

The Role of the Prefrontal Cortex in the Maintenance of Verbal Working Memory: An Event-Related fMRI Analysis

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Neuroimaging studies have been inconclusive in characterizing the role of the prefrontal cortex (PFC) for maintaining increasingly larger amounts of information in working memory (WM). To address this question, the authors collected event-related functional MRI data while participants performed an item-recognition task in which the number of to-be-remembered letters was parametrically modulated. During maintenance of information in WM, the dorsolateral and the ventrolateral PFC exhibited linearly increasing activation in response to increasing WM load. Prefrontal regions could not be distinguished from one another on the basis of load sensitivity, but the dorsolateral PFC had stronger functional connectivity with the parietal and motor cortex than the ventrolateral PFC. These results suggest an increasingly important role for the PFC in actively maintaining information as the amount of that information increases.

Keywords: verbal working memory, prefrontal cortex, executive processing, fMRI, encoding, maintenance, retrieval, parietal cortex, neuroimaging

Working memory (WM) involves the active maintenance of goal-relevant information for a short period of time. This process is fundamental to higher cognitive tasks, such as problem solving, reasoning, and language (Baddeley, 1986). Single-unit recordings in nonhuman primates have found neurons within the prefrontal cortex (PFC) and the posterior parietal cortex that are persistently active across a delay period during delayed match-to-sample tasks (Goldman-Rakic, 1987), and physiological studies in nonhuman primates have identified a network of brain areas in the PFC that are involved in WM (Fuster, 1997; Kubota & Niki, 1971). Functional brain imaging studies of WM in humans have found consistent activation in similar brain regions, including the dorsolateral PFC (i.e., middle frontal gyrus), ventrolateral PFC (i.e., inferior frontal gyrus), and posterior parietal cortex (Awh, Smith, Koeppe, Schumacher, & Katz, 1996; Cohen et al., 1997; Fletcher

& Henson, 2001; Paulesu, Frith, & Frackowiak, 1993; Prabhakaran, Narayanan, Zhao, & Gabrieli, 2000; Smith & Jonides, 1999).

WM appears to comprise distinct subsystems: (a) *maintenance*, or the process of keeping information in mind in the absence of an external stimulus, including subsystems responsible for active rehearsal and storage, and (b) *manipulation*, or executive processes that involve reordering and updating of information maintained in WM (reviewed in Fletcher & Henson, 2001). Several studies have attempted to dissociate the maintenance and manipulation components of WM (D'Esposito, Postle, Ballard, & Lease, 1999; Postle, Berger, & D'Esposito, 1999; Veltman, Rombouts, & Dolan, 2003). A distinction between the ventrolateral and dorsolateral regions of the PFC has been proposed, whereby the ventrolateral regions are primarily involved in active maintenance, and the dorsolateral regions are differentially recruited when information must be manipulated in addition to being maintained (D'Esposito, Postle, & Rypma, 2000; Petrides, 1994, 1996).

WM resources in the brain must be taxed increasingly as larger amounts of information need to be maintained in WM. Accordingly, neuroimaging studies that have increased WM load, or the number of items to be maintained in WM, have observed greater activations in both the ventrolateral PFC and the dorsolateral PFC as a consequence of increased WM load (Braver et al., 1997; Bunge, Ochsner, Desmond, Glover, & Gabrieli, 2001; Manoach et al., 1997; Rypma, Prabhakaran, Desmond, Glover, & Gabrieli, 1999; Veltman et al., 2003). However, these studies do not provide direct insights into activations associated specifically with the maintenance of increasingly larger amounts of information. Many of the studies involved blocked designs or complex tasks (e.g., the *n*-back task) that invoke executive processing, such as updating and monitoring items being maintained in WM. Such designs preclude determination of whether this load-dependent activation was related specifically to active maintenance versus additional encoding, retrieval, and executive processes involved in task performance.

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A task that enables the dissociation of WM processes is the item-recognition paradigm (Sternberg, 1966), in which a target set is actively maintained across a delay period, and participants must judge whether a probe matches the target set. Separate functional MRI (fMRI) responses can be calculated for (a) encoding of the target set, (b) maintenance of the target set in WM, and (c) retrieval of the target set in order to compare it with the probe. Furthermore, WM load can be varied as a function of the size of the target set. Latency of response time to the probe letter and error rate have been shown to increase parametrically with WM load (Sternberg, 1966).

Event-related designs that have used this paradigm have thus far been inconclusive regarding the role of the PFC in maintenance of WM information as a function of the amount of information to be maintained. Rypma and D'Esposito (1999) reported no increase in the activation of either the dorsolateral or the ventrolateral PFC with increasing WM load during maintenance. Postle et al. (1999) did not find reliable increases in the activation of the dorsolateral PFC during maintenance when load was increased from two to five letters. Similarly, Rypma, Berger, and D'Esposito (2002) reported inconsistent increases in the PFC with increasing WM load during maintenance across participants. For high-capacity participants, activation in both the dorsolateral and the ventrolateral PFC increased as a function of WM load during maintenance; in contrast, for low-capacity participants, activation in the ventrolateral PFC did not increase with WM load during maintenance, and activation in the dorsolateral PFC increased with WM load only during the last 4 s of the maintenance period. In nonverbal WM tasks, the role of the PFC in maintenance of WM information as a function of load also remains unclear. In an item-recognition study with faces, Jha and McCarthy (2000) did not find evidence for a load-sensitive WM process related to the maintenance of WM information because increases in activation of both the dorsolateral PFC and the ventrolateral PFC with increased WM load did not span the maintenance period in their task. In contrast, in an item-recognition task involving spatial locations, Leung, Gore, and Goldman-Rakic (2002) found activation in both the dorsolateral PFC and the ventrolateral PFC that increased with WM load during maintenance. From these studies, it is difficult to characterize the role of the PFC in maintenance of WM information because these studies did not reliably find increases in PFC activation with WM load.

To test whether the role of the PFC increases as the amount of WM information to be maintained is increased, in the present study we parametrically manipulated the number of letters to be maintained between three and six letters in an item-recognition paradigm while collecting event-related fMRI data. We expected that regions involved directly in active maintenance would increase their activity as WM load was increased during the maintenance phase of the task.

Method

Participants

Twelve healthy, right-handed, native-English-speaking volunteers were recruited from Stanford University's student population (5 women and 7 men, ages 19–26 years, $M = 20.6$) and paid for their participation. All studies were done in full compliance with the guidelines of the Institutional Review Board of Stanford University, and each participant provided informed consent.

Task and Stimuli

PsyScope (Cohen, MacWhinney, Flatt, & Provost, 1993) was used to generate stimuli and collect responses. Stimuli were back-projected onto a screen located above the participant's head via a magnet-compatible LCD projector. Stimuli were viewed from a mirror mounted above the participant's head. Participants performed a verbal WM item-recognition task while fMRI data were acquired. Each trial had successive encoding, maintenance, and retrieval phases. During encoding, a target set of three, four, five, or six uppercase letters was presented for 2.16 s in a single row in the center of the stimulus field. Target set letters were selected randomly from all possible consonants (excluding y), and letters appearing in a target set (including incorrect probes) did not appear in the immediately preceding trial. During maintenance, participants were instructed to actively maintain the memory set over a delay period of 6.48 s, throughout which the stimulus field was blank. During retrieval, participants then viewed a single, lowercase probe letter presented in the center of the stimulus field for 2.16 s and had to press one of two buttons to indicate whether or not the probe letter corresponded to one of the letters in the target set. After retrieval, there was a 10.8-s intertrial interval. Twenty-four trials of each memory load were presented over four successive sessions in randomized order. For each WM load, half of the probe letters matched the target set, and half did not. The order of the four sessions was counterbalanced across participants.

Data Acquisition

The fMRI data were collected with a 1.5 Tesla whole-body MRI scanner (Signa, Rev. 5.5; General Electric Medical Systems, Chalfont, St. Giles, UK). Head movement was minimized by means of a custom-built bite bar. Images for each participant were obtained from 16 T1-weighted anatomic images (field of view [FOV] = 20×20 cm², slice thickness = 7 mm, in-plane resolution = 3.125 mm) acquired parallel to the anterior commissure–posterior commissure line. A T2* sensitive gradient echo spiral sequence was used for functional imaging (Glover & Lai, 1998; return time [TR] = 2,160 ms, echo time = 40 ms, flip angle = 83°, FOV = 20 cm, in-plane resolution = 3.125 mm², and sampling interval = 2.16 s). With a TR of 2,160 ms, encoding comprised one scan, maintenance comprised three scans, retrieval comprised one scan, and the intertrial interval comprised five scans.

fMRI Analysis

Image reconstruction was performed offline by transferring the raw data to Pentium II computers running Red Hat Linux (Red Hat, Durham, NC). Image analyses were conducted with SPM99 (Wellcome Department of Imaging Neuroscience, 1999) running in Matlab v5.2 (Mathworks, Natick, MA). Images were corrected for differences in temporal acquisition between slices, realigned, motion-corrected, and normalized. Images were sinc interpolated to $2 \times 2 \times 2$ -mm³ voxels and spatially smoothed with a Gaussian filter (7 mm full width half maximum). Images were high-pass filtered at 48 s to remove low-frequency drifts in blood oxygen level-dependent signal.

An event-related approach (D'Esposito, Zarahn, & Aguirre, 1999; Friston et al., 1998) was used to model encoding, maintenance, retrieval, and the intertrial interval. Regressor functions were generated by convolving a boxcar corresponding to each event with the canonical hemodynamic response function (Josephs, Turner, & Friston, 1997). Because regressor functions were generated in this way, there was an a priori possibility of contamination of the variance of one phase contributing to subsequent phases. Incorrect trials were excluded. Contrasts between the phase of interest (encoding, maintenance, or retrieval) and the intertrial interval were computed. Group data were analyzed by means of a random effects analysis. Statistical parametric maps were created for each contrast by transforming T maps to normal z distributions. Data are reported for

clusters of activation that survived a statistical threshold of $p < .001$ (uncorrected and with a cluster size greater than five voxels). Foci of activation are reported in Montreal Neurological Institute (MNI) coordinates.

Region of Interest (ROI) Analysis

ROIs were drawn in relevant Brodmann areas (BA) according to labels for the Talairach Daemon database (<http://ric.uthscsa.edu/projects/talairachdaemon.html>; Lancaster et al., 2000; Talairach & Tournoux, 1988) and transformed into MNI space. Raw signal intensities over the course of the experiment were extracted at each time point by averaging signal intensities from suprathreshold voxels (above $p < .001$) during encoding, maintenance, and retrieval. The time course of the fMRI signal was extracted from each ROI according to methods described in detail elsewhere (Christoff et al., 2001). To minimize noise-related artifacts, we processed the raw fMRI signal by linear detrending, bandpass filtering (using a second-order Butterworth filter between 0.25 Hz and 0.048 Hz) to reduce low-frequency confounds and high-frequency noise, scaled across sessions. The fMRI signal from each participant was then decomposed into 21.68-s trial blocks corresponding to the task structure and re-sorted according to WM load. The resulting fMRI signal was trial-averaged within participants. Median time courses across participants were interpolated to generate plots of group fMRI signal from all participants in each ROI at each WM load.

To quantify the effects of WM load, we averaged T statistics corresponding to the degree of fit of fMRI signal in a particular voxel with a regressor of interest (encoding, maintenance, or retrieval at WM loads of three, four, five, and six letters; see Figure 1) among all suprathreshold voxels in each ROI from each participant. Repeated measures analyses of variance (ANOVAs) were performed to test for both main effects of WM load and for linear effects of WM load (using a linear contrast of $[-3 -1 1 3]$) across 12 participants in each ROI during encoding, maintenance, and retrieval.

To investigate the functional connectivity between pairs of brain regions (Hampson, Peterson, Skudlarski, Gatenby, & Gore, 2002; Lowe, Mock, & Sorenson, 1998; Sakai, Rowe, & Passingham, 2002), we correlated the activity of several pairs of ROIs in each participant. We calculated a correlation coefficient (Pearson's R) between the time courses from two given ROIs in each participant by means of the following equation (Lowe et al., 1998):

$$R = \frac{\sum(S_1 - M_{S1})(S_2 - M_{S2})}{\sqrt{\sum(S_1 - M_{S1})^2 \sum(S_2 - M_{S2})^2}}$$

where S_1 and S_2 are the signals from two ROIs, and M_1 and M_2 are their respective means. Significance of R can be assessed by converting R to a T distribution (Spiegel, Schiller, & Srinivasan, 2000); however, because small values of R are significant as a result of the high degrees of freedom involved ($df = 960$, $R = .1$, $T = 3.076$, $p < .001$), this test was used only to identify ROIs whose time courses of activation were *not* significantly correlated. To test whether one pair of ROIs was more strongly correlated than another pair of ROIs across participants, we converted R values from each of 12 participants for a particular pair of ROIs to an approximately normal distribution with Fisher's z transformation (Sakai, Rowe, & Passingham, 2002) and compared them by means of a one-way T test with similarly transformed R values from other pairs of ROIs across participants.

Results

Behavioral Data

Repeated measures ANOVAs were performed to examine the effects of memory load on accuracy and response time (RT; see Figure 2). Mean RT increased linearly with load across participants, $F(3, 11) = 6.02$, $p < .01$. Participants had a mean RT slope of 55 ± 30 ms/letter and a mean intercept of 94 ± 198 ms. Accuracy tended to decrease with load, $F(3, 11) = 2.99$, $p < .08$, and accuracy at Loads 3 and 4 was significantly higher than accuracy at Loads 5 and 6, $T(11) = 3.94$, $p < .0005$.

Imaging Data

To identify all brain regions activated during encoding, maintenance, or retrieval of WM irrespective of load, we performed random effects analyses contrasting encoding, maintenance, and retrieval for all WM loads with the intertrial interval (see Figure 3).

Encoding activation was observed bilaterally in the thalamus, primary visual cortex (BA 17), higher visual areas (BA 19), angular gyrus (BA 39), left-lateralized middle frontal gyrus (BA 9), inferior frontal gyrus (BA 44/45), anterior cingulate gyrus (BA 32), and striatum (see Table 1).

Maintenance activation was observed in the left-lateralized premotor cortex (BA 6), superior frontal gyrus (BA 8), middle frontal gyrus (BA 9/46), inferior frontal gyrus (BA 44/45), anterior cin-

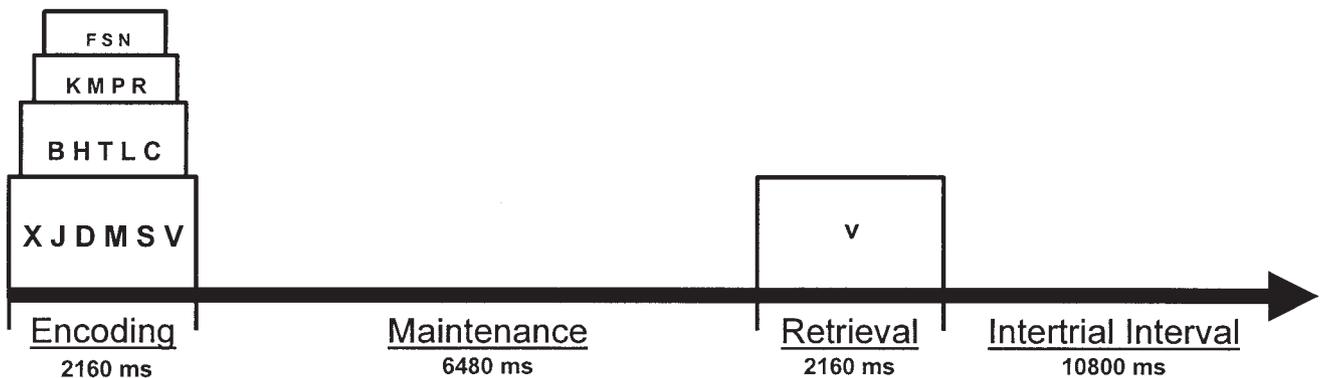


Figure 1. Verbal working memory item-recognition task (Sternberg, 1966). During functional imaging, participants encoded a memory set of three, four, five, or six letters for 2,160 ms. Participants maintained the memory set for a 6,480-ms delay and had to judge whether a single probe letter matched the memory set for each trial. A 10,800-ms intertrial interval separated each trial.

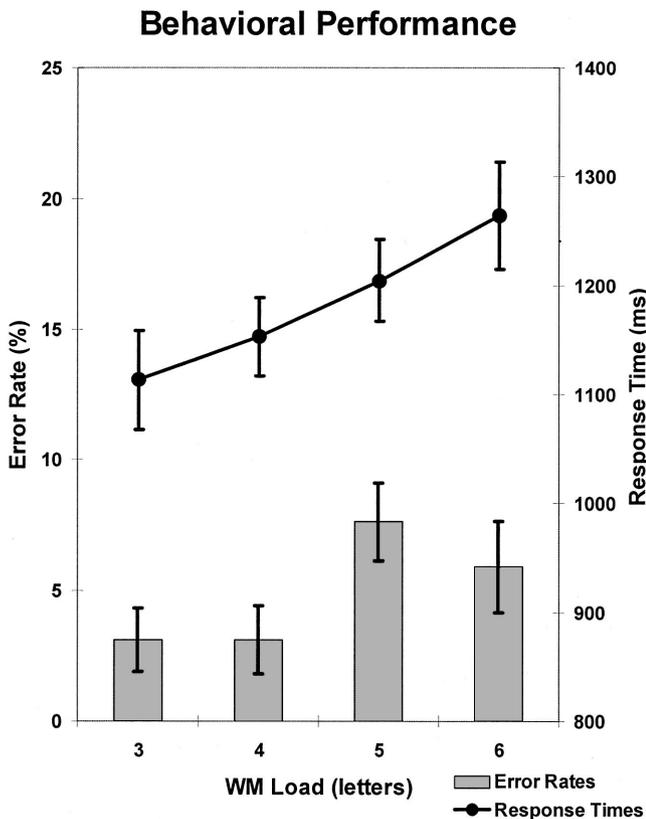


Figure 2. Mean (\pm SEM) error rates with increasing working memory (WM) load. Behavioral response time increased linearly with WM load, and behavioral accuracy decreased with load.

gulate gyrus (BA 24/32), parietal cortex (BA 40), and caudate (see Table 2).

Retrieval activation was observed in the left-lateralized left superior frontal gyrus (BA 8), left middle frontal gyrus (9/46), inferior frontal gyrus (BA 44/45), anterior cingulate gyrus (BA 24), left parietal cortex (BA 40/7), and thalamus. Additional regions of activation were found in the right inferior frontal gyrus (BA 44/45), right insula, left primary motor cortex (BA 4/5), left somatosensory cortex (BA 0.5/3), bilateral claustrum, and right insula (see Table 3).

Load Effects Within ROIs

To investigate the effect of WM load on fMRI signal in ROIs, we investigated T statistics corresponding to the degree of fit of fMRI signal from suprathreshold voxels with a regressor of interest (encoding, maintenance, or retrieval at WM loads of three, four, five, or six letters). To test for main effects and for linear effects of WM load, we performed repeated measures ANOVAs on T statistics in ROIs selected for being involved in WM (middle frontal gyrus, inferior frontal gyrus, and posterior parietal cortex) and in ROIs selected for visuomotor controls (visual cortex and left precentral gyrus; see Figure 4).

Principal areas increasing with WM load during encoding included the primary visual cortex: BA 17, $F(3, 11) = 18.13$, $p <$

$.10 \times 10^{-7}$, in which an increasing linear effect of load was observed: BA 17, $F(3, 11) = 15.09$, $p < .0003$. Motor regions, left middle frontal and inferior frontal gyri, parietal regions, anterior cingulate gyrus, and the thalamus were not sensitive to load during encoding.

Principal areas that showed increased activity with increasing WM load during maintenance included the left middle frontal gyrus: BA 9, $F(3, 11) = 5.38$, $p < .001$; bilateral superior frontal gyrus: BA 8, $F(3, 11) = 4.80$, $p < .01$; and left inferior frontal gyrus: BA 44, $F(3, 11) = 2.84$, $p < .05$; BA 45, $F(3, 11) = 3.10$, $p < .04$. Of these areas, linear effects of load were observed in the left middle frontal gyrus: BA 9, $F(3, 11) = 3.77$, $p < .05$; left superior frontal gyrus: BA 8, $F(3, 11) = 3.82$, $p < .04$; and left inferior frontal gyrus: BA 44, $F(3, 11) = 3.07$, $p < .07$; BA 45, $F(3, 11) = 3.52$, $p < .05$. The parietal regions, right inferior frontal gyrus, motor cortex, and subcortical regions did not reliably increase activation as a function of load.

The superior frontal gyri, middle frontal gyri, inferior frontal gyri, motor cortex, visual regions, parietal cortex, and subcortical regions were not sensitive to WM load during retrieval.

In summary, load effects were observed in the bilateral visual cortex during encoding and in the left superior, middle, and inferior gyri during maintenance.

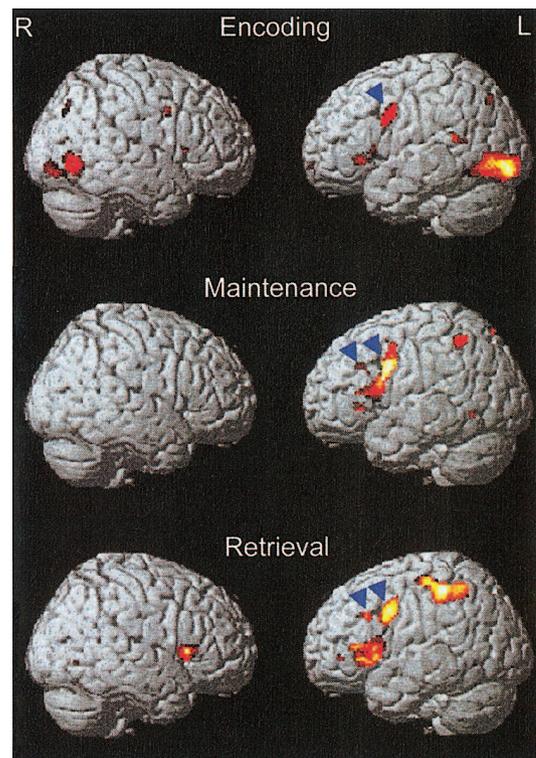


Figure 3. Statistical parametric maps of encoding, maintenance, and retrieval independent of load, from a random effects model from 12 participants rendered on whole brain volumes. All voxels that are correlated with encoding, maintenance, and retrieval as determined by the general linear model above $p < .001$ (uncorrected) are shown. Blue arrows indicate activations in the dorsolateral prefrontal cortex (Brodmann areas 9 or 46). R = right; L = left.

Table 1
Foci of Activation During Encoding

Brain region	Brodmann area	Maxima			Volume (mm ³)	T value
Frontal						
Left middle frontal gyrus	9	-50	2	32	8,432	5.78
Left inferior frontal gyrus	44/45	-34	26	0	15,300	7.26
Anterior cingulate	24/32	-10	-18	44	4,434	4.86
Parietal						
Left angular gyrus	39	-44	-78	2	1,564	5.28
Right angular gyrus	39	42	-70	2	1,496	6.62
Occipital						
Left visual cortex	17	-12	-86	-6	7,208	8.37
Right visual cortex	17	8	-88	-6	5,644	5.38
Left lateral occipital	19	-28	-84	-2	17,272	7.43
Right lateral occipital	19	42	-66	-2	7,480	8.23
Subcortical						
Thalamus		-10	-26	-2	20,128	8.68
Striatum		-20	10	2	7,480	5.15
Left claustrum		-32	26	-2	1,564	7.19

Correlated Activity Between ROIs

To investigate functional relationships between brain regions, we correlated the fMRI signal from selected ROIs with the fMRI signal from other ROIs within each participant (see Figure 5). The left middle frontal gyrus activation was correlated with activation in the left inferior frontal gyrus (BA 9 vs. BA 44: $R = .46 \pm .01$). The left middle frontal gyrus activation was also correlated with the left parietal activation (BA 9 vs. BA 40: $R = .47 \pm .01$). In comparison, the left inferior frontal gyrus activation was less strongly correlated with parietal activation (BA 44 vs. BA 40, $R = .25 \pm .01$), and this difference in correlation strength for the two PFC subregions was significant, $T(11) = 6.86, p < 6.76 \times 10^{-7}$.

The left middle frontal gyrus activation was also correlated with the left primary motor cortex (BA 9 vs. BA 4: $R = .43 \pm .02$), and this correlation was significantly stronger than that between the primary motor cortex and the left inferior frontal gyrus (BA 44 vs. BA 4: $R = .28 \pm .02$); difference, $T(11) = 3.23, p < .004$. However, the left parietal cortex was even more strongly correlated with the left motor cortex than it was with the left middle frontal gyrus (BA 40 vs. BA 4, $R = .60 \pm .01$); difference, $T(11) = 2.49, p < .02$.

The fMRI signal from the prefrontal regions and left parietal cortex was not significantly correlated with the fMRI signal from the visual cortex (BA 9 vs. BA 17: $R = .02 \pm .01, T = .62, p < .26$; BA 44 vs. BA 17: $R = .00 \pm .01, T = .62, p < .26$; BA 40 vs. BA 17: $R = -.01 \pm .01, T = .30, p < .38$).

Correlation strength between pairs of ROIs was not affected by the size of ROIs. The mean correlation between R values and ROI size in voxels across participants was small ($R_{\text{avg}} = .007$). No correlations were significantly affected by WM load.

Discussion

The present study used event-related brain imaging to investigate whether the PFC showed any influence of WM load during maintenance of WM information by identifying brain regions involved specifically in the active maintenance of verbal WM and characterizing the response of these brain regions to an increase in verbal WM load. Maintenance was associated with load-sensitive activation of the left inferior, middle, and superior frontal gyri. The left-hemisphere lateralization of activations likely reflects the verbal nature of the material. In addition, encoding was associated with load-sensitive activation in the visual cortex and load-inde-

Table 2
Foci of Activation During Maintenance

Brain region	Brodmann area	Maxima			Volume (mm ³)	T value
Frontal						
Left premotor cortex	6	-58	4	32	14,284	6.91
Left superior frontal gyrus	8	-6	24	46	8,364	6.59
Left middle frontal gyrus	9/46	-58	6	30	17,612	6.00
Left inferior frontal gyrus	44	-50	10	18	2,448	4.87
Left inferior frontal gyrus	45	-36	26	2	2,720	5.21
Left anterior cingulate	24/32	-4	22	46	10,744	6.26
Parietal						
Left posterior parietal cortex	40	-34	-50	52	8,228	5.97
Subcortical						
Left caudate		-12	10	6	3,740	5.32

Table 3
Foci of Activation During Retrieval

Brain region	Brodmann area	Maxima			Volume (mm ³)	T value
Frontal						
Left superior frontal gyrus	8	-46	6	38	2,516	5.95
Left middle frontal gyrus	9	-50	22	32	2,108	6.99
Left middle frontal gyrus	9	-46	2	40	15,436	6.46
Left middle frontal gyrus	46	-50	30	8	2,312	5.00
Left inferior frontal gyrus	45	-34	24	2	27,540	10.73
Left inferior frontal gyrus	44	-40	16	6	5,780	6.94
Right inferior frontal gyrus	44/45	40	16	2	18,224	7.52
Left anterior cingulate	24	-8	6	52	1,972	5.44
Left premotor cortex	6	-46	0	40	12,240	6.61
Left motor cortex	4/5	-38	-48	54	1,972	5.44
Right insula		40	18	4	14,824	7.69
Parietal						
Left posterior parietal cortex	40/7	-44	-36	48	35,836	5.84
Left primary sensory cortex	1/2/3	-44	-34	48	11,288	5.72
Subcortical						
Left thalamus		-4	-28	0	31,212	7.67
Left claustrum		-34	24	-2	3,196	8.35
Right claustrum		36	18	4	1,156	6.03

pendent activation of the left inferior frontal gyrus. Retrieval was associated with load-independent activation in the bilateral inferior frontal gyrus, left middle frontal gyri, left superior frontal gyri, and parietal cortex. Regions in the left PFC could not be distinguished from one another by their load sensitivity, but a functional connectivity analysis revealed correlations between PFC activations and both parietal and motor activations that were significantly stronger for the left middle frontal gyrus than for the left inferior frontal gyrus.

The Ventrolateral PFC in WM

In the present study, the left ventrolateral PFC exhibited load sensitivity during the maintenance of verbal WM information. This finding is consistent with the greater ventrolateral PFC activation for higher WM loads than for lower WM loads found by Rypma et al. (1999); however, that study used a blocked design that could not discriminate between encoding, maintenance, and response phases of the task.

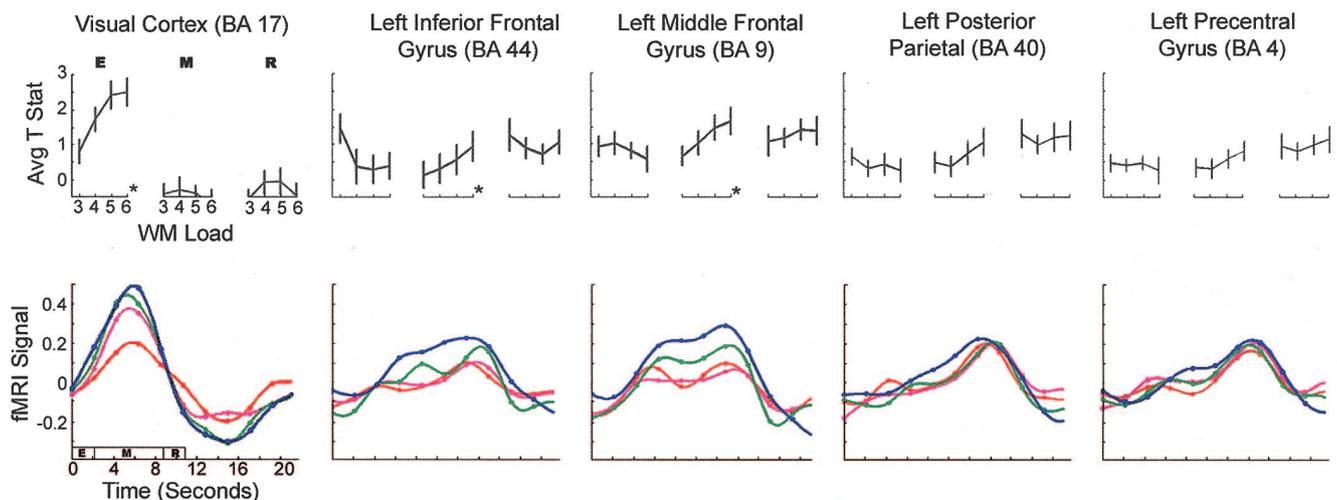


Figure 4. Load effects and normalized functional MRI (fMRI) signal in regions of interest (ROIs). Top row: Average *T* statistics (Avg *T* Stat) and standard errors corresponding to the degree of fit of fMRI signal in each ROI with a regressor of interest (encoding [E], maintenance [M], or retrieval [R]) at working memory [WM] load of three, four, five, or six letters). Asterisks indicate significant load effect ($p < .05$). Bottom row: Normalized and interpolated group fMRI time courses from 12 participants at four WM loads. Load 3: red; Load 4: magenta; Load 5: green; Load 6: blue. BA = Brodmann area.

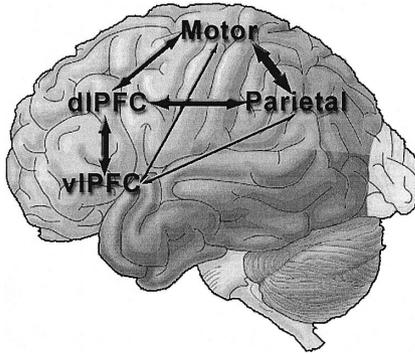


Figure 5. Significant correlations of functional magnetic resonance imaging (fMRI) signal between regions of interest (ROIs). Arrow size is proportional to the strength of the correlation between fMRI signals between ROIs. dIPFC = dorsolateral prefrontal cortex; vIPFC = ventrolateral prefrontal cortex.

Prior event-related studies have found inconsistent evidence of load sensitivity in the ventrolateral PFC during the maintenance of verbal WM information. Rypma and D'Esposito (1999) found that the ventrolateral PFC was not load sensitive during maintenance when WM load was increased from two to six letters. A subsequent study (Rypma et al., 2002) found that the ventrolateral PFC was load sensitive when WM load was parametrically increased from one to eight letters only for high-performing participants during the middle 4 s of a 12-s maintenance period, and not load sensitive either for low-performing participants or during other phases of the maintenance period. In both of these studies, the first 4 s of the maintenance period was excluded.

In contrast to these studies, we found evidence for a linear increase in the activation of the left ventrolateral PFC with a parametric increase in WM load. Our finding is in agreement with Veltman et al. (2003), who found that activation in the ventrolateral PFC was linearly load sensitive when WM load was increased parametrically from two to seven letters in an item-recognition task. Our result is consistent with a view that the left ventrolateral PFC, particularly Broca's area, is involved in the subvocal rehearsal of verbal information (Awh, Smith, Koeppel, Schumacher, & Katz, 1996; Paulesu et al., 1993; Smith, Jonides, Marshuetz, & Koeppel, 1998). Rehearsal processes may be hypothesized to intensify at higher verbal WM loads.

The Dorsolateral PFC in WM

The dorsolateral PFC also exhibited load sensitivity during maintenance. This finding differs from some previous event-related studies of verbal WM load. Rypma and D'Esposito (1999) found no load-sensitive activation in the dorsolateral PFC during maintenance when WM load was increased from two to six letters in an item-recognition task. Postle et al. (1999) found load-sensitive activation in the dorsolateral PFC in only 2 of 5 participants when WM load was increased from two to five letters during an item-position recognition task. However, our results are consonant with those of Veltman et al. (2003), who found that the dorsolateral PFC was linearly load sensitive when WM load was parametrically increased from two to seven letters in an item-recognition task, and with those of Rypma et al. (2002), who found load-

sensitive increases in the dorsolateral PFC when WM load was parametrically increased from one to eight letters in an item-recognition task. Our results suggest that the dorsolateral PFC is involved directly in WM maintenance in the absence of overt manipulation requirements, especially as the WM load increases. We also observed load sensitivity during maintenance in the superior frontal gyrus (BA 8), an area that includes presupplementary motor areas. This region may be a component of the rehearsal circuitry involved in actively maintaining WM information (Smith & Jonides, 1999).

The dorsolateral PFC has been found to be sensitive to load during maintenance in nonverbal WM tasks. In a study involving an item-recognition task with spatial locations and a maintenance period of 24 s, Leung et al. (2002) observed significant activation increases during maintenance in the dorsolateral PFC as WM load was increased from three to five spatial locations. These results suggest that the dorsolateral PFC is sensitive to load during maintenance across multiple WM modalities.

Differences exist between the present study and previous event-related studies that have examined load sensitivity in the dorsolateral PFC during the maintenance of WM information. Rypma and D'Esposito (1999) reported an absence of load-sensitive activation in the dorsolateral PFC during maintenance of WM information. In a subsequent study (Rypma et al., 2002), load sensitivity in the PFC during maintenance was found only in low-performing participants. In both of these studies, the authors constructed structural ROIs of the entire dorsolateral PFC, combining several Brodmann areas from both hemispheres. When large ROIs were used for both hemispheres, the significant activation of a population of load-sensitive voxels may have been masked when pooled together with a large number of load-insensitive voxels. For instance, in the present study, only 10% of all voxels in the bilateral dorsolateral PFC were significantly active during maintenance. Furthermore, most of the activation involved in maintenance occurred in the left hemisphere, so that pooling results with homologous right-hemisphere regions could have obscured left-hemisphere activation. Examination of activation within functionally defined ROIs (such as left BA 9, 46, 44, and 45) that were shown to be engaged in the task may have been more sensitive for detecting prefrontal regions with load-sensitive characteristics during the maintenance of information in WM.

One study, which found that frontal regions were generally load insensitive during maintenance (Postle et al., 1999), used ROIs of similar size as those used in our study; however, this study included only 5 participants; manipulated load between only two points (two letters and five letters); and used an item-position recognition task, which includes a nonverbal WM component. The present study may have been more sensitive to manipulations of verbal WM load because it included a larger participant pool, manipulated WM load parametrically, and used an item-recognition task in which only verbal information is maintained.

Another difference between the present study and previous studies by Rypma and D'Esposito (1999) and Rypma et al. (2002) is the role of the PFC during the encoding phase. The inconsistent load sensitivity observed in these studies during the encoding period (Rypma & D'Esposito, 1999, found that the dorsolateral PFC increases with WM load during encoding, and Rypma et al., 2002, found that the ventrolateral PFC decreases with WM load during encoding) may be an artifact of the 4-s encoding period

used in their task, during which maintenance processes may begin and interfere with the encoding period regressors. Although the time of initial encoding can be controlled by the time of initial presentation, participants may begin maintenance-related rehearsal in parallel with encoding. This overlapping psychological processing of encoding and maintenance (such as maintaining the first three letters and encoding the second three letters for a stimulus set of six letters) may be more pronounced at higher loads. In the present study, which used an encoding period of 2.16 s, inferior frontal regions were observed to be active but load insensitive during encoding, suggesting that active maintenance begins very soon after, or even during, the encoding of WM information.

These results offer insight into the posited functional dissociation within the PFC, whereby the ventrolateral PFC is hypothesized to be more involved in maintenance of WM information, and the dorsolateral PFC is hypothesized to be differentially recruited in manipulation of information in WM (D'Esposito, Postle, & Rypma, 2000; Petrides, 1994, 1996). The finding that the dorsolateral PFC response is linearly related to WM load differs from the suggestion—derived from previous blocked design studies—that the PFC is divided functionally, with the dorsolateral PFC primarily recruited at high WM loads (i.e., exhibits a step function), when additional executive processes, such as “chunking” of WM information, may be recruited to assist maintenance processes (Bunge et al., 2001; Rypma & D'Esposito, 1999). Rather, the present data are consistent with prior studies (Bunge, Klingberg, Jacobsen, & Gabrieli, 2000; Jha & McCarthy, 2000; Leung et al., 2002; Postle et al., 1999; Rypma et al., 1999, 2002) in which dorsolateral activation was observed during performance of tasks with no overt manipulation. Our results are in agreement with those of Veltman et al. (2003), who found that maintenance (item-recognition) and manipulation (*n*-back) tasks activate the same distributed network of WM areas. Taken together with the present results, these data show that activation in the dorsolateral PFC is recruited during maintenance and is not solely attributable to nonmnemonic processes associated with encoding or retrieval.

Parietal Cortex in WM

The absence of a reliable load effect in the left parietal cortex is somewhat surprising given other evidence that the parietal cortex may serve as the storage site for information held in verbal WM (Awh et al., 1996; Paulesu et al., 1993) and given neuroimaging studies that found increased activation with increased WM load in parietal regions (Postle et al., 1999; Veltman et al., 2003). Indeed, there was a clear trend for the parietal cortex to show increased activation across Loads 4–6 (see Figure 4), but the variability was such that activation did not reliably increase across loads. This may reflect limited power of measurement. Furthermore, there has been some controversy regarding the location of the phonological store within the left parietal lobe (Becker, MacAndrew, & Fiez, 1999), and it is possible that our ROI analysis of the parietal lobe included multiple parietal subregions and resulted in decreased sensitivity to manipulations of WM load. Thus, our findings about the parietal cortex are better viewed as ambiguous than as evidence against a load effect on parietal activation during the maintenance of verbal information in WM.

Functional Connectivity of Areas in WM

In the present study, functional connectivity was analyzed simply as the correlation of activation between brain regions during task performance. The contributions of the dorsolateral and ventrolateral PFC regions to WM could not be distinguished on the basis of load dependence during maintenance, but a difference between these regions was observed in their functional connectivity. The dorsolateral PFC exhibited a significantly higher correlation with parietal and motor cortices than did the ventrolateral PFC. The finding that the dorsolateral PFC and parietal cortex are strongly correlated is consistent with neuroanatomical data from nonhuman primates showing rich interconnections between the dorsolateral PFC and parietal–premotor regions (Friedman & Goldman-Rakic, 1994; Pandya & Seltzer, 1982; Petrides & Pandya, 1984).

The correlation analyses suggest that the dorsolateral PFC is more related to behavioral response than is the ventrolateral PFC. Motor activations presumably reflected response processes related to button pressing in response to the retrieval probe. Motor and parietal activations were strongly correlated to one another, and both activations were significantly more correlated with the dorsolateral than the ventrolateral PFC activation. This finding is consistent with that of Sakai, Ramnani, and Passingham (2002), in which the dorsolateral PFC was found to be involved in preparatory motor action, and that of Rowe and Passingham (2001), in which the dorsolateral PFC and parietal regions were found to be involved in response selection. Activation of primary motor areas increased during the maintenance period, particularly for high loads (see Figure 4), suggesting that the activation of the preparatory motor action–response selection network may begin concurrently with maintenance processing. In sum, these correlations suggest that both the ventrolateral and the dorsolateral PFC regions supported the increased demands of maintaining increased amounts of information in WM, but that the processes mediated by the dorsolateral cortex were more directly involved in maintaining information relevant to response selection and execution at retrieval.

Separation of WM Phases

A limitation of the event-related approach in the present study is the inability to separate the activation of successive encoding, maintenance, and retrieval phases of the item-recognition paradigm. Because participants encoded the same number of letters that they maintained, modeling of maintenance regressors may have been influenced by activation that occurred during encoding. However, load effects were not observed during the encoding phase in any brain region outside of the visual cortex. Because frontal regions demonstrated load-insensitive activation during encoding, encoding-related contamination of maintenance regressors was likely to be similar at all loads. Consequently, it is unlikely that activation during the encoding phase influenced the load effects observed during maintenance, and encoding-related contamination of maintenance regressors cannot explain the strong load sensitivity observed during maintenance in frontal regions.

A study by Manoach, Greve, Lindgren, and Dale (2003) used an event-related approach in which trials with only encoding and retrieval phases were subtracted from trials with a maintenance phase in addition to encoding and retrieval phases. This approach,

coupled with use of a finite impulse response to avoid modeling the hemodynamic response function, enabled separation of maintenance processes from encoding and retrieval processes. Future studies might use such techniques to avoid confounds inherent in the present study and better resolve the impact of increasing WM load on isolated maintenance processes.

Conclusion

In summary, the present study identified several regions of the PFC involved in the active maintenance of WM information that are sensitive to load, including the dorsolateral PFC and the ventrolateral PFC. Functional connectivity analysis suggested a dissociation between the processing in the dorsolateral versus the ventrolateral PFC, with the dorsolateral PFC more involved in interactions with other brain areas during response selection-motor output, including parietal and motor cortices.

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