1 ORIGINAL PAPER

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

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8 **Abstract** There is evidence that the human prefrontal
9 cortex is asymmetrically involved in long-term episodic
10 memory processing. Moreover, abstract and concrete words
11 processing has been reported to differentially involve pre-
12 frontal and parietal areas. We implemented a two-stages
13 functional magnetic resonance imaging (fMRI)–repetitive
14 transcranial magnetic stimulation (rTMS) paradigm to
15 investigate the role of the dorsolateral prefrontal cortices
16 (DLPFCs) and parietal cortices (PARCs) in encoding and
17 retrieval of abstract and concrete words. Using this paradigm
18 we could select areas to be stimulated on the basis of single-
19 subject (SS) anatomical and functional data, investigating
20 the usefulness of this integration approach. With respect to
21 fMRI, abstract and concrete words differed only for a
22 greater left fusiform gyrus activation for concrete words. In
23 turn, significant rTMS effects were found, but only for the
24 retrieval of abstract words. Consistent with previous find-
25 ings, repetitive stimulation of the right DLPFC had a specific
26 impact on episodic retrieval. Memory retrieval performance
27 was also disrupted when rTMS was applied to the left PARC.
28 Finally, we found a significant positive correlation between
29 the effect sizes of SS right PARC activations for abstract
30 word retrieval and the consequent rTMS interference effects.
31 Taken together these data provide for the first time evidence
32 that also the PARC has a necessary role in episodic retrieval
33 of abstract words. Importantly, from a methodological per-
34 spective, our data demonstrate that fMRI-guided rTMS with
35 a SS approach provides a powerful tool to investigate the
36 neural underpinnings of cognitive functions.

38 **Keywords** rTMS · Single-subject · Memory ·
39 Combining · Youngs

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Introduction 40

While functional magnetic resonance imaging (fMRI) data 41
reveal the correlations existing between brain functions and 42
behavior, transcranial magnetic stimulation (TMS) is a 43
complementary technique that can address hypotheses 44
about the necessity of one or more brain areas to a par- 45
ticular aspect of cognitive performance (Walsh and Pasc- 46
ual-Leone 2003), and it has been extensively used to map 47
the flow of information across different brain regions 48
during the execution of a cognitive task (Walsh and 49
Rushworth 1999). The use of combined TMS–fMRI has 50

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 off-line (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. underlined the critical importance of the left Brodmann area 9 in a reading span test (Osaka et al. 2007). 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

157 suggested that parietal activations accompanying the
158 memorization processes reflect the engagement of a
159 widespread brain attentional network (Rossi et al. 2006).
160 The main goal of our study was to adopt the SS com-
161 bined experimental approach, consisting in an fMRI-based
162 target area selection on an individual basis followed by
163 rTMS, to verify the usefulness of this approach in the study
164 of cognitive functions and, in particular, in the assessment
165 of the causal role of prefrontal and parietal areas in
166 memory encoding and retrieval of abstract and concrete
167 words.

Materials and Methods

Subjects

170 A group of 11 subjects [mean age = 30 years (range:
171 25–40); mean education = 18.5 years (range: 18–22)]
172 participated in the experiment. All subjects were native
173 Italian speakers and had normal or corrected-to-normal
174 visual acuity. Participants reported being free of neuro-
175 logical disorders or history of seizures. All were right
176 handed, with a mean score on the Edinburgh Handedness
177 Inventory (Oldfield 1971) of +88% (range = + 44 to
178 +100%). Participants were informed about the procedures
179 and the possible risks of rTMS and informed consent was
180 obtained after a safety screening. The experimental meth-
181 ods had ethical approval from the locals Human Ethics
182 Committees (Ethics Committee of the San Raffaele Sci-
183 entific Institute, Milano, Italy and Ethics Committee of the
184 IRCCS San Giovanni di Dio Fatebenefratelli, Brescia,
185 Italy). Each subject first underwent an fMRI investigation,
186 followed by rTMS.

Stimuli

188 For the encoding condition, a total of 90 abstract words and
189 90 concrete words were selected from the "Corpus e Lessico
190 di Frequenza dell'Italiano Scritto (CoLFIS)" (Laudanna
191 et al. 1995). For the retrieval condition, we further selected
192 45 abstract and 45 concrete "new" words. The retrieval word
193 list thus consisted of half of the old words (45 concrete, 45
194 abstract) and 90 new words (45 concrete, 45 abstract). The
195 mean word length was 6.4 ($\pm$1.5) letters, and 2.7 ($\pm$0.6)
196 syllables. Abstract and concrete words were balanced for
197 word length and for variables known to influence memory
198 performance, i.e., word frequency (mean $\pm$ standard devi-
199 ation = 59 $\pm$ 20) and familiarity (mean $\pm$ standard devia-
200 tion = 6.0 $\pm$ 0.7). There were significant differences with
201 respect to concreteness (concrete = 6.0; abstract = 3.6;
202 $t(1, 99) = 29.47, P < 0.05$) and imageability (concrete = 5.7;

203 abstract = 3.3; $t(1, 99) = 30.89$, $P < 0.05$) based on
204 CoLFIS (Laudanna et al. 1995).
205 In total, both the encoding and the retrieval word lists
206 included 180 words. Each word list (encoding and retrie-
207 val) was then divided into 9 blocks of concrete words and 9
208 blocks of abstract words, each block consisting of ten
209 words. Each encoding block was associated with one
210 retrieval block containing an equal number of "new" and
211 "old" words in randomized order. Each encoding block
212 began with the instruction "Encode", while each retrieval
213 block began with the instruction "Old or new?". The
214 experimental paradigm also included a baseline condition,
215 with one baseline block associated to each encoding or
216 retrieval block. In the baseline blocks, the instruction
217 "Count" was presented, followed by a cross remaining on
218 the screen for the entire block duration: the subject were
219 instructed to fixate the cross and to covertly count from one
220 to ten in a non-stop mode. The baseline condition served
221 both as a subtraction baseline for fMRI, and as a delay
222 period between the stimulations of different cortical areas
223 for rTMS. All blocks (encoding, retrieval or baseline)
224 lasted for 30 s, preceded by 4000 ms of instructions.
225 The blocks were grouped into three encoding and three
226 associated retrieval sessions for each word category
227 (abstract or concrete). Each encoding session included 3
228 encoding and 3 baseline blocks, while each associated
229 retrieval session comprised the 3 corresponding retrieval
230 blocks and 3 baseline blocks. This behavioural paradigm,
231 reflecting a 2 by 2 factorial combination of task (encoding
232 or retrieval) and word category (abstract or concrete),
233 resulted in four experimental conditions (encoding of
234 abstract words: EncA; encoding of concrete words: EncC;
235 retrieval of abstract words: RetA; retrieval of concrete
236 words: RetC). Since each encoding or retrieval block was
237 associated to one baseline block, four baseline conditions
238 were also constructed, reflecting one level of a further two-
239 levels experimental factor (experimental condition: mem-
240 ory or baseline). The four baseline conditions were formed
241 by dividing the baseline blocks according to the session
242 (encoding or retrieval of abstract or concrete words) in
243 which they were included (baseline for encoding of
244 abstract words: BasEncA; baseline for encoding of con-
245 crete words: BasEncC; baseline for retrieval of abstract
246 words: BasRetA; baseline for retrieval of concrete words:
247 BasRetC). This allowed us to treat the 4 baseline condi-
248 tions as orthogonal data sources and to compute interaction
249 and conjunction effects (see below). The three associated
250 encoding and retrieval abstract sessions were presented
251 consecutively followed (half of the subjects) or preceded
252 (the other half of the subjects) by the three associated
253 encoding and retrieval concrete sessions. Between each
254 encoding and retrieval sessions, we included a 5 min delay
255 to allow for working memory wash out and trace

256 consolidation. During this delay, the subjects simply rested
257 in the scanner listening to music (Fig. 1b).

Behavioural Task

259 The stimuli were presented using Presentation software
260 (Version 10.3, www.neurobs.com). All stimuli were pre-
261 sented in black lower-case letters on a white background.
262 During the fMRI phase, stimuli were projected from out-
263 side the magnet room onto a translucent screen placed at
264 the foot of the magnet bore. A mirror attached to the top of
265 the head coil allowed the participants to view the translu-
266 cent screen from inside the magnet. During both the fMRI
267 and rTMS studies, the subjects were told to read and
268 encode the presented words. After a delay, they were asked
269 to decide whether the presented word was from the

270 previously encoded word list or not, by making a speeded
271 decision via a two-choice button press, using the right and
272 left forefingers (hands counterbalanced across subjects).
273 During both encoding and retrieval, each word was pre-
274 sented for 1500 ms in the centre of the screen and was
275 followed by a 1500 ms delay (Fig. 1a).

276 At the end of the experimental phase (both during the
277 fMRI and the rTMS phase) subjects were asked to fill in an
278 "Encoding strategies questionnaire". This questionnaire
279 comprised twelve possible strategies that could be used
280 during the task and subjects had to assign a score from 1 to
281 ten (1 = never, 10 = always) to each strategy according to
282 how often they had used each strategy during the task. The
283 12 listed strategies were: (i) to use words' initials, (ii) to
284 create sentences including some of the presented words,
285 (iii) to imagine the pictures corresponding to the presented

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
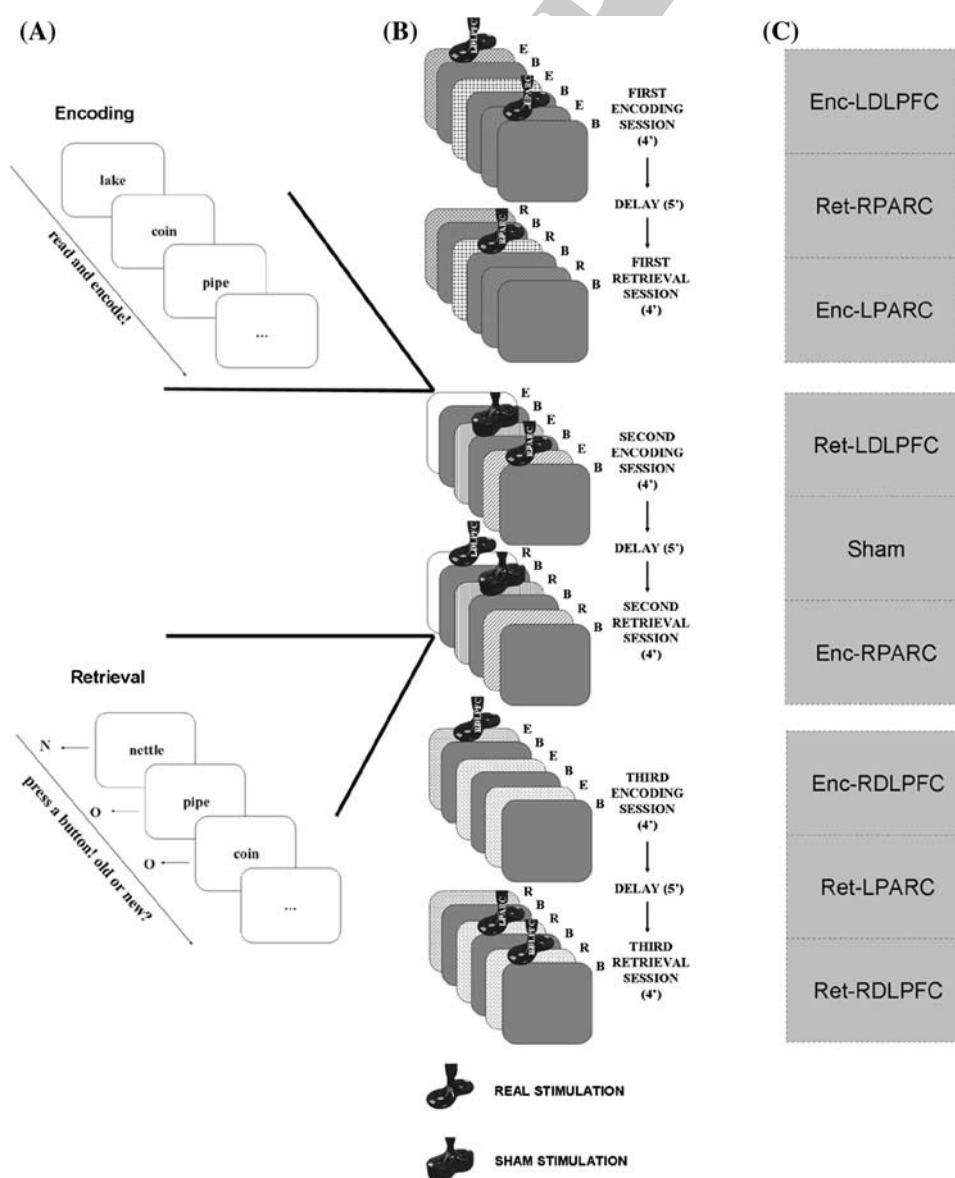
286 words, (iv) to repeat the words, (v) to create songs
287 including some of the presented words, (vi) to create
288 rhymes between the displayed words, (vii) to translate the
289 words in a foreign language, (viii) to create associations of
290 words, (ix) to create a brief story including the presented
291 words, (x) to associate each word to a personal event, (xi)
292 to classify each word as easy/difficult, abstract/concrete,
293 positive/negative, etc., (xii) to imagine the words' sound,
294 color, shape, etc.

295 fMRI Study

MRI Data Acquisition

297 MRI scans were acquired on a 3T Achieva Philips body
298 scanner (Philips Medical Systems, Best, NL) using an 8
299 channels-sense head coil (sense reduction factor = 2).
300 Whole-brain functional images were obtained with a T2*-
301 weighted gradient echo, echo-planar sequence, using
302 blood-oxygenation-level-dependent contrast. Each func-
303 tional image comprised 30 contiguous axial slices (4 mm
304 thick), acquired in interleaved mode, and with a repetition
305 time of 2000 ms (echo time: 30 ms; field of view:
306 240 mm × 240 mm; matrix size: 128 × 128). The para-
307 digm was a block-design each participant underwent 12
308 functional scanning sessions (half encoding sessions and
309 half for concrete words). Baseline blocks were included in
310 all sessions. The duration of each session was 110 scans,
311 preceded by 10 dummy scans that were discarded prior to
312 data analysis.
313 For anatomical localization of brain activations and pre-
314 cise coil positioning during rTMS phase, we acquired one
315 high-resolution whole-brain structural T1 weighted scan
316 (resolution 1 mm × 1 mm × 1 mm) of each participant. In
317 order to maximize the coil localization accuracy, we
318 acquired 200 slices, covering on average the entire brain and
319 skull down to the midbrain. The normalized structural ima-
320 ges of all participants were then averaged in one single image
321 for visualization of group brain activations.

Data Analysis

323 *Behavioural Data* Behavioural data were analyzed eval-
324 uating both accuracy and reaction times (RTs) during the
325 retrieval sessions. It is important to consider that the
326 behavioural performance measured in this way may reflect
327 the functioning during both the encoding and the associated
328 retrieval sessions. For both accuracy and RTs data, we
329 conducted a paired *t*-Student test between abstract and
330 concrete words, verifying if, as predicted by the concrete-
331 ness effect, concrete words were recognized better and
332 faster than abstract words. We also calculated two-one-way
333 ANOVAs (one for each word category) with the block as a

334 factor, in order to exclude performance differences
335 between blocks.

337 *Functional MRI Data* Statistical parametric mapping
337 (SPM5, Wellcome Department of Imaging Neuroscience,
338 London, UK) was used for image realignment (Andersson
339 et al. 2001), normalization to the Montreal Neurological
340 Institute (MNI) standard space (yielding normalization
341 parameter files used for the inverse definition of individual
342 rTMS loci in the subject native brain space, see below),
343 smoothing by a 6 mm FWHM Gaussian kernel, and
344 General Linear Model statistical analysis (Friston et al.
345 2002). We adopted both: (i) a fixed-effects single subject
346 analysis, in order to identify individual stimulation loci; (ii)
347 a two-stage random-effects group analysis approach, in
348 order to identify group brain activations.

349 Fixed-effects Single Subject Analysis

350 At the first stage, the time series of each participant were
351 high-pass filtered at 67 s and pre-whitened by means of an
352 autoregressive model AR(1) (Friston et al. 2002). Global
353 normalization was performed to account for the global
354 between sessions effects confounding the comparisons
355 between experimental conditions. For each participant, we
356 modelled a 2 (experimental condition: memory or base-
357 line) × (tasks: encoding or retrieval) × 2 (word category:
358 abstract or concrete) factorial design with 12 separate
359 sessions, reflecting conditions EncA, EncC, BasEncA,
360 BasEncC, RetA, RetC, BasRetA, BasRetC. We then calcu-
361 lated effect-specific interaction contrasts and, for the purpose
362 of the random effects group analysis only, a set of condition-
363 specific contrasts, each contrast including a weight of one
364 for a particular condition of interest and a weight of zero
365 for all the other conditions. The *t*-Student effect-specific
366 interaction contrasts included: (i) activations for encoding
367 versus retrieval of abstract words: (EncA − BasEncA)
368 − (RetA − BasRetA) inclusively masked by (EncA
369 − BasEncA), (ii) activations for encoding versus retrieval of
370 concrete words: (EncC − BasEncC) − (RetC − BasRetC)
371 inclusively masked by (EncC − BasEncC), (iii) activations
372 for retrieval versus encoding of abstract words: (RetA
373 − BasRetA) − (EncA − BasEncA) inclusively masked by
374 (RetA − BasRetA), and (iv) activations for retrieval versus
375 encoding of concrete words: (RetC − BasRetC) − (EncC
376 − BasEncC) inclusively masked by (RetC − BasRetC).

377 Second-level Random Effects Group Analysis
378 Approach

379 At the second stage of analysis, the contrast images
380 obtained at the SS level were used to compute a within-
381 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,

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

maximum radius = 20 mm) around the group-level stereotactic coordinates of the four brain regions (see 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.

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

Accordingly, at the time of rTMS delivery, all subjects 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 neuronavigational 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. At each stimulation site, the coil was oriented with its longer side perpendicular to the scalp midline. 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, with its longer side perpendicular to the scalp midline, 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

553 stimulation in retrieval); RDLPFC-Enc; LPARC-Enc;
554 RPARC-Enc; LDLPFC-Ret; RDLPFC-Ret; LPARC-Ret;
555 RPARC-Ret; sham (i.e., sham rTMS in both the phases).
556 The stimulation conditions were pseudo-randomly ordered.
557 No data are available for one subject who could not tolerate
558 RDLPFC stimulation (all the others areas could be stimu-
559 lated without any problems).

560 *Data Analysis*

561 In order to assess the effects of stimulated area during each
562 memory tasks and for each word category, we analyzed
563 both accuracy and RTs.
564 In order to exclude repetition effects, we first calculated
565 separately for each word category a paired *t*-Student test
566 comparing the performance during fMRI with the perfor-
567 mance during sham stimulation in the rTMS experiment.
568 Subsequently, we calculated the effects induced by rTMS
569 in two different ways. The performance in a stimulated
570 block (for encoding, in the associated retrieval block) was
571 either (i) compared to the performance in the sham stim-
572 ulation blocks or (ii) compared to the performance in the
573 same block in the fMRI experiment. The second, less
574 conventional, comparison was motivated by the fact the
575 experimental design was the same for both the fMRI and
576 rTMS phases, and by the SS localization approach. The
577 latter approach corresponded to a 5 (stimulations, including
578 sham) × 2 (phase: fMRI and rTMS) repeated measures
579 ANOVA for each memory task (encoding and retrieval)
580 and for each word category.

581 **Results**

582 Encoding Strategies Questionnaire

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

595 fMRI: Behavioural Performance

596 On average, during the fMRI study, the subjects correctly
597 recognized 82.3% (standard deviation (SD) = 13.4) of all

598 abstract words and 90.8% (SD = 10.7) of the all concrete
599 words. A paired sample *t*-Student test showed that concrete
600 words were better recognized than abstract words
601 ($t = 5.60$, $P = 0.0001$). We also looked for performance
602 differences between blocks, with two-one-way ANOVAs
603 (one for each word category) with blocks as factor. No
604 effects of block on accuracy were found for either abstract
605 [$F(1, 8) = 1.50$, $P = 0.17$] or concrete [$F(1, 8) = 0.60$,
606 $P = 0.78$] words. Regarding reaction times (RTs), the
607 subjects responded on average after 867 ms (SD = 13.1)
608 for abstract words and after 835 ms (SD = 12.5) for con-
609 crete words. A paired sample *t*-Student test showed that, as
610 predicted by the concreteness effect, concrete words were
611 recognized faster than abstract words ($t = 3.40$,
612 $P = 0.001$). No effects of block on RTs were found for
613 either abstract [$F(1, 8) = 0.355$, $P = 0.94$] or concrete
614 [$F(1, 8) = 0.260$, $P = 0.98$] words.

615 fMRI: Group Analysis Results

616 The first aim of our study was to assess the main effects of
617 word category independently of the level of the memory
618 task. Compared to abstract words, concrete words were
619 associated with a higher signal only in the left fusiform
620 gyrus. Abstract words, in turn, did not produce any dif-
621 ference in activation compared to concrete words.
622 Given the lack of substantial activation differences
623 between word categories, we only computed the specific
624 interaction effects between experimental condition (mem-
625 ory versus baseline) and task (encoding versus retrieval). In
626 other words, we did not further consider the word category
627 factor. We looked at the null conjunctions of these inter-
628 action effects between abstract and concrete words. The
629 *encoding* conjunction showed that the encoding task,
630 compared to the retrieval task, was associated with a higher
631 signal in the left inferior frontal gyrus (*pars orbitalis*) (see
632 Table 1A; Fig. 2). The *retrieval* conjunction, in turn,
633 showed a higher signal for retrieval compared to encoding
634 in the right middle frontal gyrus (RDLPFC), in the right
635 superior medial frontal gyrus, in the right middle cingulate
636 cortex, in the right thalamus, and in the left postcentral
637 gyrus. In addition, the cerebellum (including vermis and
638 cerebellar hemispheres), the insula, the precuneus, and the
639 supramarginal gyrus bilaterally (LPARC and RPARC)
640 were also activated (Table 1B; Fig. 2).

641 *Selected Group Target Areas*

642 Based on the important role in episodic encoding and
643 retrieval of the dorsolateral prefrontal cortices and of the
644 inferior parietal lobules (supramarginal and angular gyri),
645 as described in the introduction, we decided to focus
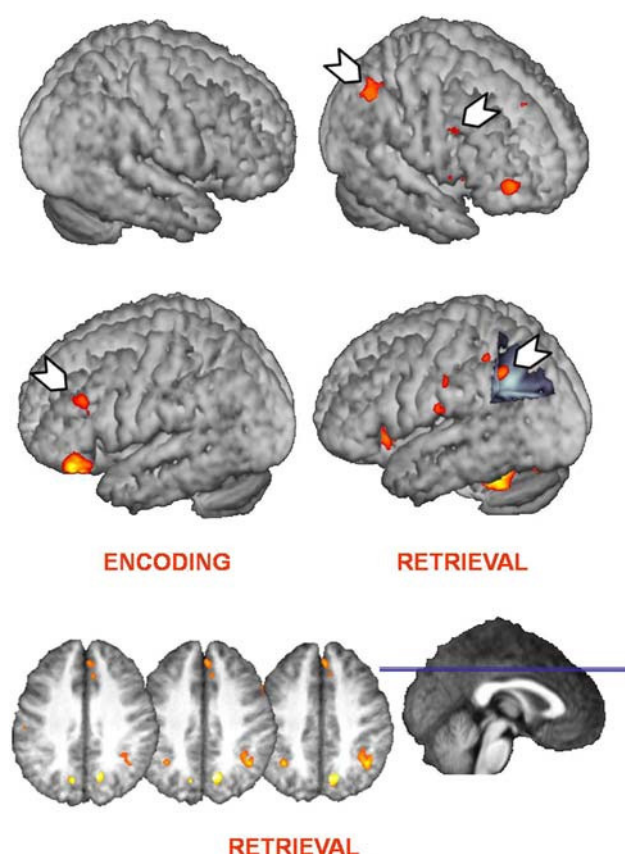646 on these anatomical structures for the rTMS study.

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

647 Consequently, of the activations reported above, we
648 selected the following stimulation target areas for retrieval:
649 RDLPFC ($x = 55$, $y = 16$, $z = 40$), RPARC ($x = 44$,
650 $y = -55$, $z = 44$) and LPARC ($x = -35$, $y = -56$,
651 $z = 43$).
652   For encoding, the stimulation of the pars orbitalis of the
653 left IFG was excluded, as we tested its stimulation in a
654 separate sample of subjects and it resulted in a remarkably
655 painful muscle contraction in the majority of them. We
656 therefore adopted a small volume correction procedure to
657 specifically look for LDLPFC activations in the *encoding*
658 conjunction, with an a priori anatomical hypothesis based
659 on previous rTMS studies on episodic encoding (Rossi
660 et al. 2001; Rossi et al. 2004; Sandrini et al. 2003). In this
661 studies, the 10–20 system was used to localize the coil on
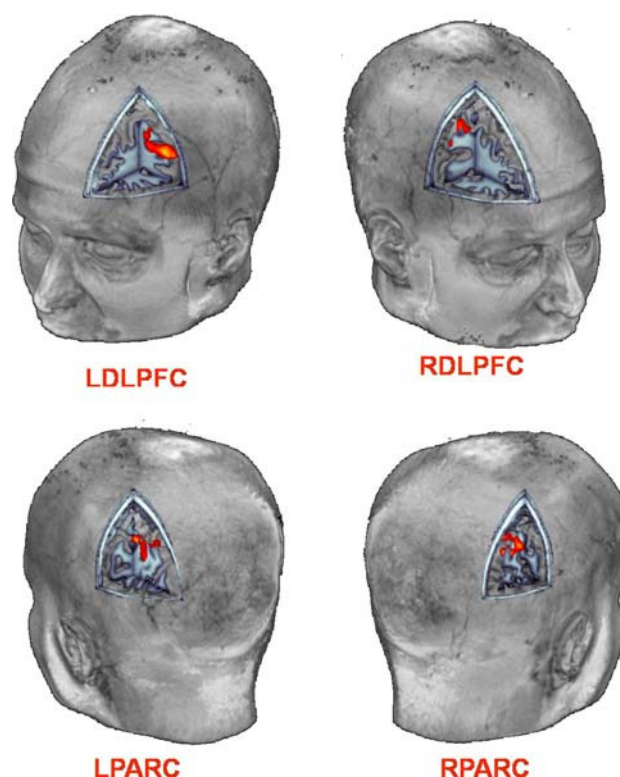662 F3 which approximately corresponds to the left DLPFC.

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

We therefore took the F3 estimated coordinate in the MNI 663
space ($x = -37$, $y = 26$, $z = 49$, see Herwig et al. 2003b) 664
as the center of a spherical volume of radius 20 mm for 665
small volume correction ($P < 0.05$, FDR corrected). We 666
found a significant activation that was selected as a stim- 667
ulation target area for encoding: LDLPFC ($x = -43$, 668
$y = 29$, $z = 34$; Z score $= 2.40$). 669

fMRI: Results of the SS Analyses 670

There was a considerable variability across subjects in the 671
topographical localization of the selected SS target areas 672
(Table 2; Fig. 3). 673

rTMS Results 674

First, we verified that for both, accuracy and RTs, perfor- 675
mance achieved during the sham condition in the rTMS 676
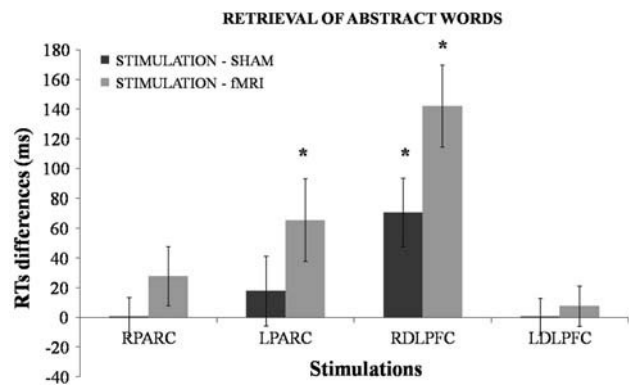
RETRIEVAL OF ABSTRACT WORDS

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, $P(\text{FDR}) = 0.0001$; comparison with fMRI baseline: 43–54 39, $P(\text{FDR}) = 0.017$] (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.
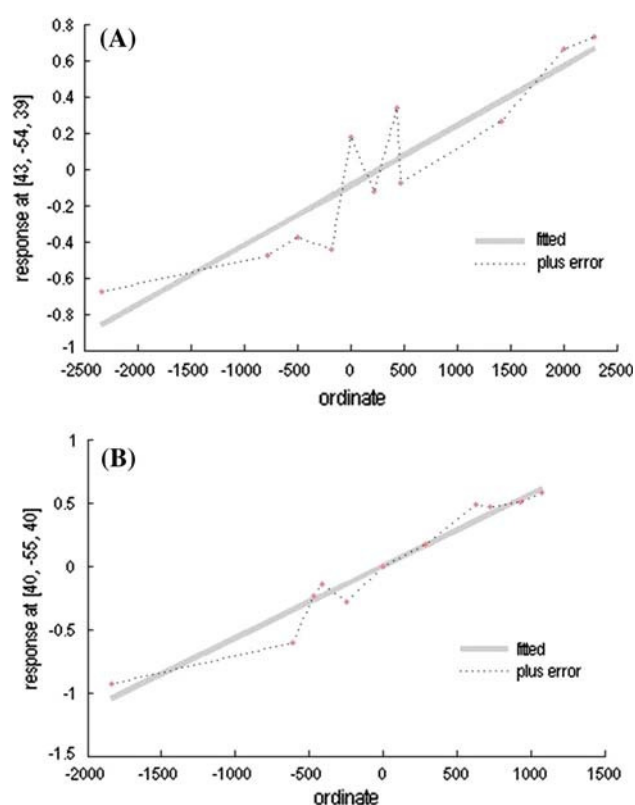
Fig. 5 Correlations between individual RPARC activation effect sizes during retrieval of abstract words and individual RPARC-Ret rTMS effects on performance (measured as differences between RTs during RPARC-Ret stimulation and RTs during fMRI (**a**) or during sham condition (**b**)

758 Moreover, since the PARCs seem to be differentially
759 involved in abstract and concrete word processing (Jessen
760 et al. 2000), we also wished to verify differences in acti-
761 vated areas (fMRI data) and in functional involvement of
762 the target areas (rTMS data) related to word category.
763 This was done using a combined fMRI and TMS
764 approach allowing the stimulation of the individual acti-
765 vation spots, thus taking into account the substantial
766 interindividual anatomo-functional differences in episodic
767 memory tasks, which might be related not only to structural
768 differences, but also to the use of different strategies.
769 Following the same logic of single subject-based analysis,
770 we also correlated the effect sizes of the SS activations
771 with the observed rTMS effect in each individual.
772 In the fMRI experiment, we found process-specific
773 (encoding versus retrieval) but no substantial category-
774 specific (abstract versus concrete) activation differences.
775 More specifically, similarly for abstract and concrete
776 words, encoding was selectively associated to an activation
777 of the LDLPFC, whereas retrieval selectively activated the
778 RDLPFC and the PARC, bilaterally. Accordingly, during
779 the rTMS experiment we stimulated these four areas for

780 both word categories. Since no category-specific activation
781 differences were found, we investigated different causality
782 of the same target areas for both word categories.
783 With respect to the results of the rTMS experiment, it
784 must be emphasized that we only found significant effects
785 when analyzing RT data; no significant effects of accuracy
786 were observed. This pattern was most likely due to the
787 relatively low level of difficulty of the task, with a small
788 number of items in each condition. RTs performance is
789 generally more sensitive than response accuracy in rTMS
790 studies. The type of effect is often related on increased
791 timing in the information processing (e.g., increased reac-
792 tion time) and, if a reduction of subject performance is
793 recorded, it is most likely explained by the complexity of
794 the processing needed to solve the task; for that reason the
795 effect of TMS may be related to the reduction of the dif-
796 ference between the signal and the noise present in the
797 system. Therefore, TMS can be regarded as an interference
798 method that can increase the timing for the information
799 processing or modify criteria for response decision. Effects
800 of rTMS on accuracy, in turn, are less frequently observed
801 (Manenti et al. 2008).
802 In addition, there were no significant rTMS effects for
803 concrete words. Consequently, it might be relevant to
804 underline that for what concerns the difference between
805 abstract and concrete words fMRI and TMS findings do not
806 fit well together. No substantial category-specific (abstract
807 versus concrete) activation differences were found during
808 the fMRI experiment, whereas a differential stimulation
809 effect between the two word categories was obtained in the
810 TMS experiment (i.e., no effect for concrete words). The
811 lack of TMS effects on concrete words could depend on the
812 higher performance level observed during the processing of
813 words of this category compared to abstract words (i.e., to a
814 ceiling effect obscuring any potential interference effect),
815 but it might also be that the areas targeted during stimulation
816 were more involved for abstract than for concrete words
817 processing, a difference not detectable using fMRI. This
818 divergence highlights, as previously suggested for a work-
819 ing memory study (Postle et al. 2006), that these two tech-
820 niques are quite different and thus likely to provide insights
821 into slightly different aspects of neural functioning.
822 For what concerned DLPFCs, our data were only par-
823 tially compatible with the DLPFC functional asymmetry
824 hypothesis in showing the functional relevance of RDLPFC
825 during retrieval (rTMS applied to these areas during
826 abstract word retrieval caused a slowing down of perfor-
827 mance), but in failing to demonstrate the functional
828 involvement of LDLPFC during encoding (Rossi et al.
829 2001; Rossi et al. 2004; Sandrini et al. 2003).
830 Several explanations could be proposed to justify the
831 lack of rTMS effects induced by the stimulation of the
832 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. with pictures (Rossi et al. 2004). 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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