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Original Investigation

Preliminary evidence of hippocampal dysfunction in adolescent MDMA ("ecstasy") users: possible relationship to neurotoxic effects

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Abstract

Rationale 3,4-Methylenedioxymethamphetamine (MDMA or ecstasy) is a potent and selective serotonin neurotoxin whose use is growing among adolescents. Although cognitive deficits among adult MDMA users are well documented, little is known of the cognitive and brain functional sequelae of MDMA use during adolescence.

Objective We tested for evidence of cognitive deficits and changes in brain function in a pilot sample of adolescent MDMA users, who were compared with adolescent non-users of MDMA.

Methods Selective and divided attention and verbal working memory were examined in six adolescent MDMA users and six non-users of MDMA who were similar in age, gender, IQ, and other substance use. Brain function was assessed during performance of the working memory task using functional magnetic resonance imaging (fMRI).

Results MDMA users had significantly prolonged reaction times during tests of selective and divided attention, and failed to deactivate the left hippocampus normally during high verbal working memory load.

Conclusions MDMA use in adolescence may be associated with cognitive

impairments and dysfunction of inhibitory circuits within the hippocampus. Further work is urgently needed to delineate the developmental impact and long-term functional and clinical significance of MDMA use during adolescence.

Keywords MDMA - Ecstasy - Adolescent - Selective attention - Divided attention - Working memory

Introduction

MDMA is a ring-substituted amphetamine derivative with structural similarities to mescaline (Anderson et al. *1978*). In light of increasing recreational use, together with growing evidence of its neurotoxicity, MDMA was designated as a schedule I controlled substance by the US Drug Enforcement Agency in 1986 (Steele et al. *1994*). In spite of this, recreational use has continued to grow, and age at initiation of use has continued to decline (Parrott *2002*; von Sydow et al. *2002*). Recent surveys of college students have demonstrated persistent and growing use of MDMA despite declining use of other drugs (Peroutka *1987*; Pope et al. *2001*; Schuster et al. *1998*), and the 2002 Monitoring the Future Survey observed a lifetime prevalence of MDMA use of 4.3% among 8th graders, 6.6% among 10th graders, and 10.5% among 12th graders in the United States (http://monitoringthefuture.org/data/02data.html).

Neurotoxicity

A consistent profile of long-lasting serotonergic damage has been demonstrated in a variety of rodent species and in New and Old World monkeys (Steele et al. *1994*; Hegadoren et al. *1999*; Parrott *2002*; Taffe et al. *2002*). MDMA produces dose-related reductions in brain concentrations of serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), in the density of 5-HT uptake sites, and in tryptophan hydroxylase activity (Stone et al. *1986*; Commins et al. *1987*; Schmidt *1987*; Schmidt et al. *1987*; O'Hearn et al. *1988*). These reductions are correlated with degeneration of serotonergic axons (Commins et al. *1987*; O'Hearn et al. *1988*; Wilson et al. *1989*). Although in both rodents and in non-human primates there is some evidence of neuronal recovery 12–18 months following MDMA exposure, reinnervation is abnormal, with cortical regions remaining denervated and some subcortical structures becoming hyperinnervated (Steele et al. *1994*; Fischer et al. *1995*; Hatzidimitriou et al. *1999*). While the dose per kg of MDMA that produces neurotoxic damage in animals are higher than those typically ingested by humans, application of interspecies drug dose scaling indicates that doses of MDMA that are neurotoxic in animals are comparable to those self administered by humans (Mordenti and Chappell *1989*; McCann and Ricaurte *2001*).

Positron emission tomography (PET) and single photon emission computed tomography (SPECT) imaging studies in humans have demonstrated sustained decrements in brain 5-HT transporter density (McCann et al. *1998*; Semple et al. *1999*; Ricaurte et al. *2000*) and in cortical 5-HT_{2A} receptor density (Reneman et al. *2002*), with 5-HT transporter density being inversely correlated with extent of previous MDMA use (McCann et al. *1998*; Reneman et al. *2001a*). After sustained abstinence, cortical 5-HT_{2A} receptor density appears to recover, and density of occipital 5-HT_{2A} receptors increases, suggesting a compensatory upregulation (Reneman et al. *2002*). Upregulation of occipital 5-HT_{2A} receptors in long abstinent MDMA users was found to be positively correlated with verbal memory performance (Reneman et al. *2000*). Effects of MDMA on 5-HT transporters may be more pronounced in women (Reneman et al. *2001a*, *2001b*) and may reverse with prolonged abstinence (Reneman et al. *2001a*, *2001b*). Reduced cerebrospinal fluid (CSF) concentrations of 5-HIAA (McCann

et al. *1994*, *1999*) and blunted prolactin and cortisol responses to *d*-fenfluramine (5-HT releasing agent and reuptake inhibitor) challenge (Gerra et al. *2000*; Verkes et al. *2001*) have also been demonstrated in abstinent MDMA users. Deficits in CSF 5-HIAA were found to be correlated with deficits in verbal and visual memory (Bolla et al. *1998*).

Cognitive correlates of MDMA use in humans

To date, studies of cognitive function in humans who self administer MDMA have focused on adult users. Human studies are frequently confounded by the presence of polysubstance abuse in the MDMA users, uncertainty regarding the quantity and purity of the MDMA ingested, and the absence of measures of cognitive function prior to MDMA exposure.

Most studies examining the cognitive function of adults with a history of MDMA use have demonstrated evidence of deficits. Deficits in verbal and visual memory have been most frequently observed (Krystal et al. *1992*; Parrott *1997*; Parrott and Lasky *1998*; Morgan *1999*; Gouzoulis-Mayfrank et al. *2000*; Bhattachary and Powell *2001*; Croft et al. *2001*; Reneman et al. *2001b*; Verkes et al. *2001*; Curran and Verheyden *2003*; Gouzoulis-Mayfrank et al. *2003*); however, prolonged reaction times during tests of attention and evidence of poorer performance on more complex and challenging tasks involving working memory have also been seen (Wareing et al. *2000*; Fox et al. *2001*; Verkes et al. *2001*). With few exceptions (Croft et al. *2001*), observed deficits have remained significant after controlling for potentially confounding group differences in other substance use (Parrott and Lasky *1998*; Gouzoulis-Mayfrank et al. *2000*; Parrott *2000*; Wareing et al. *2000*; Fox et al. *2001*; Verkes et al. *2001*). Further supporting the association between cognitive deficits and MDMA use has been the observation of significant relationships between quantity of MDMA use and severity of cognitive deficits (Bhattachary and Powell *2001*; Croft et al. *2001*; Fox et al. *2001*; Verkes et al. *2001*). As noted above, significant correlations between MDMA related alterations in cortical 5-HT_{2A} receptor density, CSF 5-HIAA concentration and cognitive function have also been found (Bolla et al. *1998*; Reneman et al. *2000*).

Although some investigators have observed positive correlations between cognitive measures and duration of abstinence from MDMA, suggesting some degree of recovery of function with cessation of MDMA use (Bhattachary and Powell *2001*), studies of MDMA users who have been abstinent for at least 1 year have demonstrated persistence of cognitive deficits (Reneman et al. *2001b*; Curran and Verheyden *2003*), suggesting that this sequela of MDMA use may be long lasting or permanent. Indeed, one longitudinal study of MDMA users demonstrated that continued use of MDMA over a 1-year follow-up period was associated with progression of deficits in immediate and delayed verbal memory (Zakzanis and Young *2001*).

Effects of MDMA on brain function

As with studies of the cognitive sequelae of MDMA use, studies of CBF and brain functional sequelae of MDMA use to date have focused exclusively on adults. Buchert and colleagues (*2001*) compared resting cerebral glucose metabolism of 93 MDMA users with that of 27 oncology patient controls using ^{18}F-fluoro-2-deoxyglucose (FDG) and PET. Normalized FDG uptake was significantly reduced in bilateral caudate and putamen and left amygdala in MDMA users. There was also a trend for reduced FDG uptake at left hippocampus. Age at first use of MDMA was positively correlated with FDG uptake at bilateral putamen, right caudate, and left hippocampus. This study was confounded by the use of oncology patients as controls, who did not have central nervous system (CNS) lesions, but may have received systemic chemotherapy with CNS toxicity (Buchert et al. *2001*; Obrocki et al. *2002*).

Gamma and colleagues (*2001*) used $H_2{}^{15}O$ PET to compare task related rCBF in MDMA users and controls performing the A-X CPT. Significant task by group interactions in rCBF were not observed. However, both the small number of control scans obtained in this study and the lack of group differences in task performance may have contributed to the failure to observe group differences in task related brain activation (Gamma et al. *2001*).

Most recently, Daumann and colleagues (*2003*) compared heavy (lifetime dose $\geq$80 tablets), and moderate (lifetime dose <80 tablets) MDMA users with controls using functional magnetic resonance imaging (fMRI) while the subjects performed a visual *n*-back task. There were no group differences in task performance and no group differences in brain activation at a conservative level of significance. However, at a more liberal significance level, heavy MDMA users showed weaker activation of left superior temporal lobe, left superior frontal gyrus, and anterior cingulate than controls during the two-back condition. Both heavy and moderate MDMA users showed greater activation of right parietal cortex than controls during one- and two-back conditions. These observations suggest that MDMA use may be associated with subtle changes in cortical activation during working memory task performance.

In summary, preclinical work has demonstrated that MDMA is toxic to serotonergic neurons even when administered in recreational doses. Deficits in memory, attentional processing, and executive cognitive function have been observed in adult MDMA users, which have remained significant in most studies after controlling for confounding variables.

Although recreational use of MDMA remains substantial among adolescents, with many teenagers beginning to experiment with this drug at the age of 14 or 15 (http://monitoringthefuture.org/data/02data.html), few published studies have systematically examined cognitive or brain functional correlates of MDMA use in this population. Substantial brain development and organization occurs during adolescence (Casey et al. *2000*). These changes appear to subserve development of cognitive capacities during this period (Casey et al. *2000*). It is possible, therefore, that use of a neurotoxic substance such as MDMA during this period could, by disrupting critical developmental processes, exert more pernicious effects on brain function than those seen in adult users. Previous work in adults highlights the importance of controlling for potentially confounding variables that can contribute to group differences in cognitive performance and brain function, including other substance use and group differences in general intelligence.

To test for evidence of MDMA associated deficits in cognition and brain function in adolescents, we assessed sustained, selective, and divided attention, and verbal working memory in adolescent MDMA users and non-users. Brain function was assessed during performance of the working memory task using fMRI.

Materials and methods

Subjects

Six adolescents with a history of MDMA use were compared with six adolescents with no history of MDMA use who were matched for age and gender. Demographic data for both groups are presented in Table 1. All participants were recruited from the community, were participants in an ongoing study of adolescent tobacco use, smoked tobacco daily, and were free of medical and psychiatric illness and substance abuse or dependence, other than nicotine dependence, as determined by structured clinical

interview (Puig-Antich et al. *1980*). The groups did not differ in number of cigarettes smoked per day, degree of nicotine dependence [assessed using the Fagerstom Test for Nicotine Dependence (Pomerleau et al. *1994*)], history of cannabis use (all but one non-user of MDMA reported a history of cannabis use and all but one subject from each group were abstinent from cannabis for at least 4 weeks prior to assessment), years of education completed, years of education completed by the parents, estimated intelligence, reading achievement, or self reported symptoms of depression and anxiety. One MDMA user had tried cocaine 1 year prior to study. MDMA users reported consuming an average of 2 (range 0–3) alcoholic drinks per week, while non-users of MDMA reported consuming an average of 0.8 (range 0–3) alcoholic drinks per week (t=1.9, df=10, P=0.09). MDMA users reported an average of ten episodes of MDMA use (range 1–25 episodes) and first used MDMA at age 15.8 years (range 15–17 years). Use of other substances beyond those described above was denied by all subjects. To reduce the disrupting effects of nicotine withdrawal on cognition, subjects were permitted to smoke during a break prior to assessment.

Table 1 Demographic data for six adolescent MDMA users and six non-users of MDMA

	MDMA users (mean±SD[a])	Non-users (mean±SD)
Age (years)	17.3±1.1	17.1±1.1
Gender	4 female/2 male	4 female/2 male
Cigarettes/day	10.3±5.3	8.8±6.1
FTND[b]	3.7±2.0	2.2±1.0
Education (years)	10.0±1.3	10.3±1.0
Parent education (years)	13.3±2.1	13.5±1.5
KBIT Composite Score[c]	96.7±8.0	96.8±10.9
WJR Word Attack SS[d]	104.7±20.1	107.3±12.5
WJR Letter-Word SS	98.3±10.9	97.0±5.1
POMS[e] Anxiety Score	0.5±3.6	2.2±4.5
POMS Depression Score	2.7±3.4	3.3±3.6

[a]SD=standard deviation

[b]FTND=Fagerstrom Test for Nicotine Dependence (Pomerleau et al. *1994*)

[c]KBIT=Kaufman Brief Intelligence Test (Bowers and Pantle *1998*)

[d]Woodcock-Johnson Revised Test of Achievement Word Attack and Letter-Word Identification subtests standard scores

[e]POMS=Profile of Mood States (Little and Penman *1989*)

Assessment of selective, divided, and sustained attention

Sustained attention was assessed using the Conners Continuous Performance Test (CPT; Chen et al. *1998*). Selective and divided attention were assessed using a simplified version of a previously described computerized word recognition task (Shaywitz et al. *2001*). Within the selective attention task, both auditory and visual selective attention are assessed.

Auditory selective attention is assessed by comparing an *auditory simple* with an *auditory select* condition. During auditory simple, subjects listen to a verbal stimulus

and press one button if the stimulus is a real word and another if it is not. Simultaneous with auditory stimulus presentation, four diagonal lines are presented on the computer screen. During auditory select, the subject must again judge whether an auditory stimulus is a real word or not. However, instead of diagonal lines, visual word or non-word distracters simultaneously appear on the screen.

Visual selective attention is assessed by comparing a *visual simple* with a *visual select* condition. During visual simple, subjects view a letter string on the computer screen and press one button if the stimulus is a real word and another button if it is not. Simultaneous with presentation of the visual stimulus, subjects hear a tone. During visual select, subjects again make a word/non-word judgment about a visually presented stimulus. However, instead of tones, auditory word or non-word distracters are played through the headphones.

During the divided attention condition, word or non-word stimuli are presented simultaneously in both auditory and visual modalities. A visual cue is then presented to indicate which word/non-word combination is correct and subjects must then press a button indicating whether the seen and heard stimuli represent the correct combination. Thus, unlike the selective attention task, the divided attention task requires that subject's fully process both auditory and visual stimuli before making a judgment.

Assessment of brain function

Brain function was assessed with fMRI while subjects performed an auditory *n*-back task with two levels of working memory load (one-back and two-back) and two levels of selective attention load (binaural and dichotic stimulus presentation). During each imaging run, subjects heard nonsense words presented in sequence and indicated with a button press whether each word presented matched the word presented one-back or two-back. A binaural three-back run was added to the imaging protocol after three non-users had been scanned. During binaural runs, the same tone and nonsense words are presented to both ears. During dichotic runs, tones are presented binaurally, but different words are presented to each ear. The binaural/dichotic manipulation modulates demands on selective attention. Subjects are instructed as to which ear to attend to prior to each dichotic run. Targets are presented only to the attended ear. *n*-Back blocks were interleaved with control blocks during which tones rising or falling in pitch were presented. Subjects indicated the direction of pitch change with a button press. This task involves perceiving sound and pressing a button but does not involve selective attention or working memory. To reduce variance related to learning or to excessive errors, subjects were trained, using different stimuli lists than those presented during scanning, until they correctly identified a minimum of 85% of targets across all conditions.

Image acquisition and processing

All subjects were prepared for scanning with a training session in a mock MRI scanner. Subjects were scanned using a 1.5 Tesla Signa LX MRI system (GE, Milwaukee, Wisc., USA). Subjects' heads were immobilized within a circularly polarized head coil by using a neck support, foam wedges, and a restraining band drawn tightly across the forehead. For optimal visualization of the hippocampus, coronal oblique T1 weighted anatomic images were acquired perpendicular to the long axis of the hippocampus using a spin echo pulse sequence (7 mm thick slices, 24 slices; echo time (TE)=14 ms, repetition time (TR)=500 ms, matrix=256×192 pixels, field of view (FOV)=20 cm^2, number of excitations (NEX)=1). Echoplanar functional images were acquired in the same relative slice locations using a single shot, gradient echo pulse sequence (TE=50 ms, TR=2500 ms, flip angle (FA)=80°, matrix=64×64, FOV=20 cm^2, NEX=1). A total of 160 functional images were collected per slice per activation condition.

Image analysis was performed using software written in Matlab (MathWorks, Natick, Mass., USA). Prior to statistical analysis, images from each scanning session were motion corrected using SPM 99 (Friston et al. *1995*). Fourteen images at each slice location were discarded per run to avoid variance stemming from hemodynamic changes that occur during task transitions. The remaining images were spatially smoothed with a 6.25 mm full width at half maximum (FWHM) Gaussian kernel. In the temporal domain, a high pass filter was applied to remove drift at frequencies lower than twice the period of the activation paradigm.

Statistical analysis

Single subject activation data were obtained by subtracting pixel intensity during the control task from pixel intensity during the experimental task and dividing this difference by the average signal intensity during the entire run to obtain normalized percent signal change for each task condition. Anatomic images and activation maps from each subject were transformed into a proportional three-dimensional grid (Talairach and Tournoux *1988*). Effects of MDMA use and task were assessed at each voxel using mixed model repeated measures analysis of variance (ANOVA) (Kirk *1982*; Woods *1996*; Holmes and Friston *1998*) with level of working memory load (one-back or two-back) and level of selective attention load (binaural or dichotic) as within subjects factors, and MDMA exposure as a between subjects factor. Because of the small sample size in the present study, the recurrent observation of memory deficits in human MDMA users (Krystal et al. *1992*; Parrott *1997*; Parrott and Lasky *1998*; Morgan *1999*; Gouzoulis-Mayfrank et al. *2000*, *2003*; Bhattachary and Powell *2001*; Fox et al. *2001*; Reneman et al. *2001b*; Verkes et al. *2001*; Zakzanis and Young *2001*; Curran and Verheyden *2003*), and evidence in animals that MDMA related damage to hippocampal neurons is severe (Ricaurte et al. *1992*; Fischer et al. *1995*; Hatzidimitriou et al. *1999*), we focused the fMRI assessment upon the hippocampus. The significance level was set at α=0.01, with a cluster-size threshold of eight contiguous voxels. Since fMRI data during the binaural three-back condition were not obtained from three non-users of MDMA, binaural three-back data were used in correlation analyses only.

Accuracy [measured by computing D' (Repp and Frost *1988*)] and reaction time during task performance were assessed with repeated measures ANOVA. The relationships between outcome measures (task performance and brain activation) and magnitude and recency of MDMA exposure and age of onset of MDMA user were examined using Pearson's correlation coefficient. Urine toxicology screen prior to assessment was negative for all but one subject in each group, both of whom had positive screens for tetrahydrocannabinol (THC). Sequential quantitative screens indicated that THC concentrations were declining in both subjects, consistent with recent abstinence from cannabis prior to assessment. Prior to study entry, all subjects provided written assent or, for 18 year-olds, consent. Parental consent was obtained for subjects 17 years of age and younger. This study was approved by the Yale University School of Medicine Human Investigation Committee.

Results

Selective, divided, and sustained attention

There were no significant group differences in accuracy of task performance, although there was a trend for MDMA users to perform less accurately during the visual selective attention task (F=3.28, df=1,10, P=0.1). There was no effect of MDMA use upon CPT or divided attention task performance accuracy. ANOVA of reaction time

data revealed that MDMA users had significantly prolonged reaction times across simple, select, and divide task conditions (F=9.9, df=1,10, P=0.01). Accuracy and reaction time data from these tasks are presented in Table 2. Effect sizes [Cohen's d (Cohen *1988*)] ranged from 1.5 to 2.1, except in the divided attention task for which the effect size was 0.4. Removal of the MDMA user and control subject with residual THC in their urine at the time of assessment did not alter this finding (F=7.2, df=1,8, P=0.03). There was a trend for MDMA users to have prolonged reaction times during the CPT (MDMA users=415.5±46.2, non-users=367.9±56.1, F=2.6, df=1,10, P=0.14).

Table 2 Accuracy and reaction time during performance of selective and divided attention tasks in adolescent MDMA users and non-users

	Auditory		Visual		Divide±SD
	Simple±SD	Select±SD	Simple±SD	Select±SD	
Proportion of button presses that were correct					
MDMA users	0.88±0.14	0.86±0.07	0.92±0.03	0.89±0.10	0.77±0.15
Non-users	0.89±0.06	0.91±0.05	0.92±0.09	0.97±0.05	0.78±0.07
Reaction time (ms)					
MDMA users	1411.2±155.2	1499.8±141.9	1272.3±212.1	1260.3±181.1	1074.0±267.0
Non-users	1208.4±119.2	1244.1±137.3	932.7±95.8	1017.8±121.0	977.1±227.9

Brain function

There was a significant group by memory load by selective attention load interaction at the left hippocampus (Talairach coordinates: X=–24.2, Y=–28.9, Z=–10.4); shown on the left in Fig. 1). Percent signal change data for each task condition obtained from a region of interest defined by left hippocampal voxels showing a significant interaction are plotted on the right side of Fig. 1. In non-users, activation of the left hippocampus decreased relative to the tone sweep control condition during the most difficult task condition (dichotic two-back). In MDMA users, activation of left hippocampus decreased during the relatively easier dichotic one-back and binaural two-back conditions and decreased less during the most difficult dichotic two-back condition. The effect size for the group difference in activation of left hippocampus during the dichotic two-back task was 0.8. When the MDMA user and control subject with residual THC in their urine at the time of assessment were removed from the analysis, activation of left hippocampus during the dichotic two-back task remained strongly negative in the controls and became positive in the MDMA users (d=1.4).

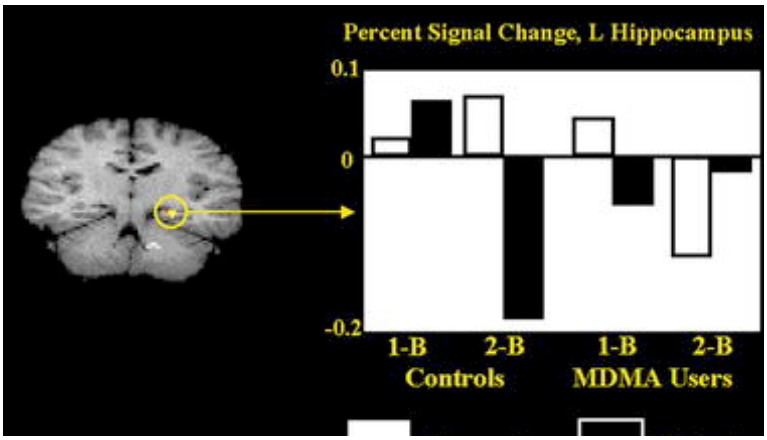

Fig. 1 On the *left* are results of the voxel by voxel ANOVA showing a significant interaction between MDMA use, working memory load, and selective attention load at left hippocampus using a significance threshold of $P \leq 0.01$ and cluster filter of eight voxels. The left side of the brain is on the right. On the *right* of the figure is graphed the mean percent signal change of the encircled, functionally defined, region of the left hippocampus associated with each task condition for MDMA users and non-users. *1-B* one-back, *2-B* two-back

Correlation analysis revealed strongly negative relationships between left hippocampal activity and time since last MDMA use across working memory task conditions (*r* values ranging from –0.53 to –0.84; Fig. 2), with the strongest negative correlation occurring in the highest memory load condition (binaural three-back: *r*=–0.84, *n*=6, *P*=0.03). Neither total number of episodes of MDMA use nor age at onset of MDMA use showed a strong or consistent relationship to hippocampal activity across working memory task conditions.

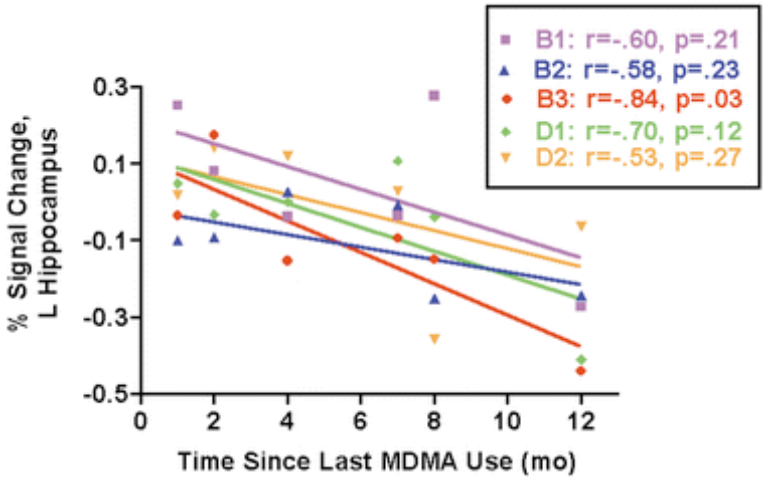

Fig. 2 Correlation between activity of the left hippocampus during each working memory task condition and duration of abstinence from MDMA for six adolescent MDMA users. *B1* binaural one-back, *B2* binaural two-back, *B3* binaural three-back, *D1* dichotic one-back, *D2* dichotic two-back

Total number of episodes of MDMA use was negatively correlated with accuracy of task performance across all working memory and attention task conditions, with the exception of the auditory simple and auditory selective attention tasks and the CPT. The correlation between number of episodes of MDMA use and performance accuracy achieved statistical significance for visual simple and divided attention tasks (*r*=–0.95, *n*=6, *P*=0.003 and *r*=–0.89, *n*=6, *P*=0.02, respectively), and for the binaural three-back task (*r*=–0.86, *n*=6, *P*=0.03). Correlations between total number of episodes of MDMA use and reaction time were weak and inconsistent across sustained, selective and divided attention tasks, but were strongly positive for the working memory task (*r* values ranging from 0.56 to 0.84). These correlations reached statistical significance for the binaural two-back and dichotic one-back task (*r*=0.84, *n*=6, *P*=0.04 and *r*=0.82, *n*=6, *P*=0.04, respectively).

Age at onset of MDMA use was inconsistently related to performance accuracy and reaction time during the attention tasks, but was consistently positively correlated with performance accuracy (*r* values ranging from 0.38 to 0.86) and consistently negatively correlated with reaction time (*r* values ranging from –0.26 to –0.78) across working

memory task conditions. However, only the relationship with performance accuracy during the binaural two-back task achieved statistical significance (r=0.86, n=6, P=0.03).

Discussion

In this pilot study of adolescent MDMA users, significant delays in reaction time during simple, selective and divided attention tasks and abnormal function of the left hippocampus during high working memory load were observed. MDMA users did not differ from controls on a number of variables that could potentially have contributed to the observed findings, including age, general intelligence, reading achievement, years of education, history of cannabis use, or tobacco use. The effect of MDMA use on both reaction time during the attention tasks and on left hippocampal function remained significant after removal of the MDMA user and control subject with residual THC in their urine at the time of assessment.

Mean delays in reaction time during the attention tasks ranged from 97 ms (divided attention task) to 340 ms (visual simple attention task). Previous studies of adult MDMA users have reported significant, but smaller, delays in reaction time during tests of selective and divided attention and during simple visual and auditory tasks, with reaction time delays ranging from 27 to 54 ms (Gouzoulis-Mayfrank et al. *2000*; Verkes et al. *2001*). In adults, reaction time delays have been found to be more severe in heavy than in moderate users of MDMA, supporting a relationship between MDMA exposure and slowing of attentional processing. However, in the present study, correlations between quantity and recency of MDMA exposure and reaction time during the attention tasks were inconsistent in direction and were non-significant.

Our observation of abnormal function of the left hippocampus during the working memory task is consistent with the large number of studies of adult MDMA users that have documented memory deficits (Krystal et al. *1992*; Parrott *1997*; Parrott and Lasky *1998*; Gouzoulis-Mayfrank et al. *2000*; Bhattachary and Powell *2001*; Fox et al. *2001*; Reneman et al. *2001b*; Verkes et al. *2001*; Zakzanis and Young *2001*; Curran and Verheyden *2003*; Gouzoulis-Mayfrank et al. *2003*), with preclinical studies demonstrating high rates of serotonergic denervation of the hippocampus and parahippocampus following MDMA administration and poor recovery with extended abstinence (Ricaurte et al. *1992*; Fischer et al. *1995*; Hatzidimitriou et al. *1999*), and with a previous study of adult MDMA users that showed a trend for abnormal glucose metabolism at left hippocampus (Buchert et al. *2001*; Obrocki et al. *2002*). Serotonergic neurons ascending from the median raphe nucleus and targeting hippocampus and related areas play a critical role in regulating shifts in the activity of hippocampal neurons between irregular activity and theta rhythmicity (Azmitia and Segal *1978*; Kohler and Steinbusch *1982*; Freund et al. *1990*; Vertes and Kocsis *1997*; Gulyas et al. *1999*). Hippocampal theta oscillations have been shown to increase synaptic plasticity within the hippocampus and to induce long-term potentiation, which is believed to be a foundation for learning and memory (Staubli and Lynch *1987*; Greenstein et al. *1988*; Huerta and Lisman *1993*; Holscher et al. *1997*). Subcortical serotonergic neurons modulate hippocampal theta activity by modulating inhibitory GABAergic interneurons within the hippocampus (Freund et al. *1990*; Varga et al. *2002*). Abnormal hippocampal function in human MDMA users may therefore result from disrupted function or ablation of serotonergic neurons that normally modulate inhibitory circuits within the hippocampus.

The consistent negative relationship between left hippocampal activity and duration of abstinence from MDMA suggests that function of inhibitory circuits within the hippocampus may recover with sustained abstinence. Negative correlations between magnitude of MDMA exposure and performance accuracy across tasks suggests that

heavier use of this substance during adolescence may be associated with more pronounced cognitive deficits, as has been observed in adult MDMA users (Bhattachary and Powell *2001*; Fox et al. *2001*; Reneman et al. *2001b*; Verkes et al. *2001*).

Limitations of the present study include small sample size and lack of measures obtained prior to MDMA exposure to exclude the possibility that group differences preceded MDMA use. However, our observation of significant decrements in cognition and brain function in these subjects, who had consumed less MDMA (1–25 tablets) than typical participants in studies of adult MDMA users (Gouzoulis-Mayfrank et al. *2000*, *2003*; Fox et al. *2001*; Daumann et al. *2003*), is consistent with the notion that the brain may be more vulnerable to MDMA induced neurotoxicity during adolescence. Larger studies with longitudinal follow-up are urgently needed to confirm these findings and to determine whether cognitive and brain functional changes associated with MDMA use in adolescence reverse with sustained abstinence.

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References

Anderson GM, Braun G, Braun U, Nichols DE, Shulgin AT (1978) Absolute configuration and psychotomimetic activity. NIDA Res Monogr 22:8–15
ChemPort PubMed

Azmitia EC, Segal M (1978) An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat. J Comp Neurol 179:641–667
ChemPort PubMed

Bhattachary S, Powell JH (2001) Recreational use of 3,4-methylenedioxymethamphetamine (MDMA) or "ecstasy": evidence for cognitive impairment. Psychol Med 31:647–658
crossref ChemPort PubMed

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

Bowers TL, Pantle ML (1998) Shipley institute for living scale and the Kaufman Brief Intelligence Test as screening instruments for intelligence. Assessment 5:187–195
ChemPort PubMed

Buchert R, Obrocki J, Thomasius R, Vaterlein O, Petersen K, Jenicke L, Bohuslavizki KH, Clausen M (2001) Long-term effects of "ecstasy" abuse on the human brain studied by FDG PET. Nucl Med Commun 22:889–897
crossref ChemPort PubMed

Casey BJ, Giedd JN, Thomas KM (2000) Structural and functional brain development and its relation to cognitive development. Biol Psychiatry 54:241–257
crossref ChemPort

Chen WJ, Hsiao CK, Hsiao LL, Hwu HG (1998) Performance of the Continuous Performance Test among community samples. Schizophr Bull 24:163–174
ChemPort PubMed

Cohen J (1988) Statistical power analysis for the behavioral sciences. Erlbaum, Hillsdale

Commins DL, Vosmer G, Virus RM, Woolverton WL, Schuster CR, Seiden LS (1987) Biochemical and histological evidence that methylenedioxymethylamphetamine (MDMA) is toxic to neurons in the rat brain. J Pharmacol Exp Ther 241:338–345
ChemPort PubMed

Croft RJ, Mackay AJ, Mills ATD, Gruzelier JGH (2001) The relative contributions of ecstasy and cannabis to cognitive impairment. Psychopharmacology 153:373–379
SpringerLink ChemPort PubMed

Curran HV, Verheyden SL (2003) Altered response to tryptophan supplementation after long-term abstention from MDMA (ecstasy) is highly correlated with human memory function. Psychopharmacology 169:91–103
SpringerLink ChemPort PubMed

Daumann J, Fimm B, Willmes K, Thron A, Gouzoulis-Mayfrank E (2003) 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
crossref ChemPort

Fischer C, Hatzidimitriou G, Wlos J, Katz J, Ricaurte G (1995) Reorganization of ascending 5-HT axon projections in animals previously exposed to the recreational drug (±)3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"). J Neurosci 15:5476–5485
PubMed

Fox HC, Parrott AC, Turner JJD (2001) Ecstasy use: cognitive deficits related to dosage rather than self-reported problematic use of the drug. J Psychopharmacol 15:273–281
ChemPort PubMed

Freund TF, Gulyas AI, Acsady L, Gorcs T, Toth K (1990) Serotonergic control of the hippocampus via local inhibitory interneurons. Proc Natl Acad Sci 87:8501–8505
ChemPort PubMed

Friston KJ, Ashburner J, Frith CD, Poline JB, Heather JD, Frackowiak RS (1995) Spatial registration and normalization of images. Hum Brain Map 2:165–189

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 [$H_2{}^{15}O$]-positron emission tomography study. J Clin Psychopharmacol 21:66–71
crossref ChemPort PubMed

Gerra G, Zaimovic A, Ferri M, Zambelli U, Timpano M, Neri E, Marzocchi GF, Delsignore R, Brambilla F (2000) Long-lasting effects of (±)3,4-methylenedioxymethamphetamine (ecstasy) on serotonin system function in humans. Biol Psychiatry 47:127–136
ChemPort PubMed

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

Gouzoulis-Mayfrank E, Thimm B, Rezk M, Hensen G, Daumann J (2003) Memory impairment suggests hippocampal dysfunction in abstinent ecstasy users. Prog Neuropsychopharmacol Biol Psychiatry 27:819–827
crossref ChemPort PubMed

Greenstein YJ, Pavlides C, Winson J (1988) Long-term potentiation in the dentate gyrus is preferentially induced at theta rhythm periodicity. Brain Res 438:331–334
crossref ChemPort PubMed

Gulyas AI, Acsady L, Freund TF (1999) Structural basis of the cholinergic and serotonergic modulation of GABAergic neurons in the hippocampus. Neurochem Int 34:359–372
crossref ChemPort PubMed

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
ChemPort PubMed

Hegadoren KM, Baker GB, Bourin M (1999) 3,4-Methylenedioxy analogues of amphetamine: defining the risks to humans. Neurosci Biobehav Rev 23:539–553
ChemPort PubMed

Holmes AP, Friston KJ (1998) Generalizability, random effects, and population inference. Neuroimage 7:S34

Holscher C, Anwyl R, Rowan MJ (1997) Stimulation on the positive phase of hippocampal theta rhythm induces long-term potentiation that can be depotentiated by stimulation on the negative phase in area CA1 in vivo. J Neurosci 17:6470–6477
ChemPort PubMed

Huerta PT, Lisman JE (1993) Heightened synaptic plasticity of hippocampal CA1 neurons during a cholinergically induced rhythmic state. Nature 364:723–725
crossref ChemPort PubMed

Kirk RE (1982) Experimental design: procedures for the social sciences. Wadsworth, Belmont, Calif.

Kohler C, Steinbusch H (1982) Identification of serotonin and non-serotonin-containing neurons of the mid-brain raphe projecting to the entorhinal area and the hippocampal formation. A combined immunohistochemical and fluorescent retrograde tracing study in the rat brain. Neuroscience 7:951–975
PubMed

Krystal JH, Price LH, Opsahl C, Ricaurte GA, Heninger GR (1992) Chronic 3,4-methylenedioxymethamphetamine (MDMA) use: effects on mood and neuropsychological function? Am J Drug Alcohol Abuse 18:331–341
ChemPort PubMed

Little K, Penman E (1989) Measuring subacute mood changes using the profile of mood states and visual analogue scales. Psychopathology 1989:42–49

McCann UD, Ricaurte GA (2001) Caveat emptor: editors beware. Neuropsychopharmacology 24:333–334
crossref PubMed

McCann UD, Ridenour A, Shaham Y, Ricaurte GA (1994) Serotonin neurotoxicity after (±)3,4-methylenedioxymethamphetamine (MDMA; “ecstasy”): a controlled study in humans. Neuropsychopharmacology 10:129–138
ChemPort PubMed

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
ChemPort PubMed

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
ChemPort PubMed

Mordenti J, Chappell W (1989) The use of interspecies scaling in toxicokinetics. In: Yacobi A, Kelly J, Batra V (eds) Toxicokinetics and new drug development. Pergamon Press, New York, pp 42–96

Morgan MJ (1999) Memory deficits associated with recreational use of “ecstasy ” (MDMA). Psychopharmacology 141:30–36
SpringerLink ChemPort PubMed

O’Hearn E, Battaglia G, De Souza EB, Kuhar MJ, Molliver ME (1988) Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence for neurotoxicity. J Neurosci 8:2788–2803
ChemPort PubMed

Obrocki J, Schmoldt A, Buchert R, Andresen B, Petersen K, Thomasius R (2002) Specific neurotoxicity of chronic use of ecstasy. Toxicol Lett 127:285–297
crossref ChemPort PubMed

Parrott AC (1997) MDMA, mood and memory: the agnosia of the ecstasy. Br Psychol Soc Proc 5:49

Parrott AC (2000) Human research on MDMA (3,4-methylenedioxymethamphetamine) neurotoxicity: cognitive and behavioural indices of change. Neuropsychobiology 42:17–24
ChemPort PubMed

Parrott AC (2002) Recreational ecstasy/MDMA, the serotonin syndrome, and serotonergic neurotoxicity. Pharmacol Biochem Behav 71:837–844

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

Peroutka SJ (1987) Incidence of recreational use of 3,4-methylenedimethoxymethamphetamine (MDMA, “ecstasy”) on an undergraduate campus. N Engl J Med 317:1542–1543

ChemPort

Pomerleau CS, Carton SM, Lutzke ML, Flessland KA, Pomerleau OF (1994) Reliability of the Fagerstrom tolerance questionnaire and the Fagerstrom test for nicotine dependence. Addict Behav 19:33–39
crossref ChemPort PubMed

Pope HG, Ionescu-Pioggia M, Pope KW (2001) Drug use and life style among college undergraduates: a 30-year longitudinal study. Am J Psychiatry 158:1519–1521
crossref PubMed

Puig-Antich J, Orvaschel H, Tabrizi MA, Chambers W (1980) The Schedule for Affective Disorders and Schizophrenia for School-Age Children-Epidemiologic Version (Kiddie-SADS-E), 3rd edn. New York State Psychiatric Institute and Yale University School of Medicine, New York

Reneman L, Booij J, Schmand B, van den Brink W, Gunning B (2000) Memory disturbances in "ecstasy" users are correlated with an altered brain serotonin neurotransmission. Psychopharmacology 148:322–324
ChemPort PubMed

Reneman L, Booij J, de Bruin K, Reitsma JB, de Wolff FA, Gunning WB, den Heeten GJ, van den Brink W (2001a) Effects of dose, sex, and long-term abstention from use on toxic effectsof MDMA (ecstasy) on brain serotonin neurons. Lancet 358:1869
crossref

Reneman L, Lavalaye J, Schmand B, de Wolff FA, van den Brink W, den Heeten GJ, Booij J (2001b) Cortical serotonin transporter density and verbal memory in individuals who stopped using 3,4-methylenedioxymethamphetamine (MDMA or "ecstasy"): preliminary findings. Arch Gen Psychiatry 58:901–906
ChemPort PubMed

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

Repp BH, Frost R (1988) Detectability of words and nonwords in two kinds of noise. J Acoust Soc Am 84:1929–1932
ChemPort PubMed

Ricaurte GA, Martello AL, Katz JL, Martello MB (1992) Lasting effects of MDMA on central serotonergic neurons in nonhuman primates: neurochemical observations. J Pharmacol Exp Ther 261:616–622
ChemPort PubMed

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

Schmidt CJ (1987) Neruotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine. J Pharmacol Exp Ther 240:1–7
ChemPort PubMed

Schmidt CJ, Levin JA, Lovenberg W (1987) In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain. Biochem Pharmacol 36:747–755
ChemPort PubMed

Schuster P, Lieb R, Lamertz C, Wittchen HU (1998) Is the use of ecstasy and hallucinogens increasing? Results from a community study. Eur Addict Res 4:75–82
ChemPort PubMed

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
ChemPort PubMed

Shaywitz BA, Shaywitz SE, Pugh KR, Fulbright RK, Skudlarski P, Mencl WE, Constable RT, Marchione KE, Fletcher JM, Klorman R, Lacadie C, Gore JC (2001) The functional neural architecture of components of attention in language-processing tasks. Neuroimage 13:601–612
crossref ChemPort PubMed

Staubli U, Lynch G (1987) Stable hippocampal long-term potentiation elicity by "theta" pattern stimulation. Brain Res 435:227–234
crossref ChemPort PubMed

Steele TD, McCann UD, Ricaurte GA (1994) 3,4-Methylenedioxymethamphetamine (MDMA, "Ecstasy"): pharmacology and toxicology in animals and humans. Addiction 89:539–551
ChemPort PubMed

Stone DM, Stahl DC, Hanson GR, Gibb JW (1986) The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) on monaminergic systems in the rat brain. Eur J Pharmacol 128:41–48
ChemPort PubMed

Taffe MA, Davis SA, Yuan J, Schroeder R, Hatzidimitriou G, Parsons LH, Ricaurte GA, Gold LH (2002) Cognitive performance of MDMA-treated rhesus monkeys: sensitivity to serotonergic challenge. Neuropsychopharmacology

27:993–1005
crossref ChemPort PubMed

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

Varga V, Sik A, Freund TF, Kocsis B (2002) GABA(B) receptors in the median raphe nucleus: distribution and role in the serotonergic control of hippocampal activity. Neuroscience 109:119–132
ChemPort PubMed

Verkes RJ, Gijsman HJ, Pieters MSM, Schoemaker RC, de Visser, S, Kuijpers M, Pennings EJM, de Bruin D, de Wijngaart GV, van Gerven JMA, Cohen AF (2001) Cognitive performance and serotonergic function in users of ecstasy. Psychopharmacology 153:196–202
ChemPort PubMed

Vertes RP, Kocsis B (1997) Brainstem-diencephalo-septohippocampal systems controlling the theta rhythm of the hippocampus. Neuroscience 81:893–926
ChemPort PubMed

von Sydow K, Lieb R, Pfister H, Hofler M, Wittchen HU (2002) Use, abuse and dependence of ecstasy and related drugs in adolescents and young adults—a transient phenomenon? Results from a longitudinal community study. Drug Alcohol Depend 66:147–159
crossref PubMed

Wareing M, Fisk JE, Murphy PN (2000) Working memory deficits in current and previous users of MDMA ("ecstasy"). Br J Psychol 91:181–199
PubMed

Wilson MA, Ricaurte GA, Molliver ME (1989) Distinct morphologic classes of serotonergic axons in prmates exhibit differential vulnerability to the psychotropic drug 3,4-methylenedioxymethamphetamine. Neuroscience 28:121–137
crossref ChemPort PubMed

Woods RP (1996) Modeling for intergroup comparisons of imaging data. Neuroimage 4:S84–S94
crossref ChemPort PubMed

Zakzanis KK, Young DA (2001) Memory impairment in abstinent MDMA ("ecstasy") users: a longitudinal investigation. Neurology 56:966–969
ChemPort PubMed