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Effects on Executive Function Following Damage to the Prefrontal Cortex in the Rhesus Monkey (*Macaca mulatta*)

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Executive function is a term used to describe the cognitive processes subserved by the prefrontal cortex (PFC). An extensive body of work has characterized the effects of damage to the PFC in nonhuman primates, but it has focused primarily on the capacity of recognition and working memory. One limitation in studies of the functional parcellation of the PFC has been the absence of tests that assess executive function or its functional components. The current study used an adaptation of the Wisconsin Card Sorting Test, a classic test of frontal lobe and executive function in humans, to assess the effects of bilateral lesions in the dorsolateral PFC on executive function in the rhesus monkey (*Macaca mulatta*). The authors used the category set-shifting task, which requires the monkey to establish a pattern of responding to a specific category (color or shape) based on reward contingency, maintain that pattern of responding, and then shift to responding to a different category when the reward contingency changes. Rhesus monkeys with lesions of the dorsolateral PFC were impaired in abstraction, establishing a response pattern to a specific category and maintaining and shifting that response pattern on the category set-shifting task.

Keywords: executive function, Wisconsin Card Sorting Test, nonhuman primate, cognition

In clinical settings, damage to the human prefrontal cortex (PFC) produces a variety of functional impairments, but impaired executive function is among the most marked. Although views on the exact components of executive function vary, it is generally agreed that it includes the abilities of abstraction, shifting of response patterns, planning, working memory, and response suppression (Trans-NIH Executive Function Workshop, 2003). Although a variety of neuropsychological tests are sensitive to damage in the PFC, the most commonly used test is the Wisconsin Card Sorting Test (WCST). The WCST was developed in 1948 by Berg and has been widely employed as a test of PFC function in clinical and research settings ever since. It is generally agreed that the WCST assesses abstract reasoning, cognitive flexibility, and the ability to maintain and shift cognitive set according to changing reward contingencies (Damasio & Anderson, 1993; Heaton, Chelune, Talley, Kay, & Curtiss, 1993; Nagahama et al., 1996).

Performance on the WCST by patients with damage limited to the PFC is typically characterized by a high incidence of perseverative errors, an inability to shift set once established, and an inability to use feedback to modify response patterns (Heaton, 1981; Milner, 1963, 1995). Milner (1963) reported that the ability to shift from one mode of response to another is more often impaired by frontal lobe damage than as a consequence of temporal or occipital damage and appears to result from an inability to derive and effectively use feedback to modify response patterns. In addition, most patients with frontal lobe damage can verbalize the correct response on the WCST but are unable to use this information to produce a correct response (Milner, 1963, 1995).

The use of nonhuman primates in cortical ablation studies, dating back to the work of Jacobsen (1935, 1936), has provided insight into the functional role of the various subdivisions of the PFC in cognition (Bachevalier & Mishkin, 1986; Butters & Panyda, 1969; Dias, Robbins, & Roberts, 1996; Gaffan & Harrison, 1989; Mishkin & Manning, 1978; Oscar-Berman, 1978; Passingham, 1985; Pohl, 1973; Woods & Knight, 1986). In one study, Passingham (1972) demonstrated that animals with lesions in the frontal cortex were impaired on nonreversal shifts. Specifically, he found that animals with damage limited to orbital regions of the PFC made more perseverative as well as nonperseverative errors in reaching criterion on a test of nonreversal shifts than animals with lesions of the lateral frontal regions. However, the animals with lesions limited to lateral frontal regions were impaired on shifts to position rather than color or size, a finding that could be attributed to a spatial impairment rather than impairment in attention or shifting abilities.

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Similarly, it has been demonstrated that lesions of the dorsolateral PFC (DLPFC) can result in deficits in visuospatial function, inability to shift set, perseverative responding, and impaired performance on conditional discrimination tasks (Butter, Mishkin, & Rosvold, 1963; Butters & Pandya, 1969; Dias et al., 1996; Goldman & Rosvold, 1970; Jacobson, Butters, & Tovsky, 1978). Later studies revealed that damage to sulcus principalis of the DLPFC results in impairment in response modification, spatial learning, and spatial memory (Butters & Pandya, 1969; Gaffan & Harrison, 1989; Mishkin & Manning, 1978; Smith & Milner, 1984). Finally, it has also been shown that lesions in ventromedial PFC result in impaired performance on visual recognition tasks (Bachevalier & Mishkin, 1986; Kowalska, Bachevalier, & Mishkin, 1991). More recently, activation studies of the DLPFC and ventrolateral PFC in humans and monkeys using fMRI and single unit recordings, respectively (Asaad et al., 2000; Lie, Specht, Marshall, & Fink, 2006; Monchi, Petrides, Petre, Worsley, & Dagher, 2001; Nakahara, Hayashi, Konishi, & Miyashita, 2002; Wallis, Anderson, & Miller, 2001; Zanolie et al., 2008), suggest the possibility that these regions may play a role in various aspects of executive function (i.e., cognitive flexibility, set shifting, and attentional control). Despite this extensive body of work, more direct evidence of whether the DLPFC plays a pivotal role in executive function is needed.

In the current study, we addressed this question by employing a cognitive task with the nonhuman primate that is an adaptation of the WCST (Moore, Killany, Herndon, Rosene, & Moss, 2005). This task, the category set-shifting task (CSST),[1] uses the same basic principles, learning criteria, definitions, and a similar subset of the stimuli as those used for the WCST. The CSST, as with the WCST, can be considered a "partial change paradigm" that requires the animal to develop and maintain an appropriate response strategy across changing stimulus characteristics. In previous publications describing the CSST, we used the term *concept* to describe the conditions used in this test (color and shape). Further consideration of the terminology used with the WCST together with that used in other tasks requiring shifting modes of responding, and with our continued experience using this task, we have more operationally defined the shifting component in this test from *concept* to *category*. The task still uses two stimulus categories (color and shape) and four conditions based on stimulus characteristics (red, triangle, blue, and star). Specifically, the CSST requires the animal to establish a pattern of responding to a specific stimulus category (color or shape) based on a reward contingency, maintain responding to that stimulus category for a period of time, and then shift to responding to a different stimulus category when the reward contingency changes. Although it is difficult to interpret the precise quality or characteristic of the stimuli (abstract or sensory) or set of stimuli that monkeys respond to in this task, it is clear that they learn to respond to a set of stimuli, maintain that response set, and then, when conditions change, shift that response pattern. Furthermore, this task represents an increased level of difficulty from traditional delayed response and discrimination reversal tests used to assess frontal lobe function in monkeys in that it requires an extradimensional shift (change in target stimulus from one dimension to another; i.e., color to shape) rather than an intradimensional shift (change in target stimulus within the same dimension; i.e., color to color, location to location, object to object, etc.). Hence, the CSST provides a sensitive assessment of various components of executive function; in the present study, it was used as such to assess the effects of bilateral lesions in areas 8, 9, 10, and 46 of the DLFPC in the rhesus monkey.

Method

Subjects

The subjects were nine, behaviorally naive, young adult, male rhesus monkeys (*Macaca mulatta*) weighing between 6.0 kg and 14.5 kg at the beginning of this study. All of the monkeys were obtained from a national primate research facility or breeding facility and had known birth dates and complete health records. Before entering the study, monkeys received medical examinations that included serum chemistry, hematology, urine analysis, and fecal analysis. All monkeys were individually housed in colony rooms where they were in constant auditory and visual range of other monkeys in the Laboratory Animal Science Center of Boston University School of Medicine. This facility is fully AAALAC approved and animal maintenance and research were conducted in accordance with the guidelines of the National Institutes of Health Committee on Laboratory Animal Resources and according to procedures approved by the Institutional Animal Use and Care Committee of the Boston University Medical Campus. Diet consisted of Purina Monkey Chow (Purina Mills Inc., St. Louis, MO) supplemented by fruit, with feeding taking place once per day, immediately following behavioral testing. Water was available continuously. The monkeys were housed under a 12-hr light–dark cycle with cycle changes occurring in a graded fashion over the course of an hour. Following a quarantine period and acclimation to the colony room, four animals (DLPFC 1–4) underwent surgery for bilateral removal of the DLPFC, and the remaining five served as unoperated controls (HM 034, 038, 043, 044, and 059). The controls monkeys, part of another ongoing study in our laboratory, were tested concurrently with the lesion animals on the identical test battery. The behavioral data for the control animals were previously published in Moore et al. (2002). At the beginning of the study, all monkeys underwent MRI to provide a baseline scan to ensure that there was no occult neurological damage and to provide a baseline for lesion reconstruction.

Surgical Procedures

Animals were sedated with ketamine hydrochloride (10 mg/kg), and cuff blood pressures and electrocardiograms were taken. An intravenous line was established via the saphenous vein and slow infusion of lactated Ringers solution was begun. A surgical level of anesthesia was induced with intravenous sodium pentobarbital (approximately 25 mg/kg) in titrated doses to effect. The animals were intubated; heart rate, respiration rate, temperature, and muscle tonus were continuously monitored throughout surgery to maintain a deep surgical level of anesthesia. Animals were mon-

[1] The CSST was referred to as the conceptual set-shifting task in previously published studies. On further consideration of the nature of the stimuli and processes employed in the completion of this task, we decided that the term *category* was more appropriate to describe the conditions of color and shape in this task.

itored throughout surgery and body temperature was maintained with a heating pad.

After opening the skin and retracting fascia and muscle, a bone flap was opened over the prefrontal cortex extending approximately 5 cm caudally from the frontal sinus and about 5 cm in width at its caudal margin. The cortical lesion was accomplished in one stage through subpial aspiration separating the superficial layers of cortex from their pial blood vessels. This results in degeneration of the cortical gray matter without the risk of damage to underlying white matter tracks. The area of the lesion included the ventral bank, floor, and dorsal bank of the sulcus principalis from the rostral edge of the arcuate sulcus caudally as far as the end of sulcus principalis at the frontal pole. Medially, the lesion extended from sulcus principalis to the dorsal limb of the arcuate sulcus caudally; more rostrally, it was continued medially across the dorsal surface and down the midline to the dorsal lip of the cingulate sulcus from the coronal plane intersecting the dorsal tip of arcuate sulcus all the way to the frontal pole. On completion of the lesion, the dura was closed, the bone flap replaced, and the incision was closed in layers.

At the conclusion of surgery, the animals were extubated and placed in an incubator until fully awake. They were also administered 600,000 units of Bicillin-LA intramuscularly to guard against infection and were administered analgesic to treat postoperative pain (Banamine 1.0 mg/kg im). Analgesia was continued for 48 to 96 hr or longer if symptoms indicated as determined by the veterinary staff. One week after surgery, the skin sutures were removed and a complete physical examination was done. Prior to behavioral testing, which began 4 weeks postoperatively, all monkeys underwent an MRI scan to characterize the locus and extent of the lesion (see Figure 1).

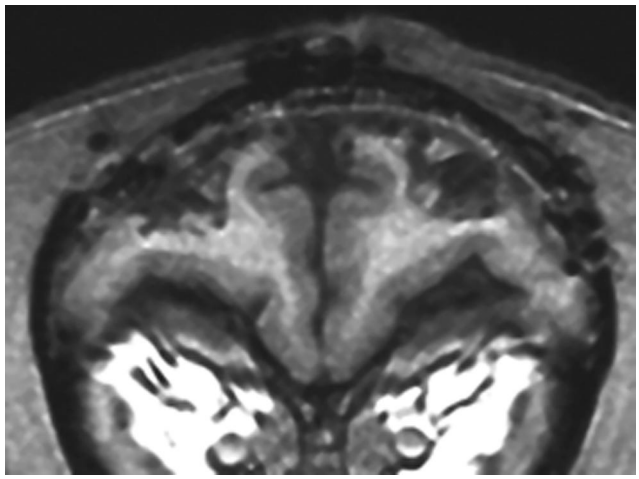

Figure 1. An example of one slice from a three-dimensional, SPGR MRI scan acquired on a 1.5T imager. For each monkey, 60 slices were acquired in the coronal plane at a thickness of either 1.3 or 1.4 mm, with no gaps between successive sections. A 512 × 384 matrix over a 16 cm × 16 cm field of view was used so that each voxel in a slice covered 0.31 mm^2 of tissue. As can be seen, this protocol produced excellent images that were relatively free of artifact and had good differentiation of gray matter, white matter, and the ventricles.

Behavioral Testing

Preoperatively, all monkeys were initially trained in a Wisconsin General Testing Apparatus (WGTA) to displace a gray plaque placed over a central or one of two lateral food wells to obtain the reward. This was to ensure that animals were able to carry out the basic response procedures necessary to complete the full battery of tasks. Postoperatively, all monkeys completed testing on two recognition memory tasks: delayed nonmatching-to-sample and delayed recognition span tests in a WGTA prior to testing on the CSST. The results of these studies are reported separately (Moore, Killany, Rosene, & Moss, 2000).

Following the completion of the delayed nonmatching-to-sample test and the delayed recognition span test, monkeys completed three tasks in an automated general testing apparatus that contained a 19-in., touch-sensitive, resistive, computer screen, driven by a Macintosh computer and an automated reward dispenser. The first task was a simple pretraining task to shape the monkeys to touch the screen. The second was a complex three-choice discrimination task to ensure that monkeys could respond to complex stimuli, and the third was the CSST. For stimulus presentation, the computer screen was organized into a 3 × 3 matrix (unmarked). The testing chamber had a darkened interior and was located in a darkened room. White noise was presented on two speakers located within the automated apparatus to mask extraneous sounds. A noncorrectional procedure was used throughout testing with M&Ms or Skittles as rewards delivered to a food cup beneath the touch screen.

The automated pretraining task required the monkey to touch a single stimulus (an image of an apple) that appeared randomly in one of nine locations on the screen to receive a food reward. Pretraining was continued for 20 trials a day until the monkey correctly responded to 20 consecutive trials in a single day. The day after the pretraining task was completed, some of the monkeys began a simple three-choice discrimination task. This task was administered to determine whether there was a group difference in the ability to discriminate among three fixed stimuli on the basis of the reward contingency. The three stimuli presented simultaneously on each trial were a pink square, an orange cross, and a brown 12-point star. The stimuli remained constant in terms of color and shape for each trial, but their spatial location varied from trial to trial in a pseudorandom order. The pink square was the positive stimulus for all trials and all monkeys. A noncorrectional procedure was used throughout this task, with the monkey being rewarded for touching the pink square on the screen. A total of 80 trials were presented each day until the monkey chose the pink square on 10 consecutive trials during one testing session to reach criterion performance. The discrimination task was only available to be given to a subset of monkeys (all DLPFC and three control animals). However, both visual inspection of the data comparing the performance of the monkeys on the CSST that had the discrimination task with the performance of those that did not demonstrated that experience on this task did not significantly alter performance on the CSST for either controls or lesion subjects. In addition, the correct stimulus in the discrimination task (a pink square) was not used as a stimulus in the CSST and was chosen specifically as it did not closely resemble any of the stimuli used in the CSST to ensure that responding to this stimulus would not impair their performance on the CSST.

Following completion of the pretraining and discrimination tasks, testing began on the CSST. The task has been described in detail by Moore et al. (2005) and further illustrated in application to studies of aging (Moore, Killany, Herndon, Rosene, & Moss, 2003) and hypertension (Moore et al., 2002) but is summarized in this section. Each day of testing consisted of 80 trials with an intertrial interval of 15 s. During each trial of the CSST, three stimuli were presented in a pseudorandom order appearing in three of nine locations on the computer touch screen (see Figure 2). The stimulus set for the CSST consisted of nine different figures representing two categories—color (red, green, or blue) and shape (triangle, star, and circle)—yielding a total of nine stimuli. On each trial, three different stimuli were presented, representing each color and each shape. All nine possible combinations of stimuli (i.e., red triangle, red star, red circle, blue triangle, etc.) were presented in a pseudorandom but balanced sequence. On each trial, if a monkey did not respond within a 1-min time limit, the screen went blank, a nonresponse was recorded, and the intertrial interval began.

Testing consisted of the initial abstraction and acquisition of the first category (color; red) and then three additional abstractions and shifts of categories, alternating shape and color (triangle, blue, and star). During the abstraction and acquisition of the first category, to obtain a food reward the monkey had to choose the red stimulus regardless of its shape, as illustrated in the top row of Figure 2. Once the monkey chose this stimulus on 10 consecutive trials, the program switched the rewarded contingency during the same testing session, without alerting the monkey. Then, to obtain a food reward, the monkey had to choose the stimulus shaped like a triangle, regardless of its color, as illustrated in the lower row of Figure 2. Again, when the monkey reached a criterion of 10 consecutive responses, the computer switched the rewarded contingency, so that the blue stimulus had to be chosen, regardless of its shape, to obtain a food reward. Finally, when criterion was reached on the blue category, the contingency was switched to the final category, star.

Perfusion and Lesion Reconstruction

Following completion of testing, monkeys were deeply anesthetized with intravenous sodium pentobarbital (15 mg/kg to effect) and were killed by exsanguination during transcardial perfusion of

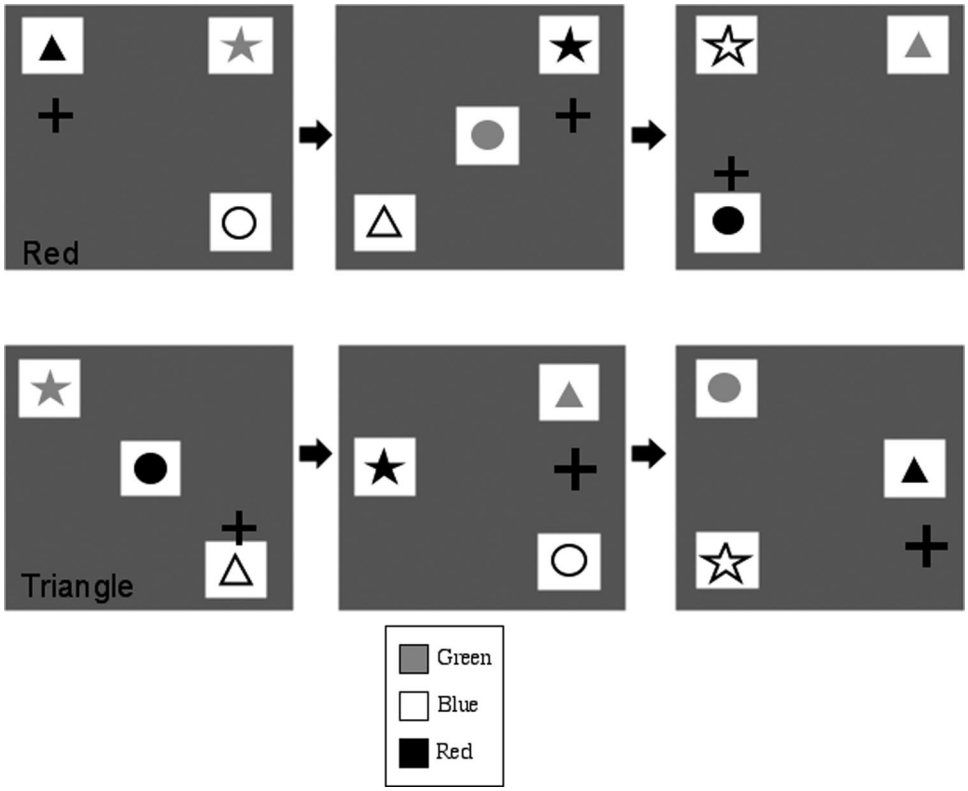

Figure 2. In this schematic of the category set-shifting task (CSST), each screen (panel) represents one trial. On each trial of the CSST, the monkey is presented with three stimuli that vary in shape and color. During the first concept condition, the monkey must choose the red stimulus regardless of its shape, as illustrated sequentially in the top three screens of this figure. Once the monkey chooses the correct stimulus on 10 consecutive trials, the computer switches the rewarded stimulus on the same testing day, without alerting the monkey. In the second concept condition, the monkey must choose the triangle-shaped stimulus regardless of the color, as illustrated in the bottom three screens of the figure. Again, when the monkey chooses the correct stimulus for 10 consecutive trials, the computer switches the rewarded stimulus on the same testing day, without alerting the monkey. Testing is continued in this same manner for the blue and star concept conditions.

the brain with 4% paraformaldehyde. Following perfusion, both hemispheres of the brain were blocked, in situ in the coronal stereotactic plane for serial sectioning, and transferred to cryoprotectant solution to eliminate freezing artifact (Rosene, Roy, & Davis, 1986). The cryoprotected blocks were then flash frozen and stored at −80 °C until they were cut on a microtome into nine interrupted series of 30-μm thick frozen sections and one 60-μm thick series. One 60-μm series and one 10-μm series were immediately mounted on microscope slides, stained with thionin, and used to reconstruct the lesions.

For lesion reconstructions, each monkey's preoperative T1 weighted three-dimensional SPGR MRI scans (1.3- or 1.4-mm thick slices) were used to create an individualized coronal section atlas of the entire frontal lobe from the arcuate sulcus to the rostral extent of the frontal pole. Walker's cytoarchotechtonic areas (as revised by Barbas & Pandya, 1989) were identified for each section of the map. This was accomplished by spatially matching the location of these regions on the figures provided by Barbas and Pandya (1989) to the MRI scans. This method allowed us to approximate these anatomical regions and provided a reliable way of assessing volumetric change within the subject across time. Area measurements (mm^2) were determined for all cytoarchotechtonic areas on each section of the map using NIH Image software (http://rsbweb.nih.gov/nih-image/).

To reconstruct the lesions, the thionin-stained sections throughout the rostral/caudal extent of the lesion were superimposed onto appropriate MRI-derived atlas drawings and the extent of the lesion was marked. This was then checked at higher power under the light microscope and adjusted accordingly. Each section was then scanned into the computer and the percentage of cortical tissue damaged was determined for each cytoarchitectonic area using NIH Image software. These relative lesion sizes are shown in Figure 3, and the percentage of tissue damage is presented in Table 1.

Based on the lesion reconstructions, we determined, as intended, that all monkeys had complete damage to areas 46, 8a, 8b, 9, and 10. However, in addition, there was slight damage in areas 6 and 12 in three monkeys and area 24 in one monkey. Because of the small group size in this study, it was not possible to determine whether the extraneous damage in areas 6, 12, and 24 was related to a greater a degree of impairment on the CSST. However, examination of the data from these four animals shows very little variability on test performance within the lesion group.

Data Analysis

The total number of errors to criterion and nonresponses for the discrimination task and the total number of errors, nonresponses, and broken sets to criterion for the red condition were recorded. For the three shift conditions (triangle, blue, and star), we determined the total number of errors, broken sets, and nonresponses. The total perseverative errors as a percentage of total shift trials

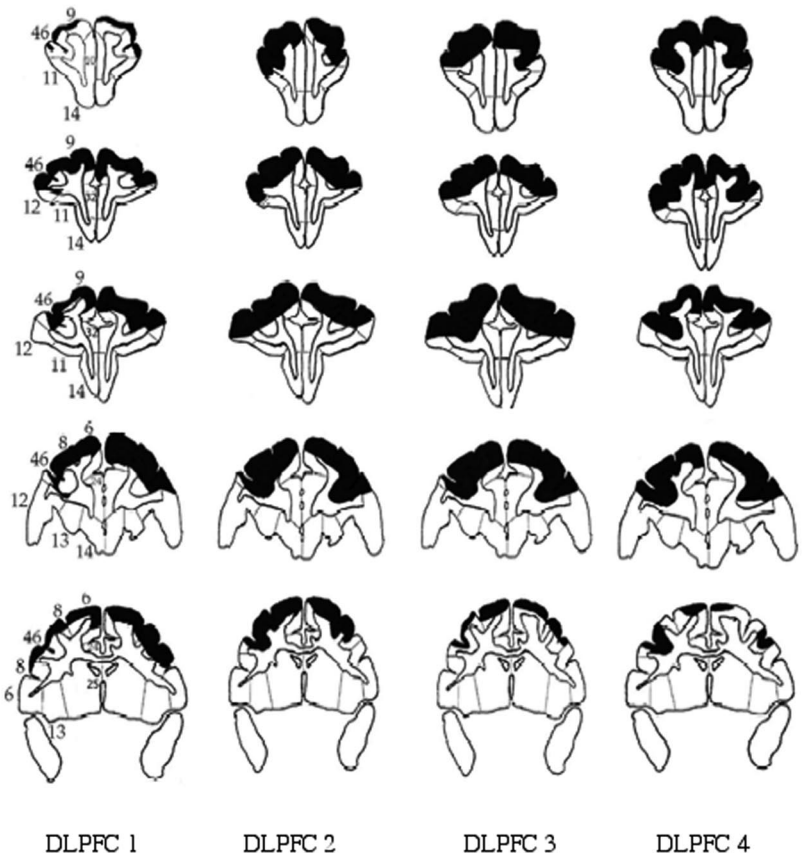

Figure 3. Lesion reconstructions of all four monkeys in the group with dorsolateral prefrontal cortex lesions (DLPFC).

Table 1
Extent of Damage in Four Monkeys With Lesions of Dorsolateral Prefrontal Cortex (DLPFC)

	Area						
Monkey	46	8	9	10	12	24	6
Intended lesion	100.00	100.00	100.00	25.00	0.00	0.00	0.00
DLPFC 1	70.8	65.73	79.27	4.03	18.30	0.00	31.63
DLPFC 2	89.92	53.64	85.54	20.13	17.26	0.00	32.29
DLPFC 3	87.47	45.70	84.30	24.35	3.18	0.00	31.55
DLPFC 4	94.33	43.92	90.34	30.29	2.99	0.00	22.18

Note. Values represent percentage of damaged tissue based on comparison of thionin-stained sections with preoperative MRI scans.

and as a percentage of shift errors were determined. Although there are many definitions of preservative responses or errors in the literature, we chose to use the "perseverated to" principle described in the instructions for the WCST (Heaton et al., 1993). In the CSST, a perseverative error was recorded during shift trials when a monkey chose a stimulus that contained the component of the previously correct category, unless the response was correct. For example, in the triangle category, a red star and a red circle would be counted as a perseverative error, whereas a red triangle (the correct response) would not be counted as a perseverative error. A broken set was recorded when a monkey achieved a span of 6 to 9 consecutive correct responses but then made an error and missed reaching the criterion of 10 consecutive correct responses. A nonresponse was recorded when a monkey failed to respond by touching the screen on any trial within 1 min of the stimuli appearing on the screen. A nonresponse was not counted as an error; however, it did result in the count of consecutive correct responses to be reset to zero (i.e., for criterion purposes). Thus, the total number of errors did not include the number of nonresponses.

Separate one-way analyses of variance (ANOVAs), with group (lesion vs. unoperated control) as the between-subjects variable were run on the number of errors on the discrimination task. For the CSST, separate one-way ANOVAs were run on the number of errors on the initial abstraction of the first category, the number of broken sets across all trials, the number of nonresponses across all trials, and the total perseverative errors as a percentage of shift trials. Separate two-way repeated measures ANOVAs with group (lesion vs. unoperated control) as a between-subjects variable and category shifts as a within-subjects variable were used to compare the performance of the two groups of monkeys in terms of errors to criterion across each shift condition.

In addition, separate one-sample *t* tests for each group were used to determine whether the percentage of errors that were perseverative across all three shifts was significantly different from the chance level of making a perseverative error (50%).

Results

Table 2 shows the performance of the monkeys on the initial three-choice discrimination task in terms of errors and nonresponses to criterion. Data for errors to criterion were analyzed separately with a one-way ANOVA. This analysis did not reveal a statistically significant difference between the groups with regard to errors, $F(1, 5) = 5.38$, $p = .07$, to criterion. Although p values

Table 2
Total Number of Errors and Nonresponses for Each Monkey on the Discrimination Task and Total Number of Nonresponses and Perseverative Errors as a Percentage of Shift Trials on the Category Set-Shifting Task (CSST)

Monkey	Discrimination task error	Discrimination task nonresponses	CSST nonresponses	CSST perseverative errors
HM 034	N/A	0.00	4.00	38.54
HM 043	6.00	0.00	0.00	21.02
HM 038	N/A	0.00	0.00	33.21
HM 044	20.00	0.00	4.00	25.38
HM 059	33.00	0.00	1.00	31.44
M	19.67	0.00	2.20	29.92
SE	6.37	0.00	1.07	2.43
DLPFC 1	4.00	0.00	2.00	32.94
DLPFC 2	5.00	0.00	12.00	39.70
DLPFC 3	7.00	0.00	2.00	38.53
DLPFC 4	0.00	0.00	6.00	23.43
M	4.00	0.00	5.50	33.65
SE	1.27	0.00	2.05	3.21

Note. HM = unoperated control monkeys; DLPFC = monkeys with dorsolateral prefrontal cortex lesions. Some of the monkeys did not complete this task; therefore, there are no data available (N/A).

approached significance, it is important to note that the DLPFC subjects actually learned the three-choice faster than control animals. All monkeys responded to all trials in the discrimination task and therefore no nonresponses were recorded.

In contrast to the spared performance on the discrimination task, impairments were seen in the performance of the DLPFC monkeys on the CSST (see Figure 4). Separate one-way ANOVAs did reveal differences between the monkeys with DLPFC lesions and unoperated controls on the number of errors, $F(1, 7) = 27.28$, $p = .001$, required to reach criterion on the abstraction of the first category.

On the three shift conditions, two-way repeated measures ANOVA revealed an overall effect of group. Furthermore, there was a significant Group × Set Shift Condition interaction for total errors, $F(2, 14) = 8.94$, $p = .003$, across shift conditions. There was no overall effect of set shift condition on the total number of errors, $F(2, 14) = 1.74$, $p = .212$.

Follow-up analyses of the set shift condition interaction for total errors with tests of simple main effects for each individual shift revealed that the monkeys with DLPFC lesions were impaired relative to the monkeys in the control group for the number of errors, $F(1, 21) = 4.64$, $p = .05$, to criterion for the triangle condition (first set shift); for the number of errors, $F(1, 21) = 41.40$, $p = .001$, to criterion for the blue condition (second set shift); and for the number of errors, $F(1, 21) = 16.24$, $p = .001$, to criterion for the star condition (third set shift; see Figure 4). Therefore, the monkeys with DLPFC lesions made significantly more errors on all shift conditions than the control monkeys.

Overall, the monkeys with lesions made more perseverative type errors than control monkeys. However, it is plausible that this increased number of overall perseverative errors is merely a reflection of the increased opportunity (i.e., more trials to criterion) to make this type of error. We tested this hypothesis by analyzing the total perseverative errors as a percentage of total shift trials with a separate one-way ANOVA (see Table 2). This analysis revealed no significant difference between the groups on the proportion of perseverative errors, $F(1, 7) = 0.222$, $p = .65$. This suggests that the monkeys with DLPFC lesions did not demonstrate a perseverative response pattern relative to the control monkeys. Finally, we used separate one-sample t tests to determine whether the percentage of errors that were perseverative across all three shifts was significantly different from the chance level of making a perseverative error (50%). This analysis revealed that monkeys in both groups made more perseverative responses than could be accounted for by chance: DLPFC lesion group, $t(3) = 10.782$, $p = .0017$; control group, $t(4) = 15.404$, $p = .0001$. These results suggest that although both groups of monkeys, based on our definition of perseveration, demonstrated a tendency to perseverate in their response pattern, there was no significant difference in the rate of perseveration between groups.

There was a significant difference between the groups in the total number of broken sets, $F(1, 7) = 10.10$, $p = .01$ (see Figure 5). This is of particular interest as it suggests that monkeys with DLPFC lesions have greater difficulty maintaining a correct response pattern despite positive feedback for correct responses, a function often associated with the DLPFC. Finally, there was no statistically significant difference between the groups for the number of nonresponses, $F(1, 7) = 1.77$, $p = .225$ (see Table 2).

Discussion

CSST and Abstraction

In the present study, we used the CSST to assess the role for the DLPFC in various components of executive function in rhesus monkeys. The results demonstrated that compared with intact controls, monkeys with DLPFC lesions were impaired on the abstraction of a specific stimulus category and shifting their response pattern to establish a new response pattern to a different stimulus category. In the CSST, the monkey must establish a response pattern to a rule or generalization that has been learned from individual trials and that can then be applied across trials to solve a problem. One can view this as requiring the monkey to identify what is the "same" across trials and to continue to respond to what was rewarded before (also analogous to a matching paradigm). In this way, the color red (or later in the task, shape) serves

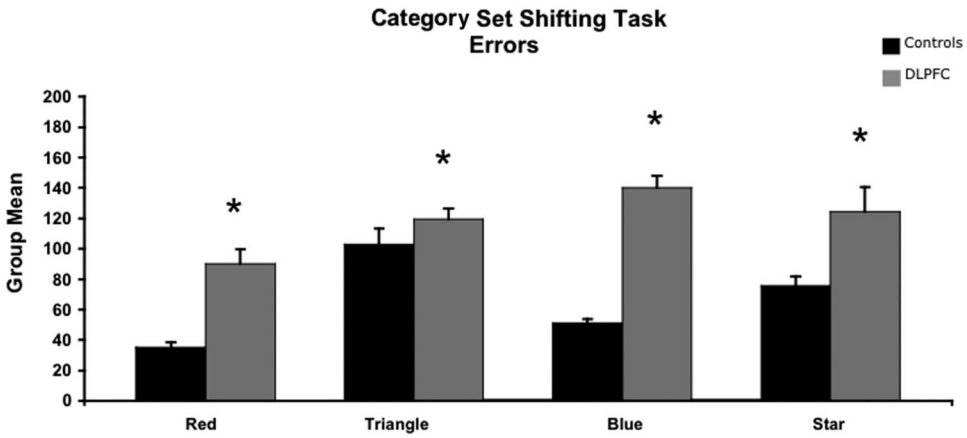

Figure 4. Group mean errors to criterion for the acquisition condition (red) and the three shift conditions (triangle, blue, and star). Asterisks indicate a significant group difference for errors ($p < .05$). Error bars represent standard error.

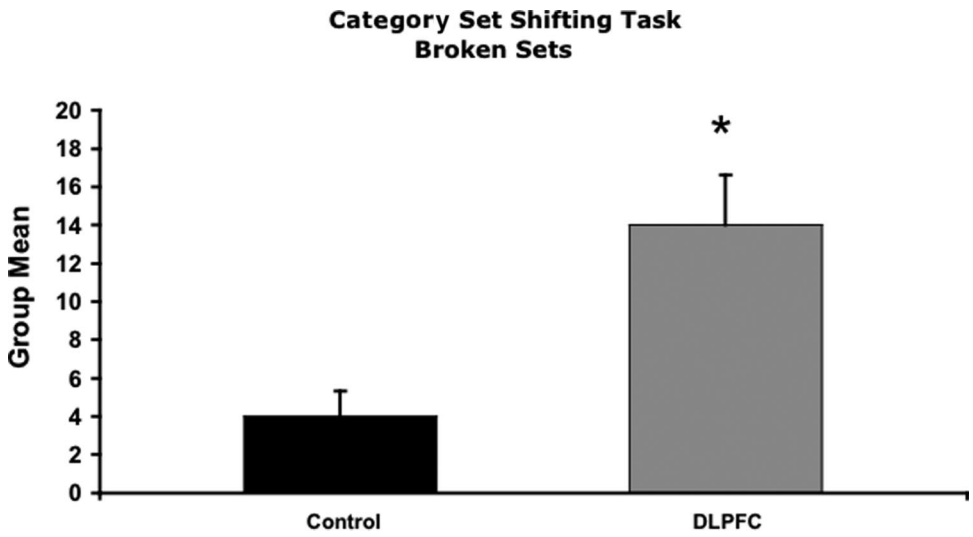

Figure 5. Group mean broken sets. The asterisk indicates a significant group difference for total broken sets ($p < .01$). Error bars represent standard error.

as both the discriminanda and the category concept. Although it cannot be determined from the performance of the monkeys in this study whether this is the sole method used to solve this task, it is likely that the control monkeys used the abstraction capacities of the PFC to successfully perform this task, whereas the monkeys with damage to the DLPFC relied on a more primitive association system to simultaneously learn all nine unique combinations of trials for each condition.

In considering the operations required to perform the CSST and the WCST, it is clear that they require the subject to use items in working memory, specifically in an abstract fashion, to determine what common feature of the stimuli is being reinforced across trials. In the case of the CSST, the common elements are either color regardless of shape or shape regardless of color. Successful performance on the CSST assumes that the animals in the control group are learning specific stimuli characteristics that require categorizing stimuli on the basis of specific perceptual similarities (i.e., red circle, red triangle, etc.). This differs from learning abstract concepts that require applying rules beyond the initial stimuli (Bodily, Katz, &Wright, 2008; Wright & Katz, 2007). Although it may be optimal to have the animals perform a test requiring learning abstract concepts (i.e., complete design change paradigm), the present test provides an assessment of the animals' ability to establish a response pattern to a stimulus characteristic or set of stimuli with a shared characteristics (i.e., color or shape), maintain that response pattern, and then when appropriate, shift that response pattern. In other words, whether they have learned to respond to a specific color or shape or learned that a set of stimuli (red star, red circle, and red triangle) is correct, they have learned those stimuli or stimuli characteristics and maintained a response pattern for a period of time. Furthermore, we assume that the control monkeys with an intact DLPFC are abstracting a category (i.e., color and shape) or stimulus characteristic (i.e., red, blue, triangle, star, etc.) and reaching criterion based on that abstraction. However, the monkeys with lesions in the DLPFC are likely unable to successfully abstract a rule and may be responding only to specific exemplars or sensory characteristics rather than a category or dimensional set.

An alternative explanation of the monkeys' performance on this test may be related to deficits in attentional abilities that are thought to be mediated in part by lateral prefrontal regions. Although impairments in attention would cause deficits in performance on tests of executive function, such as the CSST, the monkeys in this study also completed a test of simple and sustained attention on which they were not impaired (unpublished data). Therefore, it is unlikely that the impaired performance on the CSST by the monkeys with DLPFC lesions is the result of difficulties with simple attention. However, their deficits in shifting their response patterns may be related to difficulties in switching their attention to previously irrelevant stimulus characteristics. This type of impairment has been demonstrated in studies investigating the neural substrates of attentional control and attentional shifting (Hampshire & Owen, 2006). However, it is important to clarify that there are different forms of shifting (i.e., attention/perceptual shifting vs. rule shifting) and that different tasks may be assessing these various forms of shifting and each likely has a different, although linked, neuroanatomical correlate (Loose, Kaufman, Tucha, Auer, & Lange, 2006; Ravizza & Carter, 2008; Zanolie et al., 2008). Furthermore, the testing paradigm used, experimenter-controlled shifting versus participant-controlled shifting, appears to affect performance on tasks of attentional or set shifting (Hampshire & Owen, 2006; Zanolie et al., 2008). These issues of attentional control and the precise nature of shifting tests (experimenter vs. participant control and perceptual vs. rule shifting) have increasingly become the focus of neuroimaging studies. Future studies will need to incorporate more precise tasks such as those proposed by Loose et al. (2006) and Zanolie et al. (2008) to facilitate understanding the specific brain regions mediating response and attention shifting.

PFC Functional Systems and Working Memory

Although it is well established that the PFC is critical for various cognitive functions such as learning, abstraction, and the establishment, maintenance, and shifting of attentional set, how different regions of PFC mediate these functions and their interactions remains unclear. Two theories have been proposed recently in the literature. The first, based primarily on the work from Goldman-Rakic's group, suggests that different areas of the PFC encode different types of content using the same basic processing functions of working memory to hold and compare information. The second theory stems from the work of Dias, Robbins, and Roberts (1997) and suggests that different areas of the PFC subserve different processing functions such as abstraction versus inhibition (O'Reilly, Noelle, Braver, & Cohen, 2002).

The working memory model of PFC function describes working memory as the process of maintaining information through continual neural firing that can be rapidly updated by changing the activation state of a set of neurons (Kubota & Niki, 1971; Miller & Cohen, 2001; O'Reilly et al., 2002). These activation-based working memories, although unstable, are flexible and can be altered to accommodate new information to allow for abstraction and set shifting. Based on this proposal, successful performance on the CSST would require the maintenance of a pattern of activity for the representation of the currently relevant stimulus characteristic in the PFC. However, when the correct stimulus characteristic changes, this pattern of activity would be updated to incorporate new information into working memory that would allow the animal to determine the newly correct concept condition (Miller & Cohen, 2001; O'Reilly et al., 2002).

The functional parcellation theory of PFC function is based on extensive work by many groups that have demonstrated dissociation of functions in different regions of the PFC using lesion, electrophysiology, and fMRI studies (Dias et al., 1996; Freedman, Black, Ebert, & Binns, 1998; Kubota & Niki, 1971; Nakahara et al., 2002; Petrides, 2000a, 2000b; Wallis et al., 2001; Wilson, Scalaidhe, & Goldman-Rakic, 1993). Recently, Dias et al. (1997) demonstrated that lesions in the orbitofrontal cortex of marmosets impaired the ability to reverse a stimulus–reward association within a perceptual dimension, and lesions on the lateral cortex impaired the ability to shift attentional set from one dimension to another. Furthermore, these impairments were observed only with novel stimuli or for the first occasion that a shift occurred.

Theoretical Considerations

O'Reilly et al. (2002) proposed a model of PFC function that integrates basic concepts from each of the two theories presented above. They suggested that there is an overall single function of the PFC of maintaining information in an active state over a period of time while different areas of the PFC process different information or dimensions. In the marmoset, they suggested that more ventral regions process more specific information and lateral regions process more abstract information. These regions function together to provide the necessary input for activation-based working memory to allow for cognitive flexibility and abstraction. They stated that this general principle likely holds across species. They suggested in the rhesus macaque and in humans, based on the literature, that dorsal–frontal areas may encode more complex and abstract information over longer delays, whereas ventral regions are involved in simpler memory processes for specific information and for information held over brief intervals (Mishkin & Manning, 1978; O'Reilly et al., 2002; Wilson et al., 1993).

The results from the present study can possibly be explained on the basis of these ideas. The monkeys with DLPFC lesions were impaired on the response maintenance and shifting, which could be accounted for by a decrease in the flexibility of activation-based working memory and the ability to encode and retain more complex information over longer periods of time.

However, the lack of perseverative response patterns in the present study is more difficult to explain. It has been suggested that perseveration occurs when an active trace in working memory for currently relevant information is insufficiently strong and previously relevant information is likely to interfere with the new information (Morton & Munakata, 2002). In the current study, it may be that the monkeys had impairments in the establishment of a response set to such a degree that they did not form a strong enough stimulus–reward association to provide the necessary basis for perseveration. This may be tested in future studies by having monkeys repeat testing on a shift condition the day after making criterion. The stimulus–reward association may be strong enough to reach criterion on a single day but not established well enough to be repeated at the next testing day. If this were true, then the association would likely also not be strong enough to cause perseveration.

Although these theories have strong arguments and considerable evidence, further study of the effects of lesions in specific regions of the PFC on tasks that require intradimensional and extradimensional shifts is still needed to further understand the precise functionality of the PFC. Furthermore, there are many methodological issues that need to be addressed for future studies. First, many of the studies investigating the functions of the PFC have used either marmosets (New World monkeys) or rhesus macaques (Old World monkeys), and it remains unclear whether the various areas of the PFC in each of these species contribute to PFC function in the same way. Second, given the considerable intra- and interconnectivity of the PFC, the contribution of other cortical structures to PFC function needs to be further addressed. Certainly, it has been demonstrated that various parts of the brain (i.e., basal forebrain, posterior parietal cortex) are likely involved in the performance on shifting tasks (Fox, Barense, & Baxter, 2003; Lie et al., 2006; Tait & Brown, 2008). Finally, lesion size and location are always an issue in lesion studies, and the direct comparison of impairments across studies with various lesions remains difficult.

Conclusions

The results of the present study argue for the DLPFC playing an important role in establishing rules for guiding behavior and flexibly altering these rules as contingencies change. Considering the results of the present study in conjunction with those of Mishkin and Manning (1978) and Bachevalier and Mishkin (1996) and the two models discussed above, we can conclude that an intact DLPFC allows for the efficient creation of abstract rules and their manipulation in working memory process. Although it is likely that the integrity of the DLPFC is pivotal to successful completion of tasks of executive function, it is likely that other regions of the PFC (e.g., ventral and orbital PFC regions) as well as other cortical

association areas may also play some role in this complex cognitive domain, the precise locus and nature of which have yet to be worked out in detail.

References

Asaad, W. F., Rainer, G., & Miller, E. K. (2000). Task-specific neuronal activity in the primate prefrontal cortex. *Journal of Neurophysiology, 84,* 451–459.

Bachevalier, J., & Mishkin, M. (1986). Visual recognition impairment follows ventromedial but not dorsolateral prefrontal lesions in monkeys. *Behavioural Brain Research, 20,* 249–261.

Barbas, H., & Pandya, D. N. (1989). Architecture and intrinsic connections of the prefrontal cortex in the rhesus monkey. *Journal of Comparative Neurology, 286,* 353–375.

Berg, E. A. (1948). A simple objective test for measuring flexibility in thinking. *Journal of General Psychology, 39,* 15–22.

Bodily, K. D., Katz, J. S., & Wright, A. A. (2008). Matching-to-sample abstract-concept learning by pigeons. *Journal of Experimental Psychology: Animal Behavioral Processes, 34,* 178–184.

Butter, C. M., Mishkin, M., & Rosvold, H. E. (1963). Conditioning and extinction of food-rewarded response after selective ablations of frontal cortex in rhesus monkey. *Experimental Neurology, 7,* 65–75.

Butters, N., & Pandya, D. (1969, September 19). Retention of delayed alteration: Effect of selective lesions of sulcus principalis. *Science, 165,* 1271–1273.

Damasio, A. R., & Anderson, S. W. (1993). The frontal lobes. In K. Heilman & E. Valenstein (Eds.), *Clinical neuropsychology* (pp. 409–448). Oxford, England: Oxford University Press.

Dias, R., Robbins, T. W., & Roberts, A. C. (1996). Primate analogue of the Wisconsin Card Sorting Test: Effects of excitotoxic lesions of the prefrontal cortex in the marmoset. *Behavioral Neuroscience, 110,* 872–886.

Dias, R., Robbins, T. W., & Roberts, A. C. (1997). Dissociable forms of inhibitory control within prefrontal cortex with an analog of the Wisconsin Card Sort Test: Restriction to novel situations and independence from "on-line" processing. *Journal of Neuroscience, 17,* 9285–9297.

Fox, M. T., Barense, M. D., & Baxter, M. G. (2003). Perceptual attentional set-shifting is impaired in rats with neurotoxic lesions of posterior parietal cortex. *Journal of Neuroscience, 23,* 676–681.

Freedman, M., Black, S., Ebert, P., & Binns, M. (1998). Orbitofrontal function, object alternation and perseveration. *Cerebral Cortex, 8,* 18–27.

Gaffan, D., & Harrison, S. (1989). A comparison of the effects of fornix transection and sulcus principalis ablation upon spatial learning by monkeys. *Behavioural Brain Research, 31,* 207–220.

Goldman, P. S., & Rosvold, H. E. (1970). Localization of function within the dorsolateral prefrontal cortex of the rhesus monkey. *Experimental Neurology, 27,* 291–304.

Hampshire, A., & Owen, A. M. (2006). Fractionating attentional control using event-related fMRI. *Cerebral Cortex, 16,* 1679–1689.

Heaton, R. K. (1981). *A manual for the Wisconsin Card Sorting Test*. Odessa, FL: Psychological Assessment Resources.

Heaton, R. K., Chelune, G. J., Talley, J. L., Kay, G. G., & Curtiss, G. (1993). *Wisconsin Card Sorting Test manual revised and expanded*. Odessa, FL: Psychological Assessment Resources.

Jacobsen, C. F. (1935). Functions of the frontal association area in primates. *Archives of Neurological Psychiatry, 33,* 558–569.

Jacobsen, C. F. (1936). Studies of cerebral function in primates: I. The functions of the frontal association areas in monkeys. *Comparative Psychological Monograph, 13,* 3–60.

Jacobson, S., Butters, N., & Tovsky, N. J. (1978). Afferent and efferent subcortical projections of behaviorally defined sectors of prefrontal granular cortex. *Brain Research, 159,* 279–296.

Kowalska, D. M., Bachevalier, J., & Mishkin, M. (1991). The role of the inferior prefrontal convexity in performance of delayed nonmatching-to-sample. *Neuropsychologia, 29,* 583–600.

Kubota, K., & Niki, H. (1971). Prefrontal cortical unit activity and delayed alternation performance in monkeys. *Journal of Neurophysiology, 34,* 337–347.

Lie, C. H., Specht, K., Marshall, J. C., & Fink, G. R. (2006). Using fMRI to decompose the neural processes underlying the Wisconsin Card Sorting Test. *NeuroImage, 30,* 1038–1049.

Loose, R., Kaufmann, C., Tucha, O., Auer, D. P., & Lange, K. W. (2006). Neural networks of response shifting: Influence of task speed and stimulus material. *Brain Research, 1090,* 146–155.

Miller, E. K., & Cohen, J. D. (2001). An integrative theory of prefrontal cortex function. *Annual Review in Neuroscience, 24,* 167–202.

Milner, B. (1963). Effects of different brain lesions on card sorting. *Archives of Neurology, 9,* 90–100.

Milner, B. (1995). Aspects of human frontal lobe function. *Advances in Neurology, 66,* 67–81.

Mishkin, M., & Manning, F. J. (1978). Non-spatial memory after selective prefrontal lesions in monkeys. *Brain Research, 143,* 313–323.

Monchi, O., Petrides, M., Petre, V., Worsley, K., & Dagher, A. (2001). Wisconsin Card Sorting revisited: Distinct neural circuits participating in different stages of the task identified by event-related functional magnetic resonance imaging. *Journal of Neuroscience, 21,* 7733–7741.

Moore, T. L., Killiany, R. J., Herndon, J, G., Rosene, D. L., & Moss, M. B. (2003). Impairment in abstraction and set shifting in aged rhesus monkey. *Neurobiology of Aging, 24,* 125–134.

Moore, T. L., Killiany, R. J., Herndon, J. G., Rosene, D. L., & Moss, M. B. (2005). A non-human primate test of abstraction and set shifting: An automated adaptation of the Wisconsin Card Sorting Test. *Journal of Neuroscience Methods, 146,* 165–173.

Moore, T. L., Killiany, R. J., Rosene, D. L., & Moss, M. B. (2000). Impaired memory function following lesions of dorsal prefrontal cortex in the rhesus monkey [Abstract]. *Society for Neuroscience*.

Moore, T. L., Killiany, R. J., Rosene, D. L., Prusty, S., Hollander, W., & Moss, M. B. (2002). Impairment of executive function induced by hypertension in the rhesus monkey. *Behavioral Neuroscience, 116,* 387–396.

Morton, J. B., & Munakata, Y. (2002). Active versus latent representations: A neural network model of perseveration, dissociation, and decalage. *Developmental Psychobiology, 40,* 255–265.

Nagahama, Y., Fukuyama, H., Yamauchi, H., Matsuzaki, S., Konishi, J., Shibasaki, H., & Kimura, J. (1996). Cerebral activation during performance of a card sorting test. *Brain, 119,* 1667–1675.

Nakahara, K., Hayashi, T., Konishi, S., & Miyashita, Y. (2002, February 22). Functional MRI of macaque monkeys performing a cognitive set-shifting task. *Science, 295,* 1532–1536.

O'Reilly, R. C., Noelle, D. C., Braver, T. S., & Cohen, J. D. (2002). Prefrontal cortex and dynamic categorization tasks: Representational organization and neuromodulatory control. *Cerebral Cortex, 12,* 246–257.

Oscar-Berman, M. (1978). The effects of dorsolateral–frontal and ventrolateral–orbitofrontal lesions on nonspatial test performance. *Neuropsychologia, 16,* 259–267.

Passingham, R. (1972). Non-reversal shifts after selective prefrontal ablation in monkeys (*Macaca mulatta*). *Neuropsychologia, 10,* 41–46.

Passingham, R. E. (1985). Memory of monkeys (*Macaca mulatta*) with lesions in prefrontal cortex. *Behavioral Neuroscience, 99,* 3–21.

Petrides, M. (2000a). Dissociable roles of mid-dorsolateral prefrontal and anterior inferotemporal cortex in visual working memory. *Journal of Neuroscience, 20,* 7496–7503.

Petrides, M. (2000b). The role of the mid-dorsolateral prefrontal cortex in working memory. *Experimental Brain Research, 133,* 44–54.

Pohl, W. (1973). Dissociation of spatial discrimination deficits following

frontal and parietal lesions in monkeys. *Journal of Comparative & Physiological Psychology, 82,* 227–239.

Ravizza, S. M., & Carter, C. S. (2008). Shifting set about task switching: Behavioral and neuronal evidence for distinct forms of cognitive flexibility. *Neuropsychologia, 46,* 2924–2935.

Rosene, D. L., Roy, N. J., & Davis, B. J. (1986). A cryoprotection method that facilitates cutting frozen sections of whole monkey brains for histological and histochemical processing without freezing artifact. *Journal of Histochemistry and Cytochemistry, 34,* 1301–1315.

Smith, M. L., & Milner, B. (1984). Differential effects of frontal-lobe lesions on cognitive estimation and spatial memory. *Neuropsychologia, 22,* 697–705.

Tait, D. S., & Brown, V. J. (2008). Lesions of the basal forebrain impair reversal learning but not shifting of attentional set in rats. *Behavioural Brain Research, 187,* 100–108.

Trans-NIH Executive Function Workshop. (2003, June). *Executive function: Current knowledge and future research opportunities*. New York.

Wallis, J. D., Anderson, K. C., & Miller, E. K. (2001, June 21). Single neurons in prefrontal cortex encode abstract rules. *Nature, 411,* 953–956.

Wilson, F. A., Scalaidhe, S. P., & Goldman-Rakic, P. S. (1993, June 25). Dissociation of object and spatial processing domains in primate prefrontal cortex. *Science, 260,* 1955–1958.

Woods, D. L., & Knight, R. T. (1986). Electrophysiological evidence of increased distractibility after dorsolateral prefrontal lesions. *Neurology, 36,* 212–216.

Wright, A. A., & Katz, J. S. (2007). Generalization hypothesis of abstract-concept learning: Learning strategies and related issues in *Macaca mulatta, Cebus apella,* and *Columba livia. Journal of Comparative Psychology, 121,* 387–397.

Zanolie, K., Teng, S., Donohue, S. E., van Duijvenvoorde, A. C., Band, G. P., Rombouts, S. A., & Crone, E. A. (2008). Switching between colors and shapes on the basis of positive and negative feedback: An fMRI and EEG study on feedback-based learning. *Cortex, 44,* 537–547.

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