A FUNCTIONAL MAGNETIC RESONANCE IMAGING STUDY OF THE EFFECTS OF PERGOLIDE, A DOPAMINE RECEPTOR AGONIST, ON COMPONENT PROCESSES OF WORKING MEMORY

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Abstract—Working memory is an important cognitive process dependent on a network of prefrontal and posterior cortical regions. In this study we tested the effects of the mixed D1–D2 dopamine receptor agonist pergolide on component processes of human working memory using functional magnetic resonance imaging (fMRI). An event-related trial design allowed separation of the effects on encoding, maintenance, and retrieval processes. Subjects were tested with spatial and object memoranda to investigate modality-specific effects of dopaminergic stimulation. We also measured baseline working memory capacity as previous studies have shown that effects of dopamine agonists vary with working memory span. Pergolide improved reaction time for high-span subjects and impaired reaction time for low-span subjects. This span-dependent change in behavior was accompanied by span-dependent changes in delay-related activity in the premotor cortex. We also found evidence for modality-specific effects of pergolide only during the response period. Pergolide increased activity for spatial memoranda and decreased activity for object memoranda in task-related regions including the prefrontal and parietal cortices.

Key words: prefrontal cortex, memory span, individual differences, spatial memory.

The catecholamine neurotransmitter dopamine is important for cognitive and motor functions, including working memory. The prefrontal cortex (PFC), an area rich in dopamine, has been linked to working memory by numerous physiological and lesion studies in both monkeys and humans (Fuster 1997). In monkeys, depletion of PFC dopamine is deleterious as lesions to this region on performance on working memory tasks (Brozoski et al., 1979). Moreover, dopaminergic augmentation improves working memory performance in monkeys (Armsten et al., 1995; Castner and Goldman-Rakic 2004; Castner et al., 2004) and in humans with frontal lesions (McDowell et al., 1998). Working memory tasks require many trial-specific subprocesses such as stimulus encoding, maintenance, and response selection, as well as task-general processes operating across all trial phases, including directed attention and maintaining task goals. It is unknown to what extent these subprocesses are sensitive to dopaminergic signaling. However, monkey electrophysiological studies have shown that augmenting dopamine increases cell firing in PFC neurons for the cue, delay, and probe periods of working memory tasks, while suppressing nonselective background activity (Sawaguchi et al., 1988, 1990a). Thus, dopamine may act as a signal-to-noise modulator in task-related PFC neurons (Cohen and Servan-Schreiber, 1993; Durstewitz et al., 2000; Sawaguchi, 1987), and these changes may have behaviorally relevant benefits for PFC function.

Few studies have investigated the effects of specific dopaminergic agonists on working memory in healthy human subjects. Luciana and Collins (1997) and Luciana et al. (1992) found improved performance on a delayed recall task for spatial locations, but not objects, when young subjects were given the D2 receptor agonist bromocriptine. This effect was present at long but not short delays, consistent with dopaminergic involvement in spatial working memory maintenance processes, rather than bottom-up sensory processes. Subsequently, Muller et al. (1998) found no performance improvement with bromocriptine on a delayed spatial recognition task, but a beneficial effect was found with pergolide, a mixed D1–D2 receptor agonist. No effects of either bromocriptine or pergolide were found on an object n-back task (Bartholomeusz et al., 2003). Thus, from these findings it remains unclear whether dopamine has modality-specific effects on working memory function, and whether different dopamine receptors (such as D1 and D2) mediate different behavioral processes.

Accumulating empirical findings from studies of animals and humans suggest that D1 and D2 receptors may have distinct functions. In monkeys performing delayed response tasks, D1 receptor agonists increase and antagonists decrease delay period activity, but D2 receptor agonists have no effect (Sawaguchi, 2000, 2001; Sawaguchi et al., 1990b). Instead, application of D2 agonists to task-related PFC neurons increases the firing rate of only those neurons responding at the time of the monkey’s memory-guided saccade, an effect which was reversible with D2 antagonists (Wang and Goldman-Rakic, 2004). Thus, it has been proposed that D1 effects may be more tonic in nature and important for working memory maintenance processes whereas D2 effects may be more phasic in nature and involved in updating these repre-
sentations (Braver and Cohen, 2000; see Bilder et al., 2002 for review).

Finally, previous studies have also revealed that the effect of dopaminergic augmentation depends on baseline working memory capacity or baseline task performance. For example, it has been observed that individuals with smaller working memory spans have improved behavioral performance after bromocriptine treatment, while high-span subjects are impaired (Kimberg et al., 1997; Mattay et al., 2000; Mehta et al., 2001). This pattern suggests remediation of sub-optimal dopamine in low-span or low-performing subjects, while relative “overdosing” of high-span subjects may contribute to performance changes. These baseline-dependent changes have also been reported following the nonspecific catecholaminergic agonists amphetamine (Mattay et al., 2000) and methylphenidate (Mehta et al., 2000). Interestingly, pergolide appears to have the opposite pattern of span-dependent effects: high-span subjects are more accurate at delayed response tasks while low-span subjects are less accurate (Kimberg and D’Esposito, 2003). A similar pattern of findings has also been observed in rat and monkey studies with dopaminergic drugs, supporting an “inverted-U” relationship between optimal dopamine levels and working memory performance (Arnsten and Goldman-Rakic, 1998; Granon et al., 2000; Murphy et al., 1996; Williams and Goldman-Rakic, 1995), and see Cools and Robbins (2004) for review.

The present study was designed to investigate the effects of the dopamine agonist pergolide on component processes of working memory. We used a delayed recognition task and an event-related design to isolate drug-related physiological changes during stimulus encoding, maintenance and retrieval processes. We included both spatial and object memoranda to additionally test for modality-specific effects. Also, working memory capacity was estimated for each subject to test for individual differences in response to pergolide, and the cortical changes that may underlie this interaction.

**EXPERIMENTAL PROCEDURES**

**Subjects**

Nine young healthy participants (six females; ages 20–39) gave written informed consent according to University of California guidelines. Subjects were screened for history of drug or alcohol abuse, use of prescription medication, neurological abnormality, blood pressure abnormality, and any other conditions that would preclude completing the study (e.g., metallic implants, difficulty with manual responses, low visual acuity). In addition, subjects were screened for current mood disturbances using the Beck Depression Inventory (Beck et al., 1961).

**Cognitive tasks**

Each subject participated in two functional magnetic resonance imaging (fMRI) scanning sessions spaced at least 14 days apart. The order of sessions was counterbalanced across subjects. Subjects were tested after administration of 0.1 mg pergolide or a lactose placebo in a double-blind design. fMRI scanning began approximately 120 min following administration of the drug or placebo as peak plasma concentrations are expected beginning at this time (Blin, 2003; Rubin et al., 1981). fMRI scans were acquired while subjects performed three types of working memory trials: delayed match to location, delayed nonmatch to location, and delayed match to object as shown in Fig. 1. All trials included the presentation of a single stimulus for encoding, followed by a 12.5 s delay interval, then a choice between two stimuli at response. Subjects indicated their choice by making a saccade to the stimulus. After the response there was an unfilled intertrial interval. Eye position was monitored in the scanner with an infrared videographic camera equipped with a telephoto lens (Model 504LRO, Applied Sciences Laboratories, Bedford, MA, USA, www.a-s-l.com) that focused on the subject’s right eye via a small mirror mounted inside the RF coil. Nine-point calibrations were performed at the beginning of each session and between runs if necessary. Eye movement data were scored offline with iLAB software (Gitelman, 2002) written for Matlab (Mathworks, Inc., Natick, MA, USA, www.mathworks.com) and verified when necessary with videotape recording of eye position acquired with the eyetracking camera. Subjects performed six 9-minute scanning runs in each session. Each run consisted of six spatial match, six...
spatial nonmatch and four object match trials, for a total of 96 trials per scanning session. A post-scan computer hardware problem affected five subjects’ placebo session behavioral data. Two subjects have incomplete records for reaction time and three subjects have incomplete records for accuracy.

Each subject was also given control tasks assessing motor speed and vigilance before and after the drug and placebo scanning sessions. Motor speed was measured with a box completion task. Subjects were presented with 10 rows of 10 line drawings of squares, each with one side missing. Subjects were asked to draw in the missing side of each square as quickly and accurately as possible. We also administered a numerical cancellation task to measure vigilance [Digit Vigilance Test (Lewis and Kupke, 1977)]. Subjects crossed out target digits from a field of 28 rows of 35 digits as quickly and accurately as possible. Finally, during a separate session subjects performed a listening version of the Daneman and Carpenter (1980) Reading Span Task to measure working memory capacity. Subjects listened to sets of two to six sentences and completed a written factual verification question for the content of each sentence. After the last sentence of each set, subjects recalled the final word of each sentence in the order in which they were presented.

**MRI data acquisition**

Functional and structural images were acquired with a Varian INOVA 4.0T scanner (Varian, Inc., Palo Alto, CA, USA, www.varianinc.com) and a TEM send-and-receive RF head coil (MR Instruments, Minneapolis, MN, USA, www.highfieldcoils.com). Head movement was restricted using a foam cushion adjusted for each subject. Stimuli were back-projected onto a screen at the subject’s waist, and viewed through a mirror mounted inside the head coil.

Functional images were acquired using a two-shot gradient echo EPI sequence, in 20 3.5-mm thick axial slices with a 0.5 mm inter-slice gap and a TR of 2200 ms. Each slice was acquired with a 22.4 cm field of view with a 64×64 matrix size resulting in an in-plane resolution of 3.5×3.5 mm. This slice prescription allowed for near whole-brain coverage. Data were acquired during six runs lasting 9 min each. The first 10 images from each run were deleted to approach steady-state tissue magnetization. High-resolution in-plane T1-weighted images were acquired using a gradient echo multislice sequence for anatomical localization. In addition, high-resolution MPFlash 3D T1-weighted scans were acquired for normalization to the Montreal Neurological Institute (MNI) reference brain.

**MRI data analysis**

Functional images acquired from the scanner were reconstructed, then image volumes were corrected for slice timing skew using temporal sync-interpolation and corrected for movement using rigid-body transformation parameters. Image preprocessing and statistical analyses were performed using SPM2 (Wellcome Department of Cognitive Neurology, London, UK, http://www.fil.ion.ucl.ac.uk/spm). Images were resampled to 2×2×2 mm and then smoothed with an 8 mm FWHM gaussian kernel. A high-pass filter was used to remove frequencies below 0.01 Hz from the data.

Structural T1-weighted images were normalized to the MNI reference brain. Transformations calculated from normalizing each subject’s structural images were applied to the functional images collected in each run. Data were analyzed using the general linear model (GLM; Worsley and Friston 1995). For each subject, the blood oxygen level dependent (BOLD) signal during the encoding, early and late delay, and response periods for each trial type was modeled as impulses of neural activity convolved with the SPM canonical HRF. The encoding covariate was placed at the onset of the cue period (first TR, Fig. 1). The early and late delay covariates were placed at the third and fifth TRs, respectively (Fig. 1). As encoding processes may continue into the early delay period, our estimate of delay activity is from the late delay covariate. The early delay period is modeled to reduce noise in the estimate of the baseline, but not included in the mapwise analysis (Zarahn et al., 1999). The response covariate was placed at the onset of the retrieval period (seventh TR, Fig. 1). These covariates were then entered into the GLM. Maps of parameter estimates were computed from the GLM to assess the magnitude of activation during each trial period. Stereotactic coordinates of peak activations were reported with respect to the MNI coordinate system.

Group random-effects analyses were performed for each contrast of interest to test whether the mean parameter estimate magnitude was significantly different from zero. Areas of significant activation in the individual session mapwise analyses were determined by identifying regions whose peak activation exceeded a threshold of t=4.30, P≤0.001, uncorrected for multiple comparisons. We then performed a conjunction analysis to isolate task-related regions common to both the drug and placebo session, as this voxel selection is independent from the test for drug-related changes. For each of the three task periods, the thresholded map of the main effect of task in the pergolide session was used to mask the thresholded map of the main effect of task in the placebo session. Regions surviving this conjunction at less than P≤0.001 uncorrected are reported. PFC activity is often variable in individual subjects (e.g. Druzel and D’Esposito, 2003), a factor affecting sensitivity in group analyses. To compare task-related PFC activity during multiple task periods, we also examined PFC regions surviving thresholds of t=3.36, P<0.005 uncorrected and t=2.33, P<0.01 uncorrected in each individual subject. We tested the parameter estimates extracted from all conjunction regions for effects of pergolide.

**RESULTS**

**Behavioral results**

**Group differences.** Behavioral data are shown in Table 1. There was no main effect of pergolide on reaction time [F(1,6)=0.10, P=0.77] and no interaction between trial type and pergolide treatment [F(2,12)=0.27, P=0.76]. There was a significant effect of trial type on reaction time [F(2,12)=7.77, P=0.007]. Planned comparisons revealed that subjects were faster to respond in the spatial match-to-location condition, when they could prepare their response during the delay period, than in the spatial nonmatch-to-location and object match conditions, when subjects could not prepare their response during the delay period [spatial match<nonmatch (t(8)=-2.79, P=0.03); spatial match<object (t(8)=-2.76, P=0.03); spatial nonmatch<object (t(8)=-0.70, P=0.51)]. As indicated by the lack of interaction between trial type and drug treatment, planned comparisons revealed that pergolide did not substantially change this pattern [spatial match<nonmatch (t(8)=-3.06, P=0.02); spatial match<object (t(8)=-1.31, P=0.23); spatial nonmatch<object (t(8)=0.59, P=0.57), Fig. 2A].

Subjects were on average greater than 88% accurate in all conditions of both sessions. Mean accuracy for the pergolide session was 90±4% and for the placebo session 92±2%. There were no effects of pergolide on accuracy for any of the trial types (P>0.43 for all).

**Individual differences.** The range of spans in our group of subjects was from 2.5–5.0 items with a mean score of 3.77±0.91 items. Subjects scoring above the
mean (four or greater items, n=6) were considered high-span individuals, while those scoring below the mean (less than four items, n=3) were considered low span. This distribution agrees well with the original report of this task by Daneman and Carpenter (1980) who observed a range of spans was two to 4.5 items with a mean of 2.95±0.72 items.

Subjects’ working memory capacity as measured by span reliably predicted the effect of pergolide on reaction time. High span subjects were faster and low span subjects slower on pergolide relative to placebo [high span: t(5)=3.78, P=0.03; low span: t(2)=-4.63, P=0.04, Fig. 2B]. To identify whether this effect was present for all trial types, we tested the drug effect in each trial type for high- and low-span subjects. This pattern was strongest for spatial match trials [high span: t(5)=6.12, P=0.009; low span: t(2)=-6.93, P=0.02], was a similar trend for spatial nonmatch trials [high span: t(5)=-2.5, P=0.82; low span: t(2)=-3.12, P=0.09], but was not evident for object trials (high span: t(5)=1.41, P=0.25; low span: t(2)=-1.22, P=0.35, Fig. 2C). Span did not predict the drug effect on accuracy for any trial type (P>0.26 for all).

Control tasks. We administered control tasks of motor speed and vigilance before and after each session. Using a within-subjects analysis of variance (ANOVA) we examined the effect of session (pergolide and placebo) and administration time (pre-scan vs. post-scan) on performance of the box completion and numeral cancellation tasks. There was no effect of session [F(1,8)=1.52, P=0.25] or administration time [F(1,8)=0.20, P=0.67] on box completion performance, and no interaction between the two factors [F(1,8)=0.81, P=0.39]. Likewise, we did not observe an effect of pergolide treatment [F(1,8)=0.002, P=0.97] or administration time [F(1,8)=0.23, P=0.65] on numeral cancellation performance, and again observed no interaction between the two factors [F(1,8)=0.30, P=0.60]. In addition, there was no effect of subjects’ listening span on control task performance for either of the two tasks [motor speed: F(1,7)=0.62, P=0.46, vigilance: F(1,7)=0.06, P=0.81].

Neuroimaging results

Task-related regions with significant effects in both pergolide and placebo sessions are shown in Table 2 and Fig. 3. Regions surviving this contrast had significantly greater activity in both sessions for all three trial types relative to the intertrial interval. The mean BOLD signal change from each region of interest was then extracted for each session to test for effects of pergolide.

Cue period effects of pergolide

Across sessions, we observed significant activation in six regions including left middle frontal gyrus [Brodmann area (BA) 46] extending to inferior frontal gyrus, right dorsal middle frontal gyrus (BA 6), left superior and inferior parietal cortex and bilateral angular gyrus (BA 7, 40) (Fig. 3). Pergolide treatment significantly reduced activity across trial types in the left middle/inferior frontal gyrus ([t(8)]=-2.67, P=0.03), and the left superior parietal cortex ([t(8)]=-2.47, P=0.04, Fig. 4). The drug effects were not significantly different for object and spatial trials (P>0.15 for all).

Delay period effects of pergolide

Across sessions, we observed significant activation in three regions during the delay period: right supplementary motor area (BA 6), left premotor cortex (BA 6) and left inferior occipital gyrus corresponding to BA 19 (Fig. 3). In addition, individual analyses revealed that seven subjects had significantly active regions in middle or inferior frontal gyrrus: four subjects, right middle frontal gyrus, one subject right inferior frontal gyrus, two subjects left middle frontal gyrus. There was no reliable effect of pergolide treatment on PFC delay period activity in this subset of subjects (Sign test, N+=5 N=2, P=0.45).

Pergolide significantly reduced delay-period BOLD signal across all trial types in the left premotor cortex ([t(8)]=-3.18, P=0.01, Fig. 5A). This effect was found for all three trial types [match: t(8)=-2.28, P=0.05; nonmatch: t(8)=-3.07, P=0.02; object: t(8)=2.53, P=0.04]. The effect of pergolide on premotor cortex BOLD signal correlated significantly with the effect of pergolide on reaction time. Subjects who were
Fig. 2. Reaction time (A) for each trial type in the pergolide (filled bars) and placebo sessions (open bars). Individual differences in working memory capacity predicted changes in reaction time following pergolide (B) for high span (n=6 left panel) and low span subjects (n=3 right panel). High span subjects were faster, and low span subjects slower after pergolide treatment, a pattern strongest for spatial trials (C).
slower after pergolide relative to placebo had reduced prefrontal cortex BOLD signal on pergolide relative to placebo, while subjects who were faster on drug compared with placebo had increased BOLD signal on pergolide relative to placebo \( (r=-0.78, P=0.04, \text{Fig. 5B}) \). In addition, there was a significant relationship between subjects’ working memory span and the drug effect on prefrontal cortex BOLD signal. The lower the subject’s working memory capacity, the larger the drug-induced decrease in prefrontal cortex activity \( (r=0.70, P=0.04, \text{see Fig. 5B}) \).

### Table 2. Regions of interest showing increased activation in both pergolide and placebo sessions

<table>
<thead>
<tr>
<th>Region</th>
<th>Size (voxels)</th>
<th>Coordinates (x y z)</th>
<th>Peak t</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cue period</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Left superior parietal cortex (BA 7)</td>
<td>27</td>
<td>-14 -72 56</td>
<td>6.10*</td>
</tr>
<tr>
<td>Left inferior parietal cortex (BA 40)</td>
<td>44</td>
<td>-46 -52 44</td>
<td>5.65*</td>
</tr>
<tr>
<td>Right angular gyrus (BA 40)</td>
<td>174</td>
<td>40 -56 52</td>
<td>10.24*</td>
</tr>
<tr>
<td>Left angular gyrus (BA 40)</td>
<td>3</td>
<td>-52 -48 32</td>
<td>4.61</td>
</tr>
<tr>
<td>Right dorsal middle frontal gyrus (BA 6)</td>
<td>11</td>
<td>42 6 58</td>
<td>4.45</td>
</tr>
<tr>
<td>Left middle frontal gyrus (BA 46)</td>
<td>12</td>
<td>-40 48 4</td>
<td>6.48</td>
</tr>
<tr>
<td><strong>Delay period</strong></td>
<td></td>
<td></td>
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<tr>
<td>Pre-SMA (BA 6)</td>
<td>57</td>
<td>8 10 56</td>
<td>15.71*</td>
</tr>
<tr>
<td>Left premotor cortex (BA 6)</td>
<td>34</td>
<td>-30 -4 48</td>
<td>16.61</td>
</tr>
<tr>
<td>Left inferior occipital cortex (BA 19)</td>
<td>12</td>
<td>-32 88 6</td>
<td>5.57</td>
</tr>
<tr>
<td><strong>Probe period</strong></td>
<td></td>
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<tr>
<td>Pre-SMA (BA 6)</td>
<td>24</td>
<td>0 6 62</td>
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<tr>
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<tr>
<td>Left inferior parietal cortex (BA 7)</td>
<td>704</td>
<td>30 -64 50</td>
<td>10.27*</td>
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<tr>
<td>Left inferior parietal cortex (BA 40)</td>
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<td>-58 -32 46</td>
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<tr>
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<tr>
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<tr>
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<td>-2 24 38</td>
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<td>38 30 34</td>
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<tr>
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<tr>
<td>Right putamen</td>
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<td>34 8 2</td>
<td>7.03</td>
</tr>
<tr>
<td>Left superior temporal pole (BA 30)</td>
<td>3</td>
<td>-48 12 2</td>
<td>6.10</td>
</tr>
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Regions marked with (*) are also significant at \( P<0.05 \) corrected, using Gaussian random field theory (Worsley et al. 1998).

To address the concern that global effects of pergolide were responsible for the effects presented here, we tested the main effect of pergolide collapsed across all task periods and all trial types at \( t=-4.30, P<0.001 \) uncorrected. One region in the right precentral gyrus \( (56 0 40) \) showed increased global activity on pergolide relative to placebo. Several regions showed decreased global activity on pergolide, including superior frontal gyrus/pre-SMA \( (-12 10 54, 22 26 60, -4 4 52) \) right inferior frontal gyrus \( (48 36 8) \), left frontal eye fields (FEF) \( (-24 260) \), right cuneus \( (16 -78 42) \), right calcarine cortex \( (8 -72 14) \), right superior occipital gyrus \( (24 -86 12) \), and right hippocampus \( (18 -34 4) \). None of these regions showing global modulation overlapped with any of the task-related regions of interest reported here, even at a lower threshold \( (t=-2.33, P<0.01 \) uncorrected).
DISCUSSION

In this study we report behavioral and brain effects of the mixed D1–D2 dopamine receptor agonist pergolide during the performance of working memory task in young healthy subjects. We identified task-related regions across both sessions during the encoding, delay and retrieval task periods and tested these regions for effects of pergolide treatment. We observed encoding-related activity in the prefrontal and parietal cortices, delay-related activity in the PFC, premotor regions, and visual cortex, and response-related activity in widespread regions including the PFC, ACC, superior and inferior parietal cortex, and temporal cortex, consistent with previous event-related fMRI studies of working memory (e.g. Braver et al., 1997; D’Esposito et al., 2000; Pessoa et al., 2002; Postle et al., 2000; Zarahn et al., 1999, see Cabeza and Nyberg, 2000; Wager and Smith, 2003 for review).

Pergolide treatment affected subjects differently in this spatial and object delayed response task depending on their working memory capacity, as measured by a listening span test (Daneman and Carpenter, 1980). Low-span subjects had slower reaction times following pergolide, while high-span subjects had faster reaction times. This finding is consistent with a prior behavioral study of pergolide that used different working memory tasks (Kimberg and D’Esposito, 2003).

This finding seems inconsistent with prior studies of effects of bromocriptine, another dopamine agonist, which have shown that this medication improves performance on working memory tasks in individuals with low span, and impairs performance in individuals with high spans (Kimberg et al., 1997). However, there are several important differences between bromocriptine and pergolide that may explain the divergent pattern of behavioral results. For example, receptor-binding studies suggest that although both drugs bind to D2 receptors, pergolide has greater affinity at the D1 receptor than bromocriptine (Miyagi et al., 1996; Piercey et al., 1996; Wachtel, 1991). In monkey studies, D1 and D2 receptor activity affect different stages of processing during delayed response task (Wang et al., 2004). For example, selective D1 receptor activation may mediate working memory maintenance processes since specific D1 antagonists suppress delay-related activity, and D1 agonists enhance delay-related activity in only those neurons with direction-selective activity (Sawaguchi, 2001; Williams and Goldman-Rakic, 1995). In contrast, D2
receptor activation may mediate more phasic working memory processes since D2 receptors agonists enhance and D2 antagonists reduce saccade-related activity in monkeys during oculomotor delayed response tasks (Wang et al., 2004). These phasic dopamine signals are transient increases in response to behavioral events, while low levels of tonic dopamine are maintained by slow irregular cell firing. Tonic dopamine levels may then regulate phasic dopamine release by acting at D2 autoreceptors (Grace, 1991).

One recent model proposes that while optimal D1 receptor stimulation may help maintain active memory representations in the PFC, D2 receptor activation may serve as a gating signal to update the contents of working memory (Cohen et al., 2002, see Bilder et al., 2002 and Cools and Robbins 2004 for review). Pergolide may therefore act presynaptically at D2 autoreceptors to reduce phasic dopamine release during the task and impact the updating or gating of information during different states of mnemonic processing like encoding, maintenance or retrieval. Thus, the opposing behavioral effects that we and others have found may reflect differences in the affinity of pergolide for the D1- and D2-family receptor subtypes compared with bromocriptine. It is notable that despite its structural similarity to D2 and D4 receptors, the D3 receptor appears to have different functional properties. For example, while typical antipsychotic drugs are D2 antagonists, D3 agonists rather than antagonists appear to have antipsychotic properties (Fink-Jensen, 2000; Lahti et al., 1998). There is also neurophysiological evidence to support the unique functional properties of the D3 receptor. The D2 agonist quinpirole mimicked dopamine-induced PFC burst firing, which was reversed by D2 antagonism with sulpiride. In contrast, a D3 agonist increased the threshold for this type of dopamine-induced PFC burst firing (Wang and Goldman-Rakic, 2004), a property more characteristic of D2 antagonists.

Importantly, in one recent investigation of agonist binding affinities for the D1 and D2 receptor families in human striatal tissue, pergolide had greater affinity for the D3 receptor subtype compared with both D1 and D2 receptors [Kᵢ reported as D1 447; D2 10.3; D3 0.86 nM], while bromocriptine did not display significant effects at D3 receptors (Gerlach et al., 2003). D3 receptors belong to the family of D2-like receptors, and are present predominantly in the limbic system, including the hippocampus, mammillary bodies, and the nucleus accumbens (Bouthenet et al., 1991). D3 signaling has been implicated in conditions with dopaminergic dysregulation, including schizophrenia and addiction (see Black et al., 2002 for review), although little work has investigated its relevance to working memory. It is notable that despite its structural similarity to D2 and D4 receptors, the D3 receptor appears to have different functional properties. For example, while typical antipsychotic drugs are D2 antagonists, D3 agonists rather than antagonists appear to have antipsychotic properties (Fink-Jensen, 2000; Lahti et al., 1998). There is also neurophysiological evidence to support the unique functional properties of the D3 receptor. The D2 agonist quinpirole mimicked dopamine-induced PFC burst firing, which was reversed by D2 antagonism with sulpiride. In contrast, a D3 agonist increased the threshold for this type of dopamine-induced PFC burst firing (Wang and Goldman-Rakic, 2004), a property more characteristic of D2 antagonists. Although pergolide has greater affinity at D1 receptors than bromocriptine, actions at D3 receptors are approximately 400 times stronger (Gerlach et al., 2003), suggesting the D3-mediated effects may dominate. Thus, the actions of pergolide at D3 receptors may have D2-family antagonist effects that explain the opposing pattern of behavioral results to agonists like bromocriptine. We have previously suggested that baseline differences in D2 receptor concentration may explain the span-dependent performance changes observed with bromocriptine (Kimberg et al., 1997). It is possible that similar differences in D3
receptor concentration may underlie the effects of pergolide reported here.

To investigate the neural mechanisms underlying the effects of pergolide, we isolated task-related networks engaged during both the drug and placebo session, and then interrogated these brain regions for drug effects. First, we found a potential neural mechanism for the observed span-dependent behavioral changes following dopaminergic...
augmentation. During the delay period of our task, the effect of pergolide depended on subjects' working memory capacity and behavioral performance. High-span subjects had drug-induced increases in premotor cortex activity, with accompanying decreases in reaction time. In contrast, in low-span subjects, pergolide decreased premotor cortex activity and slowed reaction time. These effects were found toward the latter part of the delay period suggesting that this premotor cortex activity reflected motor preparatory processes.

Neurophysiology studies of primate delayed response tasks show that firing rates of premotor cortical neurons were negatively correlated with reaction time, such that increased motor preparatory activity predicted faster reaction times (Riehle and Requin, 1993). Thus, trials with enhanced delay-period preparatory cortical signals were associated with improved behavioral performance. We observed a similar pattern following pergolide treatment, with enhancements in premotor cortex activity for subjects with behavioral improvements. Lateral premotor cortex has been implicated in externally-cued movements, as opposed to the supplementary motor area and pre-SMA involved in internally-cued movements (Deiber et al., 1991; Mushiake et al., 1991). As these enhancements were seen for all trial types, the preparation is not likely to be specific to an oculomotor program, but rather a task-general response readiness or motor set.

Dopamine may be an important signal modulating the maintenance and preparation of a behaviorally relevant upcoming motor action. For example, dopamine is involved in reinforcement learning (Ljungberg et al., 1991), and changes in both striatal and cortical activations in dopaminergic regions have been demonstrated in subjects learning a rewarded task (Knutson et al., 2001; McClure et al., 2004; Ramnani et al., 2004; Ullsperger and von Cramon, 2003). In an fMRI study, monetary reward improved reaction time when subjects could prepare for the upcoming motor response. Performance improvements were accompanied by preparatory-period changes in premotor cortical activity (Ramnani and Miall, 2003). Likewise, monkeys performing a memory-guided saccade task are faster and more accurate when cued that large rewards are to be given at the end of the trial compared with a cue signaling small rewards. This behavioral enhancement is accompanied by greater delay-period activity in motor cortical regions compared with PFC, which suggests that motor readiness improved performance (Roesch and Olson, 2003, 2004). A similar mechanism may explain the behavioral facilitation we observed in high-span individuals.

We also found that pergolide treatment reduced activity during the presentation of the cue across all trial types in the PFC and parietal cortex. Prior work with primates showed dopamine applied to PFC neurons increased cell firing rates during the cue, delay and response periods of a spatial-delayed response task (Matsumura et al., 1990). However, signal enhancement was specific to task-related neurons with direction-selective memory fields. Dopamine reduced cell firing rates in those pre-cue and delay-period cells without memory fields (Sawaguchi et al., 1988). Dopaminergic modulation occurred via task-related firing increases which were larger than background activity changes (Sawaguchi et al., 1990a). Applying dopamine antagonists to the PFC reduced this effect, and did so by reducing task activity more than background activity (Sawaguchi, 2001; Sawaguchi et al., 1990b). These findings from single-cell studies suggest that dopamine may act as a signal-to-noise modulator, with optimal levels of dopamine resulting in optimal signal-to-noise during task-relevant processes. Pergolide may thus have increased signal-to-noise in subpopulations of PFC and parietal task neurons that facilitated encoding performance. At the population level, this may appear as a net reduction in task-related activity (e.g. Mattay et al., 2002; Mehta et al., 2000). Most subjects were improved following pergolide (high-span, n=6), and all these subjects had numerical decreases in each encoding region of interest. In contrast, the low-span subjects (n=3) had numerical increases in five of six encoding regions, suggesting that encoding modulation may play a role in behavioral performance following pergolide treatment.

Finally, we found evidence for modality-specific neural effects of pergolide during the presentation of the probe. Several studies have shown performance enhancements following dopamine agonists that were selective for spatial memoranda (Bartholomeusz et al., 2003; Luciana and Collins, 1997; Mehta et al., 2001; Muller et al., 1998). For example, Luciana et al. (1997) found improved performance on a delayed spatial location task when young subjects were given bromocriptine, but performance on a delayed nonspatial task was unchanged. However, as suggested by Luciana and colleagues (1997), it is possible that this apparent differential sensitivity of spatial and object working memory to D2 stimulation in fact reflects differential demands for action preparation processes. Thus, Luciana et al.'s (1997) spatial working memory task required reproduction of locations and thereby enabled the preparation of a motor trajectory, whereas the non-spatial WM task did not allow such preparation. Therefore, this selective effect of bromocriptine on spatial working memory tasks may well be due to a specific effect of D2 receptor stimulation on the guidance of movement or the preparation of a motor set as opposed to the maintenance of (spatial versus non-spatial) representations. In keeping with this hypothesis, recent observations by Wang et al. (2004) revealed that D2 receptor agents iontophoretically applied to monkey PFC neurons dose-dependently modulated memory-guided saccade-related activity, but did not affect delay-related neuronal activity. Also, in a human behavioral study, Muller and colleagues (1998) did not find improvements with bromocriptine on a task that required delayed recognition for spatial dot patterns where subjects could not prepare for the action during the delay period.

In our study, we included conditions with balanced oculomotor response demands, but subjects could only prepare the saccade vector during the delay period in the spatial match condition. In the spatial nonmatch condition, subjects attended and responded to spatial information,
but had to wait until the presentation of the probe stimulus to prepare their saccade. Likewise, in the object match condition, subjects attended to object information, but could not prepare their saccade until the presentation of the probe stimulus. The drug-related increase in probe activity we found was evident for both spatial match and spatial nonmatch trials, irrespective of whether subjects could (match) or could not (nonmatch) prepare a saccade in advance. Thus, pergolide appears to have a greater effect when selecting responses based on spatial than nonspatial memoranda. This finding will require further investigation to disentangle the specific probe-related processes that are being influence by pergolide.

We did not find significant behavioral effects of pergolide treatment for all trial types. It is possible that, despite the 12.5-second delay used here, recognition of a single target item or location was not sufficiently difficult. We may have suffered from ceiling effects, particularly in measuring changes in accuracy rates which here were high for all conditions. In other studies, maximal behavioral effects have been shown only for the most difficult trial conditions [e.g. 3-back trial types on the n-back paradigm, (Mattay et al., 2003)]. Subsequent studies may also benefit from including conditions with greater memory demands to examine how cortical differences explain behavioral changes.

It is also possible that we did not have enough power to detect behavioral differences across all conditions. For example, Kimberg and colleagues’ (2003) significant improvements for high-span and impairments for low-span subjects were found in a group of 31 participants. Sample sizes of the magnitude we report here (n=9, including six high-span and three low-span subjects) are common to other pharmacological imaging paradigms in young healthy subjects (e.g. Furey et al., 2000; Hariri et al., 2002; Kimberg et al., 2001; Knutson et al., 2004; Mehta et al., 2001), even in paradigms explicitly discussing individual differences (e.g. Mattay et al., 2003; Mehta et al., 2000). Nevertheless, future studies with larger sample sizes may benefit from balanced recruitment of high- and low-span subjects to further investigate the mechanism of span-dependent effects of pergolide on working memory performance.

There were no effects of pergolide on control tasks measuring motor speed or vigilance. In addition, there was no interaction of drug effect with listening span group for these control tasks, suggesting changes in these processes were not responsible for the drug effects we report here. Global modulation of the fMRI BOLD signal by pharmacological agents may indicate vascular changes or nonspecific modulation outside of the task network. To address this concern, we identified regions showing nonspecific global increases or decreases during the pergolide session relative to the placebo session. There was no overlap between the task-specific regions we report here and regions displaying global signal changes across all task periods and all trial types.

CONCLUSION

In summary, in this study of working memory processes in young healthy subjects on and off the mixed D1–D2 family agonist pergolide we have found evidence of individual differences in behavioral response to the drug, with improvements for high-span subjects and impairments for low span subjects. Patterns of performance change were most strongly associated with brain activity changes during the delay period: increased motor preparatory activity in the premotor cortex predicted improved performance for the high-span subjects, with the reverse pattern for low-span subjects. This suggests that the mechanism of performance enhancement by pergolide may be to enhance preparation for the motor set required to successfully complete the task. During the response period, we found evidence of modality specificity with pergolide, with increased activity during spatial trials and reduced activity for object trials in task-related regions. Together, our findings suggest that dopamine can influence different component processes of working memory, perhaps due to effects on different classes of dopamine receptors.

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