# Effects of Left Inferior Prefrontal Stimulation on Episodic Memory Formation: A Two-Stage fMRI—rTMS Study

# Effects of Left Inferior Prefrontal Stimulation on Episodic Memory Formation: A Two-Stage fMRI–rTMS Study

**Stefan Köhler[1], Tomáš Paus[2], Randy L. Buckner[3], and Brenda Milner[2]**

## Abstract

■ Successful recovery of words from episodic memory relies strongly on semantic processes at the time of encoding. Evidence from several functional magnetic resonance imaging (fMRI) studies has shown that changes in neural activity in the left inferior prefrontal cortex (LIPFC) during semantic encoding predict subsequent memory performance. This evidence has been taken to suggest that LIPFC plays a critical role in memory formation. Functional neuroimaging findings, however, do not establish a causal brain–behavior relationship. To determine whether there is a causal link between LIPFC involvement at encoding and subsequent success in memory performance, we conducted a two-part study in which we first used fMRI to localize encoding-related activation in LIPFC and then employed repetitive transcranial magnetic stimulation (rTMS) to manipulate neural processes in LIPFC during semantic encoding. To demonstrate the neuroanatomical specificity of any observed effect and to control for nonspecific rTMS side effects, we also stimulated neural processes in two control sites. Using frameless stereotaxy, we positioned the stimulation coil to target (1) the LIPF region that was activated during fMRI (mean $xyz = -48\ 35\ 5$); (2) the homologous right-hemisphere region; and (3) an additional left parietal control site. At each site, ''stimulated'' items (600 msec of 7-Hz rTMS with Cadwell Round Coil) were intermixed with items presented without concurrent stimulation. Subsequently, subjects performed a recognition memory task for the words encountered. We found support for the predicted causal brain–behavior relationship, which was specific to LIPFC. When comparing recognition scores for stimulated items, normalized for variations in performance on nonstimulated trials, we found that words encoded under LIPFC stimulation were subsequently recognized with higher accuracy than words encoded under stimulation in the two cortical control sites. By contrast, no performance difference emerged when the two control sites were compared with each other. Based on additional analyses of the rTMS effects observed directly at the time of encoding (i.e., on semantic-decision performance), we suggest that LIPFC stimulation may have produced its effect on recognition memory, at least in part, through the triggering of more extensive processing of the stimulated items and an ensuing gain in item distinctiveness. Physiological processes of facilitation probably also contributed to the observed memory benefit. Together, these findings suggest that LIPFC does play a causal role in episodic memory formation. ■

## INTRODUCTION

Behavioral studies have shown that cognitive processes engaged during the initial experiencing, or encoding, of an event strongly influence how well such an event will be remembered later. This phenomenon is most clearly reflected in the levels-of-processing effect (Craik & Lockhart, 1972): In most situations, semantic processing of words during encoding (e.g., deciding whether a word describes something animate or inanimate) leads to superior recovery of these words on a subsequent memory test compared to perceptual processing of the same stimuli (e.g., deciding whether a word is written in upper- or lowercase letters). Neuroimaging studies with positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) have shown that this effect has neural correlates in the prefrontal cortex. Numerous experiments have been reported in which semantic encoding of words was accompanied by higher activation in the left inferior prefrontal cortex (LIPFC) than perceptual or phonological encoding of the same words (e.g., Kapur et al., 1994; Petersen, Fox, Posner, Mintun, & Raichle, 1988). Typically, these studies have relied on experimental designs in which trials from the two different encoding conditions were grouped in blocks and activity was compared across blocks, thus precluding a direct comparison between encoding trials that lead to successful memory performance and those that do not. More recently, however, the advent of event-related fMRI has allowed investigators to examine brain activity more directly in relation to memory formation. Wagner et al. (1998), extending from paradigms developed using event-related potentials (ERPs; Paller & Wagner, 2002; Rugg 1995; Paller et al., 1987), showed that words that could later be recognized successfully were associated with higher LIPFC activation during semantic encoding than those that could not. This pattern of results, which is referred to as the subsequent memory effect, mirrored previously reported data from

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ERP studies, which demonstrated different EEG responses over the frontal cortex for subsequently remembered and subsequently forgotten words during semantic encoding (see Rugg, 1995, for a review). Evidence from recent fMRI research also indicates that there is an overlap between brain regions in the prefrontal cortex that show the levels-of-processing effect and those that show the subsequent memory effect for semantic encoding (Baker, Sanders, Maccotta, & Buckner, 2001; Otten, Henson, & Rugg, 2001; Otten & Rugg, 2001; Wagner et al., 1998). This overlap has been observed most consistently, although not exclusively, in the most anterior and ventral aspects of the LIPFC.

The summarized findings have been taken as support for the notion that the LIPFC plays an important role in memory formation of semantically processed information (Wagner, 2002; Fletcher, Shallice, & Dolan, 2000; Buckner, Kelley, & Petersen, 1999; Gabrieli, Poldrack, & Desmond, 1998; Nyberg, Cabeza, & Tulving, 1997; Tulving, Kapur, Craik, Moscovitch, & Houle, 1994). However, it is commonly acknowledged that functional neuroimaging data only point to brain regions that are involved in a given behavior; correlations between localized brain activity and task performance cannot establish a causal brain–behavior relationship. Thus, with respect to the findings described, it is unclear whether activation in LIPF during semantic encoding determines causally the level of performance on a subsequent memory task for the encoded stimuli. To address this issue, we conducted a two-part study on semantic encoding of words, which employed fMRI and repetitive transcranial magnetic stimulation (rTMS). The latter technique allows for the reversible experimental manipulation of neural activity in brain regions of interest while individuals engage in a cognitive task (Jahanshahi & Rothwell, 2000; Walsh & Cowey, 2000). Here, we were interested in examining whether stimulation of the LIPF with rTMS during semantic encoding would affect memory formation and, as a result, subsequent memory performance.

We first used fMRI to localize activation in the LIPFC related to semantic encoding in 12 subjects. We then administered rTMS in the same subjects during performance of the same semantic encoding task; using frameless stereotaxy, we targeted the most anterior and ventral aspect of the LIPFC that was activated in fMRI in these subjects. To demonstrate the neuroanatomical specificity of any observed effect and to control for nonspecific rTMS side effects, such as head-muscle twitches, discomfort, or intersensory facilitation associated with loud noise and tactile stimulation of the scalp (Rossi et al., 2001; Terao et al., 1997), we also stimulated the homologous right-hemisphere region (referred to as RIPFC) and an additional site in the left superior parietal cortex (LPC). These regions allowed for the control of side effects across lobes and hemispheres; they were chosen because they have usually failed to show activation related to the levels-of-processing effect in past neuroimaging research. Following stimulation, subjects performed a recognition task for the words encountered across all experimental conditions; the resulting accuracy data provided the critical information for the examination of rTMS effects on memory formation.

We predicted that if there is a causal relationship between neural processes in the LIPFC during semantic encoding and subsequent memory performance, manipulation of these neural processes with rTMS at the time of encoding should have a unique effect on subsequent recognition performance that is distinguishable from any nonspecific side effect of rTMS estimated in the control sites. In other words, evidence to support this prediction would come from a pattern of results in which recognition performance for words encoded under LIPFC stimulation differs from performance in both control sites, while both control sites do not differ from each other. It is well established that rTMS can have either facilitatory or inhibitory effects on behavior, including on memory (see Grafman & Wassermann, 1999); the precise conditions, however, that determine the direction of the effect are not fully understood at present. For the specific hypothesis tested here, the direction of the predicted effect was not critical; support for our hypothesis could come from results showing that rTMS in the LIPFC improves or worsens subsequent memory performance as compared to stimulation in the control sites.

## RESULTS

### fMRI Findings

In line with past research findings, we observed a robust increase in the fMRI BOLD signal (''activation'') in the LIPFC when semantic encoding was compared to nonsemantic encoding. In the group analysis, four distinct peaks were identified in the left prefrontal cortex for this comparison; two of these peaks were found in dorsolateral aspects of the prefrontal cortex ($xyz = -52\ 18\ 32$; $t = 9.45$; $xyz = -50\ 28\ 18$; $t = 7.81$), whereas the other two were located more ventrally in the LIPFC ($xyz = -52\ 28\ 6$; $t = 9.21$; $xyz = -50\ 36\ 0$; $t = 8.83$). The more anterior of these LIPFC peaks was chosen as the target site for rTMS because this aspect of LIPFC has consistently been reported to show differential activation related to semantic encoding (replicated here) as well as activation reflecting the subsequent memory effect in past research (Baker et al., 2001; Otten et al., 2001; Wagner et al., 1998). When the fMRI results for individual subjects were examined, we found anterior LIPFC peaks in the immediate vicinity of this anterior group peak in 10 of the 11 subjects for whom valid fMRI data were available. Importantly, as predicted, neither the homologous right frontal region (RIPFC) nor the left superior parietal region (LPC) that served as control sites for rTMS showed significant

**Table 1.** Talairach Coordinates for the Center of the Targeted Three Stimulation Sites Averaged Across Subjects

| | *X* | *Y* | *Z* |
|---|---|---|---|
| LIPFC | −48 (−57, −40) | 35 (24, 46) | 5 (−3, 11) |
| RIPFC | 45 (32, 57) | 35 (24, 46) | 5 (−9, 13) |
| LPC | −43 (−43, −43) | −63 (−63, −63) | 34 (34, 34) |

Minima and maxima across subjects are shown in parentheses.

activation related to semantic encoding in the present group of subjects.

### rTMS Findings: Effects on Subsequent Recognition Performance

Table 1 shows the standardized stereotaxic coordinates (Talairach & Tournoux, 1988), averaged across subjects, for the stimulation sites in the LIPFC, RIPFC, and LPC that were used for the administration of rTMS during semantic encoding. The location of the LIPFC peaks in individual subjects and the group mean are shown in Figure 1a; Figure 1b illustrates the positioning of the rTMS coil during LIPFC stimulation in relation to fMR activation for one subject. In each of the three testing blocks that correspond to the different stimulation sites, items that were presented in combination with rTMS were intermixed with items presented without concurrent stimulation, while subjects made semantic decisions (Figure 2A and B). To examine stimulation effects on subsequent recognition performance, we concentrated on the percentage of previously encountered items that subjects recognized with high confidence. This measure is thought to be a more reliable marker for the examination of subsequent memory effects than total recognition accuracy, which does not take confidence into

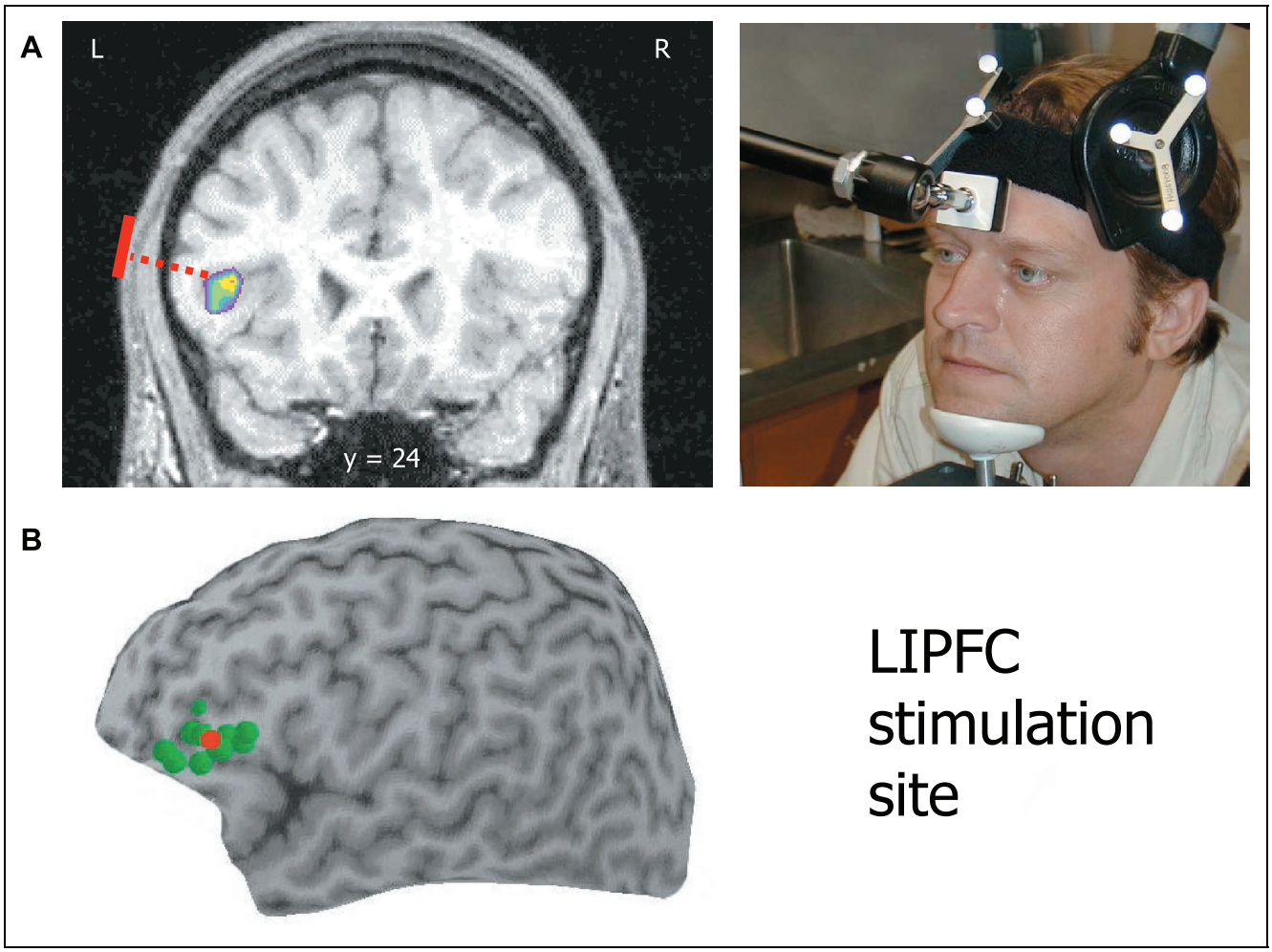


**Figure 1.** Positioning of the rTMS coil during LIPFC stimulation. (A) Positioning of coil in relation to fMRI activation in 1 of the 12 subjects examined. The left image shows the most anterior fMRI peak in the LIPFC for the semantic versus nonsemantic encoding comparison. The right image shows the positioning of the coil to target this site. Note that the tear-shaped part of the coil is positioned directly over the target and that the upper edge of the coil is tilted away from the skull. (B) LIPFC target sites in the 12 individual subjects (green) and the mean target site averaged across subjects (red). Locations were rendered onto a partial 3-D curvilinear reconstruction of a template brain in Talairach and Tournoux (1988) space with the surface shown 15 mm below the outer cortical surface. The image was reconstructed using Brainsight software for image-guided coil positioning.

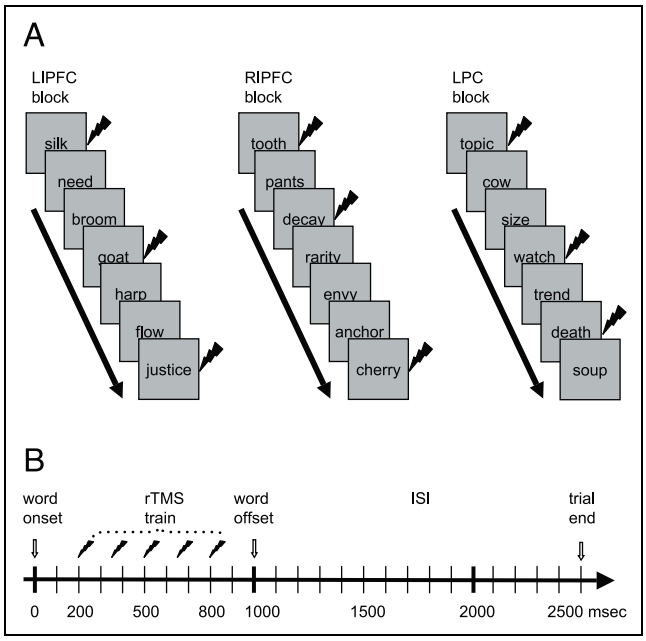


**Figure 2.** Details of the stimulation protocol in the rTMS part of the study. (A) rTMS was administered during behavioral testing in three different blocks corresponding to three different stimulation sites. Subjects performed the same semantic decision task across all blocks. In each block, trials including concurrent stimulation (indicated with an arrow) were intermixed with trials including no stimulation. One to three nonstimulated items intervened between the presentations of neighboring stimulated trials. (B) The diagram shows the timing of events within all stimulated trials. In each rTMS train, five pulses were delivered at a frequency of 7 Hz; each train started 200 msec after stimulus onset and finished 200 msec before stimulus offset; 1500 msec elapsed between stimulus offset and the beginning of the next trial.

account (for a discussion, see Paller & Wagner, 2002). However, results for recognition scores that included the total number of responses are also reported for completeness; the outcomes of the statistical analyses were the same for both types of scores.

Behavioral data for the nonstimulated trials served as baseline for each stimulation site; stimulation effects were estimated by normalizing the data for stimulated trials to these baseline scores, i.e., by computing difference scores between stimulated and nonstimulated trials on a subject-by-subject basis for each site. This normalization served to control for variations in performance across the different sites that were unrelated to the timed experimental manipulation of specific target items. The baseline scores used for normalization are presented in Table 2. This table also provides the rate of false alarms for the recognition test. Given that all items (stimulated and nonstimulated) from the different stimulation sites were tested together in an intermixed format, the same single false-alarm rate holds across all conditions. Consequently, this rate does not need to be considered when examining stimulation effects on recognition performance. Statistical comparison of the hit rates with the false-alarm rate showed significant differences at all stimulation sites regardless of whether only high confidence or all responses were examined: all $t(11) > 11$; all $p < .001$; this pattern indicates that subjects generally discriminated previously studied from novel words at a high level of accuracy.

Figure 3A depicts the stimulation effects on recognition performance. It shows that the normalized high-confidence hit rate was positive for the LIPFC, reflecting numerically higher performance for previously stimulated than nonstimulated trials; by contrast, it was negative for the other two sites. The same pattern emerged when the total number of hits, rather than of high-confidence hits, was considered (means for normalized total hit rates were 2.45, −4.08, and −2.32 for the LIPFC, RIPFC, and LPC, respectively). A one-way ANOVA yielded a significant effect of stimulation site on these hit rates, $F(2,22) = 4.24$; $p = .028$ for high-confidence responses; $F(2,22) = 3.52$; $p = .047$ for total responses. To follow up on this result and test our hypothesis directly, we also used a planned linear contrast in which we compared LIPFC with the two control sites; the hit rate for the LIPFC was significantly higher than those for the RIPFC and the LPC, $F(1,11) = 7.49$; $p = .019$ for high-confidence responses; $F(1,11) = 7.04$; $p = .022$ for total responses. Importantly, an additional planned pairwise comparison yielded no difference in performance between the two control sites ($t(11) = 1.007$; $p = .336$ for

**Table 2.** Means of Baseline Scores for Nonstimulated Items Across Different Stimulation Sites and Behavioral Measures (*SEM* in Parentheses)

| | *LIPFC* | *RIPFC* | *LPC* |
|---|---|---|---|
| *Recognition* | | | |
| Percent high-confidence hits | 63.5 (2.2) | 67.9 (3.4) | 66.4 (2.9) |
| Percent high-confidence false alarms | 10.7 (2.7) | 10.7 (2.7) | 10.7 (2.7) |
| Percent total hits | 81.8 (2.2) | 87.6 (1.7) | 86.5 (1.9) |
| Percent total false alarms | 31.1 (3.9) | 31.1 (3.9) | 31.1 (3.9) |
| RTs for high-confidence hits | 1001 (46) | 1032 (56) | 1006 (40) |
| RTs for total hits | 1100 (47) | 1126 (67) | 1114 (60) |
| *Encoding* | | | |
| Percent correct semantic decisions | 93.0 (1.3) | 91.1 (1.4) | 93.1 (1.4) |
| RTs for correct semantic decisions | 750 (25) | 759 (18) | 774 (19) |

RTs are mean (across subjects), medians (across items within subjects) in msec. The same false-alarm rate applies to all stimulation sites for recognition.

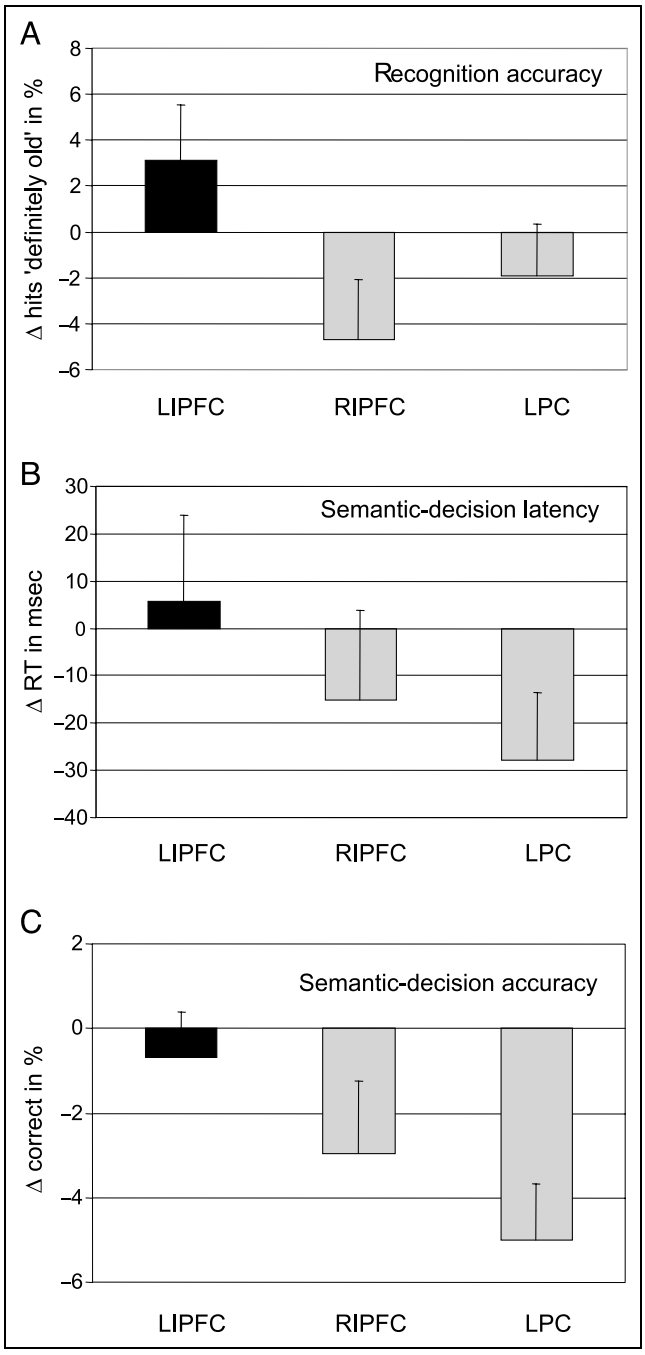


**Figure 3.** Effects of rTMS at encoding on different behavioral performance measures as a function of stimulation site. Note that all measures reflect performance on stimulated trials normalized for performance on nonstimulated trials. The stimulation site shown in black is the target site, whereas those in gray are control sites. (A) Effects on recognition accuracy for decisions made with high confidence. (B) Effects on latency of correct semantic (encoding) decisions made at the time of stimulation. (C) Effects on accuracy of semantic (encoding) decisions made at the time of stimulation.

high-confidence responses; $t(11) = -0.663$; $p = .521$ for total responses). Finally, we also explored the response times (RTs) for recognition judgments and found no evidence indicating an effect of stimulation site, $F(2,22) = 1.43$; $p = .799$ for high-confidence responses; $F(2,22) = 0.60$; $p = .596$ for total responses. Together, the analyses of our recognition data revealed that stimulation in the LIPFC at encoding resulted in improved subsequent memory accuracy as compared to stimulation in the two control sites, without having any effect on the corresponding RTs.

### rTMS Findings: Effects on Semantic Decisions at Encoding

To elucidate the possible mechanism underlying the differential improvement in memory performance following LIPFC stimulation, we also examined the rTMS effect on performance of the semantic decisions at encoding that were made directly at the time of stimulation. The baseline scores used for the normalization of accuracy and RTs of the semantic decisions on stimulated trials are shown in Table 2. Figure 3B shows the most important finding regarding semantic decisions; it depicts the data for stimulation effects on the normalized RTs for correct semantic decisions. An ANOVA performed on these scores revealed a significant effect of stimulation site, $F(2,22) = 3.60$; $p = .044$; the planned contrast showed that LIPFC stimulation slowed down RTs as compared to stimulation in the two control sites, $F(1,11) = 9.72$; $p = .010$. In addition, a planned pairwise comparison revealed no difference between the two control sites, $t(11) = 0.872$; $p = .402$. Figure 3C shows the stimulation effects at the three different sites for the accuracy of semantic decisions. Although an ANOVA performed on the normalized scores failed to yield a significant effect of stimulation site, $F(2,22) = 2.41$; $p = .113$, the planned linear contrast revealed a statistical trend, showing that accuracy scores for the LIPFC tended to be larger than those for the RIPFC and LPC, $F(1,11) = 3.55$; $p = .086$. At the same time, there was no difference in accuracy between the RIPFC and LPC, $t(11) = 1.045$; $p = .319$. Together then, we observed slowed RTs and a trend for improved accuracy for semantic decisions made under LIPFC stimulation at encoding. These data suggest that words were processed more extensively under LIPFC stimulation than under stimulation in the two control sites. We will return to this point in the Discussion.

## DISCUSSION

The results of the present study provide support for the hypothesis that there is a causal link between LIPFC involvement at encoding and subsequent memory performance. We found that manipulating neural processes in the LIPFC with rTMS during encoding of words had a beneficial effect on subsequent recognition performance that was specific to this brain region and could be distinguished, based on comparisons with two control sites, from nonspecific side effects.

### Critical Analyses Yielding rTMS Effects

Our main finding emerged from analyses that were carried out on recognition hit rates for items encoded under stimulation; a consideration of false-alarm rates was not required because the same rate applied to all stimulation conditions. The recognition hit rates were normalized for baseline performance, defined as recognition hits for nonstimulated items in each stimulation block. This normalization effectively increased the sensitivity to detect effects of rTMS on those cognitive processes that occurred directly at the time of stimulation in response to item presentation (see Devlin et al., 2003; Schluter et al., 1999, for the use of similar normalization procedures). It allowed for the control of longer-lasting effects of rTMS within stimulation blocks and of variability in encoding processes that was unrelated to the experimental manipulation, including the buildup of proactive interference.

### Interpretation of rTMS Effects across Stimulation Sites

The present study was designed, based on a hypothesis derived from neuroimaging, in such a way that the LIPFC served as the target site for stimulation while the RIPFC and LPC served as two independent control sites to estimate side effects of stimulation across lobes and hemispheres. Considering that the noise associated with rTMS was comparable at all stimulation sites, the critical finding of improved accuracy following LIPFC stimulation cannot be explained as a nonspecific side effect reflecting intersensory facilitation (Rossi et al., 2001; Terao et al., 1997). Similarly, the pattern of results observed across the three stimulation sites weighs against explanations in terms of other nonspecific side effects of rTMS.

A visual inspection of the observed recognition performance might at first glance suggest that the critical effect of stimulation arose in the right rather than the left prefrontal cortex; the difference in hit rates between stimulated and nonstimulated items was numerically largest at the RIPFC. However, such an interpretation does not take the full pattern of results obtained in the present study into account. Given the design of the study, any inferences to be drawn must be based on comparisons that consider the data for all three stimulation sites. Notably, while recognition scores associated with LIPFC stimulation were significantly higher than those obtained in the other two sites, RIPFC scores did not differ significantly from those in the LPC. This pattern of results makes it difficult, if not impossible, to argue that RIPFC stimulation had a unique effect on semantic encoding that was specific to this brain region and could be distinguished from side effects. Our fMRI results provide additional evidence that speaks against assigning a special role to the RIPFC; at the selected threshold, 10 of the 11 subjects who were scanned successfully and underwent rTMS showed a significant BOLD increase for semantic processing in the LIPFC target site. By contrast, not a single subject showed a BOLD increase in the RIPFC stimulation site. Even at an overly lenient threshold of $t = 1.96$, only 4 of the 11 subjects showed a BOLD effect in the targeted RIPFC site. When comparing these subjects with those that did not show a BOLD effect, we found no difference in the normalized recognition performance following RIPFC stimulation ($p = .40$). Together, these results suggest that the critical processes linking semantic processing at encoding to subsequent memory performance occur, and were manipulated successfully in our study, in the left rather than in the right prefrontal cortex.

### Role of Other Brain Regions not Targeted by rTMS Directly

Research combining rTMS with functional neuroimaging techniques has shown that rTMS can also have distant effects in brain regions that are neuroanatomically connected with the area targeted by stimulation (Paus et al., 1997; reviewed in Paus, 2002). In light of such findings, one needs to consider that the region-specific effect of rTMS in the LIPFC on memory performance might have been boosted by indirect manipulation of activity in regions connected with the LIPFC. One possible candidate would be the left dorsal prefrontal cortex, which also showed differential activation related to semantic processing in the fMRI part of the current study and in past neuroimaging research. Results from an fMRI study by Otten et al. (2001) would argue against such an interpretation; these authors found that no prefrontal region other than the one we targeted showed both differential activation related to semantic processing and the crucially relevant subsequent memory effect. Other fMRI studies, however, have sometimes observed subsequent memory effects for semantically encoded words in left prefrontal regions situated posterior and dorsal to the one targeted by our stimulation (Baker, Sanders, Maccotta, & Buckner, 2001; Otten and Rugg, 2001; Wagner et al., 1998). In light of these findings, we cannot rule out with certainty that other regions in the left prefrontal cortex contributed to the rTMS effect reported here.

An additional brain region that has exhibited differential activation related to semantic processing of words and a subsequent memory effect in past fMRI research is the left medial temporal lobe (Otten et al., 2001; Wagner et al., 1998). Given the prominent neuroanatomical connections between the prefrontal cortex and the medial temporal lobes (Petrides & Pandya, 1994), it is possible that our stimulation not only affected local processing in the LIPFC but also functional interactions between the LIPFC and the left medial temporal lobe; such interactions have been suggested to be essential for episodic memory formation (Kirchhoff, Wagner, Maril, &

Stern, 2000; Buckner et al., 1999; Tulving, Markowitsch, Craik, Habib, & Houle, 1996; Moscovitch, 1992). It is important to realize, however, that even when interpreting our findings in terms of such changed interactions, the LIPFC would be assigned a crucial functional role in memory formation.

### Possible Cognitive Mechanisms Mediating the rTMS Effect in LIPFC

To gain an understanding of the cognitive mechanisms mediating the observed memory benefit associated with LIPFC stimulation, we also examined whether there was an effect of rTMS on the semantic decisions made directly at the time of stimulation. After analyzing the effects of rTMS on memory performance, we hypothesized that the memory benefits observed with LIPFC stimulation might be the result of more extensive processing, perhaps signifying further elaboration, of the stimulated words in the context of the semantic decisions that led to memory encoding. In support of this hypothesis and in line with evidence from another recent rTMS study (Devlin, Matthews, & Rushworth, 2003), we found that RTs for correct semantic decisions made under LIPFC stimulation were significantly slower than RTs for decisions made under stimulation in the two control sites. In addition, an analysis of the accuracy scores revealed a statistical trend in the same direction (i.e., an increase) as the effect described for the subsequent recognition judgments. Together, both aspects of our results, i.e., slowed RTs and the trend towards increased accuracy for the semantic decisions, are consistent with the idea that words encountered under LIPFC stimulation received more extensive semantic processing than words encountered under stimulation in the control sites.

How could the summarized processing difference have resulted in the observed subsequent memory benefit? It is possible that rTMS trains delivered in the LIPFC during the semantic analysis of words led to brief interruptions of processing that triggered partial reanalyses with further elaborations; these reanalyses may in turn have produced a gain in distinctiveness of the stimulated items, which could form the basis of the observed subsequent memory benefit (see Klein & Saltz, 1976; Moscovitch & Craik, 1976, for related evidence from cognitive studies). Of course, the results of a single first study with a methodological approach as complex as the present one cannot establish with certainty that these cognitive mechanisms were indeed at work. For example, the observed rTMS effect in LIPFC on semantic decisions can also be interpreted as reflecting a shift in response criteria, i.e., a difference in speed–accuracy tradeoff, at encoding. The rTMS findings reported by Devlin et al. (2003) provide some evidence that speaks against this interpretation by showing that rTMS in the LIPFC can slow RTs for semantic decisions even in the absence of any changes in accuracy. Nevertheless, it is clear that further research is necessary to rule out this alternative interpretation with certainty.

### Possible Physiological Mechanisms Mediating the rTMS Effect in LIPFC

Although this is to our knowledge the first study that has shown a beneficial effect of LIPFC stimulation at encoding on subsequent memory performance, it is worth noting that enhanced behavioral performance has been reported in several past studies as the result of TMS during or prior to performance of other cognitive tasks (e.g., Grosbras & Paus, 2002, in press; Boroojerdi et al., 2001; Hilgetag, Theoret, & Pascual-Leone, 2001; Topper, Mottaghy, Brugmann, Noth, & Huber, 1998; Walsh, Ellison, Battelli, & Cowey, 1998). Whether the effects of rTMS on behavior are disruptive or enhancing is thought to depend on a number of factors whose complex interplay is not well understood; frequency of stimulation appears to be one of the most important factors in this regard. Physiological studies of the motor cortex have shown that rTMS at frequencies of 5 Hz and above increases cortical excitability transiently, whereas slow rTMS at 1 Hz depresses excitability (Hallett, 2000). Although it is still unknown whether the same holds for regions other than the motor cortex (but see Paus et al., 2001), this research raises the possibility that with the 7 Hz frequency used in the present study we may have effectively increased cortical excitability in the LIPFC during or immediately following stimulation. Such a transient increase in cortical excitability at the time of encoding could have facilitated cortical interactions related to memory formation and thus contributed to the beneficial effect of LIPFC stimulation on subsequent memory performance.

At first glance, this account of our findings in terms of cortical excitability appears to be incompatible with the cognitive mechanisms we propose to be at work. How could prolonged RTs for semantic decisions under LIPFC stimulation, which purportedly reflect interruptions in processing and ensuing reanalyses, be compatible with the notion that the stimulation increased cortical excitability? Recent findings reported by Grosbras and Paus (2002) indicate that TMS can produce either disruptive or enhancing effects, in the same location and with identical stimulation parameters, depending on the type of cognitive process examined and the pattern of cortical interactions involved. Grosbras and Paus showed that TMS over the right frontal eye field can facilitate visual detection performance but interfere with shifting of attention. In light of such evidence, the present findings can be interpreted as reflecting an immediate disruptive effect of rTMS on the semantic decisions combined with an enhancing effect on processes of episodic memory formation. Notably, the latter processes are thought to rely on interactions between cortical regions different from those involved in the semantic decisions themselves

(Bookheimer, 2002; Paller & Wagner, 2002; Martin & Chao, 2001; Buckner et al., 1999). These considerations suggest that the two accounts discussed for the present findings are not incompatible.

A final comment regards the question of whether rTMS administered in the LIPFC during semantic encoding should be expected to improve subsequent memory performance under all circumstances. Our account of the cognitive mechanisms involved would predict a strong ''no'' as the answer to this question. It is important to note that with the present parameters, rTMS did not interfere with the semantic analysis at encoding to the extent that the decisions could not be performed accurately; it only slowed the processes involved. Findings from an intraoperative study conducted with direct cortical stimulation suggest that stronger manipulation of neural activity in this region may prevent the semantic processes supported by the LIPFC from being completed (Klein et al., 1997). Our interpretation of the present findings would predict no subsequent memory benefit for the items processed under such conditions. Rather, building on the causal link between LIPFC involvement and subsequent memory performance established here, we would predict that these conditions produce a subsequent memory impairment, reflecting the lack of semantic elaboration and ensuing lack of distinctiveness of items encountered under stimulation. Future research conducted with stimulation parameters that produce interference effects on semantic processing comparable to those in Klein et al.'s (1997) study will be instrumental for testing these predictions and, more generally, will help to elucidate further the role of the LIPFC in episodic memory formation.

### Conclusions

Motivated by recent neuroimaging evidence suggesting that the LIPFC plays a role in memory encoding, we conducted an rTMS study in which we stimulated anterior LIPFC in the context of a semantic encoding task and examined the effect on subsequent memory performance. Our goal was to determine whether the LIPFC plays a crucial role in memory formation in that the degree of LIPFC involvement at the time of encoding is causally linked to subsequent levels of recognition success. We found evidence in support of such a causal link; words encoded under LIPFC stimulation were recognized with higher accuracy than words encoded under stimulation in two cortical control sites. To explain the mechanisms that mediated the beneficial memory effect of LIPFC stimulation in the present study we offer two accounts that are not mutually exclusive. Based on our results regarding rTMS effects on the semantic decisions at encoding, we suggest that LIPFC stimulation may have produced its effect on recognition memory through the triggering of more extensive processing of the stimulated items and an ensuing gain in item distinctiveness. At the same time, physiological processes of facilitation may have also contributed to the observed memory benefit by boosting functional interactions between the LIPFC and the medial temporal lobes. Regardless of the relative contributions of each of these mechanisms, the present findings suggest that the LIPFC plays a causal role in episodic memory formation.

## METHODS

### Subjects

Twelve right-handed, healthy volunteers participated in the study (ages 18–38 years; 6 women). All subjects took part in the fMRI section and the rTMS section of the study. For one subject, however, fMRI data were unusable due to a problem with the experimental software that synchronized fMR image acquisition and stimulus presentation during scanning. All subjects gave their written informed consent before participation. The study protocol was approved by the Research Ethics Board of the Montreal Neurological Institute and Hospital.

### Stimulus Material

A total of 1350 nouns, between 4 and 12 letters in length, served as the stimuli for the study. The nouns were obtained from the MRC Psycholinguistic Database (Version 2) together with normative data on word frequency and concreteness. For the fMRI section, 480 of these items were used. The set was split into 12 lists of 40 nouns that were matched for word frequency, word length, and concreteness ratings. The other 870 items were used for the rTMS section. This set of items was split into 10 lists, again matched in terms of word frequency, word length, and concreteness ratings. Six of these lists served as critical items, each containing 60 items. Three further lists, with 50 items each, served as fillers. The remaining list of 360 items served as lures for the recognition memory test following the rTMS session. Across both sections of the study, half of the items in each list were concrete (rating $\geq$498), and half were abstract (rating $\leq$378).

### Behavioral Procedures in fMRI

The experiment was conducted with subjects lying in the MR scanner. The stimuli were projected with an LCD projector onto a screen that was visible to participants through a mirror. Participants performed the behavioral tasks in six separate blocks corresponding to the six runs of fMR scanning. Within each set, three different tasks were administered that included semantic encoding of words, nonsemantic encoding of words, and a fixation-baseline task. In the semantic encoding task, participants were required to judge for each word presented whether it referred to something abstract or

concrete. In the nonsemantic encoding task, they were asked to judge for each whether it was written in upper- or in lowercase letters. In both tasks, subjects were asked to press one of two mouse buttons with their right hand to indicate their response. Words were presented for 1 sec each with an ISI of 1 sec. In the fixation-baseline task participants were simply asked to fixate on a crosshair presented continuously in the middle of the screen. During each fMRI run, both encoding tasks were administered in an alternating sequence of 40-sec periods that were interleaved with the fixation-baseline task. The order of tasks and the assignment of stimulus lists to tasks were counterbalanced across participants.

### fMR Image Acquisition

Scanning was performed on a 1.5-T Siemens Vision magnet and began with the acquisition of 3-D high-resolution T1-weighted anatomical scans (voxel size $1 \times 1 \times 1$ mm). Following this scan, six runs of functional T2* gradient-echo images (mosaic with $64 \times 64$ matrix size, TE = 50 msec, in-plane resolution $5 \times 5$ mm) that were sensitive to BOLD contrast were acquired while participants performed the behavioral tasks. Each run involved the acquisition of 128 volumes obtained at a rate of one volume every 2 sec. Each volume consisted of 17 contiguous slices (7 mm thick) that were acquired parallel to the AC–PC line and were positioned to maximize coverage of the frontal lobes.

### fMR Image Analysis

BOLD images were corrected for head motion and transformed into standardized stereotaxic space using an automated linear image-registration method (Collins, Neelin, Peters, & Evans, 1994) and the MNI-305 template for the standardized stereotaxic space (Talairach & Tournoux, 1988). Following transformation, images were smoothed with a 3-D Gaussian Kernel with FWHM of 6 mm. The statistical analysis was carried out, on a subject-by-subject basis and for the entire group, with in-house software (fmristat; Worsley et al., 2002) using a variant of the general linear model and a weighted random-effects analysis. To account for the temporal lag of the BOLD response, image signals were convolved with a hemodynamic response function that was modeled as the difference between two gamma density functions, using the parameters published by Glover (1999). Drift was removed by adding a third-order polynomial covariate to the design matrix; a 3-D Gaussian Kernel was used to adjust the data for autocorrelation (see Worsley et al., 2002). Task-related activity was measured by examining the contrast between the semantic and nonsemantic encoding tasks in the specified model; results were expressed as $t$ statistic maps. Statistic maps for individual subjects were thresholded at $t > 4.00$; the map for the entire group was thresholded at $t > 4.48$ based on Gaussian random field theory (Worsley et al., 1996).

### Behavioral Procedures during and following rTMS

Participants were tested on a separate day in the rTMS section of the study, after their fMR data had been analyzed. Behavioral testing under rTMS was carried out in three different blocks corresponding to the three different stimulation sites. In each testing block, subjects were presented with a list of words (different from those used in fMRI) and asked to perform the same semantic encoding task that was used in fMRI; they were required to judge for each word whether it referred to something abstract or concrete. They indicated their response by pressing one of two mouse buttons. Words were presented for 1 sec with an ISI of 1.5 sec. Within each testing block, 170 words were presented, consisting of two critical lists of 60 items and an additional list of 50 filler items. rTMS was administered only during presentation of one of the 60-item lists (''stimulated items''); the other 60-item list did not involve stimulation and served for comparison (''nonstimulated items''; see Figure 2A). Filler items were also presented without stimulation; they were included in the testing block to allow for a safe intertrain interval between neighboring stimulated items and for making the order between stimulated and nonstimulated items unpredictable to the subjects. The 170 items from all three lists within a testing block were presented in pseudorandom order with the constraint that neighboring stimulated items were at least 5 sec but no more than 10 sec apart from each other. Testing of the entire block of 170 items at each stimulation site took approximately 10 min, including at least two short breaks to allow for cooling of the rTMS coil. Assignment of the six different 60-item lists to the different experimental conditions (stimulated vs. nonstimulated items in three different sites) was counterbalanced across subjects, as was the order of stimulation sites.

Memory testing for the stimulated and nonstimulated items encountered in the semantic encoding task began 10 min after the completion of the last rTMS testing block. Memory performance was tested with a yes–no recognition test for which the 360 target items were intermixed with 360 novel lures in pseudorandom order with the constraint that each quarter of the test include an equal number of items of each type. Subjects were asked to decide for each word whether they had encountered it in the rTMS section of the study on that same day and how confident they were in this decision. They indicated their response in a single step by pressing one of four buttons on a response box (yes/no paired with high or low confidence). They were also informed that no words from the fMRI section were included in this test and that the novel lures were items

never encountered in any part of the study. The task was subject paced and included three short breaks.

### rTMS Protocol

A Cadwell high-speed magnetic stimulator and Cadwell Round Coil with an external diameter of 9 cm were used to administer rTMS. Each rTMS train associated with a word presentation consisted of five pulses delivered at 7-Hz frequency, i.e., with one pulse every 150 msec (see Figure 2B). The start of each train occurred 200 msec poststimulus onset and was triggered by the computer that controlled word presentation; this timing was chosen to ensure that the stimulation covered the time window between 400 and 800 msec post word onset, during which reliable differences in EEG signal between subsequently remembered and forgotten words typically emerged in past ERP research (e.g., Paller et al., 1987). Intensity of stimulation was set to 100% of motor threshold, except when this threshold exceeded 60% of the maximum Cadwell output. In the latter case, which occurred in two subjects, stimulation intensity was set to 60%. Motor threshold was defined as the level at which stimulation of motor cortex with single pulses elicits a visible and reproducible finger twitch while the subject's hand is in a resting position and fingers are slightly flexed.

The LIPFC target site for rTMS was determined based on the fMRI data obtained in individual subjects. It was the most anterior and most ventral left prefrontal peak observed in each subject for the specified encoding contrast (i.e., higher activation in the semantic than in the nonsemantic encoding task). For 2 of the 12 subjects, we used the group mean coordinates rather than individually determined fMRI peaks because in one of them the fMRI data were corrupted and in another one no anterior LIPFC peak was observed at the chosen statistical threshold. In addition to the LIPFC, two control sites were selected for rTMS. They included the homologous region in the right inferior prefrontal cortex (RIPF) and a more posterior region in the left parietal cortex (LPC).

Accurate placement of the rTMS coil position in relation to the cortical target and control sites was achieved by using frameless stereotaxy (Paus, 1999). For this purpose, the cortical target and control sites were first marked on each subject's anatomical MRI scan. The scan was then coregistered with the subject's head position using Brainsight software (Rogue Research, Montreal, Canada) and the Polaris infrared motion-tracking device (Northern Digital, Waterloo, Canada) based on a set of four scalp landmarks. Upon completion of the coregistration, the tracking device was used to position the tear-shaped part of the rTMS coil over the cortical site of interest; it was positioned such that the handle of the coil pointed upward and the top part tilted away (by ~2 cm) from the head. The direction of current in the part of the coil placed over the target site was from front to back. To stabilize head position during rTMS and behavioral testing, subjects positioned their head on a chin rest.


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The data reported in this experiment have been deposited in the fMRI Data Center (http://www.fmridc.org). The accession number is 2-2003-11452.

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