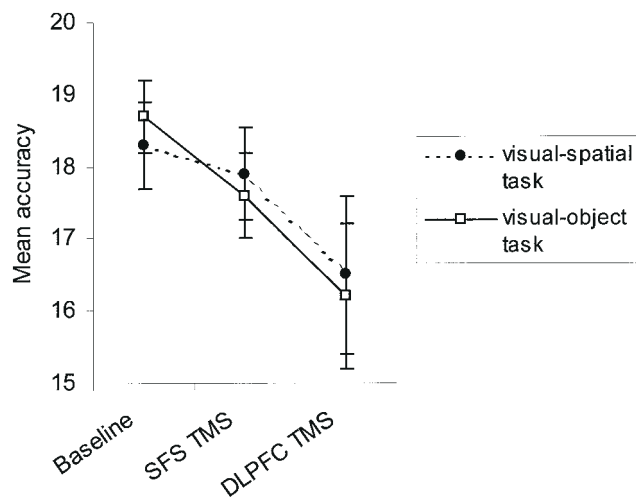


Figure 5. Mean level of accuracy (number of correct responses) in visual-object versus visual-spatial WM tasks as a function of the experimental condition in experiment 4. The data refer to SOA of 600 ms. Error bars indicate 1 SEM.

[$F(1,13) = 3.7$; $P = 0.07$]. The difference between the two TMS conditions turned out to be significant [$F(1,13) = 5.72$; $P = 0.03$].

The main effect of Task [$F(1,13) = 0.01$; $P = 0.89$] and the Task \times Condition interaction [$F(2,26) = 0.35$; $P = 0.70$] were not significant. These findings are indicative of a parallel reduction in the performance level induced by TMS of the DLPFC relative to both baseline and SFG TMS in the two WM tasks (Fig. 5).

Summarizing, TMS of the SFG specifically prolonged RTs in the visual-spatial WM task, whereas TMS of the DLPFC interfered with both WM tasks, both by prolonging RTs and by increasing the number of errors. These effects were observed when TMS was delivered at a SOA of 600 but not of 300 ms from the offset of the visual stimulus.

Experiment 5: Effects of DLPFC and SFG TMS, Delivered during the Encoding and Motor Response Phases of Visual-object and Visual-spatial WM Tasks

The main results of this experiment are summarized in Table 5.

The analysis of RTs revealed only a significant main effect of Task, both for blocks with 0 ms [$F(1,5) = 48.77$; $P < 0.001$] and for those with 3300 ms SOA [$F(1,5) = 327.9$; $P < 0.001$]. No

significant effects of Condition or Task \times Condition interaction were found at either SOA.

Similarly, when analysing accuracy, no significant main effects or interactions between the two factors were found at either SOA.

In sum, TMS of frontal cortical areas, when delivered in the study phase or in the response phase of each trial, had no perceptual or motor disruptive effects on visual-spatial and visual-object WM tasks.

Discussion

The present study used TMS to transiently interfere with the function of posterior and frontal cortical areas, in order to provide evidence of distinct cortical systems for the temporary ‘on line’ retention and active manipulation of spatial versus object visual information in normal humans.

The main results showed some topographic and temporal specific TMS effects, depending on the WM task: bilateral parietal TMS selectively disrupted spatial WM, whereas bilateral temporal TMS was associated with a decline in visual-object, but not visual-spatial, WM performance. On the other hand, at the level of frontal cortical areas, TMS of the SFG selectively disrupted visual-spatial WM performance, whereas TMS of the DLPFC interfered with both WM tasks. TMS of the DLPFC was able to affect performance accuracy in contrast to TMS of the other cortical regions, which interfered only with RTs. In addition, TMS over the frontal cortical areas had an influence on WM processes when applied during a later period of memorization compared with TMS delivered on posterior cortical areas. Finally, our results showed that TMS of either cortical area had a disruptive effect limited to the delay phase of the tasks.

The neuronal mechanisms and the brain organization of visuospatial WM have been extensively studied in humans with a variety of experimental procedures, including positron emission tomography (PET), functional magnetic resonance imaging (fMRI) and ERP recordings (Jonides *et al.*, 1993; McCarthy *et al.*, 1994, 1996; Smith *et al.*, 1995; Courtney *et al.*, 1996, 1998; Rama *et al.*, 1997; Ruchkin *et al.*, 1997; D’Esposito *et al.*, 1998; Owen *et al.*, 1999). These studies have not, however, yielded uniform results, especially concerning the organization of WM functions in the frontal lobe. In this connection, TMS, working as a ‘virtual lesion’ method in normal humans, can provide a link between neuropsychological studies of brain-damaged patients and the cortical activations evidenced in PET/fMRI studies, by showing the necessity of a given brain region for a specific WM accomplishment.

Few studies have addressed the issue of selectively disrupting short-term/WM functions by means of single-pulse or repetitive TMS (rTMS) (Duzel *et al.*, 1996; Jahanshahi *et al.*, 1998; Grafman *et al.*, 1999; Mottaghy *et al.*, 2000; Kessels *et al.*, 2000). To our knowledge, the present study is the first in which TMS was employed to contrast the interfering effects on visual-object versus visual-spatial WM operations in the same subjects. For this purpose, we have adopted a particular experimental procedure, the *n*-back task (Owen *et al.*, 1996, 1998, 1999; Carlson *et al.*, 1998; D’Esposito *et al.*, 1998) elaborated to ensure the performance of a series of computations on information temporary maintained on line (which is the basic difference between the general concept of short-term memory and that of WM). Differently from most of the commonly used short-term memory tests (e.g. memory spans), requiring a discrete response after the serial presentation of a definite number of stimuli, the *n*-back task consists of a continuous sequence of stimuli, in which the

Table 5Mean level of accuracy (no. of correct responses \pm SD) and RTs in the two WM tasks during bilateral TMS of frontal cortical areas at SOAs of 0 and 3300 ms

	Baseline		SFG TMS		DLPFC TMS	
	SOA: 0	SOA: 3300	SOA: 0	SOA: 3300	SOA: 0	SOA: 3300
Accuracy						
Visual-spatial WM	18.3 \pm 1.5	19.0 \pm 0.9	17.8 \pm 2.9	18.5 \pm 1.4	18.8 \pm 0.8	18.5 \pm 1.2
Visual-object WM	18.5 \pm 1.8	18.7 \pm 1.5	18.7 \pm 0.8	17.7 \pm 1.5	18.7 \pm 1.4	17.0 \pm 2.5
RT (ms)						
Visual-spatial WM	443.2 \pm 219.1	369.3 \pm 131.5	411.5 \pm 181.1	329.2 \pm 58.4	418.0 \pm 185.1	366.3 \pm 121.2
Visual-object WM	728.7 \pm 130.5	714.3 \pm 101.2	715.7 \pm 119.9	708.0 \pm 126.7	698.3 \pm 127.3	688.7 \pm 131.2

response phase has to be constantly updated. On the other hand, the WM task employed in the present study was a modified version of the *n*-back task that has been applied previously in fMRI research (Owen *et al.*, 1998). As for the visual-spatial task, the modification was such that the subjects need not compare a new stimulus with the one presented *n* trials back. Therefore, there was one cognitive process less (comparison of stimuli) than in the conventional *n*-back paradigm. This modification implies that the subject can decide immediately after having seen the stimulus location which button to push after *n* trials, since the target button is in the location corresponding to where the stimulus was presented. Therefore, instead of keeping in mind the stimulus location during the delay period, the subject may be preparing for the response movement and keeping that in mind. Conversely, in the visual-object task, the subject will know only after *n* trials (in the response phase) which of the three buttons correspond to the memorized pattern. This aspect could also partly explain the shorter RTs obtained in the visual-spatial compared with visual-object task in all experiments.

Moreover, the nature of the spatial task makes it probable that the TMS interference with the visual-spatial task could also be due to the disruption of the motor preparation/memory for the ensuing movement rather than visual-spatial memory only. This interpretation is consistent with the view assigning to visual-spatial WM a role in the short-term maintenance and manipulation of information for ensuring the performance of complex tasks, including the motor acts toward a selected target location. This implies a close link between the visual-spatial representational system and the movement planning system (Logie, 1986; Laquaniti *et al.*, 1997; Goodale, 1998), such that a transient disruption of the first can result in an impairment of the latter, even if they rely on partially separate anatomical mechanisms.

TMS of parietal and temporal regions

The overall pattern of results observed after parietal and temporal TMS is concordant with previous neuroimaging and ERP studies in indicating that posterior cortical areas are organized in a ventral/dorsal fashion subserving the temporary storage of 'what' and 'where' information (Wilson *et al.*, 1993; Haxby *et al.*, 1994; Courtney *et al.*, 1996; Ruchkin *et al.*, 1997; Smith and Jonides, 1997, 1999; Ungerleider *et al.*, 1998).

The current WM model postulates that posterior cortical areas are involved in both visual information (object or spatial) perceptual processing and in the transient storage of the same information within WM. It is therefore presumable that a lesion involving posterior cortical areas both interferes directly with the perceptual function in question and limits their capacity of information storage in WM. On the other hand, it is difficult with neuroimaging techniques, which entail the summing of brain

activity over extended time intervals, to distinguish between activity related to perception and that related to memory in a WM task. In this context, the temporal resolution of TMS makes it a choice technique for combining an interference paradigm with precise timing of stimulation (Walsh and Rushworth, 1999; Pascual-Leone *et al.*, 2000), and so for dissociating memory and perceptual aspects of WM, as can be predicted from clinical observations and neuropsychological investigations (Stark *et al.*, 1996; Belger *et al.*, 1998; Carlesimo *et al.*, 2001). Despite this optimal temporal resolution of TMS, the distinct roles of the various components of a WM process are not strictly temporally defined in *n*-back paradigms, making it difficult to dissociate perceptual, memory and motor aspects along with a continuous task. Therefore, our finding of selective TMS interfering effects limited to the delay phase of the WM tasks does not imply univocally a disruption of memory components. It is instead conceivable that the performance deficits observed after TMS of posterior cortical regions are due to any of the component processes involved in WM. In particular, a TMS disruption of the motor preparation, especially in the case of the spatial task, cannot be excluded at all in the paradigms adopted. Future studies will be necessary to determine which one, if, indeed, only one, of these subprocesses is affected by TMS in the delay period of such WM tasks.

Lateralization patterns in posterior cortical areas

Data from some neuroimaging studies suggest that spatial WM is a strongly right-hemisphere function, whereas object WM is bilateral or left-lateralized (Smith and Jonides, 1997; Ungerleider *et al.*, 1998). This conclusion was also supported by a recent study showing greater disruption of spatial WM following rTMS of the right compared with the left parietal cortex (Kessels *et al.*, 2000), and by the recent report of a patient with severe impairment of visual-spatial WM due to a lesion confined to the right hemisphere (Carlesimo *et al.*, 2001). Our results, in failing to show a significant interference on WM tasks during unilateral TMS, seem most concordant with those of other studies reporting substantial activation of both hemispheres in both spatial and object WM tasks (Ruchkin *et al.*, 1997; Smith and Jonides, 1997). As a possible account for these discrepancies in data regarding hemispheric lateralization in WM competencies, it could be hypothesized that any difference in lateralization is likely quantitative rather than qualitative, and that it can be a function of the task employed, being less evident for more complex tasks (Smith and Jonides, 1997). Another explanation is that some degree of hemispheric lateralization is present, especially for spatial WM, but it can only be detected in the presence of unilateral brain lesions strong enough to produce a dissociation of performance on behavioural tests (Carlesimo *et al.*, 2001). Conversely, the limited interference caused by TMS could allow

for a relative compensation by the opposite (non-stimulated) hemisphere during a WM task, such that a significant disruptive effect might emerge only in the case of bilateral, simultaneous TMS of homologous areas. Further studies, examining verbal WM components (that appear to be more strongly left lateralized), should better clarify these issues.

TMS of frontal cortical areas

Perhaps the major theoretical issue concerning the role of frontal lobes in WM is whether discrete regions (modules) of the frontal cortex are dedicated to specific WM operations and, if so, the characterization of those processes, including their domain- or process specificity.

Neurophysiological studies in monkeys supporting the domain-specific model indicate that the DLPFC within and surrounding the principal sulcus (BA 46/9) is involved primarily in WM for spatial locations, whereas cortices below area 46 – on the inferior convexity – are more involved in processing the features and identity of objects within WM (Goldman-Rakic, 1987; Funahashi *et al.*, 1993; O'Scalaidhe *et al.*, 1997). On the other hand, neuroimaging evidences supporting a distinction between human spatial and object WM have revealed a selective activation of the SFG (Courtney *et al.*, 1996, 1998; Carlson *et al.*, 1998; Haxby *et al.*, 2000), in addition to that of the DLPFC (McCarthy *et al.*, 1994, 1996; Sweeney *et al.*, 1996; Carlson *et al.*, 1998), during spatial WM tasks.

As opposed to this theoretical framework, a neurophysiological study (Rao *et al.*, 1997), comparing directly spatial and non-spatial delay units using the same task, reported that more than half of the neurons with delay activity around the principal sulcus of the monkey showed both spatial and non-spatial tuning. Moreover, most functional brain imaging studies on WM tasks involving non-spatial (i.e. verbal and visual object) information also activate the DLPFC (Fiez *et al.*, 1996; Smith *et al.*, 1996; Courtney *et al.*, 1996; Cohen *et al.*, 1997). Therefore, the existence of a functional specialization of prefrontal cortex in humans has been questioned. According to this view, a 'process-specific' model has been proposed in which there are two executive processing systems, one dorsal and the other ventral, within the lateral PFC. The ventral prefrontal cortex (BA 45/47) would be the site where information is initially received from posterior association areas and where active comparisons of information held in WM are made, as in the case of memory spans or delayed response tasks. In contrast, the DLPFC (BA 46/9) would be recruited only when 'monitoring' and 'manipulation' within WM is required, as in the *n*-back tasks (Petrides, 1994, 1995; Owen *et al.*, 1996, 1999). This model implies a hierarchical scheme of different levels of processing across prefrontal areas, whereas the domain-specific hypothesis argues that storage and processing functions are integrally related within the same region (McCarthy *et al.*, 1994, 1996; Sweeney *et al.*, 1996).

Our results cannot provide a clear interpretation regarding the theoretical issues of domain- or process-specific models. In fact, even if the selective interference produced by TMS of the SFG with the visual-spatial WM task seems to argue for a domain specificity of this region, a strong possibility exists that the SFG is mainly involved in motor preparation, which is most critical in the visual-spatial task. In fact, in this task the subjects can anticipate/rehearse/prepare the response throughout the delay and subsequent trial. This aspect, together with the deficits in RT but not in accuracy observed, makes an interference with motor

programming as likely a scenario as that there is a WM deficit after TMS of the SFG.

Another possibility is that the SFG region could be the anatomical substrate of spatial rehearsal. By this account, spatial rehearsal would involve the covert shifting of attention from one location to another, a process that could require recruitment of an attentional circuit, including the premotor cortex (Awh and Jonides, 1997). Support for this hypothesis comes from neuroimaging studies of spatial WM and spatial attention, showing an overlap in activation in a right premotor site (Awh and Jonides, 1997).

As regards the DLPFC, the significant interference provided by TMS of this region with both visual-spatial and visual-object WM tasks would be more consistent with the process-specific model. This assigns to the DLPFC the role of a multimodal monitoring area of information in WM, provided that the particular tasks being performed demand the type of executive processing subserved by that area.

It is also worth noting the different pattern of TMS interference observed after TMS of the DLPFC compared with other cortical areas. In fact, whereas TMS of the parietal/temporal cortices and of the SFG affected only RTs, TMS of the DLPFC was more disrupting, as it also affected performance accuracy in both WM tasks. In addition to confirming that reaction time performance is most sensitive for the effects of TMS compared with response accuracy (Kessels *et al.*, 2000), these findings are consistent with the hypothesis of a different role of the various network components involved in WM computations. In particular, the DLPFC is supposed to be involved both in sustaining the transient patterns of neural activity of posterior cortical regions that maintain information available on line, and in executive control processes not specific for WM (Cohen *et al.*, 1997; D'Esposito *et al.*, 1998; Levy and Goldman-Rakic, 1999; Postle *et al.*, 1999; D'Esposito and Postle, 1999; Rowe *et al.*, 2000). The observed greater interference on WM tasks produced by TMS of the DLPFC compared with other cortical areas fits well with a transient disruption of both storage and central executive mechanisms.

However, our results do not exclude the possibility that different areas of the prefrontal cortex can support *per se* domain-specific executive operations (Levy and Goldman-Rakic, 1999). In fact, considering that cortical areas presumably involved in the dorsal and ventral streams are much closer to each other in the frontal cortex than are the parietal and temporal lobe territories compared, the spatial resolution of TMS falls short of addressing the domain-specific hypothesis at this level (see also the next section).

Concerning hemispheric lateralization, although a tendency toward segregation of visual-object WM in the left hemisphere and of visual-spatial WM in the right hemisphere was observed in some studies (Smith *et al.*, 1995; Belger *et al.*, 1998), frontal involvement in visual-spatial WM has more often reported to be bilateral (Courtney *et al.*, 1996, 1998; Smith and Jonides, 1997; Carlson *et al.*, 1998; D'Esposito *et al.*, 1998; Owen *et al.*, 1998). For this reason, given also the results of experiments 1 and 2, we used only bilateral TMS of frontal cortical sites. Further studies, employing rTMS paradigms, could be of better value for clarifying the issue of hemispheric lateralization in frontal areas.

Methodological Issues

A crucial point in this study was the choice of the cortical regions to interfere with during the execution of WM tasks. We did not target ventrolateral PFC regions that, due to their ventral

location in the brain, are not easily accessible to TMS effects. This implies that we cannot exclude the possibility of some functional differentiation based on stimulus material between the DLPFC and VLPFC. Moreover, even if the selection of stimulation sites on MRIs of the subjects' brains and the choice of a focal coil certainly improved the localization, it is known that the physiological effects of TMS are not restricted to the small region at which one is aiming and whose outer perimeters are indicated in Figure 2. In fact, within 30 ms after stimulation, there is a spread of effects to nearby areas and to areas that are anatomically interconnected (Ilmoniemi *et al.*, 1997; Paus *et al.*, 1997). Given these concerns on spatial resolution, it cannot be excluded that TMS of the DLPFC spanned, at least in part, even the region around the inferior frontal sulcus, and therefore could have affected both visual-spatial and visual-object representation domains in the prefrontal cortex.

Another critical issue concerns the nature of the memory tasks employed. On the one hand, non-face object perception may require some participation of spatial perception to represent the spatial configuration of the parts of the object (Postle and D'Esposito, 1999). On the other hand, the spatial task employed in the present study did not use truly spatial stimuli (i.e. presented in peripheral vision with central fixation), as subjects focused on both spatial stimuli and visual patterns. This implies that the two tasks may not be so distinct from the encoding/WM perspective. Taken together, these mechanisms could partly explain the activation of the DLPFC by visual patterns, and might blur the dissociations between object and spatial WM at the level of this region.

Time-dependent activation of frontal compared with posterior cortical areas during WM tasks

It has been suggested that a critical time for TMS to interfere with the function of a human brain area is when large populations of neurons of that area are synchronously active for a task. This period appears to coincide with the time when single unit responses can first be recorded from the homologous areas of the macaque brain, or when ERPs are recorded from the human scalp (Walsh and Rushworth, 1999). In this connection, the time scale of ERP studies of visual spatial WM is concordant with our finding of a critical time interval of 300 ms from visual stimulus presentation for interfering with parietal/temporal areas during WM tasks (Ruchkin *et al.*, 1997). TMS over the frontal cortex had an influence on both visual-object and visual-spatial WM tasks when applied during a later period of memorization compared with TMS of posterior cortical regions.

Our results concur with those of another TMS study examining parieto-frontal interactions during the execution of memory guided saccades (Muri *et al.*, 1996). Moreover, other studies have suggested that frontal cortical areas show a sustained activity throughout the retention interval of a WM task, as opposed to transient activity of posterior cortical areas (Cohen *et al.*, 1997). These findings can be interpreted in different ways regarding the role played by the frontal versus posterior association cortices in WM operations. In fact, it is possible that the later involvement during the delay interval reflects the recruitment of amodal, central executive processes. Contrary to this view is the finding of a time-dependent activation of the SFG, provided that this region mediates spatial rehearsal, supposed to be an ongoing process, sustained throughout the memorization period and not limited to a particular time window.

Conclusions

This is the first study attempting to use TMS to investigate the neural bases of both visual-object and visual-spatial WM processes in healthy humans. The main findings relate to the discrimination of a dorsal and ventral pathway from the parieto-temporal to the frontal areas, and indicate that it is easier to discriminate these pathways in posterior cortical regions than in frontal ones. In addition, the results stress the need to consider WM performance as a result of the concerted, time-dependent activation of multiple regions in a widely distributed cortical network.

Notes

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