Psychopharmacology (2004) 177: 185–194
DOI 10.1007/s00213-004-1908-5

ORIGINAL INVESTIGATION

F. Gerard Moeller · Joel L. Steinberg · Donald M. Dougherty · Ponnada A. Narayana · Larry A. Kramer · Perry F. Renshaw

Functional MRI study of working memory in MDMA users

Received: 3 February 2004 / Accepted: 7 April 2004 / Published online: 18 June 2004
© Springer-Verlag 2004

Abstract *Rationale:* Methylene-dioxymethamphetamine (MDMA) is known to cause degeneration of serotonin nerve terminals after acute doses in animals. Similarly, behavioral studies in human MDMA users regularly find abnormalities in memory, mood, and impulse control. However, studies of brain function using brain imaging in MDMA users have been less consistent. *Objectives:* The purpose of this study was to determine, using functional magnetic resonance imaging (fMRI), whether individuals with a self-reported history of MDMA use would differ from non-MDMA using controls on activation while performing a working memory task. *Methods:* Fifteen MDMA using subjects and 19 non-MDMA using controls underwent fMRI scanning while performing the immediate and delayed memory task (IMT/DMT). The study was based on a block design in which the delayed memory task (DMT) alternated with the immediate memory task (IMT), which served as a control condition. FMRI scans were acquired on a 1.5 T scanner, using a gradient echo echoplanar pulse sequence. *Results:* Random effects SPM99 analysis showed significantly greater activation (whole volume corrected cluster $P<0.05$) during the DMT relative to the IMT in the MDMA subjects compared with the control subjects in the medial superior frontal gyrus, in the thalamus extending into putamen, and in the hippocampus. *Conclusions:* Although these effects could be due to other drugs used by MDMA users, these results are consistent with behavioral problems that are associated with MDMA use, and with animal studies on the effects of MDMA on brain function.

Keywords MDMA · Functional magnetic resonance imaging · Working memory · Substance abuse

F. G. Moeller (✉) · J. L. Steinberg · D. M. Dougherty
Department of Psychiatry and Behavioral Sciences, University of Texas Health Science Center Houston,
1300 Moursund,
Houston, TX 77030, USA
e-mail: frederick.g.moeller@uth.tmc.edu
Tel.: +1-713-5002858
Fax: +1-713-5002634

P. A. Narayana · L. A. Kramer
Department of Radiology, University of Texas Health Science Center Houston,
Houston, Tex., USA

P. F. Renshaw
McLean Hospital and Harvard Medical School,
Boston, Mass., USA

Introduction

Numerous preclinical studies have found evidence of long-term changes in serotonergic neurons after MDMA administration (Schmidt and Taylor 1987; Pan and Wang 1991; Colado et al. 1999; Hatzidimitriou et al. 1999; Ricaurte et al. 2000a, b). Based on the animal findings of chronic effects of MDMA on serotonin nerve terminals, investigators have attempted to determine the long-term cognitive and behavioral effects of MDMA use in humans. The majority of these studies found differences between MDMA users and non-users on measures of memory (Bolla et al. 1998; Parrott et al. 1998; McCann et al. 1999; Gouzoulis-Mayfrank et al. 2000; Rodgers 2000), mood (Cohen 1995; Parrott and Lasky 1998; Parrott et al. 2000), and impulsivity (Morgan 1998; Schifano 2000; Moeller et al. 2002). However, there is controversy surrounding these findings, since other studies found that differences between MDMA users and controls on memory were primarily due to other drugs used by MDMA users, such as marijuana (Croft et al. 2001; Simon and Mattick 2002; Dafters et al. 2003).

Studies using functional brain imaging have not always produced consistent results in showing MDMA causes long-term neuronal effects in humans. Results of positron emission tomography (PET) studies have been mixed, with some studies showing altered brain metabolic glucose rate in MDMA users (Obrocki et al. 1999), while another study found no difference in brain blood flow between MDMA users and controls during a simple version of the continuous performance task (Gamma et al. 2001). Several studies have examined serotonin transporter binding in

MDMA users. McCann et al. (1998) found decreased global and regional brain 5-HT transporter binding in MDMA users compared with controls. Similar results were reported by Thomasius et al. (2003), with reduced serotonin transporter availability in the mesencephalon and caudate nucleus. In another study by the same group, current MDMA users were found to have reduced transporter binding in the mesencephalon and thalamus, but former MDMA users showed transporter binding very similar to controls (Buchert et al. 2003). Other studies using single photon emission computed tomography (SPECT) of MDMA users have found increased 5-HT_{2a} receptor binding (Reneman et al. 2002) and decreased serotonin transporter binding (Semple et al. 1999) in the cerebral cortex of MDMA users.

To date, there have been three published studies comparing MDMA users and controls using fMRI. In one study, the authors compared the response to an *n*-back cognitive task on fMRI of 22 MDMA users (divided into heavy and moderate users) with 11 healthy controls. This study found no statistically significant differences between heavy MDMA users (n=11), light MDMA users (n=11), and controls (n=11) on random effects analysis after correction for multiple comparisons (Daumann et al. 2003a). In a second study by the same authors (Daumann et al. 2003b), eight "pure" MDMA users were compared with eight MDMA users who reported using other drugs along with MDMA, and eight non-drug using controls. This study reported differences between MDMA users and controls on fMRI in the premotor cortex, inferior temporal region, angular gyrus region and striate cortex. However, this study used a statistical analysis which did not correct for the multiple comparisons, which makes interpretation of the results of this study difficult. Correcting for multiple comparisons within the whole brain volume has been described as being necessary to control the experiment-wise type I statistical error rate (Poline et al. 1997; Petersson et al. 1999). Most recently, a study comparing MDMA-using adolescents with non-MDMA using adolescents, which focused on the hippocampus using an auditory *n*-back task, found greater fMRI activation in adolescent MDMA users (Jacobsen et al. 2003).

In order to examine further whether MDMA users differ from non-MDMA users as measured by functional neuroimaging, the present study scanned a group of MDMA users and controls with blood oxygen level dependent (BOLD) fMRI while performing a working memory task. The hypothesis of the study was that MDMA users would show differences in BOLD activation on fMRI compared with non-MDMA using controls.

Materials and methods

Subjects

MDMA using subjects and non-drug abusing controls were recruited through advertisements in a local entertainment newspaper seeking research participants to study the effects of ecstasy use on behavior. Written informed consent was obtained from all subjects prior to participation. MDMA using subjects were required to have used MDMA on multiple occasions, and non-MDMA using subjects were required never to have used MDMA. Subjects received monetary compensation of approximately $8.00 per hour for behavioral testing and $50 for fMRI testing for participation.

Exclusion criteria included lifetime presence of any axis I disorder other than substance abuse or dependence, or substance induced mood disorder. Subjects were also excluded if they had any non-psychiatric medical condition, were taking any prescribed psychotropic medication, or if any alcohol was found on a breath alcohol measurement. Additional exclusion criteria for control subjects included past or present substance use disorder, or positive urine drug screen for any substance of abuse.

Diagnostic assessment

All subjects were screened for psychiatric illness using the structured clinical interview for DSM-IV (SCID) (First et al. 1996). The diagnostic assessments were performed by a bachelors degree level research assistant who had undergone extensive training on the use of the SCID including observing videotaped and live patient interviews. Diagnostic interviews were intermittently monitored by a board certified psychiatrist (F.G.M.) who has also undergone extensive SCID training. Results of all SCID interviews were also reviewed by the same psychiatrist. All subjects also underwent a medical history and physical examination. This study was approved by the Committee for the Protection of Human Subjects of the University of Texas Health Science Center at Houston and conformed to the Helsinki Declaration of 1975.

MRI scans

Scans were performed on a 1.5 T general electric (GE) Echospeed LX NVi scanner. Prior to MRI, sessions were conducted with an MRI simulation device (mock MRI) in the behavioral laboratory. A Vac Fix Immobilization Device (VF-160 bag) was used to restrict head motion. The Vac Fix bag consists of a reusable, cushion-like airtight bag filled with small polystyrene spheres. The cushion is molded to provide a custom fit to the back and sides of the subject's head. After air is evacuated from the bag with a vacuum pump, the spheres inside adhere to each other such that the bag becomes a rigid shell, while maintaining the individualized form-fitting molded shape that is comfortable to the subject. A separate, soft comfortable strap passes under the gantry and over the sides of the shell to be joined over the subject's forehead with a hook and loop closure, and thereby anchors the shell to the gantry. Care is taken to make the subject comfortable and not to obstruct the subject's vision. In addition to mechanical restriction of head motion,

mathematical correction of head motion was performed during post-processing (see below). Sessions with greater than 2.0 mm head motion were eliminated from analysis.

Following a T1 weighted sagittal localizer; T1 weighted high-resolution images using GE 3D-SPGR pulse sequence, 256×256 matrix on 240×240 mm field of view, 120 slices, 1 mm slice thickness were acquired. The fMRI scans were axial slices acquired using a single-shot gradient echo echoplanar imaging pulse sequence that is sensitive to BOLD hemodynamic response (Ogawa et al. 1990), flip angle 90°, TR 2500 ms, TE 40 ms, receiver bandwidth 62.5 kHz, 24 slices, 3 mm slice thickness, 1.5 mm spacing between slices, 64×64 matrix, and 240×240 mm field of view. A total of 250 repetitions were acquired in each series while the subject participated in the IMT/DMT protocol. The duration of each fMRI series was 10 min 35 s.

Behavioral protocol during fMRI

The subjects performed the immediate and delayed memory task (IMT/DMT) (Dougherty 1999) that had been modified for fMRI scanning. The IMT is a continuous performance test in which a series of visual stimuli are presented on a screen at a rate of one stimulus per second, and each stimulus consists of a horizontal string of multiple numeric digits. Subjects were trained to press a button when a number (probe stimulus) was an identical match with the stimulus that immediately preceded it (target stimulus). A different target stimulus was presented in each trial. The DMT was similar to the IMT, except that in the DMT, a distracter stimulus was presented 3 times during a delay period between target and probe stimulus (see Fig. 1). The IMT/DMT shares features with the "*n*-back" sequential letter memory test because there is a contrast between a longer working memory delay period (DMT) and a shorter delay period (IMT). One advantage of the IMT/DMT is that it allows the experimenter to manipulate the task difficulty independently of the duration of the working memory delay. The IMT/DMT also varies working memory demand by changing the number of digits per stimulus to alter task difficulty independently from changing the working memory delay. Thus, both the memory delay and the number of digits per stimulus can be varied parametrically.

The distracter stimulus was a multi-digit string consisting of all zeros. Thus, the DMT was similar to the "*n*-back" test, which in this case would be a 1-back condition with a greater delay than the IMT. Between individual trials of the DMT, a multi-digit string consisting of all ones was shown on the screen to indicate that one trial ended and another was about to begin. The string of all ones was shown four times between individual trials during IMT blocks, so that the number of non-salient stimuli (the sum of all the distracter and inter-trial stimuli) was the same in both the IMT and DMT blocks. Also, the number of trials was the same in both the IMT and DMT blocks. The percentage of correct detections or "hits" was calculated,

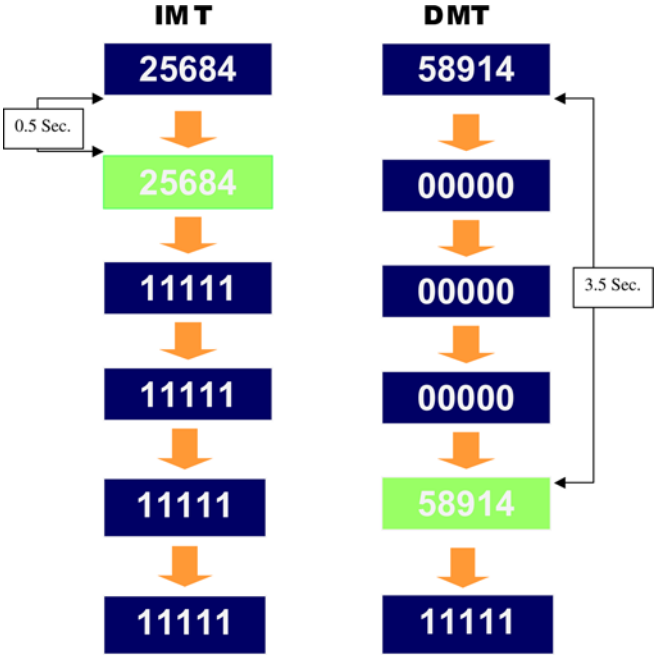

Fig. 1 Immediate and delayed memory task

as well as errors of omission and commission and response latency.

The methodology was a parametric block design, which presented three different values for the number of digits per stimulus (3, 5, and 7) within each of two different delay periods: IMT=0.5 s, and DMT=3.5 s (see Fig. 1). All three levels of the variable-digit condition (3, 5, 7 digits) and two levels of the variable-memory-delay condition (either 3.5 or 0.5 s) were presented in alternating blocks within each session. The order of the variable-digit conditions within each session was counterbalanced between subjects. The duration of each behavioral block was 42 s, and there was a 10-s rest between each block to reduce carry-over effects between conditions. There was a 20-s rest period at the beginning of the session to allow magnetization to achieve equilibrium at the start of the fMRI scanning.

Image processing

After image reconstruction and prior to any other post-processing steps, Fourier interpolation was used to time-shift each slice to correct for the different slice acquisition times within a TR, utilizing SPM99 (Friston et al. 1995a). Next, 3D registration with AFNI software (Cox 1996) was used to realign the fMRI images and to determine the degree of head motion for each volume. Sessions with head motion >2.0 mm were eliminated from analysis. AFNI software was also used to transform the high-resolution 3D anatomical volume for each subject to Talairach stereotaxic coordinates using the subject's own anatomical landmarks according to the original definitions of the Talairach atlas (Talairach and Tournoux 1988).

Images were then resliced to 3 mm isotropic voxels using AFNI.

fMRI data analysis

The data were analyzed with statistical parametric mapping using software (SPM99) from the Wellcome Department of Cognitive Neurology, London, UK (Friston et al. 1995a), implemented in Matlab (Mathworks Inc., Sherborn, Mass., USA). The images were spatially smoothed using a 6 mm isotropic Gaussian filter prior to fixed effects analysis, plus an additional 4 mm isotropic Gaussian filter prior to random effects analysis, in order to ensure that assumptions were met about the smoothness of the random field (i.e. ≥3× the resolution). The condition, subject, and covariate effects were estimated according to the general linear model at each voxel (Friston et al. 1995a).

The regression parameters for the IMT and DMT blocks were estimated using delayed boxcar models that were convolved with the SPM99 canonical hemodynamic response function (hrf). Each subject's performance scores as measured by the A' accuracy scores (Donaldson 1992) per each block were entered as covariates in the general linear model. Global effects were removed using proportional scaling. Cosine functions with a period greater than 210 s were added to the model as covariates to serve as a temporal high pass filter in order to reduce low frequency noise. In order to improve the signal to noise ratio, the time series was convolved with the canonical hrf, which served as a low pass temporal filter.

Random effects analysis

The random effects procedure for SPM99 provides for a mixed model analysis of fixed and random effects (Holmes and Friston 1998). This consisted of a two-stage procedure. First, the data were collapsed to one single contrast image per subject calculated from the fixed effects DMT parameter estimate minus the IMT parameter estimate after removal of confounds. The contrast images for each subject were then entered into the SPM99 Basic Models two-sample *t*-test module. The resulting set of voxel values for each contrast (control group minus MDMA group, and MDMA group minus control group) constituted a statistical parametric map of the one-tailed *t* statistic. The criterion for statistical significance at the voxel and cluster levels of inference used the SPM99 correction for multiple comparisons across the whole volume, derived from random field theory (Adler 1981; Friston et al. 1995b).

In addition to the SPM99 correction, all probability levels except post hoc testing of effects of other drugs of abuse were multiplied by a factor of 2 in order to account for two-tailed hypothesis testing. After significant clusters had been identified using SPM99, the mean value of the parameter contrasts within the cluster were determined using Marsbar software (Brett et al. 2002).

Urine testing

Urine drug screening was performed on all subjects using an immunochromatographic assay for THC, opiates, cocaine (benzoylecgonine), amphetamine, and benzodiazepines (Syva Company). All subjects were free of alcohol at the time of testing as determined by an Intoximeter Alcosensor III breathalyzer (Intoximeters Inc., St Louis, Mo., USA).

Results

Subject characteristics

Twenty-four MDMA using subjects and 25 controls underwent fMRI scans. Of these, scans from two MDMA using subjects were excluded because of excessive head motion or large body movements observed during the scan. An additional five scans from MDMA using subjects and three controls were eliminated due to corruption of the raw data files. One control and two MDMA users were dropped from all analyses because of no better than chance performance on the IMT/DMT. Finally, two control subjects were excluded for significant symptoms of depression.

The final subject groups in the analysis included 15 MDMA users and 19 non-drug using controls. Demographic characteristics of MDMA users and controls are shown in Table 1.

MDMA users did not differ statistically from controls in age (t=0.40, df=32, P=0.69). or gender (Pearson chi square=1.87, df=1, P=0.171), but there was a significant difference in education (t=3.52, df=1,28.9, P=0.001), with the majority of MDMA users having only partly completed college, while the majority of controls had completed college or were in graduate school. No controls reported ever using MDMA. Among the MDMA users, the number of self-reported uses of MDMA averaged 193.5±239.7 times, ranging from 8 to 1000 lifetime uses. MDMA users reported using MDMA 6.5±4.9 times per month, and the last MDMA use was 37±51 days prior to the fMRI scan, with a minimum of 3 and a maximum of 194 days prior to the scan. As has been noted in previous studies (McCann et al. 1999; Morgan et al. 2002), MDMA using subjects tend to use a number of other drugs along with MDMA. The subjects included in this study also had a lifetime history of several other drugs (see Table 1).

Urine drug screen results

On the day of the fMRI, breath alcohol screens were negative for all subjects. Urine drug screens were negative for five of the MDMA using subjects.

Of the other ten MDMA using subjects, four subjects were positive for THC, six subjects were positive for cocaine, and two subjects were positive for amphetamine,

Table 1 Demographics and lifetime history of drug use by group. Drug use is shown as percent of subjects ever using the drug and the mean±SD of number of lifetime uses (MDMA) or the number of uses in the last year (all other drugs)

Group	Age	Gender	Years of education	MDMA		Alcohol		Opiates		Cocaine		Barb.		Benzo.		THC	
MDMA (n=15)	24.7±3.4	12 M, 3F	12.8±1.2	100%	194±240	100%	106±105	40%	28±94	80%	30±52	33.3%	18±36	53.3%	10±16	86.7%	124±157
Control (n=19)	25.4±6.0	11 M, 8 F	14.9±2.2	0%	0	68.4%	57±92	0%	0	0%	0	0%	0	0%	0	31.6%	1±6

methamphetamine, or MDMA. Urine drug screens were negative for all of the controls.

Behavioral performance

Using the SAS mixed procedure implementation of repeated measures ANOVA, MDMA users were compared with controls for behavioral data while in the scanner. The results of that analysis showed that MDMA users and controls did not differ on the non-parametric discrimination score (A') (Donaldson 1992), correct detections, or the number of commission errors (false alarms), for 3, 5, or 7 digits. However, there was a significant group by stimulus type interaction effect (F=12.53, df=1, 63.9, P=0.0008) for response latency. MDMA users had significantly shorter latency for commission errors (mean±SE=570±28 ms) than controls (689±24 ms). There was no significant difference between the two groups on latency for correct detections.

fMRI results

Overall pattern of activation

SPM99 random effects analysis of the main effect across both groups combined showed significantly greater clusters of activation during the DMT relative to the IMT as revealed by significant clusters (whole volume corrected two-tailed cluster level P<0.05) in the following regions (Talairach coordinates represent the maximum t value within each cluster; left is positive): superior frontal gyrus (3, 6, 51), middle frontal gyrus (−36, 33, 33), superior parietal lobule (24, −54, 45), precuneus (−30, −63, 39), and insula (−33, 18, 6). These regions, with the exception of the insula, are often reported as being activated in working memory studies of normal individuals (D'Esposito et al. 1998).

Differences in activation between groups

SPM99 random effects analysis showed that MDMA users had significantly greater BOLD activation, at the cluster level corrected for multiple comparisons and corrected for two-tailed hypothesis testing, compared to the control group during the DMT (relative to the IMT baseline) in three separate clusters (Table 2, Fig. 2).

The first cluster (labeled cluster A in Table 2) was in the left medial and superior frontal gyrii, with activation extending into in the right medial and superior frontal gyrii, bilateral anterior cingulate gyrii, and the right middle frontal gyrus. The second cluster (labeled cluster B in Table 2) was in the left thalamus, with activation extending into the left caudate and putamen, left parahippocampal gyrus, left hippocampus, and left insula. The third cluster (labeled cluster C in Table 2) was in the right parahippocampal gyrus, extending into the right

Table 2 Clusters that show statistically significant greater activation in MDMA-using subjects versus control subjects during DMT relative to IMT. SPM99 random effects analysis. Cluster *P*(cor) signifies cluster level probability values corrected for multiple comparisons over whole brain volume (43,753 voxels, smoothness FWHM=3.9, 3.9, 3.3 voxels) and also corrected for two-tailed hypothesis testing. Voxel threshold t=2.14, df=32, uncorrected threshold $P \leq 0.020$. Tailarach atlas coordinates in *bold* font signify the voxel that showed the maximum value of the t statistic within each significant cluster. Talairach atlas coordinates in *non-bold* font signify local maxima >8.0 mm apart within each significant cluster

Cluster label	Talairach atlas coordinates (mm) (left is positive)			Number of voxels in cluster	Mean activation of all voxels within cluster (% of mean global BOLD signal)	Standard error of activation of all voxels within cluster	Cluster *P*(cor)
	x	*y*	*z*				
A	**9**	**51**	**15**	380	0.1113	0.0958	0.020
	−18	51	12				
	−24	51	3				
B	**15**	**−27**	**6**	846	0.1453	0.1142	0.002
	33	−18	6				
	27	3	−3				

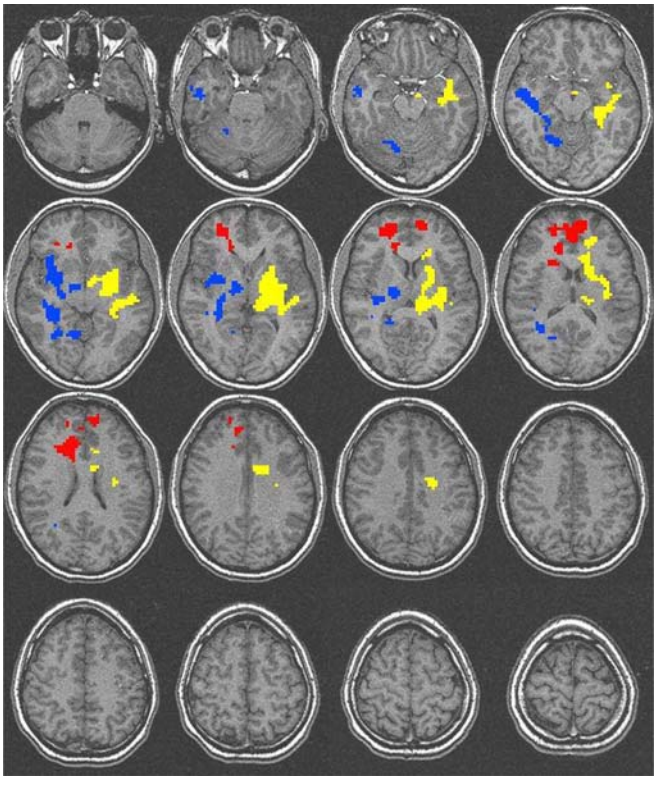

Fig. 2 Areas of statistically significant activation in MDMA users compared with controls. *Red, yellow, and blue areas* signify cluster A, B, and C in Table 2, respectively, overlayed on a representative single control subject's Talairach SPGR in *gray*

hippocampus, right thalamus, right lentiform nucleus, right putamen, right insula, and right temporal cortex (see Table 2, Fig. 2). No voxels were statistically significant at the individual voxel level of inference (Friston et al. 1995b).

There were no statistically significant voxels or clusters in which control subjects showed greater activation than MDMA using subjects. Since MDMA users differed from controls on the number of years of education, an SPM99 random effects regression analysis was performed to determine if there were any areas of BOLD activation which correlated with education. In that analysis, there were no areas of activation which correlated positively or negatively with education (P<0.05 corrected) at either the cluster or voxel level of inference.

A separate SPM99 random effects regression analysis was performed within MDMA users to determine if there was a correlation between self-reported number of uses of MDMA and areas of activation on fMRI. Number of uses was log-corrected prior to regression analysis. The results of that analysis showed no statistically significant clusters or voxels of activation that correlated positively or negatively with number of uses of MDMA or maximum amount of MDMA used during an episode of use. Similarly, there was no statistically significant positive or negative correlation between length of time (whether log transformed or not) since last MDMA use and BOLD activation at any level of task difficulty (i.e. 3, 5, or 7 digits per stimulus), even when using a similar criterion as in Jacobsen et al. (2003) of eight contiguous voxels greater than a threshold of uncorrected alpha=0.01 within a hippocampal region of interest.

Since some MDMA users had a positive urine drug screen (UDS) and others did not, an SPM random effects ANOVA was performed comparing MDMA users who had a negative UDS with control subjects. Results of that analysis are shown in Table 3.

MDMA users with a negative urine drug screen showed greater activation than controls in three regions. The first region had maximal activation in the right caudate, extending into the left caudate and left medial frontal gyrus and Brodman area 10. The second region had maximal activation in the left parahippocampal gyrus, extending into the left basal ganglia, thalamus and hippocampus. The third region had maximal activation in the right inferior temporal gyrus, extending into the right basal ganglia, thalamus and hippocampus. These three regions overlapped with the three regions of increased activation seen in the comparison of the larger group of MDMA users to controls (Table 3).

Further exploratory post hoc analyses were performed to examine the effects of other drugs of abuse upon the difference in brain activation between groups. A separate SPM random effects ANCOVA was performed with amount of use over the past year of each of the following drugs as the covariate: barbiturates, benzodiazepines, hallucinogens, alcohol, cocaine, and marijuana. Results of these ANCOVAs are listed in Table 4. MDMA users continued to show greater brain activation compared to

Table 3 Post hoc analysis results of clusters that show greater activation in MDMA-using subjects who had a negative urine drug screen at the time of scanning versus control subjects during DMT relative to IMT. SPM99 random effects analysis. Cluster *P*(cor) signifies cluster level probability values corrected for multiple comparisons over whole brain volume (43,753 voxels, smoothness FWHM=3.9, 3.9, 3.3 voxels) (one tail). Voxel threshold t=2.14, df=32, uncorrected threshold $P \leq 0.020$. Tailarach atlas coordinates signify the voxel that showed the maximum value of the t statistic within each significant cluster. *Percent overlap* indicates the percent of voxels in each cluster (A, B, C) in Table 2 that are also present in the aggregate of the significant clusters from this analysis, which was calculated using Marsbar software according to the following formula: [the number of voxels in the logical intersection of the {union of all of the significant clusters from this analysis} with the {set of voxels in an individual cluster (A, B, or C) from Table 2}] multiplied by 100 divided by the number of voxels in the individual cluster (A, B, or C) from Table 2

Talairach atlas coordinates (mm) (left is positive)			Number of voxels in cluster	One tail cluster *P*(cor)	Percent overlap with each cluster (A, B, C) from Table 2
x	*y*	*z*			
21	0	24	383	0.005	A 8
21	−9	−3	233	0.06	B 36
−9	−9	−3	597	0.01	C 33

controls during the DMT relative to the IMT after covarying for the extent of use of each of the other drugs. There was a large percentage of overlap (Table 4) between areas with greater activation in MDMA users in analyses that did or did not include extent of other drug use as a covariate.

However, after including marijuana use as a covariate, there was no significant increase in activation in the prefrontal cortex in MDMA users. Also, due to a decrease in signal to noise ratio when quantity of benzodiazepine use was included as a covariate, the SPM voxel-level t threshold was empirically decreased from 2.14 to 1.70 in order to detect a statistically significant group difference at the cluster level of inference (Table 4).

Discussion

This study found statistically significant differences during DMT relative to IMT between MDMA users and controls on BOLD fMRI using a random effects analysis with corrections for multiple comparisons over the whole brain. This finding of greater BOLD activation in MDMA users is consistent with several prior studies using BOLD fMRI with a working memory task in substance abusing populations (see Table 5).

Since BOLD fMRI compares the relative change in activation between a baseline "resting" or control state and an "active" state, increased BOLD activation seen in substance abusing groups may possibly be due to substance abusers having less cerebral blood volume or cerebral blood flow at baseline (Volkow et al. 1988).

Thus, the net change in BOLD activation would be greater in these groups due to a greater dynamic range for the BOLD signal between the two conditions. This explanation for the greater BOLD signal in MDMA users would be consistent with a previous study that found significantly increased BOLD activation in the occipital cortex after photic stimulation in cocaine users compared with controls (Lee et al. 2003). There are two studies suggesting that at least acute MDMA administration can decrease regional cerebral blood flow (rCBF). Chang et al. (2000) found that MDMA users did not have significantly less rCBF compared to non-MDMA using controls as measured by SPECT at baseline. However, after taking MDMA there was a significant decrease in rCBF in MDMA users. This finding is similar to a recent study in rats in which significant decreases in rCBF were seen in 28 of the 50 brain areas analyzed after acute MDMA administration (Quate et al. 2004). Another possible explanation for the greater BOLD activation in MDMA users is that MDMA using subjects had less "efficiency" in performing the working memory task and thus showed a greater increase in neuronal activity in order to perform the task at the same level of performance as control subjects. Similar findings have been seen in other studies in patients at risk for dementia (Bookheimer et al. 2000; Ernst et al. 2002).

The main shortcoming of this study is the fact that other drug use was not controlled. Thus, it cannot be determined whether the greater BOLD activation seen in this study was specifically due to MDMA use. It is possible that the finding of increased activation could be due to concomitant drug use, since a study in "pure" MDMA users (Daumann et al. 2003b) found lower activation. However, the two studies cannot be directly compared due to differing methodology, since the study by Daumann et al. did not control for multiple comparisons over the whole brain volume.

Post hoc analyses comparing the smaller sample of MDMA users with a negative urine drug screen at the time of scan to controls were consistent with the findings comparing the larger group of MDMA users to controls, although the overlap in areas of activation was relatively small in the prefrontal cortical region. Likewise, the inclusion of quantity of other drug use as a covariate in the analyses produced similar results to the overall analysis comparing MDMA users to controls. However the region of increased activation in the prefrontal cortex was no longer significant after inclusion of quantity of marijuana use as a covariate.

The areas of differences in activation found in the present study (including prefrontal cortex, hippocampus, thalamus, and basal ganglia) are consistent with clinical

Table 4 Clusters that showed significantly greater activation in MDMA-using subjects versus control subjects during DMT relative to IMT, after including the amount of concomitant drug use (number of uses in the past year) as a covariate. For each drug covariate, a separate ANCOVA was performed using SPM99 random effects analysis. Cluster *P*(cor) signifies cluster level probability values corrected for multiple comparisons over whole brain volume (one-tail contrast between groups). Tailarach atlas coordinates signify the voxel that showed the maximum value of the voxel *t* statistic within each significant cluster. Percent overlap indicates the percent of voxels in each cluster (A, B, C) in Table 2 that are also present in the significant clusters from each ANCOVA, and was calculated using MARSBAR software according to the following formula: [number of voxels in the logical intersection of the {union of all of the significant clusters from each ANCOVA} with the {set of voxels in an individual cluster (A, B, or C) from Table 2}] multiplied by 100 divided by the number of voxels in the individual cluster (A, B, or C) from Table 2

Drug covariate in ANCOVA	Clusters that show greater activation in MDMA-using subjects in ANCOVA after covarying for the number of uses in the past year of another drug. Talairach atlas coordinates (mm) (left is positive)			Number of voxels in cluster (voxel threshold *t*=2.14, except where indicated)	One-tail cluster *P*(cor)	Percent overlap with each cluster (A, B, C) from Table 2
	x	*y*	*z*			
Barbiturates	12	51	12	377	0.011	A 73
	15	−6	6	1961	0.001	B 78
						C 72
Benzodiazepines	9	51	15	696 (1.70)	0.043	A 78
	33	−18	6	1479 (1.70)	0.001	B 80
	−15	−66	3	974 (1.70)	0.007	C 78
Cocaine	33	−18	6	2921	0.001	A 51
						B 84
						C 78
Hallucinogens	−24	51	3	499	0.002	A 82
	−30	−30	−3	2298	0.001	B 90
						C 85
Opiates	−9	30	18	3000	0.001	A 99
						B 95
						C 90
Alcohol	15	−27	6	1657	0.001	A 74
						B 74
						C 73

Table 5 BOLD fMRI studies using working memory task in substance abusing subjects and controls. *ROI* region of interest

Study	Population	Stimulus	Analysis method	Finding
Tapert et al. (2003)	Ten alcohol dependent women and ten control women	Spatial working memory task	AFNI. Random effects. Whole brain correction for multiple comparisons	Controls>alcoholics right inferior and superior parietal lobule, postcentral gyrus, middle frontal gyrus, left thalamus, superior frontal gyrus, cerebellum. Alcoholics>controls right inferior occipital gyrus
Pfefferbaum et al. (2001)	Ten alcohol dependent men and seven controls	Spatial *n*-back task	SPM96. Random effects. Whole brain correction for multiple comparisons	Controls>alcoholics bilateral middle and inferior frontal gyri, inferior parietal lobe, left DLPFC, angular gyrus, extrastriate cortex, right middle frontal gyrus
Desmond et al. (2003)	Ten alcoholic men and 13 control men	Verbal working memory task	SPM99. Random effects. Small volume correction for multiple comparisons within ROI's	Alcoholics>controls in left prefrontal cortex (trend) and right superior cerebellum
Jacobsen et al. (2003)	Six adolescent MDMA users and six controls	Auditory *n*-back task	Matlab. Random effects. Planned comparisons restricted to hippocampus ROI	MDMA users>controls in left hippocampus
Daumann et al. (2003a)	11 heavy MDMA users, 11 moderate MDMA users, and 11 controls	Visual *n*-back task	SPM99. Random effects. Whole brain correction for multiple comparisons	No statistically significant differences between groups after correction for multiple comparisons
Daumann et al. (2003b)	Eight "pure" MDMA users, eight polydrug MDMA users, and eight controls	Visual *n*-back task	SPM99. Random effects. Not corrected for whole brain multiple comparisons	Pure MDMA users>controls (uncorrected) in superior parietal lobule. Controls>pure MDMA users (uncorrected) in posterior cingulate, inferior temporal gyrus, angular gyrus, and striate cortex

studies showing deficits in working memory and impulse control in MDMA users (Bolla et al. 1998; Morgan 1998; Parrott and Lasky 1998; Parrott et al. 1998, 2000; Gouzoulis-Mayfrank et al. 2000; Schifano 2000; Moeller et al. 2002), with the caveat that many prior studies of working memory also did not control for other drug use. The regional differences in activation in MDMA users are also consistent with prior rodent studies showing long-term changes in serotonin levels in the hippocampus, striatum and cortex after MDMA administration (Colado et al. 2001; McGregor et al. 2003). The greater activation in the hippocampus in the present study is also consistent with a prior study in adolescent MDMA users (Jacobsen et al. 2003).

Increased BOLD fMRI activation in the hippocampus may be relatively specific to MDMA use, since this finding has not been reported in alcoholics (see Table 3). Unlike the prior fMRI study by Jacobsen, we found no significant correlation between length of time since last MDMA use and BOLD activation. One possible explanation for this difference in findings is due to the fact that the MDMA users in the present study were older, used more MDMA, and used MDMA more recently than the prior study. It is also possible that the use of MDMA in combination with other drugs could have a bearing on the relationship between length of time since last MDMA use and BOLD activation, since MDMA users in the present study also reported using a number of other drugs.

Bearing in mind the limitations of the present study, MDMA users show differences in BOLD activation in the prefrontal cortex, hippocampus, thalamus and striatum while undergoing a working memory task compared to controls. Further research is warranted to study changes in perfusion and cerebral blood volume in MDMA users as well as to compare MDMA users to other drug using groups in order to determine the specific etiology of the greater activation seen in MDMA users.

Acknowledgements This study was supported by National Institute on Drug Abuse grants R01-DA15345, R01-DA08425, K02-DA00403 (F.G.M.) and the Charles A. Dana Foundation Clinical Hypothesis in Neuroimaging Grant and RO1-MH61927 (J. L.S.).

References

Adler RJ (1981) The geometry of random fields. Wiley, New York

Bolla KI, McCann UD, Ricaurte GA (1998) Memory impairment in abstinent MDMA ("ecstasy") users. Neurology 51:1532–1537

Bookheimer SY, Strojwas MH, Cohen MS, Saunders AM, Pericak-Vance MA, Mazziotta JC, Small GW (2000) Patterns of brain activation in people at risk for Alzheimer's disease. N Engl J Med 343:450–456

Brett M, Anton J-L, Valabregue R, Poline J-B (2002) Region of interest analysis using an SPM toolbox [abstract]. Presented at the Eighth International Conference on Functional Mapping of the Human Brain, June 2–6, 2002, Sendai, Japan. NeuroImage 16

Buchert R, Thomasius R, Nebeling B, Petersen K, Obrocki J, Jenicke L, Wilke F, Wartberg L, Zapletalova P, Clausen M (2003) Long-term effects of "ecstasy" use on serotonin transporters of the brain investigated by PET. J Nucl Med 44:375–384

Chang L, Grob CS, Ernst T, Itti L, Mishkin FS, Jose-Melchor R, Poland RE (2000) Effect of ecstasy [3,4-methylenedioxymethamphetamine (MDMA)] on cerebral blood flow: a co-registered SPECT and MRI study. Psychiatry Res 98:15–28

Cohen RS (1995) Subjective reports on the effects of the MDMA ("ecstasy") experience in humans. Prog Neuropsychopharmacol Biol Psychiatry 19:1137–1145

Colado MI, Granados R, O'Shea E, Esteban B, Green AR (1999) The acute effect in rats of 3,4-methylenedioxyethamphetamine (MDEA, "eve") on body temperature and long term degeneration of 5-HT neurones in brain: a comparison with MDMA ("ecstasy"). Pharmacol Toxicol 84:261–266

Colado MI, O'Shea E, Esteban B, Green AR (2001) Studies on the neuroprotective effect of the enantiomers of AR-A008055, a compound structurally related to clomethiazole, on MDMA ("ecstasy")-induced neurodegeneration in rat brain. Psychopharmacology 157:82–88

Cox RW (1996) AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. Comput Biomed Res 29:162–173

Croft RJ, Mackay AJ, Mills AT, Gruzelier JG (2001) The relative contributions of ecstasy and cannabis to cognitive impairment. Psychopharmacology 53:373–379

Dafters RI, Hoshi R, Talbot AC (2003) Contribution of cannabis and MDMA ("ecstasy") to cognitive changes in long-term polydrug users. Psychopharmacology Aug 30 [EPub ahead of print]

Daumann J, Fimm B, Willmes K, Thron A, Gouzoulis-Mayfrank E (2003a) Cerebral activation in abstinent ecstasy (MDMA) users during a working memory task: a functional magnetic resonance imaging (fMRI) study. Cognit Brain Res 16:479–487

Daumann J, Schnitker R, Weidemann J, Schnell K, Thron A, Gouzoulis-Mayfrank E (2003b) Neural correlates of working memory in pure and polyvalent ecstasy (MDMA) users. Neuroreport 14:1983–1987

Desmond JE, Chen SH, DeRosa E, Pryor MR, Pfefferbaum A, Sullivan EV (2003) Increased frontocerebellar activation in alcoholics during verbal working memory: an fMRI study. Neuroimage 19:1510–1520

Donaldson W (1992) Measuring recognition memory. J Exp Psychol [Gen Sect] 121:275–277

Dougherty DM (1999) IMT/DMT immediate memory tasks & delayed memory task: a research tool for studying attention and memory processes. Neurobehavioral Research Laboratory and Clinic, University of Texas Houston Health Science Center, Houston

D'Esposito M, Aguirre GK, Zarahn E, Ballard D, Shin RK, Lease J (1998) Functional MRI studies of spatial and nonspatial working memory. Brain Res Cognit Brain Res 7:1–13

Ernst T, Chang L, Jovicich J, Ames N, Arnold S (2002) Abnormal brain activation on functional MRI in cognitively asymptomatic HIV patients. Neurology 59:1343–1349

First MB, Spitzer RL, Gibbon M, Williams JB (1996) Structured clinical interview for DSM-IV axis I disorders patient edition. Biometrics Research Department, New York State Psychiatric Institute, New York

Friston KJ, Holmes, Worsley KJ, Poline JP, Frith CD, Frackowiak RSJ (1995a) Statistical parametric maps in functional imaging: a general linear approach. Hum Brain Mapp 2:189–210

Friston KJ, Holmes AP, Poline JB, Price CJ, Frith CD (1995b) Detecting activations in PET and fMRI: levels of inference and power. Neuroimage 40:223–235

Gamma A, Buck A, Berthold T, Vollenweider FX (2001) No difference in brain activation during cognitive performance between ecstasy (3,4-methylenedioxymethamphetamine) users and control subjects: a [H2(15)O]-positron emission tomography study. J Clin Psychopharmacol 21:66–71

Gouzoulis-Mayfrank E, Daumann J, Tuchtenhagen F, Pelz S, Becker S, Kunert HJ, Fimm B, Sass H (2000) Impaired cognitive performance in drug free users of recreational ecstasy (MDMA). J Neurol Neurosurg Psychiatry 68:719–725

Hatzidimitriou G, McCann UD, Ricaurte GA (1999) Altered serotonin innervation patterns in the forebrain of monkeys treated with (±)3,4-methylenedioxymethamphetamine seven years previously: factors influencing abnormal recovery. J Neurosci 19:5096–5107

Holmes A, Friston KJ (1998) Generalisability, random effects & population inference. Neuroimage 7:S754

Jacobsen LK, Mencl WE, Pugh KR, Skudlarski P, Krystal JH (2003) Preliminary evidence of hippocampal dysfunction in adolescent MDMA ("ecstasy") users: possible relationship to neurotoxic effects. Psychopharmacology Nov 28 [Epub ahead of print]

Lee JH, Telang FW, Springer CS Jr, Volkow ND (2003) Abnormal brain activation to visual stimulation in cocaine abusers. Life Sci 73:1953–1961

McCann UD, Szabo Z, Scheffel U, Dannals RF, Ricaurte GA (1998) Positron emission tomographic evidence of toxic effect of MDMA ("ecstasy") on brain serotonin neurons in human beings. Lancet 352:1433–1437

McCann UD, Mertl M, Eligulashvili V, Ricaurte GA (1999) Cognitive performance in (±) 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") users: a controlled study. Psychopharmacology 143:417–425

McGregor IS, Clemens KJ, Van der Plasse G, Li KM, Hunt GE, Chen F, Lawrence AJ (2003) Increased anxiety 3 months after brief exposure to MDMA ("ecstasy") in rats: association with altered 5-HT transporter and receptor density. Neuropsychopharmacology 28:1472–1484

Moeller FG, Dougherty DM, Steinberg JL, Swann AC, Silverman PB, Ruiz P, Barratt ES (2002) Heavy "ecstasy" use is associated with increased impulsivity. Addict Disord Treat 1:47–52

Morgan MJ (1998) Recreational use of "ecstasy" (MDMA) is associated with elevated impulsivity. Neuropsychopharmacology 19:252–264

Morgan MJ, McFie L, Fleetwood H, Robinson JA (2002) Ecstasy (MDMA): are the psychological problems associated with its use reversed by prolonged abstinence? Psychopharmacology 159:294–303

Obrocki J, Buchert R, Vaterlein O, Thomasius R, Beyer W, Schiemann T (1999) Ecstasy—long-term effects on the human central nervous system revealed by positron emission tomography. Br J Psychiatry 175:186–188

Ogawa S, Lee TM, Kay AR, Tank DW (1990) Brain magnetic resonance imaging with contrast dependent on blood oxygenation. Proc Natl Acad Sci USA 87:9868–9872

Pan HS, Wang RY (1991) The action of (±)-MDMA on medial prefrontal cortical neurons is mediated through the serotonergic system. Brain Res 543:56–60

Parrott AC, Lasky J (1998) Ecstasy (MDMA) effects upon mood and cognition: before, during and after a Saturday night dance. Psychopharmacology 139:261–268

Parrott AC, Lees A, Garnham NJ, Jones M, Wesnes K (1998) Cognitive performance in recreational users of MDMA of "ecstasy": evidence for memory deficits. J Psychopharmacol 12:79–83

Parrott AC, Sisk E, Turner JJ (2000) Psychobiological problems in heavy "ecstasy" (MDMA) polydrug users. Drug Alcohol Depend 60:105–110

Petersson KM, Nichols TE, Poline JB, Holmes AP (1999) Statistical limitations in functional neuroimaging. II. Signal detection and statistical inference. Philos Trans R Soc Lond B Biol Sci 354:1261–1281

Pfefferbaum A, Desmond JE, Galloway C, Menon V, Glover GH, Sullivan EV (2001) Reorganization of frontal systems used by alcoholics for spatial working memory: an fMRI study. Neuroimage 14:7–20

Poline JB, Holmes A, Worsley K, Friston KJ (1997) Making statistical inferences. In: Frackowiac RSJ, Friston KJ, Frith CD, Dolan RJ, Mazziotta JC (eds) Human brain function. Academic, San Diego, pp 85–106

Quate L, McBean DE, Ritchie IM, Olverman HJ, Kelly PA (2004) Acute methylenedioxymethamphetamine administration: effects on local cerebral blood flow and glucose utilization in the dark agouti rat. Psychopharmacology Feb 20 [Epub ahead of print]

Reneman L, Endert E, de Bruin K, Lavalaye J, Feenstra MG, de Wolff FA, Booij J (2002) The acute and chronic effects of MDMA ("ecstasy") on cortical 5-HT_{2A} receptors in rat and human brain. Neuropsychopharmacology 26:387–396

Ricaurte GA, McCann UD, Szabo Z, Scheffel U (2000a) Toxicodynamics and long-term toxicity of the recreational drug, 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). Toxicol Lett 112–113:143–146

Ricaurte GA, Yuan J, McCann UD (2000b) (±)3,4-Methylenedioxymethamphetamine ("ecstasy")-induced serotonin neurotoxicity: studies in animals. Neuropsychobiology 42:5–10

Rodgers J (2000) Cognitive performance amongst recreational users of "ecstasy". Psychopharmacology 151:19–24

Schifano F (2000) Potential human neurotoxicity of MDMA ("ecstasy"): subjective self-reports, evidence from an Italian drug addiction centre and clinical case studies. Neuropsychobiology 42:25–33

Schmidt CJ, Taylor VL (1987) Depression of rat brain tryptophan hydroxylase activity following the acute administration of methylenedioxymethamphetamine. Biochem Pharmacol 36:4095–4102

Semple DM, Ebmeier KP, Glabus MF, O'Carroll RE, Johnstone EC (1999) Reduced in vivo binding to the serotonin transporter in the cerebral cortex of MDMA ("ecstasy") users. Br J Psychiatry 175:63–69

Simon NG, Mattick RP (2002) The impact of regular ecstasy use on memory function. Addiction 97:1523–1529

Talairach J, Tournoux P (1988) Co-planar stereotaxic atlas of the human brain, 1st edn. Thieme, New York

Tapert SF, Cheung EH, Brown GG, Frank LR, Paulus MP, Schweinsburg AD, Meloy MJ, Brown SA (2003) Neural response to alcohol stimuli in adolescents with alcohol use disorder. Arch Gen Psychiatry 60:727–735

Thomasius R, Petersen K, Buchert R, Andresen B, Zapletalova P, Wartberg L, Nebeling B, Schmoldt A (2003) Mood, cognition and serotonin transporter availability in current and former ecstasy (MDMA) users. Psychopharmacology 167:85–96

Volkow ND, Mullani N, Gould KL, Adler S, Krajewski K (1988) Cerebral blood flow in chronic cocaine users: a study with positron emission tomography. Br J Psychiatry 152:641–648