Differential Anterior Prefrontal Activation during the Recognition Stage of a Spatial Working Memory Task

Neuroimaging studies commonly show widespread activations in the prefrontal cortex during various forms of working memory and long-term memory tasks. However, the anterior prefrontal cortex (aPFC, Brodmann area 10) has been mainly associated with retrieval in episodic memory, and its role in working memory is less clear. We conducted an event-related functional magnetic resonance imaging study to examine brain activations in relation to recognition in a spatial delayed-recognition task. Similar to the results from previous findings, several frontal areas were strongly activated during the recognition phase of the task, including the aPFC, the lateral PFC and the anterior cingulate cortex. Although the aPFC was more active during the recognition phase, it was also active during the delay phase of the spatial working memory task. In addition, the aPFC showed greater activity in response to negative probes (non-targets) than to positive probes (targets). While our analyses focused on examining signal changes in the aPFC, other prefrontal regions showed similar effects and none of the areas were more active in response to the positive probes than to the negative probes. Our findings support the conclusion that the aPFC is involved in working memory and particularly in processes that distinguish target and non-target stimuli during recognition.

Keywords: delayed-response, encoding, fMRI, frontopolar, human, retrieval

Introduction

Working memory refers to the active maintenance and processing of information to fulfill ongoing task demands. A number of frontal regions, including the dorsolateral prefrontal cortex (PFC), ventrolateral PFC, premotor cortex and anterior cingulate cortex (ACC), are consistently activated during various kinds of cognitive tasks that involve working memory (see reviews by Goldman-Rakic, 1987; Fuster, 1989; Smith and Jonides, 1999; Duncan and Owen, 2000). One common type of task that has been applied to study working memory in humans and non-human primates is delay-response. The delayed-response tasks can be separated into three distinct phases: cue (encoding), delay (maintenance) and response (recall/recognition). While many studies have focused on investigating the roles of frontal regions in maintenance, it is unclear whether the same regions are also involved in encoding and recognition in working memory.

Electrophysiological studies of non-human primates have demonstrated that neurons in the principal sulcus of dorsolateral PFC show changes in firing specific to the cue, delay or response phases of the oculomotor delayed-response task (e.g. Funahashi et al., 1989, 1990). Nonetheless, many prefrontal neurons are reportedly responsive to multiple phases of delayed-response tasks (Niki, 1974; Funahashi et al., 1989; Takeda and Funahashi, 2002). Besides, Miller et al. (1996) have indicated that some prefrontal neurons exhibit selective responses to the testing stimuli depending on whether or not they matched the sample stimuli in a delayed-match-to-sample task.

In human neuroimaging studies, it has been shown that a number of frontal as well as posterior brain areas are active during all three phases of delayed-recognition tasks, albeit to different degrees (e.g. Courtney et al., 1997; Petit et al., 1998; Haxby et al., 2000; Leung et al., 2002). To our knowledge, none of these studies directly examined the difference between encoding and recognition in working memory, although a recent study reported that the anterior portion of the PFC [aPFC, Brodmann area (BA) 10] is more active during the recognition of faces than during the encoding and maintenance of faces (Ranganath et al., 2003). Interestingly, the aPFC has been implicated to play a unique role in retrieval of episodic memory (Tulving et al., 1994; Lepage et al., 2000) as well as in higher order cognition (Owen et al., 1996; Koechlin et al., 1999; Bunge et al., 2000; Christoff and Gabrieli, 2000). Furthermore, Druzgal and D’Esposito (2001) recently reported greater activity in dorsolateral PFC for stimuli that matched the sample stimuli than those that did not, and the opposite effect was observed for the ACC. Therefore, the roles of various frontal regions in recognition during working memory tasks are still unclear.

In a previous report, we showed widespread frontal and posterior brain activations during the encoding and retention periods of a spatial working memory task, and most of these regions reactivated during the response/recognition period (Leung et al., 2002). In this investigation, we examined and compared activations in the PFC during the recognition phase with activations during the encoding phase of the same spatial working memory task. We also compared brain activations in response to the positive (target) and negative (non-target) probes of the task. We were particularly interested in delineating the specific role played by aPFC in working memory.

Materials and Methods

Subjects

Twelve right-handed healthy adults (six females and six males, aged 24–33 years) were recruited from the Yale University community. All participants had normal or corrected-to-normal vision and none reported a history of neurological disorder or psychiatric illness. All participants gave informed consent before participation. Participants practiced the behavioral task for 15 min immediately before the scanning session.

Behavioral Paradigm

We used a spatial working memory task and a control task. Figure 1 shows the memory task which was in the same format of spatial delayed-recognition as it was in our previous study (Leung et al., 2002, 2005).
A trial began with an initial fixation of 6 s at the fixation cross (at the center of the projection screen). A sequence of five target cues was then presented at five different locations. Each target cue was displayed for 1 s and the inter-stimulus interval was 250 ms. After a delay period of 18 s, a probe stimulus was displayed at either one of the target locations or a different location for 1 s. The inter-trial interval (ITI) was 14 s. This allowed the fMRI signal to return to baseline before the next trial. Faces were used to mark the target locations and participants were instructed to remember the five target locations and to respond by pressing one of two buttons to indicate whether or not the probe was at a remembered location. For the memory tasks, positive probes (targets that matched one of the cue locations) and negative probes (non-targets) were randomly presented in each run. For the control task, scrambled faces were used and participants were instructed not to remember any of the stimuli locations and to respond by pressing both buttons in response to the probe presentation. While target locations varied across trials, the visual stimuli used to mark the locations did not vary. Control and memory trials were presented alternately, and their order was counterbalanced across runs.

Stimuli locations were randomly selected from a pool of 36 locations, which were defined in a range of ±3.6° and ±1.5° from the center of the screen. Negative probes were −2.5° or more away from the target cues. All visual stimuli were presented against a black background and were back-projected onto a screen positioned at the front of the magnet bore opening. Participants viewed the screen through a mirror mounted on the head coil. Prior to the experiment, participants were told to fixate at the fixation cross for the entire trial. Faces were used to mark the target locations and to respond by pressing one of two buttons to indicate whether or not the probe was at a remembered location. For the memory tasks, positive probes (targets that matched one of the cue locations) and negative probes (non-targets) were randomly presented in each run. For the control task, scrambled faces were used and participants were instructed not to remember any of the stimuli locations and to respond by pressing both buttons in response to the probe presentation. While target locations varied across trials, the visual stimuli used to mark the locations did not vary. Control and memory trials were presented alternately, and their order was counterbalanced across runs.

Images showing significant motion (RMS > 0.5 pixels of center of mass) were removed from all analyses. Low intensity voxels were also removed from the analysis by simple thresholding.

Details of the fMRI analyses that were performed for this study were presented elsewhere (Leung et al., 2002). Here, we focused on analyzing the response/recognition period. For each data set, pixel-by-pixel activation maps for each phase (cue, delay and response) of the memory and control tasks were generated by calculating the average percentage changes of signal from fixation baseline. Linear drifts were corrected in these comparisons (Skudlarski et al., 1999). For each task, the fMRI signal of fixation baseline was computed using two images prior to and one image after the onset of the first cue. The cue phase was composed of four images, starting from the second image after the cue-onset. The encoding or early-delay phase was composed of the subsequent four images after the cue phase. The rest of the delay was divided into two phases (mid- and late-delay) and each composed of four images. Similarly, the response phase was composed of four images starting from the second image after probe-offset. The memory trials were further divided into two recognition conditions according to the positive probe and the negative probe presentations. Average percentage changes of signal from fixation baseline were calculated for the response phase of each probe condition.

Group composite maps were generated after transforming the individual maps into a standardized coordinate system (Talairach and Tournoux, 1988). A bootstrapping randomization technique (applied in Marois et al., 2000; Leung et al., 2002) was used to calculate the contrast maps such as positive-probe versus negative-probe conditions. Under the null hypothesis of no signal change, the expected value for a particular comparison was equal to zero. The randomization created a population distribution for each voxel by calculating multiple values for the comparison in which randomly chosen subsets of subjects’ data were assigned reversed contrast weights. The randomization was performed 2000 times in order to generate an adequate sampling distribution. By comparing the experimental data to this distribution, the significance of each measurement was estimated. The composite maps, such as those shown in Figure 2A, were cluster-filtered (20 contiguous pixels) and thresholded (P < 0.005) to reveal only pixel clusters with percent signal change values that fall above the 99.5 percentile of the random sampling distribution.

Figure 1. A schematic diagram for a task trial. Both memory and control trials have the same time line. Each visual stimulus lasts for 1 s and the gap between two adjacent stimuli is 250 ms. The delay interval is 18 s. The ITI is 14 s (not indicated in the figure).
Activation region-of-interests (ROIs) were defined based on the frontal activations obtained from the comparison between the response phases of the memory and control tasks (Fig. 2A). The same ROIs were used in all other comparisons. The frontal ROIs included the aPFC, ACC, middle frontal gyrus (MFG), inferior frontal gyrus (IFG) and precentral sulcus (PrCS). For comparison purposes, we included two non-frontal ROIs: the thalamus and superior parietal lobe (SPL). The average percent signal change from baseline was calculated for each ROI for both response and early-delay phases of the memory task. Percentage change of signal was calculated using \( \frac{A - B}{B} \times 100 \), where \( A \) and \( B \) were the averaged signals of the response (or early-delay) phase and the baseline of the memory task, respectively. Similar calculations were made for the response phases of the positive-probe and negative-probe conditions. Significant signal differences between the two probe conditions were determined using paired \( t \)-tests.

The time course of each task was determined for each ROI. For each individual, the average percentage changes in signal at each time point were calculated relative to the mean signal during baseline. Time differences in slice acquisition were adjusted for each slice. Before performing the interpolation and resampling, all time course data were time-smoothed by a Gaussian filter (full-width at half maximum of 1.5 s).

**Results**

Behavioral results showed significantly longer reaction times to both positive and negative probes of the memory task (1849 and 1542 ms, respectively) than to the probes of the control task (985 ms). Only correct trials were included in the analysis. The differences in response to the two probe conditions of the memory task were significant for reaction times \( [t(11) = 2.72, P < 0.05] \) but not for response accuracy (average 78%, \( P > 0.05 \)).

**Activations during the Response Stage**

During the memory tasks, response-related activations were observed in multiple frontal regions, including the MFG (BA 9/46 and BA 6/8), IFG (BA 45 and BA 47), ACC (BA 24/32) and aPFC (BA 10 and BA 10/46), in comparison with the control task.

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**Figure 2.** Contrast maps and time courses. (A) Composite maps of response-related activations were generated by contrasting signals from the response phases of the memory and control tasks. Red/yellow and blue/purple indicate percent signal change above and below threshold, respectively. Cluster filter is set at 20 contiguous voxels and activation threshold is set at \( P < 0.005 \), uncorrected. R, right; L, left. (B) Time courses for memory (red line) and control (blue line) tasks of the ROIs selected from the activation maps (numbers shown in A). Cue onset starts at the first time point. The first vertical line marks the end of the cue presentation and the second line marks the onset of the response probe presentation. The time between the two lines is the delay period (18 s). The black bars on the x-axis mark the times of which hemodynamic responses were used to calculate the encoding/early-delay and response-related signals. Baseline was calculated using the first and last two time points. See Table 1 for abbreviations.
Response Phase versus Encoding Phase

Stronger and more widespread activations were generally observed during the response phase in comparison with other phases of the memory task (Fig. 2B, Table 1). In order to visualize the extent of overlapping brain activations in response to encoding/early-delay and response/recognition requirements, we overlaid the activation maps from the two phases after correcting the control-related activations (Fig. 3, P < 0.005, uncorrected). Because the encoding phase and the response phase were different in sensory and motor demands, control-related activations were subtracted with the purpose of removing simple sensory and motor effects. As illustrated in the figure, both task events activated mostly the same frontal and non-frontal regions (yellow). Although the aPFC, ACC, IFG (BA 45 and BA 47) and MFG (BA 6/8) were more activated than the control during the response phase (red), the thalamus, occipital gyrus, precentral sulcus and a part of the SPL were more activated than the control during the encoding/early-delay phase (blue). However, as with any other subtractive comparisons, these data should be interpreted with care as there were probably other processes (such as response selection) that may also contribute to the differences in activation patterns.

Negative Probe versus Positive Probe

We focused on examining the difference between the two probe conditions (positive and negative) of the memory task. Similar frontal and non-frontal regions were activated above the fixation baseline for both probe conditions. Direct contrast between the two probe conditions revealed only one suprathreshold region, the right ventral aPFC (BA 10, the average center of mass at the Talairach coordinates 30, 52, 9), that was more activated in response to the negative probe than to the positive probe (P < 0.005, uncorrected; see Fig. 4A). This activation in the ventral portion of the aPFC overlapped with the part of aPFC that showed a response-related fMRI signal (see above and Fig. 2A). No brain areas showed greater responses to the positive probe than to the negative probe even when the threshold was lowered to P < 0.05 (uncorrected).

In order to determine whether the activation in the aPFC showed by the group composite analysis was dissociable from other response-related activations in the frontal lobe, separate ROI analysis was conducted. Activations in the selected ROIs were compared across the probe conditions (Fig. 4B). A region [ACC, IFG (BA 45), IFG (BA 47), dorsal aPFC (BA 10/46) and ventral aPFC (BA 10)] by probe (positive and negative) interaction indicated that these frontal regions were differentially engaged across the probe conditions [F(4, 44) = 3.37, P < 0.005]. We performed paired t-tests to confirm that the responses of the ventral aPFC (BA 10) to the negative probe and the positive probe were significantly different [t(11) = 2.64, P < 0.05]. The dorsal aPFC [BA 10/46, t(11) = 2.05, P = 0.07] and the ventral IFG [BA 47, t(11) = 1.88, P = 0.09] also showed marginally significantly greater changes in signal in response to the negative probe than to the positive probe. Other frontal regions, including the ACC and IFG (BA 45), showed little or no difference in response to the two probe conditions.

Anterior PFC

We examined the average percent signal change from baseline in the ventral part of the aPFC (BA 10) for both memory and control tasks across the different task events. The right aPFC was activated above the baseline during all phases (i.e. cue, early-, mid-, late-delays and response) of the memory task (Fig. 2B, region 4). Both phase effect [F(4, 44) = 12.93, P < 0.0001] and task effect [F(1, 11) = 8.21, P = 0.02] were significant. The phase and task interaction [F(4, 44) = 2.59, P < 0.05] was also significant. Post-hoc tests indicated that signal change during the response phase was significantly greater than all other phases of the memory task (P < 0.05). The delay-related activations in the aPFC during the memory task were also significantly greater than during the control task (paired t-tests, P < 0.05). The left aPFC showed similar effects, with significant phase effect [F(4, 44) = 13.95, P < 0.01], task effect [F(1, 11) = 13.71, P < 0.005] and their interactions [F(4, 44) = 10.43, P < 0.0001].

Discussion

In the present experiment, we used event-related fMRI to examine activity in the prefrontal cortex during the response/recognition phase of a spatial working memory task. As expected, we observed widespread brain activations during the response phase of the spatial delayed-recognition task that largely overlapped with activations during the encoding or early-delay phases. In the current study, we focused on examining the role of aPFC during the response phase of working
memory since this region has been particularly emphasized in the retrieval of long-term memory. While we found that the right aPFC was more active in response to negative probes than to positive probes, the aPFC (bilateral) was also active throughout all phases of the spatial delayed-recognition task. Our findings thus converge with previous evidence in supporting the proposal that the aPFC is involved in working memory, and further suggest that this region is especially involved in processes that distinguish target and non-target stimuli during recognition in working memory.

It was surprising that we observed only greater brain activity in response to the negative probes than to the positive probes and did not find the opposite even when we lowered the activation threshold. This was unexpected because findings of a single-unit study in non-human primates has indicated that the prefrontal neurons exhibit selective responses to both positive and negative probes, with more neurons showing enhanced activity in response to positive probes that matched the sample stimuli (Miller et al., 1996). Using a similar design, an fMRI study in humans has demonstrated that the inferior PFC is more responsive to testing stimuli that match the sample stimuli than those that do not match (Jiang et al., 2000). However, only the positive probes were behaviorally relevant in these former studies, whereas our participants made responses to both positive and negative probes. Our results thus extend previous studies by showing the prefrontal regions may be more involved in rejecting non-targets (see below).

The finding of greater aPFC activity to negative probes than to positive probes was also different from a recent study that found differential dorsolateral PFC and ACC activations in response to positive and negative probes in a face working memory study (Druzgal and D'Esposito, 2001). The differences in findings probably stem from task-specific differences. It is possible that our participants adopted different recognition strategies than those in the Druzgal and D'Esposito study because we used a longer delay. However, similar aPFC activations were observed

Figure 3. Comparisons of supra-threshold activations for encoding/early-delay and response phases of the memory task. Early-delay or encoding-related activations above control are shown in blue and recognition-related activations above control are shown in red. Areas that showed activations during both phases are in yellow. Only positive activations are shown.

Figure 4. (A) Activation differences between the negative and positive probe conditions. The right ventral aPFC (x = 30, y = 52, z = 9) showed greater activation in response to the negative probes than to the positive probes. (B) Average percentage changes of signal from baseline are plotted for responses to positive probes (white bars) and negative probes (gray bars). All ROIs are in the right hemisphere. Standard error bars are shown in the figure. See Table 1 for abbreviations and Talairach coordinates.
in previous studies of verbal (Zhang et al., 2003) and spatial (Leung and Zhang, 2004) working memory using a different design and shorter delays (2 s). Another possibility is that the type of visual material used differs between the two studies: spatial locations in the current study and faces in the Druguza and Desposito study. Furthermore, the ACC may be involved in more general selection and monitoring processes that are common in both response conditions. Activation of the ACC is consistently observed in tasks that require response selection and monitoring (e.g. Stroop; Taylor et al., 1997; Leung et al., 2000; Go/No-go: Liddle et al., 2001). Rowe and Passingham (2001) also demonstrated activation in the ACC, MFG and IFG related to a selection demand during the response stage of a spatial working memory task where subjects moved a joystick to target locations selected from working memory. We also observed activity in the MFG (BA 6/8 and 9/46) during the recognition phase, but we did not find significant probe-related response differences for these areas.

The response-related aPFC activation in the current study coincides with activations consistently found during retrieval in long-term memory studies (see review by Duncan and Owen, 2000; but see MacLeod et al., 1998). Although previous studies of retrieval in episodic memory have reported both right lateralized (Tulving et al., 1994; Buckner et al., 1996; Nyberg et al., 2000; Grady et al., 2001) and bilateral anterior PFC activations (Andreasen et al., 1995; Rugg et al., 1996), several recent studies have also found aPFC activations in both working memory and long-term memory tasks (Braver et al., 2001; Nyberg et al., 2003; Ranganath et al., 2003; but see Cabeza et al., 2002). For example, Ranganath and colleagues observed activations in the bilateral aPFC during face recognition in working memory and in the left aPFC during face recognition in episodic memory using parallel designs for both memory tasks (Ranganath et al., 2003). The loci of the bilateral aPFC activations in the current study were slightly anterior but very close to the aPFC activations in this latter study. It is possible that similar strategies are implemented during delayed-recognition in working memory and recognition in long-term memory, and hence similar findings of aPFC activations are observed. However, our results showed that the aPFC was active not only during recognition but also during other critical events in working memory, such as encoding and maintenance. Results of the present study thus provide further evidence to support the postulate that the anterior portion of PFC is involved in memory systems besides long-term memory.

In long-term memory studies, some have associated aPFC activity with the success of retrieval (e.g. Rugg et al., 1996; McDermott et al., 2000). The part of the right aPFC that showed differences in response to negative (non-target) and positive (target) probes in our study is in the near vicinity of activations identified for various recognition responses in long-term memory studies (e.g. McDermott et al., 2000; Rugg et al., 2003). McDermott and colleagues found that activations in the right aPFC are stronger in response to non-target words (that overlapped with target words) and target words than to new words. Similarly, Rugg and colleagues showed greater activations in the left aPFC in response to correct rejection of previously studied non-target words than to correct rejection of new words (Rugg et al., 2003). Interestingly, part of the activations in aPFC appeared to be numerically stronger (but insignificant) in relation to non-target items than to target items in both studies (see fig. 2 in McDermott et al., 2000; fig. 2C in Rugg et al., 2003).

Besides, other long-term memory studies have demonstrated similar aPFC responses to new words as well as to target words (e.g. Buckner et al., 1998). The negative probes, or non-targets, in our experiment are more compatible with the previously studied non-target items than with the new items in long-term memory studies. This is because the sample size for stimuli used in working memory studies is usually very small and task items including the negative probes are often presented multiple times such as those in the current study. Besides, the retrieval or reactivation of target items in episodic memory has been considered to require working memory (Baddeley, 2000). Taking findings from both working memory and long-term memory studies, it appears that the aPFC may support recognition in both types of memory in a similar way and may be responsive to both targets and non-targets but to different extents depending on the task.

Activation of aPFC has also been related with control processes that involve evaluation during retrieval or recognition in memory (e.g. Nolde et al., 1998; Raye et al., 2000; Dobbins et al., 2002). Perhaps the main function of aPFC is to compare the probe with the on-line information maintained in working memory or retrieved information from long-term memory. If that is the case, the current findings would suggest that greater aPFC activity may result from greater mismatch between the probe and the on-line information. Alternatively, the comparison process may be in larger demand for the recognition of non-targets in working memory even though our participants showed shorter reaction times for the negative probes than for the positive probes. Similar predictions were made by the behavioral theories regarding differences in reaction times to positive and negative probes (Sternberg, 1966; Ratcliff, 1985).

One caveat is that error trials were not removed from the current analyses to maintain the statistical power. To clarify this potential problem, we eliminated the error trials and found the same patterns of activations but the effects were weaker. Therefore, it is unlikely that the differences in activity between the probe conditions can be solely accounted for by response errors. In addition, it could be due to a lack of statistical power that we did not find any brain regions to show stronger signal in response to targets than to non-targets. The aPFC may not be the only region involved in working-memory recognition considering that a few other PFC areas showed similar but weaker effects in the current study. Since we concentrated on the aPFC in the current study, further investigations should include a larger sample of subjects and determine whether or not our findings can be generalized to other brain areas and other types of working memory tasks.

Notes

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Address correspondence to Hoi-Chung Leung, SUNY Stony Brook, Department of Psychology, Stony Brook, NY 11794-2500, USA. Email: hoi-chung.leung@sunysb.edu.

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