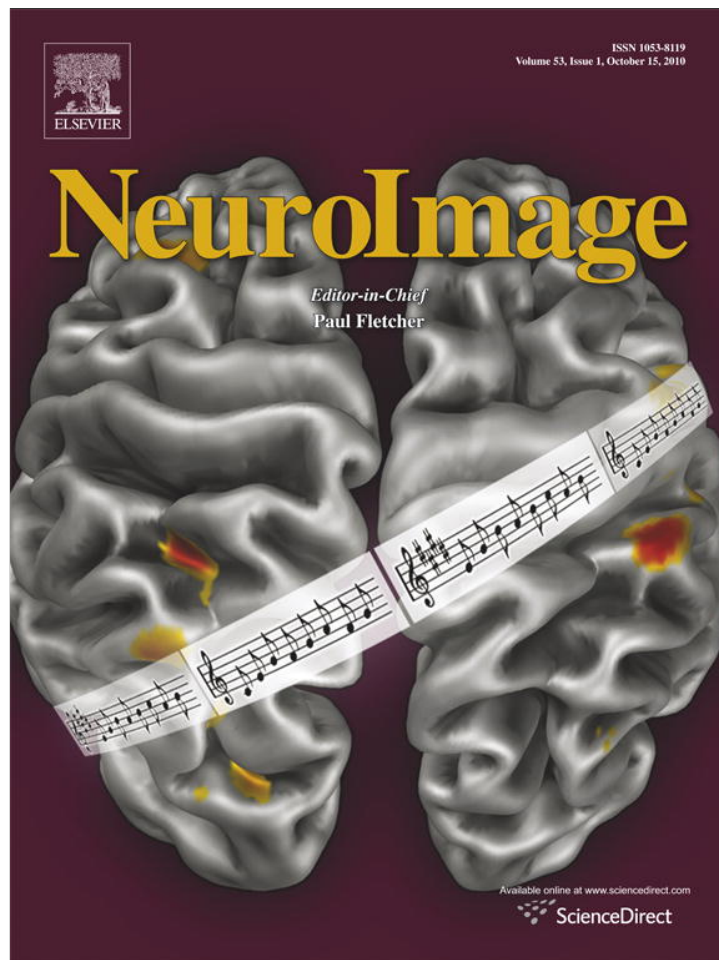


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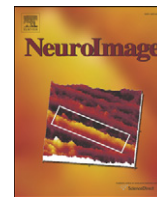
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## Event-related rTMS at encoding affects differently deep and shallow memory traces

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### ABSTRACT

The “level of processing” effect is a classical finding of the experimental psychology of memory. Actually, the depth of information processing at encoding predicts the accuracy of the subsequent episodic memory performance. When the incoming stimuli are analyzed in terms of their meaning (semantic, or deep, encoding), the memory performance is superior with respect to the case in which the same stimuli are analyzed in terms of their perceptual features (shallow encoding).

As suggested by previous neuroimaging studies and by some preliminary findings with transcranial magnetic stimulation (TMS), the left prefrontal cortex may play a role in semantic processing requiring the allocation of working memory resources. However, it still remains unclear whether deep and shallow encoding share or not the same cortical networks, as well as how these networks contribute to the “level of processing” effect. To investigate the brain areas casually involved in this phenomenon, we applied event-related repetitive TMS (rTMS) during deep (semantic) and shallow (perceptual) encoding of words. Retrieval was subsequently tested without rTMS interference. rTMS applied to the left dorsolateral prefrontal cortex (DLPFC) abolished the beneficial effect of deep encoding on memory performance, both in terms of accuracy (decrease) and reaction times (increase). Neither accuracy nor reaction times were instead affected by rTMS to the right DLPFC or to an additional control site excluded by the memory process (vertex). The fact that online measures of semantic processing at encoding were unaffected suggests that the detrimental effect on memory performance for semantically encoded items took place in the subsequent consolidation phase. These results highlight the specific causal role of the left DLPFC among the wide left-lateralized cortical network engaged by long-term memory, suggesting that it probably represents a crucial node responsible for the improved memory performance induced by semantic processing.

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### Introduction

The ability to consciously remember an experience requires its initial encoding and its subsequent retrieval from long-term memory. Psychological studies have shown that the level of processing at encoding, in terms of semantic or perceptual analysis of the incoming information, affects the probability of a successful retrieval (Lockhart and Craik, 1990). For example, by processing the meaning or implications of words (deep, semantic processing), it leads to a better memory retrieval than a shallow processing, which consists in a superficial judgment based on the perceptual analysis of the visual form or phonological structure.

Several brain regions [including prefrontal lobe, medial temporal lobe (MTL) and posterior association cortices] have been implicated in encoding by functional magnetic resonance studies (Simons and Spiers, 2003). A higher level of activation in the ventral portion of the prefrontal cortex (PFC) has been associated with deep processing (Fletcher et al., 2003; Kapur et al., 1994; Otten et al., 2001; Wagner et al., 1998). The PFC has been suggested to exert a top-down hierarchical control on MTL in encoding operations (Simons and Spiers, 2003). Within this region, the ventral area (VPFC), which includes the classical Broca's language area, is a good candidate for a role in semantic processing. In particular, there is extensive imaging evidence for a crucial role of the inferior frontal gyrus (IFG) in semantic access and lexical-semantic selection (Badre and Wagner, 2007; Schnur et al., 2009; Thompson-Schill et al., 1997). In contrast, the dorsolateral prefrontal cortex (DLPFC) is a good candidate for a supervising role in encoding, through the selection, organization and manipulation of the material to be remembered (Simons and Spiers,

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2003). The level of activation found in the left DLPFC is higher during semantic than nonsemantic encoding (Petersen et al., 1988; Kapur et al., 1994), and left prefrontal engagement decreases in parallel with the level of memory performance when semantic encoding is defective (Grady et al., 1995; Fletcher et al., 2003).

Functional magnetic imaging or positron emission tomography do not allow one to decide whether these activations are functionally necessary for successful remembering or whether they simply reflect epiphenomena of the level of memorization. Causality can be unveiled by the temporary interference that repetitive transcranial magnetic stimulation (rTMS) allows in a variety of cognitive processes (Hallett, 2007; Rossi and Rossini, 2004; Walsh and Cowey, 2000). A role of the DLPFC in verbal and visuospatial encoding is supported by several studies (Floel et al., 2004; Kahn et al., 2005; Kohler et al., 2004; Rami et al., 2003; Rossi et al., 2001, 2006; Sandrini et al., 2003), and its role is maintained throughout the entire life span (Rossi et al., 2004). Moreover, recent evidences suggest that the left DLPFC actively participates to the semantic categorization of objects (Viggiano et al., 2008), as well as to verbal working memory processes required for the semantic comprehension of sentences (Manenti et al., 2008) or for the reading span test (Osaka et al., 2007).

Therefore, the aim of this study was to assess the possible contribution of the DLPFC to semantic encoding. This has been postulated on the basis of previous event-related fMR investigations (Wagner et al., 1998), but not yet confirmed in a causal manner. Moreover, as an original approach, we contrasted online rTMS applied at encoding during both deep and shallow memorization tasks. To this end, four blocks of encoding were run, one without rTMS and three with rTMS delivered to the left or right DLPFC, or to a control site (i.e., the vertex), which is excluded by the memory process, to check for unspecific effects of the rTMS. Stimulation coincided with the presentation of each memorandum. Test items were an equal number of words requiring a standardized deep (discrimination between living or non-living things) or a shallow phonological encoding (identify whether the word contained the letter 'e') judgment. Encoding was followed by four corresponding blocks of incidental retrieval, free from rTMS application, each one containing an equal number of already seen (test) and new (distractors) words, both matched as semantic or perceptual content. Thus, the behavioral performance in each retrieval block allowed to causally address both the lateralization and functional specificity of left and right PFC in relation to the semantic/perceptual task at encoding.

## Materials and methods

### Subjects

Eighteen healthy volunteers (nine women; mean age 27.7 years, range 19–36 years) with normal or corrected-to-normal vision and no history of implanted metal devices or neurological disease were included in the study. All subjects were right-handed according to the Edinburgh handedness inventory (mean dexterity index 90%, range 75–100%), except one (dexterity index 35%). The study was performed according to the Declaration of Helsinki and the local ethics committee approved the use of rTMS. All subjects gave their written informed consent and were asked to report adverse effects experienced during or after rTMS.

### Experimental protocol

Subjects were seated in a comfortable chair in front of a 17-in monitor. The experimental protocol consisted of four blocks of incidental encoding and four blocks of retrieval tasks. Encoding was obtained during two different categorization tasks, whereas retrieval was assessed by a recognition memory task. Stimuli were 320 Italian words of high frequency, ranging between 4 and 12 letters in length.

Words were presented on the center of a computer screen for 500 ms, with an inter-trial interval of 5000 ms and were preceded by a fixation point for 300 ms. Four blocks of 40 words matched for frequency and length were used for encoding. In each block, stimuli were arranged in two lists of 20 words corresponding to two levels of processing (semantic and perceptual processing, respectively). In the semantic task (deep encoding), subjects had to judge whether the presented word indicated living or non-living things, by pressing with the index finger of the dominant hand one of the two mouse buttons (left, living; right, non-living) as quick as possible after categorization. In the perceptual task (shallow encoding), subjects had to decide whether the word contained the letter 'e' (left button, containing e; right, non-containing e). Each list of words was selected such that the proportion of living and non-living words containing the letter e was identical. In each block, the order of the word presentation and the order of the tasks were randomized.

Ten minutes after the encoding phase, subjects performed an unexpected memory test (incidental retrieval phase). The four encoding blocks had four corresponding retrieval blocks. Each retrieval block consisted of 40 words presented in the encoding phase (20 deep encoded and 20 shallow encoded) and 40 novel words (distractors) randomly displayed. The timing of warning, word presentation, and inter-trial intervals were the same as in the encoding phase. Subjects were requested, in a yes–no recognition task, to answer whether the presented word had been already shown in the encoding phase by pressing one of the two buttons (left, "old"; right, distractor) as quickly as possible after recognition.

### Procedures of transcranial magnetic stimulation

Repetitive TMS was delivered using a Magstim SuperRapid stimulator with a biphasic current waveform (Magstim Co., UK), connected to an eight-shaped coil (outer diameter of each wing, 7 cm). The coil was placed tangentially to the scalp, with the handle pointing backwards and 45° away from the midline. Prior to rTMS, single magnetic pulses were delivered by the same coil and stimulator to the hand area of either primary motor cortex to establish the individual excitability threshold for the first dorsal interosseous muscle (FDI). Then, stimulator output was set to an intensity of 90% threshold of the FDI contralateral to the stimulated hemisphere (mean intensity  $\pm$  SD:  $48.3 \pm 5.6\%$  of the maximal stimulator output, without significant interhemispheric differences). When required by the experimental design, 500 ms trains of 10-Hz rTMS were delivered simultaneously to the presentation of each word. Such a combination between intensity and length of stimulation, inter-train intervals and number of trains falls within safety limits of rTMS application (Rossi et al., 2009).

In the encoding phase, both tasks (deep and shallow processing) were performed in four different experimental conditions (one block of 40 words for condition): no rTMS (Baseline), rTMS of the left DLPFC, rTMS of the right DLPFC, and rTMS delivered on the Vertex, as a control site to check for unspecific rTMS effects. The left and right DLPFCs were stimulated on the scalp regions corresponding to the Brodmann area 9, according to a previously detailed anatomical localization procedure (Rossi et al., 2006). No rTMS application was scheduled by the protocol during the retrieval phase.

The order of experimental conditions and coupling between blocks of words and experimental conditions were randomized and counter-balanced across subjects. The experimental phase was preceded by a training phase to familiarize subjects with rTMS and the task (different words were used in this phase).

### Data analysis

For each participant, the behavioral performances were separately evaluated in each experimental condition and type of encoding (deep

and shallow) through measures of accuracy and by the mean RT between the beginning of the presented word and the motor response.

For the encoding phase, the measure of accuracy was the mean percentage of correct responses.

For the retrieval phase, d-prime (d') and criterion (C), two psychometric measures derived from signal detection theory, were calculated (Macmillan and Kaplan, 1985; Macmillan and Creelman, 1997). These measures reflect the ability to distinguish between 'true' items and distractors and to reject distractors during a recognition memory task. In the present tasks, d' can be interpreted as the ability to discriminate between already seen and novel words and C can be considered as an index of the 'willingness' of a subject to endorse words as old.

Then, dependent variables (accuracy and RT for encoding and d', C and RT for the recognition task, respectively) were entered in separate two-way repeated-measures ANOVA with Experimental condition (4 levels) and Encoding (two levels: deep and shallow) as within-subject factors.

To correct violations of the sphericity assumption, Greenhouse–Geisser corrections were applied when necessary. Post hoc tests were performed using the Tukey test.

To investigate the relationship between individual memory performance as assessed by d' measure, RT and C, Pearson correlation coefficient was calculated by data cumulated from all experimental conditions. For all analyses significance was set at  $p < 0.05$ .

## Results

None of the participants reported adverse or side effects during or after rTMS application. Table 1 summarizes results: during the encoding phase, the behavioral performance, as indexed by the accuracy (percentage of correct responses) and the reaction time (RT) of the responses, varied with the level of processing at encoding, as tested by using two different categorization tasks (accuracy:  $F_{1,17} = 10.615$ ,  $p = 0.005$ ; RT:  $F_{1,17} = 7.796$ ,  $p = 0.013$ ). In general, subjects were slightly less accurate but faster in performing the shallow encoding compared to the deep encoding task (Table 1). Repetitive TMS did not influence the ability of the subjects to perform the two tasks, as shown by the absence of a main effect of the different experimental conditions on the behavioral performance (accuracy:  $F_{3,17} = 0.802$ ;  $p = 0.499$ ; RT:  $F_{3,17} = 0.983$ ;  $p = 0.408$ ) and by the lack of interaction between experimental condition and task (accuracy:  $F_{3,51} = 0.478$ ;  $p = 0.699$ ; RT:  $F_{3,51} = 0.460$ ;  $p = 0.711$ ).

In the retrieval phase, the behavioral performance was indexed by d-prime (d') and, as an ancillary measure, by criterion (C), two measures of the signal detection theory reflecting hit rates and false alarm rates. Namely, d' estimates the ability to discriminate between 'already seen' and 'never seen' words and C is inversely correlated to the rate of false positives (i.e. when a subject erroneously answers

that a distractor has been seen in the encoding phase). RT of the responses were also recorded and analyzed.

Both d' and RT, as well as C, were influenced by the level of processing (d':  $F_{1,17} = 26.829$ ,  $p < 0.001$ ; C:  $F_{1,17} = 26.809$ ,  $p < 0.001$ ; RT:  $F_{1,17} = 14.289$ ,  $p = 0.001$ ). As expected, subjects were less accurate and slower retrieving the shallowly encoded than the deeply encoded words (Table 1, Figs. 1 and 2). The main effect of experimental condition on d' and C was not significant (d':  $F_{3,17} = 1.464$ ,  $p = 0.235$ ; C:  $F_{3,17} = 0.734$ ,  $p = 0.536$ ).

However, the effect of rTMS on both measures definitely emerged by the interaction between the experimental condition and the categorization task (d':  $F_{3,51} = 3.793$ ,  $p = 0.038$ ; C:  $F_{3,51} = 3.791$ ,  $p = 0.038$ ). Post hoc comparisons revealed that, specifically for deeply encoded words, d' was significantly lower with rTMS to the left DLPFC with respect to Baseline, right DLPFC, and Vertex conditions ( $p = 0.011$ ,  $p = 0.014$ , and  $p = 0.045$ , respectively), whereas C was significantly higher with rTMS to the left DLPFC with respect to the Baseline alone ( $p = 0.015$ ) (Table 1 and Fig. 1). In contrast, d' and C did not vary across experimental conditions for shallow encoded words (Table 1 and Fig. 1).

The main effect of experimental condition on RTs was significant ( $F_{3,17} = 3.428$ ,  $p = 0.024$ ). Post hoc comparisons showed that RT was significantly longer with rTMS to the left DLPFC with respect to right DLPFC alone ( $p = 0.022$ ) (Table 1 and Fig. 2, panel A). As longer RTs with rTMS of the left DLPFC were seen for both deep and shallow encoded words (Table 1 and Fig. 2, panel A), the interaction between the experimental condition and the categorization task was not significant ( $F_{3,51} = 2.247$ ;  $p = 0.116$ ).

When data from all experimental conditions were pooled, d' significantly correlated with RTs ( $r = -0.178$ ,  $p = 0.033$ ), suggesting that a lower memory performance in retrieval was associated with longer RTs and with a less efficient recognition strategy (Fig. 2, panel B). The correlation between d' and RT reached a closely significance level when the left DLPFC was stimulated ( $r = -0.306$ ;  $p = 0.07$ ).

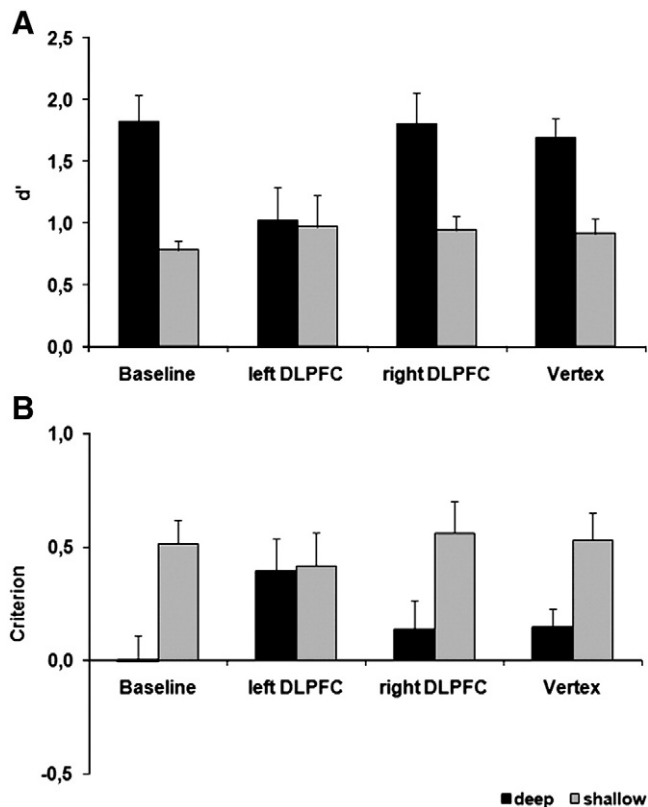
## Discussion

The main result of this study is that rTMS stimulation of the left DLPFC at encoding abolishes the beneficial effect of semantic analysis on accuracy at retrieval. The effect is specific for memory performance, and extends previous TMS results pointing to an involvement of the left DLPFC in the working memory operations required for semantic categorization and sentence comprehension (Osaka et al., 2007; Manenti et al., 2008). As encoding accuracy per se was not affected, the interference probably took place in the subsequent consolidation phases of the memory trace, in agreement with the results of a recent study in which the temporal dynamics of episodic encoding have been specifically addressed (Rossi et al., in press).

When no rTMS was applied, or when rTMS had been applied to a control brain region not engaged in the memory process, subjects

**Table 1**  
Behavioral data (mean ± sd) in the different experimental conditions for encoding and retrieval phases.

	Baseline (no rTMS)		Left DLPFC rTMS		Right DLPFC rTMS		Vertex rTMS	
	Deep	Shallow	Deep	Shallow	Deep	Shallow	Deep	Shallow
<i>Encoding</i>								
Accuracy (%)	97 ± 3	95 ± 5	97 ± 3	95 ± 4	95 ± 5	94 ± 7	97 ± 3	94 ± 5
RT	772 ± 145	688 ± 187	767 ± 157	729 ± 190	805 ± 162	746 ± 195	778 ± 127	710 ± 193
<i>Retrieval</i>								
Hits (%)	77 ± 14	45 ± 18	54 ± 26	52 ± 22	71 ± 20	47 ± 24	74 ± 16	48 ± 21
False alarms (%)		21 ± 13		23 ± 17		18 ± 13		18 ± 12
d'	1.82 ± 0.93	0.78 ± 0.34	1.02 ± 1.16	0.97 ± 1.08	1.80 ± 1.09	0.94 ± 0.46	1.69 ± 0.68	0.91 ± 0.53
Criterion C	-0.01 ± 0.49	0.51 ± 0.45	0.39 ± 0.61	0.42 ± 0.60	0.13 ± 0.55	0.56 ± 0.60	0.14 ± 0.36	0.53 ± 0.49
RT	1027 ± 300	1114 ± 18	1223 ± 16	1238 ± 86	950 ± 268	1099 ± 88	1020 ± 90	1118 ± 323



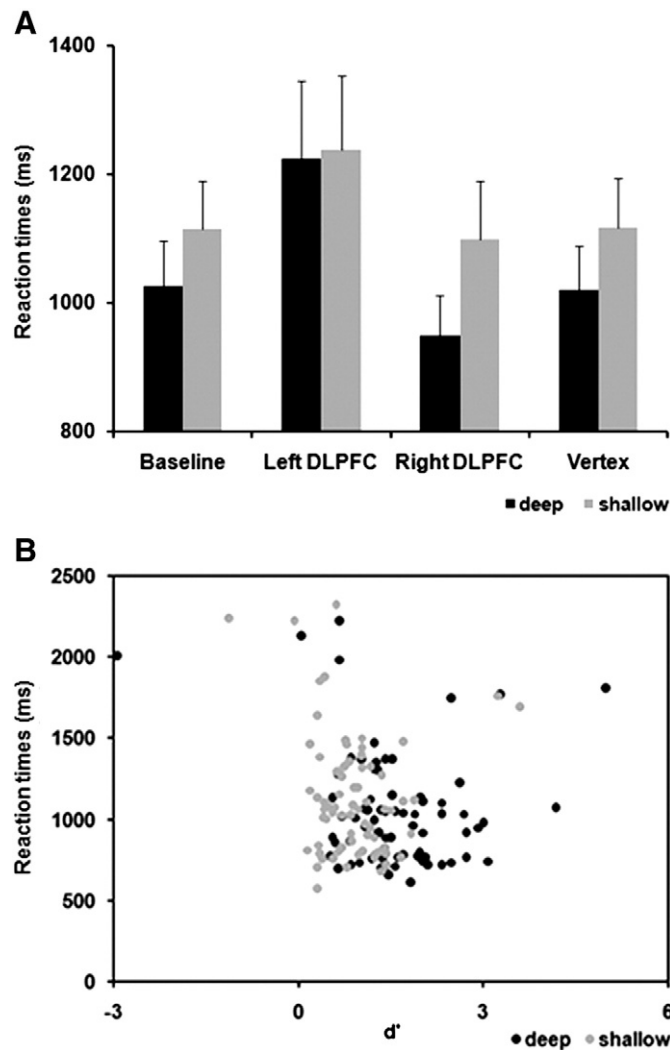
**Fig. 1.** Results of accuracy. Accuracy in retrieval (pooled subjects) for semantically (black bars) and perceptually (grey bars) encoded words in the different conditions. Accuracy drop ( $d'$ ) and changes of C are evident only for semantically encoded words after left DLPFC stimulation.

were slower in encoding words requiring a semantic (deep) rather than a perceptual (shallow) process, and were more accurate and faster retrieving them (see Table 1, baseline column and vertex TMS column, and Fig. 2, panel A).

When rTMS had been applied to the left DLPFC at encoding, RTs at retrieval were longer for both semantically and perceptually encoded words, while the ability to distinguish old/new stimuli, as indexed by the  $d'$  measure, dropped significantly only in case of semantically encoded words. In addition, the drop of accuracy and lengthening of RTs were also correlated (Fig. 2, panel B).

In contrast, rTMS interference applied on the right DLPFC or to a control site at encoding did not affect either the time required to retrieve test items or the accuracy. This makes it very unlikely that the performance decline could be attributed to inadvertent blinking or eye movements due to rTMS applied to the DLPFCs during stimulus presentation, resulting in a reduction of the time of stimulus observation. In addition, we visually monitored the subjects throughout the session. In fact, a perfect symmetry between the coil positioning in the two hemispheres cannot be assumed, and even a minimal jitter may theoretically elicit facial movements. No facial movements ipsilateral to the TMS site were not observed, confirming that the stimulation was subthreshold.

The interference of the rTMS delivered on the left DLPFC was relevant in terms of effect size, and appears to “reset” the high-functioning neural network recruited by deep encoding processes to a lower level of performance, comparable to the effects of shallow encoding (Fig. 1A). This concept is also supported by the unexpected finding of a selective increase of the ancillary index C induced by left DLPFC stimulation for deeply encoded words, which raised up to the same level of the shallowly encoded stimuli (Fig. 1B). It can be hypothesized that the left DLPFC stimulation during encoding modified the subjects’ recognition strategy, making them generally



**Fig. 2.** Results of reaction times. Panel A): Reaction times in retrieval (pooled subjects) for semantically (black bars) and perceptually (grey bars) encoded words in the different conditions. Bars are standard error. Left DLPFC rTMS increases RTs irrespective of the deep or shallow encoding. Panel B): Correlation between accuracy ( $d'$ ) and average response time (pooled conditions) for each subject, during retrieval of deep (black symbols) and shallow (grey symbols) encoded words.

more conservative towards false alarms. In other words, this effect underscored that the lower memory performance was exclusively due to a decay of the memory trace for the encoded test items rather than an increase of false alarms rate.

Taken together, these findings causally suggest that the left DLPFC in encoding is engaged for both semantically and phonologically processed items, as indicated by the lengthening of RTs at retrieval for both conditions. Additionally, the interference with left DLPFC at encoding abolished the “level of processing effect” induced by semantic demands, leaving unaltered the memory performance at retrieval for the phonologically encoded words. These findings are in line with the notion that the behavioral rTMS impact is strictly state-dependent (Silvanto et al., 2008; Rossi et al., 2009; Thut and Pascual-Leone, 2010): the more a region is engaged in a given task, the more the rTMS-induced interference is likely to impact that task.

Previous correlational neuroimaging investigations put forward the hypothesis of a left-sided specificity of the PFC in building up an efficient memory trace selectively for semantically encoded words (Fletcher et al., 1998; Wagner et al., 1998; Lidaka et al., 2000). The majority of these studies suggested a primary role for the ventral part of the PFC [or left inferior PFC (LIPFC)] during semantic processing (Buckner et al., 1999; Fletcher et al., 2000; Gabrieli et al., 1998; Nyberg

et al., 1996; Tulving et al., 1994), as indicated by higher activation levels of the LIPFC when compared to those induced by perceptually or phonologically encoded cues (Kapur et al., 1994; Petersen et al., 1988), in agreement with the well-known role of this region in phonological, lexical-semantic and morphosyntactic processing (Dobbbins et al., 2002; Grodzinsky and Santi, 2008). However, a recent meta-analysis suggested that lesions of the left PFC (Brodmann areas 8 and 9) may specifically affect self-guided, goal-directed retrieval of semantic information (Binder et al., 2009).

The causal role of the DLPFC indicated by our study is open to different interpretations. Episodic memory functioning requires continuous PFC-MTL interactions, with a top-down hierarchical control from PFC to MTL during encoding, which may be actually modulated by the DLPFC (Simons and Spiers, 2003). Indeed, causal evidences are accumulating that the supervising role of the left PFC in encoding is supra-modal, since it has been described for complex pictures (Rossi et al., 2001, 2004, 2006), faces (DLPFC, Turriziani et al., 2008; IFG, Feurra et al., 2010) and purely verbal stimuli (Floel et al., 2004; Rami et al., 2003; Sandrini et al., 2003). It is thus likely that interfering with rTMS on the left DLPFC disrupts hierarchically the building up of an efficient memory trace. This would also imply that the disturbing action of the rTMS does not necessarily take place only at the point of application, but that trans-synaptic effects might spread to the whole PFC-MTL anatomic-functional complex. Future comparative studies should better address whether similar rTMS trains applied on the VLPFC, rather than on the DLPFC, will have more effect on semantic memory impairment. Currently available literature in this sense is largely contradictory: Kohler and co-workers, using a non focal coil and 7 Hz rTMS at encoding, found that encoded words during left VLPFC rTMS were subsequently recognized with higher accuracy than those under stimulation of two additional cortical control sites (Kohler et al., 2004), whilst both single-pulse (Kahn et al., 2005) and paired-pulse (Machizawa et al., 2010) TMS applied at different delays to the left VLPFC at encoding, impaired subsequent recognition performance. Furthermore, TMS interference with VPFC, and in particular with left IFG, has a detrimental effect on semantic processing per se (Gough et al., 2005), which would have biased the main experimental hypothesis of the current study. Therefore, at this stage of the research project, such divergent effects of VLPFC TMS led us to target the left DLPFC, an approach that had already provided more consistent information on episodic memory mechanisms (see above).

In alternative, the functional specialization of PFC sub-regions could be relative rather than absolute (Duncan, 2001). They might be commonly recruited together, adapting their function depending on the nature of the task being undertaken (Duncan and Owen, 2000; Duncan, 2001) and, according to this state-dependency, changing their susceptibility to the interfering effect of the rTMS.

Along this reasoning vein, there is emerging evidence that deep encoding, in the frame of an incidental learning task as in the current study, is associated with power decrease of alpha (8–12 Hz) and beta (12–20 Hz) oscillatory activity, while shallow encoding processing are associated with increased power of theta (4–8 Hz) oscillations in the PFC (Freedman et al., 2001). Most importantly, semantically encoded, successfully remembered, items are associated with increase of gamma (55–100 Hz) activity (Hanslmayr et al., 2009), suggesting a potential functional specificity of brain rhythms (Thut and Miniussi, 2009). Of note, rhythmic brain stimulation has been shown to selectively improve cognitive/perceptual processes when synchronously applied at individual alpha rhythms (Kanai et al., 2008; Klimesch et al., 2003), the so called “entrainment” phenomenon (Thut and Miniussi, 2009). It can thus be hypothesized that the higher is the difference between the frequency of brain stimulation (10 Hz in the current study) and the one of the regional oscillatory activity associated with a given cognitive task (such as gamma range in semantic encoding), the higher is the probability of a successful interference during event-related, online rTMS. In other words,

disproportionately out-of-phase rTMS with respect to the intrinsic oscillatory brain activity seems a plausible mechanism to explain how online rTMS may inject neural noise – or random depolarization of neurons (Harris et al., 2008) in the cognitive process being undertaken, thereby temporarily disrupting task performance. Future combined TMS-EEG studies, which are disclosing complex effects of rTMS on endogenous patterns of network-level oscillations (Hamidi et al., 2009), are required to definitely test this hypothesis.

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