

Maintenance and Manipulation in Spatial Working Memory: Dissociations in the Prefrontal Cortex

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Two experiments were conducted to compare theories of the functional organization of spatial working memory within the human prefrontal cortex. In Experiment I, memory set size for locations was parametrically varied, allowing for the assessment of BOLD signal across maintenance requirements. In the second experiment, manipulation of spatial information held in working memory was contrasted with simple maintenance of that information. Both experiments evoked significant activity in a distributed spatial working memory network. Although dorsolateral prefrontal activation increased monotonically with memory set size, this region was differentially engaged in task conditions involving explicit manipulation of internal representations. Activation in the superior frontal sulcal region was associated with maintenance of spatial information, increasing with memory set size. In contrast, ventrolateral prefrontal activation was present only at the highest memory set size, possibly due to the differential use of organizational strategies with more complex stimuli. These results support claims that the dorsolateral prefrontal cortex is involved in the manipulation of internal representations and that the superior frontal sulcal region is involved in the maintenance of spatial information, but they suggest a complex role for the ventrolateral prefrontal region. © 2002 Elsevier Science (USA)

INTRODUCTION

Working memory refers to the temporary storage and manipulation of information in the service of “higher” cognitive processing. Though there is considerable evidence that the lateral prefrontal cortex is recruited in the formation, maintenance, and manipulation of internal representations of information held in working memory (Fuster, 1997; Ungerleider *et al.*, 1998), the potential functional segregation of this re-

gion and the principal activities of these subregions remain controversial (Owen *et al.*, 1998; Duncan and Owen, 2000). Based on lesion and electrophysiological recording studies in nonhuman primates, two theories have been proposed specifying different functional dissociations of the cytoarchitecturally distinct dorsolateral prefrontal (DLPFC; Brodmann areas (BA) 46 and 9, 9/46) and ventrolateral prefrontal (VLPFC; BA 44, 45, and 47) cortices. The first theory argues that the topography of the frontal cortex is organized according to information modality, with the DLPFC principally involved with spatial information and VLPFC with nonspatial information (Goldman-Rakic, 1995, 1996). The second theory, proposed initially by Petrides, argues that prefrontal cortex is functionally segregated according to the nature of processing performed, with the VLPFC specialized for active memory process, including selection, comparison, and judgment of maintained information, while the DLPFC is specialized for the active manipulation and monitoring of information within working memory (Petrides *et al.*, 1995; Petrides, 1996). Based on functional neuroimaging experiments in humans, others have simplified the process-specific model, suggesting that the VLPFC supports processes necessary for maintaining information over a delay (i.e., storage), while DLPFC supports processes necessary for use of the stored representation in relation to a goal (i.e., executive processes) (Smith and Jonides, 1997; D’Esposito *et al.*, 1998). However, the role of VLPFC in maintenance processing may depend on the nature of the information being maintained. While maintenance of nonspatial information has been associated with activation within VLPFC (D’Esposito *et al.*, 1998; Owen, 2000), several researchers have failed to find activation there during spatial maintenance tasks (Courtney *et al.*, 1996; Klingberg *et al.*, 1997; Zarahn *et al.*, 1999; Diwadkar *et al.*, 2000; Rowe *et al.*, 2000). Indeed, Courtney and colleagues (1998) proposed that

an area of the superior frontal sulcus (SFS; the junction of BA 6 and 8b (Petrides and Pandya, 1994)) is specialized for maintenance of spatial information in humans, a finding replicated by others (Petit *et al.*, 1998; Zarahn *et al.*, 1999; Nystrom *et al.*, 2000; Rowe *et al.*, 2000). Thus, maintenance processing may be subject to an information modality dissociation, with VLPFC serving nonspatial (verbal) information and SFS serving spatial information (but see Postle *et al.*, 2000). In the current study, we applied two spatial working memory tasks designed to elucidate the relative specialization of these regions by varying memory set size and explicit manipulation demands.

The link between DLPFC activity and manipulation is complicated by the complex nature of manipulation or "central executive" processing. There are many potentially dissociable ways in which a stored representation may be utilized by executive processes in the orchestration of behavior. For example, within the verbal domain, D'Esposito and colleagues (1999) employed a task that contrasted simple maintenance of letter strings with the alphabetization of letters held in memory; this study observed VLPFC activity in both conditions and increased DLPFC activation specifically during alphabetization (i.e., manipulation) trials. Within the visual spatial domain, Rowe and colleagues (2000) demonstrated that DLPFC activation was specific to response selection during a delayed-response task, in contrast to SFS activity, which was more closely linked to storage. While the task used by Rowe and colleagues did not have an overt manipulation requirement, their result emphasizes that even relatively simple delayed-response tasks require some level of executive processing. Contrasting spatial-temporal manipulation with spatial-temporal maintenance, Postle and colleagues (2000) reported increased DLPFC activity during manipulation relative to maintenance trials. In this study, subjects were presented with varying numbers of locations, presented one at a time, and were required either to maintain those locations in the order presented (spatial-temporal maintenance) or to reorder and rearrange the location information to fit a predetermined spatial configuration (spatial-temporal manipulation). While Postle and colleagues report condition-specific DLPFC activity, neither the VLPFC nor the SFS was activated significantly above baseline in this experiment, leaving the role of these regions in doubt. Furthermore, linking visual spatial information over time may involve additional executive processing not typically present in simple delayed-response tasks. A spatial working memory task that requires subjects to explicitly manipulate location information held in memory would allow for more direct contrast of the possible processing differences in the DLPFC, VLPFC, and SFS regions.

Although initial investigations of the maintenance of location information suggested right VLPFC involve-

ment (Jonides *et al.*, 1993; Baker *et al.*, 1996), this lateralization of the spatial maintenance has not been replicated (Postle and D'Esposito, 1999) and may have been dependent upon sample demographics (Reuter-Lorenz *et al.*, 2000) or methodological disparities (e.g., control condition used (Klingberg *et al.*, 1997) or sampling restriction due to imaging technique). Furthermore, as discussed above, several researchers have not reported activation in VLPFC during spatial maintenance tasks (Courtney *et al.*, 1996, 1998; Klingberg *et al.*, 1997; Zarahn *et al.*, 1999; Diwadkar *et al.*, 2000). At issue may be task difficulty, in which higher processing demands or larger memory set sizes seemingly require bilateral activation and in which lower processing demands or smaller memory set sizes may be linked to unilateral activation (Jha and McCarthy, 2000). Yet such differences may be more an artifact of arbitrary thresholds applied during image analysis than true laterality differences (see (Jonides *et al.*, 1997) for a discussion of this matter). Regardless of the laterality issues, there is a more fundamental question concerning VLPFC processing during spatial working memory tasks. Namely, is VLPFC activity linked to the maintenance of spatial information (D'Esposito *et al.*, 1998), monitoring of information currently in the environment (Petrides, 1996), or both functions (D'Esposito *et al.*, 2000)?

Here, we present two experiments, one designed to detect regions involved in spatial maintenance by parametrically varying memory set size (Experiment I) and the other designed to elucidate regions differentially involved in maintenance plus manipulation of spatial information compared with maintenance alone (Experiment II). We tested the hypotheses that significant DLPFC activation will be present only when task demands include manipulation of spatial location information, that VLPFC activity will be more closely linked to maintenance processing when task difficulty increases, and that SFS activation will be closely related to the maintenance of spatial information across different task demands. Comparison of the results across experiments should augment insight into potential specialization of prefrontal subregions for the processing of visuospatial information.

EXPERIMENT I: MAINTENANCE OF INCREASING AMOUNTS OF SPATIAL INFORMATION

To study spatial maintenance processing, we developed a task wherein the number of to-be-remembered locations was varied parametrically across conditions. Generally, functional neuroimaging studies that vary levels of a cognitive function parametrically are well suited to investigate the dynamic response of active regions to changing task demands. Investigating areas implicated in maintenance processing, we hope to dissociate regions in which signal increases monotonically

with increasing memory set sizes from those in which signal changes are not linked as clearly to the storage of internal spatial representations.

Methods

The sample consisted of 14 (36% female) right-handed healthy volunteers, average age 50.38 ± 3.5 (range 45.2–57.3). Prior to functional MRI evaluation, participants signed informed-consent forms approved by the institutional review boards of the University of California (Los Angeles) and the National Public Health Institute of Finland. The same subjects were used in Experiments I and II and data were collected during the same scanning session.

Task Paradigm

For Experiment I, participants performed a spatial delayed response task (SDRT). For each trial, subjects were shown a target array of 1, 3, 5, or 7 yellow circles positioned pseudo-randomly around a fixation point on a computer screen. After a fixed delay, they were shown a single green circle and were required to indicate if that circle was in the same position as one of the previously shown target circles (Fig. 1A). A 2-s stimulus presentation was used to allow the subject to fully encode the target array, even on the highest load level, to minimize potential encoding by set size interaction. Likewise, decision or selection requirements were kept constant across load levels to reduce possible effects of set size on response processes (Rowe *et al.*, 2000). Trial events included a 500-ms initial period of blank screen, 500 ms of a fixation point, a 2-s target array presentation, a 3-s delay period (with fixation), and a 3-s fixed response interval (Fig. 1A). Fifty percent of the trials were true positive and 50% of the trials were true negative. Responses (yes or no) and reaction times were recorded for each trial. The number of correct responses, mean reaction time, median reaction time, and number of omissions were calculated for each memory set size.

Prior to scanning, task instructions were presented on the computer screen and read and explained to subjects by trained administrators. In addition, subjects performed a series of example trials during which feedback was provided. During scanning, subjects performed 48 total trials (12 trials per memory load), presented in six cycles lasting a total of 7:20 min. Each cycle consisted of four blocks, which in turn contained 2 trials with the same memory requirement. Within each cycle, the blocks were ordered randomly so that the order of the load conditions was not identical for any two cycles. Hence, the task was administered as a blocked parametric task (Aguirre and D'Esposito, 1999), in which blocks consisted of two trials of identical memory requirements lasting 18 s. In pilot behavioral testing ($n = 25$), accuracy decreased linearly

(from 97.40 to 79.12%; $F[3,22] = 20.72$, $P < 0.0001$) as memory requirements increased.

Scanning Procedures

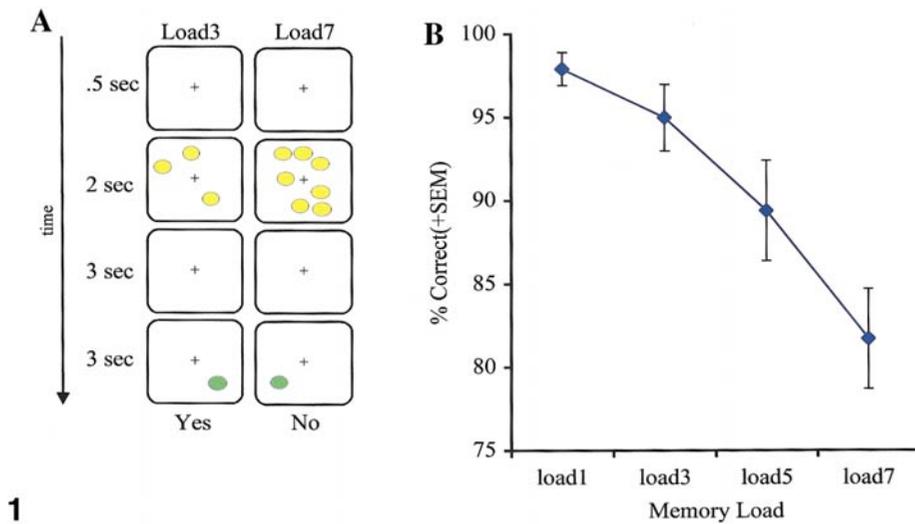
Imaging was carried out in the Department of Radiology, University of Helsinki, on a Siemens (Erlangen, Germany) 1.5-T Vision whole-body scanner equipped with a fast gradient system for echoplanar imaging. Prior to each functional study, a high-resolution axial T1-weighted series was obtained for each subject and used for anatomical reference (TR = 720 ms, TE = 14 ms, flip angle 90° , 24 axial slices, FOV 256 matrix). A gradient echo, echoplanar (EPI) sequence was used to acquire data sensitive to the BOLD signal (Kwong, 1995) at a TR of 3000 ms and TE of 64 ms, 64×64 matrix. Resolution for the 24 contiguous axial EPI slices was 4 mm isotropic (field of view 256 cm). These images were acquired parallel to the AC-PC line. Through a mirror mounted on the head coil, subjects observed stimuli generated on a Macintosh laptop and back-projected via an LCD projector onto a screen placed at the subjects' feet. Behavioral responses were recorded through a hand-held fiber-optic response box connected directly to the Macintosh, allowing for both button presses and reaction time to be recorded. The order of tests was counterbalanced across subjects to equate the effects of fatigue.

Image Analysis: Single Subject

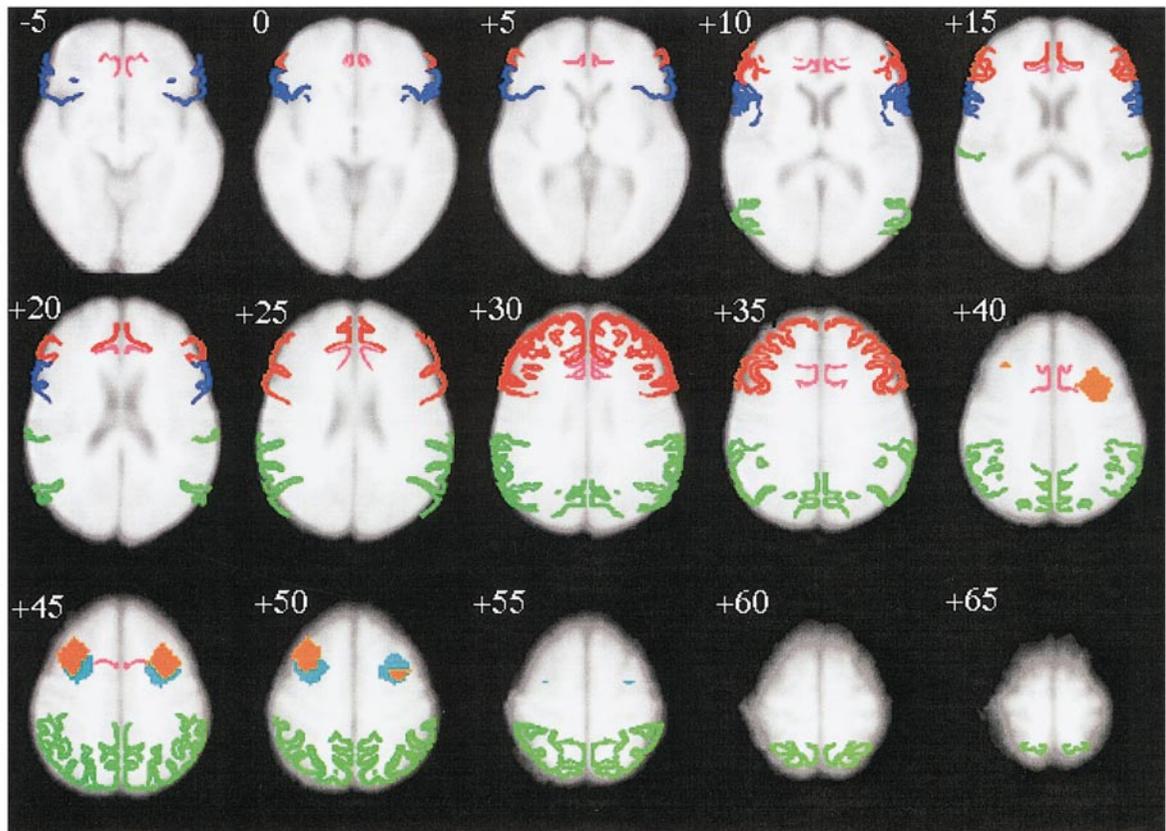
To combat potential motion artifacts each BOLD image in a time series underwent a 3D coregistration (6-parameter rigid-body automated algorithm (Woods *et al.*, 1998)) to the first data point in the time series. The data sets were smoothed with a nonlinear algorithm designed to preserve image structure by smoothing over only voxels thought to be of the same tissue type (5-mm kernel) (Smith and Brady, 1997). Each data set was subjected to a multiple-regression analysis, using a prewhitening technique (Bullmore *et al.*, 1996) to account for the intrinsic temporal autocorrelation of BOLD imaging (Zarahn *et al.*, 1997). For each intracranial voxel, least-squares coefficients were generated independently for predictors reflecting each of the four memory set sizes in Experiment I (Woolrich *et al.*, 2001). Four magnitude images, composed of the linear combination of these least-squares coefficients, were generated (1) reflecting a monotonic (linear) increase in BOLD signal with increase with increasing memory set size (e.g., -2, -1, 1, 2) and (2–4) contrasting each larger memory set's size with memory for a single location (e.g., three locations vs one location).

Image Analysis: Creation of a Common Space

To facilitate multisubject analysis, a common brain was defined which approximated the average size,



1



2

FIG. 1. Description of the activation paradigm (A) and behavioral data (B) collected from Experiment I. (A) SDRT paradigm. In Experiment I subjects were required to maintain increasing numbers of locations, denoted by yellow circles, in memory. After a fixed delay interval, a single location was cued, with a green circle, and the subject determined if that location was part of the target array. Depicted are trials from the load 3 (true positive) and load 7 (true negative) conditions. (B) SDRT performance. Plot of behavioral performance collected during fMRI measurements (mean \pm standard error of the mean). As memory set size increased, there was a near-linear decrease in accuracy (percentage correct).

FIG. 2. Anatomical regions of interests representing DLPFC (BA 9 and 46; red), VLPFC (BA 44, 45, and 47; blue), anterior cingulate (BA 32, pink), posterior parietal lobe (BA 7, 39, and 40; green), the SFS region (orange), and the frontal eye fields (cyan), overlaid on the model brain representing the approximated average size, shape, and orientation of each subject's higher resolution T1-weighted image.

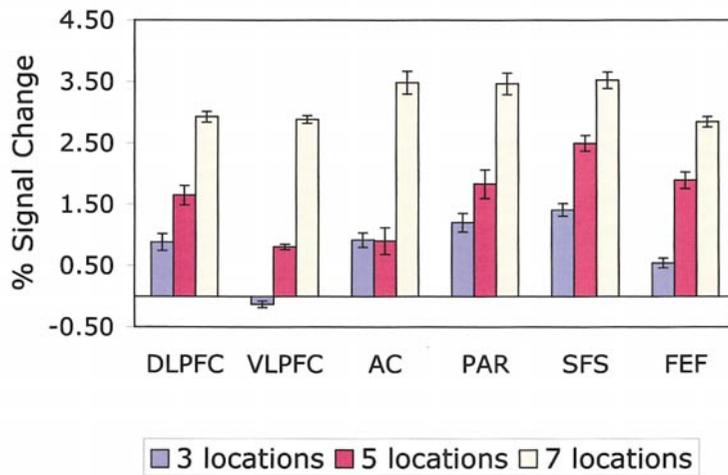
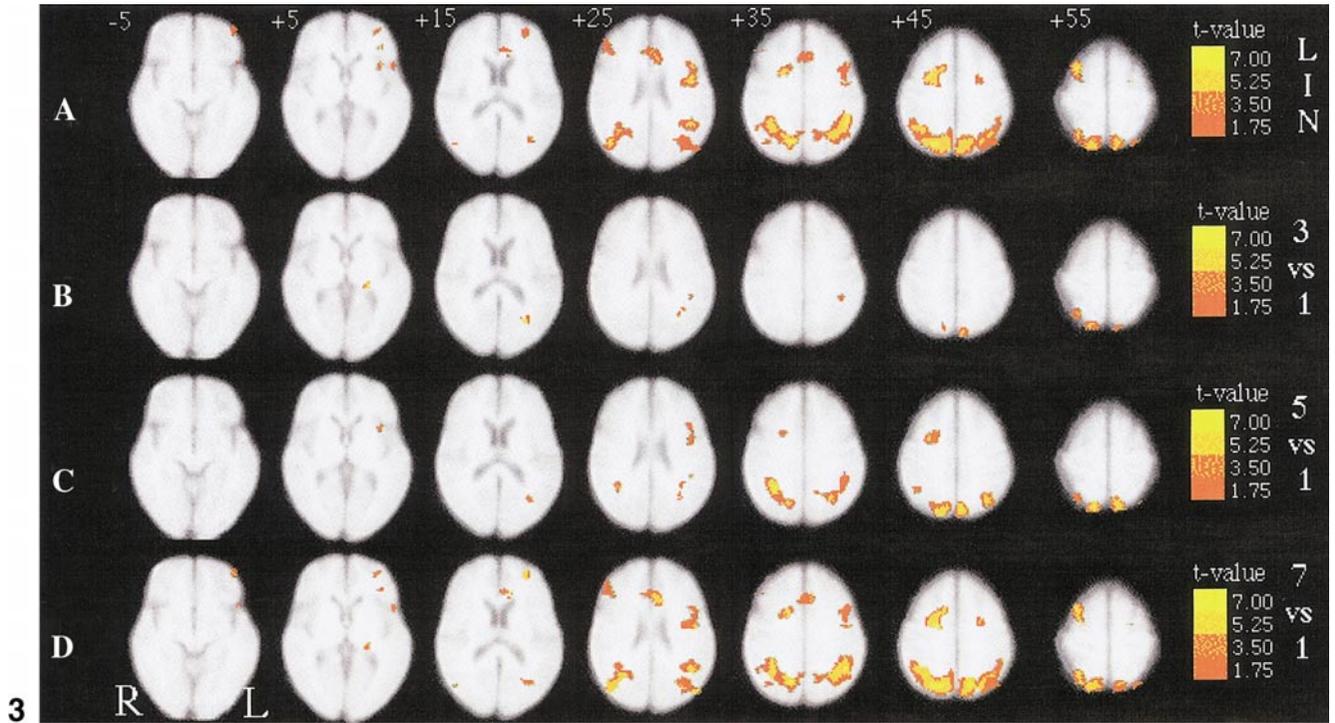


FIG. 3. Experiment I group level activation maps reflecting the monotonic contrast (A) and the three-location (B), five-location (C), and seven-location (D) contrasts. With few exceptions, the monotonic contrast captures those regions found to be active in the individual memory set contrasts. Images are left–right reversed and presented at 10-mm increments.

FIG. 4. Plot of the percentage signal change in the anatomically defined regions of interest with increasing memory set size in Experiment I. AC, anterior cingulate (BA 32); PAR, posterior parietal cortex (BA 7, 39, and 40); FEF, frontal eye fields.

shape, and orientation of each subject's higher resolution T1-weighted image (Woods *et al.*, 1999). Based on the parameters created from the T1-weighted image, each of the statistical images described above were normalized into this common space, allowing for voxel-level comparisons between subjects. To make the results from these group comparisons comparable with others in the literature, the common brain was normalized into a standard stereotactic space (Talairach and Tournoux, 1988), using AFNI (Cox, 1996).

Anatomically based regions of interest (ROIs), defined by Brodmann's areas in the common space (Lancaster *et al.*, 1999), delineated the following areas: DLPFC (BA 9 and 46), VLPFC (BA 14, 45, and 47), posterior parietal lobe (BA 7, 39, and 40), anterior cingulate cortex (AC; BA 32), SFS, and frontal eye fields (FEF; see Fig. 2). The final two regions were created to reflect those regions reported by Courtney and colleagues (1998) to be associated with spatial maintenance and eye movement, respectively. The an-

TABLE 1
Experiment I Behavioral Performance

	Percentage correct	Reaction time (ms)
1 location	97.3 ± 1	745 ± 40
3 locations	95.0 ± 2	955 ± 51
5 locations	89.4 ± 3	1041 ± 60
7 locations	81.7 ± 3	1159 ± 57

Note. Mean ± standard error.

atomical ROIs, refined by the group activation maps, were applied to each subject's memory set size contrast images, which reflect the percentage signal change associated with larger memory set sizes (three, five, or seven locations) compared to the nominal memory condition (one location). For each ROI, statistics, including average intensity and maximum intensity, were calculated for each magnitude image.

Image Analysis: Group Comparisons

Spatially normalized magnitude images from the monotonic contrast were entered into a second-order (random-effects) analysis (a one-way Student *t*). This analysis tested the hypothesis that task-related signal change in a voxel was significantly different from zero, across subjects, indicating that it had a monotonic relationship with memory set size. This *t* map was thresholded based on the magnitude ($t = 4.2$, $P < 0.001$) and extent (volume 608 mm³) of activation (Forman *et al.*, 1995; Poline *et al.*, 1997). In Monte Carlo simulations, these height and extent values controlled Type I error to $\alpha < 0.05$, given the number of multiple comparisons, smoothness, and search volume of the data.

Results

Behavioral

When Experiment I was administered during fMRI evaluation, the subject's accuracy level decreased linearly with increasing load ($F[3,11] = 6.97$, $P < 0.005$) from about 97% at one location to 82% for seven loca-

tions (Fig. 1B and Table 1). Reaction time increased with increasing memory set size ($F[3,11] = 30.52$, $P < 0.0001$) from 748 ± 43 (mean ± standard error) in one location to 1156 ± 60 for seven locations.

fMRI

Multisubject voxel-level analysis revealed a network of seven spatially distinct brain regions sensitive to maintaining increasing amounts of spatial information (Table 2 and Fig. 3A). These regions included: (1) a bilateral posterior activation (center of mass (CM) in Talairach coordinates -2 , -60 , 40 ; encompassing portions of BA 39, 40, and 7) extending from the superior-most portion of the superior temporal gyrus to the inferior and superior parietal lobules, (2) a right medial frontal region (CM -27 , 6 , 47 ; BA 6 and 8) including the middle frontal gyrus and the junction of the middle frontal gyrus and superior frontal gyrus, (3) a left dorsolateral prefrontal region (CM 42 , -6 , -29 ; BA 9 and 6) extending from the left middle frontal gyrus to the inferior frontal gyrus to the precentral gyrus, (4) a region of the anterior cingulate (CM 3 , 27 , 27 ; BA 32), (5) a left anterior frontal region (CM 36 , 50 , 5 ; BA 10) including the middle and superior frontal gyri, (6) a right dorsolateral prefrontal region (CM -46 , 34 , 27 ; BA 9 and 46) within the middle frontal gyrus, and (7) a left ventrolateral prefrontal region (CM 49 , -17 , 2 ; BA 47) including inferior frontal gyrus and left insular cortex.

The previous analysis identified regions showing a monotonic relationship between memory set size and degree of activation. That regions involved in maintenance should increase in activity with increasing set size is defensible based on the inverse linear relationship of performance with memory load, but the results are incomplete to the extent that physiologic systems behave in nonlinear ways in relation to increased task demands. Hence, three additional analyses were conducted comparing each memory set size (three, five, or seven locations) with the nominal load level (one location). These were performed as second-order (random-effect) two-way *t* tests testing the hypotheses of increasing activation at each memory set size inde-

TABLE 2
Brain Regions Sensitive to Increasing Memory Set Size in Experiment I

Region	Volume	CM <i>x</i>	CM <i>y</i>	CM <i>z</i>	Max <i>t</i>	Max <i>t_x</i>	Max <i>t_y</i>	Max <i>t_z</i>
1	49,902	-2	-60	40	6.7	-19	-59	33
2	5,892	-26	6	47	6.2	-29	3	47
3	4,670	42	-6	29	6.1	41	3	31
4	3,510	3	27	27	5.1	6	25	28
5	2,800	36	50	5	4.0	36	35	6
6	1,378	-46	-34	27	3.7	-44	-34	31
7	622	49	-17	2	3.3	49	16	1

pendently and are presented in Figs. 3B–3D. These analyses indicate that one region, including the left thalamus and pulvinar (CM: 22, –25, 6), was found active in all three contrasts, but was not detected by the monotonic contrast, suggesting that the region is involved in the task, but that its signal change does not depend on memory set size. However, the most striking result of these analyses is their consistency with the results of the monotonic contrast in showing increased BOLD signal, in a distributed set of brain regions with increasing memory requirements. To further elucidate the relationship between memory set size and activation, we performed an anatomically based ROI analysis. As can be seen in Fig. 4, each of the regions studied showed increased signal with each increase in memory set size, with the exception of the anterior cingulate, which showed a significant signal increase only at the highest load level (seven locations).

Discussion

The regions activated during Experiment I are similar to those reported in previous functional neuroimaging studies of spatial working memory (e.g., Duncan and Owen, 2000; Smith and Jonides, 1997). Consistent with findings reported by Courtney and colleagues (1998) we found activation in the right SFS region during a spatial delayed response task and extended her original observation by providing evidence that this region is sensitive to increasing memory set size. Indeed, the ROI analysis suggested that activation in this region was present in the lowest load condition (three locations) and monotonically increased with increasing memory set sizes (a near-linear increase).

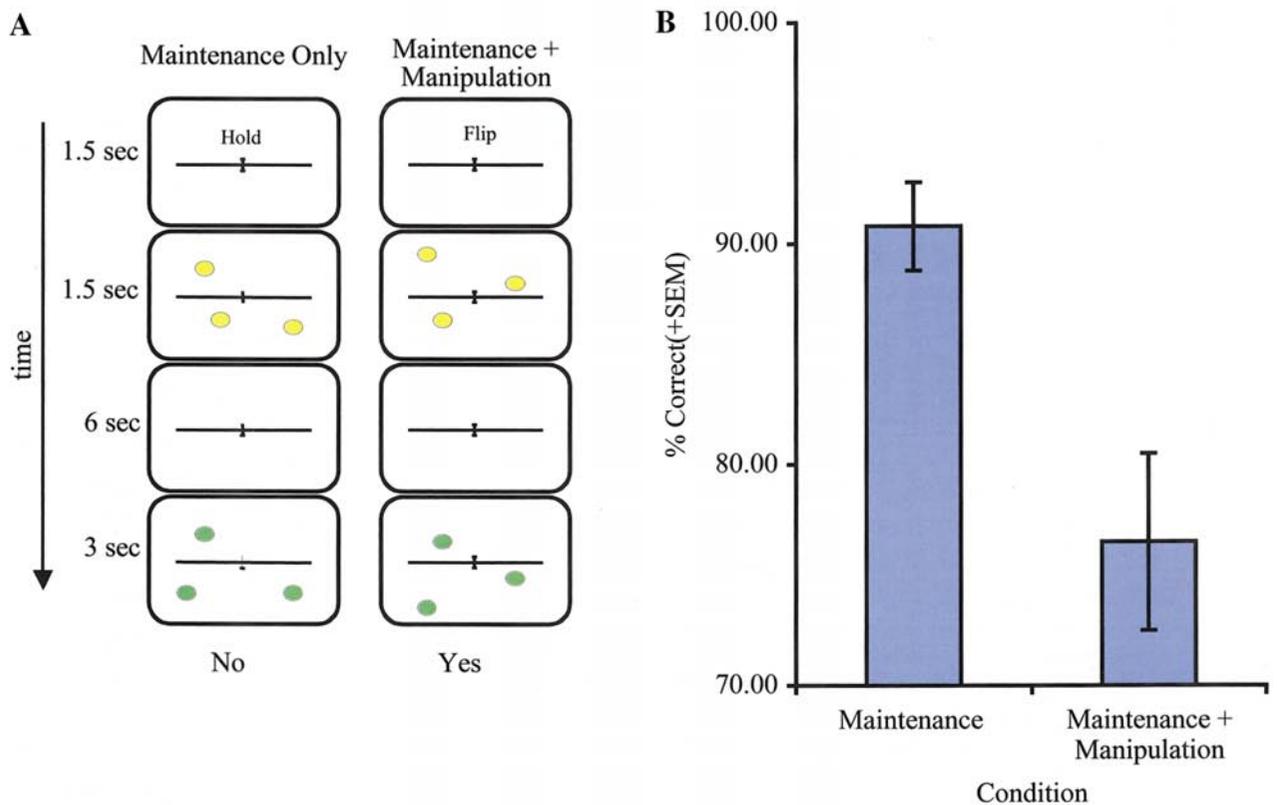
While SFS activation was closely linked to memory set size, VLPFC activation was significant only at the highest load levels (five and seven locations). These findings suggest either that VLPFC activity is very similar for the three-location and control condition or that this region is not engaged until the memory set size is large, in which case there may be a critical threshold of memory demands for recruitment of VLPFC, which may not have been reached in some prior studies of spatial working memory that failed to observe VLPFC activity (e.g., Courtney *et al.*, 1998; Zarahn *et al.*, 1999; Rowe *et al.*, 2000). However, the insensitivity of the VLPFC to small maintenance demands as observed in the present study raises the possibility that this region may not be involved in maintenance per se, rather it may be involved in other memory processes that are differentially affected by task difficulty or large memory demands (e.g., task conditions requiring greater organizational strategies). Yet such an interpretation should be tempered by possible Type II error, which is not well characterized in this study or in functional MRI in general.

Although the task used in Experiment I did not have an explicit manipulation requirement, we found bilateral DLPFC activation that increased monotonically with memory load, indicating that this region is very likely to participate in short-term maintenance of spatial information. At one level, the observation of DLPFC activation in a spatial maintenance task is consistent with the information-specific model (Goldman-Rakic *et al.*, 1996). Yet, we also observed VLPFC activation, which is inconsistent with this framework. Although the activation paradigm used here was a simple delayed-response task, it is likely that some strategic or selection processing was involved in task performance (e.g., Rowe *et al.*, 2000). Rypma and colleagues (1999) found that conditions requiring maintenance of large but not small amounts of verbal information were associated with DLPFC activation. In a subsequent study, DLPFC activation was shown to be limited to the encoding phase of trials (Rypma *et al.*, 1999). However, these studies used only two memory set sizes (high and low) and thus do not indicate DLPFC involvement in midlevel memory demands (e.g., monotonic increase or critical threshold). Alternately, the DLPFC activation observed in the present study could reflect increased selection processing and thus could be linked to response-phase activity (e.g., Rowe *et al.*, 2000). The blocked design of the current study does not allow us to segregate these possibilities. However, contrasting the results of this experiment with the next, which has a clear manipulation requirement, provides a basis for understanding whether the DLPFC is differentially involved in manipulations of the spatial working memory store, compared with the other key components of the network.

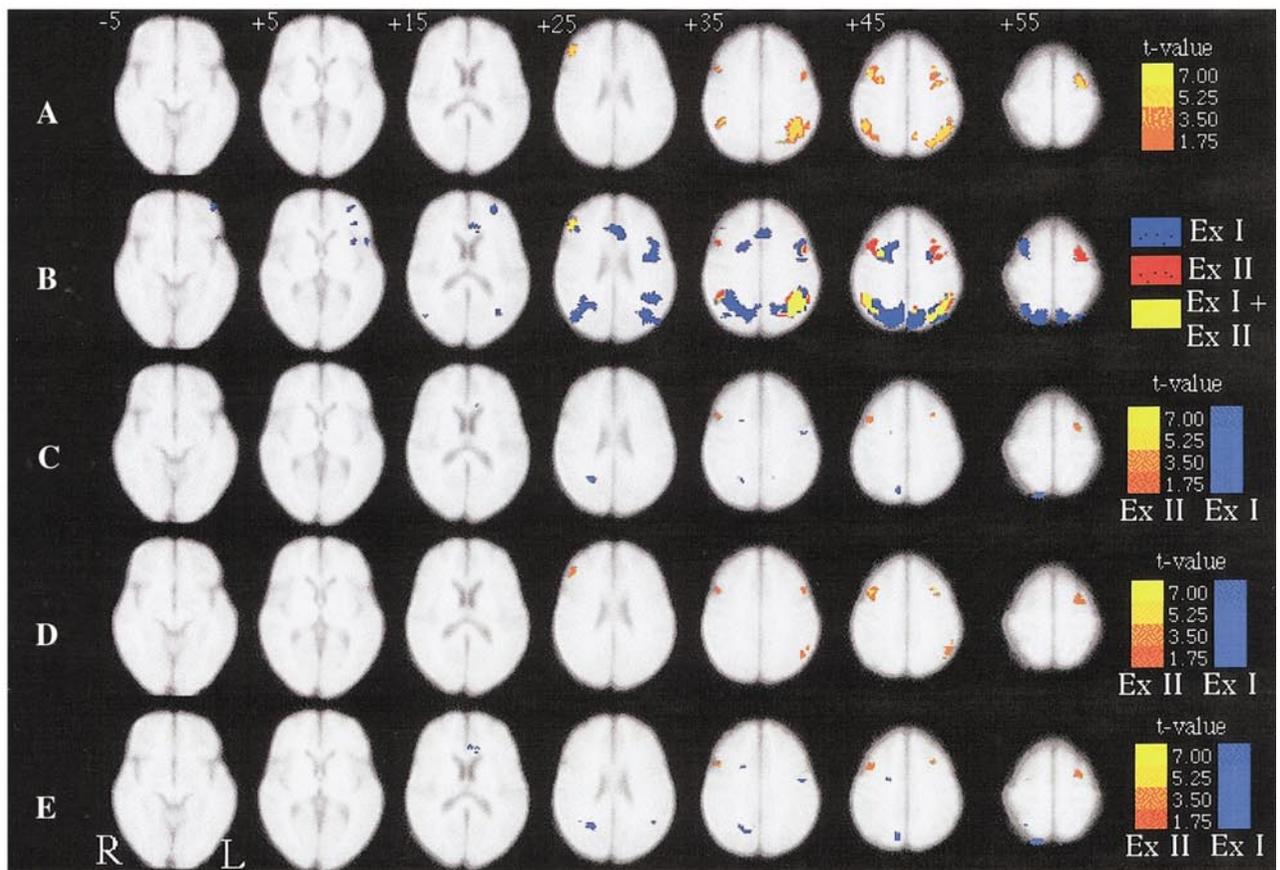
The AC was the only region examined in which BOLD signal did not significantly differ between the three-location and the five-location conditions. Indeed, although this region was nominated by the monotonic contrast (Fig. 3A), the AC was significantly involved only at the highest load level (Fig. 3D). The difference in the activation patterns across memory sets between the AC and the other regions studied suggest that the role of the AC in this task is somewhat different from those of these other regions. It is possible that the AC is sensitive to task difficulty (Barch *et al.*, 1997) and not directly involved in delayed response task performance.

EXPERIMENT II: MAINTENANCE AND MANIPULATION OF SPATIAL INFORMATION

For Experiment II, we developed a novel paradigm to contrast the maintenance and manipulation of spatial information with the simple maintenance of that information (Kim *et al.*, submitted for publication). In the “maintenance alone” condition, subjects were presented with a fixed number (3) of locations and asked to



5



6

remember those locations. In the “maintenance and manipulation” condition, subjects were instructed to flip the maintained representation over an explicitly drawn horizontal meridian. Hence, manipulation was operationalized as mental rotation, a cognitive process shown previously to activate the prefrontal cortex (Cohen *et al.*, 1996). Here, our goal was to dissociate regions associated with manipulation processes from others in the spatial working memory network.

Methods

The sample and scanning procedures were identical to those described above.

Task Paradigm

In this task (Fig. 5A), two trial types were utilized: maintenance alone and maintenance plus manipulation. In maintenance-only trials, subjects were shown three yellow circles positioned pseudo-randomly on a computer screen. After a delay, a probe stimulus containing three green circles appeared. Subjects were required to indicate if all three probe circles were in exactly the same locations as those in the target stimuli. During the delay period of maintenance-plus-manipulation trials, subjects were instructed to “flip” the locations of the target circles over the horizontal meridian (demarcated by a horizontally elongated fixation point). In this condition, subjects were required to indicate if the probe stimulus matched the *inverted* target stimulus exactly. Responses (yes or no) and reaction times were recorded for each trial. The number of correct responses, mean reaction time, median reaction time, and number of omissions were calculated for each condition. Trial events included a 500-ms initial period of blank screen, 1000 ms of a fixation point, a 1.5-s target array presentation, a 6-s delay period (with fixation), and a 3-s fixed response interval (Fig. 5A).

As in Experiment I, subjects performed a series of example trials during which feedback was provided. During scanning subjects performed 40 trials (20 trials per condition), presented in 20 cycles. Each cycle consisted of two blocks, which in turn contained 2 trials with the same condition. The task lasted a total of 8 min.

TABLE 3

Experiment II Behavioral Performance

	Percentage correct	Reaction time (ms)
Maintenance	90.8 ± 2	1052 ± 40
Maintenance plus manipulation	76.5 ± 4	1291 ± 45

Note. Mean ± standard error.

Image Analysis

Image analysis followed the same preprocessing procedures as described for Experiment I. Multiple-regression analysis modeled the maintenance-only and maintenance-and-manipulation conditions independently, and a single magnitude image reflecting the contrast between maintenance plus manipulation and maintenance only was generated for each subject. A between-subjects analysis was carried out with a one-way Student *t*, testing the hypothesis that a voxel was differentially active during the maintenance-and-manipulation compared to maintenance-alone condition.

Results

Behavioral

As in our pilot work, the subjects' accuracy in Experiment II decreased ($F[1,13] = 23.54$, $P = 0.0004$) and reaction time increased ($F[1,13] = 75.38$, $P = 0.0001$) for the maintenance-plus-manipulation condition relative to the maintenance-alone condition (Fig. 5B, Table 3).

fMRI

As seen in Fig. 6A and Table 4, compared to the maintenance-alone condition, the maintenance-and-manipulation condition is associated with higher activity in a functional network of five spatially dissociable regions. These include: (1) a left posterior region (CM 36.5, -54, 40; BA 39, 40, and 7) extending from the most superior portion of the superior temporal gyrus to the inferior and superior parietal lobules, (2) a left medial frontal region (CM 31, 5, 49; BA 6 and 8) including the middle frontal gyrus and the junction of the

FIG. 5. Description of the activation paradigm (A) and behavioral data (B) collected from Experiment II. (A) In Experiment II, subjects were presented with three locations and told to maintain those locations in memory (maintenance; true negative) or invert those locations about the horizon (manipulation plus maintenance; true positive). (B) Plot of behavioral performance collected during fMRI measurements (mean ± standard error of the mean). Accuracy for the manipulation plus maintenance condition was significantly lower than for the maintenance condition alone.

FIG. 6. Experiment II group-level activation maps reflecting the maintenance-plus-manipulation vs the maintenance-alone contrast (A) and the overlap of activation patterns for Experiment II and the monotonic contrast of Experiment I (B). Rows C–E reflect group-level paired *t* tests contrasting the magnitude images from Experiment II and the monotonic (C), three-location (D), and seven-location (E) contrasts from Experiment I.

TABLE 4

Brain Regions Sensitive to Explicit Manipulation Requirements in Experiment II

Region	Volume	CM <i>x</i>	CM <i>y</i>	CM <i>z</i>	Max <i>t</i>	Max <i>x</i>	Max <i>y</i>	Max <i>z</i>
1	8395	37	-55	40	4.9	41	-49	38
2	4389	31	5	49	5.1	31	11	46
3	2814	-45	-49	42	4.2	-48	-46	45
4	2418	-38	9	45	4.2	-42	11	42
5	806	-48	35	23	5.1	-48	35	23

middle frontal gyrus and superior frontal gyrus, (3) a right posterior region (CM -45, 49, 42; BA 39, 40, and 7) similar to but smaller than the analogous activation in the left hemisphere (region 1), (4) a right medial frontal region (CM -38, 9, 45; BA 6 and 8) similar to but smaller than the analogous activation in the left hemisphere (region 2), and (5) a right DLPFC region (CM -48, 35, 23; BA 9 and 46) within the middle frontal gyrus. These regions are remarkably similar to those nominated by the monotonic contrast in Experiment I; Fig. 6B provides a joint display that clearly illustrates this pattern of overlapping activations.

The results from Experiments I and II were compared formally using paired *t* tests in separate analyses for the monotonic (Fig. 6C), the three-location (Fig. 6D), and the seven-location (Fig. 6E) contrasts of Experiment I and the maintenance-plus-manipulation vs maintenance-only contrast of Experiment II. These *t* maps, which were limited to regions found in either Experiment I or Experiment II (see Fig. 6B), consistently show that the right DLPFC region (CM -41, 13, 43; BA 46 and 9) was significantly more active when explicit manipulation of internal spatial representations was required (i.e., as in Experiment II), while the other components of the network were not.

GENERAL DISCUSSION

The primary focus of this study was the comparison of theoretical models of the functional organization of working memory within the human prefrontal cortex. Although the functional dissociation of anatomical regions is central to most neuroimaging experiments, the interpretation of these studies is necessarily bound to the idiosyncrasies of the task design. In the domain of working memory, maintenance-related processes and manipulation or central executive (CE)-related processes, while theoretically distinct, are nevertheless difficult to isolate perfectly experimentally. A strength of the current study is that we assayed BOLD signal over several memory set sizes and with and without explicit manipulation requirements, within the same subjects, and compared the magnitude of activation across these different conditions.

The primary regions showing monotonic increases in activity with increasing memory set size in Experiment

I (i.e., DLPFC, VLPFC, SFS, and parietal cortex) were the same regions that were more active in the maintenance-plus-manipulation compared to the maintenance-only condition in Experiment II (with the exception of the VLPFC region). These regions are consistent with those previously suggested as participating in a large-scale distributed network serving working memory functions (Fuster, 1997; Smith and Jonides, 1997). That all of these regions were activated in both tasks is at one level surprising, given that such a pattern argues against specialized functions among the key components of the network and tends to refute the notion that storage and CE-related processes are functionally segregated. However, at another level, when one considers that even tasks without explicit manipulation requirements involve CE-related processes and that conditions requiring manipulation of the working memory store probably tax maintenance-related functions to a greater extent than delayed response task without such a requirement, overlap in the regions activated by such conditions would be expected. Only when the magnitude of activation among these regions was compared directly across task conditions did evidence of process dissociation emerged. Thus, when contrasting the magnitude images from Experiment II with the monotonic, three-location, five-location, and seven-location conditions from Experiment I, the most striking finding is that the right DLPFC activation in Experiment II was significantly stronger than in any condition of Experiment I, suggesting that the DLPFC activation required for explicit manipulation of internally held information is greater than DLPFC activation for delayed response tasks that do not explicitly require manipulation, an interpretation that relies upon the assumptions of cognitive subtraction (Posnet *et al.*, 1988). That this was the only component of the network showing this pattern suggests that DLPFC is differentially involved in manipulation or CE-related processes in spatial working memory.

As noted previously, the results of Experiment I suggest that VLPFC is not straightforwardly involved in spatial maintenance. Rather, the pattern of activation in this area across memory set sizes suggests that it may be recruited only at higher levels of stimulus complexity that may demand greater organizational strategies. Nevertheless, that VLPFC was not signifi-

cantly more active in Experiment II than in the higher load conditions of Experiment I suggests that its role is not specifically related to spatial manipulation (mental rotation), as was true for DLPFC, but rather, may pertain to organizational–strategic processes that are needed for maintenance alone. Yet, as discussed above, the interpretation of null results is problematic in functional MRI research and further research is needed to address this issue.

In the current study, saccadic eye movements were not monitored during scanning. The data reported in the SFS region of interest was, at the individual subject level, always anterior to the precentral sulcus and BA 4 (the location for the FEF region (Paus, 1996)). When Courtney and colleagues (1998) proposed that this SFS region is specialized for the maintenance of spatial information, they contrasted facial and spatial working memory tasks and a saccadic eye movement task and found that SFS activity was specific to the maintenance of spatial, and not facial, information and that activity in this region was functionally distinct from and just anterior to the frontal eye fields, an area known to govern orienting and exploratory eye movements (Bruce *et al.*, 1985). Given the proximity of these regions, this dissociation seemed critical because SFS activity might simply reflect increased eye movements during the spatial working memory task. In contrast to Courtney's findings, Postel and colleagues (2000) used a more rigorous saccadic eye movement task and failed to find any specificity in the SFS for delay rather than eye movement processing. Yet, the working memory task applied by Postel and colleagues involved the temporal ordering of spatial information, and the activation associated with this task was significantly less robust than in the task utilized by Courtney and colleagues and may not have had the power necessary to show the dissociation. Furthermore, although the FEF has been implicated in voluntary eye movements in human and nonhuman primates (Anderson, 1994; Fox *et al.*, 1985; Pierrot-Deseilligny *et al.*, 1991; Paus, 1996), these findings do not imply that functional activation in the FEF necessitates eye movements (Zarahn *et al.*, 1999). The spatial information coded in the FEF (Bruce *et al.*, 1985; Funahashi *et al.*, 1989) may be involved in some aspect of spatial working memory processing. It is important to note that although cortical areas may preferentially support one cognitive function over another, there is likely not a one-to-one association of cortical region and function (Mesulam, 1990) and neurons in both FEF and SFS may be involved in spatial maintenance processing as well as saccadic eye movements. Furthermore, anatomical heterogeneity in these regions make ROI analyses based on atlases or normalization procedures imperfect with potential contamination of regions. Here we have observed increased activity in both SFS and FEF with increasing memory set size.

Despite the fact that the subjects utilized in this study are significantly older (mean 50 ± 4 years) than those in most functional imaging experimentation (e.g., 20–30 years of age), the regions nominated by Experiments I and II are generally consistent with previous reports in the literature for spatial working memory. Currently, there are no reports comparing activation patterns between younger and older healthy subjects performing a spatial working memory task. However, Rypma and D'Esposito (2000) have reported age-related DLPFC activation differences in a verbal working memory task, without evidence of VLPFC differences between groups. To the extent that these results can generalize to the current study, we might predict more DLPFC activation in younger subjects, possibly making the maintenance-and-manipulation distinction more pronounced than in our sample.

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