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The Neural Bases of Word Encoding and Retrieval: A fMRI-Guided Transcranial Magnetic Stimulation Study

**R. Manenti · M. Tettamanti · M. Cotelli ·
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Abstract There is evidence that the human prefrontal cortex is asymmetrically involved in long-term episodic memory processing. Moreover, abstract and concrete words processing has been reported to differentially involve prefrontal and parietal areas. We implemented a two-stages functional magnetic resonance imaging (fMRI)–repetitive transcranial magnetic stimulation (rTMS) paradigm to investigate the role of the dorsolateral prefrontal cortices (DLPFCs) and parietal cortices (PARCs) in encoding and retrieval of abstract and concrete words. Using this paradigm we could select areas to be stimulated on the basis of single-subject (SS) anatomical and functional data, investigating the usefulness of this integration approach. With respect to fMRI, abstract and concrete words differed only for a greater left fusiform gyrus activation for concrete words. In turn, significant rTMS effects were found, but only for the retrieval of abstract words. Consistent with previous findings, repetitive stimulation of the right DLPFC had a specific impact on episodic retrieval. Memory retrieval performance was also disrupted when rTMS was applied to the left PARC. Finally, we found a significant positive correlation between the effect sizes of SS right PARC activations for abstract word retrieval and the consequent rTMS interference effects. Taken together these data provide for the first time evidence that also the PARC has a necessary role in episodic retrieval of abstract words. Importantly, from a methodological perspective, our data demonstrate that fMRI-guided rTMS with a SS approach provides a powerful tool to investigate the neural underpinnings of cognitive functions.

Keywords rTMS · Single-subject · Memory · Combining · Youngs

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Introduction

While functional magnetic resonance imaging (fMRI) data reveal the correlations existing between brain functions and behavior, transcranial magnetic stimulation (TMS) is a complementary technique that can address hypotheses about the necessity of one or more brain areas to a particular aspect of cognitive performance (Walsh and Pascual-Leone 2003), and it has been extensively used to map the flow of information across different brain regions during the execution of a cognitive task (Walsh and Rushworth 1999). The use of combined TMS–fMRI has been therefore receiving a growing interest over the last decade, with the development of both an online (TMS applied at the same time of fMRI acquisition) and an offline (TMS and fMRI separated in time) approach. fMRI

can be followed by rTMS and this type of combination might be particularly promising for the study of memory and other cognitive functions, in which there is high interindividual anatomo-functional variability. By submitting each experimental subject to a fMRI investigation before rTMS, it becomes possible to define the anatomical site of rTMS on the subject-specific (SS) activations rather than on the mean group activations. The importance of this aspect has been confirmed by a recent methodological study that highlighted an elevated discrepancy between the effects induced by rTMS in cognitive tasks according to target area selection (Sack et al. 2009). Sparing et al. addressed this question by evaluating the accuracy and efficiency of different localization strategies for the primary motor cortex and found the highest accuracy with SS fMRI-guided stimulation (Sparing et al. 2008). Coil positioning becomes even more important when applying TMS to "silent" areas (i.e., areas in which the stimulation does not induce a visible behavioral effect, such as muscle contraction) that are typically involved in higher-order cognitive functions, including memory.

In the last few years a number of studies have been conducted to study memory using fMRI-guided stimulation. Herwig et al. (2003a) used for the first time a combined fMRI and rTMS approach to study the functional role of premotor and parietal areas during phonological rehearsal. The results of this study showed that the premotor cortex was the only necessary brain region for rehearsal, probably because of its implication in top–down control processes (Herwig et al. 2003a). More recently, Osaka et al. (2007) underlined the critical importance of the left Brodmann area 9 in a reading span test. The combined experimental approach has also been systematically applied by another research group to the study of working memory (Feredoes and Postle 2007; Feredoes et al. 2006, 2007; Postle et al. 2006). These series of studies showed that only the stimulation of SS loci yielded an accuracy effect (Feredoes and Postle 2007; Feredoes et al. 2007).

The fMRI literature on long term memory emphasized the functional asymmetry of the frontal lobes. The HERA (Hemispherical Encoding Retrieval Asymmetry) model was based on the observation of the predominance of left prefrontal cortex activation during encoding and of the prevalence of right prefrontal cortex activation during retrieval (Nyberg et al. 2000; Tulving et al. 1994). In the last few years, it has become evident that the HERA model may be an oversimplification, as both the nature of the encoded material and the type of task also need to be taken into account (Kelley et al. 1998; Wagner et al. 1998a; Wagner et al. 1998b). Furthermore, differences between encoding and retrieval are not restricted to the prefrontal cortex. Neuroimaging studies have demonstrated the involvement of a distributed neural network constituted by the dorsolateral prefrontal cortices (DLPFCs), the medial temporal lobes, the parietal cortices (PARCs), and the precuneus (Buckner and Wheeler 2001; Cabeza et al. 2003; Cabeza and Nyberg 2003; Fletcher and Henson 2001; Rugg and Wilding 2000; Simons and Spiers 2003; Wagner et al. 1998a).

With respect to the nature of the encoded material, a number of fMRI studies have shown different activations for abstract and concrete words during episodic memory tasks. A recent fMRI study analyzed the concreteness effect during both encoding and recognition, and highlighted a greater effect of stimulus type compared to the effect of process (Fliessbach et al. 2006). The intentional encoding of abstract words compared to concrete words was associated to a stronger activation of the left IFG, while the recognition of concrete words compared to abstract words was associated to a stronger activation of the bilateral inferior parietal cortex and of the angular gyrus (Fliessbach et al. 2006). The authors concluded that the parietal areas showing a concreteness effect were more engaged during the recognition task than during the encoding task because of their role in item identification (Weis et al. 2004).

The first study based on the "interference" approach with repetitive TMS (rTMS) to assess the prefrontal cortex functional asymmetries during encoding and retrieval was conducted by Rossi et al. (2001). During encoding, subjects were asked to discriminate between complex coloured pictures (indoor–outdoor), whereas during retrieval subjects had to recognize the previously seen pictures from new ones. The right DLPFC was found to be crucial for the retrieval of the encoded pictorial information, whereas the left DLPFC was specifically involved during encoding. Moreover, Sandrini et al. (2003) tested the influence of the material by studying the encoding and the retrieval of words, and found that the encoding of verbal material was disrupted by both right and left prefrontal cortex stimulation, whereas the retrieval was disrupted by right prefrontal cortex stimulation. In conclusion, rTMS data concur with neuroimaging data, in indicating that both the nature of the material and the type of memory process may affect the lateralization of frontal activation during memory tasks (Fletcher and Henson 2001). With respect to posterior brain areas, in a more recent work, Rossi et al. (2006) investigated the functional role of the parietal cortex in encoding and retrieval, and found that the activity of the intraparietal sulci, unlike that of the DLPFC, is not causally engaged in the encoding and retrieval of visual scenes. The authors suggested that parietal activations accompanying the memorization processes reflect the engagement of a widespread brain attentional network (Rossi et al. 2006).

The main goal of our study was to adopt the SS combined experimental approach, consisting in an fMRI-based target area selection on an individual basis followed by rTMS, to verify the usefulness of this approach in the study of cognitive functions and, in particular, in the assessment of the causal role of prefrontal and parietal areas in memory encoding and retrieval of abstract and concrete words.

Materials and Methods

Subjects

A group of 11 subjects [mean age = 30 years (range: 25–40); mean education = 18.5 years (range: 18–22)] participated in the experiment. All subjects were native Italian speakers and had normal or corrected-to-normal visual acuity. Participants reported being free of neurological disorders or history of seizures. All were right handed, with a mean score on the Edinburgh Handedness Inventory (Oldfield 1971) of +88% (range = + 44 to +100%). Participants were informed about the procedures and the possible risks of rTMS and informed consent was obtained after a safety screening. The experimental methods had ethical approval from the locals Human Ethics Committees (Ethics Committee of the San Raffaele Scientific Institute, Milano, Italy and Ethics Committee of the IRCCS San Giovanni di Dio Fatebenefratelli, Brescia, Italy). Each subject first underwent an fMRI investigation, followed by rTMS.

Stimuli

For the encoding condition, a total of 90 abstract words and 90 concrete words were selected from the "Corpus e Lessico di Frequenza dell'Italiano Scritto (CoLFIS)" (Laudanna et al. 1995). For the retrieval condition, we further selected 45 abstract and 45 concrete "new" words. The retrieval word list thus consisted of half of the old words (45 concrete, 45 abstract) and 90 new words (45 concrete, 45 abstract). The mean word length was 6.4 (±1.5) letters, and 2.7 (±0.6) syllables. Abstract and concrete words were balanced for word length and for variables known to influence memory performance, i.e., word frequency (mean ± standard deviation = 59 ± 20) and familiarity (mean ± standard deviation = 6.0 ± 0.7). There were significant differences with respect to concreteness (concrete = 6.0; abstract = 3.6; $t(1, 99) = 29.47$, $P < 0.05$) and imageability (concrete = 5.7; abstract = 3.3; $t(1, 99) = 30.89$, $P < 0.05$) based on CoLFIS (Laudanna et al. 1995).
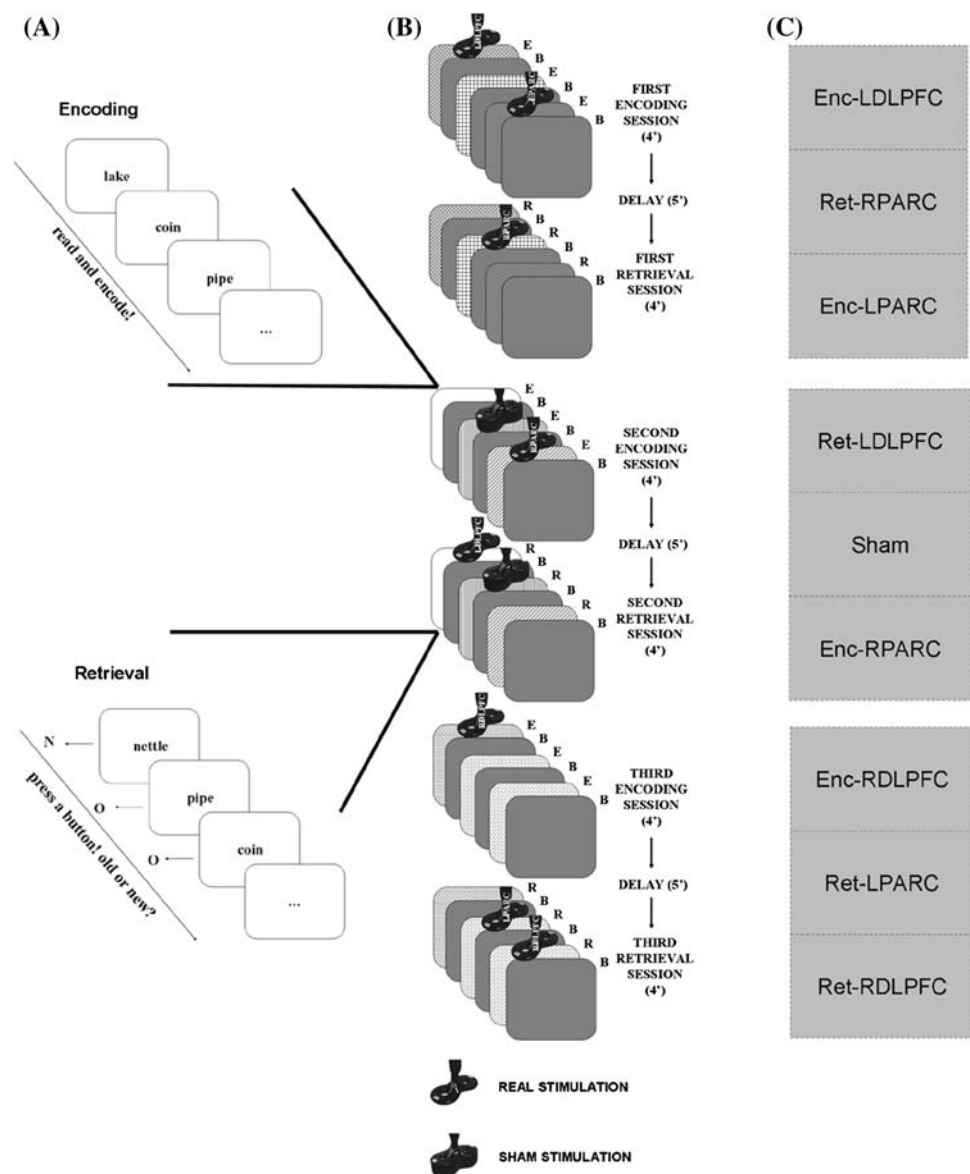
In total, both the encoding and the retrieval word lists included 180 words. Each word list (encoding and retrieval) was then divided into 9 blocks of concrete words and 9 blocks of abstract words, each block consisting of ten words. Each encoding block was associated with one retrieval block containing an equal number of "new" and "old" words in randomized order. Each encoding block began with the instruction "Encode", while each retrieval block began with the instruction "Old or new?". The experimental paradigm also included a baseline condition, with one baseline block associated to each encoding or retrieval block. In the baseline blocks, the instruction "Count" was presented, followed by a cross remaining on the screen for the entire block duration: the subject were instructed to fixate the cross and to covertly count from one to ten in a non-stop mode. The baseline condition served both as a subtraction baseline for fMRI, and as a delay period between the stimulations of different cortical areas for rTMS. All blocks (encoding, retrieval or baseline) lasted for 30 s, preceded by 4 s of instructions.

The blocks were grouped into three encoding and three associated retrieval sessions for each word category (abstract or concrete). Each encoding session included 3 encoding and 3 baseline blocks, while each associated retrieval session comprised the 3 corresponding retrieval blocks and 3 baseline blocks. This behavioural paradigm, reflecting a 2 by 2 factorial combination of task (encoding or retrieval) and word category (abstract or concrete), resulted in four experimental conditions (encoding of abstract words: EncA; encoding of concrete words: EncC; retrieval of abstract words: RetA; retrieval of concrete words: RetC). Since each encoding or retrieval block was associated to one baseline block, four baseline conditions were also constructed, reflecting one level of a further two-levels experimental factor (experimental condition: memory or baseline). The four baseline conditions were formed by dividing the baseline blocks according to the session (encoding or retrieval of abstract or concrete words) in which they were included (baseline for encoding of abstract words: BasEncA; baseline for encoding of concrete words: BasEncC; baseline for retrieval of abstract words: BasRetA; baseline for retrieval of concrete words: BasRetC). This allowed us to treat the 4 baseline conditions as orthogonal data sources and to compute interaction and conjunction effects (see below). The three associated encoding and retrieval abstract sessions were presented consecutively followed (half of the subjects) or preceded (the other half of the subjects) by the three associated encoding and retrieval concrete sessions. Between each encoding and retrieval sessions, we included a 5 min delay to allow for working memory wash out and trace consolidation. During this delay, the subjects simply rested in the scanner listening to music (Fig. 1b).

Behavioural Task

The stimuli were presented using Presentation software (Version 10.3, www.neurobs.com). All stimuli were presented in black lower-case letters on a white background. During the fMRI phase, stimuli were projected from outside the magnet room onto a translucent screen placed at the foot of the magnet bore. A mirror attached to the top of the head coil allowed the participants to view the translucent screen from inside the magnet. During both the fMRI and rTMS studies, the subjects were told to read and encode the presented words. After a delay, they were asked to decide whether the presented word was from the previously encoded word list or not, by making a speeded decision via a two-choice button press, using the right and left forefingers (hands counterbalanced across subjects).

During both encoding and retrieval, each word was presented for 1500 ms in the centre of the screen and was followed by a 1500 ms delay (Fig. 1a).

At the end of the experimental phase (both during the fMRI and the rTMS phase) subjects were asked to fill in an "Encoding strategies questionnaire". This questionnaire comprised twelve possible strategies that could be used during the task and subjects had to assign a score from 1 to ten (1 = never, 10 = always) to each strategy according to how often they had used each strategy during the task. The 12 listed strategies were: (i) to use words' initials, (ii) to create sentences including some of the presented words, (iii) to imagine the pictures corresponding to the presented words, (iv) to repeat the words, (v) to create songs including some of the presented words, (vi) to create rhymes between the displayed words, (vii) to translate the

Fig. 1 Experimental design. a Experimental task: during encoding subjects had to read the presented words and were instructed to memorize them; during retrieval they had to decide if a word was old (O) or new (N). b fMRI and rTMS sessions: the displayed scheme was repeated twice (one time for each word category, i.e., abstract and concrete, during both fMRI and rTMS phase). Each encoding session was composed of 3 encoding blocks (E) alternating with 3 baseline blocks (B); the same pattern was also used for the retrieval sessions, with alternating retrieval blocks (R) and baseline blocks. Coil icons represent the sequence of rTMS stimulation conditions, reported for clarity in c, in the example of one of the experimental subjects. The combination between the kind of stimulation applied during the encoding and the one applied during the retrieval (see b) results in the experimental condition (see c). The words blocks were presented exactly in the same order in fMRI and rTMS experiments

words in a foreign language, (viii) to create associations of words, (ix) to create a brief story including the presented words, (x) to associate each word to a personal event, (xi) to classify each word as easy/difficult, abstract/concrete, positive/negative, etc., (xii) to imagine the words' sound, color, shape, etc.

fMRI Study

MRI Data Acquisition

MRI scans were acquired on a 3T Achieva Philips body scanner (Philips Medical Systems, Best, NL) using an 8 channels-sense head coil (sense reduction factor = 2). Whole-brain functional images were obtained with a T2*-weighted gradient echo, echo-planar sequence, using blood-oxygenation-level-dependent contrast. Each functional image comprised 30 contiguous axial slices (4 mm thick), acquired in interleaved mode, and with a repetition time of 2000 ms (echo time: 30 ms; field of view: 240 mm × 240 mm; matrix size: 128 × 128). The paradigm was a block-design each participant underwent 12 functional scanning sessions (half encoding sessions and half for concrete words). Baseline blocks were included in all sessions. The duration of each session was 110 scans, preceded by 10 dummy scans that were discarded prior to data analysis.

For anatomical localization of brain activations and precise coil positioning during rTMS phase, we acquired one high-resolution whole-brain structural T1 weighted scan (resolution 1 mm × 1 mm × 1 mm) of each participant. In order to maximize the coil localization accuracy, we acquired 200 slices, covering on average the entire brain and skull down to the midbrain. The normalized structural images of all participants were then averaged in one single image for visualization of group brain activations.

Data Analysis

Behavioural Data Behavioural data were analyzed evaluating both accuracy and reaction times (RTs) during the retrieval sessions. It is important to consider that the behavioural performance measured in this way may reflect the functioning during both the encoding and the associated retrieval sessions. For both accuracy and RTs data, we conducted a paired *t*-Student test between abstract and concrete words, verifying if, as predicted by the concreteness effect, concrete words were recognized better and faster than abstract words. We also calculated two-one-way ANOVAs (one for each word category) with the block as a factor, in order to exclude performance differences between blocks.

Functional MRI Data Statistical parametric mapping (SPM5, Wellcome Department of Imaging Neuroscience, London, UK) was used for image realignment (Andersson et al. 2001), normalization to the Montreal Neurological Institute (MNI) standard space (yielding normalization parameter files used for the inverse definition of individual rTMS loci in the subject native brain space, see below), smoothing by a 6 mm FWHM Gaussian kernel, and General Linear Model statistical analysis (Friston et al. 2002). We adopted both: (i) a fixed-effects single subject analysis, in order to identify individual stimulation loci; (ii) a two-stage random-effects group analysis approach, in order to identify group brain activations.

Fixed-effects Single Subject Analysis

At the first stage, the time series of each participant were high-pass filtered at 67 s and pre-whitened by means of an autoregressive model AR(1) (Friston et al. 2002). Global normalization was performed to account for the global between sessions effects confounding the comparisons between experimental conditions. For each participant, we modelled a 2 (experimental condition: memory or baseline) × (tasks: encoding or retrieval) × 2 (word category: abstract or concrete) factorial design with 12 separate sessions, reflecting conditions EncA, EncC, BasEncA, BasEncC, RetA, RetC, BasRetA, BasRetC. We then calculated effect-specific interaction contrasts and, for the purpose of the random effects group analysis only, a set of condition-specific contrasts, each contrast including a weight of one for a particular condition of interest and a weight of zero for all the other conditions. The *t*-Student effect-specific interaction contrasts included: (i) activations for encoding versus retrieval of abstract words: (EncA − BasEncA) −(RetA − BasRetA) inclusively masked by (EncA − BasEncA), (ii) activations for encoding versus retrieval of concrete words: (EncC − BasEncC) − (RetC − BasRetC) inclusively masked by (EncC − BasEncC), (iii) activations for retrieval versus encoding of abstract words: (RetA −BasRetA) − (EncA − BasEncA) inclusively masked by (RetA − BasRetA), and (iv) activations for retrieval versus encoding of concrete words: (RetC − BasRetC) −(EncC − BasEncC) inclusively masked by (RetC −BasRetC).

Second-level Random Effects Group Analysis Approach

At the second stage of analysis, the contrast images obtained at the SS level were used to compute a within-subjects one way ANOVA assessing their significance at the group-level (*n* = 11 participants). The ANOVA included the set of all first-level contrast images, one image per

participant, per experimental condition. The *t*-Student contrasts assessed at the second level included: (i) Main effect of abstract versus concrete words: (EncA + RetA) − (EncC + RetC); (ii) Main effect of concrete versus abstract words: (EncC + RetC)−(EncA + RetA); (iii) Main effect of encoding versus retrieval: (EncA + EncC) − (RetA + RetC); (iv) Main effect of retrieval versus encoding: (RetA + RetC) − (EncA + EncC); (v) Interaction effect assessing the activations for encoding versus retrieval of abstract words: (EncA − BasEncA) − (RetA − BasRetA) inclusively masked by (EncA − BasEncA), (vi) Interaction effect assessing the activations for encoding versus retrieval of concrete words: (EncC − BasEncC) − (RetC − BasRetC) inclusively masked by (EncC − BasEncC), (vii) Interaction effect assessing the activations for retrieval versus encoding of abstract words: (RetA − BasRetA) −(EncA − BasEncA) inclusively masked by (RetA − BasRetA), and (viii) Interaction effect assessing the activations for retrieval versus encoding of concrete words: (RetC − BasRetC) − (EncC − BasEncC) inclusively masked by (RetC − BasRetC). Two conjunction contrasts, reflecting the commonalities between abstract and concrete words in the comparison between encoding and retrieval were also calculated: (ix) Encoding conjunction: common activations for encoding versus retrieval: conjunction between interaction (EncA − BasEncA) − (RetA − BasRetA) and interaction (EncC − BasEncC) − (RetC − BasRetC) inclusively masked (*P* = 0.05) by the conjunction between (EncA −BasEncA) and (EncC − BasEncC), (Table 1A) and x) Retrieval conjunction: common activations for retrieval versus encoding: conjunction between interaction (RetA −BasRetA) − (EncA − BasEncA) and interaction (RetC −BasRetC) − (EncC − BasEncC) inclusively masked (*P* = 0.05) by the conjunction between (RetA − BasRetA) and (RetC − BasRetC) (Table 1B) .

All reported effects relate to voxel-level statistics (*P* < 0.05, false discovery rate (FDR) error type correction).

SS Target Areas Selection

The two conjunction contrasts ix and x estimated in the group analysis were then also assessed for each subject in the fixed-effects single subject analysis to select SS target areas, including the left prefrontal cortex "hot-spot" found in the encoding and the right prefrontal cortex and the left and right parietal cortex "hot-spots" found in the retrieval null conjunction. To extract SS hot-spots, we used a small volume correction procedure. The *t*-Student conjunction contrasts were first thresholded at *P* = 0.05, uncorrected. We then defined spherical volumes (starting radius = 6 mm, maximum radius = 20 mm) around the group-level stereotactic coordinates of the four brain regions (see

Table 1 Group stereotaxic coordinates for the two null conjunction effects

Anatomical location	x	y	z	Z-value
A: encoding conjunction				
L inferior frontal gyrus (PO)	−46	36	−13	3.76
L middle frontal gyrus	**−43**	**29**	**34**	**2.40[a]**
B: Retrieval conjunction				
L postcentral gyrus	−62	−19	25	3.77
L supramarginal gyrus	−58	−23	41	3.64
L supramarginal gyrus	**−35**	**−56**	**43**	**4.32**
L supramarginal gyrus	−35	−45	52	3.78
L insula	−30	20	−5	4.11
L cerebellar hemisphere	−30	−52	−27	6.15
L precuneus	−12	−71	38	4.84
R/L cerebellar vermis	1	−70	−33	4.86
R middle frontal gyrus	**55**	**16**	**40**	**3.40**
R middle frontal gyrus	42	49	3	4.20
R middle cingulate cortex	8	26	37	3.76
R superior medial frontal gyrus	4	37	41	3.97
R supramarginal gyrus	**44**	**−55**	**44**	**4.63**
R insula	35	24	−2	5.19
R thalamus	8	−20	8	3.37
R cerebellar hemisphere	34	−46	−32	4.97
R precuneus	13	−67	39	5.82

The reported effects relate to voxel-level statistics (*P* < 0.05, FDR error type correction). The coordinates reported in bold correspond to the selected group target areas. [a]This area was identified using a small volume correction procedure, as described in the Results. *PO* pars orbitalis

Table 2), and extracted the maximum activation peak for each subject (see Table 2). We also checked that the subject-specific coordinates identified through this procedure actually corresponded to the same anatomical location represented by the group-level coordinates. In each subject, four SS regions were selected as rTMS targets based on a combination of the following criteria: (i) the region showed a task-related activation; (ii) the region was the nearest activation locus to the corresponding group coordinates; (iii) the region was accessible to the stimulating coil (with respect to skull-brain conformation, i.e., superficial enough, and to pain of stimulation) (Postle et al. 2006).

rTMS Study

rTMS was delivered to each subject after the fMRI investigation. A time delay between the two experimental phases (mean: 21 weeks, range 16–37) was motivated by the time required for fMRI SS and group data analyses, and to minimize repetition effects with the presented stimuli. Accordingly, at the time of rTMS delivery, all subjects

Table 2 Selected SS target areas

Subject	x	y	z	z-score	Radius	Anatomical location
A: right parietal target area						
Subject01	44	−55	50	2,41	6	Right supramarginal gyrus
Subject02	45	−60	46	3,16	6	Right angular gyrus
Subject03	52	−51	46	5,7	6	Right supramarginal gyrus
Subject04	43	−54	49	4,02	6	Right supramarginal gyrus
Subject05	48	−52	45	4,23	6	Right supramarginal gyrus
Subject06	42	−53	54	4,37	6	Right supramarginal gyrus
Subject07	47	−51	47	2,07	6	Right supramarginal gyrus
Subject08	47	−50	45	4,93	6	Right supramarginal gyrus
Subject09	47	−58	51	5,27	6	Right supramarginal gyrus
Subject10	44	−51	52	9,72	6	Right supramarginal gyrus
Subject11	42	−62	42	2,47	6	Right angular gyrus
B: left parietal target area						
Subject01	−34	−60	52	3,8	6	Left supramarginal gyrus
Subject02	−38	−52	51	3,24	6	Left supramarginal gyrus
Subject03	−40	−58	55	4,22	6	Left supramarginal gyrus
Subject04	−33	−59	46	4,48	6	Left supramarginal gyrus
Subject05	−30	−59	40	3,19	6	Left supramarginal gyrus
Subject06	−30	−53	42	2,92	6	Left supramarginal gyrus
Subject07	−32	−48	40	3,38	6	Left supramarginal gyrus
Subject08	−35	−56	45	3.04	6	Left supramarginal gyrus
Subject09	−39	−57	45	3,13	6	Left supramarginal gyrus
Subject10	−35	−47	41	2,51	6	Left supramarginal gyrus
Subject11	−34	−46	43	2,26	12	Left supramarginal gyrus
C: right frontal target area						
Subject01	46	20	38	5,56	6	Right middle frontal gyrus
Subject02	49	24	40	3,93	6	Right middle frontal gyrus
Subject03	52	23	45	4,45	6	Right middle frontal gyrus
Subject04	50	17	42	1,98	6	Right middle frontal gyrus
Subject05	41	8	60	5,05	20	Right middle frontal gyrus
Subject06	50	27	35	4,14	6	Right middle frontal gyrus
Subject07	49	26	40	3,48	12	Right middle frontal gyrus
Subject08	50	23	44	2,46	12	Right middle frontal gyrus
Subject09	56	19	43	3,52	6	Right middle frontal gyrus
Subject10	44	32	43	5,83	12	Right middle frontal gyrus
Subject11	46	22	37	4,63	12	Right middle frontal gyrus
D: left frontal target area						
Subject01	−43	30	39	1,99	6	Left middle frontal gyrus
Subject02	−42	30	31	3,07	6	Left middle frontal gyrus
Subject03	−52	27	35	5,99	6	Left middle frontal gyrus
Subject04	−48	24	35	5,21	6	Left middle frontal gyrus
Subject05	−40	22	47	4,32	12	Left middle frontal gyrus
Subject06	−40	16	38	3,33	12	Left middle frontal gyrus
Subject07	−40	33	33	4,66	6	Left middle frontal gyrus
Subject08	−44	38	27	5,5	12	Left middle frontal gyrus
Subject09	−38	29	31	4,43	6	Left middle frontal gyrus
Subject10	−44	29	38	2,98	6	Left middle frontal gyrus
Subject11	−42	34	32	4,01	6	Left middle frontal gyrus

Target areas are reported as MNI coordinates. Radius is the radius of the sphere of the small volume correction necessary to find the group target areas in the SS data

reported that they did not remember any of the words presented during the preceding fMRI investigation (see also "Data Analysis" and "Results").

rTMS was applied using a Magstim Rapid with a figure-of-eight (double 50 mm) coil with the handle perpendicular to the two coil wings and the two wings on the same plane. Before rTMS, individual resting motor excitability thresholds (MT) of stimulation were determined by stimulating the left motor cortex and inducing a contraction evoked by a single TMS pulse in the contralateral first *interosseus dorsalis* muscle. The threshold was defined as the minimum intensity that induced a visible contraction in the tested muscle, as agreed by two experimenters on at least 3 out of 6 trials. The stimulation intensity used during the encoding/retrieval experiment was set at 100% of each subject's threshold. For one subject the MT reached the 70% of the maximum stimulator output: in this case the stimulation intensity during the experiment was set at the 60% of the maximum stimulator output to ensure the safety of the high-frequency stimulation protocol used (Wassermann 1998). The mean stimulation intensity was 54% (min 43%, max 60%) of the maximum of the stimulator output. During the experiment, rTMS was delivered using a train of eleven pulses with a frequency of 10 Hz (i.e., lasting a total of 1000 ms), starting with the trial onset.

Target Areas Localization

We localized the target areas using the SofTaxic Evolution navigator system (Version 1.0, www.emsmedical.net) on the individual T1-weighted MRI scans. This frameless stereotaxic neuronavigational system registered the relative positions of landmarks on the head and the position of the stimulation site, which can be identified on the individual MRI scans. The system is constituted by a graphic user interface and an Optical Tracking System (NDI Polaris Vicra, www.ndigital.com), having an head reference and a coil reference each with four passive markers (11.5 mm diameter spherical retro-reflective markers) and one stylus (four markers passive probe). The head reference was set firmly in front of the subject in order to control for head movements. The coil reference was applied on the coil in order to continuously verify the coil position, while the stylus was used to locate some additional reference points on the scalp of the subject. The precision of this neuro-navigational method is within millimetres, depending: (i) on the resolution of the MRI scans, (ii) on the properties of the electric field and its effect on the cortex, and (iii) on the precision of the craniometric referencing procedure of the head, respectively in the subject and in the MRI brain spaces (Bastings et al. 1998). A fitting procedure which optimizes the correspondence between the two analogous sets of fiducial points (MRI and craniometric points) was

carried out in order to improve accuracy when integrating the spatial data. Navigation was carried out in the SS brain space, thus avoiding any geometric transformations of the native space MRI scans. In order to inversely convert the coordinates of the SS hot-spots in the MNI space (Table 2), identified on the basis of the group analysis (Table 1) with a small volume correction procedure (see above), into the SS native brain space, we first generated a deformation map representing the SS normalization parameters. We then derived the target voxel in the SS native brain, as the voxel whose deformation according to the deformation map yields the corresponding SS hot-spot in the MNI space. This procedure was used to localize the target rTMS stimulation loci for the four selected brain areas.

Coil Positioning

To stimulate the target areas, we placed the junction of the two coil wings above the marked positions on the skullcap, while the neuronavigation system was switched on and monitored coil-scalp movements. For the sham control condition we applied a 3-cm thick plywood to a 50 mm figure-of-eight coil (Rossi et al. 2007). In this way, no magnetic fields reach the cortex. During the sham condition, we placed the junction of the two coil wings above CZ, using the same procedure as for real rTMS. We checked in a debriefing session that the participants could not reliably distinguish sham from true TMS.

rTMS Experimental Procedure

During rTMS, we used exactly the same protocol as for fMRI, including the same task sessions and blocks presented in the same order. For all experimental sessions (encoding or retrieval of concrete or abstract words), real rTMS was applied during four of the nine blocks, sham stimulation was applied during one block, while for the remaining four blocks no stimulation was applied. When a word block was stimulated in an encoding session, the corresponding block in the retrieval session was not stimulated, in order to assess the specific effects of rTMS on encoding. Vice versa, when a word block was not stimulated in an encoding session, the corresponding block in the retrieval session was stimulated, in order to assess the specific effects of rTMS on retrieval. The four stimulated blocks within every session corresponded each to a different target area: left dorsolateral prefrontal cortex: LDLPFC; right dorsolateral prefrontal cortex: RDLPFC; left parietal cortex: LPARC; right parietal cortex: RPARC (Fig. 1c). Thus, the resulting experimental conditions were: LDLPFC-Enc (i.e., LDLPFC rTMS in encoding, no stimulation in retrieval); RDLPFC-Enc; LPARC-Enc; RPARC-Enc; LDLPFC-Ret; RDLPFC-Ret; LPARC-Ret; RPARC-

Ret; sham (i.e., sham rTMS in both the phases). The stimulation conditions were pseudo-randomly ordered. No data are available for one subject who could not tolerate RDLPFC stimulation (all the others areas could be stimulated without any problems).

Data Analysis

In order to assess the effects of stimulated area during each memory tasks and for each word category, we analyzed both accuracy and RTs.

In order to exclude repetition effects, we first calculated separately for each word category a paired *t*-Student test comparing the performance during fMRI with the performance during sham stimulation in the rTMS experiment. Subsequently, we calculated the effects induced by rTMS in two different ways. The performance in a stimulated block (for encoding, in the associated retrieval block) was either (i) compared to the performance in the sham stimulation blocks or (ii) compared to the performance in the same block in the fMRI experiment. The second, less conventional, comparison was motivated by the fact the experimental design was the same for both the fMRI and rTMS phases, and by the SS localization approach. The latter approach corresponded to a 5 (stimulations, including sham) × 2 (phase: fMRI and rTMS) repeated measures ANOVA for each memory task (encoding and retrieval) and for each word category.

Results

Encoding Strategies Questionnaire

First, we found no differences between the scores assigned during the fMRI and the rTMS phases, treating all the 11 subjects as a group. Crucially, we did not find any evidence of inter-subject variability, as all the subjects relied on similar strategies. Overall, subjects preferentially used a combination of three different encoding strategies: (i) Building sentences or stories linking together the studied words; (ii) Creating images representing the studied words; and (iii) Focusing on item features. Given the lack of differences in the reported encoding strategies, we did not consider dividing the experimental subjects into sub-groups for the fMRI and TMS data analysis.

fMRI: Behavioural Performance

On average, during the fMRI study, the subjects correctly recognized 82.3% [standard deviation (SD) = 13.4] of all abstract words and 90.8% (SD = 10.7) of the all concrete words. A paired sample *t*-Student test showed that concrete words were better recognized than abstract words ($t = 5.60$, $P = 0.0001$). We also looked for performance differences between blocks, with two-one-way ANOVAs (one for each word category) with blocks as factor. No effects of block on accuracy were found for either abstract [$F(1, 8) = 1.50$, $P = 0.17$] or concrete [$F(1, 8) = 0.60$, $P = 0.78$] words. Regarding reaction times (RTs), the subjects responded on average after 867 ms (SD = 13.1) for abstract words and after 835 ms (SD = 12.5) for concrete words. A paired sample *t*-Student test showed that, as predicted by the concreteness effect, concrete words were recognized faster than abstract words ($t = 3.40$, $P = 0.001$). No effects of block on RTs were found for either abstract [$F(1, 8) = 0.355$, $P = 0.94$] or concrete [$F(1, 8) = 0.260$, $P = 0.98$] words.

fMRI: Group Analysis Results

The first aim of our study was to assess the main effects of word category independently of the level of the memory task. Compared to abstract words, concrete words were associated with a higher signal only in the left fusiform gyrus. Abstract words, in turn, did not produce any difference in activation compared to concrete words.

Given the lack of substantial activation differences between word categories, we only computed the specific interaction effects between experimental condition (memory versus baseline) and task (encoding versus retrieval). In other words, we did not further consider the word category factor. We looked at the null conjunctions of these interaction effects between abstract and concrete words. The *encoding* conjunction showed that the encoding task, compared to the retrieval task, was associated with a higher signal in the left inferior frontal gyrus (*pars orbitalis*) (see Table 1A; Fig. 2). The *retrieval* conjunction, in turn, showed a higher signal for retrieval compared to encoding in the right middle frontal gyrus (RDLPFC), in the right superior medial frontal gyrus, in the right middle cingulate cortex, in the right thalamus, and in the left postcentral gyrus. In addition, the cerebellum (including vermis and cerebellar hemispheres), the insula, the precuneus, and the supramarginal gyrus bilaterally (LPARC and RPARC) were also activated (Table 1B; Fig. 2).

Selected Group Target Areas

Based on the important role in episodic encoding and retrieval of the dorsolateral prefrontal cortices and of the inferior parietal lobules (supramarginal and angular gyri), as described in the introduction, we decided to focus on these anatomical structures for the rTMS study. Consequently, of the activations reported above, we selected
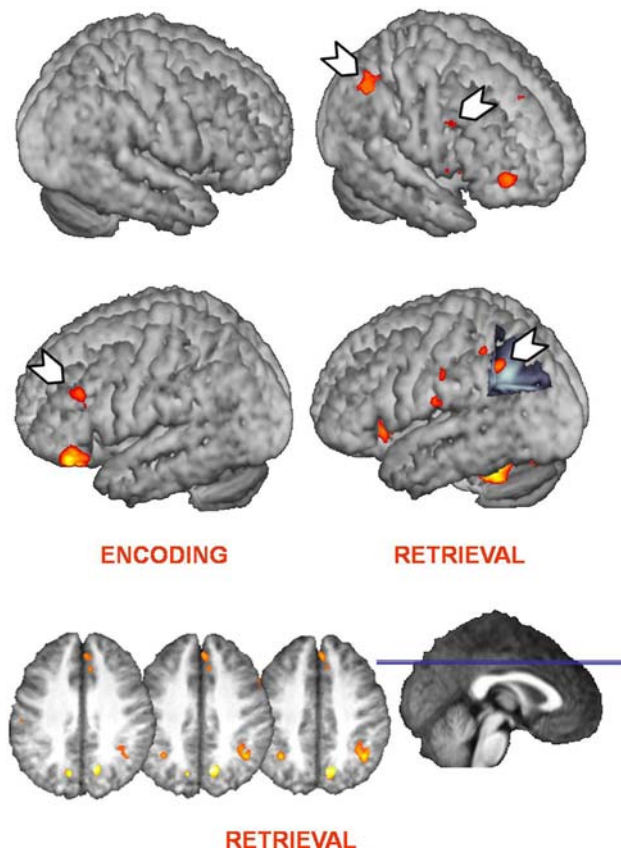
Fig. 2 Areas of activation ($P < 0.05$, FDR corrected for multiple comparisons) for the two main reported contrasts (i.e., encoding and retrieval, both representing the conjoined effects between abstract and concrete words) are displayed on cortical renderings of the participants' average anatomical image. A section into the left parietal cortex reveals an activation in the left supramarginal gyrus (LPARC) that was chosen as a stimulation target area. The other two stimulation target regions for retrieval (RPARC and RDLPFC), and the one for encoding (LDLPFC), are visible on the cortical surface. The four target areas are indicated by arrows. A set of axial slices reveals further activations for retrieval in the precuneus, bilaterally, in the anterior cingulate cortex, and in the medial frontal cortex

Fig. 3 The positioning of the rTMS coil on the head of each experimental subject was guided by fMRI-based target area selection on an individual basis (SS analysis). As an example, we show the head of one of the experimental subjects, reconstructed from the T1 structural image, with sections through the skull in correspondence of the SS stimulation target regions. The sections reveal the individual activations ($P < 0.05$, uncorrected; see methods) of this particular subject for the two main conjunction effects. These SS activations were selected as stimulation target regions, and were, the LDLPFC, the RDLPFC, the RPARC, and the LPARC

the following stimulation target areas for retrieval: RDLPFC ($x = 55$, $y = 16$, $z = 40$), RPARC ($x = 44$, $y = -55$, $z = 44$) and LPARC ($x = -35$, $y = -56$, $z = 43$).

For encoding, the stimulation of the pars orbitalis of the left IFG was excluded, as we tested its stimulation in a separate sample of subjects and it resulted in a remarkably painful muscle contraction in the majority of them. We therefore adopted a small volume correction procedure to specifically look for LDLPFC activations in the *encoding* conjunction, with an a priori anatomical hypothesis based on previous rTMS studies on episodic encoding (Rossi et al. 2001; Rossi et al. 2004; Sandrini et al. 2003). In this studies, the 10–20 system was used to localize the coil on F3 which approximately corresponds to the left DLPFC.
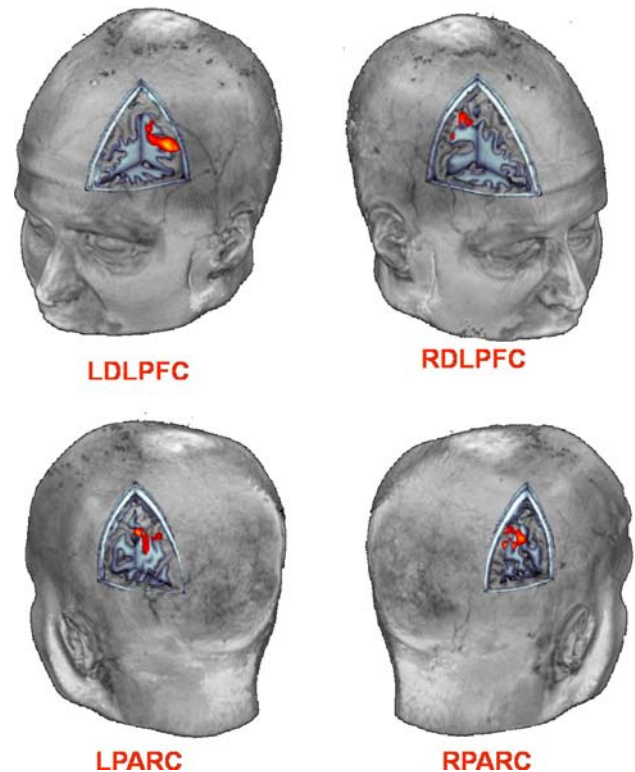
We therefore took the F3 estimated coordinate in the MNI space ($x = -37$, $y = 26$, $z = 49$, see Herwig et al. 2003b) as the center of a spherical volume of radius 20 mm for small volume correction ($P < 0.05$, FDR corrected). We found a significant activation that was selected as a stimulation target area for encoding: LDLPFC ($x = -43$, $y = 29$, $z = 34$; Z score = 2.40).

fMRI: Results of the SS Analyses

There was a considerable variability across subjects in the topographical localization of the selected SS target areas (Table 2; Fig. 3).

rTMS Results

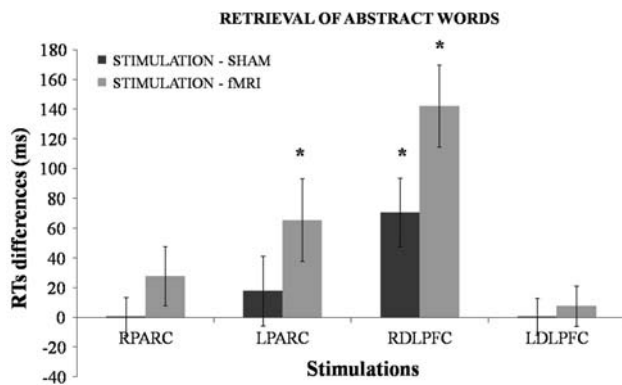First, we verified that for both, accuracy and RTs, performance achieved during the sham condition in the rTMS

RETRIEVAL OF ABSTRACT WORDS

Fig. 4 Behavioural results. The graph shows the differences between the RTs during the four experimental condition (RPARC, LPARC, RDLPFC, LDLPFC) and the RTs achieved during the sham condition (dark grey) or during fMRI (light grey). 95% standard error bars are indicated. Only data for stimulation during retrieval of abstract words are displayed. Asterisks indicate significant differences

phase was comparable with the one obtained in the fMRI phase [Accuracy: abstract words $t = 1.21$, $P = 0.25$; concrete words $t = 0.03$, $P = 0.98$; RTs: abstract words: $t = 0.94$, $P = 0.37$; concrete words: $t = 0.17$, $P = 0.86$]. The lack of significant differences was consistent with the absence of repetition effects reported by the subjects in the debriefing during the rTMS phase.

With respect to the stimulation effects as measured by accuracy, we did not find any significant result, either in terms of differences between real stimulation and sham or between real stimulation and performance in the fMRI phase.

Considering RTs, however, significant effects were found for the retrieval of abstract words. The interaction between stimulation and phase (fMRI, rTMS) was significant [$F(4, 40) = 3.44$, $P = 0.016$]. Post-hoc analyses (LSD Fisher) showed significant differences when comparing real stimulation in the RDLPFC with sham stimulation: stimulation produced longer RTs than sham stimulation [$P = 0.023$; RDLPFC-Ret = 969 (SD = 151), SHAM = 898 (SD = 125)]. Significant differences were also found when comparing the performance during real stimulations in the rTMS phase with the performance achieved in the corresponding blocks of the fMRI phase: both LPARC-Ret and RDLPFC-Ret stimulations induced longer RTs than the fMRI baseline conditions [LPARC-Ret: $P = 0.034$, LPARC-Ret (rTMS) = 916 (SD = 156), LPARC-Ret (fMRI) = 850 (SD = 123); RDLPFC-Ret: $P = 0.00003$, RDLPFC-Ret (rTMS) = 969 (SD = 151), RDLPFC-Ret (fMRI) = 828 (SD = 117)] (Fig. 4).

No significant RTs effects were found for neither the encoding of abstract words nor the encoding and retrieval of concrete words.

fMRI–rTMS Correlational Results

rTMS Effects–fMRI Activations Correlations

As a post-hoc analysis motivated by the results of the stimulation versus sham and stimulation versus fMRI-performance analyses, an additional analysis was conducted in order to investigate the presence of correlations between the individual fMRI activation effect sizes in the target areas and the individual effect of rTMS stimulation (in terms of an increase of RTs in the rTMS phase compared to either sham or the fMRI phase) for the experimental subjects. Correlations were assessed between the effect sizes in LDLPFC during encoding of abstract words and the rTMS effects after Enc-LDLPFC stimulation, and between the effect sizes in RDLPFC, in LPARC, and in RPARC during retrieval of abstract words and the corresponding rTMS effects after Ret-RDLPFC, Ret-LPARC, and Ret-RPARC, respectively. The condition-specific differences in RTs between rTMS and sham or fMRI for each subject were entered as covariates of interest in a set of second-level random effects one sample t-Student test analyses in SPM5, which included the contrast images for the effects of interest (e.g., the contrast 'RetA-BasRetA' for the rTMS effect in the Ret-RDLPFC condition). Positive correlations between fMRI activation effect sizes and rTMS effect measured by an increase of RTs were assessed using a small volume correction procedure, with a sphere of a 6 mm radius around the corresponding target group analysis coordinates (Table 1) at $P < 0.05$ FDR corrected.

A significant positive correlation was found between the effect size in the RPARC during the retrieval of abstract words and the corresponding increase of RTs during its stimulation with rTMS [comparison with sham: 40–55 40, P(FDR) = 0.0001; comparison with fMRI baseline: 43–54 39, P(FDR) = 0.017] (Fig. 5a, b).

Discussion

Our study was designed to investigate the usefulness of a SS combined experimental approach, consisting in an fMRI-based target area selection on an individual basis followed by rTMS, in the study of encoding and retrieval memory processes. This subject-specific procedure is particularly important in memory tasks, in which there are substantial interindividual anatomo-functional differences. Accordingly, we applied this approach to evaluate the role of the DLPFCs and of the PARCs in the encoding and retrieval of abstract and concrete words. Specifically, we wished to verify both the functional hemispheric asymmetry of the DLPFC and the causal role of both the DLPFCs and the PARCs in an episodic memory task.
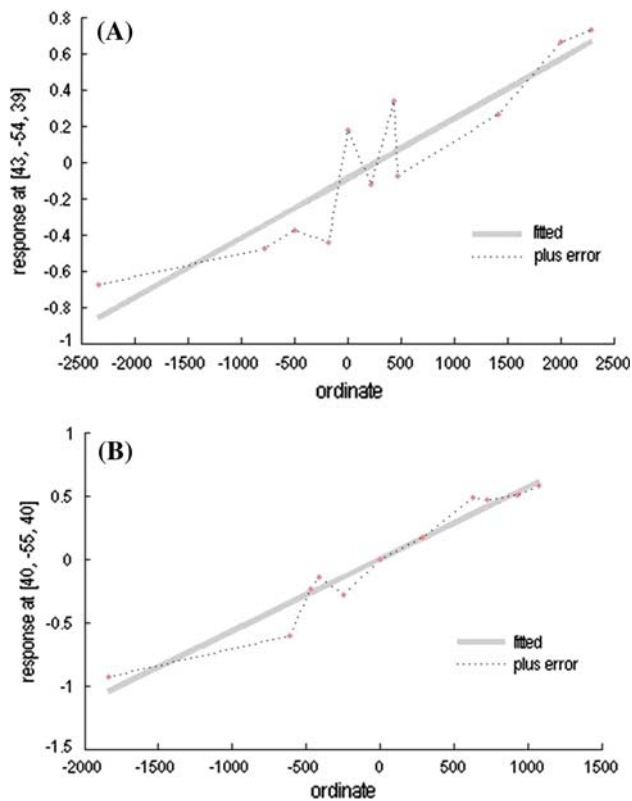
Fig. 5 Correlations between individual RPARC activation effect sizes during retrieval of abstract words (abscissa) and individual RPARC-Ret rTMS effects (ordinate) on performance (measured as differences between RTs during RPARC-Ret stimulation and RTs during fMRI (**a**) or during sham condition (**b**))

Moreover, since the PARCs seem to be differentially involved in abstract and concrete word processing (Jessen et al. 2000), we also wished to verify differences in activated areas (fMRI data) and in functional involvement of the target areas (rTMS data) related to word category.

This was done using a combined fMRI and TMS approach allowing the stimulation of the individual activation spots, thus taking into account the substantial interindividual anatomo-functional differences in episodic memory tasks, which might be related not only to structural differences, but also to the use of different strategies. Following the same logic of single subject-based analysis, we also correlated the effect sizes of the SS activations with the observed rTMS effect in each individual.

In the fMRI experiment, we found process-specific (encoding versus retrieval) but no substantial category-specific (abstract versus concrete) activation differences. More specifically, similarly for abstract and concrete words, encoding was selectively associated to an activation of the LDLPFC, whereas retrieval selectively activated the RDLPFC and the PARC, bilaterally. Accordingly, during the rTMS experiment we stimulated these four areas for

both word categories. Since no category-specific activation differences were found, we investigated different causality of the same target areas for both word categories.

With respect to the results of the rTMS experiment, it must be emphasized that we only found significant effects when analyzing RT data; no significant effects of accuracy were observed. This pattern was most likely due to the relatively low level of difficulty of the task, with a small number of items in each condition. RTs performance is generally more sensitive than response accuracy in rTMS studies. The type of effect is often related on increased timing in the information processing (e.g., increased reaction time) and, if a reduction of subject performance is recorded, it is most likely explained by the complexity of the processing needed to solve the task; for that reason the effect of TMS may be related to the reduction of the difference between the signal and the noise present in the system. Therefore, TMS can be regarded as an interference method that can increase the timing for the information processing or modify criteria for response decision. Effects of rTMS on accuracy, in turn, are less frequently observed (Manenti et al. 2008).

In addition, there were no significant rTMS effects for concrete words. Consequently, it might be relevant to underline that for what concerns the difference between abstract and concrete words fMRI and TMS findings do not fit well together. No substantial category-specific (abstract versus concrete) activation differences were found during the fMRI experiment, whereas a differential stimulation effect between the two word categories was obtained in the TMS experiment (i.e., no effect for concrete words). The lack of TMS effects on concrete words could depend on the higher performance level observed during the processing of words of this category compared to abstract words (i.e., to a ceiling effect obscuring any potential interference effect), but it might also be that the areas targeted during stimulation were more involved for abstract than for concrete words processing, a difference not detectable using fMRI. This divergence highlights, as previously suggested for a working memory study (Postle et al. 2006), that these two techniques are quite different and thus likely to provide insights into slightly different aspects of neural functioning.

For what concerned DLPFCs, our data were only partially compatible with the DLPFC functional asymmetry hypothesis in showing the functional relevance of RDLPFC during retrieval (rTMS applied to these areas during abstract word retrieval caused a slowing down of performance), but in failing to demonstrate the functional involvement of LDLPFC during encoding (Rossi et al. 2001; Rossi et al. 2004; Sandrini et al. 2003).

Several explanations could be proposed to justify the lack of rTMS effects induced by the stimulation of the LDLPFC during encoding.

A first reason might be related to the task. In previous studies deep encoding tasks were used (internal/external categorization or semantic related/unrelated categorization, Rossi et al. 2001; Sandrini et al. 2003), while in our study we used a more shallow encoding task. A more widespread frontal network seems to be involved during deep versus shallow encoding: for instance, the comparison between a "deep" semantic task and a "shallow" orthographic task revealed a significant greater activation in the left prefrontal cortex for the semantic compared to the orthographic task (Kapur et al. 1994). Therefore, the cerebral activations induced in the present study may have been anatomically more restricted compared to the one associated to previous rTMS studies.

An alternative explanation for the lack of LDLPFC stimulation effects during encoding could be found in the method used. Compared to previous studies (Rossi et al. 2001; Sandrini et al. 2003), in our rTMS experiment we used both a smaller coil (50 mm versus 70 mm figure of eight coil) and a more precise coil positioning, thus probably generating a more localized and restricted stimulation effect. Previous works (Rossi et al. 2001; Sandrini et al. 2003) suggested that it is difficult to ascertain whether some remote effects of DLPFC stimulation might extend to more ventral regions through extant functional connections. Rossi and coworkers proposed that this spreading effect might also explain the relatively low specificity of the effects induced by rTMS of the DLPFC, since the same site of brain stimulation may lead to interference with other aspects of memory function, including working memory (Mottaghy et al. 2002a; Mottaghy et al. 2002b; Mottaghy et al. 2000; Mottaghy et al. 2003), procedural learning (Pascual-Leone et al. 1996) and semantic memory (Flitman et al. 1998). The latter point could be a possible reason why previous rTMS studies, but not our study, were able to find an effect after stimulation of LDLPFC during encoding as a larger coil may be more liable to create spreading effects to ventral areas. We propose that in our study the reduced stimulation did not induce remote effects in more ventral regions, preventing the appearance of stimulation effects during encoding. Within the LDLPFC, the left IFG has been found to be engaged in a variety of tasks, including word encoding (Jessen et al. 2000) and we also found a strong involvement of the left IFG (*pars orbitalis*) during word encoding. The *pars orbitalis* of the left IFG might be the really causally involved area in memory encoding. One possibility is that the left IFG may have been stimulated in previous works thanks to spreading effects from LDLPFC to more ventral regions and not stimulated in our work in which spreading effects were less likely to occur. Unfortunately, the unpleasant activation of nocireceptors prevented the possibility of stimulation of this area.

In sum, the absence of LDLPFC effects induced by rTMS during encoding in our study could be due both to the use of a smaller coil that prevented the stimulation to spread to the IFG, which could not be directly stimulated for technical reasons, and to the choice of a shallow encoding task that activated more restricted frontal areas. A further possible explanation of this null result could concern the experimental procedure. The words to be encoded remained the same for both the fMRI and the TMS experiments and, even if the two sessions took place several months apart and no repetition effects were found, it may still be that the results of the rTMS encoding sessions were partially confounded by the previous exposure to the word lists.

With respect to the involvement of the PARC in episodic memory tasks, we demonstrated the causal role of the LPARC during abstract word retrieval (longer RTs after the stimulation of this area). However, this effect was only found in comparing the performance measured after real rTMS with the one measured during fMRI, and not comparing real with sham stimulation. The choice to perform these two different types of comparison was motivated by the use of two different control conditions (sham and baseline) in previous rTMS works (Rossi et al. 2001; Rossi et al. 2004; Sandrini et al. 2003). A rTMS study on episodic memory in young and elder subjects showed differences comparing baseline and sham performances (Rossi et al. 2004): consequently, different results were found when comparing real rTMS stimulation to one or to the other control condition. The authors interpreted this difference as an aspecific alerting effect, probably linked with the auditory stimulation in the sham condition. Consequently, the use of both the control conditions is recommended. In our study, since the paradigm was exactly the same in fMRI and in rTMS phases, the baseline condition (i.e., no stimulation) was represented by the fMRI phase and each real stimulation in the rTMS phase was associated to the corresponding fMRI baseline block (i.e., the block with the same items that were presented in the same sequence position). The two different types of comparison (versus sham or versus fMRI) produced the same results for abstract word retrieval with respect to the RDLPFC but not with respect to the LPARC (i.e., higher RTs during Ret-LPARC stimulation than during fMRI baseline but lack of this effect when real stimulation was compared to the sham one). The explanation of the difference between the results of these two comparisons may be reconducted to the reduction of confounding variables using the comparison between real stimulations and fMRI baseline (i.e., the comparison between real stimulation and fMRI performances reflected exactly the same items, while sham stimulation concerned different items). Further studies will be required to clarify this issue. The rTMS effect on

LPARC is relevant for the issue of the causal involvement of left parietal areas during retrieval of abstract words, and it stands in contrast to the findings of Rossi et al. (2004) with pictures. This recent rTMS work showed that the interference of PARC stimulation on encoding and retrieval performance was negligible, suggesting that the activity of the intraparietal sulci, previously found in several fMRI studies on memory, would be not causally involved in episodic memory encoding and retrieval (Rossi et al. 2006).

In our study, however, we also investigated more specifically the effect of this stimulation by the correlation analysis between the effect size of the SS activations in the RPARC during abstract word retrieval and the size of the interference effect (as measured by RTs slowing) found after rTMS stimulation of this brain region. The results of this correlation analysis, showing that a significant positive correlation was present in RPARC, suggest that the RPARC activation was probably associated with an inter-subject variability that did not allow to find a mean causal effects using rTMS. A causal relationship, however, may exist in those subject that activate the RPARC to a significant extent during word retrieval. Taken together our data on PARC may be taken to suggest a causal involvement of the parietal cortices in retrieval processes. The SS approach used in the present work could justify the discrepancy between the results of the present study and previous results obtained by adopting a more conventional approach: while fMRI studies consistently reported a parietal activation (Buckner and Wheeler 2001; Cabeza et al. 2003; Cabeza and Nyberg 2003; Fletcher and Henson 2001; Rugg and Wilding 2000; Simons and Spiers 2003; Wagner et al. 1998a), the considerable variability across subjects may have prevented from finding a mean parietal effect in retrieval processes in rTMS studies. Further studies will of course be necessary to better elucidate the involvement of parietal areas in retrieval processes.

In conclusion, the combined fMRI and rTMS approach demonstrated to be useful in several respects: (i) it enables the use of the SS MRIs for the coil positioning using neuronavigation systems, thus allowing to consider SS anatomical differences; (ii) it ensures that the activated area is activated at a SS level, thus allowing to consider also SS functional differences and (iii) it provides a direct investigation of causal involvement of an area in a task, permitting to verify the correlation between cerebral activation and rTMS effect, thus explaining, at least in part, SS rTMS effects variability.

It has been underlined that, in terms of causality, a brain region can only be considered functionally relevant for a certain cognitive performance if this region is (i) activated during the performance of a particular task (e.g., with fMRI), and (ii) a controlled manipulation, e.g., with rTMS, of this regional activity results in a modulation of task

performance (Sack and Linden 2003). Nevertheless, for what concerns the first point, it is important to underline here that often different neuroimaging techniques implicate different brain regions, as proved by a recent work (Liljestrom et al. 2009) that analyzed the convergence between fMRI and magnetoencephalography (MEG) data acquired during a naming task. Future studies may be aimed at clarifying which are the optimal imaging techniques to be used in combination with rTMS and which are the most useful ways to combine these techniques. Clearly, the manipulation with rTMS can only be meaningful if the target area can be accurately localized on an individual basis. The repetition of the same behavioral paradigm, once with fMRI and once with rTMS, allows the assessment of correlations between cerebral activity and the induced rTMS effects. Specifically, in our study the availability of correlational results, allowed to verify the causality of right parietal cortex during retrieval of abstract words that could be hidden in the mean results analysis. This type of analysis may prove to be really crucial in the evaluation of anatomo-functional causality, particularly in those cognitive tasks, such as in episodic memory, where interindividual differences are particularly relevant.

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