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ORIGINAL INVESTIGATION

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Processing efficiency of a verbal working memory system is modulated by amphetamine: an fMRI investigation

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Abstract *Rationale:* Working memory performance may be improved or decreased by amphetamine, depending on baseline working memory capacity and amphetamine dosage. This variable effect suggests an optimal range of monaminergic activity for working memory, either below or above which it is compromised. We directly tested this possibility with human participants by varying amphetamine dosage and measuring the efficiency of cortical processing in brain regions associated with working memory. *Objectives:* The modulation of cortical processing in a verbal working memory network by dextroamphetamine (D-amph) was examined using BOLD functional magnetic resonance imaging (fMRI) with healthy participants. The goal of the study was to test the hypothesis of an inverted U-shaped relationship between D-amph dose and processing efficiency of a verbal working memory system. *Methods:* D-amph dosage was increased cumulatively every 2 h across four scanning sessions collected in a single day. The primary measure used for analyses in this study was the extent of activation in brain regions empirically defined as a working memory network. *Results:* An inverted U-shaped relationship was observed between the amount of D-amph administered and working memory processing efficiency. This relationship was specific to brain areas functionally defined as working memory regions and to the encoding/maintenance phase (as opposed to the response phase) of the task. *Conclusion:* The results are consistent with the hypothesis that the neurochemical effects of amphetamine modulate the efficiency of a verbal working memory system. The effect of amphetamine on working memory in healthy individuals may provide insight regarding the working memory deficits seen in schizophrenia, given the overlap between neurochemical systems affected by amphetamine, and those disordered in schizophrenia.

Keywords Amphetamine · Working memory · fMRI · Dose–response relationship · Monoamines · Dopamine · Schizophrenia · Cognition

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Introduction

Working memory impairments are observed in many psychiatric disorders, including depression and schizophrenia (Gold et al. 1997; Goldberg et al. 1998; Pelosi et al. 2000; Landro et al. 2001). In fact, disturbance of working memory has been described as one of the cardinal cognitive deficits in schizophrenia (Goldman-Rakic 1994). Recent functional magnetic resonance imaging (fMRI) work has demonstrated that these working memory deficits are manifested as abnormal patterns of cortical BOLD activations during the acute phase of schizophrenia that "normalize" with treatment. Mendrek et al. (2004) demonstrated that in acutely ill patients with schizophrenia, the extent of cerebral activation during performance of a simple working memory task is exacerbated initially, but returns to normal levels after 6 weeks of treatment with antipsychotic medication. The apparent over-activity of the working memory network during the acute phase of schizophrenia, coupled with behavioural working memory impairments, is consistent with a deficit in the efficiency of neural processing in these cortical regions.

Dopaminergic over-activity, either via increased D2 receptor density, increased dopamine (DA) release, or ele-

vated synthesis, is one of the best supported biochemical hypotheses for the acute symptoms of schizophrenia (Soares and Innis 1999; Laruelle et al. 1996; Laurelle and Abi-Dargham 1999). More recently, however, over-activity of other monoamines, including serotonin, has been shown to play a key role (Abi-Dargham et al. 1997). The possibility exists, then, that abnormalities in monoaminergic function underlie the reduced working memory-related cortical processing efficiency associated with the acute phase of schizophrenia.

The present study directly tested whether monoaminergic activity modulates working memory processing efficiency. We manipulated monoaminergic activity in healthy individuals using varying doses of dextroamphetamine (D-amph). Amphetamine is an indirect DA agonist that also enhances serotonin and norepinephrine activity (Leyton et al. 2002), all of which are neurotransmitters that are implicated in schizophrenia.

Recent investigations of human working memory have shown that the up-regulation of monoaminergic activity has differential effects depending on an individual's baseline working memory ability. Enhancement of monoaminergic activity with either bromocriptine (Kimberg et al. 1997) or D-amph (Mattay et al. 2000) can improve working memory performance in people with low baseline ability and can impair working memory performance in people with high baseline ability. Given individual differences in endogenous monoamine levels, these results suggest the possibility that working memory performs optimally within a specific range of monoaminergic activity, and that deviation both below and above this range will impair working memory function.

The present study was conducted to directly test the hypothesis of an inverted U-shaped relationship between an amphetamine-related increase in monoaminergic function and working memory processing efficiency. We examined the role of D-amph in the modulation of working memory efficiency using BOLD fMRI with participants carrying out a variable load working memory task. Based on the results of Kimberg et al. (1997) and Mattay et al. (2000), we hypothesised that for a working memory task of low to moderate difficulty, a low dose (2.5 mg) of D-amph would improve working memory efficiency, but as D-amph dose was increased, monoamine levels would exceed their optimal range and working memory processing efficiency would again decline.[1]

Consistent with other studies of cortical processing efficiency (e.g. Mattay et al. 2003), the primary measure used for analyses in this study was the extent of neural activation in working memory cortical regions at each dosage level. The use of this metric employs the logic that a more inefficient brain needs to recruit a more extensive set of neurons to perform the same operation. For two instances of the same task, then, a decrease in activation extent can be interpreted as an increase in processing efficiency, and conversely, an increase in activation extent can be interpreted as a decrease in processing efficiency.[2] Therefore, we tested specifically for a U-shaped (quadratic) relationship between D-amph dosage and activation extent in working memory regions.

Materials and methods

Subjects

Eighteen healthy people (6 females, mean age 24 years) consented to participate in the study, which was approved by the Clinical Research Ethics Board of the University of British Columbia. All participants were right-handed, had no personal history of mental illness, no generalized medical conditions requiring treatment, no personal history of neurological conditions including migraine, no history of illicit substance abuse or dependence, no family history of any psychotic disorder, and no contraindication for MR scanning.

Pharmacological procedure

Participants were randomized into either an amphetamine (N=12) or a control group (N=6) in a single-blind fashion. Although it is unlikely that our primary outcome measures (including BOLD activation and self-administered questionnaires) were susceptible to observer bias, we must note that the use of a single-blind as opposed to a double-blind approach is a limitation of the present study. All participants were told that they would receive either an amphetamine or a placebo tablet three times over the course of the day. Each participant was scanned in four separate sessions of 30 min each.

For the amphetamine group, the first session was a baseline condition, in which no compound was administered. For the next three sessions, increasing dosages of D-amph were given in tablet form, 90 min prior to scanning, and immediately after finishing the previous scanning session. The second session was the low dose (2.5 mg) condition, the third was the medium dose (5.0 mg) condition, and the fourth was the high dose (12.5 mg) condition.[3] The

[1] We based these hypotheses both on previous research showing that a low dose of amphetamine can improve working memory performance, as well as on pilot work from our own lab that showed a 20-mg dose of amphetamine greatly hindered working memory performance and exacerbated cortical processing efficiency on a similar task. We want to emphasize that these predictions were specific to the particular task employed in this experiment and acknowledge that a given dose of amphetamine will have differential effects depending on the individual and the specific task employed.

[2] For example, Uftring et al. (2001) found that a 20-mg dose of amphetamine increased the extent of activation in task-related cortical regions, but did not have any effect on reaction time or accuracy. To achieve the same level of behavioural performance, more cortex was recruited during the task—a reduction in cortical processing efficiency.

[3] We would like to clarify that the use of the terms "low dose", "medium dose", and "high dose" is relative to the dosage levels used in the present study. A 12.5-mg dose of D-amph is not in fact a particularly high dose (25 mg would typically be considered a high dose), but it was the highest dose employed here.

dosages were always administered in this order so as to allow scanning under multiple dosage conditions in a single day. For control group subjects, no compound was given for the baseline scan, and for the remaining three sessions, a placebo tablet was given 90 min prior to scanning. Heart rate, blood pressure, and responses to a subjective side-effect questionnaire were recorded every 30 min for each participant to monitor for any adverse reactions.

Data acquisition and working memory task

Functional MRI data were collected on a standard clinical GE 1.5-T system fitted with a Horizon Echo-Speed upgrade. Functional image volumes were collected with a gradient echo pulse sequence (TR=3,000 ms, TE=40 ms, 90° flip angle, field of view (FOV) 24×24 cm, 64×64 matrix, 62.5 kHz bandwidth, 3.75×3.75 mm in-plane resolution, 5.00-mm slice thickness, 29 slices), which is sensitive to BOLD contrast.

The working memory task was programmed and presented on a personal computer running presentation software (Neurobehavioral Systems, San Francisco, CA, USA). An event-related fMRI design was used, which allowed the pseudo-random intermixed presentation of trials from four working memory load conditions. A modified Sternberg Item Recognition task, which our lab has previously used to identify task-specific components of working memory (Cairo et al. 2004), was employed. Participants were asked to commit to memorise a target consonant string of either 2, 4, 6, or 8 letters during a 4-s encoding phase, maintain this string across a 3, 4, or 5-s delay period, and then respond as to whether or not a single probe letter presented for 1 s was a member of the target string. Participants responded by pressing buttons with their right index and middle fingers, corresponding to "yes" and "no" responses on a fiber-optic response device (Lightwave Medical, Vancouver, B.C., Canada). The probability of the test letter having been in the target string was 0.5. Eighteen trials of each memory load condition were given over two 10-min scanning runs. Each scanning run consisted of 196 scans (covering the entire brain).

Image processing and data analysis

Functional images were reconstructed off-line. Statistical Parametric Mapping software (SPM99, Wellcome Institute of Cognitive Neurology, London, UK) was used for image reorientation, realignment, normalization into modified Talairach anatomical space, and smoothing using a Gaussian kernel (8 mm FWHM; Friston et al. 1995). A low pass filter (cutoff period 6.25 s) was used to remove noise associated with fluctuations not due to the BOLD effect. BOLD responses for the encoding and maintenance phase of the task were modelled in SPM99 as the convolution of a 6-s box-car epoch (beginning at the onset of the target letter) and a synthetic impulse hemodynamic response function. The response phase of the task was modelled as an event synchronized to the onset of the probe letter. A high pass filter (cutoff period 165 s) was incorporated into the model to remove noise associated with low frequency confounds. The parameters of the modelled hemodynamic responses were adjusted to fit the observed BOLD signal time course in each voxel, employing the General Linear Model, using SPM99. Contrast images representing the parameter estimates of the encoding/maintenance and response portions of the working memory task were created for each subject for each of the four memory load conditions for each of the four scanning sessions (this lead to a total of 32 contrast images per subject—16 for the encoding/maintenance phase, and 16 for the response phase).

Load-dependent mask

To identify working memory regions likely to be sensitive to variations in monoaminergic activity, a region of interest mask consisting of all voxels that showed a statistically significant (height threshold $p<0.05$ corrected for whole brain) increase in BOLD signal with increasing load was created from an independent sample of 20 healthy control participants (data from Cairo et al. 2004). This mask was used as an independent benchmark against which we could compare the BOLD activation in all conditions in the present study. The mask (Fig. 1) contained a total of 2,371 voxels that included regions previously shown to be activated during working memory tasks, including the medial prefrontal cortex (PFC) (BA 6, 10, 32), dorsolateral PFC (BA 9) the lateral PFC (BA 46), the ventrolateral PFC (BA 44), a large portion of the superior parietal lobule (BA 7), a small area in the intraparietal sulcus region (BA 40), as well as the primary visual cortex (BA 17), and the secondary visual cortex (BA 18).

For each encoding/maintenance contrast image, the number of suprathreshold voxels ($t=3.10$, $p<0.001$, uncorrected) within the mask region was calculated. The voxel count (i.e. activation extent) results were taken into further statistical analyses. The primary working hypothesis of this study predicted that there would be a U-shaped relationship between amphetamine dosage and suprathreshold voxel count. This U-shaped relationship was directly tested by assessing the significance of the quadratic weighting of the voxel count data across sessions with a one-sample t test.

Focal region of interest analyses

In conjunction with testing for the hypothesised U-shaped relationship between amphetamine dose and activation extent in an a-priori-defined working memory network, independent voxel counts for the encoding/maintenance and response phases of the task were obtained for bilateral prefrontal and primary motor cortex regions of interest for the amphetamine group. The two regions of interest were bilateral, with left and right spheres centered on local maxima obtained from the same data used to generate the load-dependent mask (Cairo et al. 2004). The voxels with

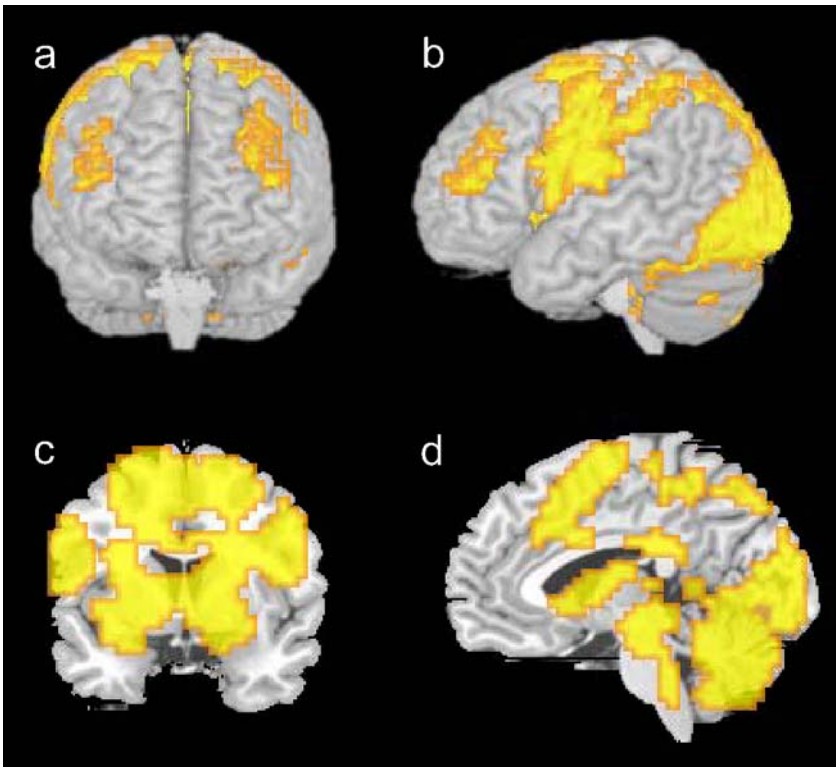

Fig. 1 A priori mask of independently defined load-dependent working memory regions. The mask is depicted here in **a** frontal and **b** sagittal surface renderings, **c** coronal, and **d** mid-sagittal cross-sections. *Highlighted areas* represent voxels in which activation increases linearly with increasing memory load ($p<0.05$, corrected) in an independent sample of 20 healthy participants (from Cairo et al, 2004). This network of activation defined the working memory regions of interest for the present study. The number of activated voxels in regions defined by this load-dependent mask was used as the primary dependent measure for the present study. The mask includes regions of the medial, dorsolateral, lateral, ventrolateral prefrontal cortex, the superior parietal lobule, the intraparietal sulcus, and the visual cortex

the highest *t* scores in left (−40, 36, 32) and right (36, 48, 28) prefrontal cortex and in left (−44, −8, 48) and right (44, −8, 48) primary motor cortex were selected as the origin for 10-mm spheres. A quadratic weighting of the mean suprathreshold ($t=3.10$, $p<0.001$, uncorrected) voxel count within the two bilateral regions of interest (prefrontal, primary motor) for each amphetamine dosage level was computed. One sample, one-tailed *t* tests were conducted to determine whether this quadratic trend was significant for the encoding/maintenance and response phases of the task in the prefrontal cortex and primary motor cortices. In addition to voxel counts, peak BOLD response amplitude estimates were computed for the most highly activated voxel in prefrontal and primary motor regions of interest, for the encoding/maintenance and response phases of the task for the amphetamine group. The same quadratic analysis performed on the voxel count data was repeated for the response amplitude data.

Additional analyses

Specific *t* tests, *F* tests, and ANOVAs were used to analyze the response time data, side-effect questionnaire data, physiological recordings, and the voxel counts based on the a-priori-defined load-dependent mask.

Results

Behavioural response time (RT) and accuracy data are presented in Tables 1a, b. RTs were analyzed with a 4×4×2-mixed model ANOVA, with memory load and session as the within-subjects factors, and group as the between-subjects factor. Due to technical difficulties during the scanning session, the behavioural data for the baseline session for one participant in the amphetamine group were lost, and this participant was dropped from the behavioural

Table 1a Behavioural data for the amphetamine group

Reaction times (in ms) are listed for each memory load condition, for each session. Accuracy data, in proportions, are presented in parentheses. Session means are presented in brackets

Memory load (number of letters)	Session (amphetamine dose in mg)				Memory load means
	1 (0)	2 (2.5)	3 (5.0)	4 (12.5)	
2	781 (.95)	805 (.98)	746 (.97)	774 (.99)	776 (.97)
4	886 (.96)	940 (.97)	873 (.89)	810 (.98)	877 (.95)
6	1,025 (.93)	1,046 (.93)	936 (.88)	873 (.91)	970 (.91)
8	1,100 (.83)	1,083 (.89)	1,011 (.80)	1,011 (.87)	1,049 (.85)
	[948 (.92)]	[968 (.94)]	[892 (.88)]	[865 (.94)]	

Table 1b Behavioural data for control group

Reaction times (in ms) are listed for each memory load condition, for each session. Accuracy data, in proportions, are presented in parentheses. Session means are presented in brackets

Memory load (number of letters)	Session				Memory load means
	1 (0 mg)	2 (0 mg)	3 (0 mg)	4 (0 mg)	
2	863 (.95)	848 (.98)	903 (.8)	761 (.99)	844 (.95)
4	865 (.97)	799 (.96)	888 (.93)	808 (.98)	840 (.96)
6	933 (.91)	835 (.98)	943 (.89)	888 (.93)	900 (.93)
8	1,036 (.75)	982 (.87)	1,079 (.85)	895 (.94)	998 (.85)
	[924 (.89)]	[866 (.94)]	[953 (.89)]	[838 (.96)]	

analysis. Main effects of memory load, $F(3,45)=26.87$, $p<0.001$, and session, $F(3,45)=3.71$, $p=0.02$, were found. In general, RT increased with increasing memory load, $F(1,15)=34.37$, $p<0.001$, and decreased across sessions, $F(1,15)=12.64$, $p=0.003$. There was no significant difference in RT between groups, $F(1,15)=0.01$, $p>0.05$. No interactions were present (all $p>0.05$). The significant memory load and session results are consistent with difficulty and practice effects, respectively.

Responses on a subjective side-effect questionnaire administered every half hour were averaged to produce a single measure (rating out of 10) for sessions 2–4 for each of anxiety, restlessness, irritability, and overall mood. Each measure was analyzed with a 2×3-mixed model ANOVA, with group (amphetamine vs control) as the between-subjects factor, and session (session 2 vs session 3 vs session 4) as the within-subjects factor. For the ratings of the negative side effects, no group or session effects were significant (all $p>0.05$). There was however, a significant Group×Session interaction for the positive mood rating, $F(2,32)=4.98$, $p<0.05$, indicating that overall mood significantly increased across sessions for the amphetamine group, but not for the control group. Importantly, however, initial mood ratings for the amphetamine group were lower than those for the control group, and climbed back up to control group levels across sessions.

Measurements of blood pressure and pulse taken every half hour were averaged for each participant to produce a single measure for each variable for sessions 2–4. A significant Group×Session interaction was found for systolic

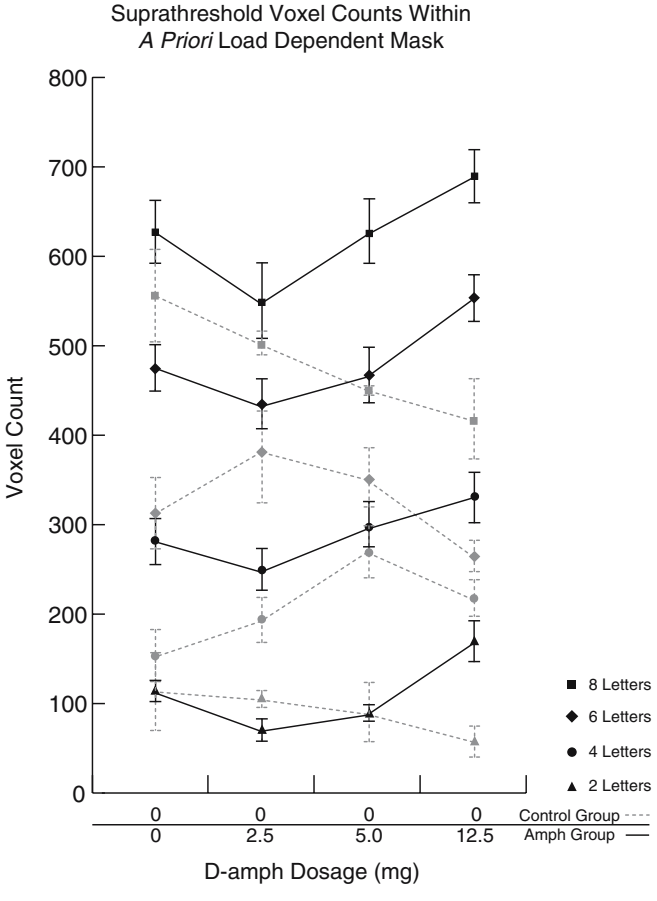

Fig. 2 Voxel counts from regions encompassed by the independently derived load-dependent mask for amphetamine (*solid lines*) and control (*dotted line*) groups, plotted for each session and for each memory load. Clearly visible for the amphetamine group is a U-shaped relationship between D-amph dosage and voxel count that does not vary across the levels of memory load. This relationship is not present for the control group. Also evident is a large linear main effect of memory load, whereby voxel counts in both groups increase sharply and linearly as memory load increases

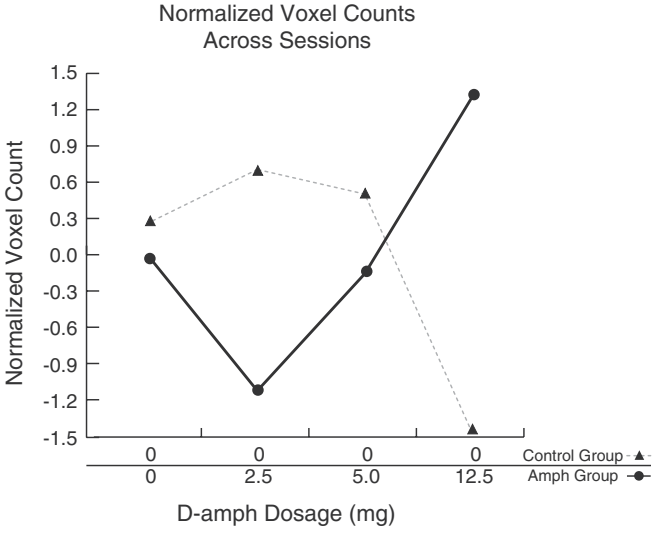

Fig. 3 Normalized voxel count scores from regions within the load-dependent mask for amphetamine and control groups, averaged over levels of memory load, are plotted for each session. *Z* scores were computed for each group separately based on each group's mean and standard deviation of the session means. Voxel counts were normalized for illustrative purposes, to show the overall pattern of change in voxel count across sessions (averaged over levels of memory load) for each group. Clearly observable here is the presence of a U-shaped modulation of working memory activation by D-amph that is absent in the control group

Table 2 Voxel count data for focal regions of interest in prefrontal and primary motor cortices for amphetamine group

		Session (amphetamine dose in mg)			
		1 (0)	2 (2.5)	3 (5.0)	4 (12.5)
Prefrontal	Encode	17 (16)	12 (14)	12 (11)	15 (12)
	Response	17 (15)	16 (17)	14 (13)	15 (13)
Primary motor	Encode	14 (11)	16 (15)	17 (14)	18 (14)
	Response	13 (8)	12 (11)	13 (12)	11 (9)

Group means for the encoding/maintenance and response phases of the task are given for each bilateral focal region of interest (averaged for left and right regions), for each session (averaged over memory load). Standard deviations are presented in parentheses

blood pressure, where blood pressure increased across sessions for the amphetamine group, $F(1,11)=8.53$, $p<0.05$, and decreased across sessions for the control group, $F(1,5)=7.70$, $p<0.05$. There were no significant differences between groups for either diastolic blood pressure or pulse (both $p>0.05$). Furthermore, suprathreshold voxel counts were not correlated with any of systolic blood pressure, diastolic blood pressure, or pulse (all $p>0.05$).

Voxel counts in regions encompassed by the a-priori working memory load-dependent mask for the amphetamine and control groups are presented in Fig. 2. The presence of a U-shaped relationship between amphetamine dose and working memory activation extent was assessed with a quadratic polynomial contrast. We used the polynomial weighting scheme (1×session 1)+(−1×session 2)+(−1×session 3)+(1×session 4) to compute a weighted aggregate of voxel counts at each dosage level representing the importance of the U-shaped quadratic trend for each subject. Averaged overall subjects and levels of load, this U-shaped aggregate was significantly different from 0 for the amphetamine group, t (11)=3.12, $p<0.01$, but not significantly different from 0 for the control group.[4] The direct between-subjects t test of this group difference was significant, t (16)=2.75, $p<0.05$. For illustrative purposes, Fig. 3 shows normalized scores for voxel counts obtained from the load-dependent mask for the amphetamine and control groups, averaged over the levels of memory load. Evident in Fig. 3 is the sharp contrast between the session effects for the amphetamine and control groups.

The focal region of interest voxel counts for dorsolateral prefrontal and primary motor cortices for the amphetamine group are presented in Table 2. The results revealed that while a significant quadratic trend across amphetamine dosage levels was found for the encoding/maintenance phase in bilateral prefrontal regions, t (11)=2.01, $p<0.05$, this trend was not present for the response phase of the task in the prefrontal regions, t (11)=0.83, $p=0.21$, and was not present for either the encoding/maintenance, t (11)=−0.08, $p=0.47$, or the response, t (11)=−0.27, $p=0.40$, phases of the task in bilateral primary motor cortical regions. Peak response amplitude estimates for these focal regions of interest are presented in Table 3. The data pattern is the same as that obtained for the focal region of interest voxel counts. The quadratic trend for the encoding/maintenance phase of the task in prefrontal regions, however, was only marginally significant, t (11)=1.44, $p=0.08$.

Inasmuch as our hypothesis centered on the pattern of modulation of voxel counts across all sessions, we thought it important to directly test whether the changes in voxel count between each session were significant. The comparison between the first and second sessions was particularly important, as we wanted to rule out the possibility that the decreased voxel count in the second session for the amphetamine group reflected a practice effect. One-tailed t tests of voxel counts in the first and second sessions revealed a significant decrease in voxel count in the second session for the amphetamine group, t (11)=1.75, $p=0.05$, but not for the control group, t (5)=−0.12, $p>0.45$. T tests of differences in voxel count between sessions 2 and 3, and sessions 3 and 4 were not significant in either the amphetamine or control groups (all $p>0.05$).

To test whether or not the presence of a U-shaped relationship between amphetamine dose and voxel count depended on memory load, a follow-up 2×4-mixed model ANOVA was carried out, with group (amphetamine vs control) as the between-groups factor, and load (2, 4, 6, or 8 letters) as the within-subjects factor. The quadratic weighted aggregate was used as the dependent variable. The results of this analysis demonstrated that the U-shaped trend did not vary over the levels of load for either group separately, $F(3,48)=1.08$, $p=0.36$, or averaged over groups, $F(3,48)=1.29$, $p=0.32$.

Table 3 Response amplitude data (beta parameter estimates) for peak activation in focal regions of interest in prefrontal and primary motor cortices for amphetamine group

		Session (amphetamine dose in mg)			
		1 (0)	2 (2.5)	3 (5.0)	4 (12.5)
Prefrontal	Encode	0.22 (0.11)	0.17 (0.09)	0.20 (0.12)	0.21 (0.08)
	Response	0.22 (0.08)	0.19 (0.11)	0.20 (0.09)	0.18 (0.07)
Primary motor	Encode	0.20 (0.07)	0.20 (0.08)	0.21 (0.08)	0.21 (0.07)
	Response	0.17 (0.05)	0.16 (0.06)	0.17 (0.07)	0.16 (0.06)

Group means for the encoding/maintenance and response phases of the task are given for each bilateral focal region of interest (averaged for left and right regions), for each session (averaged over memory load). Standard deviations are presented in parentheses

[4] We must note that these data are an average of both men and women. As one reviewer pointed out, men and women may differ in their response to amphetamine. To ensure that no such differences were present in this study, we conducted an ANOVA for the amphetamine group with sex as a between-subjects factor, and dose and load as within-subjects factors, using the voxel counts obtained from the a-priori mask as the dependent measure. There were no significant main or interaction effects of sex (all ps>0.05), indicating that in the present study, amphetamine affected brain activity in a similar manner for both men and women.

To explore the pattern of the memory load effect and its interaction with other factors, a 4×4×2-mixed model ANOVA was carried out, with memory load and session as the within-subjects factors, and group as the between-subjects factor. The main effect of memory load was highly significant $F(3,144)=77.07$, $p<0.001$. This significant result can be attributed solely to a linear effect, $F(1,16)=121.27$, $p<0.001$, such that the voxel count increased linearly with load. Memory load did not interact with any other factors (all $p>0.05$).

Discussion

The results of the present study are consistent with the hypothesised U-shaped relationship between D-amph dosage and working memory activation extent. The overall voxel counts showed a significant quadratic relationship in the number of activated voxels in working memory regions across the D-amph dosages employed. The reduced voxel count following administration of a 2.5-mg dose of D-amph is interpreted as an increase in processing efficiency—an effect that was reversed as increasing dosages of D-amph were adiministered. As amphetamine dosage was further increased, the voxel count grew.

Specific focal region of interest analyses were conducted to determine whether the observed U-shaped relationship between D-amph dosage and voxel count was specific to working memory functioning per se (as opposed to being a global effect of amphetamine). Given that this relationship was observed in the prefrontal cortex for the encoding/maintenance phase of the task, but not for the response phase, and was not observed in the primary motor cortex for either phase of the task, we conclude that the amphetamine-related modulation of cortical processing efficiency was specific to working memory. That is, increasing dosages of D-amph produced a U-shaped modulation of activation extent that was specific to cortical regions known to be involved in working memory, and specifically associated with the cognitive operations involved in the storage of information over a delay period (as opposed to the decision and motor processes involved in a response).[5]

Overall, the behavioural data indicated that working memory performance improved steadily across the four sessions for both groups, indicating that there was no unique effect of amphetamine on task performance. This dissociation between BOLD effects and behavioural measures highlights the important distinction between cortical processing efficiency and absolute task performance. Although a low dose of amphetamine did not uniquely affect behavioural performance, it did reduce the number of suprathreshold voxels associated with the working memory task. The results suggest that under the low dose amphetamine condition, the cortical processing of the working memory system became more efficient, requiring less cortical activation to achieve the same level of performance seen in the baseline session. This result contrasts with studies that show concomitant changes in cortical activation and behaviour for a given task. For example, Daniel et al. (1991) showed that in schizophrenic patients treated for 6 weeks on haloperidol, a 0.25 mg/kg-dose of amphetamine improved accuracy on a Wisconsin Card Sorting Test (WCST), and also increased activation in left dorsolateral PFC (DLPFC). Because accuracy improved, the increased activation in this study should be interpreted as an engagement of the cortical region in processing that was previously omitted rather than a decrease in processing efficiency.

The present finding of improved working memory processing efficiency with a low dose of D-amph is consistent with other studies showing that a low dose of amphetamine (or other DA agonist) generally improves working memory performance in humans (Mattay et al. 1996; Elliott et al. 1997; Muller et al. 1998). Indeed, many studies have shown improved task performance after having received a larger dose (15 mg+) of amphetamine (e.g. Servan-Schreiber et al. 1998). In most studies showing task improvement with a dose of amphetamine 20 mg or larger, sustained performance was measured under sleep deprivation conditions (e.g. Baranski and Pigeau, 1997; Magill et al. 2003; Shappell et al. 1992). The particular doses of amphetamine that help and hinder performance will vary depending in the task conditions at hand. In addition, an individual's response to a given pharmacological manipulation of monoamine activity will vary depending on intrinsic monoamine levels.

Although the quadratic relationship between D-amph dose and voxel count was statistically significant, direct t tests of the increase in the number of suprathreshold voxels between sessions as a result of the medium and high D-amph doses were not significant. It is possible that this study did not utilize D-amph dosages high enough to produce a large decrease in working memory efficiency. Mattay et al. (2000) used a single 20-mg dose of D-amph in their behavioural study of working memory performance. While we administered 20 mg of D-amph over the course of the day, it is possible that the titrated schedule that we used may have produced some tolerance effects, thereby reducing the effectiveness of the medium and high dosages.

The possibility of a drug adaptation effect over the course of the day was a risk that we, at the outset of the study, were willing to take in favor of the practical benefits of having participants come in for a single day of testing. We reasoned that any washout effect would induce a conservative bias by reducing the effectiveness of the higher doses, thereby providing a strong test for the hypothesised quadratic relationship between amphetamine dose and

[5] A similar data pattern was obtained for the peak response amplitude estimates for these focal regions of interest. That is, a U-shaped relationship between BOLD response amplitude and amphetamine dose was present only for the encoding/maintenance phase of the task, and only in the dorsolateral prefrontal cortex. The quadratic trend for the response amplitude data, however, did not quite reach significance, $t(11)=1.44$, $p=0.08$. Where as voxel counts and response amplitude estimates are certainly not independent, this result highlights the fact that the two measures are dissociable. Given that the present research question centers on cortical efficiency, we felt activation extent rather than peak amplitude was the appropriate measure.

voxel count. This reasoning, however, was speculative, so we should note that the administration of all four amphetamine doses in a single day is a limitation of the present study. As such, we must emphasize that our results and conclusions are based on cumulative dosage effects rather than absolute levels. It is entirely possible that drug effects from earlier testing sessions were carried over into later testing sessions. Although the primary hypothesis of the present study was satisfactorily addressed with cumulative dosages for a precise and absolute dose–response relationship to be determined, the appropriately randomized study needs to be conducted.

There is some evidence that global effects may be a concern in neuroimaging protocols that utilize amphetamine. The results of positron emission tomography (PET) and single photon emission tomography (SPECT) studies of global effects of amphetamine are mixed. Such studies have reported global decreased glucose metabolism (Wolkin et al. 1987), a small global increased glucose metabolism (Schmidt et al. 1996), regionally specific increases and decrease in blood flow (Devous et al. 2001), and task-specific increases and decreases in glucose metabolism (Ernst et al. 1997; Mattay et al. 1996) after receiving amphetamine. Importantly, however, the results of the present study were not subject to global effects confounds. The contrast images used for the voxel counts were generated by comparing task-specific BOLD signal to an overall session baseline. In this manner, all global effects induced by amphetamine were removed from the analysis.

The aim of this study was to directly test whether monoaminergic activity could modulate cortical processing efficiency in a working memory system. Although amphetamine affects several neurotransmitter systems, it is possible that a fluctuation of dopaminergic activity in particular was responsible for the observed modulation of working memory processing efficiency. Preclinical studies provide compelling evidence that DA is critical in the modulation of working memory function. Studies of the medial PFC have revealed a critical role for DA in working memory and other executive functions (Robbins 2000). Loss of DA terminals in the medial PFC of both primates (Brozoski et al. 1979) and rats (Bubser and Schmidt 1990) severely impairs performance on delayed response tasks at short delay intervals (i.e. 10–60 s). Similarly, infusion of selective DA D1 receptor antagonists into the medial PFC of rats (Seamans et al. 1998) or monkeys (Sawaguchi and Goldamn-Rakic 1991) selectively impairs spatial working memory. Furthermore, DA release in the medial PFC of rats is greater and task performance is better during a food retrieval task at a relatively short delay (30 min), compared to a long delay (6 h), in which both medial PFC DA release and task performance are diminished (Phillips et al. 2004). DA D1 receptors in the medial PFC are particularly important for performance on tasks requiring short-term memory or executive processes, as intermittent D1 stimulation reverses working memory impairments in juvenile monkeys, following down-regulation of D1 receptors by chronic neuroleptic treatment (Castner et al. 2000). Working memory in aged monkeys can also be enhanced by sensitisation induced by intermittent administration of a selective D1 receptor agonist (Castner and Goldman-Rakic 2004).

In line with human research suggesting an inverted U-shaped relationship between monoaminergic activity and working memory ability, there is animal research demonstrating that cognitive processes mediated by the medial PFC are optimized within a specific range of DA activity (Arnsten 1998; Williams and Goldman-Rakic 1995; Zahrt et al. 1997). Administration of DA agonists into medial PFC impair delayed responding in animals displaying optimal performance prior to drug treatment, and this deficit can be reversed with the administration of DA antagonists (Romanides et al. 1999; Zahrt et al. 1997). Other work has shown that for rats, a low dose of amphetamine (0.25 mg/kg) can improve delayed T-maze performance, whereas higher doses impair such performance (Aultman and Moghaddam 2001). Aged monkeys display a biphasic response to D1 agonist treatment with cognitive performance facilitated by low doses and impaired with higher doses (Arnsten et al. 1994). In addition, noise stress, which increases DA release in PFC of monkeys, is detrimental to performance on a delayed-response task but not for a no-delay control task. These deficits are ameliorated by drugs that block DA receptors or reduce stress-related DA release (Arnsten and Goldman-Rakic 1998).

Recent work by Mattay et al. (2003) provides strong evidence that the effect of D-amph in the present study may well be underlain by its action on dopaminergic signaling in the PFC. They found that for people with a *val/val* catechol *O*-methyltransferase genotype, whose intrinsic levels of prefrontal DA are lower, baseline prefrontal functioning was less efficient than for people with a *met/met* genotype, whose intrinsic prefrontal DA levels are higher (see also Egan et al. 2001). Amphetamine enhanced prefrontal cortical efficiency for people with the *val* allele and reduced prefrontal cortical efficiency for people with the *met* allele. These individual differences in the brain response to amphetamine can therefore be understood with respect to individual differences in prefrontal dopaminergic activity.

The present study, in conjunction with the other research we have reviewed, underscores the possibility that working memory deficits associated with schizophrenia are underlain by impoverished cortical processing efficiency brought about by abnormal dopaminergic signaling in the prefrontal cortex. Recent research into the psychopharmacology of schizophrenia has uncovered complexities in the neural substrates of the disorder, including roles for glutamatergic (Kegeles et al. 2000; Laruelle et al. 2003) and adrenergic (Arnsten 2004; Cai and Arnsten 1997) modulation of DA activity. We are not suggesting that DA dysfunction alone brings about the working memory disturbances seen in schizophrenia, but rather that monoaminergic systems, and possibly the DA system in particular, do seem to play an important role. Further complexity is added by findings that DA D1 receptor concentrations in the prefrontal cortex of patients with chronic schizophrenia are decreased (Friedman et al. 1999; Laruelle et al. 2003). It

is therefore possible that diminished DA D1 activity in the prefrontal cortex is to blame for the working memory disturbances seen in schizophrenia (see also Meyer-Lindenberg et al. 2002).

Future research should address whether working memory deficits seen in schizophrenia are yoked to fluctuations in monoamine levels that occur over the course of the illness and its treatment. Of particular interest would be to examine whether initial improvements in working memory processing efficiency with neuroleptic treatment (e.g. Mendrek et al. 2004) are sustained or reversed during prolonged chronic phases of the illness during which time overmedication may in some cases be a possibility.

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