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Research Report

Organization of mnemonic and response operations within prefrontal cortex

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ABSTRACT

There is mounting evidence that prefrontal cortex (PFC) is activated during mnemonic operations such as working memory maintenance and also during response-related operations. In the current study, we examine the neural organization of mnemonic and response operations with respect to each other within PFC. Stimulus-evoked and sustained functional MRI activity was recorded during performance of a mental calculation task. The presence or absence of mnemonic and response demands was manipulated in a 2 × 2 factorial design with conditions requiring: (1) memory encoding and maintenance (M+); (2) response selection and execution (R+); (3) encoding, maintenance, and response execution (M+R+); (4) neither mnemonic nor response-related processes (M–R–). The first step of the analyses identified PFC voxels exhibiting differential activity during (M+) vs. (R+) trials. Within these voxels, we then examined activity during multiple phases of (M+R+) trials. Greater stimulus-evoked and sustained activity was observed within the anterior extent of dorsolateral prefrontal cortex (BA 46) during R+ vs. M+ trials. In contrast, greater activity was observed in the posterior extent of dorsolateral PFC during M+ vs. R+ trials. Importantly, both regions were activated during (M+R+) trials. Activity levels during all of these conditions exceeded levels observed during (M–R–) control trials. These results suggest that integrative functions of PFC that allow past information to guide future actions may emerge from communication between discrete subregions supporting mnemonic and response operations.

1. Introduction

Goal-directed behavior requires mnemonic operations that allow for short-term encoding, maintenance, and manipulation of information, referred to as working memory (Baddeley, 1986). In addition, there must be quick access to response operations so that stored goals can be instantiated into action (Quintana and Fuster, 1992, 1999). Many recent models of prefrontal organization have suggested that prefrontal cortex may perform integrative functions that link mnemonic and response operations in the service of behavioral goals (Quintana and Fuster, 1999; Curtis and D'Esposito, 2003; Wood and Grafman, 2003; Ramnani and Miall, 2004). Yet, very little is known about the functional neural organization of these operations with respect to each other within prefrontal cortex in humans.

Previous studies investigating mnemonic functions of the PFC use delayed-recognition and delayed-response tasks

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(DRT). In these tasks, trials begin with the presentation of the memory array (S1) which is followed by a brief delay interval, and end with presentation of a memory probe item (S2). The subject's task is to determine if S2 is a member of the S1 memory array on that trial. Two key findings in nonhuman primates have been used to support the view that PFC subserves mnemonic functions. First, monkeys with lesions to PFC are impaired during DRT tasks (Funahashi et al., 1993b). Second, specialized cells within PFC are active during delay intervals of spatial DRTs, and different cells code for different spatial locations that are to be maintained (Goldman-Rakic, 1990, 1995). While the power of DRTs is that they allow temporal segregation of maintenance-related activity from S1- and S2-evoked activity, maintenance may not be the only process engaged during the delay interval. For example, in oculomotor DRTs, monkeys are instructed to move their eyes to the position of S1 when S2 is presented at the end of the delay. Delay-interval activity could reflect retrospective maintenance of S1 or prospective response planning to move eyes to the S1 position (Funahashi et al., 1991; Funahashi et al., 1993a,b).

Some neuroimaging studies have attempted to isolate maintenance and response-related delay activity by manipulating when response information is delivered during spatial working memory paradigms (Rowe et al., 2000; Pochon et al., 2001; Curtis and D'Esposito, 2003). Greater delay-interval activity was reported within the posterior extent of dorsolateral PFC (dlPFC), corresponding to BA 8 (Petrides and Pandya, 1999), during passive maintenance of spatial information when no response planning was possible. In contrast, greater delay activity was reported within anterior dlPFC, corresponding to BA 46 (Rajkowska and Goldman-Rakic, 1995), when responses could be prepared vs. when they could not. A similar functional organization of PFC has been observed in single-unit studies in monkeys. Cells within the posterior third of the principal sulcus coded for spatial positions that were to be maintained in working memory even when these locations were incongruent with response locations (Funahashi and Takeda, 2002; Takeda and Funahashi, 2002). In contrast, activity of cells within the anterior extent of the principal sulcus coded for motor response locations (Sawaguchi and Yamane, 1999). Based on this evidence, it has been suggested that while posterior PFC appears to be involved in "simple maintenance", anterior PFC may be involved in transforming sensory information into a response code. Importantly, the bulk of findings supporting this proposal are limited to studies of spatial working memory (Passingham and Sakai, 2004).

In the current study, we examined the organization of mnemonic and response functions within PFC while subjects performed a mental calculation task in which numbers were the main stimulus class. We tested the hypothesis that the functional organization of delay activity corresponding to mnemonic and response functions within PFC may be invariant across stimulus domains. If so, in the current task, we should observe greater anterior dlPFC delay activity during tasks requiring response processing and greater posterior dlPFC delay activity during tasks requiring maintenance.

The bulk of studies using DRTs to examine the functional organization of PFC emphasize delay activity profiles. Yet, in addition to delay activity, there is robust evoked activity within PFC during DRTs. Single-unit recording studies report that while evoked activity within some PFC cells is tuned to sensoriperceptual features of memory items, the activity of other cells is tuned to response features (Quintana et al., 1988; Yajeya et al., 1988). Similarly, neuroimaging studies report stimulus-evoked activity within dlPFC that is sensitive to mnemonic demands such as memory load (Rypma et al., 1999; Jha and McCarthy, 2000), as well as response demands such as response conflict, expectancy, and response execution(Huettel et al., 2002; Schumacher et al., 2003; Huettel and McCarthy, 2004). While delay activity may correspond to maintenance and response preparation processes, evoked activity may correspond to stimulus encoding and memory comparison processes (see Rypma et al., 1999 for discussion) and response selection and execution processes. Thus, examination of both delay-interval activity and evoked activity is likely to bolster understanding of the functional organization of PFC.

In the current study, the presence or absence of mnemonic and response demands was manipulated in a 2 × 2 factorial design during a mental calculation task with 4 conditions requiring: (1) memory encoding and maintenance (M+); (2) response selection and execution (R+); (3) encoding, maintenance, and response execution (M+R+); (4) neither mnemonic nor response-related processes (M−R−). The (M+R+) trials were equivalent to standard delayed-recognition trials whereas (M−R−) trials served as control trials requiring only passive viewing of numbers and mathematical symbols. We compared activity profiles during (M+) and (R+) trials to answer three main questions. First, is there an anterior–posterior organizing axis within dlPFC similar to that described in spatial working memory studies? Second, if discrete regions supporting mnemonic and response functions are identified, is process-specific activity present for both stimulus-evoked and delay-interval activity? Finally, are these regions coactivated during multiple phases of (M+R+) delayed-recognition trials?

2. Results

2.1. Behavioral results

2.1.1. Behavioral data analysis

Response times (RT) and percent correct measures were entered into analysis of variance (ANOVA) tests with main factors of Condition: (M+), (R+), M+R+, and Solution Type: Correct vs. Incorrect. In addition to ANOVAs, paired *t* tests were conducted. No main effect of condition was revealed by the ANOVA on either accuracy or RT. In addition, no significant effects were observed during paired tests between (R+) (mean: 95.49%, SD = 0.44), (M+) (mean: 95.83%, SD = 0.06), M+R+ (mean: 98.26%, SD = 0.01) trials. However, paired tests revealed that RTs were significantly faster for (M+R+) (mean: 1061.76 ms, SD = 268.56 ms) relative to each of the other conditions ($P < 0.01$), but (R+) (mean: 1710.88 ms, SD = 388.01 ms) and (M+) (mean: 1522.02 ms, SD = 311.23 ms) did not differ from each other ($P > 0.5$).

2.2. *Neuroimaging results*

Stimulus-evoked and delay-interval covariate effect scores were obtained for the contrast (M+) vs. (R+) for correct trials. These scores were entered into separate two-way ANOVAs for each ROI. For the dorsomedial PFC (dmPFC), there was no main effect of slice band (1, 2, 3, and 4), or hemisphere (Left, Right), nor was there an interaction between these factors for stimulus-evoked or delay-interval covariates ($P > 0.3$). For the dorsolateral PFC (dlPFC), there was a main effect of slice band for stimulus-evoked ($F(3,7) = 5.52$, $P < 0.006$) and delay-interval covariates ($F(3,7) = 6.25$, $P < 0.003$), but no effect of hemisphere or slice by hemisphere interaction for either covariate ($P > 0.7$). For the ventrolateral PFC (vlPFC), there was no effect of slice band or a slice by hemisphere interaction ($P > 0.6$) for either stimulus or delay covariate. There was a significant effect of hemisphere for stimulus-evoked activity ($F(1,7) = 39.8$, $P < 0.001$) and a near-significant effect of hemisphere for delay-interval covariates ($F(1,7) = 5.15$, $P < 0.07$).

Next, effect scores for each covariate were entered into one-sample *t* tests to determine if the magnitude of effects significantly differed from zero. An effect score of zero indicates that there is no difference between activity levels observed during (M+) and (R+) trials. It is important to note that zero does not indicate that absolute activity levels were near-baseline (i.e. % change score of zero). Activity levels were robust during all experimental conditions of interest which is not surprising given how the functional anatomical ROIs were determined. In fact, stimulus-evoked ($P < 0.01$) and delay-interval activity ($P < 0.05$) were greater during (M+), (R+), and (M+R+) trials than Control trials (M–R–) during which neither mnemonic nor response demands were present.

For the dmPFC, a one-sample *t* test was performed on effect scores for the region as a whole, collapsed across slices and hemisphere since no slice or hemisphere effects were observed in the analyses described above. This result was not significant for either stimulus-evoked or delay-interval covariate ($P > 0.4$).

For the dlPFC, since scores were found to differ across slices but not hemisphere, effect scores for each slice collapsed across hemisphere were entered into one-sample *t* tests (see Figs. 1B and C). Slice 1 significantly differed from zero for the stimulus-evoked ($P < 0.03$) and delay-interval covariate ($P < 0.01$). Importantly, effect score values were negative indicating that activity in this region was greater during (R+) relative to (M+) trials. In contrast, Slice 4 significantly differed from zero for the stimulus-evoked ($P < 0.01$) and delay-interval covariate ($P < 0.05$). In contrast to Slice 1, Slice 4 had a positive mean value indicating the activity was greater during (M+) vs. (R+) trials. Slices 2 and 3 did not significantly differ from zero for either covariate ($P > 0.3$). A direct paired comparison of effect scores for Slice 1 vs. Slice 4 was significant for both stimulus-evoked ($F(1,8) = 8.11$, $P < 0.03$) and delay-interval ($F(1,8) = 7.00$, $P < 0.02$) covariates.

For the vlPFC, for which a significant effect of hemisphere was observed, effect scores were collapsed across all slice bands and analyzed separately for the left and right hemispheres. Neither left hemisphere nor right hemisphere effect scores were significantly different from zero for the stimulus-evoked or delay-interval covariate ($P > 0.5$).

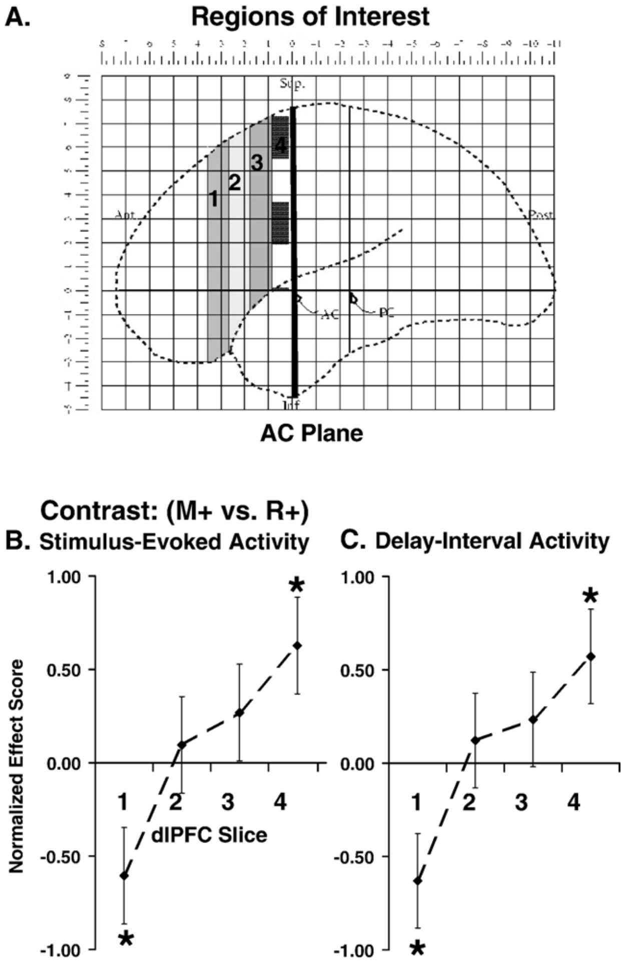

Fig. 1 – Regions of interest within dorsomedial, dorsolateral, and ventrolateral prefrontal cortex were drawn on a slice-by-slice subject by subject basis (A). All regions were indexed relative to the distance from the anterior commissure (AC), which allowed for across-subject comparisons without spatial normalization. Four slice bands within the superior, middle, and inferior frontal gyrus were investigated. (B) The line graph shows normalized parameter estimates for slice bands 1 through 4 within the left and right middle frontal gyrus for the contrast between (M+) and (R+) separately for the Stimulus covariate (B) and the delay-interval covariate (C). Positive values denote greater activity during (M+) relative to (R+) trials, whereas negative values indicate the opposite. Error bars denote standard error of the mean across subjects. These results show that there is an anterior–posterior gradient within dlPFC during stimulus-evoked and delay-interval activity such that anterior regions are more active during trials emphasizing response demands whereas posterior regions are more active during trials emphasizing mnemonic demands. An effect score of zero indicated that no differences in activity were observed across (M+) and (R+) trials. As indicated by the asterisks, mean activity within Slices 1 and 4 significantly differed from zero indicated that activity within these regions was modulated by mnemonic vs. response demands.

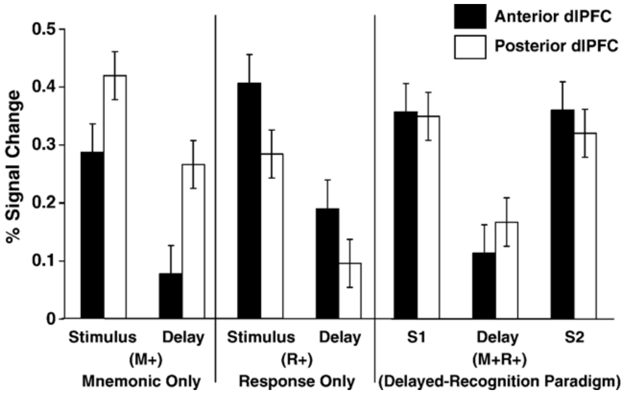

Fig. 2 – The bar graph shows percent signal change values for stimulus-evoked and delay-interval activity within the Anterior dlPFC (black bar) and posterior dlPFC (white bar) for the (M+), (R+), and (M+R+) delayed-recognition trials. Error bars denote standard error of the mean across subjects. While (M+) trials robustly activated Posterior but not Anterior MFG, (R+) trials robustly activated Anterior but not Posterior MFG during both stimulus-evoked and delay-interval activity. Importantly, the delayed-recognition trials activated Anterior and Posterior extents of the MFG during S1-evoked, delay-interval, and S2-evoked activity.

Next we examined activity during (M+R+) trials within Slices 1 and 4 of dlPFC which were the only regions demonstrating differential activity across (M+) and (R+) trials. Robust activity was observed within both subregions during S1-evoked activity; delay-interval activity and S2-evoked activity (see % signal change values in Fig. 2). Paired contrasts between (M+) vs. (M+R+) and (R+) vs. (M+R+) did not result in effect scores that significantly differed from zero for S1, delay-interval, or S2-evoked activity ($P > 0.12$).

Thus, two subregions of the dlPFC were significantly modulated by mnemonic vs. response demands. Whereas the anterior extent (Slice 1) was more active during trials emphasizing response processes, the posterior extent (Slice 4) was more active during trials emphasizing mnemonic encoding and maintenance. Further both subregions were comparably engaged during S1-evoked, delay-interval, and S2-evoked time periods during (M+R+) delayed-recognition trials.

3. Discussion

In the current study, we examined the organization of prefrontal cortex to determine if discrete subregions of PFC support mnemonic and response operations. In studies of spatial working memory, anterior PFC is activated during response selection, preparation, and execution (Rowe et al., 2000; Pochon et al., 2001; Rowe and Passingham, 2001; Curtis and D'Esposito, 2003). In contrast posterior PFC is activated during mnemonic encoding and passive maintenance of spatial stimuli (for review see Passingham and Sakai, 2004). We observed a similar organization for mnemonic and response functions within PFC during a mental calculation task with numerical stimuli. The anterior extent of the dlPFC was more active during (R+) trials requiring response selection and execution relative to (M+) trials that required memory encoding and maintenance. We also found that posterior dlPFC was more active during (M+) relative to (R+) trials. Importantly, stimulus-evoked and inter-stimulus-interval delays exhibited this pattern. Finally, both anterior and posterior dlPFC subregions were active during S1-evoked, delay-interval, and S2-evoked time periods in (M+R+) trials.

The study design aimed to unconfound mnemonic and response requirements while holding constant the need to perform simple mental calculation across conditions. We isolated mnemonic encoding and maintenance from response requirements in (M+) trials by requiring encoding and maintenance for each stimulus but requiring a response only after the presentation of 6–8 consecutive stimuli. We isolated response selection and execution from mnemonic requirements during (R+) trials by instructing subjects to simply respond once they determined if a mathematical solution was correct and incorrect. The need to mnemonically encode or maintain items was eliminated because each item was to be independently evaluated.

Unlike these conditions, (M+R+) DRT trials engaged multiple processes. S1 required mental calculation and memory encoding of the solution. In addition, S1 served as a temporal cue indicating that a response would be required after a brief delay. However, S1 did not provide information about the specific response to be executed at S2. During the delay, subjects maintained items in working memory and also prepared for an upcoming unspecified action to be executed at S2. At S2, subjects performed mnemonic comparison processes to determine if S2 was a match or nonmatch and executed a corresponding button press. If discrete foci were activated during (M+) and (R+) conditions, we anticipated that during "confounded" DRT trials we would see coactivation of both foci. Indeed, (M+) and (R+) activated the posterior and anterior extent of dlPFC, respectively. In addition, both of these foci were activated during (M+R+) trials. Below we discuss possible explanations for these results.

Numerous studies have reported delay-interval activity within posterior PFC in "simple maintenance" tasks (Courtney et al., 1998; Zarahn et al., 1999; Postle et al., 2000; Rowe et al., 2000). In studies requiring memory for spatial information, activation foci were typically within dorsal regions of PFC, such as BA 8 (Courtney et al., 1998; Sakai and Passingham, 2003). In contrast, during memory for letters, ventral regions, such as inferior frontal gyrus (BA 44), were activated (Sakai and Passingham, 2003). While such a domain-differentiated organization of PFC for verbal and spatial information is well supported for posterior PFC (see Passingham and Sakai, 2004 for review), very few studies have investigated the neural regions supporting maintenance for numerical stimuli—which have been treated as a special class of verbal stimuli (Gruber et al., 2001). In the current study, we observed maximal activity within the posterior dlPFC, specifically middle frontal gyrus (MFG), corresponding to BA 8 (see Petrides and Pandya, 2002), during (M+) conditions emphasizing maintenance of numerical stimuli. It is interesting to note that other studies have reported a correspondence between regions activated during tasks involving spatial stimuli and simple arithmetic of numerical material, specifically within parietal cortex (Gruber et al., 2001). Consistent with such findings, we observed activation within posterior dlPFC subregions previously reported to support maintenance of spatial information during our presentation of numerical stimuli.

Several studies have reported that response-related processes are supported by activity in anterior dlPFC (Rowe et al., 2000; Rowe and Passingham, 2001; Passingham and Rowe, 2002; Rowe et al., 2002). In the current study, many regions were activated during (R+) trials. Yet, there was more activity during (R+) relative to (M+) trials within the anterior extent of dlPFC. It has been suggested that this portion of dlPFC corresponds to BA 46 (Petrides and Pandya, 1999). While tasks emphasizing response requirements activate BA 46, Passingham and Sakai (2004) have argued that this region subserves transformation and manipulation of information in addition to response-related processes (Passingham and Sakai, 2004). As support, they site studies reporting delay-interval activity within BA 46 in DRTs only when memory load is high. They argue that only high load trials necessitate transformation of information to keep memory traces active. Under high load conditions, for example, subjects are more likely to engage in rehearsal, recoding, chunking, or other mnemonic strategies. In contrast, transformation of information is not necessary and may not enhance memory representation when load is low. In tasks emphasizing response selection and execution, BA 46 may subserve transformation of a sensoriperceptual code to a motoric response code. Perhaps the stimulus-evoked response-related activity observed during (R+) trials in our study reflects subjects transforming their evaluation of each mathematical solution into a response code (e.g., The solution is incorrect so I should press right button). It is interesting to note that when stimuli did not necessitate recoding and could be simply encoded and maintained during (M+) trials, minimal activity was observed within BA 46.

In our study, we observed robust stimulus-evoked and delay-interval activity during (M+R+) trials within anterior and posterior dlPFC. S1 presentation may have initiated memory encoding within posterior dlPFC, and nonspecific response selection within anterior dlPFC. During the delay interval, mnemonic maintenance processes may be subserved by posterior dlPFC, whereas processes related to response preparation and planning may be subserved by anterior dlPFC. Finally at S2, mnemonic comparison processes may be subserved by posterior dlPFC, whereas response execution and/or the transformation of mnemonic codes into motoric responses may be subserved by anterior dlPFC.

In conclusion, there is little debate that PFC is involved in complex cognitive behavior (Koechlin et al., 2003; Wood and Grafman, 2003; Ramnani and Miall, 2004). Nonetheless, the subjective experience of fluid behavior in which past events guide future actions appears to be supported by multiple nodes organized in both a process- and domain-differentiated fashion within PFC (Passingham and Sakai, 2004; Sala et al., 2003). When damage to these nodes occurs, there are corresponding impairments in specific operations and behavior becomes disjointed (Ferreira et al., 1998). While our study investigated the organization of mnemonic and response operations within PFC, other studies have investigated these operations within parietal cortex (Bunge et al., 2002; Todd and Marois, 2004). Future studies should elaborate on how PFC, parietal cortex, and other brain regions interact to support mnemonic and response organization.

4. Experimental procedures

4.1. *Experimental methods*

Ten healthy volunteers participated (7 female, 3 male; mean age 21 ± 10 years). The University of Pennsylvania Institutional Review Board approved this study, and each subject provided informed consent. One subject was removed from analyses due to a data recording error. Another subject was removed due to excessive head motion (>2 mm). Thus, results from 8 subjects will be discussed herein. All stimuli were created and presented using E-prime (Psychology Software Tools). During fMRI recording, stimuli were displayed using a Dell Inspiron 4100 laptop, and were projected with an Epson LCD projector onto a screen placed at the subject's feet and viewed through an angled mirror mounted within the head coil. All experimental conditions contained variants of simple mathematical equations similar to those used by Gruber et al. (2001). Four conditions comprised a 2 × 2 factorial design matrix in which mnemonic and response demands were present or absent (see Fig. 3).

4.1.1. *Control condition (M–R–)—no mnemonic and no response demands*

Each stimulus display contained numbers and mathematical symbols in pseudorandom nonmathematical combinations. Each display was presented for 1.5 s and followed by a 16.5 s inter-stimulus delay interval. A fixation cross appeared in the center of the screen throughout delay intervals during this and all other conditions. Subjects were instructed to passively view each stimulus display and rest their eyes on the fixation cross during delay intervals. Thirty-six stimulus displays were presented across each of two experimental runs in this condition.

4.1.2. *Mnemonic condition (M+)*

The first stimulus display in each trial was an unsolved mathematical equation presented for 1.5 s. Subjects were instructed to solve the equation and maintain its solution in working memory over a 16.5 s inter-stimulus delay interval until the next display appeared. The next display contained another unsolved equation in which one of the operands was replaced with a "#". Subjects were to mentally substitute "#" with the maintained solution from the previous problem and then solve the equation. Subjects were cautioned to maintain the number only "in your mind—subvocally" during the delay interval. A series of 6–8 equations were presented. At the end of this series of equations, a single number was presented to the right of an "=". The subject's task was to determine if this number was the correct solution from the previous equation and then make the corresponding response. Each of two experimental runs consisted of three such trials each separated by an 18 s inter-trial interval. Thus, subjects made button-press responses a total of 6

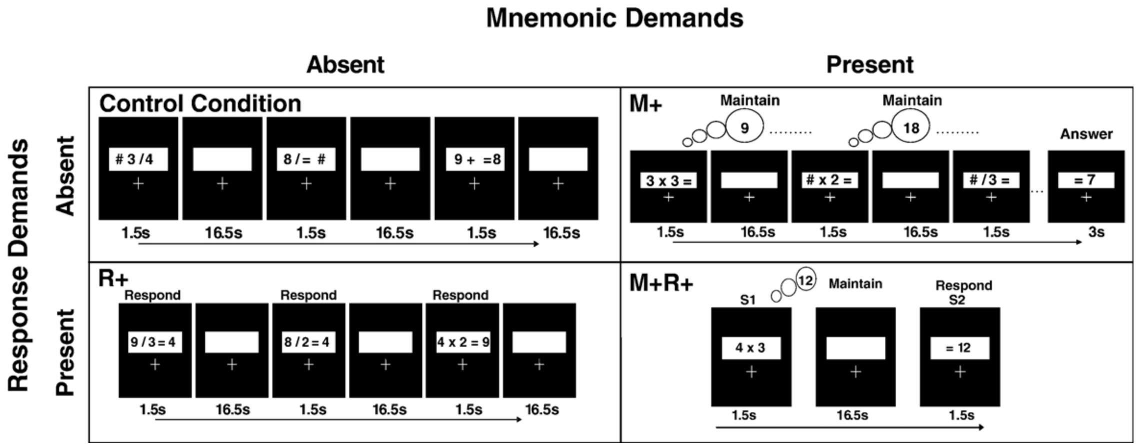

Fig. 3 – Experimental conditions and timing. The absence or presence of mnemonic and response requirements was varied across four experimental conditions during performance of a mental calculation task. Top row: Control trials (M–R–) did not require subjects to remember or respond during stimulus presentation. Each stimulus display, which contained numbers and mathematical symbols in pseudorandom nonmathematical combinations, was presented for 1.5 s and followed by a 16.5 s inter-stimulus delay interval. Subjects were instructed to passively view each display and rest their eyes on the fixation cross during delay intervals. Mnemonic demands (M+): The first stimulus display in each trial was an unsolved mathematical equation presented for 1.5 s. Subjects were instructed to solve the equation and maintain its solution in working memory over a 16.5 s inter-stimulus delay interval until the next display appeared. The next display contained another unsolved equation in which one of the operands was replaced with a "#". Subjects were to mentally substitute "#" with the maintained solution from the previous problem and then solve the equation. At the end of this series of equations, a single number was presented to the right of an "=". The subject's task was to determine if this number was the correct solution from the previous equation and then make the corresponding response. Bottom row: Response demands (R+): each stimulus display contained a solved mathematical equation displayed for 1.5 s and was followed by a 16.5 s inter-stimulus delay interval. The subject's task was to determine if the equation was solved correctly or incorrectly. All equations were simple and required no multiplication or division beyond stored table facts. Mnemonic and response demands (M+R): Each delayed-recognition trial began with an S1 memory array presented for 1.5 s, comprising an unsolved mathematical equation. Subjects were to solve the equation and told to maintain the solution over a 16.5 s uninterrupted S1–S2 delay interval. In addition, they were told to prepare to make a quick and accurate button press at the end of each delay. At the end of the delay, S2 was presented for 1.5 s and provided a solution for the equation to which subjects executed a response. Half of the S2 items were correct solutions and the other half were incorrect.

times during this condition. Importantly, subjects could not predict exactly when a response was required since the number of equations was randomly varied between 6 and 8 across trials. There were a total of 36 stimulus-delay pairs in this condition that required mnemonic encoding and maintenance of a number. On this and all other conditions requiring responses, responses were made by pressing one of two keys on a button box held in the right hand; a left key indicated that the solution was "Correct" and a right key indicated that the solution was "Incorrect". Correct and incorrect solutions were presented equaprobably and randomly intermixed. Subjects were instructed to respond as quickly and accurately as possible.

4.1.3. *Response condition (R+)*

Each stimulus display contained a solved mathematical equation displayed for 1.5 s and was followed by a 16.5 s inter-stimulus delay interval. The subject's task was to determine if the equation was solved correctly or incorrectly. All equations were simple and required no multiplication or division beyond stored table facts. This condition consisted of two runs with 18 stimulus displays in each run for a total of 36 displays.

4.1.4. *Mnemonic and response condition (M+R+)*

This condition was modeled after standard delayed-recognition tasks. Each trial began with an S1 presented for 1.5 s, comprising an unsolved mathematical equation. Subjects were to solve the equation and told to maintain the solution "in their minds—subvocally" over a 16.5 s uninterrupted S1–S2 delay interval. In addition, they were told to prepare to make a quick and accurate button press at the end of each delay. At the end of the delay, S2 was presented for 1.5 s and provided a solution for the equation to which subjects executed a response. Half of the S2 items were correct solutions and the other half were incorrect. The inter-trial-interval (ITI) was 15 s. A total of 36 trials were presented over two runs.

4.2. *MRI acquisition and processing*

Anatomical and functional data were acquired using a 1.5 T GE Signa scanner with a standard head coil. High-resolution axial T1-weighted images were obtained from each subject prior to functional imaging. 124 axial slices consisting of near-isotropic voxels (0.94 × 0.94 × 1 mm) were acquired (254 × 256 × 124 voxel matrix, flip angle = 20°). Blood oxygenation level-dependent (BOLD) echo-planar functional images (TR = 3000 ms, TE = 50 ms) were acquired axially for all experimental runs. Each brain volume consisted of 30 neighboring 3.8-mm slices, with an in-plane resolution of 64 × 64, yielding a voxel size of 3.75 × 3.75 × 3.8 mm for full-brain coverage.

4.3. *Behavioral data analysis*

Behavioral data were analyzed independently of functional data. Response time (RT) and percent correct measures were entered into separate analysis of variance (ANOVA) tests to determine averages and statistically significant differences as a function of condition.

4.4. *Imaging data analysis*

The VoxBo software package (www.voxbo.org) was used for all fMRI analyses. Standard data preprocessing included slice-time correction, six-parameter motion correction (Friston et al., 1995), and conversion of orientation to the coronal plane. Spatial smoothing and spatial normalization were not conducted, as hypotheses were tested in anatomically and functionally defined regions of interest.

The general linear model (GLM) was used to statistically characterize fMRI signal changes observed in the time-series analyses as a series of amplitude-scaled and time-shifted covariates (Fig. 4). This method has been extensively used and thoroughly described elsewhere (Zarahn et al., 1997a,b; Postle et al., 2000). Briefly, changes in BOLD signal were modeled as independent regressors for each stage of each condition of the experimental tasks. A single regressor time-locked to stimulus onset was used to model stimulus-evoked activity (0–3 s following stimulus-onset). The delay interval following stimulus presentation was modeled by 3 regressors at 7 s, 12 s, and 15 s following stimulus-onset. Each regressor was convolved with a canonical hemodynamic response function (HRF). Data were proportionally normalized to account for differences in mean-signal values, and correct for linear signal drift within scanning runs. Additional nuisance covariates were included to model the intercept and trial-specific baseline shifts. The convolution matrix included a time domain representation of the 1/f power structure (Zarahn et al., 1997a,b) and a filter to remove frequencies above and below the prominent task frequencies. For each subject, normalized effect scores (e.g., beta values; regression weights) yielded by the GLM for the stimulus displays and delay time periods were extracted for each covariate and averaged within each functionally defined ROI (see below). These normalized effect scores served as dependent measures for across-subjects random-effects analyses. Because our hypotheses concerned activity changes specific to stimulus displays and delay intervals, analyses centered on covariates modeling these task periods.

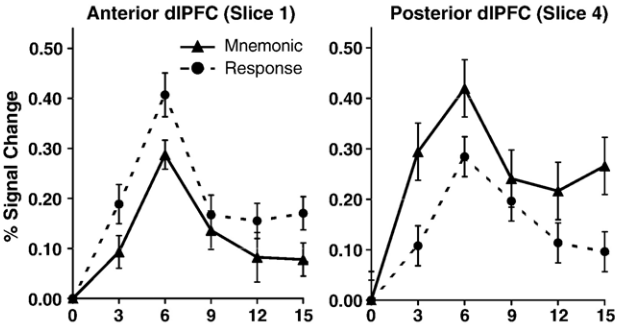

Fig. 4 – The time-series presented in this figure is the average percent change in fMRI signal within anterior dlPFC (A) and posterior dlPFC (B) during (M+) (solid line) and (R+) (dotted line) trials. Error bars denote standard error of the mean across subjects. These results graphically depict the significant differences observed between M+ and R+ trials within this region during stimulus-evoked activity (0–6 s following stimulus onset) as well as delay activity (9–15 s following stimulus onset).

4.5. *Regions of interest (ROI) analyses*

Activity was examined within three functionally defined ROIs that were first defined by anatomical boundaries on each subject's high-resolution coronal anatomical images by experienced technicians. ROIs were drawn on left and right superior frontal gyri (SFG), middle frontal gyri (MFG), and inferior frontal gyri (IFG), to correspond to dorsomedial, dorsolateral, and ventrolateral PFC regions, respectively. These regions were drawn over 3 contiguous slices to create 4 discrete slice bands (Fig. 1A: Slices 1, 2, 3, 4). ROIs were indexed by the distance in mm from each subject's anterior commissure (AC) so that summary activation waveforms by slice region could be created across subjects for each region. These prefrontal ROIs ranged from 0 to 45.6 mm anterior to the AC. Slice bands were selected a priori based on regions reported to be activated in previous studies examining mnemonic and response functions within PFC (Rajkowska and Goldman-Rakic, 1995; D'Esposito et al., 2000; Rowe et al., 2000; Pochon et al., 2001; Rowe and Passingham, 2001; Curtis and D'Esposito, 2003; Sala et al., 2003). Specifically, Slice 1 of the MFG was drawn to correspond to Brodmann Area (BA) 46 whereas Slice 4 of this gyrus was drawn to correspond to BA 8 as suggested in previous studies investigating the correspondence between cytoarchitectonics and anatomical locations guided by the Talairach Coordinate system (Rajkowska and Goldman-Rakic, 1995).

Next, an ROI was defined as all voxels within these regions that exhibited statistically significant task-related activity ($P < 0.05$, uncorrected) during any phase of the task. This method of identifying functional-anatomical ROIs has been recently used to examine prefrontal and posterior perceptual activity (Jha et al., 2004; Ranganath et al., 2004). An important aspect of the statistical contrasts used to functionally define ROIs is that they were orthogonal to, and therefore did not bias, the subsequent statistical contrasts conducted.

4.6. *fMRI planned contrasts*

BOLD responses were separately analyzed for functional–anatomical ROIs within right and left dorsomedial (dmPFC), dorsolateral (dlPFC), and ventrolateral (vlPFC) prefrontal cortex for correct and incorrect trials. We modeled our data to independently assess activity associated with stimulus-evoked and delay-interval activity in all conditions. We used a long delay-interval duration (15 s) to ensure that our contrasts of interest regarding delay-spanning activity were uncontaminated by stimulus-evoked activity.

Posterior dlPFC (corresponding to BA 8av and 8ad) has previously been reported to subserve mnemonic encoding and maintenance during delayed-recognition paradigms (for review see Passingham and Sakai, 2004). In contrast, anterior dlPFC (corresponding to BA 46) has been reported to be activated during response selection, preparation, and execution as well as manipulation and transformation of information during working memory (for review see Passingham and Sakai, 2004). We hypothesize that posterior dlPFC would be more active during (M+) trials whereas anterior dlPFC would be more active during (R+) trials. Thus, our primary contrast of interest compared stimulus-evoked and delay-interval activity for (M+) vs. (R+) trials. Separate analyses were conducted on each of the PFC regions (dorsomedial, dorsolateral, and ventrolateral PFC). Within each region, the normalized effect scores were first entered into two-way ANOVAs to determine if the effects significantly differed across slice bands (1, 2, 3, 4) or hemisphere (right vs. left). This analysis allowed us to determine if distinct subregions were maximally activated for different tasks. Once peak-activity regions were identified (with either positive or negative normalized effect scores—see below), effect scores for those regions were entered into one-sample *t* tests to determine if they significantly differed from zero.

It is important to note that regions whose activity profiles were significantly greater during high mnemonic demand trials (i.e., M+ trials) were predicted to produce normalized effect scores with positive values for the contrast (M+) vs. (R+), whereas regions whose activity profiles were significantly greater during high response demand trials (i.e., R+ trials) were predicted to produce normalized effect scores with negative values for the same contrast. Thus, the direction of the effect scores revealed the condition during which a region was most active. As such, peak activity regions were identified as the slice band with the highest positive value as well as a slice band with the highest negative value for the normalized effect scores. For regions in which there were no significant differences across the (M+) vs. (R+) conditions, normalized effect scores were not predicted to differ from zero, indicating no difference in activity across these two conditions. To be clear, this is not equivalent to concluding that there was no activity in a particular region—rather that there was no differential activity across the two conditions examined in this contrast.

Once (M+)- and (R+)-sensitive peak regions of PFC were identified, activity during discrete phases of (M+R+)-delayed-recognition trials was investigated within those regions. We hypothesized that (M+)- and (R+)-sensitive PFC subregions would be activated during all trial phases of the delayed-recognition trials (i.e., S1-, delay-interval, and S2-evoked activity). This activity might correspond to mnemonic encoding, maintenance, and retrieval within (M+)-sensitive regions and response planning, preparation, selection, and execution within (R+)-sensitive regions. Thus, contrasts comparing activity during (M+) and (M+R+), and (R+) vs. (M+R+) were predicted to result in effect scores not significantly different from zero.

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REFERENCES

Baddeley, A.D., 1986. Working Memory. Clarendon, Oxford.

Bunge, S.A., Hazeltine, E., Scanlon, M.D., Rosen, A.C., 2002. Dissociable contributions of prefrontal and parietal cortices to response selection. NeuroImage 17, 1562–1571.

Courtney, S.M., Petit, L., Maisog, J.M., Ungerleider, L.G., Haxby, J.V., 1998. An area specialized for spatial working memory in human frontal cortex. Science 279, 1347–1351.

Curtis, C.E., D'Esposito, M., 2003. Persistent activity in the prefrontal cortex during working memory. Trends Cogn. Sci. 7, 415–423.

D'Esposito, M., Ballard, D., Zarahn, E., Aguirre, G.K., 2000. The role of prefrontal cortex in sensory memory and motor preparation: an event-related fMRI study. NeuroImage 11, 400–408.

Ferreira, C.T., Verin, M., Pillon, B., Levy, R., Dubois, B., Agid, Y., 1998. Spatio-temporal working memory and frontal lesions in man. Cortex 34, 83–98.

Friston, K.J., Ashburner, J., Frith, C.D., Poline, J.B., Heather, J.D., 1995. Spatial registration and normalization of images. Hum. Brain Mapp. 3 (3), 123–134.

Funahashi, S., Takeda, K., 2002. Information processes in the primate prefrontal cortex in relation to working memory processes. Rev. Neurosci. 13, 313–345.

Funahashi, S., Bruce, C.J., Goldman-Rakic, P.S., 1991. Neuronal activity related to saccadic eye movements in the monkey's dorsolateral prefrontal cortex. J. Neurophysiol. 65, 1464–1483.

Funahashi, S., Chafee, M.V., Goldman-Rakic, P.S., 1993a. Prefrontal neuronal activity in rhesus monkeys performing a delayed anti-saccade task. Nature 365, 753–756.

Funahashi, S., Bruce, C.J., Goldman-Rakic, P.S., 1993b. Dorsolateral prefrontal lesions and oculomotor delayed-response performance: evidence for mnemonic "scotomas". J. Neurosci. 13, 1479–1497.

Goldman-Rakic, P.S., 1990. Cellular and circuit basis of working memory in prefrontal cortex of nonhuman primates. Prog. Brain Res. 85, 325–335.

Goldman-Rakic, P.S., 1995. Cellular basis of working memory. Neuron 14, 477–485.

Gruber, O., Indefrey, P., Steinmetz, H., Kleinschmidt, A., 2001. Dissociating neural correlates of cognitive components in mental calculation. Cereb. Cortex 11, 350–359.

Huettel, S.A., McCarthy, G., 2004. What is odd in the oddball task? Prefrontal cortex is activated by dynamic changes in response strategy. Neuropsychologia 42, 379–386.

Huettel, S.A., Mack, P.B., McCarthy, G., 2002. Perceiving patterns in random series: dynamic processing of sequence in prefrontal cortex [see comment] Nat. Neurosci. 5, 485–490.

Jha, A.P., McCarthy, G., 2000. The influence of memory load upon delay-interval activity in a working-memory task: an event-related functional MRI study. J. Cogn. Neurosci. 12, 90–105.

Jha, A.P., Fabian, S.A., Aguirre, G.K., 2004. The role of prefrontal cortex in resolving distractor interference. Cogn. Affect. Behav. Neurosci. 4, 517–527.

Koechlin, E., Ody, C., Kouneiher, F., 2003. The architecture of cognitive control in the human prefrontal cortex. Science State of the Planet November 14 302, 1181–1185.

Passingham, R.E., Rowe, J.B., 2002. Dorsal prefrontal cortex: maintenance in memory of attentional selection? In: Stuss, D.T., Knight, R.T. (Eds.), Principles of Frontal Lobe Function. Oxford Univ. Press, pp. 221–232.

Passingham, D., Sakai, K., 2004. The prefrontal cortex and working memory: physiology and brain imaging. Curr. Opin. Neurobiol. 14, 163–168.

Petrides, M., Pandya, D.N., 1999. Dorsolateral prefrontal cortex: comparative cytoarchitectonic analysis in the human and the macaque brain and corticocortical connection patterns. Eur. J. Neurosci. 11, 1011–1036.

Petrides, M., Pandya, D.N., 2002. Comparative cytoarchitectonic analysis of the human and the macaque ventrolateral prefrontal cortex and corticocortical connection patterns in the monkey. Eur. J. Neurosci. 16 (2), 291–310.

Pochon, J.B., Levy, R., Poline, J.B., Crozier, S., Lehericy, S., Pillon, B., Deweer, B., Le Bihan, D., Dubois, B., 2001. The role of dorsolateral prefrontal cortex in the preparation of forthcoming actions: an fMRI study. Cereb. Cortex 11, 260–266.

Postle, B.R., Zarahn, E., D'Esposito, M., 2000. Using event-related fMRI to assess delay-period activity during performance of spatial and nonspatial working memory tasks. Brain Res. Brain Res. Protoc. 5, 57–66.

Quintana, J., Fuster, J.M., 1992. Mnemonic and predictive functions of cortical neurons in a memory task. NeuroReport 3, 721–724.

Quintana, J., Fuster, J.M., 1999. From perception to action: temporal integrative functions of prefrontal and parietal neurons. Cereb. Cortex 9, 213–221.

Quintana, J., Yajeya, J., Fuster, J.M., 1988. Prefrontal representation of stimulus attributes during delay tasks: I. Unit activity in cross-temporal integration of sensory and sensory-motor information. Brain Res. 474, 211–221.

Rajkowska, G., Goldman-Rakic, P., 1995. Cytoarchitectonic Definition of Prefrontal Areas in the Normal Human Cortex: II. Variability in Locations of Areas 9 and 46 and Relationship to Talaraich Coordinate System.

Ramnani, N., Miall, C., 2004. A system in the human brain for predicting the actions of others. Nat. Neurosci. 7, 85–90.

Ranganath, C., DeGutis, J., D'Esposito, M., 2004. Category-specific modulation of inferior temporal activity during working memory encoding and maintenance. Cogn. Brain Res. 20, 37–45.

Rowe, J.B., Passingham, R.E., 2001. Working memory for location and time: activity in prefrontal area 46 relates to selection rather than maintenance in memory NeuroImage 14, 77–86.

Rowe, J.B., Toni, I., Josephs, O., Frackowiak, R.S.J., Passingham, R.E., 2000. The prefrontal cortex: response selection or maintenance within working memory? Science 288, 1656–1660.

Rowe, J., Friston, K., Frackowiak, R., Passingham, R., 2002. Attention to action: specific modulation of corticocortical interactions in humans. NeuroImage 17, 988–998.

Rypma, B., Prabhakaran, V., Desmond, J.E., Glover, G.H., Gabrieli, J.D., 1999. Load-dependent roles of frontal brain regions in the maintenance of working memory. NeuroImage 9, 216–226.

Sakai, K., Passingham, R.E., 2003. Prefrontal interactions reflect future task operations. Nat. Neurosci. 6, 75–81.

Sala, J.B., Rama, P., Courtney, S.M., 2003. Functional topography of a distributed neural system for spatial and nonspatial information maintenance in working memory. Neuropsychologia 41, 341–356.

Sawaguchi, T., Yamane, I., 1999. Properties of delay-period neuronal activity in the monkey dorsolateral prefrontal cortex during a spatial delayed matching-to-sample task. J. Neurophysiol. 82, 2070–2080.

Schumacher, E.H., Elston, P.A., D'Esposito, M., 2003. Neural evidence for representation-specific response selection. J. Cogn. Neurosci. 15, 1111–1121.

Takeda, K., Funahashi, S., 2002. Prefrontal task-related activity representing visual cue location or saccade direction in spatial working memory tasks. J. Neurophysiol. 87, 567–588.

Todd, J.T., Marois, R., 2004. Capacity limit of visual short-term memory in human posterior parietal cortex. Nature 428, 751–754.

Wood, J.N., Grafman, J., 2003. Human prefrontal cortex: processing and representational perspectives. Nat. Rev., Neurosci. 4, 139–147.

Yajeya, J., Quintana, J., Fuster, J.M., 1988. Prefrontal representation of stimulus attributes during delay tasks: II. The role of behavioral significance. Brain Res. 474, 222–230.

Zarahn, E., Aguirre, G., Desposito, M., 1997a. A trial-based experimental design for fMRI. NeuroImage 6, 122–138.

Zarahn, E., Aguirre, G.K., Desposito, M., 1997b. Empirical analyses of BOLD fMRI statistics. NeuroImage 5, 179–197.

Zarahn, E., Aguirre, G.K., D'Esposito, M., 1999. Temporal isolation of the neural correlates of spatial mnemonic processing with fMRI. Cogn. Brain Res. 7, 1–7.