# Neural Correlates of Preparation for Action Selection as a Function of Specific Task Demands

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## Abstract

■ Our behavior is frequently guided by rules, or prescribed guides for action. The prefrontal cortex (PFC) has been implicated in the ability to retrieve and use rules in a conscious, effortful manner. Several functional magnetic resonance imaging (fMRI) studies have examined the role of the PFC in rule representation; however, the precise PFC subregions implicated in this function vary from study to study. This observation raises the question of whether there are distinct classes of rules that are represented differentially in the brain. To address this question, an fMRI study was conducted in which participants performed two tasks, each at two levels of difficulty, during acquisition of event-related fMRI data. The response competition task was based on the Stroop paradigm: Participants were cued to determine either the ink color or color name associated with a word stimulus. In contrast, the paired associates task evaluated participants' memory for either one or four previously memorized pairs of words. On each trial, an instructional cue appeared briefly on the screen, followed by an 8-sec delay and a probe period. The left ventrolateral PFC (VLPFC) and the left supplementary motor area (SMA)/pre-SMA were engaged during the delay period for all conditions, consistent with a general role in rule representation. In contrast, different parts of the dorsolateral PFC, the anterior PFC, and the right VLPFC were preferentially engaged by one or both of the more challenging rules, consistent with the idea that rules are represented by partially distinct brain structures according to their content. ■

## INTRODUCTION

Our actions are frequently guided by explicit rules for behavior, or ''prescribed guide[s] for conduct or action'' (Merriam–Webster Dictionary, 1974). Neuroscientific studies in humans and nonhuman primates implicate the prefrontal cortex (PFC) in the ability to learn and use rules to control behavior (for reviews, see Bunge et al., 2005; Bunge, 2004; Murray, Bussey, & Wise, 2000; Passingham, Toni, & Rushworth, 2000). The PFC represents goal-relevant information through its interactions with a number of other brain regions. The involvement of the PFC in rule use is particularly important when rules are not yet highly overlearned or automatic (e.g., Grol, de Lange, Verstraten, Passingham, & Toni, 2006), and when ad hoc rules must be formulated to govern behavior in an unfamiliar setting (Miller, 2000). Indeed, patients with damage to the PFC have particular difficulty planning and controlling their behavior when faced with novel challenges (Stuss & Alexander, 2000).

Lesion studies in nonhuman primates demonstrate that the ventrolateral PFC (VLPFC) plays a critical role in rule learning and rule representation. VLPFC lesions in monkeys severely impair learning on visuomotor conditional tasks, which require that they use one of several arbitrary stimulus–response (S–R) mappings to respond to a visual stimulus (Murray et al., 2000; Passingham et al., 2000). These lesions impair both the ability to use associations learned preoperatively and to rapidly learn new associations within a single session. The VLPFC receives its visual input from the inferotemporal cortex (Pandya & Yeterian, 1998), and therefore, disruption of the white matter tracts connecting the VLPFC and the ipsilateral temporal cortex also leads to impaired visuomotor learning (Bussey, Wise, & Murray, 2002; Parker & Gaffan, 1998). VLPFC lesions in monkeys lead to a deficit in learning a match-to-sample rule, indicating that the VLPFC is important for learning complex rules as well as simple associations (Bussey et al., 2002).

In contrast to VLPFC damage, dorsolateral PFC (DLPFC) damage causes little to no impairment on visuomotor conditional tasks in either humans or nonhuman primates (Murray et al., 2000), with the exception of the posterior DLPFC in humans (BA 8; Amiez, Kostopoulos, Champod, & Petrides, 2006; Petrides, 1997). However, neuroimaging studies in humans (Crone, Wendelken, Donohue, & Bunge, 2006; Bunge, Kahn, Wallis, Miller, & Wagner, 2003; MacDonald, Cohen, Stenger, & Carter, 2000) and electrophysiological recordings in nonhuman primates (Mansouri, Matsumoto, & Tanaka, 2006; Wallis, Anderson, & Miller, 2001; Asaad, Rainer, & Miller, 2000) implicate both the VLPFC and the mid-DLPFC (BA 9, 46) in rule representation.

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These apparent discrepancies with respect to the mid-DLPFC raise several possibilities: First, that the mid-DLPFC represents some types of rules but not others, and/or second, that this region is engaged during rule representation without being required for adequate task performance. One additional possibility is that the mid-DLPFC may be engaged during learning of a rule, but it may not be necessary after the rule is learned, as suggested from lesion studies (Stuss & Alexander, 2000; Petrides, 1985; Shallice, 1982) and neuroimaging data (Boettiger & D'Esposito, 2005).

In considering the types of rules that the DLPFC may represent, two possibilities are suggested by the extant literature. First, the DLPFC may be important for representing rules that require overriding a prepotent response tendency. Indeed, one study showed sustained mid-DLPFC (BA 9) activation while participants prepared to perform the Stroop task (MacDonald et al., 2000), and another showed that the DLPFC (but not the VLPFC) was more active when subjects were able to prepare to withhold a response on a go/no-go task than when they received no advanced warning (Hester et al., 2004). Instead or additionally, the DLPFC may not be engaged for low-level rules such as S–R associations, but may be recruited for more complex rules. Such a finding would be consistent with the idea that the DLPFC is recruited as needed to manage, monitor, or manipulate information kept active by the VLPFC (e.g., Owen et al., 1999).

The principal aim of the present study was to test the hypothesis that the VLPFC and the DLPFC contribute differentially to rule representation. Additionally, we examined brain activation in the anterior PFC (aPFC; BA 10) on the basis of prior work implicating this region in representation of rules and task-sets (see Bunge & Zelazo, 2006; Crone et al., 2006; Bunge et al., 2003; Sakai & Passingham, 2003).

More generally, the aim of the study was to investigate whether rules of different kinds are represented differentially in the brain. Prior neuroimaging research suggests that verbal working memory is a means by which we maintain relevant task rules in mind (e.g., Bunge et al., 2003). The question of interest in this study was whether a different set of regions would be involved when subjects are asked to prepare to implement an inhibitory rule with minimal working memory demands.

Electrophysiological research from Miller and colleagues has revealed that adjacent neurons in PFC can represent different rules (e.g., Wallis et al., 2001). Given the intermingling of such neurons, we were concerned that it might be impossible to detect any differences between rule types with functional magnetic resonance imaging (fMRI). As such, we included a manipulation of task difficulty for each of the two tasks (i.e., the inhibitory task and the noninhibitory task), so as to be able to determine whether different regions in the PFC were modulated by one or both manipulations.

To this end, participants performed two distinct tasks, each at two levels of difficulty, during acquisition of event-related fMRI data. In the response competition task—referred to below as the Stroop task, from which it was adapted—participants were cued to determine either the ink color or color name associated with a word stimulus. The ink condition was more challenging than the word condition because it involved overriding the automatic tendency to focus on the word's meaning. The paired associates task—referred to below as the memory task—tested participants' memory for pairs of color words (e.g., red–blue, yellow–gray). Participants had to retrieve four word pairs from long-term memory for each of two instructional cues (Set A or Set B), and had to retrieve one word pair for each of two additional cues (Set C or Set D). As such, Sets A/B and C/D corresponded to high and low memory load conditions, respectively. On each trial, an instructional cue appeared briefly on the screen, followed by an 8-sec delay and a probe period during which a response occurred.

Our analyses focused primarily on cue- and delay-period activation in the lateral PFC, as a function of task (Stroop vs. Memory task) and level of difficulty (referred to below as an effect of ''level'': Ink vs. Word and High load vs. Low load). It should be noted that ''difficulty'' is a general term used to refer to relative differences in task demands. In the Stroop task, the more difficult condition (Ink vs. Word) involves response competition, whereas in the memory task, the more difficult condition (High load vs. Low load) places greater demands on long-term memory retrieval and working memory maintenance.

## METHODS

### Subjects

Paid volunteers were recruited from the University of California at Davis and the surrounding local community. Sixteen healthy, right-handed adults were included in the study (11 women, 5 men; 18–34 years old, mean age = 22.8 years). Three additional subjects were excluded on the basis of excessive head motion (greater than 4 mm in any direction within a scan). Informed consent was obtained from all subjects, and all procedures were approved by the Internal Review Board at UC Davis.

### Experimental Conditions

For the memory task, participants were asked to memorize four different stimulus sets (Sets A–D) prior to the scan (Figure 1). Sets A and B each consisted of four word pairs (the high load condition), and Sets C and D each consisted of a single word pair (the low load condition). During scanning, participants were cued to recall a specific set of word pairs, and were asked whether two words were correctly paired together. None of the stimuli overlapped between sets, and the specific pairings were

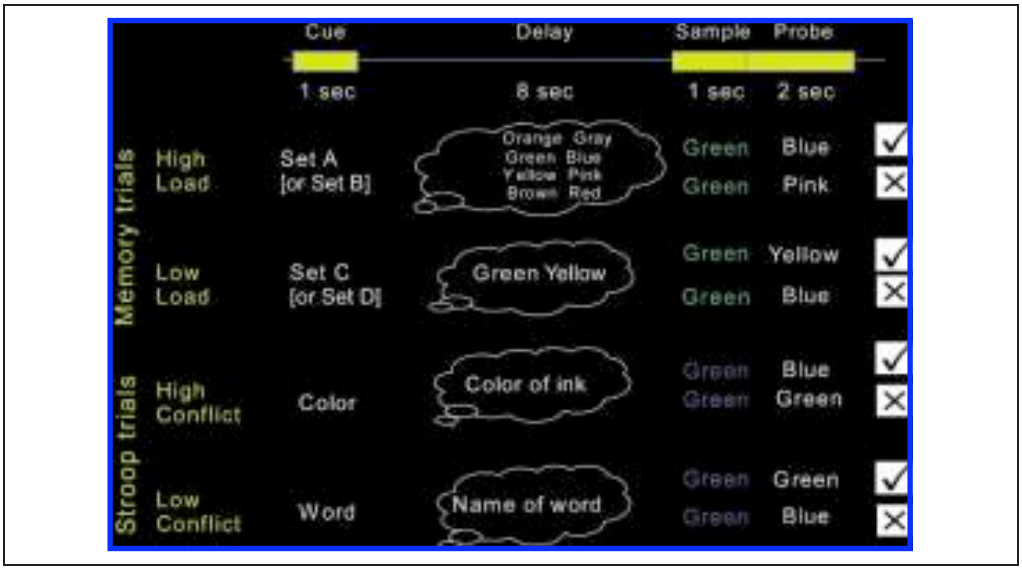


**Figure 1.** Task design. Participants were given a 1-sec cue followed by an 8-sec delay and a 3-sec sample–probe period at which time a response was required. Examples of each of the four different trial types are shown. The different trial types and stimuli were intermixed and jittered fixation occurred between each trial. The check marks denote a correct response and the *X*s denote an incorrect response on a given trial.

randomized and counterbalanced across subjects. Each of the words was printed in a congruent ink color (e.g., the word ''Green'' printed in green.)

For the Stroop task, each of eight different words representing color names was printed in an incongruent color, that is, an ink color that did not match the word. On some trials, participants were asked to identify the ink color of a word (ink condition). On other trials, participants were asked to identify the written word (word condition). These conditions are analogous to color Stroop and word Stroop conditions, respectively, although the task differs substantially from the classic Stroop paradigm. As with studies involving the Stroop paradigm, we expected that participants would experience more response competition on the color task than the word task, specifically during the probe period. Importantly, however, the study focused primarily on the cue and delay periods, during which the relevant task rules (e.g., to focus on the color word, or to focus on the color of the ink) are retrieved and maintained.

## Task

Each trial consisted of a cue period (1 sec), a delay period (8 sec), a sample stimulus (1 sec), and a probe stimulus (2 sec; Figure 1). The sample word stimulus was a color name printed in a congruent or incongruent ink color. The probe word stimulus was a color name printed in white. Participants were instructed to make a yes/no button press during the probe period. In the memory task, the cue corresponded to the name of a stimulus set (e.g., ''Set A''). Participants were instructed to retrieve the relevant set of word pairs and maintain them throughout the delay period. They then viewed a sample stimulus (e.g., the word ''Green,'' written in green ink) and then a probe stimulus (e.g., the word ''Blue'' written in white ink), and pressed one of two buttons to indicate whether or not the sample–probe pair corresponded to one of the pairings from the cued set that they had learned prior to scanning. In the Stroop paradigm, the cue was either the word ''Color'' or the word ''Word.'' When participants viewed the ''Color'' cue, they were instructed to prepare to identify the color of the ink of the ensuing word stimulus. When they viewed the ''Word'' cue, they were instructed to prepare to identify the color name represented by the word stimulus. They then viewed a sample stimulus (e.g., the word ''Green'' written in blue ink) and a probe stimulus (e.g., the word ''Green'' written in white ink), and determined whether the probe matched the stimulus in terms of ink color or in terms of word name, depending on the instruction.

Participants performed a total of 160 trials (40 trials/condition), distributed equally over four runs of approximately 10 minutes in length. The trial order within each scan was specified with an algorithm (optseq2) designed to maximize the separability of different conditions in a rapid event-related fMRI design (Dale, 1999). Periods of fixation lasting between 2 and 8 sec, jittered in increments of 2 sec, were interleaved with the experimental trials as determined by the optimization program.

## fMRI Data Acquisition

fMRI data were collected with a standard whole head coil on a 1.5-T MRI scanner (General Electric Signa Advantage, Medical Advances, Milwaukee, WI, USA) at the University of California at Davis Imaging Research Center. Visual stimuli were projected onto a screen that was viewed through a mirror mounted above the MRI head coil. Participants responded by pressing one of two buttons on a response box with fingers of their left hand, which corresponded to their nondominant hand

(given that all participants were right-handed). We asked participants to use their left hand to respond because the region in the premotor cortex controlling dominant (right) hand movements is located near the left posterior VLPFC, and we sought to facilitate the identification of this region of interest (ROI).

Functional data were acquired using a gradient-echo, echo-planar pulse sequence (TR = 2 sec, TE = 40 msec, 24 axial slices, 3.125 × 3.125 × 5 mm, 308 volumes per run). The first four volumes acquired were discarded to allow for T1-equilibration effects, yielding a total of 304 volumes per participant for fMRI data analysis. High-resolution T1-weighted coronal anatomical images were collected. Head motion was restricted using foam inserts that surrounded the head.

### fMRI Data Analysis

Data were preprocessed with SPM2 (Wellcome Department of Cognitive Neurology, London). Images were corrected for differences in timing of slice acquisition, and were submitted to rigid-body motion correction with sinc interpolation. Structural and functional volumes were spatially normalized to T1 and EPI templates, respectively. Templates are based on the MNI305 stereotaxic space (Cocosco, Kollokian, Kwan, & Evans, 1997), an approximation of Talairach space (Talairach & Tournoux, 1988). The normalization algorithm involved a 12-parameter affine transformation together with a nonlinear transformation involving cosine basis functions, and resampling of the volumes to $3 \times 3 \times 3$ $mm^3$ voxels. Functional volumes were spatially smoothed with an 8-mm FWHM isotropic Gaussian kernel.

Statistical analyses were performed on individual subjects' data with the general linear model implemented in SPM2. The fMRI time-series data were modeled as a series of events convolved with a canonical hemodynamic response function (HRF). The cue and sample–probe periods were modeled as impulses, whereas the delay period was modeled as a 3-sec epoch in the middle of the 8-sec delay period (i.e., onset times for cue, delay, and probe regressors were 0 sec, 4 sec, and 9 sec, respectively, where each trial began at 0 sec and ended at 12 sec.)

The purpose of modeling only the middle portion of the delay period was to minimize contamination of the delay-period regressor by cue-period or response-period activation (Yoon, Curtis, & D'Esposito, 2006; Postle, Zarahn, & D'Esposito, 2000; Zarahn, Aguirre, & D'Esposito, 1997). Although this approach did not allow for the perfect separation of cue, delay, and probe periods, it allowed us to test for differences in activation between conditions at various points in the trials. The resulting functions were used as covariates in a general linear model, along with a basis set of cosine functions that high-pass filtered the data, as well as a covariate for session effects. The least-squares parameter estimates of height of the best-fitting synthetic HRF for each condition were used in pairwise contrasts.

The resulting contrast images, computed on a subject-by-subject basis, were submitted to group analyses. Incorrect trials were modeled as a separate condition, although there were insufficient incorrect trials to warrant further analysis. At the group level, whole-brain contrasts between conditions were computed by performing one-tailed $t$ tests on the contrast images, treating subjects as a random effect. Results are reported for clusters larger than 10 contiguous voxels meeting an uncorrected $p$ value threshold of $p < .001$.

### Conjunction Analysis

Conjunction analyses were performed in SPM2 to identify regions that were active across several conditions. Two or more contrast images were selected from each participant (e.g., ink-fixation and high load-fixation during the cue period), and the minimum contrast value of each voxel across contrast images was calculated separately for each participant. For each target conjunction, the relevant single-subject conjunction images obtained from these calculations were submitted to a one-sample $t$ test to produce group-level maps of conjunctive activation. Significant conjunctive activity is reported for a threshold of $p < .005$, spanning at least 10 contiguous voxels; this threshold enabled us to identify regions in the lateral PFC that were not evident at $p < .001$. This conservative procedure reports activation that is present at the specified threshold in *all* of the involved contrasts. (This procedure to test the ''conjunction null'' hypothesis is described by Nichols, Brett, Andersson, Wager, & Poline, 2005.) ROIs were identified from these conjunction analyses to fully characterize the activation profile across all four conditions—that is, to test for differences between conditions that would not be evident based on the conjunction analysis alone.

### Region-of-interest Analyses

ROI analyses served to better characterize the patterns of activation across the four conditions and three task periods. The ROI analyses were performed on functionally defined regions with the MarsBar toolbox in SPM2 (Brett, Anton, Valabregue, & Poline, 2002; http://marsbar.sourceforge.net/). ROIs that spanned several functional brain regions were subdivided by sequentially masking the functional ROI with each of several MarsBar anatomical ROIs. Specific contrasts were used to identify regions with one of the following three patterns: (1) engaged generally across rule types; (2) engaged preferentially by the high or ink conditions; and (3) engaged preferentially by the harder condition for both tasks. Unless otherwise stated, the threshold used to extract the ROIs was $p < .001$.

Mean contrast values for each subject and condition were extracted for each ROI and submitted to analyses of variance (ANOVAs) and post hoc comparisons. Effects were considered significant at $p < .008$, so as to correct for multiple comparisons across six ROIs ($\alpha = .05$). Although the periods of greatest interest for the present investigation were the cue and delay periods, during which participants retrieved and maintained relevant task rules, we also examined activation levels in the probe period, during which participants implemented the rules.

## RESULTS

### Behavioral Data

Accuracy and response times (RTs) were analyzed behaviorally to test whether each task manipulation was effective. The RT measure consisted of median RTs for correctly performed trials, measured from the onset of the probe stimulus. Subjects were highly accurate across all conditions (Figure 2A). As predicted, there was a main effect of level of difficulty, such that subjects were significantly more accurate on the low load and word conditions than on the high load and ink conditions [$F(1, 15) = 29.4$, $p < .001$]. Post hoc $t$ tests revealed that subjects were significantly more accurate on the ink than the word condition [$t(15) = 2.68$, $p < .05$] and on the high load than low load condition [$t(15) = 3.47$, $p < .005$]. Additionally, accuracy was significantly higher for the memory task than for the Stroop task [$F(1, 15) = 17.4$, $p < .005$]. However, there was no significant interaction of Task × Level with respect to accuracy [$F(1, 15) < 1$], indicating that the two task manipulations affected accuracy similarly.

RTs did not significantly differ between the memory and Stroop tasks [Figure 2B; $F(1, 15) = 1.5$, $p > .05$]. There was an effect of level of difficulty on RTs [$F(1, 15) = 50.8$, $p < .001$], but this difference was driven by the slower responses in the high load condition relative to low load, as confirmed by the significant Task × Level interaction [$F(1, 15) = 38.5$, $p < .001$]. In summary, the Stroop manipulation was effective in terms of accuracy (lower accuracy for ink than word), but not in terms of RTs, whereas the memory manipulation was effective both in terms of accuracy and RTs (slower and less accurate performance on high load than low load trials).

Thus, these results suggest that the experiment successfully manipulated level of difficulty in both the Stroop task and the memory task. The differences in performance between tasks (in particular, marginally lower accuracy but faster RTs for high load than ink trials) are important to keep in mind when interpreting the fMRI results. However, it is also important to note that the fMRI analyses focused on the cue and delay periods, during which participants represented rules in a way that could not be used to plan a specific behavioral response. Thus, accuracy and RTs with respect to the probe stimuli could not directly account for differences in activation between tasks during the cue and delay periods. Indeed, if different brain regions were recruited during the retrieval and maintenance of Stroop and memory tasks, this would suggest that inhibitory and noninhibitory rules are differentially retrieved and maintained.

### Regions Identified from a General Contrast

ROIs in the left anterior VLPFC (BA 45) and left pre-SMA/SMA (BA 6; $y$ coordinates in MNI space ranged from −7 to +14) were identified from an unbiased whole-brain contrast (ink, word, high load, low load > fixation, across cue and delay periods; $p < .001$ uncorrected; Figure 3). A 2 × 2 ANOVA (Task × Level) was conducted for each region and task period to determine whether the left VLPFC (Figure 3A) and pre-SMA/SMA (Figure 3B) were sensitive to the type of task being performed

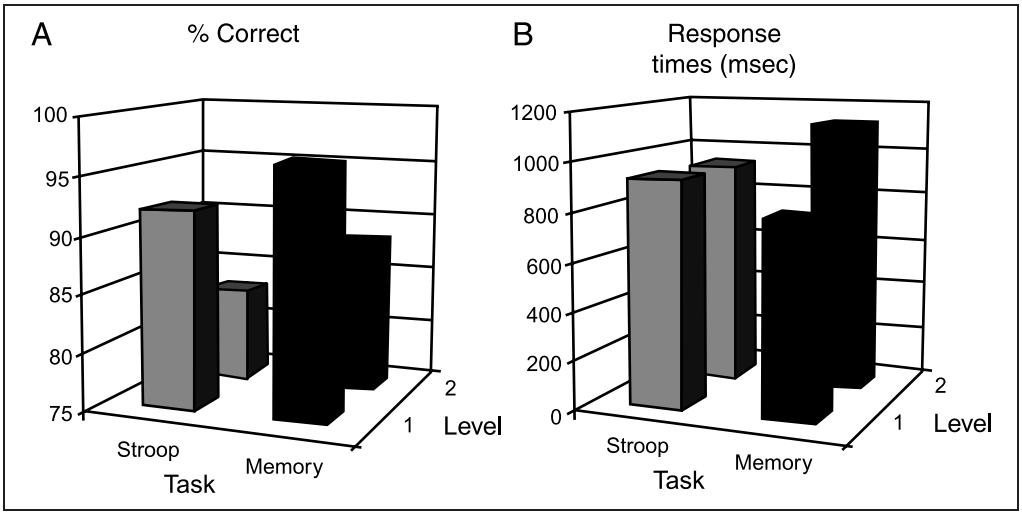


**Figure 2.** Behavioral data. For this and all subsequent figures, the high load and ink tasks are classified as Level 2, and the low load and word tasks are classified as Level 1. (A) Mean accuracy on the four different trial types. The within-subjects standard error term ($SE_w$) was 2.423. (B) Plot of median reaction times (msec) for correctly performed trials. Average RTs ± standard deviation for low load: 799.1 ± 159.5 msec; high load: 1125.7 ± 230.4 msec; word: 929.1 ± 243.3 msec; ink: 927.5 ± 186.6 msec. The $SE_w$ was 26.466.

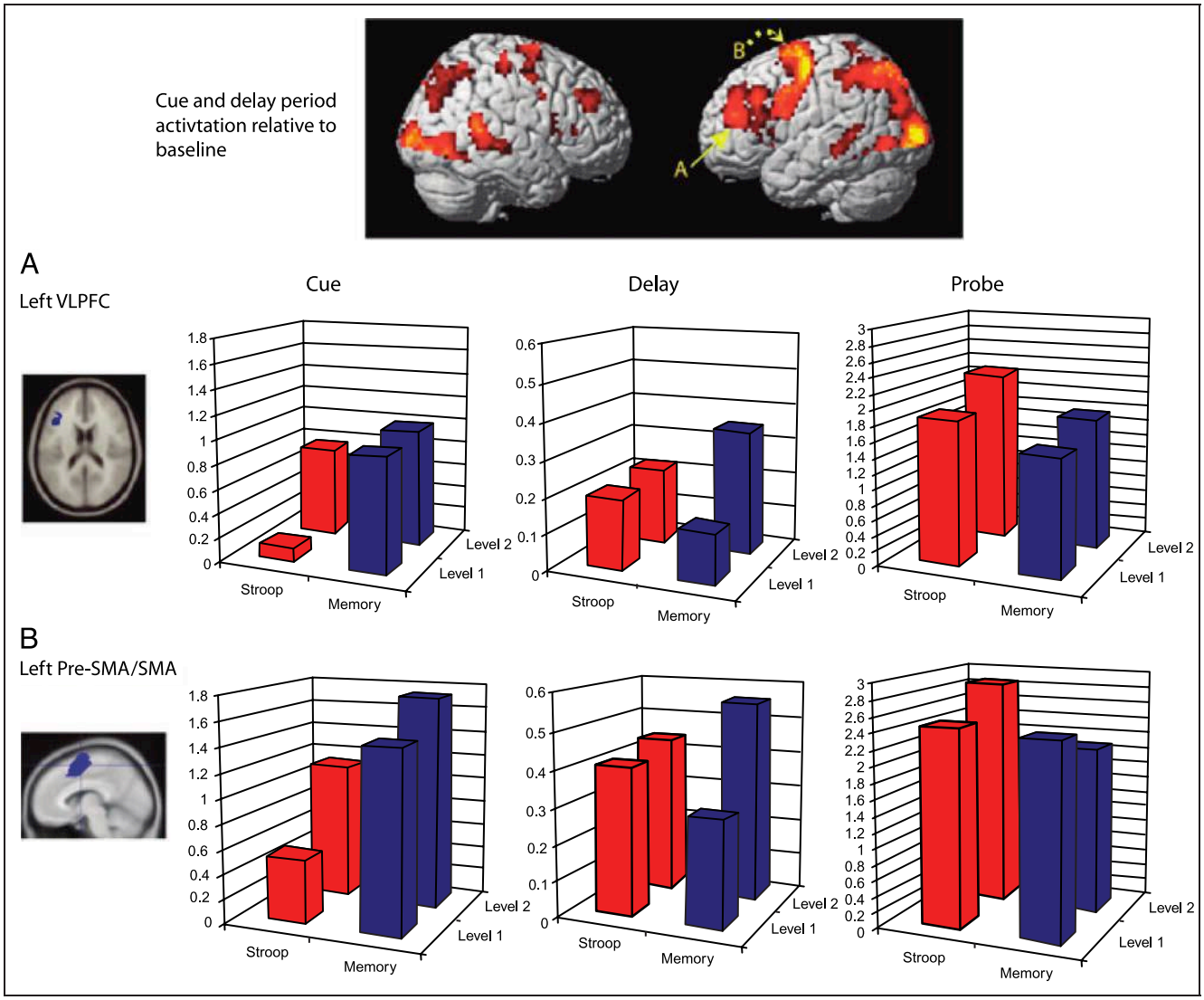


**Figure 3.** (Top) Whole-brain contrast of all correct conditions in the cue period and delay period versus fixation at $p < .001$. (A) ROI analysis for the cue, delay, and probe periods for the left (anterior) VLPFC (−40 26 18) obtained from all cue + delay correct fixation. The pattern of activation during the cue period showed a significant effect of condition [Memory > Stroop, $F(1,15) = 7.0$, $p < .05$] and within the Stroop condition, the ink condition was significantly greater than the word condition [$t(15) = 2.262$, $p < .05$]. Delay period activity showed the greatest engagement for the high load condition. The within-subjects standard error ($SE_w$) was 0.147, 0.041, and 0.137 for the cue, delay, and probe periods, respectively. (B) ROI analysis for the cue, delay, and probe periods for the left pre-SMA/SMA (−6 6 63) obtained from all cue + delay correct fixation. The vertical cross-hair in the image of this region denotes $Y = 0$. This region did not significantly differ from the left VLPFC during the cue and delay periods. The $SE_w$ was 0.159, 0.052, and 0.127 for the cue, delay, and probe periods, respectively. Note the difference in scale for the cue, delay, and probe periods as activation was of different magnitudes across the different phases of the trial.

(Stroop vs. Memory), and/or the level of difficulty of one or both tasks (Ink vs. Word, and/or High vs. Low memory load). A 2 × 2 × 2 ANOVA (ROI × Task × Level) run separately for the cue and delay periods revealed no differences in the pattern of activation between the two ROIs (all $p$ values > .10) for interactions involving ROI); therefore, we report the results for both regions in parallel below.

During the cue period, the left VLPFC and pre-SMA/SMA showed a marginally significant (VLPFC) and highly significant (pre-SMA/SMA) effect of task, with greater activation for the memory task than the Stroop task. Further, these regions were differentially engaged by the two Stroop conditions, exhibiting increased activity for the ink condition relative to the word condition (Figure 3A and B, Table 1). During the delay period, by contrast, these regions were engaged more strongly for the high load condition than the low load condition (Figure 3A and B). During the probe period, the left VLPFC was significantly more engaged for the ink condition than the word and low load conditions, with a trend toward greater activation for ink than high ($p = .04$, uncorrected for multiple comparisons). The pre-SMA/SMA was more strongly engaged by the ink condition than all other conditions. In summary, both the left VLPFC and the pre-SMA/SMA were modulated by rule

**Table 1.** Significant Activity for Six Frontal ROIs as a Function of Task, Level, and Period

| *Region* | *x* | *y* | *z* | *Task × Level × Period* | *p Values* | *Period* | *Task × Level* | *p Values* | *Activation Pattern* |
|---|---|---|---|---|---|---|---|---|---|
| *From All Cue Delay—Fixation* | | | | | | | | | |
| L VLPFC | −41 | 22 | 18 | **Task** | | **Cue** | *Task* | † | **Low, High > Word** |
| | | | | **Level** | * | | *Level* | | |
| | | | | **Period** | *** | | *Task × Level* | | |
| | | | | **Task × Level** | | **Delay** | *Task* | | |
| | | | | **Task × Period** | *** | | *Level* | * | **High > Low** |
| | | | | **Level × Period** | | | *Task × Level* | | |
| | | | | **Task × Level × Period** | | **Probe** | *Task* | ** | **Ink > Word, Low** |
| | | | | | | | *Level* | | |
| | | | | | | | *Task × Level* | | |
| L pre-SMA and SMA | −6 | 6 | 63 | **Task** | | **Cue** | *Task* | ** | **Low, High > Word** |
| | | | | **Level** | | | *Level* | | |
| | | | | **Period** | *** | | *Task × Level* | | |
| | | | | **Task × Level** | | **Delay** | *Task* | | |
| | | | | **Task × Period** | ** | | *Level* | * | **High > Low** |
| | | | | **Level × Period** | | | *Task × Level* | | |
| | | | | **Task × Level × Period** | † | **Probe** | *Task* | † | **Ink > Word, High** |
| | | | | | | | *Level* | | |
| | | | | | | | *Task × Level* | † | |
| *From All Cue Delay High Load—Ink, Word, Low Load* | | | | | | | | | |
| L ant PFC | −34 | 54 | 5 | **Task** | | **Cue** | *Task* | | |
| | | | | **Level** | ** | | *Level* | † | **High > Word** |
| | | | | **Period** | *** | | *Task × Level* | | |
| | | | | **Task × Level** | | **Delay** | *Task* | | |
| | | | | **Task × Period** | ** | | *Level* | ** | **High > Ink, Word, Low** |
| | | | | **Level × Period** | | | *Task × Level* | * | |
| | | | | **Task × Level × Period** | | **Probe** | *Task* | | |
| | | | | | | | *Level* | | |
| | | | | | | | *Task × Level* | | |
| R DLPFC | 34 | 26 | 35 | **Task** | | **Cue** | *Task* | | |
| | | | | **Level** | * | | *Level* | * | **High > Word, Low** |
| | | | | **Period** | *** | | *Task × Level* | | |
| | | | | **Task × Level** | | **Delay** | *Task* | | |
| | | | | **Task × Period** | ** | | *Level* | * | **High > Low** |
| | | | | **Level × Period** | | | *Task × Level* | * | |
| | | | | **Task × Level × Period** | ** | **Probe** | *Task* | | |
| | | | | | | | *Level* | | |
| | | | | | | | *Task Level* | | |

**Table 1.** (*continued*)

| *Region* | *x* | *y* | *z* | *Task × Level × Period* | *p Values* | *Period* | *Task × Level* | *p Values* | *Activation Pattern* |
|---|---|---|---|---|---|---|---|---|---|
| *From All Cue Delay Ink—High Load, Word, Low Load* | | | | | | | | | |
| R VLPFC | 52 | 35 | 7 | **Task** | | **Cue** | *Task* | | |
| | | | | **Level** | | | *Level* | | |
| | | | | **Period** | *** | | *Task × Level* | | |
| | | | | **Task × Level** | | **Delay** | *Task* | ** | **Ink > High**; Word > High |
| | | | | **Task × Period** | | | *Level* | | |
| | | | | **Level × Period** | | | *Task × Level* | | |
| | | | | **Task × Level × Period** | | **Probe** | *Task* | | |
| | | | | | | | *Level* | | |
| | | | | | | | *Task × Level* | | |
| R DLPFC | 24 | 48 | 32 | **Task** | ** | **Cue** | *Task* | † | **Ink > Low**; Ink > High |
| | | | | **Level** | | | *Level* | | |
| | | | | **Period** | ** | | *Task × Level* | | |
| | | | | **Task × Level** | ** | **Delay** | *Task* | *** | **Ink, Word > High**; Ink > Low |
| | | | | **Task × Period** | | | *Level* | | |
| | | | | **Level × Period** | | | *Task × Level* | | |
| | | | | **Task × Level × Period** | ** | **Probe** | *Task* | | |
| | | | | | | | *Level* | | |
| | | | | | | | *Task × Level* | ** | **Ink, Low > High** |

Bold font indicates statistics that survived correction for multiple comparisons ($p < .008$ for each of six regions of interest; $\alpha = .05$).

*$p < .01$.

**$p < .005$.

***$p < .001$.

†$p = .01$.

type, but the patterns differed across the three phases of the trial. No significant differences were observed during any period of the task (all $p > .1$) between the left VLPFC and a nearby region in the right VLPFC obtained from the same contrast (MNI coordinates for the left and right VLPFC: −41, 22, 18; 39, 27, 15). These analyses extend prior findings implicating the VLPFC and the pre-SMA in rule representation by showing that these regions represent several types of rules. Although these ROIs, and the others described below, were engaged during the cue and delay periods, it bears mention that they were most strongly engaged during the probe period (i.e., at the time of rule implementation).

## ROIs Identified as Being Preferentially Engaged by One Rule Type

To identify brain regions specifically engaged by either inhibitory or noninhibitory rules, we additionally obtained ROIs from a contrast identifying regions that were most active for the ink condition during the cue and/or delay periods, and from a similar contrast focusing on regions most strongly engaged by the high load condition (Figure 4; Table 1). Unlike the left VLPFC, these ROIs could not be identified from a general contrast collapsing across conditions because they were not consistently engaged across the different rule types. The contrasts used were: Ink > Other conditions and High load > Other conditions ($p < .005$, uncorrected). Notably, this threshold was more liberal than that used to identify the left VLPFC ROI, as activity for these contrasts was neither as extensive nor as robust as it was for the left VLPFC.

These specific contrasts yielded ROIs in several lateral PFC subregions. For the contrast of High load > Other conditions (Figure 4), ROIs were defined in the right DLPFC (middle frontal gyrus, BA 9) and the left aPFC, (BA 10, Figure 4A). For the contrast of Ink > Other conditions (Figure 4), ROIs were defined in a region of the right DLPFC (superior frontal gyrus, BA 9) and in the right VLPFC (BA 45, Figure 4B). A 2 × 2 (Task × Level) ANOVA was conducted on each of the identified regions

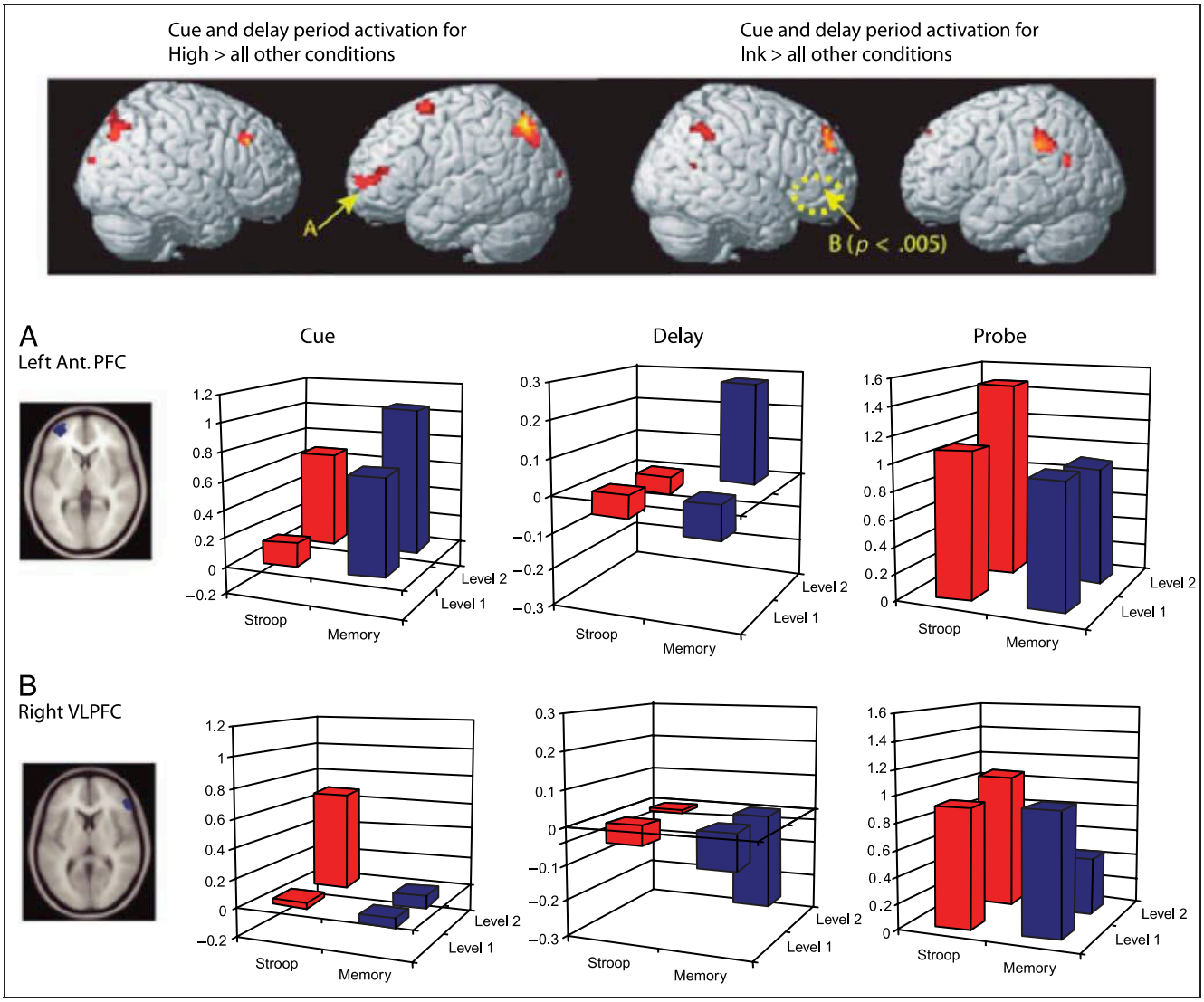


**Figure 4.** (Top, left) Whole-brain contrast of Cue + Delay High > All other conditions at $p < .001$. (Top, right) Whole-brain contrast of Cue + Delay High > All other conditions at $p < .001$. (A) ROI analysis for cue, delay and probe periods for the left aPFC obtained from Cue + Delay High load > Other conditions (BA 10; −34, 54, 5) at $p < .005$. $SE_w = 0.169$, 0.057, and 0.188 for the cue, delay, and probe periods, respectively. (B) The right VLPFC (52, 35, 7) obtained from Cue + Delay Ink > Other conditions. $SE_w = 0.187$, 0.045, and 0.167 for the cue, delay, and probe periods, respectively. These regions were engaged by different conditions through different phases of the trial. Note the difference in scale for the cue, delay, and probe periods.

separately for the cue, delay, and probe periods. Additional brain regions identified from these whole-brain contrasts as being modulated by rule type are reported in the Supplementary Table. Additional tables of activation are available upon request.

### *High > Other Conditions*

The region in the right DLPFC (BA 9) obtained from the contrast of High load > Other conditions was found to be differentially engaged across all three periods of the trial (Table 1). During the cue period, this region was marginally sensitive to the level of task difficulty (i.e., Ink > Word as well as High load > Low load; $p = .009$), but not to the type of task. During the delay period, the right DLPFC was engaged only by the high load. For the probe period, activation on the ink condition was numerically stronger than on the other conditions, but no significant effects were observed. In summary, the right DLPFC was engaged by the more difficult conditions during the cue and delay periods, without a clear effect of rule type.

The ROI in the left aPFC (Figure 4A), identified from the same contrast as the region in the right DLPFC, showed similar effects to the right DLPFC (Table 1). However, the left aPFC showed a more dramatic difference than the right DLPFC in delay-period activation between high load and the other conditions [ROI × Task: $F(1, 15) = 6.71$, $p < .05$; ROI × Level: $F(1, 15) = 6.00$, $p < .05$; ROI × Task × Level: $F(1, 15) = 5.70$, $p < .05$].

*Ink > Other Conditions*

The ROI in the right VLPFC obtained from cue and delay Ink > All conditions was analyzed across the different phases of the trial (Figure 4B). This region was specifically engaged by ink during the cue period (see Table 1). In contrast, it was deactivated relative to fixation for all conditions during the delay period, although less so for the Stroop task than for the memory task. During the probe period, there were no significant effects of task or level. A similar pattern of activation was observed in the right anterior DLPFC (BA 9; coordinates: 24, 48, 32), an ROI identified from the same contrast (Table 1). Indeed, an ANOVA comparing the right VLPFC ROI with this right DLPFC ROI revealed no significant differences between these regions.

### Identifying Common Areas: Conjunction Analysis

To identify regions that were engaged across two different types of rules, we performed a conjunction analysis of the two conditions that placed the greatest demands on rule representation: ink and high load. A conjunction analysis of Ink versus Fixation and High load versus Fixation was performed separately for the cue (Figure 5) and delay periods, again using a threshold of $p < .005$, uncorrected. During the cue period, a number of areas were active during both conditions, including the bilateral anterior DLPFC (middle frontal gyrus; BA 9/10), the SMA and pre-SMA (BA 6), the left dorsal premotor cortex (BA 6), the bilateral superior and inferior parietal lobules (BA 7, 40), the basal ganglia, and the right superior and middle temporal gyrus (BA 22). At a more liberal threshold, the left anterior VLPFC was also observed in this cue-period contrast. During the delay period, common activation was observed in the left anterior VLPFC (frontal operculum and adjacent anterior insula, BA 45/13), the posterior VLPFC and ventral premotor cortex (BA 44/6), and the bilateral pre-SMA/SMA (BA 6).

### Anterior DLPFC ROIs Identified from a Conjunction of the More Challenging Conditions

The regions in the anterior DLPFC (middle frontal gyrus; BA 9/10) identified from the conjunction of Ink versus Fixation and High load versus Fixation were submitted to ROI analyses (Figure 5A and B). The effects of level were more robust in the right anterior DLPFC [cue: $F(1, 15) = 10.95, p < .005$; delay: $F(1, 15) = 8.99, p < .01$] than in the left anterior DLPFC [cue: $F(1, 15) = 5.12, p < .05$; delay: $F(1, 15) = 7.51, p < .05$], although the patterns in the two regions did not differ statistically from one another. No effect of task (Stroop vs. Memory task) was observed in these regions during either the cue or delay period. Thus, the left and right anterior DLPFC were engaged preferentially for the more demanding tasks, regardless of the specific task demands.

## DISCUSSION

The goal of this study was to determine whether different types of task rules are differentially represented in the brain, and in particular, in the PFC. To this end, fMRI activation was measured while participants performed an inhibitory task rule (Stroop ink condition), a non-inhibitory rule (high load memory condition), and less demanding versions of each of these tasks (Stroop word and low load memory conditions, respectively). By isolating cue-period and probe-period activity, and measuring delay-related activation at the mid-point of the delay period, it was possible to identify regions engaged during rule retrieval and implementation, as well as maintenance.

As predicted, the left VLPFC was engaged during the maintenance of all four rule types. Although it was most strongly engaged by the high load condition, for which the greatest amount of information had to be maintained, it did not show an overall effect of task. This result points to the general role of the left VLPFC in rule maintenance. In effect, this region has been heavily implicated in verbal working memory, and it is likely that one tends to rely on verbal working memory to maintain task rules that are to be implemented imminently (Bunge et al., 2003). The fact that the VLPFC is strongly implicated in rule representation in nonhuman primates (Murray et al., 2000; Passingham et al., 2000), who lack verbal abilities, suggests that this region plays a fundamental role in preparing for upcoming tasks. Our prior work suggests that left VLPFC activation is largely prospective when it is necessary to maintain task rules over a delay period, that is, it is sensitive to the nature of the upcoming task rather than to the characteristics of the stimulus that cued the task (Bunge et al., 2003).

Additionally, the left pre-SMA/SMA was engaged during the maintenance of all four rule types. Indeed, this was the only region identified in the whole-brain conjunction analysis of High load > Low load and Ink > Word during the delay period. Consistent with these findings, the pre-SMA/SMA has been previously implicated in general task maintenance (e.g., Dosenbach et al., 2006) and in the anticipation of upcoming events (e.g., Ruff & Driver, 2006), potentially reflecting prospective activity (Shima & Tanji 2000), like the left VLPFC.

### Regions Engaged by the More Demanding Rules

During both the cue and delay periods, regions in the bilateral anterior DLPFC (middle frontal gyrus; BA 9/10) identified from a conjunction analysis were engaged more strongly by ink and high load than by word and low load. These data are consistent with MacDonald et al. (2000), who showed stronger engagement of left BA 9 during preparation to perform the Stroop ink

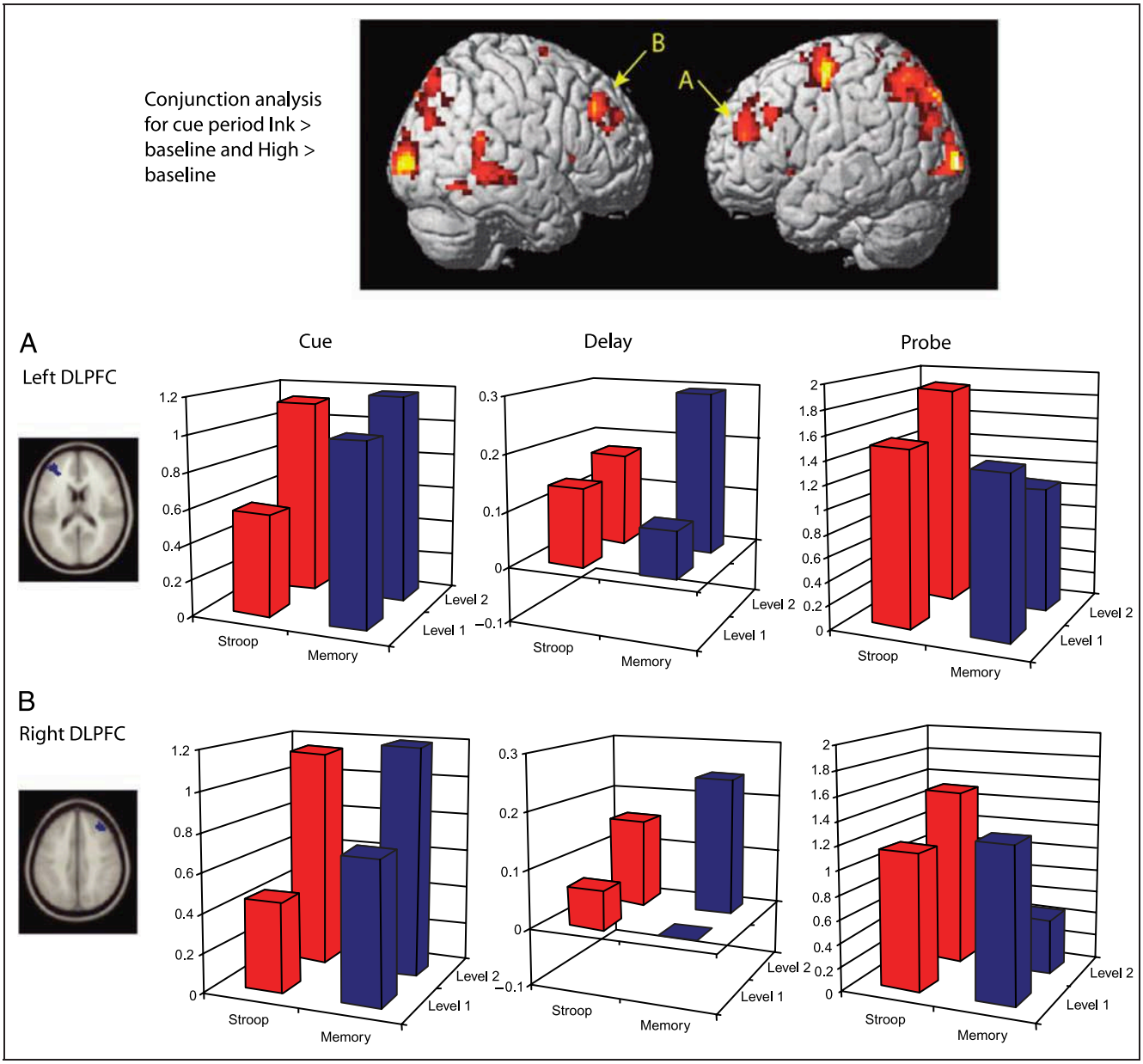


**Figure 5.** Conjunction analyses. (Top) Common activations during the cue period for Ink > Fixation and High load > Fixation. (A) ROI analysis for cue, delay, and probe periods for the left DLPFC (BA 9/10 coordinates: −39, 24, 33) obtained from the conjunction analysis shown in A. $SE_w$ = 0.125, 0.044, and 0.117 for the cue, delay, and probe periods, respectively. (B) ROI analysis for cue, delay, and probe periods for the right DLPFC obtained from conjunction analysis shown in A (BA 9/10 coordinates: 33, 48, 33). $SE_w$ = 0.02, 0.04, and 0.14 for the cue, delay, and probe periods, respectively. Note the difference in scale for the cue, delay, and probe periods.

condition than the word condition. These and other data suggest that the DLPFC assists in preparing to carry out attention-demanding cognitive tasks.

### Regions Engaged by Response Competition

During the cue period, both the right VLPFC and the right DLPFC (in the superior frontal gyrus) were engaged exclusively by the ink condition. This activity is likely to be related to retrieval of the inhibitory rule rather than task preparation per se, as the activation did not persist into the delay period. There is strong evidence that the right VLPFC plays a critical role in response inhibition (Aron, Robbins, & Poldrack, 2004); the present finding indicates that this region is involved not only in inhibiting responses but also in formulating a plan to inhibit a response. The preferential response to the ink condition in the right VLPFC and DLPFC did not persist into the delay period, which may detract from the interpretation that these regions are involved in preparing to inhibit a prepotent response tendency. However, as noted in the Introduction, adjacent neurons in the PFC often represent different rules during performance of a task. It is possible that during the presentation of the cue, we were

able to detect differences in activation between the ink rule type and others with fMRI because of increased demands placed on the neurons representing the ink rule at that time. However, by the delay period, the demands placed on these neurons may have been no greater than those placed on the neurons representing other rules. Further electrophysiological research is needed to examine the neuronal representation of inhibitory task rules.

### Regions Engaged by the Memory Task

These data indicate that the right DLPFC supports the maintenance of a large, structured set of information (the high load condition) over a delay, consistent with prior research on structured working memory representations (Wendelken, Bunge, & Carter, 2005; Bor, Duncan, Wiseman, & Owen, 2003). Right DLPFC activation is also observed during the maintenance of large sets of information (Volle et al., 2005; Wendelken et al., 2005; Rypma, Prabhakaran, Desmond, Glover, & Gabrieli, 1999), and it is thought to be related to the need to organize or chunk information in such a way that it can be remembered.

Like the right DLPFC, the left aPFC (BA 10) was strongly engaged by the high load condition during the delay period—again, consistent with our study of structured working memory representations (Wendelken et al., 2005). In this prior study, we had found that this region was insensitive to a load manipulation, but sensitive to whether the items to be remembered were organized in a specific way. These results suggest that the aPFC maintains structured mental representations—a function that can be called upon to represent a set of task contingencies (Sakai & Passingham, 2003, 2006; Crone et al., 2006; Boettiger & D'Esposito, 2005; Bunge et al., 2003).

One potential caveat in the analysis and interpretation of the data is that it is a challenge to isolate activation associated with specific phases of a trial. In an attempt to measure relatively uncontaminated cue and delay period activation, we modeled the delay period onset at 4 sec following the cue onset, and the probe period onset at 5 sec following the onset of the delay period regressor. In a previous study, we had used variable-duration intertrial interval to identify regions involved in rule retrieval versus maintenance (Bunge et al., 2003). In the present study, our primary goal was to dissociate distinct rule types, rather than to cleanly distinguish between cue-period and delay-period activation. In future studies, it would be interesting to examine the time courses of the representation of these different types of rules.

### Conclusion

The main goals of the present study were to determine whether the VLPFC and the DLPFC contribute differentially to rule representation, and—more generally—whether different types of task rules are represented differentially in the brain. As predicted, we found that the left VLPFC was generally involved in rule maintenance across all four conditions, in a manner that was load-dependent. In contrast, the right VLPFC, bilateral DLPFC, and left aPFC were preferentially engaged by one or more specific rule types. These results suggest that rules of different types have partially overlapping neural underpinnings. For the purposes of this investigation, we have examined only inhibitory and noninhibitory rules, in the context of a visual task with closely matched conditions. Future studies should consider whether the brain honors other distinctions between rule types, and whether the roles of the various lateral PFC subregions in rule representation generalize to other stimulus modalities. In this study, we have focused primarily on cue- and delay-period activation associated with task preparation. However, prefrontal activation at the probe was as much as 10-fold higher than at the cue and delay periods. This observation underscores the point that the PFC is geared toward the control of action (Fuster, 2007).


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