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Cortical Effects of Bromocriptine, a D-2 Dopamine Receptor Agonist, in Human Subjects, Revealed by fMRI

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Abstract: Studies of human subjects performing cognitive tasks on and off dopaminergic drugs have suggested a specific role of dopamine in cognitive processes, particularly in working memory and prefrontal "executive" functions. However, the cortical effects of these drugs have been poorly understood. We used functional magnetic resonance imaging (fMRI) to examine both task-specific and general changes in cortical activity associated with bromocriptine, a selective agonist for D-2 dopamine receptors. Bromocriptine resulted in task-specific modulations of task-related activity in three cognitive tasks. Across tasks, the overall effect of the drug was to reduce task-related activity. We also observed drug effects on behavior that correlated with individual differences in memory span. We argue that bromocriptine may show both task-specifc modulation and task-general inhibition of neural activity due to dopaminergic neurotransmission. *Hum. Brain Mapping 12:246–257, 2001.* © 2001 Wiley-Liss, Inc.

Key words: working memory; cognition; fMRI; dopamine; neuroimaging

INTRODUCTION

Dopamine plays an important role in working memory and in higher cognitive functions that depend on the prefrontal cortex. The role of dopamine in prefrontal-dependent working memory processes is suggested by findings that pharmacological blockade of prefrontal dopamine receptors can lead to working memory impairments in monkeys [Brozoski et al., 1979; Sawaguchi and Goldman-Rakic, 1991, 1994; Sawaguchi, Matsumura, and Kubota, 1990]. Pharmacological studies of the role of dopamine in normal human cognition logically follow from the monkey literature, the most direct available method being drug challenge combined with behavioral testing.

Studies of dopaminergic agents in human subjects performing working memory tasks have yielded suggestive but inconsistent results. Luciana et al. [1992] tested 8 subjects on and off bromocriptine, a D-2 dopamine receptor agonist, and found that the drug facilitated performance at a visuospatial working memory task. In a subsequent study, Luciana and Collins [1997] found that working memory for spatial but not object information was improved by a 1.25-mg dose of bromocriptine (but not a 2.5-mg dose). Correspondingly, they found that haloperidol, a nonspecific dopamine antagonist, had opposite effects on only the spatial working memory task.

In a subsequent study [Kimberg, D'Esposito, and Farah, 1997], we found that normal subjects show a response to bromocriptine that depends on their base-

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line working memory capacity, or reading span (as indexed by the reading span test) [Daneman and Carpenter, 1980]. On a composite measure of five tests supposed to be sensitive to prefrontal function, low-span subjects showed a benefit from the drug, while high-span subjects showed a decrement in performance. No such pattern was observed in nonprefrontal-dependent tasks, and no main effect of bromocriptine on spatial working memory was observed. This result is consistent with dose-dependent effects demonstrated by Arnsten [1995, 1997] and by Williams and Goldman-Rakic [1995] in monkeys, in which small doses of a D-2 agonist improve performance, while larger doses impair performance. Mehta et al. [2000] have shown a similar relationship between WM span scores and the effects of methylphenidate on spatial working memory.

In a more recent study, Müller et al. [1998] compared the effects of pergolide, a D-1/D-2 agonist, with the effects of bromocriptine, as a pharmacological subtraction to identify D-1-specific effects in a group of 32 normal subjects. In a visuospatial working memory task, they found that pergolide, but not bromocriptine, improved working memory performance at long (16-sec) delays. This was interpreted by Müller et al. as consistent with a more prominent role for D-1 receptors in working memory.

While these studies have been helpful in understanding the behavioral effects of selective dopaminergic drugs, our knowledge of the cortical bases of these effects is derived only indirectly from our understanding of the dopaminergic system, or from studies with rats [Pizzolato, Soncrant, and Rapoport, 1985] and monkeys [Murphy et al., 1996; Williams and Goldman-Rakic, 1995]. Functional neuroimaging methods can be used to examine more directly the changes in cortical activity associated with pharmacological challenges. While these techniques do not identify loci of direct drug effects, they can be used to identify changes in cortical activity (as indexed by blood flow) due to drug administration.

Previous imaging studies have used drugs that broadly affect catecholamine receptors [Fletcher et al., 1996; Mattay et al., 1996]. Using selective dopamine agonists, we can potentially examine the roles of particular receptor subtypes in cognitive function. As this technique is relatively new, we can also learn to what extent it is possible to observe changes in cortical activity due to these subtler drug effects.

Because of our previous results showing an interaction between the effects of bromocriptine and verbal working memory capacity, we are here interested in both main effects of the drug as well as interactions with reading span scores. It is possible that these behavioral interactions correspond to both main drug effects (i.e., consistent cortical effects that are expressed differently in different subjects) and interactions (i.e., cortical effects that depend on WM capacity). In the study described here, we used fMRI to examine the cortical effects of bromocriptine. This study provides a first step toward understanding the relationship between cortical and behavioral effects of this drug.

METHODS

Subjects

Eleven young healthy volunteers participated in return for monetary compensation. Volunteers were screened for history of neurological abnormality, blood pressure, and for anything that would preclude completing the study (e.g., metallic implants or difficulty with manual responses). Two additional subjects had to discontinue partway through the study due to adverse reactions to the drug (nausea, dizziness), and were excluded. Of the 11 included subjects, the first two were not given reading span measures or the motor task, and were therefore excluded from analyses depending on these measures. Note that because the primary goal of this study was to examine main drug effects (not span-dependent effects, as observed in our previous study), subjects were recruited without consideration of reading span. By chance, of the nine subjects from whom we recorded reading span scores, five were high-span and four low-span, by the same criteria used in our larger earlier study, making it possible to look for these interactions.

Cognitive tasks

Subjects were tested on and off bromocriptine in a double-blind design. Each subject was tested on three occasions. The first session was for practice, to minimize the effects of learning between the subsequent two sessions.

During the practice session, subjects were also given the Daneman and Carpenter [1980] reading span test. In our version of this task, subjects were shown sentences on a computer screen, one at a time, and asked to recall the last word of each sentence. Subjects were told to begin recalling words when the screen blanked. Initially, five sets of two sentences were presented, considered practice. Then, five sets of three sentences, five sets of four, and five sets of five sentences were presented. Subjects were warned not to pause between

sentences, to read ahead to the last word, or to report the final last word first.

During the second and third sessions, subjects were given either 2.5 mg bromocriptine or a placebo (order randomized) orally, followed by a 90-min delay. Following the delay, subjects underwent BOLD fMRI while performing three cognitive tasks. Each of these tasks was performed in a blocked design in which 40-sec blocks of task performance alternated with 40-sec blocks of a control task. Each run, defined as an uninterrupted sequence of data acquisition, consisted of eight blocks—four control alternating with four task blocks.

The first task was a "two-back" test of working memory in which subjects saw a series of letters appear on the screen and were required to indicate with a right-hand button press any time the letter matched the one two earlier. Each letter appeared on the screen for 500 ms, followed by 900 ms of blank screen. In the control condition for this task (presumably imposing little or no working memory demand), subjects were to respond any time the letter X appeared on the screen. Each subject performed four runs of this task in each scanning session. This task was included because it has elicited reliable patterns of prefrontal activation in a variety of paradigms [D'Esposito et al., 1998], although there are no data suggesting sensitivity to bromocriptine.

The second task was a speeded version of the Wisconsin Card Sorting Test (WCST) [Grant and Berg, 1948]. In this task, subjects have to match a card presented at the bottom of the screen to one of four constant reference cards at the top of the screen. Each card consisted of an arrangement of triangles, circles, stars, or crosses, numbering from one to four, in one of four colors. Subjects were given feedback according to a changing criterion—the cards could be matched according to color, shape, or number. Subjects were not told the criterion in advance, but were given visual right/wrong feedback after each trial. The criterion was changed without indication after eight consecutive correct sorts (it was decided that the typical clinical criterion of 10 would have resulted in too few shifts per block). Subjects were given 2 sec to sort each card, after which a timeout was recorded and the next card presented. This pacing served to control the number of trials per block and to induce subjects to respond quickly. As well, the speeded administration served to increase the number of trials per block, presumably reducing the amount of time spent off task. A control version of this task included only cards identical to one of the four reference cards and therefore did not require subjects to learn or maintain any criterion. Four runs were performed in each scanning session. This task was included because we previously observed a substantial interaction between reading span and drug effect [Kimberg et al., 1997].

Finally, subjects performed a simple bimanual motor response task, in which a central fixation point (X) changed to the letter O every 2 sec. Subjects were to respond as quickly as possible to each "O" by depressing buttons with both thumbs. As with the other tasks, this task alternated in 40-sec blocks of a control task, during which the letter O did not appear. Only one run of this task was included per session. Because this task is not considered cognitively demanding, and might be expected to engage different neural mechanisms from the two more complex tasks, it was included to help discriminate between general effects of the drug and effects that are specific to tasks that engage higher cognitive functions.

fMRI scanning and apparatus

All scanning was done with a GE Signa 1.5 Tesla GE SIGNA Scanner (GE Medical Systems), using gradient-echo echoplanar imaging (EPI). A standard RF head coil was used. Head movement was restricted using a form-fitting vacuum cushion. Stimuli were rear-projected onto a screen near the subject's feet, and viewed through a mirror.

Functional data were acquired in 21 5-mm axial slices, with a TR of 2,000 ms and a TE of 50 ms. Resolution was 64×64 pixels in a 24-cm field of view (3.75 mm in-plane resolution). High-resolution (.9375 mm in-plane) T1-weighted structural images were also acquired in both axial and sagittal planes. Twenty seconds of "dummy" gradient and RF pulses preceded data acquisition to approach steady state tissue magnetization.

Data preprocessing

Off-line processing of all data was performed using software written in Interactive Data Language (Research Systems, Boulder, CO). All functional images were first converted from frequency space to cartesian space, using a distortion correction technique to correct for field inhomogeneities.

Sinc interpolation in time was used to correct for the order of acquisition of the slices [Zarahn, Aguirre, and D'Esposito, 1997a]. A motion correction algorithm [Friston et al., 1995] was applied to realign all functional images to an EPI scout image acquired immediately after the T1-weighted structural images. A slice-wise motion compensation method was used to

remove spatially coherent signal changes [Zarahn et al., 1997b].

Data analysis

Data were analyzed using the modified general linear model [Worsley and Friston, 1995], incorporating a 1/f model of temporal autocorrelation [Zarahn et al., 1997b]. A notch filter was used to remove high and low frequencies from the data. The reference function used to model task activity was convolved with a standard hemodynamic response function.

Maps of t-statistics were calculated for each task effect (e.g., the two-back vs. the control task) in each subject, separately for the drug and placebo sessions, using an estimate of effective degrees of freedom derived using the method described by Worsley [1994] (185 for the two-back and card-sorting tasks, 48 for the motor task). The resulting statistical maps (22, or two for each of 11 subjects, for the two-back and card sorting tasks; 18, or two for each of 9 subjects, for the motor task) were normalized to a standard template [Friston et al., 1995] and smoothed using a 15-mm FWHM Gaussian kernel. The large smoothing kernel was chosen to maximize sensitivity to larger effects. Given that there is no prior comparable imaging data, we felt that it would be most appropriate to look for larger effects first. Voxels for which data were not available from all subjects were excluded. These data (consisting of t-values) were then entered into a separate linear model, which included covariates coding for subject data and for the drug treatment. The general analysis path is depicted in Figure 1.

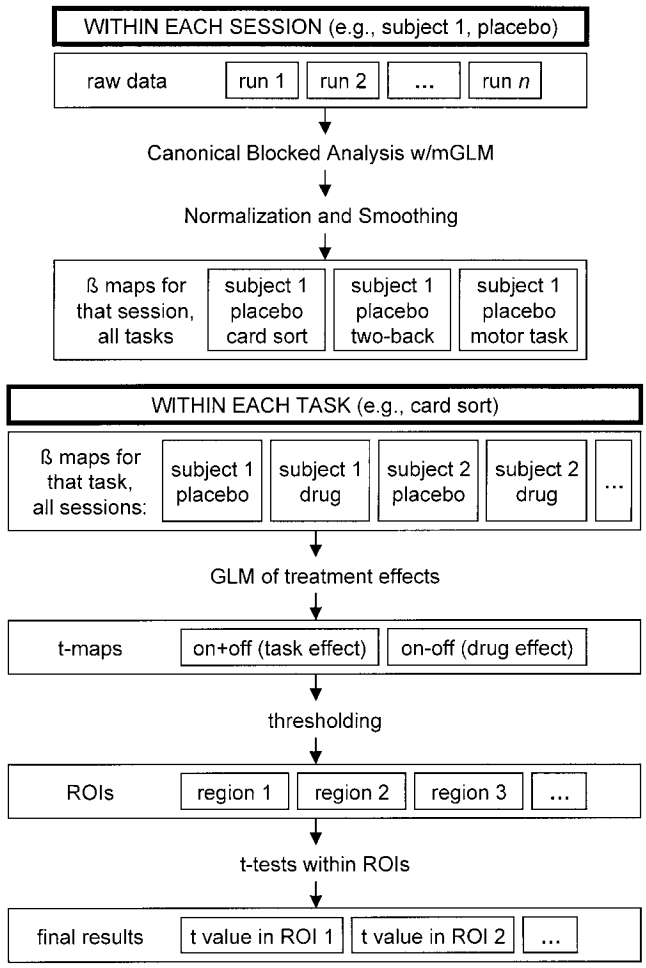

Figure 1.
Analysis path.

Maps of the main effect of each task were calculated using the sum of all the individual subject covariates, effectively comparing the mean task effect to zero. These task effect maps were thresholded at $t(10) > 8.65$ (one-tailed) for the two-back and card-sorting tasks, and $t(8) > 10.5$ for the motor task, thresholds corrected to $\alpha = 0.05$ using a method that has been empirically validated to control the map-wise false positive rate to the desired value [Aguirre, Zarahn, and D'Esposito, 1997; Zarahn et al., 1997b]. Discrete regions of interest (ROIs) were defined as regions of contiguous suprathreshold voxels within these thresholded maps. Only areas of greater activity during the experimental task (compared to the control task) were considered—regions showing less activity (i.e., deactivations) were discarded for the present analyses, as potentially reflecting cognitive components specific to the control task. Within each ROI, the effect of drug on the size of the task effect was tested using a t-test contrasting drug and placebo sessions, averaged across all of the voxels in each region.

RESULTS

Cognitive behavioral results

The effect of bromocriptine on performance was assessed separately for each task, using repeated measures ANOVA to assess the effect of drug treatment on error rates and reaction times. Note that speed was not emphasized in the instructions for the card-sorting test, although subjects perform the task more quickly with practice. Order of drug administration was included in all analyses to factor out residual learning/practice effects that were not eliminated by practice. Two subjects were omitted from behavioral analyses because the reading span test (used to categorize sub-

jects) had not been administered. Note that no additional behavioral main effects (which did not depend on reading span scores) were present with these two subjects included.

The reading span test was scored by summing the number of words correctly recalled from the five sets each of three, four, and five sentences, for a maximum of 60. By chance, the subjects divided well (5 and 4) along the same median used in our previous study, of 42.

No main effects of bromocriptine were observed in any of the tasks ($P > .2$ in all cases; see Table II), consistent with our previous results. A significant interaction of drug treatment and reading span was present in the card-sorting error score ($F(1,5) = 18.9$; $P < .01$), such that the drug was harmful (behaviorally) for low-span subjects and beneficial for high-span subjects. This interaction is opposite in direction to the one found in our earlier study [Kimberg et al., 1997]. To break down this effect further, we examined specific submeasures of the WCST. Similar interactions were found in categories ($F(1,5) = 17.9$; $P < .01$) and loss of set ($F(1,5) = 6.5$; $P = .051$). A similar trend was observed in mean reaction times ($F(1,5) = 4.6$; $P = .085$), arguing against a speed/accuracy trade-off. Perseverative errors showed the opposite pattern numerically, although non-significantly ($F(1,5) = .69$, $P > .4$).

No such interactions were observed in the two-back or motor response measures ($P > .05$ in all cases).

Neuroimaging results

In order to examine the effect of the drug on task-specific patterns of cortical activity, we first identified ROIs (as described above) for each task that showed greater activity during the task than during the corresponding control, and then tested the interaction of task and drug effects—i.e., the effect of the drug on the effect of the task—within each such region. Because these ROIs are treated here as prior hypotheses, the ROI analyses are not corrected for multiple comparisons.

Figure 2 shows these ROIs for each of the three tasks, and the directions of the significant drug effects. Table I shows the local maxima of each ROI.

In the two-back task, nine ROIs were identified. This pattern is fairly consistent with previous studies using this task, in our lab and others [D'Esposito et al., 1998]. Of these nine regions, one showed a significant interaction of drug and task. This was a posterior parietal region on the left side, including 261 voxels and extending from the middle occipital gyrus (area 19) to the superior parietal lobule (area 7) ($t(10) = -2.40$, $P = .037$). The difference between task and control activation in this region was smaller on bromocriptine than on placebo. Examination of this same ROI in the other two tasks yielded no significant drug interaction or trend.

In the card-sorting test, nine ROIs were identified. Again, this pattern is broadly consistent with previous studies using this task [Berman et al., 1995; Goldberg et al., 1998; Nagahama et al., 1996]. Of these nine regions, again one showed a significant interaction of drug and task, in the same direction as in the two-back task—task vs. control differences were reduced on the drug. This region included 5 voxels in the left insular region ($t(10) = -3.17$, $P = .01$). Again, this same area did not show a significant drug by task interaction in the other two tasks.

In the motor response task, 11 ROIs were identified. Of these 11 regions, two showed significant interactions of drug and task. A region of 10 voxels in Brodmann's area 19 on the left showed a drug-related decrease in task-related activity ($t(8) = -3.54$, $P = .008$). A single voxel in right premotor cortex showed a significant increase in task-related activation on bromocriptine ($t(8) = 2.35$, $P = .047$). This voxel was the only region to show a significant effect in this direction in any task. As with the previous tasks, these two ROIs did not yield significant drug effects in the other tasks.

Because of the significant behavioral interaction observed in the card-sorting test, we performed a corresponding analysis with the neuroimaging data. ROIs were identified for the areas of main task effect in this subset of nine subjects, and the interaction between reading span and drug effects was assessed in each identified region. Of 12 such regions tested, none yielded a significant interaction ($P > .2$ in all cases).

Because of the high concentration of D-2 receptors in the caudate nucleus [Lidow et al., 1989], we performed an additional ROI analysis of the caudate across subjects. Voxels in the caudate were identified by visual inspection of anatomical scans for each subject, and by comparison to a standard atlas [Talairach and Tournaux, 1988]. T-values were derived for each subject, and these t-values were entered into a group paired t-test. This analysis was repeated using the data from each of the three behavioral measures. In all three cases, there was no significant effect of drug on activity in the caudate ($P > .2$).

Finally, to test the hypothesis that bromocriptine had a consistent effect across task-relevant brain regions, we entered the t-values for each ROI into a sign test. Of 29 such regions, 25 showed less activity on

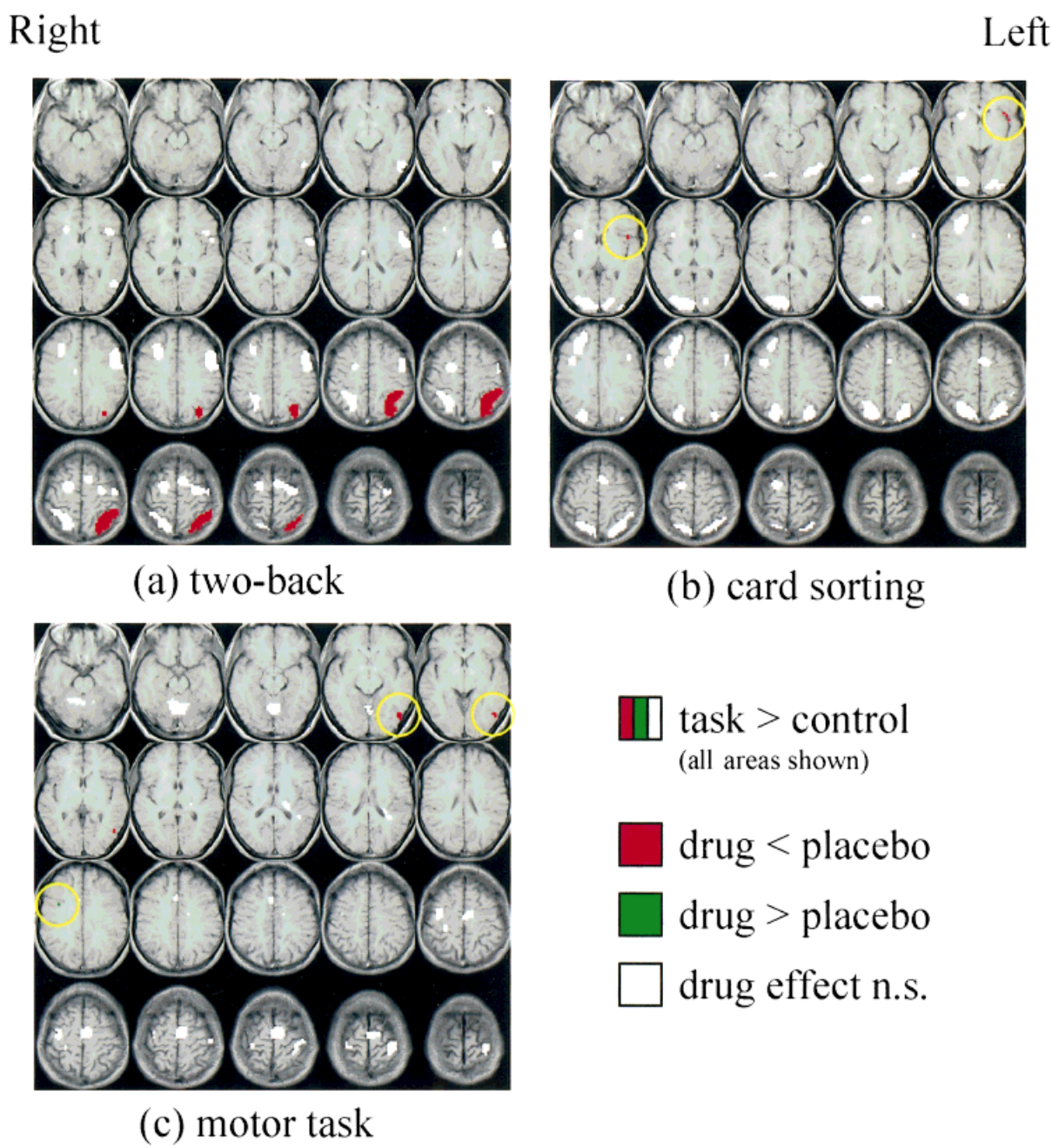

Figure 2.

Task and drug effects. In each panel, all regions displayed showed significant task-related activity (compared to the control task). Colored regions indicate main effects of task in the two-back task (**a**), card-sorting test (**b**), and motor task (**c**). The directions of drug effects (interaction of drug and task effects) are indicated by color. Regions in red showed a decrease in task-related activity on the drug. Regions in green (a single voxel in the motor task) showed an increase in task-related activity on the drug. Regions in white showed no significant drug-related modulation of task-related activity. Yellow circles are used to highlight smaller, less visible regions.

TABLE I. Coordinates of regions of interest (ROIs) for each of the three tasks*

Two-back			
Voxels	Drug effect (t)	Maximum	Brain region
6	−0.65	−26 23 0	R anterior insula
22	0.66	41 19 0	L anterior insula
12	0.53	−11 −15 20	R caudate
56	−0.92	−34 23 30	R 9/44 - inferior/middle frontal gyrus
75	−1.23	−23 −8 50	R 6/8 - superior frontal sulcus
327	−0.40	43 0 37	L 6 - precentral sulcus
		11 −4 60	L 6 - medial frontal gyrus
		26 −11 60	L 6 - medial frontal gyrus
		60 0 20	L 6 - precentral gyrus
51	−0.74	53 −68 −10	L 19 - middle occipital gyrus
176	−0.95	−29 −61 47	R7 - superior parietal lobule
		−26 −60 40	R40 - inferior parietal lobule
		−41 −49 45	R7/40 - superior/inferior parietal lobule
261	−2.40([a])	37 −64 47	L 7 - superior parietal lobule
		34 −75 40	L 19 - middle occipital gyrus
		41 −60 55	L 7 - superior parietal lobule
WCST			
Voxels	Drug effect (t)	Maximum	Brain region
5	−3.17([a])	43 16 −3	L anterior insula
		45 11 −5	L insula
		41 19 −5	L anterior insula
27	−0.38	−34 15 0	R anterior insula
174	−1.40	−34 27 27	R 9/46 - middle frontal gyrus
		−45 4 30	R 44 - inferior frontal gyrus
		−34 26 25	R 9/46 - middle frontal gyrus
26	−0.86	−26 −4 60	R 6 - precentral gyrus
18	−1.53	48 11 26	L 44 - inferior frontal gyrus
		49 8 30	L 44 - inferior frontal gyrus
		45 15 20	L 44/45 - inferior frontal gyrus
1	−1.82	49 38 25	L 44/46 - inferior frontal sulcus
34	−0.37	4 8 45	L 24 - anterior cingulate
565	−1.19	−15 −83 18	R 18/19 - middle occipital gyrus
		−34 −94 −5	R 18 - inferior occipital gyrus
		19 −90 0	L 18 - inferior occipital gyrus
		−8 −98 5	R 18 - inferior occipital gyrus
		−34 −90 15	R 19 - middle occipital gyrus
194	−1.38	30 −74 45	L 7 - superior parietal lobule
		34 −71 50	L 7 - superior parietal lobule
		39 −90 17	L 19 - middle occipital gyrus
		49 −53 50	L 40 - inferior parietal lobule
Motor			
Voxels	Drug effect (t)	Maximum	Brain region
93	−1.85	−19 −49 −25	cerebellum
10	−3.54([a])	48 −73 −7	L 19 - medial occipital gyrus

TABLE I. (continued)

Motor (continued)			
Voxels	Drug effect (t)	Maximum	Brain region
21	−0.32	26 −37 12	L 39 - middle temporal gyrus
		19 −26 10	L thalamus
		30 −45 15	L 39 - middle temporal gyrus
		41 −45 10	L 39 - middle temporal gyrus
12	−2.04	−8 11 35	R 24 anterior cingulate
1	2.35([a])	−41 4 25	R 6 precentral gyrus
2	1.52	15 −11 30	L 6 precentral gyrus
29	−0.52	−41 −15 50	R 4/6 precentral sulcus
8	−0.37	−30 −34 45	R 4 precentral gyrus
102	−0.23	8 −11 65	L 6 medial frontal gyrus
51	−0.14	34 −34 70	L 4 precentral gyrus
9	−0.64	−35 −32 63	R 4 precentral sulcus

* For regions with multiple local maxima, the center of mass (weighted by t value) is first listed, followed by the local maxima on the following lines. Structural localization was obtained by plotting coordinates on the standard template and visual comparison to standardized atlases [Talairach and Tournaux, 1988].

[a] Regions of significant drug effects, as described in the text. Positive t-values correspond to increased activity in the drug condition, negative t-values reflect reduced activity.

bromocriptine than on placebo, a difference significant at $P < .01$.

DISCUSSION

While previous studies have suggested cortical effects of bromocriptine in human subjects, the present study offers more direct evidence of changes in cortical activity that follow bromocriptine administration. The changes may be task specific—regions of apparent drug effects from one task did not generalize to the other two tasks tested. However, because the ROI interaction effects were not corrected for multiple comparisons, the probability of at least one false positive error may be greater than .05.

TABLE II. Behavioral results (means and standard errors)*

	Errors (SEM)	RT (ms)
WCST:		
Placebo	61.6 (3.2)	807.1 (33.3)
Drug	63.4 (2.9)	800.9 (24.9)
Two-back:		
Placebo	13.6 (1.8)	646.9 (16.8)
Drug	15.6 (2.1)	635.7 (20.1)
Motor:		
Placebo	0.3 (0.24)	303.0 (26.2)
Drug	0.9 (0.59)	300.1 (22.0)

* Errors are in absolute error count for WCST and motor tasks, percentages for two-back.

These results suggest that the selective dopamine agonist bromocriptine can cause systematic changes in cortical activity and that these changes are detectable with BOLD fMRI. Behaviorally, the drug's effect appeared (in one task) to interact with working memory capacity. However, this effect was opposite to the behavioral effect we observed earlier [Kimberg et al., 1997]. The significance of this is discussed below.

The most straightforward explanation for task-specific effects is that areas less active in the service of a given task are less sensitive to the level of dopaminergic neurotransmission. If bromocriptine's general effect were to reduce activity along dopaminergic pathways, we would expect to see little effect of the drug in areas that are not involved in a particular task. This is especially clear if the drug's net effects are inhibitory—regions with little task-related neural activity will be only weakly affected by inhibitory influences. In principle, this would seem to predict that regions active in the service of multiple tasks should show similar drug effects in all tasks. However, in practice the sensitivity of this comparison may differ between tasks. The magnitude of a drug effect, under this account, would likely be related to the magnitude and extent of task-related neural activity. Thus, even though there were inferior parietal ROIs in both the two-back task and the card-sorting task, the fact that we observed a drug effect only in the two-back disputes this account only weakly. However, it is also possible that the observed effects are due to more complex interactions, e.g., involving a convergence of

projections from dopamine-rich areas and task-relevant areas that do not have significant dopaminergic activity.

Although it is difficult to argue for task-general effects based on a study of only three tasks, a suggestive pattern emerges from the present study. Across the three tasks used, we looked for drug effects in a total of 29 regions of interest. The numerical direction of the drug effects was negative in 25 of these regions (reflected in negative t-values in Table I). That is, a significant majority of regions showed numerically less task-related activity on bromocriptine than on the placebo. This suggests that bromocriptine served more generally to reduce task-specific cortical activity and is consistent with predominantly presynaptic inhibitory effects of the drug that are observed early in the drug's time course [Pizzolato et al., 1985]. In this respect, the results are consistent with the results of Fletcher et al. [1996], in which a net antagonistic effect of low-dose apomorphine was attributed to presynaptic effects. However, such an interaction could also arise from the reduction in blood pressure commonly associated with bromocriptine [Mehta and Tolis, 1979] or via direct effects on cortical microcirculation [Iadecola, 1998; Krimer et al., 1998].

In light of this observation, it may be important to ask whether selecting ROIs on the basis of both the drug and the placebo sessions may have reduced our ability to detect task-related activity. Areas of signal attenuation would be less likely to show up as ROIs. However, selection on the basis of placebo data alone would have biased our target comparison of drug minus placebo—the two contrasts are not orthogonal. An alternative method would have been to select ROIs on the basis of previous results with these tasks. However, the regions of interest we identified were extensive and not grossly discrepant with previous findings. So although we acknowledge the possibility that additional (or larger) regions may have shown task-related activity, we feel the present method is reasonably sensitive.

The functional significance of the four task-specific regions identified suggests only that regions sensitive to bromocriptine are likely to be a subset of regions involved in task performance. Although the present results do not constrain our understanding of the functional role of these regions, in each case the regions identified are those that have been associated with their respective tasks in previous studies. This raises the issue of blood flow effects more directly. This kind of result might be expected simply from scaling of neural effects with globally reduced blood flow. Although we believe a priori that bromocriptine may have selective cortical effects due to its agonist properties, it is likely that circulatory effects underlie the present results to some extent.

Behavioral effects

As in our previous study [Kimberg et al., 1997], the present behavioral results suggest that the effects of bromocriptine may be sensitive to the working memory capacity of the subject. As with our previous study, we observed no main effects of bromocriptine on behavioral measures. We suggest that, as in our previous study, averaging across subjects with different capacities may have the effect of masking distinct but opposing effects.

However, the interaction observed in the present study contrasts strikingly with our previous results. Where previously we observed a more beneficial effect of the drug for low-span than high-span subjects, in the present study high-span subjects benefited from the drug, while low-span subjects were harmed (behaviorally). This result was unexpected, although there are several differences between the studies that may explain the differences. First, it is important to note that the task differed somewhat between the two studies. The deadline was somewhat shorter in the present study (2 sec vs. 3.5 sec), and the short block length necessitated by the fMRI procedure may have made the present version somewhat more difficult. While difficulty was not directly addressed in either study, it likely interacts with other factors that modulate the effects of bromocriptine.

Second, it may be important to note that in our previous study, the behavioral interaction in the WCST was reflected in both perseverative and nonperseverative errors. In the present study, the Drug $\times$ Span interaction was reflected in a variety of measures, including loss of set errors, but was notably absent in perseverative errors. Numerically, the effect on perseverative errors was consistent with the previous study (though note the present study, with a third the number of subjects, has much less power). The increased difficulty of loss-of-set errors in the present study may reflect the greater difficulty in avoiding this type of error given that the task is performed in 19-trial blocks rather than continuously.

Finally, differences in the timing of the two studies may also be important. In the present study the card-sorting test was the first test administered, at approximately 90 min following drug administration. In the previous study, the card-sorting test was administered substantially later in the protocol. If the time course of bromocriptine is indeed biphasic in the way described

by Pizzolato [1985], then we might expect more of an inhibitory effect in the present study and more of an excitatory effect in the earlier study. (Although the two-back task was administered at a time more comparable to the testing time of the previous study, we have no direct evidence from either study that two-back performance is sensitive to bromocriptine.) As described above, the neuroimaging evidence offers some tentative support for part of this observation. If this is the case, these mirror image results may be due solely to differences in the effects of the drug on cortical activity—an inhibitory effect might be more beneficial to high-span subjects while an excitatory effect might be more beneficial to low-span subjects. However, more detailed examination of the time course of both the neural and the cognitive effects of bromocriptine will be required to evaluate this account.

The absence of a behavioral drug effect in the two-back task may hint that the drug's effect is via some process required by the card-sorting test but not present in the two-back paradigm. Because the card sorting test is intuitively much more complex, this would not be surprising. However, it is worth considering the power limitations of the present design. Although we have no basis for predicting the size of bromocriptine's hypothesized effects on two-back performance, from previous studies we know that the drug's effects on performance at typically tuned cognitive paradigms are relatively subtle. Unless the two-back task were substantially more sensitive to bromocriptine than the tasks used in previous studies, we would suggest that the power to detect behavioral effects in the present study should be regarded as low.

Because we observed no behavioral main effect of bromocriptine, we cannot tell whether any observed cortical effects are consistently related to performance. It is possible that this is a result of our small sample size or a selection of tasks that are too insensitive to bromocriptine. It is also possible that the two-back and motor tasks did not tax dopamine-dependent systems as much as the card-sorting test and were therefore less sensitive in general to our manipulation. However, in the case of the card-sorting test, we observed a span-dependent drug effect (albeit one inconsistent with our previous study). In combining subjects spanning a broad range of verbal working memory capacity, we may have effectively averaged across subpopulations with opposing drug effects. Because this was the first study to examine the effects of bromocriptine with neuroimaging, the design was more appropriate to examining general (not span-dependent) effects of the drug.

Finally, the present results do not exclude the possibility that there are cortical effects of the drug that may be related directly to task performance. However, tests for such relationships were inconclusive in this small sample.

The weakness of the present results may stem from several related causes. First, the total number of subjects included is probably inadequate to detect any but the strongest and most consistent effects. More subtle effects of bromocriptine, both behavioral and neural, would be unlikely to reach statistical threshold in a study of 11 subjects. The cognitive effects of bromocriptine on young healthy subjects have indeed so far proved elusive—as reviewed earlier, results from three independent groups [Kimberg et al., 1997; Luciana and Collins, 1997; Luciana et al., 1992; Muller et al., 1998] have suggested that such effects are at best difficult to detect. These previous findings also suggest that the behavioral effects of dopamine agonists may be sensitive to a variety of factors, including dosage, timing of administration, the difficulty of the task, and the baseline ability of the subject. The present study included no attempt to explore this large design space. It therefore remains possible that the tasks used here were either poorly suited or poorly calibrated to be sensitive to bromocriptine. In light of the present weak findings, we would suggest that future attempts to examine the effects of selective dopamine agonists with fMRI should include more subjects, be more incisive with respect to the cognitive processes involved, and focus on parametric manipulation of factors of interest (e.g., dosage or timing).

The results presented here may be situated within an overall picture of the functional neuroanatomy of the dopaminergic system. This picture may include both areas rich in dopamine receptors, that are affected directly by dopminergic drugs, and areas with few dopamine receptors, but that are situated downstream from areas rich in dopamine receptors, and that may be affected indirectly. The scarcity of D-2 receptors in the neocortex argues against direct effects in the case of bromocriptine. However, cortical D-2 receptors in the prefrontal cortex may be most highly concentrated in layer V [Goldman-Rakic, Lidow, and Gallager, 1990], which may position them well to influence behavior in delayed response paradigms [Luciana et al., 1992]. Downstream effects of bromocriptine—i.e., those due to projections from areas rich in

D-2 receptors—may dominate the cortical effects of bromocriptine, as D-2 receptors are not as concentrated in the neocortex as they are in the striatum. In the context of studies examining behavioral effects of such drugs, these indirect effects may be more significant than effects due to direct modulation of neural activity, as the entire pattern of change in cortical activity is potentially relevant to behavior. However, both direct and indirect drug effects may be highly task-specific.

To the best of our knowledge, the present work is the first study to examine the effects of a selective dopamine agonist on cognitively evoked patterns of cortical activity using fMRI. Previous researchers [Mattay et al., 1996] have demonstrated that cortical effects of less-selective catecholaminergic agents can be detected using functional neuroimaging techniques. The present results suggest that effects of more selective dopaminergic drugs such as bromocriptine can be observed as well. The weakness of the present results, combined with the general weakness of behavioral findings with bromocriptine in young healthy subjects, suggests that these effects may be relatively subtle, and that future studies should be directed toward parametric exploration of factors that interact with drug effects.

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