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Repetitive Transcranial Magnetic Stimulation Dissociates Working Memory Manipulation from Retention Functions in the Prefrontal, but not Posterior Parietal, Cortex

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Abstract

■ Understanding the contributions of the prefrontal cortex (PFC) to working memory is central to understanding the neural bases of high-level cognition. One question that remains controversial is whether the same areas of the dorsolateral PFC (dlPFC) that participate in the manipulation of information in working memory also contribute to its short-term retention (STR). We evaluated this question by first identifying, with functional magnetic resonance imaging (fMRI), brain areas involved in manipulation. Next, these areas were targeted with repetitive transcranial magnetic stimulation (rTMS) while subjects performed tasks requiring only the STR or the STR plus manipulation of information in working memory. fMRI indicated that manipulation-related activity was independent

of retention-related activity in both the PFC and superior parietal lobule (SPL). rTMS, however, yielded a different pattern of results. Although rTMS of the dlPFC selectively disrupted manipulation, rTMS of the SPL disrupted manipulation and STR to the same extent. rTMS of the postcentral gyrus (a control region) had no effect on performance. The implications of these results are twofold. In the PFC, they are consistent with the view that this region contributes more importantly to the control of information in working memory than to its STR. In the SPL, they illustrate the importance of supplementing the fundamentally correlational data from neuroimaging with a disruptive method, which affords stronger inference about structure–function relations. ■

INTRODUCTION

One perspective on the role of the prefrontal cortex (PFC) is that it supports short-term retention (STR, alternatively referred to as “storage” or “maintenance”) functions (e.g., Courtney, 2004; Leung, Seelig, & Gore, 2004; Goldman-Rakic & Leung, 2002; Pessoa, Gutierrez, Bandettini, & Ungerleider, 2002; Constantinides, Franowicz, & Goldman-Rakic, 2001; Funahashi, Chafee, & Goldman-Rakic, 1993), and can thus be viewed as supporting the buffers of a multiple-component working memory system such as that proposed by Baddeley (2000) and Baddeley and Logie (1999). An alternative perspective denies an important role in STR for the PFC (e.g., Lebedev, Messinger, Kralik, & Wise, 2004; Petrides, 2000; D’Esposito & Postle, 1999), ascribing these functions instead to activity in the non-PFC regions that have evolved to accomplish sensory-, representation-, and action-related functions (e.g., Postle, 2006; Jonides, Lacey, & Nee, 2005; Pasternak & Greenlee, 2005; Theeuwes, Olivers, & Chizk, 2005). One implication of the latter view is that working memory may be

better understood as an emergent property of the mind and brain, rather than as a system that can be readily localized (as can, e.g., vision to the occipital cortex or skeletomotor control to the precentral gyrus).

One way to operationalize working memory retention processes is by varying the number of items that must be retained on different trials—brain regions whose delay-period activity is sensitive to such variations in memory load are presumed to contribute to retention-related processes. To date, the functional magnetic resonance imaging (fMRI) literature on the load sensitivity of the dorsolateral PFC (dlPFC) delay period activity for verbal stimuli has been mixed, with some studies finding evidence for (Narayanan et al., 2005; Zarahn, Rakitin, Abela, Flynn, & Stern, 2005; Veltman, Rombouts, & Dolan, 2003), and some finding evidence against (Feredoes & Postle, 2005; Postle, Berger, & D’Esposito, 1999; Rypma & D’Esposito, 1999). The same study by Postle et al. (1999) also reported evidence that some load-insensitive regions of the dlPFC were nonetheless sensitive to the requirement to reorder the contents of working memory during the delay period—an operationalization of manipulation. These results, in turn,

have been challenged by Veltman et al. (2003), who reported that “maintenance” (what we refer to in this article as retention) and manipulation “activate virtually identical systems” (p. 247). Clearly, the neuroimaging literature related to this issue is at an impasse.

The present study was intended to address the specific question of whether STR and manipulation functions colocalize in the dlPFC and to do so with a method that would support stronger inference than have the neuroimaging studies performed to date. This approach entailed supplementing fMRI with repetitive transcranial magnetic stimulation (rTMS), a method that produces the temporary disruption of the function of a local region of cortex. Although fMRI data are limited to testing hypotheses about brain–behavior correlations, rTMS can address hypotheses about the necessity of a brain area to a particular aspect of cognitive performance (Walsh & Pascual-Leone, 2003). An additional goal of this study was to broaden the investigation of manipulation in working memory beyond the PFC, to reflect the general consensus that most examples of high-level cognition, including the control of working memory, are supported by broadly distributed networks that extend beyond this one brain region. Our two-step procedure entailed first, acquiring fMRI data while subjects performed delayed recognition with different loads and different manipulation requirements, and second, delivering rTMS to fMRI-identified areas of the dlPFC and superior parietal lobule (SPL) with rTMS while the same subjects performed the same task. (The SPL has also been implicated in executive control, e.g., Garavan, Ross, Li, & Stein, 2000, and has shown manipulation-related activity in previous studies, unpublished observation). We predicted that delay-period rTMS of manipulation-sensitive regions of the dlPFC and SPL would produce selective deficits on trials requiring the manipulation, but not the simple retention, of items in working memory.

METHODS

Subjects

The 12 adults (6 men and 6 women; mean age = 22 years, $SD = 2.7$) whose data are presented here had no psychiatric or neurologic disorders, as determined by physical examination, a structured psychiatric diagnostic screening interview (Mini-International Neuropsychiatric Interview; Sheehan et al., 1998), and a mood assessment (Hamilton Depression Rating Scale; Hamilton, 1960), all administered by a psychiatrist.

Behavioral Task

The task, requiring delayed recognition of item position, was identical to that used by Postle et al. (1999). Each trial began with the simultaneous presentation of two or five consonant letters (all in a single row), followed by

instructions (“forward” or “alphabetize”), followed by an 8-sec delay period, followed by a memory probe comprising an item from the memory set and a digit. On forward trials, subjects were to retain a memory of the two or five letters in the order in which they were presented. On these trials, the probe digit represented (with $p = .5$) the ordinal position in which the probed letter had appeared in the initial stimulus display. On alphabetize trials, subjects were to reorder the letters into alphabetical order. On these trials, the probe digit represented (also with $p = .5$) the alphabetical position of the probed letter with respect to the other four letters in the memory set.

Functional Magnetic Resonance Imaging

Data Acquisition and Preprocessing

Whole-brain images were acquired with a 3-T scanner (GE Signa VH/I, Waukesha, WI). High-resolution T1-weighted images (30 axial slices, $0.9375 \times 0.9375 \times 4$ mm) were obtained in all participants, and a gradient-echo, echoplanar sequence (TR = 2000 msec, TE = 50 msec) was used to acquire data sensitive to the blood oxygen level dependent (BOLD) signal (Kwong et al., 1992; Ogawa et al., 1992) within a 64×64 matrix (30 axial slices coplanar with the T1 acquisition, $3.75 \times 3.75 \times 4$ mm, no skip). Scans of the delayed-recognition task were preceded by a scan in which we derived an estimate of the hemodynamic response function (HRF) for each participant. During this scan, each participant performed a simple reaction-time task that required a bimanual button press once every 20 sec in response to a brief change in shape of the fixation stimulus. A partial F test associated with a Fourier basis covariate set (Josephs, Turner, & Friston, 1997) was used to evaluate the significance of task-correlated activity in each voxel of primary somatosensory and motor cortical regions of interest (ROIs). An HRF estimate was extracted from the suprathreshold voxels of these ROIs by spatially averaging their time series, filtering the resultant averaged fMRI time series to remove high (> 0.244 Hz) and low (< 0.05 Hz) frequencies, adjusting it to remove the effects of nuisance covariates (Friston, Holmes, Poline, Heather, & Frackowiak, 1995), and trial averaging. The HRF characterizes the fMRI response resulting from a brief impulse of neural activity (Boynton, Engel, Glover, & Heeger, 1996) and can vary markedly across subjects (Handwerker, Ollinger, & D’Esposito, 2004; Aguirre, Zarahn, & D’Esposito, 1998). The subject-specific HRFs were used to convolve independent variables entered into the modified general linear model (GLM; Worsley & Friston, 1995) that we used to analyze the data from the scans of the working memory task. The eight scans of the working memory task each lasted 6 min 20 sec (6 min of task preceded by 20 sec of dummy pulses to achieve a steady state of tissue magnetization).

Analyses

The fMRI time series analysis modeled the signal change associated with each discrete epoch of the trial with a covariate comprised of a BOLD HRF shifted along the timeline of the task in order best model the trial epoch in question (Postle, Zarahn, & D'Esposito, 2000; Zarahn, Aguirre, & D'Esposito, 1997). The least-squares solution of the GLM of the fMRI time series data yielded parameter estimates that were associated with each covariate of interest. The smoothness of the fMRI response to neural activity allows fMRI evoked responses that arise from temporally dependent events to be resolved on the order of 4 sec (Zarahn et al., 1997). Load-sensitive and alphabetization-sensitive voxels were identified with the contrasts [$\text{Delay}_{\text{Forward } 5} - \text{Delay}_{\text{Forward } 2}$] and [$\text{Delay}_{\text{Alphabetize } 5} - \text{Delay}_{\text{Forward } 5}$], respectively, thresholded at a mapwise level of $p = .05$, Bonferroni-corrected for multiple comparisons.

Because the principal function of the fMRI data for this study was to provide activation maps that would guide the rTMS, the principal analyses were performed as single-subject analyses. (This first step was a precise replication of the procedure from Postle et al., 1999.) Transforming a subject's anatomical and functional data into a "normalized" atlas space would not be appropriate with this approach for the simple reason that rTMS can only be applied to a subject's brain in its "native" configuration (i.e., it is not possible to apply rTMS to a composite, group-normalized statistical volume; for a similar approach, see the work of Herwig et al., 2003). Before the rTMS session, the whole-brain alphabetization statistical map was coregistered and merged with a high-resolution T1-weighted anatomical scan. The three-dimensional reconstruction of this merged image would be used to guide rTMS.

To provide a sense of aggregate trends in activity produced by our task, we also performed a spatial normalization-based group analysis. This analysis was performed by first warping unthresholded statistical volumes from each subject to a template in MNI space, smoothing them to 8-mm full width half maximum, then evaluating the reliability of these statistical maps across subjects with a "second-level" analysis implemented with a GLM that treated subject as a random variable. The resultant group maps were thresholded at $p = .01$, uncorrected for multiple comparisons.

Transcranial Magnetic Stimulation

Procedure

The behavioral task used in the rTMS session only included Alphabetize 5 and Forward 5 trials that occurred with equal probability in a randomly determined order. An intertrial interval of 10 sec separated each trial.¹ Orthogonal to the factor of instructions was that of rTMS (present, absent; each also occurring randomly with $p =$

.5). An entire rTMS study comprised 12 twelve-trial blocks, with four consecutive blocks performed for each stimulation site: middle frontal gyrus (MFG) of the dlPFC, SPL, and postcentral gyrus (PCG). Order of stimulation site was counterbalanced across subjects. Within each block, the orthogonal factors of instructions (alphabetize, forward) and rTMS (present, absent) were randomized such that each trial type occurred three times during each block. Probe validity also varied independently of the two principal factors of interest, such that an equal number of valid and invalid probes occurred during each block, no more than two trials of any type (e.g., an "alphabetize, rTMS absent" trial) featured a valid probe within a single block, and an equal number of valid and invalid probes (i.e., three) had occurred for each trial type upon completion of each even-numbered block.

Apparatus

TMS was delivered with a Magstim Standard Rapid magnetic stimulator fit with a 70-mm figure-8 stimulating coil (Magstim, Whitland, Wales, UK). The first step of the TMS session was to determine the minimal intensity at which a single pulse through the TMS coil, positioned over the motor cortex, reliably produced a motor-evoked potential of $\geq 50 \mu\text{V}$ in the abductor pollicis brevis in 5 of 10 successive stimuli. This "motor threshold" was the intensity at which the subsequent rTMS was performed.²

As stated in the Introduction section, the logic of the experiment was to target portions of the dlPFC and SPL that showed alphabetization sensitivity (i.e., $\text{Delay}_{\text{Alphabetize } 5} > \text{Delay}_{\text{Forward } 5}$) in the fMRI scan. This was accomplished via coregistration, with infrared-based frameless stereotaxy (eXimia Navigated Brain Stimulation [NBS]; Nexstim, Helsinki, Finland), of the subject's head with his/her MRI data. The TMS coil was also fitted with infrared-reflecting beacons, thereby permitting us to target regions identified in the fMRI data with rTMS. NBS works from the understanding that TMS preferentially stimulates neurons located in the area where the induced current is strongest (Thielscher & Kammer, 2002). The system displays the cortical area likely to be maximally stimulated by TMS by displaying the electric field maximum in the cortex after calculating the estimated distribution and strength of the intracranial electric field. This computation takes into account the exact shape of the copper wiring inside the TMS coil, the three-dimensional position and orientation of the coil, and the overall shape of the head and the brain.

Target Selection

In each subject, only one site was stimulated in each of three regions: dlPFC, SPL, and a control site in the PCG. We opted to target the "hotspots" from each individual subject's fMRI data, rather than areas defined by composite, group-averaged statistical maps, because of growing

evidence for high levels of intersubject topographical variability in many domains of cognition (e.g., Swallow, Braver, Snyder, Speer, & Zacks, 2003; Tsao, Freiwald, Knutsen, Mandeville, & Tootell, 2003; Miller et al., 2002), including the STR of information (Feredoes & Postle, 2005), but relatively lower intrasubject variability over time (i.e., good test-retest reliability, Feredoes & Postle, 2005; Peelen & Downing, 2005; Tsao et al., 2003; Miller et al., 2002). (One manifestation of high intersubject topographic variability in the present data set was that the mean alphabetization effect size from the dlPFC was an order of magnitude larger in the single-subject analyses, 2% signal change, Figure 1, than in the group-averaged analysis, 0.33 % signal change, averaged across the two hemispheres; Table 1.) Based on this, it may be that a single-subject statistical map provides a better estimate of the true anatomical location of task-related activity for that subject than would a map derived from a group average. (If it were the case that we were applying rTMS to subjects for whom we did not have fMRI data, however, our best estimate would come from group-averaged data.)

In some instances, there were multiple foci of alphabetization-sensitive activity in a particular region, and in these cases the focus selected was the one whose stimulation was judged by the experimenters to be most likely to be tolerated by the subject. In particular, targeting regions of the MFG located relatively ventrally and anteriorly increases the likelihood of stimulating the superior auricularis muscle, which can produce involuntary wincing and discomfort. In such instances, if a different focus of alphabetization sensitivity were located in a more

dorsal and/or posterior portion of the MFG, we would target this latter focus. In particular, we found that stimulation sites near or overlapping the inferior bank of the superior frontal sulcus were well tolerated by subjects.³ Another constraint was that, for each individual subject, all three stimulation sites were in the same hemisphere. This meant that, for each subject, the hemisphere to be stimulated was determined by the location of alphabetization-sensitive activity in the dlPFC and SPL. PCG was chosen as the control region for this study because it was presumed to have no direct involvement in any component of the working memory task, and thus could serve as a region to control for nonspecific effects of cortical rTMS. Therefore, PCG stimulation sites were expressly selected for the absence of either alphabetization- or load-sensitive activity. At each stimulation site, the stimulating coil was oriented with the handle pointing posteriorly with respect to the subject's head, and roughly parallel to the midline, so as to induce current in the brain in the posterior-to-anterior direction.

Stimulation Parameters

On stimulation-present trials, the system was programmed to deliver 30 equally spaced pulses during a 6-sec epoch, beginning 2 sec after the offset of the instructions and lasting for the remaining 6 sec of the delay period (i.e., a 6-sec-long train of 5 Hz rTMS). However, a programming error was detected after the experiment was completed, and inspection of the stimulation logs indicated that for three subjects, the 30 pulses were actually delivered over

Figure 1. Group load and alphabetization effects from the fMRI data. Effects were extracted in a two step process. First, load-sensitive and alphabetization-sensitive voxels were identified in each of the three types of region. Second, the magnitude of load and alphabetization sensitivity was determined for the voxels identified in Step 1. The topography of load sensitivity varied across subjects (see text), and the effects displayed here were drawn from the functionally defined load sensitive region of each subject. Because load sensitivity was only observed in two subjects in the dlPFC (Subjects 16 and 21) and in two subjects in the SPL (Subjects 21 and 27), these results are plotted as single data points, rather than as means. Error bars represent 95% confidence intervals.

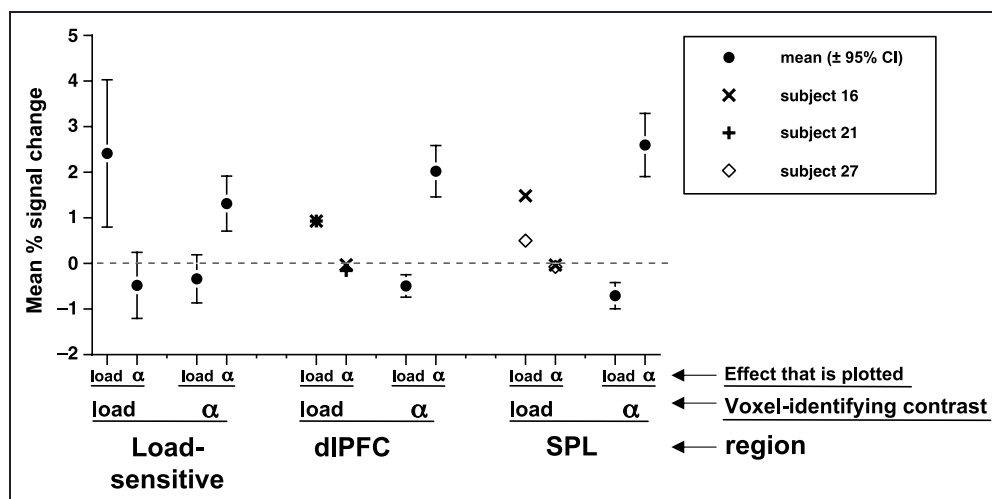


Table 1. Tabulation of Activity Identified in Spatial Normalization-based Group Analyses

Region of Activation	MNI Coordinates (mm)			Effect Size (Mean Percent Signal Change)	Volume of Activation (mm ³)
	x	y	z		
Alphabetization contrast					
Right PFC	31.88	22.5	36	0.37	3825.0
Left PFC	26.3	28.1	34	0.29	2137.5
Anterior cingulate cortex	−2	10.5	40	0.34	562.5
Superior frontal gyrus	7.7	1.9	66	0.46	956.3
Right SPL	3.8	−63.8	48	0.63	900.0
Right intraparietal sulcus	33.8	−60	44	0.42	506.3
Left SPL	−15	−56.25	56	0.39	506.3
Left SPL	−7.5	−60	44		
Left inferior parietal lobule	−37.5	−45	56	0.38	281.3
Right head of the caudate nucleus	11.25	11.25	12	0.47	900.0
Left head of the caudate nucleus	−11.25	7.5	14	0.45	1012.5
Load contrast					
Left inferior frontal gyrus/MFG	−45	−7.5	44	0.13	5.6
Right central sulcus	30	−41.25	56	0.14	5.6
Right PCG	18.75	−33.75	64	0.21	5.6
Right SPL	26.25	−71.25	48	0.23	56.3
Right inferior frontal gyrus	67.5	7.5	32	0.73	56.3
Right fusiform gyrus	48.75	−45	−24	0.42	56.3
Right amygdala	22.5	−3.75	−20	0.41	56.3
Right medial temporal lobe	18.75	−18.75	−16	0.43	56.3

The coordinates reported here indicate the centers of clusters of activity identified within each anatomical region. Identification of anatomical regions was confirmed via conversion of MNI coordinates to Talairach coordinates with the *mmi2tal* Matlab routine of Matthew Brett (<http://www.mrc-cbu.cam.ac.uk/Imaging/mnispaces.html>) and looking them up in the atlas of Talairach and Tournoux (1988).

a 6.3-sec epoch (therefore, at a rate of 4.8 Hz), and for the remaining nine subjects, the 30 pulses were delivered over a 6.8-sec epoch (therefore, at a rate of 4.3 Hz). The result was that, for each subject, rTMS was delivered during the final 6 sec of the delay period and continued during the first few hundred msec after the onset of the probe. This “spillover” of rTMS into the probe portion of the trial, although unfortunate, does not complicate our interpretation of our results, for reasons summarized in the Results section.

RESULTS

Functional Magnetic Resonance Imaging

Single-subject Analyses

Alphabetization-sensitive voxels were identified in the dlPFC in 11 subjects and in the SPL in all 12 subjects.

Load-sensitive voxels were identified in 11 subjects, in the following regions (with *n* corresponding to the number of subjects in which it was detected): left superior temporal gyrus and/or inferior parietal lobule, *n* = 5; right inferior parietal lobule, *n* = 1; SPL, *n* = 2; left dorsal extrastriate cortex, *n* = 1; left central sulcus, *n* = 2; right central sulcus, *n* = 1; anterior cingulate gyrus, *n* = 1; left dlPFC, *n* = 2; right temporal pole, *n* = 1. To accommodate this topographical variability, the “load sensitivity region” was defined exclusively from functional properties and permitted to vary topographically across subjects. To address the question of whether alphabetization sensitivity and load sensitivity are seen in the same voxels, we extracted estimates of these two effects (see Methods section, Functional Magnetic Resonance Imaging, Analyses) from alphabetization-sensitive voxels found in the three ROIs (load-sensitive, dlPFC, and SPL) as well as from load-sensitive voxels found in these ROIs (e.g.,

from alphabetization-sensitive voxels in the dlPFC, we extracted an estimate of the alphabetization sensitivity and of the load sensitivity.) Inspection of mean effect sizes and their associated 95% confidence intervals indicated that in none of the ROIs did alphabetization-sensitive voxels display significant load sensitivity or, in any ROI, did load-sensitive voxels demonstrate alphabetization sensitivity (Figure 1). Indeed, in the dlPFC and SPL, alphabetization-sensitive voxels showed reliably negative load effects (mean and 95% confidence interval < 0). These results replicate and extend the findings of Postle et al. (1999) and stand in contrast to the conclusions of Veltman et al. (2003).

Group Analyses

Alphabetization-sensitive activity identified by the spatial normalization-based group analyses was extensive and spanned large extents, bilaterally, of the PFC, anterior cingulate cortex, superior frontal cortex, SPL, and the caudate nucleus (Table 1). In both hemispheres of the PFC, these voxels showed a significant negative effect of load [right: $t(11) = -3.4$, $p < .01$; left: $t(11) = -4.4$, $p < .005$]. Load-sensitive activity identified by this analysis was markedly more sparse, consisting only of individual voxels in the right and left frontal cortex, right central sulcus and parietal cortex, and three locations in the right temporal lobe (Table 1). In neither hemisphere of the PFC did these load-sensitive voxels show a significant alphabetization effect [right: $t(11) = -0.5$, ns ; left: $t(11) = 1.5$, ns]. Therefore, the results of the group analyses also demonstrated a dissociation of retention from manipulation effects (as with the single-subject analyses, consistent with Postle et al., 1999, and inconsistent with Veltman et al., 2003).

Repetitive Transcranial Magnetic Stimulation

rTMS was performed in the left hemisphere in seven subjects and in the right hemisphere in five (Figure 2). Analyses of variance (ANOVAs) indicated that rTMS had its greatest effects on Alphabetize 5 performance at the dlPFC and SPL sites and on Forward 5 performance at the SPL (Figure 3). An initial omnibus ANOVA found no effect of hemisphere of stimulation, $F(1,10) = 0.1$, ns , and so all subsequent analyses collapsed across this variable. Omnibus ANOVA of the accuracy data revealed main effects of trial type, $F(1,11) = 11.2$, $p < .005$, and rTMS, $F(1,11) = 7.6$, $p < .05$, but not of region, $F(2,22) < 1.0$, ns , interactions Region \times rTMS, $F(2,22) = 4.9$, $p < .05$, and Trial Type \times rTMS, $F(1,11) = 5.42$, $p < .05$ (no other interactions achieved significance, $F_s < 1.0$). ANOVA of the accuracy data from dlPFC alone confirmed a selective effect of rTMS on alphabetization performance, with significant main effects of trial type, $F(1,11) = 10.4$, $p < .01$, and rTMS, $F(1,11) = 9.5$, $p < .05$, and a Trial type \times rTMS

interaction, $F(1,11) = 5.1$, $p < .05$. In contrast, ANOVA of the accuracy data from the PCG control region did not show evidence for comparable selectivity of rTMS. Instead, it revealed a main effect of trial type, $F(1,11) = 5.7$, $p < .05$, but no main effect of rTMS or Trial type \times rTMS interaction ($F_s < 3.5$). To confirm the differential effects of rTMS on these two regions, ANOVA directly comparing the dlPFC with PCG was performed. It revealed a main effect of trial type, $F(1,11) = 10.6$, $p < .001$, Region \times rTMS interaction, $F(1,11) = 7.1$, $p < .05$, and Trial Type \times rTMS interaction, $F(1,11) = 9.3$, $p < .05$.

In contrast with the results from the dlPFC, ANOVA of the accuracy data from SPL stimulation revealed main effects of trial type, $F(1,11) = 5.1$, $p < .05$, and rTMS, $F(1,11) = 5.1$, $p < .05$, but no Trial Type \times rTMS interaction, $F(1,11) < 1$, ns . The absence of this interaction indicated that rTMS effects on Alphabetize 5 and Forward 5 performance were comparable.

Analyses of RT data (Figure 3) confirmed that there were no effects of interest in these data (including no effect of hemisphere of stimulation; $F_s \leq 2.0$, ns). Note that the mean RT, collapsed across all trial types and regions, was roughly double that of the 800-msec intrusion of rTMS, experienced by nine of the subjects, into the probe epoch of the trial. This, and the fact that in no region was RT sensitive to rTMS, gives us confidence that the error effects reported above were because of the disruption of processes engaged during the delay period.

DISCUSSION

The results from the dlPFC are clear: Alphabetization-related fMRI activity is independent of (indeed, perhaps negatively correlated with) load-related activity, and delay-period rTMS of loci of alphabetization-related activity produces a decrement on Alphabetize 5 trials, but not on Forward 5 trials. This portion of the results therefore confirms our hypothesis that disruption of manipulation-sensitive regions of the dlPFC would disrupt manipulation-dependent, but not retention-dependent, performance. (Note that these dlPFC results cannot be attributed to the disparity in difficulty between the two tasks because of the results from the SPL.) Our preferred interpretation of this aspect of our results is that they were produced by rTMS disruption of delay-period manipulation processes. We cannot rule out, however, the possibility that the spillover of rTMS into the first 300–800 msec of the probe epoch may have interfered with probe perception and/or evaluation-related processes. Nonetheless, this ambiguity does not lessen the result of principle theoretical import, which is that rTMS manipulation-sensitive regions of the dlPFC did not significantly disrupt the STR of information.

The results from the SPL are more complex and, indeed, illustrate the value that disruptive techniques

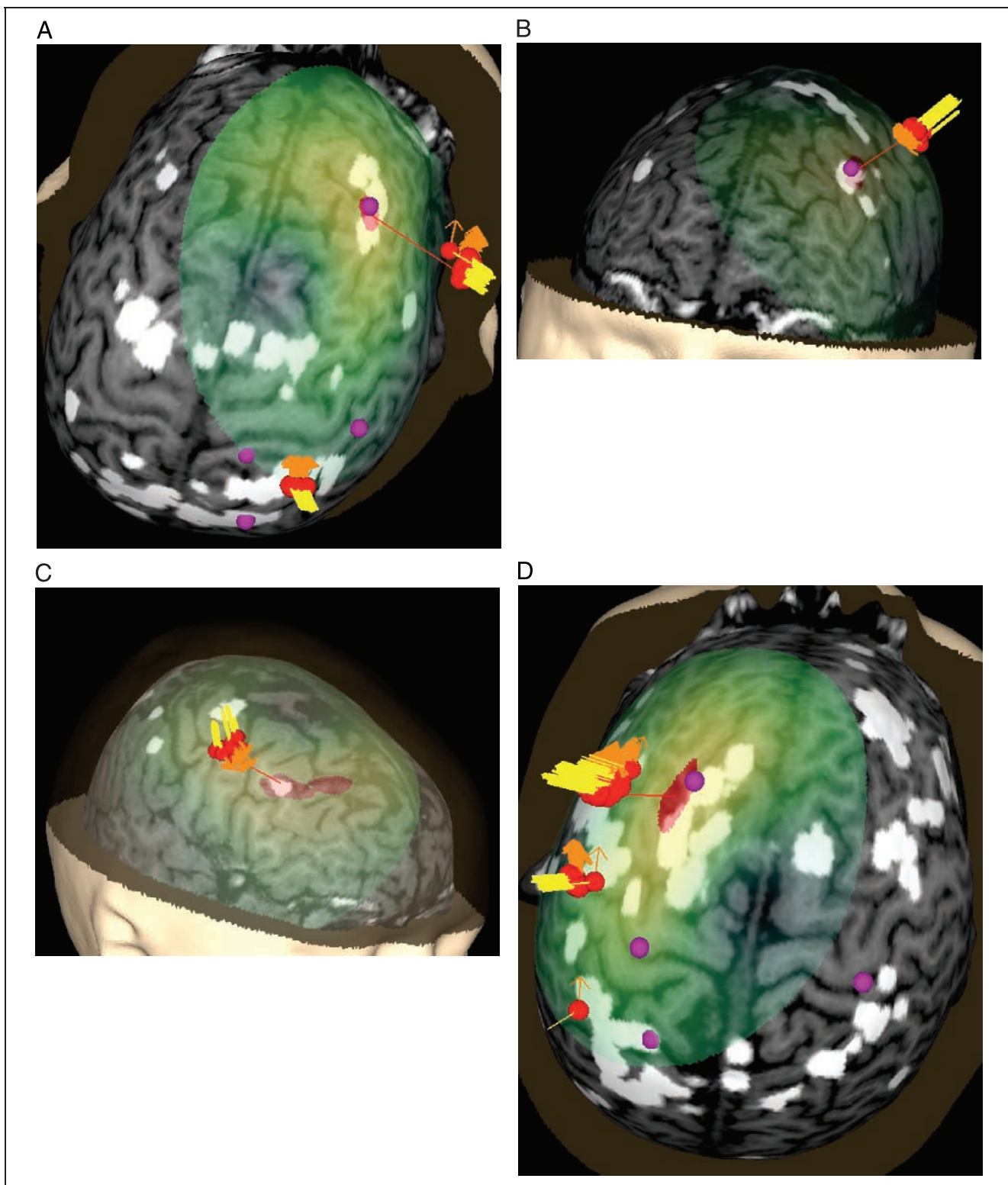


Figure 2. (A) Illustration of an rTMS experiment, from Subject 14 as displayed by the NBS system. The brain is displayed as though looking down from above, with the nose at the top of the image. The right hemisphere appears on the right side of the image. Skin and bone have been removed to below the level of the Sylvian fissure, and the cortex has been “peeled” to the depth that best displays the fMRI information (white blobs indicate regions showing alphabetization sensitivity). Each red sphere indicates the location on the scalp at which an rTMS train was delivered; the corresponding yellow spike indicates the orientation of the induced magnetic field for that stimulation train; the corresponding orange arrow indicates the estimated direction of current induction. Purple spheres indicate the targeted portions of the dlPFC and SPL. For this subject, PCG was the last region stimulated, and the splash of purple color at the end of the red spike indicates the estimated area of maximal intensity of the single rTMS train that is captured in this image. (B–H) Analogous images, highlighting rTMS of the dlPFC, are presented for seven additional subjects. Images for the rTMS sessions of the remaining four subjects were lost during a software upgrade. (B) Illustration of the left dlPFC rTMS of Subject 3. Display conventions are the same as those in (A). (C) Illustration of the right dlPFC rTMS of Subject 8. Display conventions are the same as those in (A). (D) Illustration of the three rTMS targets of Subject 16, including the left dlPFC. Display conventions are the same as those in (A).

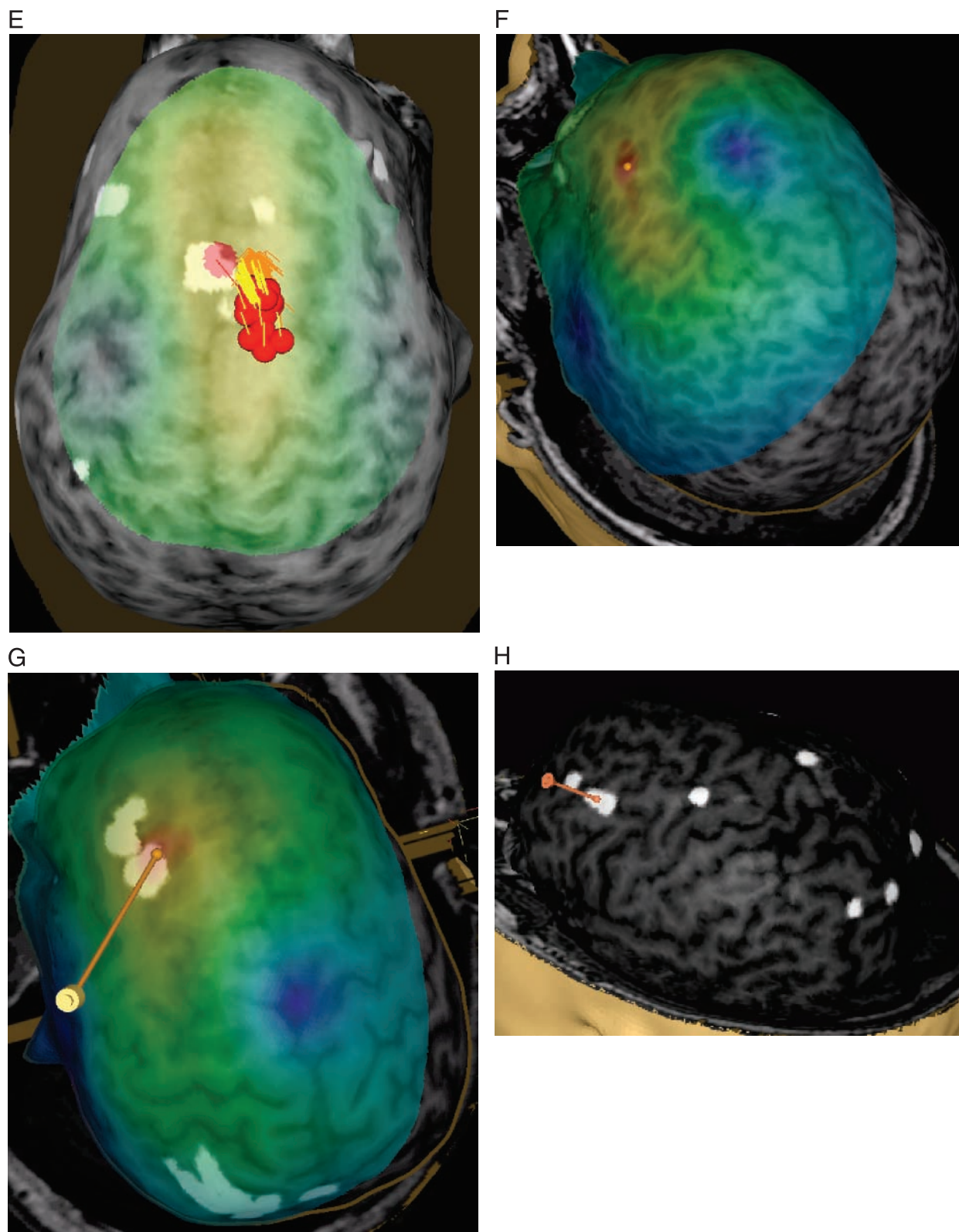


Figure 2. (*continued*) (E) Illustration of frontal-lobe rTMS target of Subject 20. This is the subject mentioned in footnote 3, who could not tolerate MFG rTMS. Display conventions are the same as those in (A). (F) Illustration of the left dlPFC rTMS of Subject 25. Display conventions differ from those in (A) in that an orange sphere represents the targeted portion of cortex and information relating to the NBS system's estimates of stimulation parameters is not shown. (G) Illustration of the left dlPFC rTMS of Subject 27. Display conventions are the same as those in (F), except that the line emanating from the targeted portion of cortex illustrates the maximal energy vector of the rTMS-induced magnetic field. (H) Illustration of the left dlPFC rTMS of Subject 17. Display conventions are the same as those in (G).

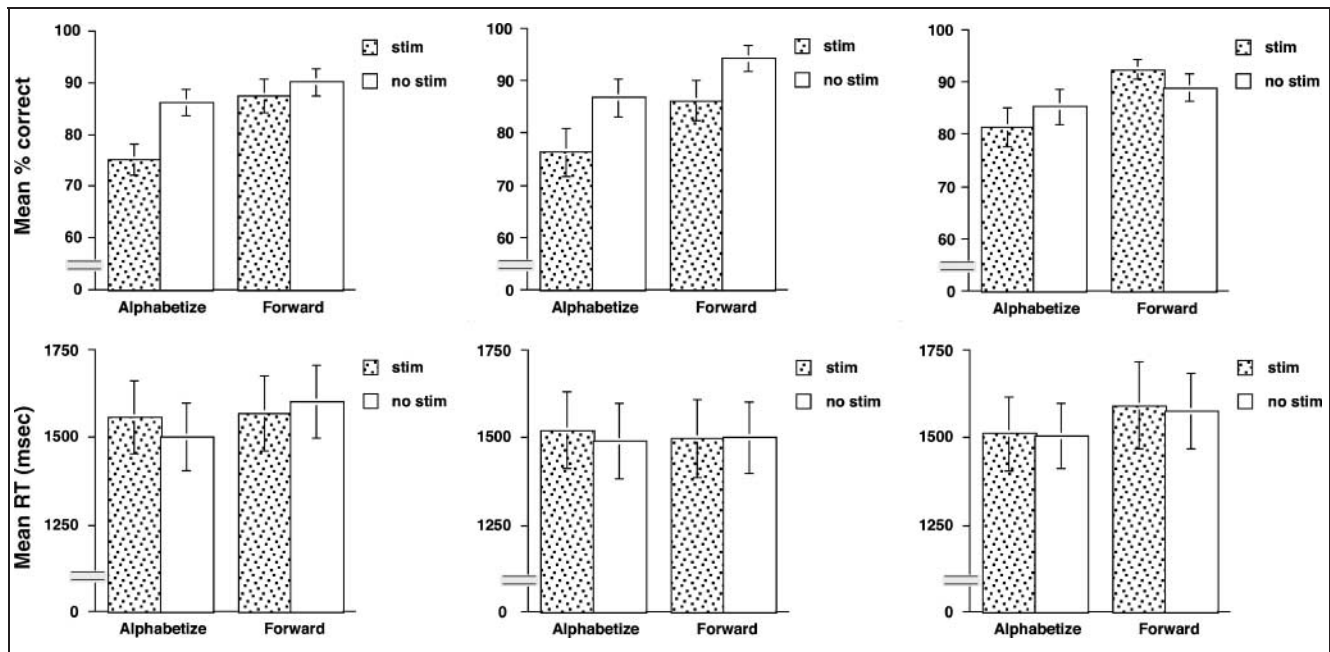


Figure 3. Results from rTMS session. Error bars indicate SEM.

can bring to systems-level analyses of cognitive functions. As with the dlPFC, fMRI data from the SPL indicated that alphabetization-related activity was independent of retention-related activity in the SPL. The rTMS data, however, led to the opposite conclusion: Disruption of activity at alphabetization-sensitive sites in the SPL yielded comparable levels of impairment on retention-requiring trials as on manipulation-requiring trials. There are at least two possible explanations for this divergence of the rTMS from the fMRI data. One is that the fMRI techniques that we employed are not sufficiently sensitive to detect retention-related effects in the SPL that are nonetheless sensitive to rTMS, a possibility that would suggest that manipulation- and retention-related functions are not independent in the SPL. This is difficult to reconcile, however, with the fact that the SPL load effects in alphabetization-sensitive voxels were not simply “not different from 0,” but were actually significantly less than 0. A second explanation, which we favor, is that the SPL voxels identified by fMRI as alphabetization-specific are just that, but that the SPL sites that we stimulated were more proximal to retention-related areas than were the analogous dlPFC sites. The proximity invoked here might be either topographical or synaptic. By the former account, the spread of the rTMS-induced electric field might be such that rTMS targeting the SPL also has disruptive effects on topographically adjacent areas, such as the angular and/or supramarginal gyri of the inferior parietal lobule, that may, themselves, support the retention of phonologically encoded information in working memory. By the latter account, the region(s) supporting retention may be only one or two synapses distant from

the SPL. It is known, for example, that TMS produces PET activation in regions that are mono- and disynaptically connected to the region that is directly targeted with TMS (Ferrarelli et al., 2004).

The absence of disruptive effects of the PFC rTMS on Forward 5 performance is consistent with the previously existing literature. Most notably, Herwig et al. (2003) used a delayed letter recognition task quite similar to our Forward 5 trials, and a similar method for guiding rTMS with fMRI data. They found no effect with a memory load of six items of delay-period rTMS targeting the dlPFC or parietal cortex (SPL or inferior parietal lobule, depending on the subject). With a load of seven items, however, delay-period rTMS targeting both the lateral premotor and parietal cortex and had disruptive effects. (They did not stimulate the dlPFC at load 7.) Other studies that have disrupted working memory performance with rTMS of the PFC have used tasks that leave ambiguous the type of information and/or process that is being disrupted. For example, delay-period activity during spatial delayed response (Brandt, Ploner, Meyer, Leistner, & Villringer, 1998; Pascual-Leone & Hallett, 1994) might be sensory, attentional, motoric, or some combination of these. The *n*-back task (Mottaghy, Gangitano, Krause, & Pascual-Leone, 2003; Mottaghy, Doring, Muller-Gartner, Topper, & Krause, 2002; Mottaghy et al., 2000) requires the simultaneous engagement of several retention- and control-related operations. The absence of laterality effect in our rTMS results is also consistent with the existing literature, which documents disruption of verbal working memory performance with right hemisphere as well as left hemisphere rTMS (e.g., Herwig et al., 2003; Mottaghy et al., 2002). This may be because of the complex and

distributed nature of the networks that support verbal working memory (e.g., Mottaghy et al., 2002, 2003) and/or that rTMS of a right hemisphere target is expected to have strong effects on the homologous contralateral region (Ferrarelli et al., 2004; Bestmann, Baudewig, Siebner, Rothwell, & Frahm, 2003).

The results of the present study are consistent with a model of segregation of executive control from STR functions in the PFC. They are also consistent with the idea that posterior regions may be more important for the STR of information than is the PFC. The contributions of the PFC to working memory may be via general-purpose control processes that are not specialized for working memory (Postle, in press).

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Notes

1. The logic of this portion of the study was that *Forward 2* trials had only been included in the fMRI study to permit evaluation of whether alphabetization-sensitive voxels (i.e., voxels significant for the [Delay_{Alphabetize} 5–Delay_{Forward 5}] contrast) also showed load sensitivity (as assessed with the contrast [Delay_{Forward 5}–Delay_{Forward 2}]). For rTMS, however, Forward 5 trials were sufficient, because if Forward 5 performance were disrupted by delay-period rTMS, one could infer that processes necessary for the STR of 5 items were disrupted. (Note that this inference would hold whether or not Forward 2 performance was disrupted by rTMS, a fact that makes clear why Forward 2 trials were not needed for the rTMS portion of this study.) The converse would be true if Forward 5 performance were not disrupted by delay-period rTMS.
2. The motor threshold offers a means of normalizing stimulation intensity across subjects, because stimulation of the (contralateral) motor cortex and PFC at motor threshold produces positively correlated evoked responses (Kahkonen et al., 2004).
3. One subject (Subject 20) could not tolerate rTMS of the MFG, because of excessive stimulation of the superior auricularis muscle. For this subject, a cluster of alphabetization-sensitive voxels along the midline, in a region judged to correspond to Brodmann's area 8, was selected as the alternative target for dlPFC stimulation (Figure 2E). The remaining two regions for this subject were stimulated in the right hemisphere.

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