Psychopharm

Journal of Psychopharmacology
20(2) (2006) 211–225

ISSN 0269-8811
SAGE Publications Ltd, London, Thousand Oaks, CA and New Delhi
10.1177/0269881106059486

Mood, cognition and serotonin transporter availability in current and former ecstasy (MDMA) users: the longitudinal perspective

R. Thomasius *Department of Psychiatry and Psychotherapy, University Hospital Hamburg-Eppendorf, Hamburg, Germany.*
P. Zapletalova *Department of Psychiatry and Psychotherapy, University Hospital Hamburg-Eppendorf, Hamburg, Germany.*
K. Petersen *Department of Psychiatry and Psychotherapy, University Hospital Hamburg-Eppendorf, Hamburg, Germany.*
R. Buchert *Department of Nuclear Medicine, University Hospital Hamburg-Eppendorf, Hamburg, Germany.*
B. Andresen *Department of Psychiatry and Psychotherapy, University Hospital Hamburg-Eppendorf, Hamburg, Germany.*
L. Wartberg *Department of Psychiatry and Psychotherapy, University Hospital Hamburg-Eppendorf, Hamburg, Germany.*
B. Nebeling *Department of Nuclear Medicine, University Hospital Hamburg-Eppendorf, Hamburg, Germany.*
A. Schmoldt *Institute of Forensic Medicine, University Hospital Hamburg-Eppendorf, Hamburg, Germany.*

Abstract

Although 3,4-methylenedioxymethamphetamine (MDMA, ecstasy) is a known serotonergic neurotoxin in different animal species, there is to date no conclusive evidence of its neurotoxicity in humans. MDMA use was associated with impairments of psychological well-being, verbal memory and altered serotonergic functioning in a number of cross-sectional studies. Due to inherent methodological limitations, such as the notorious polydrug use of ecstasy users and lack of control of possible pre-existing differences between ecstasy users and control participants, researchers have called for well-controlled, prospective longitudinal studies to shed more light on the issue of MDMA neurotoxicity to the human brain.

This longitudinal study investigated whether mood, cognition and central serotonin transporters (SERT) would deteriorate with continued MDMA use and whether or not they would recover over increasing periods of MDMA abstinence.

In a repeated-measures design, 11 current and ten ex-ecstasy users, and 11 polydrug (but not MDMA) and 15 drug-naive controls participated three times within approximately two years. Both ecstasy user groups reported a polydrug use pattern besides heavy ecstasy use. Subjective reports of ecstasy use or abstinence were verified by toxicological analyses. On each trial, the participants underwent a cognitive test battery and filled in the Symptom Check List. The availability of central SERT was assessed with positron emission tomography using the McN5652 ligand for all groups at t1, and only for the ecstasy user groups on follow-ups.

The factor Group yielded significant results in the SCL-90 scales Global Severity Index, Anxiety, Obsessive/compulsive and Interpersonal sensitivity, with the ex-ecstasy users reporting the highest symptom scores. There were significant Group effects in all measures of verbal memory, with the lowest performance in the group of ex-ecstasy users. The repeated-measures analyses yielded no significant Group×Time interactions in any SCL-90 scales or measures of memory performance, with the exception of AVLT 1 immediate recall. Thus the ex-ecstasy users' psychopathological symptoms and memory performance failed to improve, and the current ecstasy users' failed to deteriorate, over time relative to the other groups. While there was a significant effect of Group in all brain regions examined (except the control region white matter), the current users´ SERT availability seems to have recovered in the mesencephalon, as indicated by a significant Group×Time interaction.

Reduced SERT availability might be a transient effect of heavy ecstasy use, since it partially recovered as the current users reduced their MDMA use. However, this measure may not necessarily be a valid indicator of the number or integrity of serotonergic neurons. Ex-ecstasy users' verbal memory showed no sign of improvement even after over 2.5 years of abstinence and thus may represent persistent functional consequences of MDMA neurotoxicity. However, alternative causes such as pre-existing group differences cannot be completely ruled out in spite of the longitudinal design.

Keywords

3,4-methylenedioxymethamphetamine, MDMA, ecstasy, psychopathology, cognitive performance, neuroimaging, serotonin transporter, longitudinal study.

Corresponding author: R. Thomasius, Department of Psychiatry and Psychotherapy, University Hospital Hamburg-Eppendorf, Martinistraße 52, 20246 Hamburg, Germany.
Email: thomasius@uke.uni-hamburg.de.

Introduction

Twenty years after the publication of the first evidence of 3,4-methylenedioxymethamphetamine's (MDMA, ecstasy) serotonergic neurotoxicity to laboratory animals (Ricaurte *et al.* 1985; Gehlert *et al.*, 1985) the recreational use of MDMA is still widespread, despite warnings of potential damage to the central nervous system (McCann *et al.*, 1999; Parrott 2000). Various signs of long-term damage to the serotonergic system were discovered following MDMA administration to laboratory animals, such as depletion of 5-HT and 5-hydroindolacetic acid concentration (Commins *et al.*, 1987), inhibition (Fleckenstein *et al.*, 1999) and loss of serotonin transporters (SERT; Battaglia *et al.*, 1987; Aguirre *et al.*, 1998), and degeneration of 5-HT axonal projections and nerve terminals (O'Hearn *et al.*, 1988). MDMA-induced neurodegeneration persists for months in rats and for years in primates (for review see Ricaurte *et al.*, 2000) and is often, but not always accompanied by behavioural changes in different animal species (review cf. Green *et al.*, 2003). Slikker *et al.* (1989) for instance report that neurotoxic MDMA doses did not necessarily produce observable behavioural changes in female rhesus monkeys. As O'Hearn *et al.* (1988, p. 2788) have proposed, MDMA is now an experimental tool for analysing the organization and function of 5-HT projections because of its ability to induce a selective degeneration of 5-HT axons in animals. Thus the animal data raise the possibility that MDMA may be toxic to the human serotonergic system. This issue has been addressed on several levels. A number of human studies investigated various aspects of serotonergic functioning in MDMA users, such as the integrity of serotonergic neurons by neuroimaging of SERT, and levels of cognitive performance and psychological well-being, e.g. memory performance or certain psychopathological symptoms.

Measures of SERT availability are generally considered to be a marker of the number or integrity of 5-HT nerve terminals, even though the validity of this assumption has been questioned (see Kish, 2002). Validation studies on animals indicate that single photon emission tomography (SPECT) with [^{123}I] β-CIT (Reneman *et al.*, 2002; De Win *et al.*, 2004b) and position emission tomography (PET) with [^{11}C](+)McN5652 (Szabo *et al.*, 2002) may be suitable to detect the neurotoxic effects of MDMA in serotonergic neurons. Human PET studies by McCann *et al.* (1998, 2005) and a SPECT study by Semple *et al.* (1999) revealed a reduced global and regional SERT availability in ecstasy users. McCann *et al.* (2005) found the binding parameters obtained with the two SERT ligands [^{11}C](+)McN5652 and [^{11}C]DASB to be highly correlated. Furthermore, a significant correlation between global SERT binding distribution volume ratios (DVR) and the duration of abstinence from ecstasy seems to indicate that SERT availability recovered over time. Similarly, in a previous PET study by our team (Buchert *et al.*, 2003; Thomasius *et al.*, 2003) and a SPECT study by Reneman *et al.* (2001a and b), SERT availability was reduced in current, but not in former, ecstasy users. In the latter sample, SERT availability did neither correlate with depressive symptoms (De Win *et al.*, 2004b), nor with neuropsychological performance (Reneman *et al.*, 2001b).

The absence of a relationship between SERT availability and cognitive performance or symptoms of depression is somewhat surprising in light of the serotonergic system´s involvement in cognition and mood. It may imply that either SERT availability is not a valid indicator of the functional integrity of the serotonergic system or that there are alternative causes for elevated depression and impaired cognitive performance in ecstasy users, such as the concomitant use of other drugs. Heavy cannabis use may aggravate depressive symptoms (Degenhardt *et al.*, 2003) and cognitive performance (Bolla *et al.*, 2002).

Clinical studies found persistent use of MDMA to be associated with significantly elevated self-reported symptoms of depression (Gerra *et al.*, 1998, 2000; Gamma *et al.*, 2001; MacInnes *et al.*, 2001; Morgan *et al.*, 2002; Hanson and Luciana, 2004; McCardle *et al.*, 2004), anxiety (Parrott *et al.*, 2000; Morgan *et al.*, 2002; Daumann *et al.*, 2004), impulsiveness (Gerra *et al.*, 1998; Parrott *et al.*, 2000; Tuchtenhagen *et al.*, 2000) and aggression (Gerra *et al.*, 1998; Parrott *et al.*, 2000; Curran and Verheyden, 2003). Studies in former ecstasy users showed subclinically elevated depressive symptoms (Gerra *et al.*, 2000; MacInnes *et al.*, 2001; Thomasius *et al.*, 2003; Verheyden *et al.*, 2003; De Win *et al.*, 2004a) or no differences to controls (Morgan *et al.*, 2002). Some authors reported psychopathology to be primarily associated with cannabis use in their sample of ecstasy users (Morgan *et al.*, 2002; Daumann *et al.*, 2004), while others did not (Thomasius *et al.*, 2003; De Win *et al.*, 2004a).

A meta-analysis of neuropsychological studies comparing ecstasy users with controls by Verbaten (2003) yielded significant effect sizes for immediate and delayed verbal recall, processing speed and attention. The effect sizes for immediate and delayed verbal recall indicated a reduction of nearly 40% in memory performance. There was no association between lifetime consumption of ecstasy and cognitive performance.The effect size for delayed verbal recall was no longer significant after controlling for the lifetime cannabis consumption. In a later study, McCardle *et al.* (2004) found that group differences in memory performance tested with the Auditory Verbal Memory Test (AVLT) were only significant for delayed, but not for immediate recall after controlling for cannabis use and depression. In another study, both current and former ecstasy users exhibited impaired working memory and verbal recall, but only former ecstasy users performed significantly worse on verbal recall in the Rivermead Behavioural Memory Test (RBMT) than both the drug-free and the polydrug control groups (Morgan *et al.*, 2002). The majority of their neuropsychological measures was best predicted by parameters of ecstasy use. Thomasius *et al.* (2003) found that only former, but not current ecstasy users performed significantly worse on AVLT immediate recall and RBMT immediate and delayed recall compared to drug-free but not polydrug controls. Regression analyses showed AVLT immediate and delayed recall to be best predicted by measures of self-reported ecstasy use, while RBMT immediate and delayed recall were best predicted by the amount of cannabis smoked over the 12 months prior to participation. Similarly, in a recent study by Dafters *et al.* (2004), heavy cannabis users were significantly impaired on RBMT immediate and delayed recall, independently of whether they used MDMA or not. A few studies reported no cognitive impairments in ecstasy users (e.g. Back-Madruga *et al.*, 2003).

In spite of extensive research, there is to date no general agreement as to the neurotoxic potential of MDMA. Green (2004, p. 4) concludes that 'none of the data obtained have produced absolute evidence that regular MDMA ingestion causes damage to serotonin nerve endings in the human brain or results in impaired physiological performance or psychiatric problems'. MDMA obeys common pharmacological principles of dose and effect and neurotoxic doses have been identified in most animal species (Green *et al.*, 2003). As the content of ecstasy tablets (Parrott, 2004a) and the amount of MDMA taken on single occasions vary, it seems likely that some recreational ecstasy users may expose themselves to neurotoxic doses at certain times. They may take ecstasy in a hot environment at low levels of plasma antioxidants, putting themselves at higher risk of neurotoxic damage. The effects of concomitant multiple drug use, which is common among ecstasy users, are not understood well enough to realistically estimate the consumer risk. Although it seems likely that multiple drug use may potentiate the risk of MDMA-induced neurotoxicity, even the possibility of neuroprotective combinations cannot be ruled out (see Parrott, 2004b).

One fundamental problem of human ecstasy research lies in the possibility that differences between ecstasy users and control participants in serotonergic functioning, psychopathological symptoms, personality and cognitive performance may coincide with the likelihood to use certain substances. Since the administration of a potentially neurotoxic substance to volunteers poses an ethical dilemma, longitudinal studies seem to be the only way to circumvent this limitation. One of the rare longitudinal studies discovered no change in self-reported depressive symptoms after one year of abstinence from ecstasy (Gerra *et al.*, 2000). Another found a significant decline in performance on RBMT immediate and delayed recall after 1 year of continued ecstasy use (Zakzanis and Young, 2001). However, in a recent study, Gouzoulis-Mayfrank *et al.* (2005) found no changes in the memory performance of 21 current and 17 ex-ecstasy users over the course of 18 months.

In this study we present the results of a follow-up on part of a larger sample described earlier (Thomasius *et al.*, 2003). The aim of this longitudinal study was to investigate how elevated psychopathology, cognitive deficits or alterations of serotonergic neurons observed in the original cross-sectional investigation would develop over time in current and ex-ecstasy users. Both groups took other drugs in addition to ecstasy, and would better be described as polydrug ecstasy users. However, they will be referred to as current and ex-ecstasy users for the sake of convenience throughout this text. According to the hypothesis of MDMA serotonergic neurotoxicity, we expected an aggravation of psychopathological symptoms, memory deficits and reduced SERT availability with continued MDMA consumption by the current ecstasy users. The question of recovery from possible MDMA-induced serotonergic dysfunction was addressed by following up on ex-ecstasy users in the course of growing periods of abstinence. Two additional groups were included to control for general effects of time and repeated measurements: drug-naive control participants and polydrug users with a pattern of drug usage similar to that of the ecstasy users except for MDMA. Moreover, the latter served to differentiate between general effects of polydrug use which is common among ecstasy users and possible MDMA-specific effects.

Material and methods

Participants

Of the original 120 participants of a cross-sectional study reported on earlier (Thomasius *et al.*, 2003), 66 participated in the longitudinal investigation. In spite of attempts to invite all participants for follow-ups, there was a considerable drop-out rate, especially among the current and ex-ecstasy users. Reasons for drop-out were: participants were no longer available under given phone number and address and could not be traced; inability to comply with the requirement to abstain from substance use for 6 days prior to investigation; lack of motivation to participate again, especially regarding the PET scan; and pregnancy. Recruitment of control participants was terminated after the control groups had reached a size comparable to those of the ecstasy groups. Both follow-ups were completed by 18 of the original 30 current polydrug ecstasy users, 16 of 31 ex-ecstasy users, 17 of 29 polydrug and 15 of 30 drug-naive controls. Participants were excluded retrospectively in case of drug-positive urine screenings on any testing day, disagreement of their subjective reports of MDMA use and hair analyses, or if they no longer fulfilled the defining criteria of their group. In order to compare two distinctive groups of current versus ex-ecstasy users, we set the criterion for 'current ecstasy user' at five tablets between follow-ups. Accordingly, current ecstasy users were excluded if they reported having taken less than five ecstasy tablets between t1 and t3. Ex-ecstasy users as well as polydrug controls were excluded if they had taken more than five ecstasy tablets between t1 and t3, or any amount of ecstasy within 6 months prior to one of the follow-ups. This procedure left a total of 47 participants for the final data analysis: 11 current ecstasy users, 10 ex-ecstasy users, 11 polydrug controls and 15 drug-naive controls.

Recruitment and procedure

For the initial assessment (t1), participants were recruited and examined as described in Thomasius *et al.* (2003). Nine to 12 months later, those who were still available and willing to participate were given an appointment for the first follow-up (t2), and 9 to 12 months after that for the second follow-up (t3). The procedure at both follow-ups was very similar to that at t1, with the exception that most participants completed the testing in 1 instead of 2 days. Those parts of the procedure which are directly relevant to the data analyses presented here are described below (for a complete description see Thomasius *et al.*, 2003).

Sociodemography, psychopathology and drug histories

Sociodemographic data were collected with a questionnaire developed by the research group. The subjective perception of psychopathological symptoms was assessed with the Symptom

Check List (SCL-90-R, Derogatis, 1994; Franke, 1995). Drug histories were obtained by trained interviewers in detailed semi-structured interviews. The participants were asked to remember the amount of drugs taken in each month, starting with the last month and then going back along the time axis. In order to aid their recall, participants were encouraged to recollect the relevant events of each month. The participants also estimated their alcohol, tobacco and medication intake of the past week. Urine samples were screened for amphetamine, methamphetamine, MDMA, MDA, MDE, barbiturates, benzodiazepines, THC, cocaine metabolites, opiates and alcohol. Hair samples were analysed for amphetamine, methamphetamine, MDA, MDMA, MDEA and MBDB.

Neurocognitive test battery

Premorbid intelligence was estimated with a German multiple-choice test of vocabulary knowledge (Mehrfachwahl-Wortschatztest (MWT-B), Lehrl, 1985). A brief news story from the Rivermead Behavioural Memory Test (RBMT, Wilson *et al.*, 1985) was used to test the immediate and 20 minute delayed recall of verbal context-bound material. A different story was used on each trial. Sequential tests of acquisition, recall and decay of verbal memory were performed with the Auditory Verbal Learning Test (AVLT, Lezak, 1983). This involves learning a list of 15 words read out loud to the participant with immediate reproduction of the learned items in each of five consecutive trials (AVLT one to five). A second list is presented on the sixth trial (AVLT 6). Interference is measured on a seventh trial by asking the participant to reproduce the original list (AVLT 7). Delayed recall of the first list is tested after 20 minutes (AVLT 8).

Positron emission tomography

PET scans were performed on all participants at t1. However, due to ethical considerations, only the current and ex-ecstasy users underwent PET scans on the follow-ups. For the control groups, there were no sufficiently urgent research questions regarding the development of SERT availability to justify repeated exposure to the tracer substance.

Four brain structures were selected for testing the hypothesis of MDMA-induced alteration of SERT availability: mesencephalon, putamen, caudate nucleus and thalamus. We focused on these subcortical regions which are rich in SERT and in which SERT availability can be reliably investigated by the probe [^{11}C](+)McN5652 (Laruelle *et al.*, 1988; Buck *et al.*, 2000; Parsey *et al.*, 2000). The relatively low SERT density in the cerebral cortex, together with the limited signal-to-noise ratio of [^{11}C](+)McN5652-PET might have compromised the validity of SERT analysis in the cerebral cortex. White matter served as a control region in which no MDMA-induced effects were expected because of its absence of SERT. The grey matter of the cerebellum was chosen as reference region for the kinetic modelling.

The SERT ligand [^{11}C](+)McN565 was synthesized according to Suehiro *et al.* (1992, 1993). Imaging was performed on a full-ring whole-body system ECAT EXACT 921/47 (Siemens/CTI, Knoxville, TN, USA; Wienhard *et al.*, 1992) in 2d-mode. This system covers an axial field-of-view of 16.2 cm by collecting 47 transversal slices with 3.4 mm slice separation.

Head movement was minimized by a thermoplastic mask (Tru-Scan Imaging, Annapolis, MD). A 15 minute transmission scan for attenuation correction was obtained before tracer injection using three rotating ^{68}Ge rod sources, about 70 MBq each. After the transmission scan 400–600 MBq of [^{11}C](+)McN5652 dissolved in 40 ml of 0.9% NaCl were injected through a vein of the left hand at a rate of 600 ml/h. At the beginning of tracer injection a dynamic scan protocol was initiated including 35 frames with a total acquisition time of 90 minutes. Subjects were asked to keep their eyes open during the whole time of acquisition. Noise in the acquisition room was kept to a minimum.

The sinograms were corrected for random coincidences, radioactive decay, dead time and varying detector efficiency. Thereafter, the sinograms were 3d-smoothed by application of a $3\times3\times3$ binomial kernel. Forty-seven transaxial slices with 64×64 voxels were reconstructed using an iterative method. The voxel size was $3.4\times3.4\times3.4\,mm^3$, in-plane spatial resolution was about 9 mm full width at half maximum (FWHM). No scatter correction was performed.

In spite of the thermoplastic mask immobilization, there was significant head movement during the acquisition in a number of subjects. This was corrected by application of the Realign-Tool of the SPM99 software package (Welcome Department of Cognitive Neurology, Institute of Neurology, University College, London; Acton and Friston 1998). In order to support standardized identification of the volumes of interest (VOIs), individual images were stereotactically normalized using the Normalize-Tool of SPM 99. A [^{11}C](+)McN5652 template created earlier served as reference for stereotactic normalization.

VOIs for the structures to be examined were predefined in the template. Each VOI was composed of circles of 4.1 mm radius, placed in an appropriate number of transversal slices (Weeks *et al.*, 1997). No individual adjustment was performed in order to guarantee reproducible results.

Kinetic modelling was performed on the level of voxels. Distribution volume ratios (DVRs) were derived by application of the graphic reference tissue method for reversible binding described by Ichise *et al.* (1996, 2001; Ichise noninvasive plot). The time-activity curve of the reference region was generated using the mean of the cerebellum-VOI. The start time for the multilinear regression analysis was fixed at $t^* = 12$ min. DVRs for the examined structures were taken to be the mean voxel values within the corresponding VOIs copied to the individual parametric images.

Statistical analysis

Data were statistically analysed with SPSS for windows (version 10). Transversal group differences at t1, t2 and t3 were tested for significance with one-way analyses of variance (ANOVA). *Post hoc* tests were performed with Scheffé tests and, in the case of variance inhomogeneity, with Tamhane's T^2. The nonparametric Kruskal-Wallis tests and pairwise Mann-Whitney U-tests for independent samples were performed whenever the Kolmogorov-

Smirnoff test indicated that the normality assumption was violated. Profile analyses (see Stevens, 2002) of the parameters of psychopathology, verbal memory performance and SERT availability were performed with the MANOVA procedure, with the between-subject factor GROUP (current ecstasy users, ex-ecstasy users, polydrug controls, drug-naive controls) and the within-subject factor TIME (t1, t2, t3).

The multitude of measures covered by our data analysis raises the issue of multiple testing. Due to the small sample size in the cross-sectional comparisons, only very large effects can yield statistical significance. Because of this already conservative testing situation we did not correct for alpha. Therefore, the probabilities given below should be viewed as the results of a descriptive, rather than confirmatory, data analysis (see Abt 1987).

Results

Participant details, alcohol and nicotine use

An overview of the relevant sociodemographic and personal characteristics of each group is presented in Table 1. The groups did not differ significantly in gender ratio, mean age, level of education or estimated premorbid IQ. There were significant group differences in the use of alcohol in the week prior to testing ($F_{3,43}$=3.05, P=0.038) at t1, but not at t2 ($F_{3,43}$=2.85, P=0.086) and t3 ($F_{3,43}$=1.56, P=0.213). Group differences in the use of nicotine in the week prior to testing were significant throughout the study (t1: $F_{3,43}$=7.00, P=0.001; t2: $F_{3,43}$=6.94, P=0.001; t3: $F_{3,43}$=8.67, P=0.000). Comparisons of the change scores t2–t1 and t3–t2 concerning the use of alcohol or cigarettes yielded no significant differences.

Ecstasy use

When entering the study, current and ex-ecstasy users did not differ significantly in their average self-reported lifetime ecstasy use (current ecstasy users: M=798.23 tablets, SD=609.29; ex-ecstasy users: M=776.25 tablets, SD=561.64, dF=19, t=−0.09, $p(t)$=0.933), duration of ecstasy use (current ecstasy users: M=49.91 months, SD=29.47; ex-ecstasy users: M=63.70 months, SD=25.66, dF=19, t=1.14, $p(t)$=0.269), or age of first ecstasy use (current ecstasy users: M=19.73 years, SD=3.95; ex-ecstasy users: M=19.20 years, SD=4.18, dF=19, t=−0.30, $p(t)$=0.770). On average, current ecstasy users had last taken ecstasy 20.45 days (SD=13.51) and ex-ecstasy users 551.90 days (SD=520.30) prior to participation.

Current ecstasy users reported to have consumed a mean of 106 ecstasy tablets (M=105.96, SD=106.30) during the interval between t1 and t2 and a mean of 83 tablets (M=83.05, SD=90.09) between t2 and t3, with a typical dose of two tablets per event (M=1.57, SD=2.11). Although this was not intended, there seems to have been a decline in the intensity of ecstasy use after enrolling in the study. While current users reported an average intake of 134.96 ecstasy tablets (SD=94.21) over the 12 months prior to t1, it was only 82.86 tablets (SD=87.35) over the 12 months prior to t2 and 87.59 tablets (SD=89.46) prior to t3. A repeated measures ANOVA confirmed that this decline was statistically significant (Pillai's Trace=0.53; F=5.07; p=0.034).

Other illicit drug use in current and ex-ecstasy users and polydrug controls

The group means and standard deviations of amounts of different drugs taken by the participants are given in Table 2. The three groups did not differ significantly in their average subjective estimates of their lifetime consumption of cocaine or LSD at t1. However, the normality assumption was violated, and Kruskal-Wallis tests yielded significant group differences in the lifetime doses of cannabis (χ^2=6.10, P=0.047) and amphetamine (χ^2=11.38, P=0.003). Pairwise comparisons with Mann-Whitney U-tests indicate that the polydrug controls had used significantly more cannabis than current ecstasy users (U=23.00, P=0.014)

Table 1 Sociodemographic characteristics, alcohol and nicotine use in the week prior to testing: frequencies, mean±SD

	Current ecstasy users	Ex-ecstasy users	Polydrug controls	Drug-naive controls
n (m/f)	11 (7/4)	10 (5/5)	11 (6/5)	15 (8/7)
Age	23.64±3.72	25.80±4.89	25.18±5.08	22.33±2.58
Education[a]	4/4/3	4/3/3	2/2/7	4/4/7
IQ[b]	101.36±8.29	107.40±15.39	107.91±9.22	105.20±15.23
cigarettes per week at t1; changes t2–t1 and t3–t2				
t1	56.82±69.48	95.80±96.94	129.36±94.08	4.73±18.06
t2–t1	−3.64±63.69	25.67±106.34	−13.55±50.87	−2.60±10.34
t3–t2	2.36±38.53	−11.56±48.47	2.18±41.60	0.07±0.59
alcohol [g] per week at t1; changes t2–t1 and t3–t2				
t1	92.22±90.77	97.56±103.77	178.32±199.61	36.18±42.17
t2–t1	14.80±113.02	−26.86±94.50	−28.25±149.93	10.96±86.18
t3–t2	8.36±154.55	−17.92±85.60	−40.13±140.79	0.48±0.80

[a] n basic (9 y)/intermediate (10 y)/college admission level (13 y)

[b] premorbid IQ estimated from MWT-B

and significantly less amphetamine than both current ecstasy users ($U=12.00$, $P=0.001$) and ex-ecstasy users ($U=26.00$, $P=0.037$).

Only the current ecstasy users took amphetamine ($M=1.40$ g, $SD=4.51$) and LSD ($M=6.82\,\mu g$, $SD=22.61$) in the month prior to t1. Kruskal-Wallis tests yielded no significant group differences concerning the use of cannabis and cocaine in the week prior to t1 (data not shown in table).

Between t1 and t3, only cannabis and cocaine were used by a majority of participants in each of the three groups. While most of the current ecstasy users reported amphetamine use between t1 and t3, only one polydrug control participant and two ex-ecstasy users took single doses of amphetamine in this time span. Use of LSD was generally rare, with only two polydrug controls and two current ecstasy users reporting that they took LSD while participating in the study (Table 2).

To assess the development of drug use in the course of the

Table 2 Illicit drug use at t1 and in the longitudinal perspective (change scores in the estimated lifetime dose and consumption in 6 months prior to each point of measurement): number of participants (*n*) reporting use before t1 and between t1 and t3; mean ± SD

	Current ecstasy users	Ex-ecstasy users	Polydrug controls	Kruskal-Wallis Test
Cannabis (*n*,lifetime/t1 to t3)	11/9	10/9	11/10	
Cannabis [g] consumed in 6 months before t1; changes t2–t1 and t3–t2				
t1	24.56 ± 71.61	105.80 ± 147.13	69.27 ± 66.45	*
t2–t1	−8.19 ± 34.18	−42.37 ± 80.06	−44.65 ± 61.79	
t3–t2	−9.83 ± 33.59	−3.89 ± 51.20	9.46 ± 14.02	
Lifetime dose of cannabis [g] at t1; changes t2–t1 and t3–t2				
t1	334.01 ± 761.04	1717.12 ± 2160.55	1307.56 ± 1351.67	*
t2–t1	40.31 ± 101.05	141.71 ± 185.18	52.14 ± 48.04	
t3–t2	73.09 ± 140.88	371.51 ± 858.60	18.94 ± 22.01	
Amphetamine (*n*,lifetime/t1 to t3)	11/9	9/2	5/1	
Amphetamine [g] consumed in 6 months before t1; changes t2–t1 and t3–t2				
t1	8.70 ± 16.33	0.08 ± 0.17	0.09 ± 0.30	***
t2–t1	−3.63 ± 8.15	−0.08 ± 0.17	0.46 ± 1.54	*
t3–t2	−9.61 ± 22.35	−0.01 ± 0.03	0.37 ± 1.24	
Lifetime dose of amphetamine [g] at t1; changes t2–t1 and t3–t2				
t1	74.76 ± 105.22	52.93 ± 112.71	5.39 ± 11.70	**
t2–t1	12.87 ± 30.15	0.06 ± 0.19	1.74 ± 5.76	***
t3–t2	27.66 ± 59.44	0.1100 ± 0.35	0.18 ± 0.60	**
Cocaine (*n*,lifetime/t1 to t3)	11/8	10/8	10/6	
Cocaine [g] consumed in 6 months before t1; changes t2–t1 and t3–t2				
t1	4.03 ± 7.61	0.59 ± 1.21	8.03 ± 18.31	
t2–t1	0.05 ± 1.88	1.93 ± 5.31	−3.67 ± 10.63	
t3–t2	0.83 ± 2.82	−0.60 ± 6.58	3.18 ± 7.24	
Lifetime dose of cocaine [g] at t1; changes t2–t1 and t3–t2				
t1	10.42 ± 15.68	97.59 ± 138.48	449.21 ± 1081.14	
t2–t1	10.26 ± 21.21	5.48 ± 10.40	8.08 ± 17.43	
t3–t2	5.72 ± 12.13	5.77 ± 17.30	1.87 ± 3.18	
LSD (*n*,lifetime/t1 to t3)	8/2	7/0	5/2	
LSD [µg] consumed in 6 months before t1; changes t2–t1 and t3–t2				
t1	17.05 ± 52.52	0	0	
t2–t1	1.14 ± 8.78	0	4.16 ± 15.08	
t3–t2	13.64 ± 45.23	0	4.55 ± 15.06	
Lifetime dose of LSD [µg] at t1; changes t2–t1 and t3–t2				
t1	1701.56 ± 4579.69	3860.00 ± 7550.68	320.46 ± 550.92	
t2–t1	18.18 ± 60.30	0	19.91 ± 35.83	
t3–t2	4.55 ± 15.06	0	9.09 ± 30.15	

*** $p<0.001$ ** $p<0.010$ * $p<0.050$

study, repeated measures ANOVAs, and whenever appropriate, Friedman tests were performed for each of the three groups regarding the reported use of amphetamine, cannabis and cocaine in the month and in the year prior to each of the points of measurement (data not shown). There were no significant changes in the use of any of these drugs in any group over time, with the exception of cannabis in the polydrug control group. The polydrug control participants smoked less cannabis in the course of their participation, as indicated by a significant decline in the values of the two measures, cannabis use in the month prior to testing (t1: $M=10.29$ g, $SD=14.08$; t2: $M=9.00$ g, $SD=9.28$; t3: $M=2.21$ g, $SD=2.76$; Pillai´s trace=0.58; $F=6.18$; $P=0.020$; linear trend: $F=3.59$, $P=0.087$) and cannabis use in the year prior to testing (t1: $M=152.82$ g, $SD=131.78$; t2: $M=96.82$ g, $SD=133.52$; t3: $M=38.44$ g, $SD=128.16$; Pillai´s trace=0.52; $F=4.89$; $P=0.026$; linear trend: $F=7.15$, $P=0.023$).

Group comparisons at t1

Psychological symptoms The results of group comparisons regarding the SCL-90 are given in Table 3. The one-way ANOVA yielded significant group differences in the SCL-90-R Global Severity Index and the subscales obsessive-compulsive and phobic anxiety. *Post hoc* tests showed no significant differences between current and ex-ecstasy users versus polydrug controls. However, ex-ecstasy users had significantly elevated obsessive-compulsive scores compared to drug-naive controls.

Verbal memory performance The relevant group means, standard deviations and group comparisons are shown in Table 4. The groups did not differ significantly in their immediate and delayed recall performance on the RBMT brief news story at t1. However, there were significant group differences in performance on all measures of the AVLT. Multiple comparisons indicated that current ecstasy users were not significantly impaired on any AVLT measure. Ex-ecstasy users performed significantly worse on AVLT 1, AVLT sum of initial trials, AVLT 6, AVLT 7 and AVLT 8 than drug-naive-controls. They also achieved significantly higher scores on AVLT 5–1 than polydrug controls and current users. This measure reflects the improvement from trial one to five. Thus the ex-ecstasy users, whose performance was significantly impaired on the first trial, seem to have caught up with the other groups by the fifth trial. Polydrug controls were impaired only in their performance on AVLT 6 relative to drug-naive controls.

Availability of serotonin transporters The [^{11}C](+)McN5652 DVRs in the white matter and putamen ($F_{3,43}=2.61$, $P=0.064$) did not differ significantly between groups. There were significant group differences in the mesencephalon, caudate nucleus and thalamus. Although the current ecstasy users had lower SERT DVRs in all four regions of interest than the other groups, multiple comparisons yielded significant results only for the mesencephalon.

Longitudinal analysis

Psychological symptoms The profile analyses (Table 6) revealed no significant TIME×GROUP interactions, indicating that the groups did not differ in their development of psychological symptoms throughout the course of the study. The effects of the between-subjects factor GROUP were significant for the scales GSI (Fig. 1), obsessive-compulsive, inter-personal sensivity, and anxiety. There were no significant effects of GROUP for the remaining scales, although most could be considered marginally significant ($p<0.10$). The ex-ecstasy users tended to have the highest scores and drug-naive controls the lowest scores on all scales at nearly all points of measurement, but these differences were not shown to be significant by Scheffé Tests (Table 3). The within-subject factor TIME was significant regarding the GSI, obsessive-compulsive, inter-personal sensivity, depression, aggression/hostility and psychoticism, with scores generally declining in the course of the study (Table 3).

Verbal memory performance With the exception of AVLT 5, no significant TIME×GROUP interactions were revealed by the profile analyses (Table 7). Thus the groups did not differ in their development of verbal memory performance over time in most measures of verbal memory. The results concerning AVLT 1 (immediate recall) and AVLT 8 (delayed recall) are shown in Figs 2 and 3, respectively. The effects of the between-subject factor GROUP were significant for all measures of verbal memory performance. The cross-sectional analysis (Table 4) indicates that ex-ecstasy users tended to have the lowest scores and drug-naive controls the highest scores on most measures at most time points. The differences between ex-ecstasy users and drug-naive controls were statistically significant at no less than two time points in the cases of AVLT 1, sum of initial trials, seven and eight. The within-subject factor TIME had significant effects on AVLT 1, AVLT 5–1, AVLT 6 and AVLT 8.

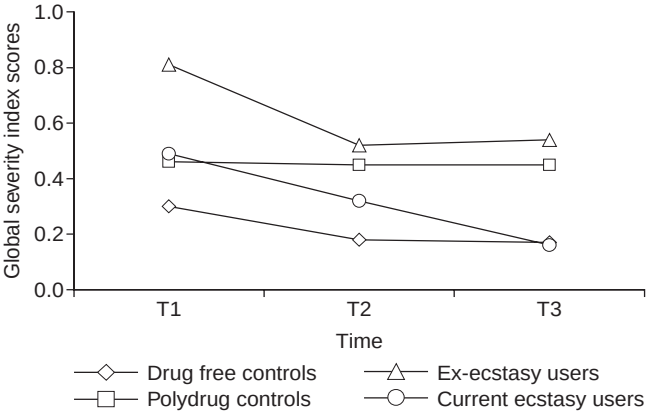

Figure 1 Global severity index (SCL-90 R): group means at t1, t2 and t3

Table 3 Means and standard deviations of the SCL-90-R scales at t1, t2 and t3 and cross-sectional group comparisons for each time point

	Drug-naive controls (DC)		Polydrug controls (PC)		Ex-ecstasy users (EE)		Current Ecstasy users (CE)		ANOVA			
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*dF*	*F*	*P(F)*	*Scheffé-Test*
Global Severity Index												
t1	0.30	0.17	0.46	0.55	0.81	0.61	0.49	0.21	3/43	3.10	0.036	n.s.
t2	0.18	0.11	0.45	0.33	0.52	0.45	0.32	0.39	3/41	2.38	0.089	n.s.
t3	0.17	0.12	0.45	0.58	0.54	0.52	0.16	0.13	3/39	2.63	0.064	n.s.
Somatization												
t1	0.36	0.31	0.41	0.34	0.70	0.66	0.33	0.19	3/43	1.92	0.141	n.s.
t2	0.35	0.34	0.38	0.49	0.53	0.40	0.38	0.50	3/41	0.40	0.751	n.s.
t3	0.22	0.22	0.45	0.65	0.45	0.52	0.22	0.18	3/40	1.06	0.376	n.s.
Obsessive-compulsive												
t1	0.38	0.25	0.63	0.84	1.06	0.54	0.90	0.51	3/43	3.66	0.019	EE > DC*
t2	0.26	0.20	0.67	0.39	0.70	0.51	0.48	0.37	3/41	3.68	0.019	n.s.
t3	0.19	0.15	0.53	0.50	0.68	0.65	0.26	0.22	3/41	3.61	0.021	n.s.
Inter-personal sensivity												
t1	0.34	0.29	0.66	0.74	1.07	1.13	0.55	0.31	3/43	2.43	0.079	n.s.
t2	0.14	0.13	0.54	0.50	0.56	0.62	0.31	0.45	3/41	2.43	0.079	n.s.
t3	0.20	0.20	0.59	0.56	0.68	0.57	0.17	0.18	3/40	4.55	0.008	n.s.
Depression												
t1	0.43	0.26	0.50	0.68	0.85	0.83	0.76	0.47	3/43	1.55	0.215	n.s.
t2	0.25	0.22	0.65	0.43	0.62	0.63	0.36	0.41	3/41	2.31	0.091	n.s.
t3	0.24	0.19	0.52	0.67	0.61	0.65	0.16	0.20	3/41	2.28	0.100	n.s.
Anxiety												
t1	0.25	0.28	0.44	0.60	0.68	0.79	0.27	0.29	3/43	1.72	0.178	n.s.
t2	0.09	0.12	0.39	0.37	0.46	0.40	0.26	0.44	3/41	2.70	0.058	n.s.
t3	0.16	0.18	0.40	0.59	0.39	0.36	0.10	0.13	3/41	2.06	0.121	n.s.
Aggression/hostility												
t1	0.28	0.27	0.64	0.94	0.83	0.60	0.55	0.35	3/43	1.99	0.130	n.s.
t2	0.10	0.11	0.45	0.42	0.57	0.67	0.38	0.54	3/41	2.33	0.089	n.s.
t3	0.19	0.23	0.36	0.62	0.59	0.85	0.20	0.28	3/41	1.35	0.271	n.s.
Phobic anxiety												
t1	0.12	0.14	0.12	0.19	0.76	0.87	0.10	0.17	3/43	6.03	0.002	n.s.
t2	0.08	0.15	0.09	0.27	0.24	0.45	0.08	0.17	3/41	.90	0.449	n.s.
t3	0.08	0.15	0.26	0.65	0.27	0.43	0.06	0.14	3/41	0.95	0.424	n.s.
Paranoid ideation												
t1	0.29	0.27	0.41	0.62	0.75	0.55	0.32	0.28	3/43	2.45	0.073	n.s.
t2	0.08	0.14	0.33	0.34	0.45	0.75	0.30	0.49	3/41	1.33	0.278	n.s.
t3	0.08	0.12	0.53	0.74	0.35	0.47	0.13	0.25	3/41	2.63	0.063	n.s.
Psychoticism												
t1	0.16	0.19	0.36	0.47	0.53	0.43	0.29	0.29	3/43	2.32	0.089	n.s.
t2	0.08	0.10	0.29	0.34	0.31	0.39	0.17	0.29	3/41	1.69	0.184	n.s.
t3	0.06	0.09	0.35	0.63	0.23	0.32	0.10	0.14	3/41	1.65	0.194	n.s.

* $p < 0.05$; n.s.: not significant ($p > 0.05$)

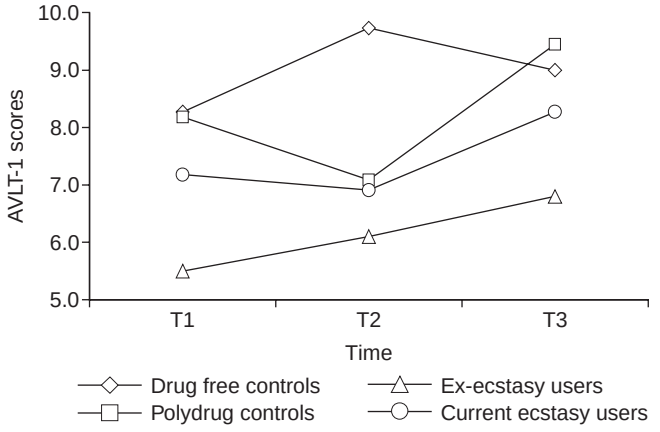

Figure 2 AVLT immediate recall: group means at t1, t2 and t3

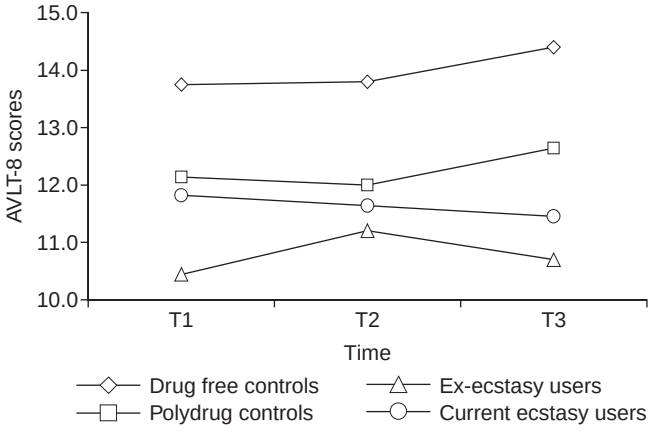

Figure 3 AVLT delayed recall: group means at t1, t2 and t3

Availability of serotonin transporters in current and ex-ecstasy users In the profile analyses (Table 8) regarding the SERT DVRs, a significant TIME×GROUP interaction was found only for the mesencephalon (Fig. 4). As shown in Table 5, the current ecstasy users, who exhibited a significantly reduced SERT availability in the mesencephalon at t1, no longer differed from the ex-ecstasy users (in this case the control group) by t3. The GROUP effect was significant in the mesencephalon, putamen, caudate nucleus and thalamus, and not in the control region white matter, with the ex-ecstasy users generally exhibiting higher DVRs than the current users. The within-subject factor TIME was significant only for the mesencephalon and thalamus.

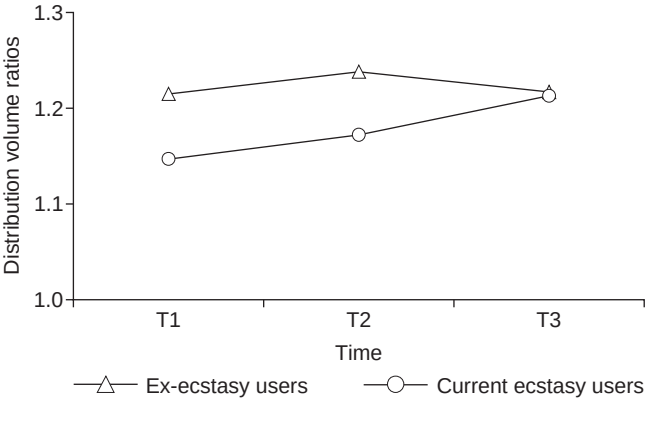

Figure 4 SERT availability in the mesencephalon: group means at t1, t2 and t3

Discussion

Differences at t1

In the initial stage of the study with the full sample of 120 participants, group comparisons revealed reduced SERT DVRs in current polydrug ecstasy users, but not in ex-users who were considerably more impaired in terms of psychopathology and verbal memory (Thomasius *et al.*, 2003). This description generally applies as well to the reduced sample of 47 participants who successfully completed this longitudinal study. One exception is that fewer group differences reached statistical significance. This might be partially accounted for by the reduced power of the cross-sectional group comparisons based on smaller sample sizes. In order to check for selection effects, we thoroughly compared the longitudinal sample of 47 with the remaining 73 of the original 120 participants who dropped out or were excluded. There were significant differences in the SCL-90 scales GSI, somatisation, paranoid ideation and psychoticism, but in no other measure of psychopathology, drug use, verbal memory or SERT availability (results not reported). Thus, those participants who could not be motivated to return for the follow-ups or failed to comply with the requirement to abstain from substance use before their appointments seem to have been more strongly impaired in terms of self-reported psychopathology than the participants who completed the long-term study.

Daumann *et al.* (2001, 2004) and Morgan *et al.* (2002) found self-reported psychopathology in (polydrug) ecstasy users to be primarily associated with the extent of cannabis, rather than ecstasy, use. We found no significant Spearman's correlations between psychopathology and several parameters of cannabis or ecstasy use, such as dose or frequency of use within the 30 days and 6 months prior to participation, and lifetime dose (results not presented). In the full cross-sectional sample of 120 described earlier (Thomasius *et al.*, 2003), psychopathology was best predicted by parameters of ecstasy use. The absence of a relationship between elevated psychopathology and cannabis use in our sample

Table 4 Means and standard deviations of AVLT and RBMT scores at t1, t2 and t3, and cross-sectional group comparisons for each point of measurement

	Drug-naive controls (DC)		Polydrug controls (PC)		Ex-ecstasy users (EE)		Current Ecstasy users (CE)		ANOVA			
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*dF*	*F*	*P(F)*	*Scheffé-Test*
AVLT 1 (immediate recall on first trial)												
t1	8.27	2.63	8.18	1.99	5.50	1.08	7.18	2.64	3/43	3.61	0.021	DC>EE*
t2	9.73	2.52	7.09	2.07	6.10	1.66	6.91	2.21	3/43	6.87	0.001	DC>(PC,EE,CE)*
t3	9.00	2.42	9.45	2.30	6.80	1.87	8.27	3.20	3/43	2.31	0.090	n.s.
AVLT 5 (immediate recall on fifth trial)												
t1	14.27	0.96	12.55	2.50	13.40	1.58	12.36	2.01	3/43	3.08	0.037	n.s.
t2	14.27	1.10	12.82	2.18	12.70	2.16	12.45	2.38	3/43	2.39	0.082	n.s.
t3	14.33	1.11	13.55	1.63	12.20	2.35	12.82	2.14	3/43	3.22	0.032	n.s.
AVLT 5–1 (improvement from trial 1 to 5)												
t1	6.00	2.36	4.36	1.43	7.90	1.97	5.18	2.04	3/43	5.86	0.002	EE>(PC, CE)*
t2	4.53	2.17	5.73	2.33	6.60	1.78	5.54	2.25	3/43	1.92	0.140	n.s.
t3	5.33	2.23	4.09	1.51	5.40	2.22	4.55	2.30	3/43	1.05	0.381	n.s.
AVLT (sum of 5 initial trials)												
t1	63.27	6.59	55.70	10.76	52.20	5.16	55.27	12.55	3/42	3.49	0.024	DC>EE*
t2	63.80	7.29	55.18	11.14	50.40	7.81	55.36	10.42	3/43	4.73	0.006	DC>EE*
t3	63.47	6.38	59.27	8.81	51.60	9.91	56.18	12.11	3/43	3.52	0.023	DC>EE*
AVLT 6 (interference list)												
t1	9.00	2.27	6.45	1.75	5.70	2.58	7.27	1.79	3/43	5.65	0.002	DC>(PC, EE)*
t2	7.67	2.29	7.09	2.17	5.20	2.25	6.18	2.09	3/43	2.81	0.051	n.s.
t3	7.87	3.09	6.27	1.62	5.40	1.78	5.36	1.86	3/43	3.49	0.024	n.s.
AVLT 7 (recall of original list after interference list)												
t1	13.80	1.42	11.82	2.36	11.40	1.84	10.64	4.90	3/43	2.94	0.044	DC>EE*
t2	13.53	1.60	12.27	2.15	10.70	3.23	11.55	3.33	3/43	2.69	0.058	n.s.
t3	14.13	1.06	12.64	2.54	10.40	2.84	11.55	3.67	3/43	4.67	0.007	DC>EE*
AVLT 8 (delayed recall)												
t1	13.75	1.82	12.14	2.34	10.44	2.35	11.82	3.12	3/35	3.21	0.035	DC>EE*
t2	13.80	1.52	12.00	2.32	11.20	1.99	11.64	3.35	3/43	3.16	0.034	n.s.
t3	14.40	0.74	12.64	2.58	10.70	3.27	11.45	3.88	3/43	4.41	0.009	DC>EE*
RBMT immediate recall												
t1	11.07	3.43	10.68	3.63	8.35	2.50	8.45	2.90	3/42	2.35	0.086	n.s.
t2	12.87	6.17	11.18	2.58	8.00	2.84	8.70	3.01	3/42	5.55	**0**.003	*DC>(EE, CE)**
t3	11.73	5.05	11.50	4.24	7.30	2.55	8.09	4.12	3/43	3.41	0.026	n.s.
RBMT delayed recall												
t1	9.96	3.52	10.18	3.35	7.05	2.18	8.25	3.34	3/41	2.37	0.085	n.s.
t2	12.10	4.15	10.86	2.00	7.05	2.57	7.80	2.78	3/42	7.07	0.001	DC>(EE, CE)* PC>EE*
t3	10.20	5.09	10.96	4.71	6.20	2.66	7.18	4.37	3/43	3.02	0.040	n.s.

* $p<0.05$; n.s.: not significant ($p>0.05$)

Table 5 Means and standard deviations of the [^{11}C](+)McN5652 distribution volume ratios at t1, t2 and t3, and cross-sectional group comparisons for each point of measurement

	Drug-naive controls (DC)		Polydrug controls (PC)		Ex-ecstasy users (EE)		Current Ecstasy users (CE)		ANOVA			
	M	*SD*	*M*	*SD*	*M*	*SD*	*M*	*SD*	*dF*	*F*	*P(F)*	*Scheffé-Test*
Mesencephalon												
t1	1.22	0.05	1.22	0.08	1.23	0.06	1.13	0.04	3/40	5.88	0.002	(DC,PC,EE) > CE*
t2					1.24	0.06	1.16	0.03	1/16	10.79	0.005	–
t3					1.23	0.07	1.21	0.05	1/16	0.43	0.522	–
Putamen												
t1	1.39	0.07	1.38	0.12	1.38	0.09	1.30	0.07	3/40	2.04	0.123	n.s.
t2					1.40	0.08	1.32	0.10	1/16	3.32	0.087	–
t3					1.41	0.07	1.33	0.09	1/16	3.96	0.064	–
Caudate Nucleus												
t1	1.23	0.05	1.23	0.10	1.25	0.08	1.15	0.09	3/40	2.95	0.044	n.s.
t2					1.25	0.08	1.18	0.12	1/16	2.05	0.171	–
t3					1.25	0.08	1.17	0.06	1/16	6.28	0.023	–
Thalamus												
t1	1.40	0.07	1.39	0.11	1.41	0.08	1.31	0.07	3/40	2.83	0.049	n.s.
t2					1.43	0.09	1.34	0.12	1/16	3.43	0.083	–
t3					1.45	0.07	1.39	0.08	1/16	3.27	0.089	–
White matter												
t1	0.57	0.05	0.54	0.06	0.57	0.05	0.53	0.08	3/40	1.18	0.329	n.s.
t2					0.61	0.05	0.58	0.04	1/16	1.04	0.323	–
t3					0.59	0.03	0.53	0.02	1/16	8.90	0.009	–

† Two-tailed t-tests were employed at t2 and t3. For the sake of a consistent representation, F-values are given throughout the table, since $t^2 = F$. * $p < 0.05$; n.s.: not significant ($p > 0.05$)

Table 6 Longitudinal analysis of the SCL-90-R scales

	TIME		GROUP	TIME×GROUP					
	dF	F	P(F)	dF	F	P(F)	dF	F	P(F)
Global Severity Index	1/37	6.64	0.014	3/37	3.78	0.018	1/37	0.98	0.423
Somatization	1/38	1.45	0.235	3/38	0.88	0.464	1/38	0.45	0.717
Obsessive-compulsive	1/39	14.62	0.000	3/39	4.65	0.007	1/39	1.77	0.170
Inter-personal sensivity	1/38	6.12	0.018	3/38	5.08	0.005	1/38	0.67	0.573
Depression	1/39	7.20	0.011	3/39	2.51	0.073	1/39	1.76	0.171
Anxiety	1/39	1.65	0.206	3/39	3.14	0.036	1/39	0.23	0.877
Aggression/hostility	1/39	4.36	0.043	3/39	2.82	0.051	1/39	0.32	0.808
Phobic anxiety	1/39	0.77	0.385	3/39	2.32	0.090	1/39	1.92	0.142
Paranoid ideation	1/39	4.23	0.460	3/39	2.55	0.070	1/39	2.01	0.128
Psychoticism	1/39	7.57	0.009	3/39	2.57	0.068	1/39	1.14	0.343

Table 7 Longitudinal analysis of the AVLT and RBMT scores

	TIME	GROUP	TIME×GROUP						
	dF	F	P(F)	dF	F	P(F)	dF	F	P(F)
AVLT 1	1/43	10.02	0.003	3/43	5.04	0.004	1/43	0.16	0.922
AVLT 5	1/43	0.12	0.727	3/43	3.30	0.029	1/43	3.77	0.017
AVLT 5–1	1/43	5.65	0.022	3/43	3.51	*0.023*	1/43	1.24	0.307
AVLT sum of initial trials	1/42	0.72	0.400	3/42	4.82	0.006	1/42	0.51	0.677
AVLT 6	1/43	8.14	0.007	3/43	4.93	0.005	1/43	1.62	0.199
AVLT 7	1/43	0.66	0.423	3/43	4.29	0.010	1/43	1.62	0.199
AVLT 8	1/35	4.90	0.033	3/35	3.78	0.019	1/35	1.61	0.203
RBMT immediate recall	1/41	0.01	0.938	3/41	5.45	0.003	1/41	0.77	0.516
RBMT delayed recall	1/40	0.23	0.633	3/40	5.24	0.004	1/40	0.52	0.671

Table 8 Longitudinal analysis of the [^{11}C](+)McN5652 distribution volume ratios

	TIME	GROUP	TIME×GROUP						
	dF	F	P(F)	dF	F	P(F)	dF	F	P(F)
Mesencephalon	1/16	6.58	0.021	3/16	14.90	0.001	1/16	5.37	0.034
Putamen	1/16	3.09	0.098	3/16	5.18	0.037	1/16	0.02	0.882
Caudate nucleus	1/16	0.17	0.686	3/16	5.86	*0.028*	1/16	0.39	0.541
Thalamus	1/16	14.97	0.001	3/16	5.75	0.029	1/16	1.45	0.246
White matter	1/16	0.73	0.406	3/16	4.26	0.056	1/16	1.23	0.284

may be related to the exclusion criteria applied. Daumann *et al.* (2001, 2004) and Morgan *et al.* (2002) did not exclude participants with THC-positive urine screenings. In the present study, however, participants with positive drug screening results were excluded in order to avoid effects of residual THC on neuropsychological testing (Pope 2001) or other measures. Possibly, some ecstasy users, who are generally polydrug users, utilize cannabis to alleviate psychopathological symptoms, or else suffer from impaired psychological well-being as a consequence of cannabis dependence. Such individuals would probably have been unable to abstain from cannabis use for several days, and thus to participate in our study, but would have been included in the above mentioned studies.

Longitudinal data

One important and unexpected fact that needs to be taken into account when interpreting the longitudinal data is that the current ecstasy users reduced their ecstasy consumption in the course of their participation in the study.

Measures of verbal memory showed mostly stable profiles over time. The relative absence of GROUP×TIME interactions indicates that while the current ecstasy users' performance did not deteriorate with continued ecstasy use, the ex-ecstasy users did not improve over increasing periods of abstinence. This is in agreement with the results of a recent longitudinal study by Gouzoulis-Mayfrank *et al.* (2005), but in contrast to a study by Zakzanis and Young (2001) who reported declining memory performance in ecstasy users over a period of 12 months. The hypothesis that the poor memory performance of the ex-ecstasy users was indeed the result of MDMA serotonergic neurotoxicity would have been strongly supported by a significant deterioration of the current ecstasy users' memory. However, since this was not the case, alternative explanations need to be considered. Fifty-five per cent of the ex-ecstasy users and 43% of the current ecstasy users (from the full cross-sectional sample) were diagnosed with a current substance-induced cognitive disorder caused by ecstasy and/or multiple substances (Thomasius *et al.*, 2005). Thus there might have been more vulnerable individuals (or simply more individuals whose memory was worse independently of MDMA use) among the ex-ecstasy users than among the current ecstasy users, possibly due to a systematic selection effect having to do with the motivation to participate in the study. Again, even in this longitudinal study, we cannot rule out the possibility of pre-existing group differences. One alternative explanation for the absence of a decline in memory performance in the group of current ecstasy users may possibly be a unique motivation to prove that ecstasy is harmless. Unlike participants from other groups, current ecstasy users tended to have a positive attitude towards ecstasy use and some expressed their hope that it would be proven harmless. Therefore, they might have tried harder than other participants. In summary, while we cannot exclude alternative causes, the lack of significant improvement of the ex-ecstasy users' memory performance, together with evidence from other neuropsychological studies (see

meta-analysis by Verbaten 2003), implies that verbal memory deficits found in ex-ecstasy users might be due to long-term effects of (polydrug) ecstasy use, indicating the possibility of long-lasting or even irreversible serotonergic damage.

We found no significant dose-effect relationships, neither between ecstasy and cannabis use and verbal memory performance at t1, nor between the changes in ecstasy and cannabis use and the changes in verbal memory performance between the three time points (data not presented). The reason may well be the reduced sample size because in the full cross-sectional sample of 120 participants (Thomasius *et al.*, 2003) stepwise regression analyses had revealed small but significant effects, with ecstasy use best predicting AVLT immediate and delayed recall and cannabis use best predicting RBMT immediate and delayed recall performance.

The initially reduced SERT DVRs of current ecstasy users showed an unexpected development over time. SERT availability did not only fail to decrease with continued ecstasy use, but on the contrary, seemed to normalize in the course of the study in the mesencephalon. The fact that SERT availability seemed to approach normal levels after a reduction in ecstasy use, together with findings of normal SERT availability in ex-ecstasy users (Reneman *et al.*, 2001a and b; Thomasius *et al.*, 2003) implies that effects of heavy ecstasy use on SERT availability may be reversible. It is not clear why this development was especially pronounced in the mesencephalon. Possibly this has to do with the mesencephalon being a region particularly rich in serotonergic nuclei.

Regarding the psychopathological symptoms, again the current ecstasy users did not show an aggravation and the ex-ecstasy users did not improve, as indicated by a lack of significant TIME×GROUP interactions. Part of these findings is in agreement with Gerra *et al.* (2000), who found no change in former ecstasy users' depressive symptoms over time. The ex-ecstasy users exhibited the highest symptom scores of all groups, but the group comparisons generally missed significance. There was an overall tendency towards declining scores in the course of the study. This might have to do with accidental therapeutic effects of participating in this study. It is also possible that mainly those participants in a psychosocially rather positive development or stable situation continued participating and those with negative developments tended to quit.

The main limitations of this study were that we could not completely rule out possible pre-existing group differences in the areas of verbal memory performance and psychopathology, or to avoid some group differences in the use of cannabis and amphetamine, with the ex-ecstasy users also being the strongest cannabis users. Perhaps most important was the reduction of MDMA consumption by the current ecstasy users. We intended to investigate users with stable or growing ecstasy use in order to maximize the chance of detecting aggravations of possible MDMA-induced impairments. We recruited only heavy users to avoid differences between the current and ex-ecstasy user groups in lifetime doses. Having taken a large amount of ecstasy, some of the current users may have developed tolerance for the desired psychological effects of ecstasy by the time of their participation and therefore lost interest in taking the drug (see Parrott 2005). The decline in average ecstasy use would have been even more pronounced had we not excluded those five current users who took less than five tablets between follow-ups. However, analyses of PET data including those five participants (Buchert *et al.*, 2005) indicate that excluding them did not change the pattern of results.

Conclusion

In summary, our results indicate that impairments of verbal memory and psychological well-being in ex-ecstasy users may persist for longer than 2.5 years after quitting ecstasy. In contrast, the reduced SERT availability of current users seems to be a transient effect of heavy ecstasy use which may recede with declining ecstasy use. However, this result might not necessarily imply a complete recovery from possible serotonergic damage, as the number and activity of SERT can be relatively flexibly up- or down-regulated and does not reflect the number of serotonergic neurons (see Kish, 2002). Therefore, cognitive and psychological impairments repeatedly found in ex-ecstasy users may be the result of serotonergic damage which cannot be detected by the neuroimaging methods employed in ecstasy research as yet. Since we were unable to exclude the possibility of premorbid group differences, this interpretation needs to be viewed with caution, because there is no conclusive evidence that memory and psychological impairments were indeed caused by MDMA in our sample.

Acknowledgements

The study was supported by a grant (No. Z12.01-68503-206) from the BfArM (Federal Institute for Drugs and Medical Devices). Thanks to Dr P-M Sack for his helpful comments on the former draft of the manuscript. Thanks to D Abed, C Berndt, C Carballo-Wandel, I Christensen, F Dobroszemsky, K Emmerich, A Goerling, F Goetz, J Hinrichsen, A Iida, S Kandel, K Marquardt, M Neukamp, M M Ouei, S Rippe, K Rossburg, B Schmidt, P Schmitter, N Schnakenberg, U Strassburger, J Tammen, B Treder and M Wenzel for their contributions to the recruitment and assessment of participants.

References

Abt K (1987) Descriptive data analysis: a concept between confirmatory and exploratory data analysis. Methods Inf Med *26*: 77–88

Acton P D, Friston K J (1998) Statistical parametric mapping in functional neuroimaging: beyond PET and fMRI activation studies. Eur J Nucl Med *25*: 663–667

Aguirre N, Ballaz S, Lasheras B, Del Rio J (1998) MDMA ('Ecstasy') enhances 5-HT1A receptor density and 8-OH-DPAT-induced hypothermia: blockade by drugs preventing 5-hydroxytryptamine depletion. Eur J Pharmacol *346*(2–3): 181–188

Back-Madruga C, Boone K B, Chang L, Grob C S, Lee A, Nations H, Poland R E (2003) Neuropsychological effects of 3,4-methylenedioxymethamphetamine (MDMA or ecstasy) in recreational users. Clin Neuropsychol *17*: 446–459

Battaglia G, Yeh S Y, O'Hearn E, Molliver M E, Kuhar M J, De Souza E B (1987) 3,4-methylenedioxymethamphetamine and 3,4-methylenedioxyamphetamine destroy serotonin terminals in rat brain: quantification of neurodegeneration by measurement of [3H]paroxetine-labeled serotonin uptake sites. J Pharmacol Exp Ther *242*(3): 911–916

Bolla K I, Brown K, Eldreth D, Tate K, Cadet J L (2002) Dose-related neurocognitive effects of marijuana use. Neurology *59*: 1337–1343

Buchert R, Thomasius R, Petersen K, Wilke F, Obrocki J, Nebeling B, Wartberg L, Zapletalova P, Clausen M (2005) Reversibility of ecstasy-induced reduction of serotonin transporter availability in polydrug ecstasy users, Eur J Nucl Med Mol Imaging (accepted)

Buchert R, Thomasius R, Nebeling B, Petersen K U, 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

Buck A, Gucker P M, Schonbachler R D, Arigoni M, Kneifel S, Vollenweider F X, Ametamey S M, Burger C (2000) Evaluation of serotonergic transporters using PET and [11C](+)McN-5652: assessment of methods. J Cereb Blood Flow Metab *20*: 253–262

Commins D L, Vosmer G, Virus R M, Woolverton W L, Schuster C R, Seiden L S (1987) Biochemical and histological evidence that methylenedioxymethylamphetamine (MDMA) is toxic to neurons in the rat brain. J Pharmacol Exp Ther *241*: 338–345

Curran H V, Verheyden S L (2003) Altered response to tryptophan supplementation after long-term abstention from MDMA (ecstasy) is highly correlated with human memory function. Psychopharmacology *169*(1): 91–103

Dafters R I, Hoshi R, Talbot A C (2004) Contribution of cannabis and MDMA ('ecstasy') to cognitive changes in long-term polydrug users. Psychopharmacology *173*(3–4): 405–410

Daumann J, Pelz S, Becker S, Tuchtenhagen F, Gouzoulis-Mayfrank E (2001) Psychological profile of abstinent recreational ecstasy (MDMA) users and significance of concomitant cannabis use. Hum Psychopharmacol *16*: 627–633

Daumann J, Hensen G, Thimm B, Rezk M, Till B, Gouzoulis-Mayfrank E (2004) Self-reported psychopathological symptoms in recreational ecstasy (MDMA) users are mainly associated with regular cannabis use: further evidence from a combined cross-sectional/longitudinal investigation. Psychopharmacology *173*(3–4): 398–404

Degenhardt L, Hall W, Lynskey M (2003) Exploring the association between cannabis use and depression. Addiction *98*: 1493–1504

Derogatis L R (1994) The SCL-90-R scoring manual. Distributed by National computer Systems, Inc., P.O. Box 1416, Minneapolis, MN 55440

De Win M M, Reneman L, Reitsma J B, den Heeten G J, Booij J, van den Brink W (2004a) Mood disorders and serotonin transporter density in ecstasy users – the influence of long-term abstention, dose, and gender. Psychopharmacology *173*(3–4): 376–382

De Win M M, de Jeu R A, de Bruin K, Habraken J B, Reneman L, Booji J, den Heeten G J (2004b) Validity of in vivo [123I]beta-CIT SPECT in detecting MDMA-induced neurotoxicity in rats. Eur Neuropsychopharmacol *14*: 185–189

Fleckenstein A E, Haughey H M, Metzger R R, Kokoshka J M, Riddle E L, Hanson J E, Gibb J W, Hanson G R (1999) Differential effects of psychostimulants and related agents on dopaminergic and serotonergic transporter function. Eur J Pharmacol *382*: 45–49

Franke G (1995) Die Symptom-Checkliste von Derogatis (SCL-90-R). Beltz Test GmbH, Göttingen

Gamma A, Buck A, Berthold T, Vollenweider F X (2001) No difference in brain activation during cognitive performance between ecstasy (3,4-methylenedioxymeth-amphetamine) users and control subjects: a (H215Oxx)-positron emission tomography study. J Clin Psychopharmacol *21*: 66–71

Gehlert D R, Schmidt C J, Wu L, Lovenberg W (1985) Evidence for specific methylenedioxymethamphetamine (ecstasy) binding sites in the rat brain. Eur J Pharmacol *119*: 135–136

Gerra G, Zaimovic A, Giucastro G, Maestri D, Monica C, Sartori R, Caccavari R, Delsignore R (1998) Serotonergic function after 3,4-methylenedioxymethamphetamine ('Ecstasy') in humans. Int Clin Psychopharm *13*: 1–9

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

Gouzoulis-Mayfrank E, Fischermann T, Rezk M, Thimm B, Hensen G, Daumann J (2005) Memory performance in polyvalent MDMA (ecstasy) users who continue or discontinue MDMA use. Drug Alcohol Depend *78*(3): 317–323

Green A R (2004) MDMA: Fact and fallacy, and the need to increase knowledge in both the scientific and popular press. Psychopharmacology *173*(3–4): 231–233

Green A R, Mechan A O, Elliott J M, O'Shea E, Colado M I (2003) The pharmacology and clinical pharmacology of 3,4-methylenedioxymethamphetamine (MDMA, 'ecstasy'). Pharmacol Rev *55*: 463–508

Hanson K L, Luciana M (2004) Neurocognitive function in users of MDMA: the importance of clinically significant patterns of use. Psychol Med *34*: 229–246

Ichise M, Ballinger J R, Golan H, Vines D, Luong A, Tsai S, Kung H F (1996) Noninvasive quantification of dopamine D2 receptors with iodine-123-IBF SPECT. J Nucl Med *37*: 513–520

Ichise M, Meyer J H, Yonekura Y (2001) An introduction to PET and SPECT neuroreceptor quantification models. J Nucl Med *42*: 755–763

Kish S J (2002) How strong is the evidence that brain serotonin neurons are damaged in human users of ecstasy? Pharmacol Biochem Behav *71*: 845–855

Laruelle M, Vanisberg M A, Maloteaux J M (1988) Regional and subcellular localization in human brain of [3H]paroxetine binding, a marker of serotonin uptake sites. Biol Psychiatry *24*: 299–309

Lehrl S (1995) Mehrfachwahl-Wortschatz-Intelligenztest, 3rd edn. (MWT-B) (Multiple Choice Verbal Intelligence Test). Medizinische Verlagsgesellschaft mbH, Balingen

Lezak M D (1983) Neuropsychological assessment, 2nd edn. Oxford University Press, New York

McCann U D, Mertl M, Eligulashvili V, Ricaurte G A (1999) Cognitive performance in (±)3,4-methylenedioxymethamphetamine (MDMA; 'ecstasy') users: a controlled study. Psychopharmacology *143*: 417–425

McCann U D, Szabo Z, Scheffel U, Dannals R F, Ricaurte G A (1998) Positron emission tomographic evidence of toxic effect of MDMA ('Ecstasy') on brain serotonin neurons in human beings. Lancet *352*: 1433–1437

McCann U D, Szabo Z, Seckin E, Rosenblatt P, Mathews W B, Ravert H T, Dannals R F, Ricaurte G A (2005) Quantitative PET Studies on the Serotonin Transporter in MDMA users and controls using [^{11}C]McN5652 and [^{11}C]DASB Neuropsychopharmacology. *30*: 1741–1750

McCardle K, Luebbers S, Carter J D, Croft R J, Stough C (2004) Chronic MDMA (ecstasy) use, cognition and mood. Psychopharmacology *173*(3–4): 434–439

MacInnes N, Handley S L, Harding G F A (2001) Former chronic methylenedioxymethamphetamine (MDMA or ecstasy) users report mild depressive symptoms. J Psychopharmacol *15*: 181–186

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

O'Hearn E, Battaglia G, De Souza E B, Kuhar M J, Molliver M E (1988) Methylenedioxyamphetamine (MDA) and methylenedioxymetham-

phetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: immunodytochemical evidence for neurotoxicity. J Neurosci *8*: 2788–2803

Parrott A C (2004a) Is ecstasy MDMA? A review of the proportion of ecstasy tablets containing MDMA, their dosage levels, and the changing perceptions of purity. Psychopharmacology *173*(3–4): 234–241

Parrott A C (2004b) MDMA (3,4-methylenedioxymethamphetamine) or ecstasy: the neuropsychobiological implications of taking it at dances and raves. Neuropsychobiology *50*: 329–335

Parrott A C (2005) Chronic tolerance to recreational MDMA (3,4-methylenedioxymeth-amphetamine) or Ecstasy. J Psychopharmacol *19*: 71–83

Parrott A C, Sisk E, Turner J J D (2000) Psychobiological problems in heavy 'Ecstasy' (MDMA) polydrug users. Drug Alcohol Depend *60*: 105–110

Parsey R V, Kegeles L S, Hwang D R, Simpson N, Abi-Dargham A, Mawlawi O, Slifstein M, Van Heertum R L, Mann J J, Laruelle M (2000) In vivo quantification of brain serotonin transporters in humans using [11C]McN5652. J Nucl Med *41*: 1465–1477

Pope H G, Gruber A J, Hudon J I, Huestis M A, Yurgelun-Todd D (2001) Neuropsychological performance in long-term Cannabis users. Arch Gen Psychiatry *58*: 909–915

Reneman L, Booij J, Habraken J B, De Bruin K, Hatzidimitriou G, Den Heeten G J, Ricaurte G A (2002) Validity of [123I]beta-CIT SPECT in detecting MDMA-induced serotonergic neurotoxicity. Synapse *46*: 199–205

Reneman L, Lavalaye J, Schmand B, De Wolff F A, van den Brink W, den Heeten G J, Booij J (2001a) Cortical serotonin transporter density and verbal memory in individuals who stopped using 3,4-methylenedioxymethamphetamine (MDMA or 'Ecstasy'). Arch Gen Psychiatry *58*: 901–906

Reneman L, Booij J, de Bruin K, Reitsma J B, de Wolff F A, Gunning W B, den Heeten G I, van den Brink W (2001b) Effects of dose, sex, and long-term abstention from use on toxic effects of MDMA (ecstasy) on brain serotonin neurons. Lancet *358*: 1864–1869

Ricaurte G A, Yuan J, McCann U D (2000) (±)3,4-methylenedioxymethamphetamine ('ecstasy')-induced serotonin neurotoxicity: studies in animals. Neuropsychobiology *42*: 5–10

Ricaurte G, Bryan G, Strauss L, Seiden L, Schuster C (1985) Hallucinogenic amphetamine selectively destroys brain serotonin nerve terminals. Science *229*: 986–988

Semple D M, Ebmeier K P, Glabus M F, O'Carroll R E, Johnstone E C (1999) Reduced in vivo binding to the serotonin transporter in the cerebral cortex of MDMA ('ecstasy') users. Br J Psychiatry *175*: 63–69

Slikker W Jr, Holson R R, Ali S F, Kolta M G, Paule M G, Scallet A C, McMillan D E, Bailey J R, Hong J S, Scalzo F M (1989) Behavioral and neurochemical effects of orally administered MDMA in the rodent and nonhuman primate. Neurotoxicology *10*: 529–542

Stevens J (2002) Applied multivariate statistics for the social sciences. Lawrence Erlbaum Associates, London

Suehiro M, Ravert H T, Dannals R F, Scheffel U, Wagner H N (1992) Synthesis of a radiotracer for studying serotonin uptake sites with positron emission tomography: [11C]McN-5652-Z. Journal of Labelled Compounds and Radiopharmaceuticals *31*: 841–848

Suehiro M, Scheffel U, Ravert H T, Dannals R F, Wagner H N (1993) [11C](+)McN5652 as a radiotracer for imaging serotonin uptake sites with PET. Life Sci *53*: 883–892

Szabo Z, McCann U D, Wilson A A, Scheffel U, Owonikoko T, Mathews W B, Ravert H T, Hilton J, Dannals R F, Ricaurte (2002) Comparison of (+)-^{11} C-McN5652 and C11-DASB as serotonin transporter radioligands under various experimental conditions. J Nucl Med *43*: 678–692

Thomasius R, Petersen K U, Zapletalova P, Wartberg L, Zeichner D, Schmoldt A (2005) Mental disorders in current and former heavy ecstasy (MDMA) users. Addiction, *100*: 1310–1319

Thomasius R, Petersen K U, 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

Tuchtenhagen F, Daumann J, Norra C, Gobbele R, Becker S, Pelz S, Sass H, Buchner H, Gouzoulis-Mayfrank E (2000) High intensity dependence of auditory evoked dipole source activity indicates decreased serotonergic activity in abstinent ecstasy (MDMA) users. Neuropsychopharmacology *22*: 608–617

Verbaten M N (2003) Specific memory deficits in ecstasy users? The results of a meta-analysis. Hum Psychopharmacol *18*: 281–290

Wienhard K, Eriksson L, Grootoonk S, Casey M (1992) Performance evaluation of the positron scanner ECAT EXACT. J Comput Assist Tomogr *16*: 804–813

Wilson B, Cockburn J, Baddeley A (1985) The Rivermead Behavioural Memory Test. Thames Valley Text Co., Reading

Zakzanis K K, Young D A (2001) Executive function in abstinent MDMA ('ecstasy') users. Med Sci Monit *7*: 1292–1298