Segregation of Areas Related to Visual Working Memory in the Prefrontal Cortex Revealed by rTMS

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The functional organization of working memory (WM) in the human prefrontal cortex remains unclear. Storage and processing functions might be segregated in ventral and dorsal areas of the prefrontal cortex, respectively. If so, storage functions might be spared, irrespective of informational domain, following damage or dysfunction in dorsolateral areas. Alternatively, WM and prefrontal function in general might be segregated according to informational domains (e.g. spatial versus object-based information). In the present study we used repetitive transcranial magnetic stimulation (rTMS) to directly test these competing hypotheses. We applied rTMS to transiently and selectively disrupt the function of the dorsomedial, dorsolateral or ventral prefrontal cortex in normal human volunteers performing either a spatial or a face-recognition delayed-response task. Performance in the spatial task was impaired by rTMS of the dorsomedial prefrontal cortex. Performance in the face-recognition (non-spatial) task was impaired by rTMS of the ventral prefrontal cortex. Transient disruption of the dorsolateral prefrontal cortex affected performance in both tasks. These findings provide evidence of domain-specific segregation of WM functions in widely separated areas of prefrontal cortex.

Introduction

The role of the prefrontal cortex in higher cognitive functions, particularly in different kinds of memory (declarative, non-declarative and working memory) is uncertain [for reviews see (Squire and Zola, 1996; Wheeler *et al.*, 1997; Smith and Jonides, 1999)]. The nature of prefrontal function (Luria, 1966; Baddeley, 1986) and the anatomical segregation of prefrontal cortex with respect to various cognitive functions (Fulton and Jacobson, 1935; Johnson *et al.*, 1968; Goldman and Rosvold, 1970; Milner, 1971; Goldman-Rakic, 1987) remain topics of debate. Information processing in the prefrontal cortex could be segregated by virtue of the cognitive function performed by different brain regions or by virtue of the nature of the stimuli processed. In keeping with the former view, a model has been proposed based on convergent evidence of several lesion studies in non-human primates (Petrides, 1994). This model is supported by some lesion (Petrides and Milner, 1982; Owen *et al.*, 1990, 1995) and neuroimaging studies in humans (Petrides *et al.*, 1993; Owen *et al.*, 1996a). The model proposes two executive processing systems within the lateral frontal cortex: the middle portion of the ventrolateral frontal cortex (Brodmann areas 45 and 47) (Brodmann, 1909) serves as one level of interaction between short-term and long-term memory systems and executive processing, while the mid-dorsolateral frontal cortex (Brodmann area 9 and 46) is assumed to constitute another level of interaction of executive processes with memory, and is recruited only when active manipulation and monitoring of information within working memory (WM) is required. Implicit in this model is the notion of being applicable irrespective of stimulus type.

On the other hand, other lesion [e.g. (Goldman and Rosvold, 1970)] and single-cell recording studies [e.g. (Funahashi *et al.*, 1989)] of non-human primates have provided strong evidence that a dorsolateral region incorporating parts of Walker's areas 46 and 8 (Walker, 1940) are highly specialized for information processing of stimuli presented in a spatial context (Funahashi *et al.*, 1989; Fuster, 1989). In contrast to these findings in dorsolateral areas, comparable studies of Walker's areas 12 and 45 on the inferior prefrontal convexity in monkeys have revealed an area in which neurons respond selectively to pictures of objects presented at the fovea but are much less responsive or completely unresponsive to spatial stimuli (Wilson *et al.*, 1993; O'Scalaidhe *et al.*, 1999). Lesions of inferior prefrontal areas in monkeys have often resulted in deficits on object discrimination reversal, object learning set and other nonspatial WM tasks [for a review see (Goldman-Rakic, 1987)]. These findings in nonhuman primates argue for a similar segregation of processing domains within the human prefrontal cortex such that the processing of spatial stimuli would take place in dorsal prefrontal regions while face stimuli would be processed in ventral prefrontal areas. Such a model would interface well with the organization of the visual system in a 'what' (i.e. intrinsic object information) and a 'where' (i.e. spatial information) processing stream (Ungerleider and Haxby, 1994; Haxby *et al.*, 2000). A comparative cytoarchitectonic analysis in the human (Brodmann areas) and the macaque brain (Walker areas) suggests a partition of the dorsolateral prefrontal cortex into three distinct regions (9, 9/46 and 46) to be able to integrate findings from functional neuroimaging studies in human subjects and experimental work in the monkey (Petrides and Pandya, 1999). Walker's areas 12 and 45 in the monkey correspond to Brodmann's areas 45 and 47 in the human. However, to date, sound evidence that might support this domain-specific hypothesis in humans has been scarce. Functional neuroimaging studies (Courtney *et al.*, 1998; Haxby *et al.*, 2000) have localized in humans a 'face area' in the frontal inferior prefrontal cortex homologous in location to that described by O'Scalaidhe and co-workers in the monkey (O'Scalaidhe *et al.*, 1999). Tasks of spatial WM have consistently activated more dorsal regions corresponding to the middle frontal gyrus [for a review see (Smith and Jonides, 1999)]. However, many studies have failed to dissociate spatial and nonspatial processes for a variety of reasons, in part due to task design and in part due to the spatial resolution of the methods used (Goldman-Rakic, 2000). The segregation of prefrontal function in humans might reflect specializations for the modality of the stimuli to be processed (spatial or object), but could be interpreted to represent a modality-independent specialization for different cognitive processes (Owen *et al.*, 1996b; Petrides, 1996; Smith and Jonides, 1999).

The aim of the present study was to investigate whether segregation of areas within the prefrontal cortex involved in a

WM task for faces or spatial locations can be demonstrated using the 'virtual lesion' potential of repetitive transcranial magnetic stimulation (rTMS) [for a review see (Pascual-Leone *et al.*, 1999)]. The feasibility of this 'virtual lesion' approach in memory studies has recently been demonstrated by using the combination of rTMS and PET. rTMS can induce blood flow changes at the stimulation site and in functionally connected brain regions that correlate with performance deterioration in a two-back working memory task (Mottaghy *et al.*, 2000). Previous studies have revealed the possibility of disrupting WM for spatial information during rTMS to the dorsolateral prefrontal cortex (Pascual-Leone and Hallett, 1994; Robertson *et al.*, 2001).

Methods

Subjects

Eight right-handed male individuals (mean age 30.4 ± 4.0 years; range 26–37 years) were studied after they gave written informed consent to the study that had been approved by the local institutional review board. None of the subjects had any neurologic, psychiatric or medical history, nor had any contraindications to rTMS (Wassermann, 1998).

Design

A delayed WM (dWM) paradigm was explored (Levy and Goldman-Rakic, 1999). Subjects were randomly presented either with a dWM trial for spatial locations or a dWM trial for unfamiliar faces. In the spatial dWM task white dots (diameter 2.4 cm) on a black screen (80 cm in front of the subjects) at 20 different locations with respect to a fixation cross at the center of the screen (maximum visual angle 11°) were presented. In the face dWM task captions (diameter 2.4 cm) of black-and-white images taken from the local student population were presented at the center of the screen, which was 80 cm in front of the subjects (visual angle ~1°). The trials were grouped into blocks of 30 trials, each containing 15 spatial dWM trials and 15 dWM trials for faces in random order. The duration of one block was ~4.5 min. Subjects individually paced the time between the dWM trials. Altogether we used 45 different spatial dWM trials and 45 different dWM trials for faces. Following the dWM tasks used in non-human primates (Levy and Goldman-Rakic, 1999), the subjects were initially presented with three stimuli in successive order. Each stimulus was presented for 300 ms and the interstimulus interval (ISI) was 150 ms. After a random delay period, of 2, 3 or 4 s, a target stimulus appeared and the subject had to respond on a computer keyboard by pressing two different keys ('n' and 'm'), whether this target stimulus was part of the initial triad or not (Fig. 1*a*). The random delay period was chosen to increase the attentional demands of the task and to avoid automatic responses. In a pilot study we tested different memory sets of 1–4 items. The error rate with memory sets of one or two items was negligible, whereas those with four items were too difficult, with a large number of random responses. Therefore we decided to use a three-item memory set. Subjects were instructed to use the index finger for the negative response ('n') and the middle finger for the affirmative response ('m'). During the ISI and the delay period a fixation cross was presented at the center of the screen to minimize eye movements. Within the 45 different dWM tasks for each stimulus type there were 15 for each delay period (2, 3 or 4 s). Each set of 15 trials contained three trials each where the first, second or third stimulus presented matched the target stimulus, together with six trials with no match. The program used for the presentation of the stimuli and the recording of the responses was SuperLab pro 1.71 for Macintosh (Cedrus Corp., San Pedro, CA). A pilot experiment was performed in a group of nine male subjects (27.4 ± 7.2 years) to determine the rate of learning effect to be expected in the two different dWM tasks. In this pilot experiment five consecutive blocks consisting of 15 trials for each stimulus type were presented (Fig. 2). We designed the rTMS study based on the results of this pilot experiment, which showed no significant learning effect in relation to accuracy after the second set of 15 trials for each stimulus type. The subjects were presented first with two blocks of dWM trials to get acquainted to the task, and then three blocks were recorded to establish an individual baseline. Thereafter, a 10 min 1 Hz rTMS train was applied over any of three different locations within the left prefrontal cortex. Immediately thereafter the subjects had to perform three more blocks of the dWM task (Fig. 1*a*).

The different locations within the left prefrontal cortex were stimulated on three different sessions at least 1 week apart. In each session, subjects received rTMS over the dorsolateral prefrontal cortex (DLPFC), the ventral prefrontal cortex (VPFC) or the dorsomedial prefrontal cortex (DMPFC). Eight subjects participated on all three sessions. None of the subjects showed adverse side effects. The order of locations was pseudorandomized and counterbalanced across subjects. Stimulation was applied to the left prefrontal cortex following the results of a recent study that found no significant hemispheric differences for dWM activity with spatial stimuli, but the most significant difference for dWM for faces versus spatial stimuli in the left inferior prefrontal cortex (Courtney *et al.*, 1998).

TMS

A tightly fitting Lycra swimming cap was placed on the subject's head in order to mark the sites for TMS. TMS was applied using a Magstim Super Rapid (Magstim Company Ltd, Whitland, UK) and an 8-shaped coil in which each wing measured 70 mm in diameter. Surface electrodes were placed on the right first dorsal interosseus muscle (FDI) and a round ground electrode was placed on the wrist and connected to a Dantec counterpoint electromyograph (Dantec, Skovlunde, Denmark). The optimal scalp site, i.e. the scalp position from which TMS induced motor evoked potentials (MEPs) of maximal amplitude in the contralateral FDI was determined. The resting motor threshold (RMT) was defined for each subject as the minimal intensity of stimulator output capable of inducing MEPs > 50 μV peak-to-peak amplitude in at least six out of 10 consecutive trials. For this purpose, TMS was applied in single pulses with an ISI of at least 7 s in order to avoid 'carry-over' effects. The coil was held flat on the scalp with the handle pointing caudally, perpendicular to the midsagittal line.

During each session subjects received a 1 Hz rTMS train at 90% RMT over the DLPFC, the VPFC or the DMPFC. Each train consisted of 600 pulses and lasted 10 min. These rTMS parameters are well within currently recommended guidelines (Wassermann, 1998). Repetitive TMS was applied at 1 Hz because such parameters have been shown to result in a relative inhibition of the excitability of the targeted cortical region for several minutes following completion of the rTMS train (Chen *et al.*, 1997; Boroojerdi *et al.*, 2000; Muellbacher *et al.*, 2000). The stimulation points (DLPFC, VPFC or DMPFC) were referred to the optimal scalp site for evoking MEPs in the FDI. The DLPFC stimulation site was marked 5 cm anterior to the reference point on a line parallel to the midsagittal line. The VPFC stimulation site was defined by going 4 cm ventral from this point along a perpendicular line to the midsagittal line. The DMPFC was determined going 2 cm mediodorsally along the same perpendicular line to the midsagittal line. For the rTMS train the coil was centered over the three marked points with the handle of the coil pointing caudally, perpendicular to the midsagittal line.

In five of the eight subjects we obtained a 3D-MPRAGE magnetic resonance imaging (MRI) scan (160 sagittal slices 1 mm apart and an inplane resolution of 256 × 256 mm^2) of their brain after the experiment. Vitamin E capsules were placed on the previously marked positions (primary motor cortex, DMPFC, DLPFC and VPFC) as well as Cz of the 10–20 EEG electrode system and the nasion, inion and bilateral tragus. The actual location of the TMS coil position on the scalp with respect to the frontal gyri was then assessed using MRIcro (Chris Rorden, UK Version 1.23). In all five subjects, we found that, as applied, the TMS scalp positions had indeed targeted the superior frontal sulcus (DMPFC), the middle frontal gyrus (and DLPFC) and the inferior frontal gyrus (VPFC). The interindividual variability of the anatomical targets was well within the presumed spatial resolution of the rTMS with the employed coil (1–1.5 cm; Fig. 1).

Analysis

The number of errors and the mean reaction time (RT) were calculated for each block of trials. The three baseline blocks before rTMS were collapsed to an overall baseline. The trials after the rTMS train were regarded as three different time points, given the likely transient effects of rTMS. RT was corrected individually with regard to the overall mean of each individual. To determine if there was a RT error rate trade-off, responses

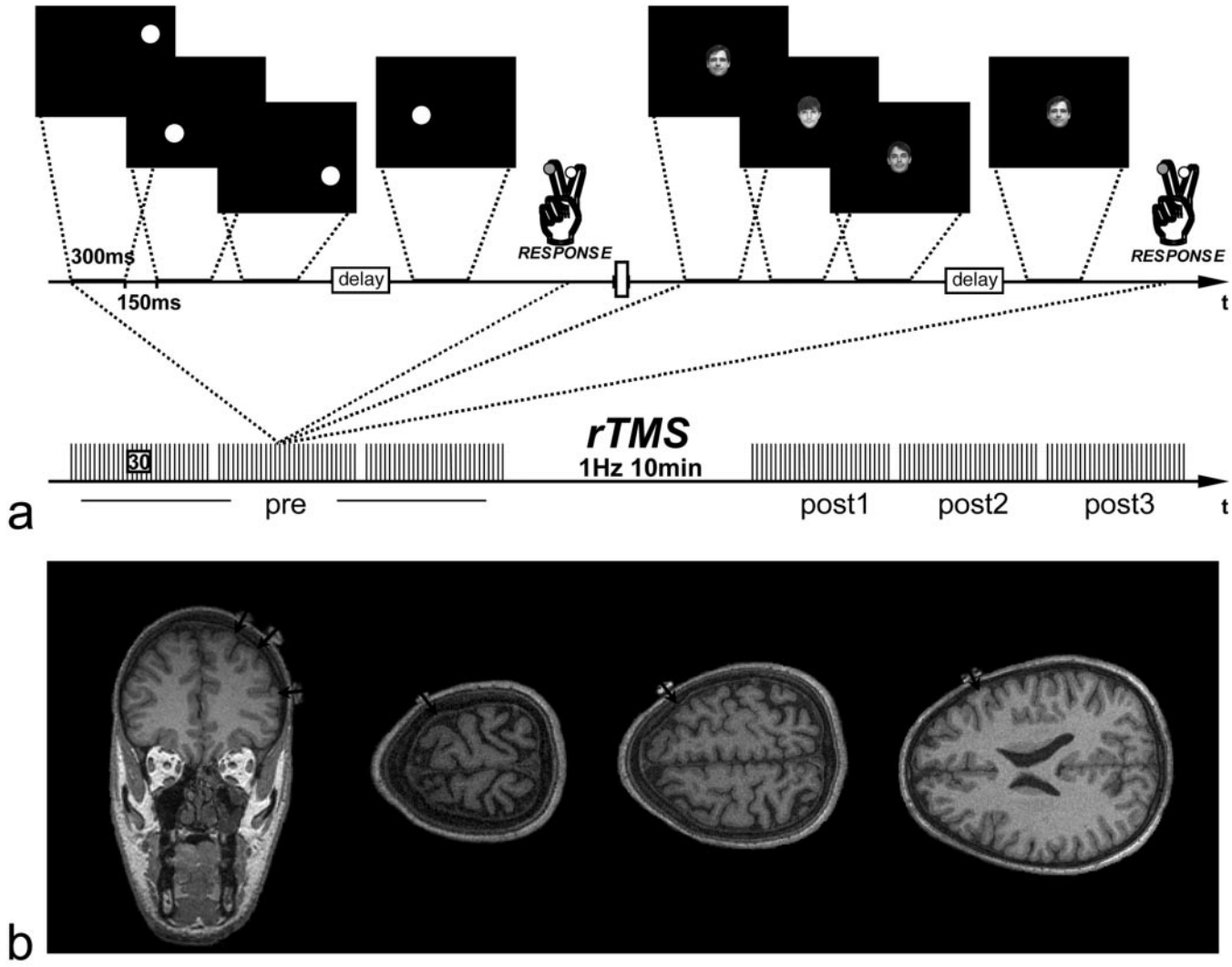

Figure 1. (*a*) In the first row the design of the delayed working memory task for spatial or face stimuli is shown. The delay period between the set of three stimuli, which were consecutively presented (300 ms each with an ISI of 150 ms), and the target stimuli was randomized between 2, 3 or 4 s. Subjects had to respond with the index ('no') or middle ('yes') finger pressing two different keys, deciding whether the target stimulus was within the set of the previously presented stimuli or not. In the second row the experimental set up is sketched (details in the text). (*b*) Positioning of the coil over the left DMPFC (Dm) DLPFC (Dl) and VPFC (V) was confirmed using a 3-D MRI sequence with vitamin E capsules in place.

were correlated. It was found that there were no significant correlations across the conditions (all $P > 0.05$). RT and errors were then analyzed separately employing a 3 × 2 × 4 repeated-measures ANOVA [prefrontal site (3): DMPFC/DLPFC/VPFC; visual stimulus (2): spatial/faces; time (4): baseline/post-rTMS 1–3]. Furthermore, *post hoc* planned comparisons (LSD-test) were performed comparing each post-rTMS block to baseline.

Results

The overall RT across all conditions was 940 ± 13 ms (mean ± SEM). RTs for each stimulation site and condition are shown in Table 1. At baseline the mean RT was 937 ± 35 ms in spatial dWM and 967 ± 38 ms in faces dWM (not significantly different; d.f. = 23; $t = 1.96$; $P = 0.09$). The three-way ANOVA for RT was found to be not significant for the overall interaction between the conditions [$F(6,63) = 0.417$, $P = 0.86$). Error rates (Table 1) were then compared. The overall error rate was 15.3% (2.3 errors in 15 trials ± 0.11). The mean number of errors at baseline across the three different stimulation sites was 1.99 ± 0.22 for the spatial dWM task and 2.04 ± 0.21 for the face stimuli, which was not significantly different (d.f. = 23; $t = 0.22$; $P = 0.83$; Fig. 2). Employing a 3 × 2 × 4 ANOVA (see Analysis), an overall significant interaction was found [$F(6,63) = 4.46$, $P < 0.001$].

Spatial delayed working memory task

The error rate for spatial dWM in the first block after rTMS to the DMPFC was significantly larger than at baseline [$F(1,21) = 6.41$, $P < 0.01$], whereas error rates in the two other blocks after rTMS were not significantly different from baseline (post-rTMS 2 and 3).

Following rTMS to the DLPFC, there was also a significant increase in error rate for the spatial dWM task between baseline and the first block of trials [post-rTMS 1; $F(1,21) = 16.01$, $P < 0.0006$]. The other two blocks of spatial dWM trials after the rTMS train to the DLPFC (post-rTMS 2 and 3) showed no significant differences with regard to the baseline.

Finally, following rTMS to the VPFC, there was no significant difference between baseline and any of the blocks after rTMS for the spatial dWM trials (post-rTMS 1, 2 or 3; all $P > 0.05$).

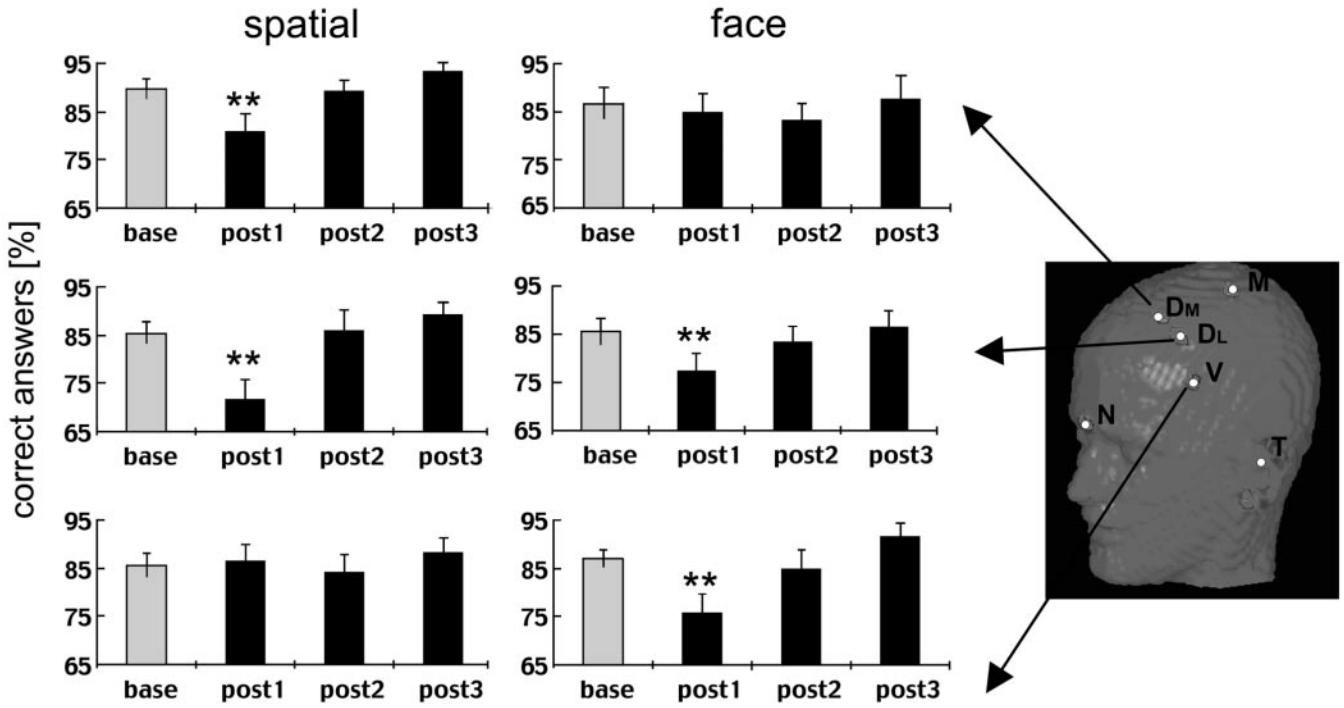

Figure 2. The mean performance rates in percent, with error bars indicating the SEM, are displayed for each stimulation site (Dm = DMPFC; Dl = DLPFC; V = VLPFC), each stimulus type (spatial; faces) and the four different time points (base = baseline; post1 = immediately after rTMS; post2 = 5 min after rTMS; post3 = 10 min after rTMS). **A significant decrease in performance ($P < 0.05$) after rTMS. One rendered brain with the corresponding points is displayed to demonstrate the locations. Other marked feducial points were the hand spot in the primary motor cortex (M), the bilateral tragus (T), the inion and the nasion (N).

Table 1
RT and number of errors (errors) out of the 15 trials per block of the delayed working memory task for spatial or face stimuli at the four different time points (baseline: base; post-rTMS 1–3)

	DMPFC		DLPFC		VPFC	
	RT ± SE (ms)	Errors ± SE (out of 15 trials)	RT ± SE	Errors ± SE	RT ± SE	Errors ± SE
Spatial						
Base	931 ± 64	1.58 ± 0.31	951 ± 56	2.21 ± 0.33	929 ± 68	2.17 ± 0.35
Post-rTMS 1	1021 ± 99	2.88 ± 0.48	974 ± 57	4.25 ± 0.59	892 ± 55	2.00 ± 0.42
Post-rTMS 2	942 ± 57	1.63 ± 0.32	937 ± 47	2.13 ± 0.61	885 ± 60	2.38 ± 0.50
Post-rTMS 3	918 ± 66	1.00 ± 0.27	905 ± 41	1.63 ± 0.38	911 ± 69	1.75 ± 0.41
Face						
Base	982 ± 71	2.00 ± 0.47	978 ± 64	2.17 ± 0.39	943 ± 69	1.96 ± 0.26
Post-rTMS 1	1064 ± 87	2.25 ± 0.53	990 ± 71	3.38 ± 0.50	936 ± 66	3.63 ± 0.56
Post-rTMS 2	973 ± 64	2.50 ± 0.50	938 ± 47	2.50 ± 0.46	924 ± 74	2.25 ± 0.53
Post-rTMS 3	874 ± 54	1.88 ± 0.69	900 ± 91	2.00 ± 0.46	920 ± 74	1.25 ± 0.37

For RT there was no significant difference across sessions (stimulation sites: DMPFC, DLPFC and VPFC) and time points, whereas the number of errors was significantly affected depending on the stimulation site and the time points (see Fig. 2).

Faces delayed working memory task

For the faces dWM task, repetitive TMS to the DLPFC resulted in a significant increase in error rates from baseline in the first block [post-rTMS 1; $F(1,21) = 5.70$, $P < 0.03$]. Error rates in the other two blocks of trials (post-rTMS 2 and 3) did not differ significantly from baseline.

The increase in errors between baseline and post-rTMS 1 was also significant following rTMS to the VPFC [$F(1,21) = 10.84$, $P < 0.0034$]. Also in this case, error rates in the post-rTMS 2 and 3 blocks did not differ significantly from baseline (Fig. 2).

Finally, error rates were not increased by rTMS to the DMPFC in any of the post-rTMS blocks as compared with baseline.

Prefrontal site of rTMS

The analysis of the effects of rTMS to the DMPFC [$F(1,21) = 0.88$, $P = 0.36$] and DLPFC depending on spatial versus face stimuli revealed no significant differences [$F(1,21) = 1.72$, $P = 0.2$]. However, there were significant differences in the effects of rTMS on spatial and face stimuli following rTMS to the VPFC [$F(1,21) = 6.97$, $P < 0.02$].

Discussion

The results of this study show that human working memory functions follow a dorso-spatial/ventro-object axis, as predicted by single-cell recording, imaging and lesion experiments (Fuster and Alexander, 1971; Funahashi *et al.*, 1989; Wilson *et al.*, 1993; Goldman-Rakic, 1995; O'Scalaidhe *et al.*, 1999; Petrides and Pandya, 1999; Haxby *et al.*, 2000).

It is clear from our results that there is a domain-specific functional segregation in the human prefrontal cortex. Stimulation to

one site affects spatial information-based but not face-based WM, while the opposite effect can be noted when rTMS is applied to a different site. However, there is no precise knowledge about the spatial resolution of rTMS, and therefore it is difficult to convert the functional segregation shown into anatomical segregation. Mapping studies within the motor or visual cortex using single-pulse TMS have argued a spatial resolution of ~0.5–1 cm at the scalp surface [for reviews see (Jahanshahi and Rothwell, 2000; Walsh and Cowey, 2000)]. However, during a prolonged train of rTMS, the spread of the effects along cortico-cortical connections may well lead to less focal effects (Pascual-Leone *et al*., 1994). Furthermore, several studies using combinations of TMS and different neuroimaging methods (Fox *et al*., 1997; Ilmoniemi *et al*., 1997; Paus *et al*., 1997, 1998) have demonstrated that TMS can exert, in addition to local effects in the directly targeted cortex, trans-synaptic effects onto functionally connected brain regions. Whether such trans-synaptic distant effects are behaviorally relevant is unclear. Regardless, it seems reasonable to consider that TMS affects a blurry volume in which the effects of the TMS tail off (similar to a Gaussian distribution) over an area of ~4–6 cm^2. This would mean that there is no or only marginal overlap of the functional lesions induced by rTMS over the DMPFC and the VPFC. The DLPFC 'virtual lesion' might disrupt two adjacent domain-specific separate areas like the ones observed in monkey (Wilson *et al*., 1993) (Fig. 3*a*).

On the other hand, it is also possible that the performance deterioration observed for both types of stimuli after applying rTMS over the DLPFC site could be explained by interference with a common WM module within the middle frontal gyrus, i.e. the DLPFC stimulation site (Fig. 3*b*). This interpretation would combine the two ideas of a functional and a modality-specific segregation within the prefrontal cortex. Such a situation would fit with the findings in non-human primates [e.g. (Wilson *et al*., 1993)] showing different neurons being involved for spatial versus object processing, and also with various neuroimaging studies in humans demonstrating a comparable segregation [e.g. (McCarthy *et al*., 1996; Belger *et al*., 1998)]. On the other hand, such a situation would also explain other neuroimaging studies proposing a segregation by function (storage, maintenance) independent of the modality (Petrides, 1994; Owen *et al*., 1996a). Other nonhuman primate studies described a rather high degree of overlap of neurons processing object ('what') or spatial ('where') information throughout the dorsolateral and ventrolateral prefrontal cortex (Rao *et al*., 1997; Rainer *et al*., 1998). Our results demonstrate modality specificity, but they do not rule out interactions between the different WM network in the prefrontal cortex and may demonstrate effective supramodal mechanisms in the DLPFC.

Another issue that has been broadly discussed is that of hemispheric specialization for different kinds of stimuli. Some studies have suggested that spatial information held in short-term memory is processed in dorsal areas of the right prefrontal cortex [for a review see (Smith and Jonides, 1999)], whereas others attribute bilateral dorsal areas around the superior frontal sulcus, including parts of the middle frontal gyrus to spatial WM (Belger *et al*., 1998; Courtney *et al*., 1998). For faces, on the other hand, there is evidence for a lateralization to the left (Courtney *et al*., 1998; Haxby *et al*., 2000). As mentioned earlier, we designed our study according to these latter findings and decided to stimulate the left rather than the right prefrontal cortex in order to differentiate the contributions of the different cortical regions to the two different WM tasks within one hemisphere.

Several studies have demonstrated a delay of memorized saccades by TMS (Priori *et al*., 1993; Muri *et al*., 1996, 2000). Single-pulse TMS applied over the supplementary motor cortex (BA6) of either hemisphere is capable of delaying the execution of memorized saccades but not of non-delayed saccades (Muri *et al*., 2000). However, in this experiment, as in previous experiments (Priori *et al*., 1993; Muri *et al*., 1996), a nonfocal round TMS coil was used and it is not possible to say to what extent BA6, the frontal eye field or adjacent areas like BA9 are stimulated (Muri *et al*., 2000). Given our previous assumption of

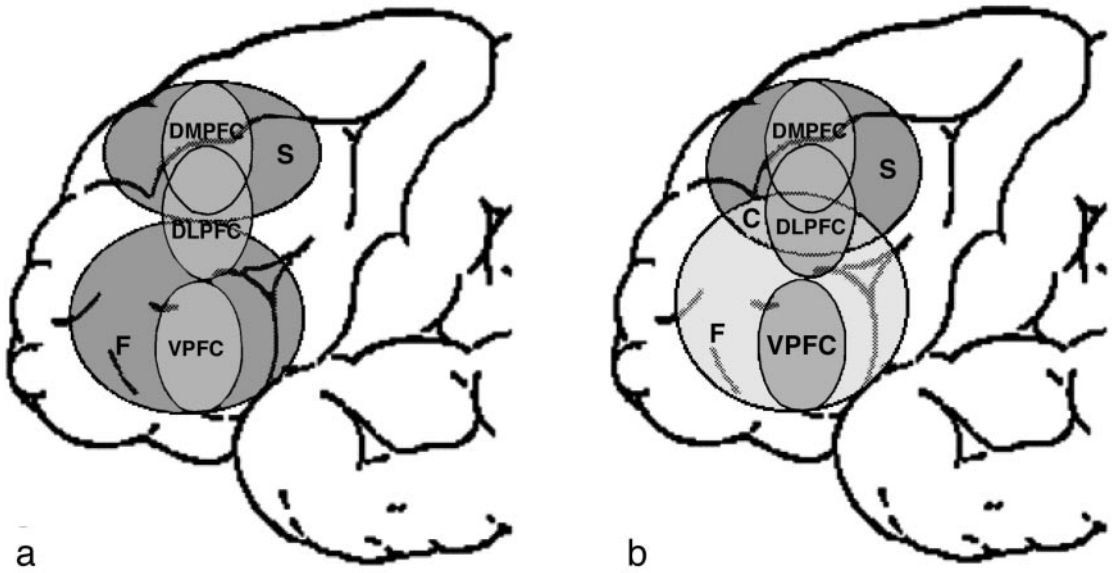

Figure 3. Two alternative models based on our data can be proposed. (*a*) There might be two different, non-overlapping functionally segregated regions within the prefrontal cortex that are domain specific (S = spatial domain; F = face domain). Repetitive TMS over the DMPFC interferes only with the processing of the spatial information. The DLPFC stimulation might have induced overlapping interference of two adjacent domain specific areas, whereas the VPFC led only to interference with the processing of the face stimuli. (*b*) The DMPFC and the VPFC interference effects can be explained in the same manner as in proposal (*a*); however, the performance deterioration over the DLPFC in this given model might be explained by the interference with information processing of a common module (C) that is employed during both types of stimulus.

the extent of the 'virtual lesion', the effect of accuracy deterioration in the spatial dWM task after rTMS to the DMPFC in our study could be explained by interference with processes within the frontal eye field, but the DLPFC stimulation should then have no or only a very slight effect due to its distance from the frontal eye field. Furthermore, in the saccade studies there was an effect on speed rather than on accuracy, and our results did not show any significant reaction time delay after the DMPFC stimulation. Recently it was demonstrated that there is a specialized area for spatial WM in the vicinity of the frontal eye field lying within/around the superior frontal sulcus and distinguishable from activations during saccadic eye movements (Courtney *et al.*, 1998). An important methodological observation of our study is the duration of the transient effect of the dWM impairment after the rTMS train. The study design employed, testing task performance before and following modulation of cortical excitability by rTMS, prevents non-specific disruptive effects of the rTMS train itself (Pascual-Leone *et al.*, 1999). However, the question of the duration of the modulatory effects of rTMS is critical. In the motor system, monitoring of the amplitude of motor-evoked potentials allows objective assessment. In this case, the decrease in cortico-spinal excitability after an rTMS train lasted up to half the time of the train itself in one study (Chen *et al.*, 1997) and persisted for twice the time of the train in another study (Muellbacher *et al.*, 2000). In the visual cortex the excitability was decreased for at least 10 min after a 15 min train (Boroojerdi *et al.*, 2000). However, the return to the baseline performance in our study was observed within 5 min after the end of the 10 min rTMS train. One explanation for this could be that the effect we observed is not due to a pure rTMS interference on the task, but is the result of rTMS on a possibly still persistent learning effect. However, in a pilot study we demonstrated that after 30 trials of the dWM task (i.e. two blocks in our design) there was only a marginal learning effect in the consecutive three blocks for each stimulus type. This strengthens our findings immediately after rTMS and underlines the assumption that the rTMS effect might already be washed out after 5 min. Yet, by the careful analysis of our findings after the rTMS train, it is obvious that there is a slight learning effect over the three post-rTMS trials in the four conditions where rTMS impaired the dWM performance, but not in the face dWM task after DMPFC rTMS or the spatial dWM task after VLPFC rTMS. Future studies using rTMS to evaluate the functional contributions of a specific brain area to a cognitive process should be carefully designed with respect to the anticipated duration of the effect of the rTMS train, taking into consideration the complexity of the task itself.

In summary, our data support the idea of modality-specific processing within the prefrontal cortex. The fact that the accuracy was impaired for both types of stimulus after rTMS over the middle frontal gyrus is compatible with two different interpretations (Fig. 3*a*,*b*) that would require additional experiments for resolution.

Notes

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