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Memory function and serotonin transporter promoter gene polymorphism in ecstasy (MDMA) users

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Abstract

Although 3,4-methylenedioxymethamphetamine (MDMA or ecstasy) has been shown to damage brain serotonin (5-HT) neurons in animals and possibly humans, little is known about the long-term consequences of MDMA-induced 5-HT neurotoxic lesions on functions in which 5-HT is involved, such as cognitive function. Because 5-HT transporters play a key element in the regulation of synaptic 5-HT transmission it may be important to control for the potential covariance effect of a polymorphism in the 5-HT transporter promoter gene region (5-HTTLPR) when studying the effects of MDMA as well as cognitive functioning.

The aim of the study was to investigate the effects of moderate and heavy MDMA use on cognitive function, as well as the effects of long-term abstention from MDMA, in subjects genotyped for 5-HTTLPR. A second aim of the study was to determine whether these effects differ for females and males.

Fifteen moderate MDMA users (<55 lifetime tablets), 22 heavy MDMA+ users (>55 lifetime tablets), 16 ex-MDMA+ users (last tablet > 1 year ago) and 13 controls were compared on a battery of neuropsychological tests. DNA from peripheral nuclear blood cells was genotyped for 5-HTTLPR using standard polymerase chain reaction methods.

A significant group effect was observed only on memory function tasks ($p = 0.04$) but not on reaction times ($p = 0.61$) or attention/executive functioning ($p = 0.59$). Heavy and ex-MDMA+ users performed significantly poorer on memory tasks than controls. In contrast, no evidence of memory impairment was observed in moderate MDMA users. No significant effect of 5-HTTLPR or gender was observed.

While the use of MDMA in quantities that may be considered 'moderate' is not associated with impaired memory functioning, heavy use of MDMA use may lead to long lasting memory impairments. No effect of 5-HTTLPR or gender on memory function or MDMA use was observed.

Keywords

MDMA, 5-HT neurotoxicity, cognition, long-term effects, 5-HTTLPR

Introduction

Though generally regarded as relatively safe, it has become increasingly apparent that the popular recreational drug 3,4-methylenedioxymethamphetamine MDMA (ecstasy') can lead to toxic effects on brain serotonin (5-HT) neurons in animals and possibly humans (McCann *et al.*, 1998; Semple *et al.*, 1999; Reneman *et al.*, 2001a, 2001b, Thomasius *et al.*, 2003). In animals, damage to 5-HT neurons has been demonstrated by reductions in various markers unique to 5-HT axons, including the density of 5-HT

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transporters (Schmidt *et al.*, 1986; Schmidt and Taylor 1987; Stone *et al.*, 1986; Commins *et al.*, 1987).

Since MDMA-induced 5-HT neurotoxic damage may lead to impairment of functions in which 5-HT is involved (e.g. memory function) (McEntee and Crook 1991; Altman and Normile, 1988; Hunter, 1989) it is not only important to study the effects of MDMA on 5-HT neurons, but on cognitive function as well. Memory function is of particular interest since several studies have found that MDMA users display significant memory impairments, whereas their performance on other cognitive tests is generally normal (Krystal *et al.*, 1992; Parrott *et al.*, 1998; Parrott 2000). In animals, MDMA severely damages 5-HT axons in brain regions involved in memory function, including the hippocampus and cerebral cortex (O'Hearn *et al.*, 1988; Steele *et al.*, 1994).

While the short-term neurotoxic effects of MDMA on 5-HT neurons and memory have been studied extensively, little is known about the long-term effects in humans. Studies in non-human primates have shown that up to 7 years after treatment with MDMA neocortical brain regions remain partially denervated while others show evidence of complete recovery (Hatzidimitriou *et al.*, 1999). Furthermore, it is unclear, whether moderate use of MDMA can produce these changes.

There is some evidence suggesting that females have increased susceptibility to psychological and neurotoxic effects of MDMA. Higher depression and anxiety scores have been observed in female MDMA users when compared to male users (Liechti *et al.*, 2001; Verheyden *et al.*, 2002). Greater reductions in cerebrospinal fluid 5-hydroxyindoleaceticacid (5-HIAA) and 5-HT transporter densities have been observed in female MDMA users compared to males (McCann *et al.*, 1994; Reneman *et al.*, 2001a; Buchert *et al.*, 2004).

Several studies suggest that 5-HT transporters may play an important role in cognitive processes such as memory function (Meneses, 1999). As a key element in the regulation of synaptic transmission in serotonergic neurons, the 5-HT transporter has become an important research target. For instance, it has been shown that selective 5-HT reuptake inhibitors in non-demented elderly depressed patients improved both mood and cognitive function (Meltzer *et al.*, 1998). Recently, a polymorphism in the 5-HT transporter promoter gene region (5-HTTLPR; Heils *et al.*, 1995) has been shown to regulate 5-HT transporter density in human cell lines (Lesch *et al.*, 1996). Besides reduced 5-HT transporter expression, the *in vitro* transcriptionally less active 5-HTTLPR *s* allele has been associated with depression and anxiety-related personality traits (Collier *et al.*, 1996; Lesch *et al.*, 1996). In line with this, Heinz and colleagues (2000) found reduced *in vivo* 5-HT transporter densities (as measured with [^{123}I]β-CIT SPECT) in healthy subjects carrying the *s* allele, although others have not observed such an association (Willeit *et al.*, 2001). There are also implications that the 5-HTTLPR genotype affects 5-HT transporter availability in disease (Du *et al.*, 1999). It is also conceivable that the 5-HTTLPR genotype modulates neurotoxic effects of drugs, such as ecstasy, on the 5-HT system (Little *et al.*, 1998). It has been argued that low 5-HT function may be a cause rather than an effect of MDMA use, since low 5-HT levels have been linked to impaired cognitive functioning and impulsivity or sensation seeking in humans (Boot *et al.*, 2000). Based on these considerations, it could be hypothesized that the *s* allele is associated with MDMA use and/or cognitive function, and therefore an important confounding variable when investigating cognitive function in users of this drug. Furthermore, it has recently been suggested that the 5-HTTLPR genotype mediates emotionally related cognitive disturbance in MDMA users, because MDMA users carrying the *s* allele, and not comparison subjects carrying the *s* allele, showed abnormal emotional processing (Roiser *et al.*, 2005).

Therefore, the present study investigated a positive association between MDMA dose and cognitive function in subjects genotyped for 5-HTTLPR. Furthermore, the effects of long-term abstention from MDMA use were analysed, as well as the effects of gender on cognitive function. We hypothesized that we would observe: (1) a negative and dose related effect of MDMA on memory function and no effect on the other cognitive domains studied, (2) no difference between memory impairment of heavy users and ex-users (3) that the *s* allele is associated with MDMA use and/or memory impairment, and that (4) female MDMA users show greater impairment in memory function than males.

A part of this study concerning the Rey Auditory Verbal Learning Test (RAVLT) in heavy MDMA users has been previously published (Reneman *et al.*, 2001b). In addition, in three other publications we reported on 5-HT and dopamine transporter densities and mood disorders in the same or a subset of subjects as in the present analysis (Reneman, 2001a, 2002; de Win *et al.*, 2004). However, in the present study the effects of MDMA on other memory tests and other cognitive domains of that population are presented, as well as possible confounding thereof by gender and 5-HTTLPR. In addition, the present analysis includes moderate MDMA users in order to investigate in more detail the dose related effects of MDMA on cognitive function.

Methods and materials

Participants

Recruitment of the participants was as previously described (Reneman, 2001a, b, 2002; de Win *et al.*, 2004). Briefly, three different groups of ecstasy users were compared with ecstasy-naive but drug using controls. Subjects were recruited with flyers distributed at venues associated with the 'rave scene' in Amsterdam with the help of UNITY, an agency which provides harm reduction information and advice. Experimental and control groups were thus recruited from the same community sources. Subjects selected were group matched for gender and age, between 18 and 45 years, otherwise healthy, and with no psychiatric history. Three different groups of ecstasy users were recruited: 15 moderate ecstasy users ('MDMA group'), 22 heavy ecstasy users ('MDMA+ group'), and 16 ex-heavy ecstasy users ('ex-MDMA+ group'). The eligibility criterion for the MDMA group was previous use of maximum 50 tablets of ecstasy, whereas the MDMA+ group had to have used at least 50 tablets prior to the study. The ex-MDMA+ group had to have taken a minimum of 50 tablets but

stopped using ecstasy for at least 1 year prior to the study. The cut-off point of 50 lifetime tablets was based on previous findings of increased risk of developing psychiatric disturbances in people with a lifetime consumption of 50 or more MDMA tablets (Schifano and Magni, 1994). The 13 controls were healthy subjects with no self-reported prior use of ecstasy.

Participants agreed to abstain from use of all psychoactive drugs for at least 3 weeks before the study, and were asked to undergo urine drug screening on the day of the study (with an enzyme-multiplied immunoassay for amphetamines, barbiturates, benzodiazepine metabolites, cocaine metabolite, opiates, and marijuana) before enrolment. Subjects were interviewed with a fully structured computer assisted diagnostic psychiatric interview (Composite International Diagnostic Interview: CIDI, version 2.1) to screen for current axis I psychiatric diagnoses. After testing urine samples, exclusion criteria were: a positive drug screen (5 subjects were excluded); pregnancy; and a severe medical or neuropsychiatric illness that precluded informed consent.

Subjects were informed that reimbursement for participation was contingent on no evidence of drug use on the urine sample. The institutional Medical Ethics Committee approved the study. After complete description of the study to the subjects, written informed consent was obtained from all participants.

Neuropsychological testing

We selected a battery of widely used tests that have been related to serotonergic functions, particularly memory (Buhot, 1997).

Test of general intelligence Dutch Adult Reading Test (DART; Schmand *et al.*, 1992). Fifty words with irregular spelling are read aloud. The number of correctly read words is transformed into an estimate of verbal intelligence (DART-IQ). The DART is the Dutch counterpart of the National Adult Reading Test (NART; Nelson, 1991). This test gives an estimate of premorbid intelligence as it is relatively insensitive to cognitive deterioration due to neurologic disorders. It was used to describe the sample and as a covariate in the statistical analyses.

Reaction speeds Reaction was tested using FePsy, an automated computerized battery of validated neuropsychological tasks (Alpherts and Aldenkamp, 1995). Reaction was evaluated separately on the non-dominant hand and dominant hand in response to simple auditive and visual stimuli, and to a Binary Choice Task.

Memory function

- Logical Memory of the Rivermead Behavioural Memory Test (Wilson *et al.*, 1985). A 21-item news message is read to the subject, who repeats as many items as he or she can remember. After a 15-minute interval he or she is asked to recall the message again. Score is the number of items recalled. In view of the limited reliability of this type of test, two messages were used and the scores were summed.
- Visual Reproduction subtest of the Wechsler Memory Scale – Revised (WMS-R; Wechsler, 1987). Four geometric figures are shown to the subject, one by one during 10 seconds. Immediately after presentation the subject draws each figure from memory. After a delay of 30 minutes he is asked to draw the figures once again. The number of correctly reproduced elements is scored. Total scores range to a maximum of 41 points.
- Rey Auditory Verbal Learning Test (RAVLT; Rey, 1964). The subject memorizes a series of 15 words in five learning trials. Following a 20-minute delay, the subject is asked to recall the word list. Raw scores are used.
- Corsi Block-tapping Test (Milner, 1971) This is a test of spatial memory span. The subject has to reproduce a series of taps on blocks that are randomly dispersed on a board. The length of the series is gradually increased until the subject consistently fails.

Tests of attention and executive functioning

- Category fluency (Luteijn and Van der Ploeg, 1983). Naming animals and occupations, for 1 minute each. Score is raw number correct in 2 minutes.
- Controlled Oral Word Association Test (COWAT; Benton and Hamsher, 1976). During 1 minute the subject must say as many words as he or she can think of that begin with a given letter. Three trials with different letters were done. Score is raw number correct in 3 minutes.
- Stroop Color Word Test (Stroop, 1935; Hammes, 1978). This test measures perceptual interference, response inhibition, and selective attention by having subject name colours, and name the colour of ink of colour-words when the words are printed in a non-matching coloured ink. Score is the time to completion in seconds for 100 items.
- Trail Making Test part A and B (Reitan, 1958, 1992). The task is to connect numbers (part A) and to connect numbers alternating with letters (part B) on a sheet of paper. This is a test of visual scanning, visuomotor and conceptual tracking, mental flexibility, and motor speed. Score is time to completion in seconds.
- Wisconsin Card Sorting Test (WCST; Heaton *et al.*, 1993). This test uses a deck of cards on which different numbers of different forms in different colours are shown. The task is to sort the cards according to one of three possible sorting rules (colour, number, or form). These rules are not told to the subject; he or she must identify the sorting rules. However, after each sort feedback is given on whether it was correct. Once a sorting rule has been found (ten correct sorts on a row), the sorting rule is changed without warning, so that the subject has to shift to a different rule. Of particular interest are perseverative errors of the kind where the subject keeps sorting according to a previously correct rule or to a rule that he or she was told to be wrong in the immediately preceding sort. The WCST is a test of concept formation and set shifting. Scores are the raw numbers of errors, perseverations and sort shifts ('categories').

Genotyping

Genotyping was performed using peripheral nuclear cells obtained by centrifugation of approximately 5 mL blood from the

antecubital vein. 5-HTTLPR *l* and *s* alleles were analysed using polymerase chain reaction as described elsewhere (Heils *et al.*, 1995; Lesch *et al.*, 1996).

Statistical analyses

Characteristics of the sample Differences in continuous variables (log transformed if necessary) between the four groups were analysed using ANOVA and Bonferroni *post hoc* analysis. Differences in the prevalence of subjects carrying the *s* alelle between MDMA users and control subjects were investigated using the Chi-square test. In addition, Pearson correlation analyses was performed between the number of s-allele 5-HTTLPR genotype and extent of previous MDMA use.

Neuropsychological testing Differences between the four groups in the three main cognitive domains (reaction speed, memory function and attention/executive functioning) were analysed using general linear model-based MANOVA. To answer our research question, our basic model included a cognitive domain (reaction speed six levels; memory function eight levels and attention/executive functioning nine levels), group (four levels), 5-HTTLPR (three levels) and gender (two levels), and the interaction between group and 5-HTTLPR, and group and gender. We extended the model by including several potential confounders, including age (continuous), DART-IQ (continuous), extent of previous cannabis use (continuous). If a significant confounding effect was observed, the variable was kept in the model. If MANOVA revealed a significant group effect, we investigated differences in cognitive parameters between groups by one-way ANOVA and Bonferroni *post hoc* analysis.

Correlations between cognitive parameters (on which the four groups differed significantly) and extent of previous MDMA use were assessed using Pearson correlation analyses. Because age, gender, verbal intelligence and extent of previous cannabis use have been shown to be highly associated with the majority of memory tests, we also performed partial correlations to control for age, gender, DART-IQ and extent of previous cannabis use. In addition, partial correlations were assessed between cognitive parameters and extent of previous cannabis use while controlling for age, gender, DART-IQ and extent of previous MDMA use.

The chance of a type I error (α) was set at 0.05. In case Bonferroni *post hoc* analyses were made, statistical significance within the text will be reported as a corrected p value. All data were analysed using SPSS version 10.0 (SPSS, Inc., Chicago, USA).

Results

Characteristics of the sample

Characteristics of the study population were described in an earlier publication (Reneman *et al.*, 2001b). The four different groups were comparable regarding age, sex distribution, premorbid IQ (NART IQ), and use of alcohol and cannabis (Table 1). MDMA users had used significantly more amphetamine than controls. Males were significantly older (on average 3.1 years) and consumed significantly more alcohol per week than females. Within the MDMA+ subgroup, males had used more MDMA tablets during lifetime and higher usual doses than females (Table 1), also when expressed per kg body weight. On average, MDMA and MDMA+ users were abstinent from ecstasy use for 3.6 and 2.3 months respectively, while the ex-MDMA+ users reported to be abstinent from ecstasy for almost 2.5 years on average (Table 1).

Neuropsychological testing

Table 2 represents the scores on the three main cognitive domains (reaction speed, memory function and attention/executive functioning) analysed. MANOVA only revealed a significant main effect of Group on memory function ($F = 1.66$, $df = 24$, $p = 0.03$), but not on reaction times ($F = 0.87$, $df = 18$, $p = 0.61$) or attention/executive functioning ($F = 0.92$, $df = 27$, $p = 0.59$).

Memory function Univariate ANOVA demonstrated a significant group effect on RAVLT immediate ($F = 7.1$, $df = 3$, $p = 0.00$) and delayed word recall ($F = 5.6$, $df = 3$, $p = 0.00$). *Post hoc* analysis showed that heavy ($p = 0.00$), but not moderate ($p = 0.11$), MDMA users recalled significantly less words when compared to controls. Ex-MDMA+ users also recalled significantly less words on the immediate RAVLT when compared to controls. Similar observations were made on the delayed RAVLT recall: heavy MDMA+ and ex-MDMA users recalled less words ($p = 0.01$ and $p = 0.04$, respectively) as compared to controls, but not moderate MDMA users ($p = 0.20$).

When analysing memory function, MANOVA revealed no significant effect of the between-group factors of 5-HTTLPR genotype ($p = 0.76$) and gender ($p = 0.13$). In addition, no significant interactions were observed between group and 5-HTTLPR ($p = 0.74$), and group and gender ($p = 0.82$). No significant covariance effect of extent of previous cannabis use (log transformed; $p = 0.21$) was observed. However a significant effect of DART-IQ ($p = 0.00$) and age ($p = 0.03$) was observed, and within groups comparisons were thus controlled for these two variables.

In the whole sample, extent of previous MDMA use (lifetime number amount of tablets; log transformed) was significantly associated with immediate ($r = -0.42$, $p = 0.00$) and delayed RAVLT scores ($r = -0.33$, $p = 0.01$) (Fig. 1). However, no significant correlations within just the three MDMA using groups were observed, suggesting that although there is a general difference between MDMA users and non-users, this difference may not be attributable to amount of use *per se*. When controlling for potential confounders (age, gender, DART-IQ, and extent of previous cannabis use) in the partial correlation analysis, the associations between extent of previous MDMA use and RAVLT scores remained significant ($r = -0.36$, $df = 61$, $p = 0.00$, and $r = -0.28$, $df = 60$, $p = 0.03$, respectively) in the whole sample. Extent of previous cannabis use (number of joints in last 3 months; log transformed) was significantly associated with immediate ($r = -0.26$, $p = 0.04$) but not delayed RAVLT scores ($r = -0.11$, $p = 0.38$). However, when controlling for age, gender, DART-IQ, and extent

Table 1 Demographics, characteristics of ecstasy use and exposure to other substances expressed as mean ± SD

	Polydrug controls ($n = 15$)		Moderate ecstasy users ($n = 15$)		Heavy ecstasy users ($n = 23$)		Former ecstasy users ($n = 16$)			
	Male (n = 7)	Female ($n = 8$)	Male ($n = 9$)	Female ($n = 6$)	Male ($n = 12$)	Female ($n = 11$)	Male ($n = 8$)	Female ($n = 8$)	*P* group[b]	*P* gender[b]
Demographics										
Age (years)	29.3 ± 6.9	23.3 ± 1.3	25.6 ± 7.5	22.7 ± 2.8	27.1 ± 6.0	25.0 ± 4.1	26.4 ± 6.2	24.1 ± 4.7	0.63	0.02
DART-IQ[a]	104.7 ± 6.2	106.9 ± 7.4	111.2 ± 11.5	112.2 ± 8.1	106.0 ± 9.0	104.5 ± 8.4	105.9 ± 11.8	102.0 ± 7.7	0.10	0.73
Ecstasy use										
Duration of use (years)	NA	NA	4.6 ± 3.1	3.3 ± 1.5	6.4 ± 3.0	4.6 ± 2.1	4.0 ± 2.0	5.1 ± 3.1	0.24	0.38
Usual dose (tablets)	NA	NA	1.33 ± 0.56	1.38 ± 0.49	2.64 ± 0.67	1.82 ± 0.46	2.00 ± 0.96	2.16 ± 1.01	0.00[d]	0.35
Lifetime dose (tablets)[c]	NA	NA	29.5 ± 17.5	27.3 ± 19.7	831.8 ± 733.0	200.9 ± 171.2	126.9 ± 91.4	409.3 ± 868.7	0.00[d]	0.25
Time since last tablet (months)	NA	NA	4.3 ± 7.5	2.7 ± 2.1	1.97 ± 2.67	2.6 ± 2.1	37.1 ± 25.4	21.0 ± 10.1	0.00[d]	0.38
Other substances										
Alcohol (no. of consumptions/week)	14.1 ± 12.8	7.1 ± 7.4	18.2 ± 14.8	5.3 ± 3.2	13.0 ± 8.2	5.8 ± 3.5	4.5 ± 3.9	7.9 ± 5.4	0.14	0.00[d]
Tobacco (cigarettes/day)	9.5 ± 3.3	10.3 ± 6.1	11.0 ± 6.5	9.4 ± 9.2	12.4 ± 13.0	6.0 ± 7.1	11.8 ± 8.5	13.3 ± 8.6	0.47	0.21
Last 3 months use of:										
Cannabis (no. of joints)[c]	2.3 ± 0.6	4.5 ± 5.0	68.1 ± 6.5	31.8 ± 51.6	94.6 ± 153.0	67.5 ± 101.9	73.1 ± 110.4	196.3 ± 369.3	0.37	0.23
Amphetamine (no. of times used)[c]	–	–	0.4 ± 0.8	–	3.8 ± 7.4	3.6 ± 5.5	–	–	0.04[d]	0.80
Usual dose amphetamine (g)	–	–	0.3 ± 0.2	0.1 ± 0.1	0.4 ± 0.3	0.3 ± 0.3	0.7 ± 0.4	1.0 ± 0.8	0.07	0.83
Cocaine[c]	–	–	1.2 ± 1.1	–	4.2 ± 2.8	4.4 ± 3.4	–	–	0.09	0.74

a DART = Dutch Adult Reading Test.
b Two way analysis of variance.
c Variables that were log transformed.
d Statistical significant differences.

Table 2 Reaction times and cognitive performance (memory and attention)*

	Controls $n=13$[a]		MDMA 15		MDMA+ 22[b]		ex-MDMA 16		p
Median Reaction Times (msec)									**0.61**
Auditive DH	242.5	(22.1)	246.7	(28.3)	245.2	(30.2)	244.1	(29.3)	
Auditive NH	244.4	(34.6)	250.1	(24.1)	245.5	(26.8)	254.3	(32.3)	
Visual DH	282.1	(52.2)	287.7	(55.2)	257.4	(30.7)	270.3	(46.6)	
Visual NH	316.0	(92.8)	298.6	(56.2)	268.7	(31.7)	279.9	(53.6)	
Binary choice task	382.9	(112.6)	368.2	(53.0)	353.7	(67.9)	368.3	(69.5)	
Binary choice (total falses)	2.5	(3.4)	2.6	(3.1)	5.0	(7.2)	2.6	(1.9)	
Memory function (total scores)									**0.03**†
Logical memory immediate	17.9	(6.1)	16.1	(5.2)	17.9	(3.8)	16.3	(5.8)	0.15
Logical memory delayed	15.3	(5.8)	12.7	(5.4)	14.4	(3.9)	13.8	(6.2)	0.12
WMS immediate	39.4	(1.9)	39.2	(1.8)	38.4	(2.6)	37.7	(3.2)	0.44
WMS delayed	36.4	(3.4)	36.2	(5.5)	35.4	(5.6)	35.9	(4.1)	0.91
RAVLT immediate	60.0	(6.8)	51.2	(8.6)	47.0	(8.6)	48.0	(12.5)	0.00‡
RAVLT delayed	13.1	(2.1)	10.7	(3.2)	9.8	(2.9)	10.1	(2.9)	0.00§
Corsi Block Span	5.2	(0.7)	5.7	(1.1)	5.6	(1.3)	5.7	(1.3)	0.59
Corsi Block Span plus one	5.6	(0.6)	5.9	(1.0)	6.0	(1.1)	6.0	(1.2)	0.51
Attention and executive functioning (total scores)									**0.59**
Category fluency (sum score)	44.3	(7.5)	47.0	(12.6)	45.1	(7.4)	41.4	(10.2)	
Letterfluency (sum score)	44.4	(9.3)	41.5	(9.8)	41.6	(12.6)	39.6	(10.4)	
Stroop colour (sec)	53.9	(9.0)	56.7	(10.5)	53.2	(9.0)	53.5	(7.9)	
Stroop colour-word (sec)	82.6	(14.4)	83.5	(12.0)	82.0	(15.5)	85.5	(14.4)	
Trailmaking A (sec)	24.8	(7.5)	20.6	(6.5)	19.9	(3.7)	24.0	(11.6)	
Trailmaking B (sec)	47.9	(12.5)	49.7	(14.5)	46.4	(15.7)	52.5	(13.5)	
WCST errors	35.3	(24.0)	36.7	(22.8)	38.8	(18.3)	35.5	(19.2)	
WCST preservations	19.3	(15.7)	15.8	(8.5)	19.7	(14.6)	15.1	(13.6)	
WCST categories	4.6	(1.5)	4.8	(1.7)	4.4	(1.6)	4.7	(2.1)	

a Two subjects missing with data.

b One subject missing with data.

*Data are expressed in mean ± SD.

†Significant group effect (MANOVA: $F_{24}=1.66$).

‡*Post hoc* analysis: Control vs MDMA group, corrected $p=0.11$, control vs MDMA+ group, Bonferroni corrected $p=0.00$, control vs ex-MDMA group, Bonferroni corrected $p=0.01$.

§*Post hoc* analysis: Control vs MDMA group, corrected $p=0.20$, control vs MDMA+ group, Bonferroni corrected $p=0.01$, control vs ex-MDMA group, Bonferroni corrected $p=0.04$.

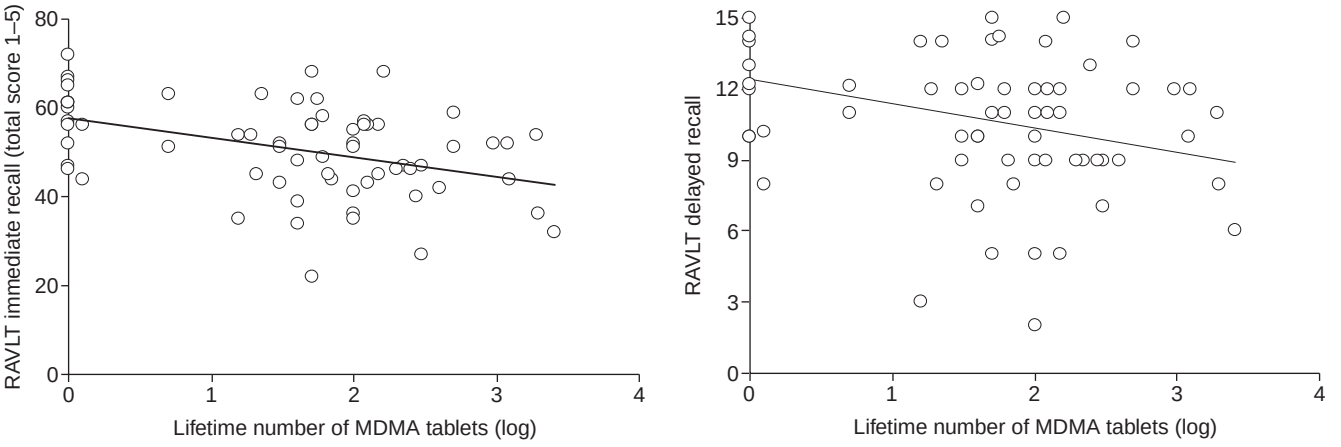

Figure 1 A. Correlation between RAVLT immediate recall scores and extent of previous MDMA use (log transformed). B. Correlation between RAVLT delayed recall scores and extent of previous MDMA use (log transformed).

of previous MDMA use, the observed association between extent of previous cannabis use and immediate recall did not remain significant (log transformed; $r = -0.14$, df = 61, $p = 0.27$), nor the delayed recall (-0.02, df = 60, $p = 0.88$).

Genotype

Genotype distribution in MDMA users was in good accordance with 5-HTTLPR genotype distribution patterns found in healthy white European subjects (Lesch *et al.*, 1996): *l/l*, 17 (32.1%); *l/s*, 28 (52.8%); *s/s*, eight (15.1%). Controls and MDMA users did not differ in genotype distribution patterns (X^2, $p = 0.38$). MANOVA demonstrated no significant effect of genotype on memory function ($p = 0.76$), nor an interaction between memory function and genotype ($p = 0.74$).

Finally, correlation analysis demonstrated no significant relationships between the 5-HTTLPR genotype and extent of previous MDMA use ($p = 0.71$). Thus, as also shown above, the 5-HTTLPR genotype is not related to performance on the memory tests and is not a confounder of the observed relation between MDMA use and memory performance.

Discussion

Our findings indicate impairments in memory function in heavy users of MDMA with relatively intact performance in reaction times and tasks of attention and executive functioning. Similar observations were made in individuals who stopped using MDMA more than 1 year ago. In contrast, no evidence of cognitive impairment was observed in subjects who indicated having used MDMA in quantities that may be considered 'moderate'. Last, our preliminary data provide no evidence for a role of 5-HTTLPR genotype in MDMA (ab)use or cognitive performance, nor gender differences in susceptibility to MDMA-induced memory impairment.

Interestingly, we observed that individuals who had stopped heavy use of MDMA for more than 1 year performed equally poor on the word recall test as recent heavy MDMA users. The persistent memory problems in ex-MDMA+ users may suggest irreversibility of MDMA-induced 5-HT neurotoxic changes in brain regions involved in memory functions. In line with this, it has been shown in non-human primates that cortical 5-HT terminal markers remain decreased up to 7 years after MDMA treatment, particularly prominent in the hippocamus (Hatzidimitriou *et al.*, 1999), although in humans hippocampal and cortical 5-HT terminals seem to be reversible (Reneman *et al.*, 2001b; Buchert *et al.*, 2004). These studies have also shown that the density of 5-HT terminal markers does not correlate with extent of memory loss. This is an intriguing observation, as discussed elsewhere (Reneman *et al.*, 2001b), and may indicate that anatomical recovery does not necessarily infer functional recovery. However, future studies will have to investigate this point. We have previously published this part of the study (Reneman *et al.*, 2001b). However, in the present study the effects of MDMA on other memory tests and other cognitive domains of that same population are presented, as well as possible confounding thereof by 5-HTTLPR. Few studies have investigated the long-term effects of MDMA on cognitive function. Persistent impairments in ex-MDMA+ users (abstinent for at least 6 months) in verbal recall performance and (visuo-spatial) working memory have been described compared to polydrug using controls (Morgan *et al.*, 2002; Daumann *et al.*, 2004; Wareing *et al.*, 2005). Their performance on other cognitive tests was generally normal, like in our study. Thomasius *et al.* (2003) only observed reduced verbal recall performance in ex-users, and not in current heavy users.

The present observations made in heavy users of MDMA are generally consistent with previous reports suggesting that recreational MDMA users display significant memory impair-

ments, whereas performance on other cognitive tests is generally normal (Spatt *et al.*, 1997; Morgan, 1998; Parrott *et al.*, 1998 Parrott, 2000; Gouzoulis-Mayfrank *et al.*, 2003; von Geusau *et al.*, 2004). Impairments have been demonstrated in immediate and delayed verbal recall (Parrott *et al.*, 1998; Bolla *et al.*, 1998; Morgan, 1999; Wareing *et al.*, 2004) and in verbal working memory (Wareing *et al.*, 2000). Presently, differences in memory function between MDMA users and controls were observed only using RAVLT. This may result from the fact that the RAVLT is known to be a very reliable test. Test re-test correlation scores (with an interval of 2 months) for RAVLT are higher than for the other memory tests administered in this study: 0.80 and 0.83 for RAVLT immediate and delayed recall, respectively (Van den Burg *et al.*, 1985). For the other memory tests administered in this study the correlation coefficient varies from 0.60 (WMS) to 0.75 (Logical memory) (derived from WMS-III, 1997; Wechsler, 1997).

Contrary to findings in previous studies in which 'novice' MDMA users (Parrott *et al.*, 1998; Bhattachary and Powell, 2001) demonstrated verbal memory deficits, we did not observe memory deficits in moderate users who had been exposed to less than 50 tablets in their life, 29 on average. Discrepancies between the previous studies may be attributed in part to the fact that subjects in our study abstained from psychoactive drugs for at least 3 weeks. Thus, acute or partial residual effects, or drug withdrawal, may have caused the memory disturbances noted in the study by Parrott *et al.* (1998). Alternatively, subjects in the previous mentioned studies may have used extremely high doses of MDMA, causing brain 5-HT neurotoxicty despite the small number of separate drug exposures. One other study reported memory problems in moderate MDMA users (Verkes *et al.*, 2001). However, the moderate users had used on average 169 tablets (lifetime), as opposed to 29 tablets in the current study. In any case, it is well known from animal studies that higher dosages of MDMA produce greater neurotoxic lesions (Steele *et al.*, 1994). In a study by Bolla and colleagues (1998) in which CSF 5-HIAA and memory function was assessed in abstinent MDMA users, only individuals with more profound decrements in CSF 5-HIAA (presumably reflecting a greater extent of 5-HT injury) had detectable difficulties with memory function. In line with this, we previously reported that post-synaptic cortical 5-HT_{2A} receptor availability was increased in MDMA users (presumably reflecting lower synaptic 5-HT levels) and correlated positively with RAVLT-recall in MDMA users (Reneman *et al.*, 2000).

We presently investigated the potentially confounding influence of heritable effects on memory function and the use of MDMA. In a small sample size we observed that the 5-HTTLPR genotype was not associated with memory function or MDMA use. Although studies observed an important role of 5-HT transporters in cognitive processes such as memory function (Meltzer *et al.*, 1998), we previously did not observe a correlation between memory function and cortical 5-HT transporter densities obtained in a subset of the same subjects presented here (Reneman *et al.*, 2001b). Although there are studies suggesting that the *s* allele is associated with depression and anxiety-related personality traits (Collier *et al.*, 1996; Lesch *et al.*, 1996), other studies failed to find such an association (Hoehe *et al.*, 1998; Mendes *et al.*, 1998). Thus, the findings of the present study suggest that the observed memory deficits in MDMA users do not result from a genetic predisposition to low 5-HT transporter densities (the *s* allele), but probably result from the use of MDMA itself, although pre-existing differences in memory performance cannot be ruled out. Furthermore, the use of MDMA does not seem to result from pre-existing differences in 5-HT transporter densities, since genotype distribution in MDMA users was in good accordance with 5-HTTLPR genotype found in healthy European subjects (Lesch *et al.*, 1996), and did not differ from the distribution found in control subjects. Furthermore, in the present study we were not able to replicate recent findings suggesting that MDMA users carrying the *s* allele are at particular risk of developing 5-HT related functional abnormalities (Roiser *et al.*, 2005). However, because of our small sample size and that in the study of Roiser, more studies are needed with larger sample sizes to address this issue.

McCann and co-workers (McCann *et al.*, 1994) observed greater reductions in 5-HIAA in female than in male MDMA users. In line with this, we (Reneman *et al.*, 2001a; Buchert *et al.*, 2004) observed greater reductions in 5-HT transporter densities in female than in male MDMA users, suggesting that females are more susceptible than males to the neurotoxic effects of MDMA. Stronger anxiety effects in response to MDMA in females compared to males have been reported (Liechti *et al.*, 2001). In addition, Verheyden *et al.* (2002) reported higher mid-week depression scores in female MDMA users. In contrast to this, we presently did not observe differences between males and females in the effects of MDMA on memory function. Other studies have failed to investigate the effect of gender on memory function in MDMA using subjects, or also did not observe an effect (Thomasius *et al.*, 2003; Rodgers *et al.*, 2003; von Gesau *et al.*, 2004), athough Bolla and colleagues (1998) reported that females were less susceptible than males to MDMA dose-related decrease in memory function.

It is common for MDMA users to consume cannabis, making it difficult to recruit MDMA users who have not also used cannabis. Recent studies have pointed out the importance of taking cannabis consumption into account when studying MDMA-related cognitive impairment (Rodgers, 2000; Croft *et al.*, 2001; Dafters *et al.*, 2004; Daumann *et al.*, 2004). However, the adverse effects of long-term cannabis use on cognitive skills appear to be short-term only. Pope *et al.* (2001) recently showed that heavy cannabis users scored significantly below control subjects on a word recall list which was detectable at least 7 days after heavy cannabis use, but not after 28 days. Furthermore, animal studies have recently shown a protective effect of cannabinoid receptor agonists on MDMA-induced 5-HT depletion, as well as on anxiety (Morley *et al.*, 2004). In the present study, three lines of evidence suggest that the deficits in the heavy recent and ex-MDMA+ users discussed above were not related to cannabis consumption. The first is that if cannabis was responsible for the observed memory impairments then a significant covariance effect of cannabis on memory function in the MANOVA analysis might be expected, which was not the case. The second piece of evidence is that no association between extent of previous cannabis use and memory function was observed after controlling for potential confounders, as was

observed for extent of previous MDMA use. Previous studies have also failed to demonstrate an association in MDMA users between extent of previous cannabis use and memory function (Morgan, 1999; Bhattachary and Powell, 2001). Finally, the poor memory performance in heavy and ex-MDMA+ using subjects is unlikely to be due to acute or partial residual effects of cannabis since all participants reported that they had abstained from use of cannabis or other psychoactive drugs for at least 3 weeks before the study, which was checked in the urine. Thus, although cannabis may have contributed to some extent to the poorer performance of heavy and ex-MDMA+ users compared with MDMA-naive subjects, cannabis is unlikely to fully account for the present findings. We cannot exclude the possibility that the use of other drugs than MDMA and cannabis (as discussed above) may have differed between groups and have contributed to the impairments observed here. We minimized the influence of other drugs than MDMA and psychosocial factors by taking a control group from the same population which the MDMA users were recruited from. This differs conspicuously from most previous studies, where controls came from a university or general population.

Unfortunately, we were not able to assure abstinence from MDMA, other than the past 3 days in the ex-MDMA+ users. In future studies, hair-sample analysis may be useful to ascertain long periods of abstinence from MDMA. In addition, follow-up studies in human subjects with known MDMA-induced neurotoxicity need to be conducted to allow definite conclusions on reversibility of memory impairments in humans.

The observed memory impairments in heavy and ex-MDMA+ users cannot readily be attributed to differences in verbal language skills, since the groups were all comparable with one and another on a measure of verbal IQ (DART). Likewise, it seems unlikely that they reflect generalized impairments of attention or concentration, since the groups did not differ on any of the tasks investigating these factors.

In summary, our data suggest dose-dependent decreases in memory function in MDMA users, which may not be reversible since individuals who had stopped using MDMA more than 1 year ago have impaired memory function, similar to recent MDMA users. In addition, our data provide no evidence for a role of 5-HTTLPR genotype in cognitive performance or MDMA (ab)use, nor gender differences in susceptibility to MDMA-induced memory impairment.

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