Prefrontal and parietal cortex in human episodic memory: an interference study by repetitive transcranial magnetic stimulation

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Summary

Neuroimaging findings, including repetitive transcranial magnetic stimulation (rTMS) interference, point to an engagement of prefrontal cortex (PFC) in learning and memory. Whether parietal cortex (PC) activity is causally linked to successful episodic encoding and retrieval is still uncertain. We compared the effects of event-related active or sham rTMS (a rapid-rate train coincident to the very first phases of memoranda presentation) to the left or right intraparietal sulcus, during a standardized episodic memory task of visual scenes, with those obtained in a fully matched sample of subjects who received rTMS on left or right dorsolateral PFC during the same task. In these subjects, specific hemispheric effects of rTMS included interference with encoding after left stimulation and disruption of retrieval after right stimulation. The interference of PC-rTMS on encoding∕retrieval performance was negligible, lacking specificity even when higher intensities of stimulation were applied. However, right PC-rTMS of the same intensity lengthened reaction times in the context of a purely attentive visuospatial task. These results suggest that the activity of intraparietal sulci shown in several funtional magnetic resonance studies on memory, unlike that of the dorsolateral PFC, is not causally engaged to a useful degree in memory encoding and retrieval of visual scenes. The parietal activations accompanying the memorization processes could reflect the engagement of a widespread brain attentional network, in which interference on a single 'node' is insufficient for an overt disruption of memory performance.

Introduction

Episodic memory is a complex set of human cognitive processes that allows the encoding, storage and intentional recollection (retrieval) of unique events associated with the context in which they occurred (Baddeley *et al.*, 2001). Therefore, correct functioning of encoding∕retrieval mechanisms is vital in forming the conscious story of our existence (Tulving, 2002). Studies in neurological patients indicate that episodic memory is dramatically disrupted by lesions of the medial temporal lobe, and in particular of the hippocampal formation and enthorinal cortex (Squire *et al.*, 2001), whereas lesions in other brain areas – such as the prefrontal cortex (PFC) – are associated only with more subtle dysfunctions of learning and memory (Alexander *et al.*, 2003). Lesions in other neocortical areas, such as the parietal lobe, appear to induce material-specific effects, largely limited to the short-term retention of information. Left-sided lesions are associated with defective performance in auditory–verbal short-term memory

tasks, and impaired spatial span performance has been found in patients with right parietal damage (De Renzi & Nichelli, 1975). Rare cases of 'retrosplenial amnesia' have been described following lesions of parietal midline structures (Valenstein *et al.*, 1987).

Other relevant evidence for the role of neocortical regions in episodic memory comes from converging neuroimaging studies in normal subjects: techniques measuring cerebral blood flow or metabolism (positron emission tomography and functional magnetic resonance), magnetoelectric brain activity [electroencephalography (EEG)∕magnetoencephalography] and event-related cortical activity (event-related potentials) are increasingly unveiling the functional correlates of human learning and memory. Overall, these studies have indicated that regional activity co-varies with encoding and retrieval tasks in several brain regions, therefore suggesting the existence of a distributed neural network governing this highly specialized process. The main functional 'nodes' of such a network include the PFCs, the medial temporal lobes, but also the parietal cortices (PCs) and precuneus (for extensive reviews see Buckner & Wheeler, 2001; Fletcher & Henson, 2001; Rugg *et al.*, 2002; Cabeza *et al.*, 2003; Simons & Spiers, 2003; Wagner *et al.*, 2005), often functionally

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coupled in complex combinations (Cabeza & Nyberg, 2000; Rugg *et al.*, 2002; Kesner & Rogers, 2004; Wagner *et al.*, 2005). The involvement of parietal areas is suggested by several neuroimaging and electrophysiological studies of visuospatial and verbal episodic memory (for reviews see Cabeza & Nyberg, 2000; Rugg *et al.*, 2002; Wagner *et al.*, 2005), although in only a few of these was PC activity specifically addressed (Henson *et al.*, 1999; Mottaghy *et al.*, 1999; Konishi *et al.*, 2000; Cabeza *et al.*, 2003; Hayes *et al.*, 2004; Mayes *et al.*, 2004).

However, neuroimaging studies, being correlational in nature, cannot disentangle whether task-related activations are truly necessary for task completion or if they are simply associated with performance. This can be better approached by applying repetitive transcranial magnetic stimulation (rTMS), a unique technique which allows us to produce, *in vivo*, a brief and reversible decline of the performance only if the stimulated area is causally engaged in the task under investigation (Walsh & Cowey, 2000; Walsh & Pascual-Leone, 2003; Rossi & Rossini, 2004). The interferential approach with event-related rTMS is increasingly used across different labs to investigate hemispheric specializations of PFCs during long-term memorization tasks concerning both visuospatial and verbal cues (Rossi *et al.*, 2001, 2004; Miniussi *et al.*, 2003; Rami *et al.*, 2003; Sandrini *et al.*, 2003; Floel *et al.*, 2004; Kohler *et al.*, 2004).

A main common finding in young adults who received rTMS on the dorsolateral PFCs (DLPFCs) was the direct confirmation of the functional specialization of these areas in episodic visuospatial memory (see Fletcher & Henson, 2001). The left DLPFC has been shown to be prevalent during encoding and the right DLPFC during retrieval of visuospatial memoranda (Rossi *et al.*, 2001, 2004), according to the neuroimaging-based HERA (Hemispheric Encoding Retrieval Asymmetry) model (Habib *et al.*, 2003). By using the same set of visuospatial stimuli as previous studies (Rossi *et al.*, 2001, 2004), a recent high-resolution EEG investigation of cortical rhythmicity during episodic memory highlighted a functional fronto-parietal connectivity by showing that the HERA predictions were fitted only by high-frequency oscillations in the gamma band (30–45 Hz), the amplitude of which was modulated in PC rather than PFC (Babiloni *et al.*, 2004). However, an *ad-hoc* analysis based on the direct transfer function method, which reflects the 'direction' of the information flow between distant but functionally connected cortical sites, successively revealed that a fronto-parietal coherence at the gamma band prevailed in the left hemisphere during the encoding and in the right hemisphere during the retrieval (Babiloni *et al.*, 2006), in line with the prefrontal HERA model prediction (Habib *et al.*, 2003).

The aim of the current event-related rTMS study is to address directly the functional relevance of the activations described in PCs during episodic encoding and retrieval. The intraparietal sulcus was targeted to this end because this region was previously found to be engaged consistently in episodic encoding and retrieval (for reviews see Mottaghy *et al.*, 1999; Cabeza *et al.*, 2003; Fujii *et al.*, 2004;

Hayes *et al.*, 2004; Mayes *et al.*, 2004; Wagner *et al.*, 2005). Moreover, our aim was to contrast results of PC interference with those obtained in a fully comparable sample of subjects who received rTMS on the DLPFCs during the same task and showed clear-cut behavioural consequences (Rossi *et al.*, 2001, 2004). Of note, the functional relevance of the PC along episodic encoding and retrieval has not yet been directly addressed by rTMS interference. Additional control experiments included active and sham rTMS of the PC with higher intensity and active and sham rTMS of the same regions during a purely visuospatial attentive task.

Subjects and methods

Subjects

Forty-two healthy volunteers took part in the study. They gave their written informed consent after approval of the protocol by the local Ethics Committee. Their medical history and objective neurological examination were normal, and they had never taken neuroactive drugs. No other significant medical conditions were present.

The core of the study was to compare the behavioural effects of rTMS delivered to the DLPFC and the PC on a standardized episodic memory task of visual scenes. To this end, two groups of ten subjects were examined (Table 1): those of the first group received active or sham rTMS to the left and right PC ('PC-rTMS group', see later for details). These were matched 1 : 1 with subjects of a second group, retrospectively selected among a cohort of 66 individuals already tested with the same memory task during active or sham rTMS on left and right DLPFC ('PFC-rTMS group', Rossi *et al.*, 2001, 2004). The matching procedure took into account mainly the baseline level of performance in the memory task under investigation (hits–false alarms of the Baseline block). When a subject of the PC-rTMS group could have been matched to more than one subject of the PFC-rTMS group, handedness, gender and age were considered as additional matching factors. These procedures resulted in the statistics reported in Tables 1 and 2.

Subjects were studied while comfortably sitting on a reclining chair, with their head stabilized, in front of a 17-inch monitor. They kept their forearms resting on armchairs, with their right index finger resting between two buttons spaced 6 cm apart. Ten minutes of training, performed with a different set of pictures, allowed the subjects to practice the task, and with both sham or active rTMS prior to the actual experimental session.

Experimental conditions

The memory task was the same previously utilized in EEG (Babiloni *et al.*, 2004, 2006) and rTMS (Rossi *et al.*, 2001, 2004) event-related experimental settings. Briefly (see Fig. 1a), the 'indoor–outdoor paradigm' included six blocks of encoding followed by six blocks of

TABLE 1. Demographics of subjects who received rTMS on prefrontal and parietal cortex

	PFC (n = 10)	PC-90% (n = 10)	PC-120% (n = 10)	Test applied	*P*-value
Age (years)	28.7; 2.3	27.3; 1.9	28.6; 1.5	ANOVA	> 0.20
Gender (F and M)	3F and 7M	4F and 6M	4F and 6M	Chi-squared	> 0.20
Handedness (median)*	93%	90%	90%	Kruskal–Wallis	> 0.20
Handedness (range)*	50–100%	45–100%	50–100%		

Group PFC, subjects who received rTMS on the dorsolateral prefrontal cortex; groups PC, subjects who received rTMS on the parietal cortex at 90% or at 120% of individual motor thresholds. *Values are median and range percentages of the Oldfield's score. Ages are given as means ± SD.

a) Experimental design

Blocks	rTMS in Encoding		rTMS in Retrieval
	1 hour		
R-Enc	right IPS		no
L-Enc	left IPS		no
Sham	left IPS		right IPS
R-Ret	no		right IPS
L-Ret	no		left IPS
Baseline	no		no

b) Experimental timing

go
cue
warning ⊞ = 500 ms of 20 Hz rTMS
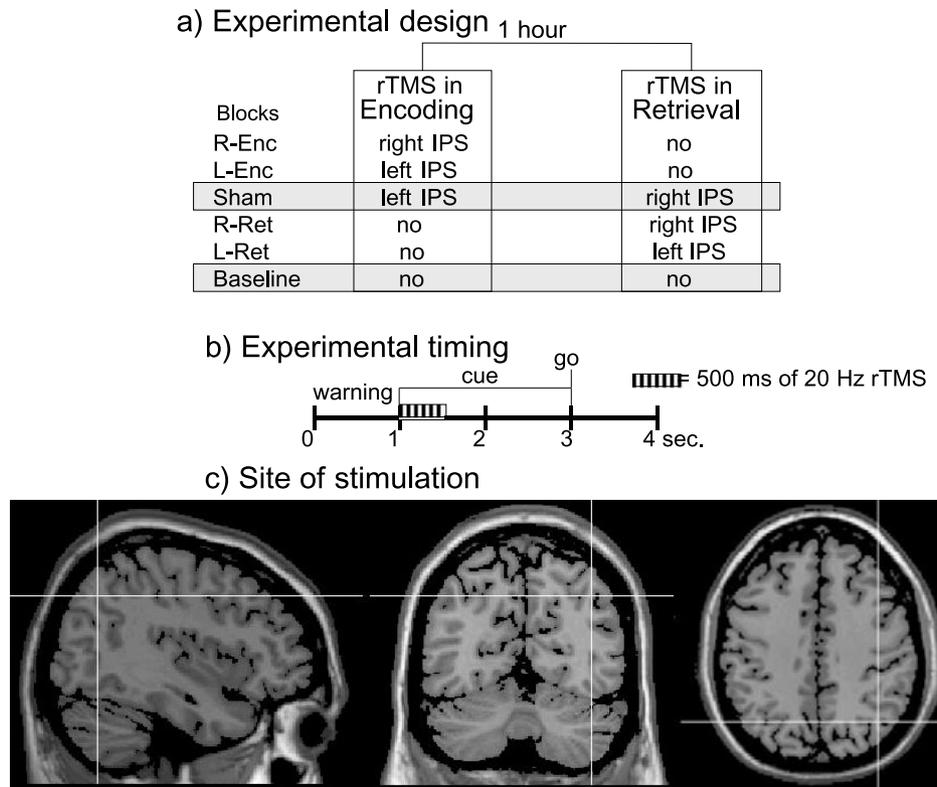
0   1   2   3   4 sec.

c) Site of stimulation

FIG. 1. Experimental details. (a) The experimental conditions. The six encoding/retrieval blocks were: R-Enc, right rTMS in encoding, no stimulation in retrieval; L-Enc, left rTMS in encoding, no stimulation in retrieval; Sham, sham rTMS (left in encoding and right in retrieval); R-Ret, right rTMS in retrieval, no stimulation in encoding; L-Ret, left rTMS in retrieval, no stimulation in encoding; Baseline, absence of stimulation. The shaded rectangles contain the 'reference' blocks (i.e. Sham and Baseline). The same block design was used for subjects who received rTMS at parietal and prefrontal sites. (b) Timing of warning, cue and go stimuli and of the rTMS train with respect to the cue. (c) Site of stimulation. MRI of a subject showing the sagittal (left), coronal (middle) and axial (right) projections on the cortical surfaces of stimulation sites in the right (P4) hemisphere, corresponding to the intraparietal suclus [Talaraich coordinates of P4 were: 40, −62, 40; Talaraich coordinates of the homologous right stimulation site P3 (not shown) were: −43, −64, 39]. The intercept of the coordinates (white lines) corresponded to BA 39 bilaterally.

retrieval, pseudo-randomized and counterbalanced between subjects as order of presentation. For each block of the encoding phase, 16 complex coloured magazine pictures (eight indoor and eight outdoor scenes) were randomly presented on the monitor for 2 s, with an intertrial interval of 10 s. Images were preceded by a visual warning stimulus (a red spot lasting 1 s). Subjects were instructed to press, with their right index finger, one of the two buttons (left, indoor; right, outdoor) after the presentation of a green circle in the middle of each picture (the 'go' signal), which appeared 1 s after the picture presentation.

The six encoding blocks had six corresponding retrieval blocks (1 h later), each containing 16 pictures of indoor scenes randomly presented: eight of them had been seen previously (tests) and eight were novel (distractors). The timing of warning and go signals, picture presentation and intervals were as for the encoding. Subjects were again asked, in a yes–no recognition task, to answer by pressing one of the two buttons (left, 'old' or test; right, distractor) after the go stimulus. Thus, the protocol excluded an overt working memory load, as pictures were presented on the screen until the subject's response. Subjects were familiarized with the task (with a different set of pictures) and with left and right rTMS (both active and sham) for about 10 min before starting the actual experiment. The six encoding/retrieval blocks were labelled according to the type (active or sham) and the side (left/right) of the rTMS applied on the PCs (see Fig. 1 for the complete labelling of the experimental conditions).

Stimulation and identification of the target regions

rTMS was delivered through a focal, figure-of-eight coil (outer diameter of each wing 7 cm) connected with a standard Mag-Stim Super Rapid stimulator (maximum output 2.2 T). As previously detailed (Rossi et al., 2001, 2004), individual resting excitability thresholds for left and right motor cortex stimulation were first determined by using the same coil and stimulator; afterwards, the intensity was reduced by 10%.

Left and right PCs were stimulated by placing the anterior end of the junction of the two coil wings on P3 or P4 (10–20 international EEG system), respectively. A mechanical arm maintained the handle of the coil angled backward at about 45° away from the midline. This position was marked on a transparent bathing cuff firmly adherent to the scalp. This allowed the correct repositioning of the coil prior to the appearance of each warning signal.

P3 and P4 locations on the subject's scalp were automatically identified using the SofTaxic Navigator system, on the basis of digitized skull landmarks (nasion, inion and two pre-auricular points) and about 40 scalp points (Fastrak Polhemus digitizer). Individual magentic resonance images were available for three subjects (see Fig. 1c); in the remaining subjects, Talairach coordinates of cortical sites underlying P3 and P4 locations were automatically estimated by the SofTaxic Navigator Stereotaxic Navigator System (E.M.S. Italy, http://www.emsmedical.net), on the basis of an MRI-constructed

stereotaxic template (estimated accuracy of ± 0.5 cm, Talairach space). These target regions (corresponding to the scalp projection of left and right intraparietal suclus) were chosen on the basis of independent neuroimaging studies that consistently showed an engagement of such brain areas in episodic encoding and retrieval (see Wagner *et al.*, 2005).

Trains of rTMS (90% of individual motor threshold, 500 ms, 20 Hz) were delivered – when required by the experimental event-related design – simultaneously with picture presentation (Fig. 1b). The same intensity and timing of rTMS was used for sham stimulation. In this case, the coil was still centred on P3 and P4, but it was held perpendicular to the scalp surface, so that scalp contact and discharging noise were quite similar to those for active stimulation, but the induced magnetic field did not activate cortical neurons.

This procedure was used for the PC-rTMS group, and was exactly the same of that used in the retrospectively selected subjects who formed the PFC-rTMS group (Rossi *et al.*, 2001, 2004).

Control experiment 1

In order to test whether rTMS of the PCs had intensity-dependent effects on memory performance, an additional group of ten healthy subjects (again matched for age, gender, handedness and basal memory performance in the Baseline condition) was tested (see Table 1 for demographics). These subjects (or 'PC-rTMS-120% group') received rTMS on the same scalp sites, at the same frequency, with the same experimental timing, but with an intensity of stimulation of 120% of the individual motor threshold.

Control experiment 2

In order to test whether the applied rTMS train (with the lower intensity, at 90% of the individual threshold) impaired specific processes of visuospatial attention, an additional 12 subjects (five males) were studied. These were again matched for age (mean 28.5 years, SD 1.6 years), handedness (Oldfield score 60–100%, mean 93%) and scholarity with those included in the other groups. Their sight was normal or corrected-to-normal.

Experimental task

The subjects kept their forearms resting on the armchairs, with the right index finger resting between two buttons of a mouse, connected to a computer monitor placed in front of them at a distance of about 100 cm. The cue stimulus was a white circle (diameter of about 0.5° of visual angle) appearing at 6° right or left of the background central white cross (diameter of 0.5°). The cue stimulus was preceded and followed by a masking visual stimulus formed by two 'Xs' (about 0.8°), located at 6° right and left of the central white cross. The sequence of the visual stimuli was as follows: (i) the masking stimulus 'Xs' lasting 5.5 s; (ii) the cue stimulus 'small circle' appearing on the right or left (50%) monitor side for 500 ms; (iii) the masking stimulus 'Xs' lasting about 2 s; (iv) a go (target) stimulus lasting about 500 ms. The go stimulus was a small green circle with a diameter of about 0.5°, which appeared at 6° right or left of the central white cross. In 80% of the cases the go stimulus was given to the same side of the cue stimulus (valid trials) (see Posner *et al.*, 1980).

The subjects had to press the left (or right) mouse button if the go stimulus appeared on the left (or right) monitor side. The computer receiving the mouse inputs registered the corresponding reaction time (RT) and the side of the button pressed.

The rTMS train, delivered during the cue stimulus presentation, was the same as used for the encoding/retrieval protocols (20 Hz, 500 ms,

90% of the individual motor threshold) and was applied over the same left or right parietal areas (P3 or P4 of the international EEG 10–20 montage). The same coil orientation was used. As a control, this paradigm was replicated using a left or right sham stimulation, as previously described. Each of the four sessions (active and sham rTMS on P3 and P4) included four blocks of 25 trials. For the statistical analysis we averaged for each subject the RT during all valid trials.

Data analysis

Main experiments and control experiment 1

Between-groups demographic characteristics were evaluated by ANOVA (age), chi-squared (gender) and Kruskal–Wallis (handedness). To analyse performance data, ANOVA for repeated measures was applied to hits–false alarms, instead of d′ and C, as previously done (Rossi *et al.*, 2001). This because we observed that many subjects of the present study did not make any false alarms or any recognition error in baseline and sham conditions. In this case, C and d′ can be computed only assuming a small proportion of errors, in order to avoid $\pm$ infinite values. This proportion should be arbitrarily small and the choice could affect the results.

A first analysis addressed the differences between the two groups of subjects (i.e. those who received rTMS on the DLPFCs and on the PCs at 90% of the individual threshold) across the six experimental conditions. This was done by means of a two-way ANOVA (Condition as within-subjects factor, Site of stimulation as between-subjects factor) with Greenhouse–Geisser corrections when necessary, and followed by planned comparisons specifically addressing the differences of L-Enc and R-Ret conditions between the PFC-rTMS and PC-rTMS groups.

A second analysis, addressing specifically the HERA model and its relationships with the site of stimulation, was based on a three-way ANOVA for repeated measures with Hemisphere (right vs. left) and rTMS Stimulation (delivered in encoding vs. retrieval) as within-subjects factors and Site of stimulation (PFC vs. PC) as between-subjects factor. Finally, ANOVA was applied to compare the cognitive performance between the PC-rTMS and PC-rTMS-120% groups.

Control experiment 2

The Reaction Time (dependent variable) was analysed by the ANOVA for repeated measures. Mauchley's test, evaluating the sphericity assumption and correction of the degrees of freedom, was used following the Greenhouse–Geisser procedure. Duncan's test was used for *post-hoc* comparisons ($P < 0.05$). The ANOVA design included the factors Hemisphere (left, right) and Condition (Sham, Active).

Results

No side-effects of stimulation were reported. rTMS, even when delivered on parietal sites at 120% of the individual motor threshold, did not evoke visible muscular responses in any subject.

Because age was very similar in the two groups (Table 1) and no subject was older than 40 years [when functional asymmetry was demonstrated to be age-dependent (Rossi *et al.*, 2004)], its effect was not taken into account.

Table 2 summarizes behavioural results (i.e. hits and hits–false alarms) in the subjects studied. The ANOVA with 'Condition' (six levels) and 'Site of stimulation' (two levels) showed a significant interaction ($F_{4.1,74.6} = 2.731$, $P = 0.034$), indicating the presence of

TABLE 2. Percentage of hits and false alarms in the different experimental conditions for the three groups of subjects

Experimental blocks	Hits (%)			Hits (%) − False alarms (%)		
	PFC ($n = 10$)	PC-90% ($n = 10$)	PC-120% ($n = 10$)	PFC ($n = 10$)	PC-90% ($n = 10$)	PC-120% ($n = 10$)
R-Enc	68 ± 21	53 ± 26	54 ± 20	43 ± 18	39 ± 23	19 ± 24
L-Enc	48 ± 25	51 ± 25	50 ± 13	30 ± 17	41 ± 25	15 ± 28
Sham	79 ± 17	71 ± 26	68 ± 14	50 ± 22	49 ± 21	34 ± 19
R-Ret	49 ± 24	65 ± 23	58 ± 20	13 ± 16	44 ± 28	28 ± 40
L-Ret	74 ± 25	61 ± 24	59 ± 18	46 ± 24	40 ± 28	24 ± 15
Baseline	66 ± 26	61 ± 27	73 ± 19	48 ± 28	46 ± 30	39 ± 18

Data are given as means ± SD. Group PFC, subjects who received rTMS on the dorsolateral prefrontal cortex; groups PC, subjects who received rTMS on the parietal cortex at 90% or at 120% of individual motor thresholds. The six encoding/retrieval blocks were: R-Enc, right rTMS in encoding, no stimulation in retrieval; L-Enc, left rTMS in encoding, no stimulation in retrieval; Sham, sham rTMS (left in encoding and right in retrieval); R-Ret, right rTMS in retrieval, no stimulation in encoding; L-Ret, left rTMS in retrieval, no stimulation in encoding; Baseline, absence of stimulation.
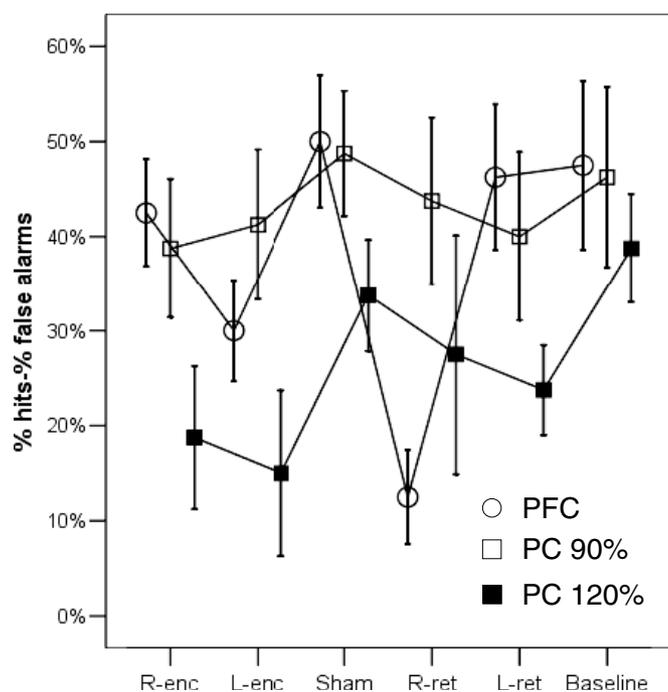

FIG. 2. Summary of the behavioural results (percentage of hits − percentage of false alarms; ± 1 SEM) in the two groups of subjects who were the core of the study (i.e. PFC-rTMS and PC-rTMS at 90% of motor threshold) across the six experimental conditions. The additional values (black squares) refer to subjects who received PC-rTMS at higher intensity. Note the similar performance in baseline (for the three groups) and sham blocks (for PFC-rTMS and PC 90%-rTMS). Prefrontal cortex rTMS worsened behavioural performance more than PC-rTMS (at both intensities) in R-Ret and, to a lesser extent, in L-Enc vs. PC 90%-rTMS.

FIG. 3. The HERA (Hemispheric Encoding Retrieval Asymmetry) pattern, which is clearly evident in the PFC-rTMS group, is absent in the PC-rTMS group.

a lack of parallelism of the two profiles across conditions (Fig. 2). This was due to the strongly significant 'Condition' effect in the PFC-rTMS group ($F_{3.1,27.7} = 5.454$, $P = 0.004$) and to the null effect in the PC-rTMS group ($F_{3.0,27.4} = 0.413$, $P = 0.748$). The matching procedure based on behavioural performance in baseline condition (resulting in a P-value of 0.925) also produced an overlap in terms of hits–false alarm rates in the sham condition ($P = 0.898$). The other planned comparisons between the two groups indicated that the only significant difference was in the R-Ret condition ($P = 0.006$).
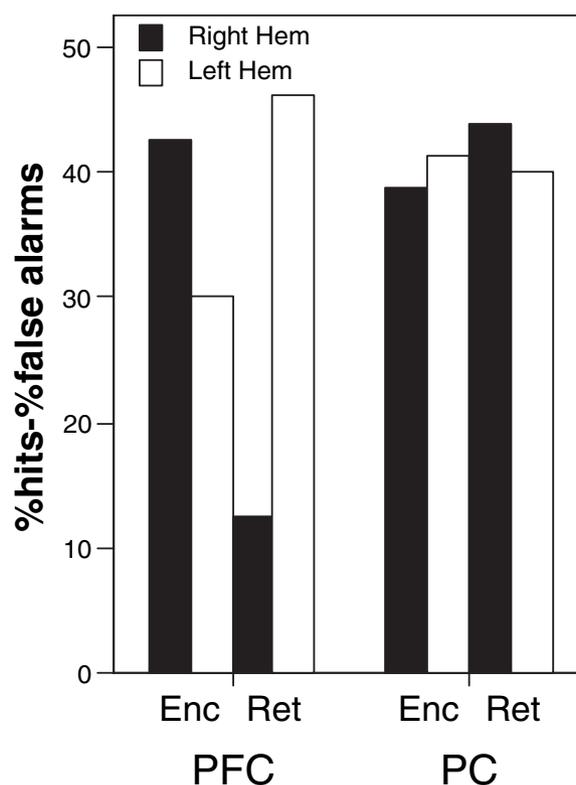
Focusing the ANOVA on the HERA model, a significant triple interaction occurred ($F_{1,18} = 8.418$, $P = 0.010$). This finding is the result of the HERA pattern in the sample drawn from the population that received rTMS on the PFC ($F_{1,9} = 23.65$, $P = 0.001$), and of the lack of such asymmetry in the PC-rTMS group ($F_{1,9} = 0.165$, $P = 0.694$) (Fig. 3).

In control experiment 1, a close descriptive inspection of recognition performance in R-Ret showed more variable effects (i.e. higher standard deviations of hits–false alarms) than that observed in the PC-rTMS group (Table 2, Fig. 2). However, ANOVA indicated that the performance was significantly lower in the PC-rTMS-120% group
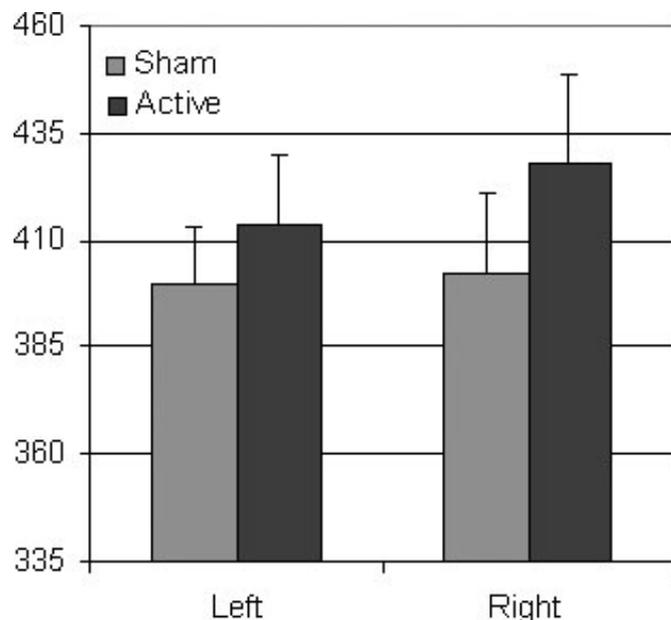
FIG. 4. Graphical representation of the RTs in control experiment 2 (12 subjects pooled), based on the Posner's test of visuospatial attention. Active rTMS delivered on the left (mean 414 ms, SEM 15.7 ms) or right (mean 428 ms, SEM 20.1 ms) IPS increases reaction times more than the corresponding sham stimulation (left: mean 399 ms, SEM 13.9 ms; right: 402 ms, SEM 18.8 ms). Rection times are significantly more delayed by active right rTMS than active left stimulation. Statistics are given in the text.

($F_{1,18} = 5.960$, $P = 0.025$), with the exception of the baseline block in which no stimulation occurred, but with profiles across the other conditions substantially parallel in the two groups ($F_{5,90} = 0.379$, $P = 0.862$). Again, any significant differences between conditions were apparent. This overall non-specific memory impairment, including the sham condition, suggests that the specific left and right rTMS effects on encoding and retrieval, respectively, are not intensity-dependent.

In control experiment 2, ANOVA analysis of the RTs showed a statistically significant interaction ($F_{1,11} = 5,27$, $P < 0.0424$) among all factors such as Hemisphere (left, right), and Condition (active, sham). *Post-hoc* comparisons (Fig. 4) showed that both left and right active rTMS slowed RTs with respect to sham stimulation ($P < 0.002$). Furthermore, the RTs evaluated during the right active stimulations were longer than during the left stimulation ($P < 0.006$).

Discussion

The current investigation was aimed to address directly, using rTMS, the functional role of a subregion of the PCs (i.e. the intraparietal sulcus) during episodic encoding and retrieval of visual scenes. The lack of significant specific recognition errors induced by the event-related rTMS interference suggests that the functional relevance of these left and right PC subregions, differently from that of DLPFCs, is poorly correlated with a successful completion of the episodic memory task, given that neither encoding nor retrieval mechanisms were significantly disrupted by left or right PC-rTMS. These negative results appear to be robust, as specific behavioural changes both in encoding and in retrieval were lacking even when the interference with rTMS was carried out with a higher intensity of stimulation. Indeed, increasing the intensity of rTMS (i.e. control experiment 1) produced

an overall non-specific memory impairment (see Table 2 and Fig. 2, hits–false alarms), which was similar in all experimental conditions, including the sham. This suggests a non-specific interference effect of rTMS on the demanding cognitive task, probably linked to a merely distraction mechanism resulting from high discharge noise.

Conversely, the same low-intensity rTMS train (i.e. 90% of the motor threshold) of the PC group, delivered on the same scalp regions, was clearly able specifically and asymmetrically to interfere with RTs in the context of a purely visuospatial attentive task (Posner *et al.*, 1980).

Before discussing the possible implications of the present results and their possible relevance for our current knowledge of the physiological mechanisms underlying episodic memory, the following methodological key points should be taken into account:

1 A similar event-related rTMS interference approach, using the same set of memoranda and carried out on a fully matched sample of subjects, was successfully utilized to demonstrate directly the functional relevance of DLPFCs in episodic memory performance, both in young adults and in normal ageing (Rossi *et al.*, 2001, 2004).

2 As a general rule for event-related rTMS studies, scalp stimulation sites for the intraparietal sulcus were chosen on the basis of independent neuroimaging studies (Walsh & Pascual-Leone, 2003), which provide the best available spatial resolution to detect eloquent regions for a given task. Indeed, brain activity in these PC regions had been previously found to be engaged consistently in episodic encoding and retrieval (Mottaghy *et al.*, 1999; Cabeza *et al.*, 2003; Fujii *et al.*, 2004; Hayes *et al.*, 2004; Mayes *et al.*, 2004; Wagner *et al.*, 2005).

3 The accuracy of TMS coil positioning (SofTax system) can be estimated to be of the order of less than 1 cm (Herwig *et al.*, 2001). Nevertheless, the high intensity of stimulation used in the PC-rTMS-120% group strongly minimizes the possibility of an insufficient interference effect due to a mismatch between the scalp site and the underlying anatomy. We should be aware, however, that both the spatial selectivity and the intimate mechanisms by which rTMS works are still not wholly understood (see Walsh & Pascual-Leone, 2003; Rossi & Rossini, 2004).

4 The same scalp positions have been previously used in TMS studies, using intensities of stimulation similar to the lower one adopted in the present study (i.e. 90% of the individual motor threshold), to interfere with cognitive functions associated with activity of the intraparietal sulcus region, such as sensory perception (Oliveri *et al.*, 1999, 2000), mental rotation (Bestmann *et al.*, 2002), grasping (Tunik *et al.*, 2005) or visuospatial and verbal working memory (Oliveri *et al.*, 2001; Mottaghy *et al.*, 2002; Koch *et al.*, 2005). Moreover, results from control experiment 2 are in line with previous rTMS studies, which suggested that the right parietal lobe plays a significant role in purely visuospatial attentional processes (Walsh *et al.*, 1999; Hilgetag *et al.*, 2001).

5 According to previous studies on DLPFC function based on the same experimental timing and setting (Rossi *et al.*, 2001, 2004), the temporal extent to which the applied rTMS train is likely to be effective in disrupting encoding and retrieval processing is at least 1.5 s, i.e. the time including that of picture display and the following yes/no motor response. Such duration is therefore largely sufficient to include all the temporal dynamics of parietal activations, as determined from event-related potentials (ERP) studies, during similar memory tasks (i.e. old/new successful recollection), which is known to emerge approximately 400 ms post-stimulus onset and to last about 400–600 ms (see Allan *et al.*, 1998; Paller & Wagner, 2002).

Keeping in mind all the abovementioned points, it is very unlikely that the lack of significant detrimental effects induced by rTMS of the PCs on memory performance could be due to experimental or methodological biases. Note that the same rTMS train used for encoding/retrieval protocols was able to interfere with a purely visuospatial attentional task in the control experiment 2.

The question of whether the PCs play a relevant role in episodic memory operations is still open. The present rTMS findings seem to challenge the results of recent neuroimaging and electrophysiological studies, which detected metabolic/cerebral blood flow increases (Henson *et al.*, 1999; Mottaghy *et al.*, 1999; Konishi *et al.*, 2000; Fujii *et al.*, 2004; Hayes *et al.*, 2004; Mayes *et al.*, 2004) and the presence of gamma EEG oscillations (Babiloni *et al.*, 2004) in specific PC regions during successful episodic retrieval. The differential sensitivity of neuroimaging methods to the diverse components of long-term episodic memory processes, as well as differences in experimental material and settings, can plausibly at least in part explain such discrepancies.

The present findings cannot offer definite proof as to why the stimulated PC subregions do not participate to a useful degree in encoding and retrieval processes in this experimental setting. However, it should be considered that the correlational nature of neuroimaging and electrophysiological findings leaves open the possibility that such activations could also be due to additional brain processes that are simply associated with, but are not crucial for, the memory challenge. Of note, it has been recently suggested (Cabeza *et al.*, 2003) that several brain regions including PFC, parietal, anterior cingulate and thalamic regions, known to be critical components of a distributed brain network for attention (Mesulam, 1990; Posner & Petersen, 1990), become active during episodic retrieval. Such widespread attentional processes, which are compatible with the post-retrieval monitoring hypothesis (Cabeza *et al.*, 2003), can be reasonably translated to the encoding phase, which also requires attention to – and successive elaboration of – the presented memoranda. Therefore, the parietal activations accompanying episodic memorization processes could reflect the engagement of attentional networks during encoding and retrieval. Interference with a single 'node' of the network, although sufficient to interfere with a spatial attention task (i.e. control experiment 2), does not result in the overt disruption of performance in the visual memory task, in which an overall analysis of a visual scene is required, rather than selective attention to the details. Anticipating the timing of rTMS with respect to memoranda presentation might address this point more directly, by considering that studies on memory-guided saccades (i.e. a reflection of spatial memory) suggest that the posterior PC is involved at a very initial stage (i.e. less than 300 ms) of visuospatial integration (Pierrot-Deseilligny *et al.*, 2002).

The current results, however, do not exclude that other regions located lateral and posterior to the intraparietal sulcus (see Wheeler & Buckner, 2004), such as the precuneus, could actively participate in episodic retrieval, as suggested by previous neuroimaging studies (Buckner *et al.*, 1996; Krause *et al.*, 1999; Mottaghy *et al.*, 1999; Cabeza & Nyberg, 2000; Kohler *et al.*, 2002; Cabeza *et al.*, 2003; Mayes *et al.*, 2004; Wheeler & Buckner, 2004). It is unlikely that the interference of focal rTMS delivered on the scalp overlying the intraparietal sulcus would spread to such relatively distant sites. This is strongly supported by the fact that previous studies demonstrated distinct neuophysiological and behavioural effects of rTMS applied to even closer cortical regions, such as primary motor and premotor areas (Munchau *et al.*, 2002; Cincotta *et al.*, 2004) or primary and non-primary visual cortical areas (i.e. V1 and V5, Silvanto *et al.*, 2005).

Another, not mutually exclusive, hypothesis that can be taken into account to explain the absence of significant rTMS interference of the stimulated region(s) (i.e. the left and right intraparietal sulcus) on encoding and retrieval mechanisms is that these areas could actually directly participate in episodic learning and memory, but at a lower level and in a complex, hierarchically organized, multinode neural network, in which medial temporal lobe structures (Cabeza *et al.*, 2003) and PFCs (Babiloni *et al.*, 2006) play a leading role. Should this be the case, then it is not surprising that interference rTMS effects of PCs are not detrimental to memory performance, as the function of the main nodes of the whole network remain free from interference. However, the lack of evidence for overt detrimental effects of parietal lesions on episodic memory militates against this interpretation.

In conclusion, the present findings suggest that activity of intraparietal sulcus, unlike that of DLPFC, does not subserve at a useful degree memory encoding and retrieval, at least under these specific experimental conditions, while it plays a causal role in purely visuospatial attentive processes. It can be hypothesized that PC activity accompanying episodic memory (as revealed by positron emission tomography, functional magnetic resonance and EEG) could be associated with parallel and more complex attentional and visuospatial integrative processes and that regional rTMS interference could be compensated for by other nodes of the distributed attentional system.

Moreover, complex cognitive phenomena – as in the case of episodic memory – provide a fitting example as to how underlying physiological mechanisms cannot be fully disclosed by investigations based on a single technique. Future studies on episodic learning and memory will take advantage of a true multimodal approach to the working brain, with the aim of combining the relative advantages provided by cerebral blood flow-based neuroimaging techniques, sush as positron emission tomography or functional magnetic resonance (i.e. spatial details), by electrophysiological methods, such as EEG and ERPs (i.e. temporal resolution) and by the behavioural interference of rTMS (to prove causal relationships).

Abbreviations

DLPFC, dorsolateral prefrontal cortex; EEG, electroencephalography; ERP, event-related potentials; HERA, Hemispheric Encoding Retrieval Asymmetry; PC, parietal cortex; PFC, prefrontal cortex; RT, reaction time; rTMS, repetitive transcranial magnetic stimulation.

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