Investigating the potential neurotoxicity of ecstasy (MDMA). An imaging approach
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Citation for published version (APA):
Chapter 8

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

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Submitted for publication
Part III | Potential functional consequences of MDMA-induced neuronal loss

Abstract

Rationale - 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 on cognitive functioning. Objectives - To investigate the effects of moderate and heavy MDMA use on cognitive function, as well as the effects of long-term abstinence from MDMA, in subjects genotyped for 5-HTTLPR. Methods - 15 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. Results - 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. Greater use of MDMA was associated with greater impairment in verbal memory. No significant covariance effect of 5-HTTLPR was observed. Conclusions - 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 on memory function or MDMA use was observed.

Introduction

Though generally regarded as relatively safe, it has become increasingly apparent that the popular recreational drug 3,4-methylenedioxymethamphetamine (MDMA or "Ecstasy") can lead to toxic effects on brain serotonin (5-HT) neurons in animals and possibly humans (McCann et al., 1988; Semple et al., 1999; Renerman et al., 2001a; 2001b). 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 transporters (Schmidt et al., 1986; 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 & Crook 1993; Altman et al., 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 seven years after treatment with MDMA neocortical brain regions remain partially denervated while others show evidence of complete recovery (Hatziidimitriou et al., 1999). Furthermore, it is unclear, whether moderate use of MDMA can produce these changes.

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 11C]-DOPAC SPECT) in healthy subjects carrying the s allele. 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 cog-
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native function, and therefore an important confounding variable when investigating cognitive function in users of this drug.

Therefore, the present study investigated the effects of moderate and heavy MDMA use on cognitive function in subjects genotyped for 5-HTTLPR. Furthermore, the effects of long-term abstinence from MDMA use were analyzed. We hypothesized that we would observe: (1) a dose related effect of MDMA on cognitive function, (2) impaired cognitive function in subjects with a long-term abstinence period from MDMA, and (3) that these effects are modified by the s allele of the 5-HTTLPR genotype.

Methods and Materials

Participants

Three different groups of ecstasy users were compared with ecstasy-naïve 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 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-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 et al., 1994). The 15 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 (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; 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 1982). 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.

Rejection Times

- Reaction times were tested using FePsy, an automated computerized battery of validated neuropsychological tasks (Alpherts & Aldenkamp 1994). Reaction times were evaluated separately on the non-dominant hand and dominant hand in response to simple auditory 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 learn-
Tests of attention and executive functioning

- Category fluency (Luteijn & Van der Plouw 1983). Naming animals and occupations, for 1 minute each. Score is raw number correct in 2 minutes.

- Controlled Oral Word Association Test (COWAT; Benton & 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 2 minutes.

- Corsi Block-tapping Test (Milner 1971). This test measures perceptual interference, response inhibition, and selective attention by having subject name colors, and name the color of ink of color-words when the words are printed in a non-matching colored 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 colors are shown. The task is to sort the cards according to one of three possible sorting rules (color, 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 I and s alleles were analyzed using polymerase chain reaction as described elsewhere (Lesch et al., 1996; Heils et al., 1995).

Statistical Analyses

Characteristics of the sample

Differences in continuous variables (log transformed if necessary) between the four groups were analyzed using ANOVA and Bonferroni post hoc analysis.

Differences in the prevalence of subjects carrying the s allele between MDMA users and control subjects were investigated using the Chi-square test. In addition, Pearson correlation analyses was performed between the 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 analyzed using general linear model-based MANOVA, with one between group factor (Group) and five potential covariants (age, gender, DART-IQ, 5-HTTLPR and extent of previous cannabis use). 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 was set at 0.05 using two-tailed tests of significance. In case Bonferroni corrections were made, statistical significance within the text will be reported as a corrected p value. All data were analyzed using SPSS Version 10.0 (SPSS, Inc., Chicago, Ill, USA).

Results

Characteristics of the sample

The three groups were similar for age, and gender distribution. The level of education was significantly lower in MDMA users. However, MDMA users did not
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Table 1. Demographics, prevalence of current depression and other recreational drug exposure

<table>
<thead>
<tr>
<th></th>
<th>Controls n = 13</th>
<th>MDMA n = 15</th>
<th>MDMA+ n = 22</th>
<th>ex-MDMA n = 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>25.0 (3.6)</td>
<td>24.6 (6.1)</td>
<td>26.2 (5.3)</td>
<td>25.3 (5.4)</td>
</tr>
<tr>
<td>Male/female</td>
<td>7/6</td>
<td>9/6</td>
<td>11/11</td>
<td>8/8</td>
</tr>
<tr>
<td>Education (y)</td>
<td>14.5 (1.3)</td>
<td>13.1 (2.3)</td>
<td>12.6 (2.2)</td>
<td>11.8 (2.4)</td>
</tr>
<tr>
<td>DART-IQ</td>
<td>105.8 (7.2)</td>
<td>111.6 (9.9)</td>
<td>105.2 (8.5)</td>
<td>103.9 (9.8)</td>
</tr>
</tbody>
</table>

Alcohol and other recreational drug use

<table>
<thead>
<tr>
<th></th>
<th>Controls n = 13</th>
<th>MDMA n = 15</th>
<th>MDMA+ n = 22</th>
<th>ex-MDMA n = 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol (no. joint)</td>
<td>12.7 (12.2)</td>
<td>15.4 (12.5)</td>
<td>11.8 (12.6)</td>
<td>14.8 (11.7)</td>
</tr>
<tr>
<td>Tobacco (cig./day)</td>
<td>9.3 (5.0)</td>
<td>8.8 (7.4)</td>
<td>10.2 (10.6)</td>
<td>13.0 (8.1)</td>
</tr>
<tr>
<td>Cannabis (no. joint)</td>
<td>3.6 (3.7)</td>
<td>53.0 (69.4)</td>
<td>81.7 (128.7)</td>
<td>114.2 (220.5)</td>
</tr>
<tr>
<td>Amphetamine (no. times used)</td>
<td>0.3 (0.6)</td>
<td>1.3 (3.5)</td>
<td>3.3 (6.3)</td>
<td>0</td>
</tr>
<tr>
<td>Cocaine (no. times used)</td>
<td>0</td>
<td>2.2 (2.1)</td>
<td>4.1 (3.0)</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2. Characteristics of MDMA use

<table>
<thead>
<tr>
<th></th>
<th>MDMA n = 15</th>
<th>MDMA+ n = 22</th>
<th>ex-MDMA n = 16</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of use (years)</td>
<td>4.1 (2.6)</td>
<td>4.6 (2.6)</td>
<td>4.6 (2.6)</td>
</tr>
<tr>
<td>Usual dose (tablets)</td>
<td>1.4 (0.5)</td>
<td>2.2 (0.7)</td>
<td>2.1 (1.0)</td>
</tr>
<tr>
<td>Lifetime dose (tablets)</td>
<td>28.6 (17.8)</td>
<td>485.0 (593.1)</td>
<td>268.1 (614.3)</td>
</tr>
<tr>
<td>Time since last tablet (months)</td>
<td>3.6 (5.9)</td>
<td>2.4 (2.4)</td>
<td>29.0 (20.4)</td>
</tr>
</tbody>
</table>

Significantly different from MDMA- (Bonferroni corrected p value = 0.00) and ex-MDMA group (Bonferroni corrected p value = 0.03)

was observed. Whereas recent MDMA users (moderate and heavy) were analyzed on average 2-4 months after taking their last tablet, this was 29 months for the ex-MDMA group (Table 2).

Neuropsychological testing

Table 3 represents the scores on the three main cognitive domains (reaction speed, memory function and attention/executive functioning) analyzed. 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 = 0.58). Since a significant covariance effect of DART-IQ (p = 0.00) and age (p = 0.03) was observed on main effect of Group on memory function in the MANOVA analysis, within groups comparisons were controlled for these covariants, but not for gender (p = 0.10), 5-HTTLPR genotype (p = 0.44) or extent of previous cannabis use (log transformed; p = 0.21).

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, but not moderate (p = 0.11), MDMA users recalled significantly less words (p = 0.00) when compared to controls. Ex-MDMA users also recalled significantly less words on the immediate RAVLT when compared to controls (p = 0.01). Similar observations were made on the delayed
RavLT recall: heavy, but not moderate (p = 0.20), MDMA and ex-MDMA users recalled less words (p = 0.01 and p = 0.04, respectively) as compared to controls.

Extent of previous MDMA use (log transformed) was significantly associated with immediate (r = -0.42, p = 0.00) and delayed RavLT scores (r = -0.33, p = 0.01) (Figure 1). 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).

Extent of previous cannabis use (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 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 (r = -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): 1/1, 17 (31.5%); 1/5, 29 (53.7%); s/s, 8 (14.8%). Controls and MDMA users did not differ in genotype distribution patterns (p = 0.38). Finally, corre-

### Table 3. Reaction times and cognitive performance (memory and attention)

<table>
<thead>
<tr>
<th></th>
<th>Controls n = 13</th>
<th>MDMA n = 15</th>
<th>MDMA+ n = 22</th>
<th>ex-MDMA n = 16</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median Reaction Times (msec)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.61</td>
</tr>
<tr>
<td>Auditive DH</td>
<td>242.5 (22.1)</td>
<td>246.7 (28.3)</td>
<td>245.2 (30.2)</td>
<td>244.1 (29.3)</td>
<td></td>
</tr>
<tr>
<td>Auditive NH</td>
<td>244.4 (34.6)</td>
<td>250.1 (24.1)</td>
<td>245.5 (26.8)</td>
<td>254.3 (32.3)</td>
<td></td>
</tr>
<tr>
<td>Visual DH</td>
<td>282.1 (52.2)</td>
<td>287.2 (55.2)</td>
<td>257.2 (30.7)</td>
<td>270.3 (46.6)</td>
<td></td>
</tr>
<tr>
<td>Visual NH</td>
<td>316.0 (92.8)</td>
<td>298.6 (16.2)</td>
<td>268.7 (51.7)</td>
<td>279.9 (53.6)</td>
<td></td>
</tr>
<tr>
<td>Bинаry choice task</td>
<td>352.9 (112.6)</td>
<td>368.2 (53.0)</td>
<td>353.7 (67.9)</td>
<td>358.3 (69.5)</td>
<td></td>
</tr>
<tr>
<td>Bинаry choice (total falses)</td>
<td>2.5 (3.4)</td>
<td>2.6 (3.1)</td>
<td>5.0 (7.2)</td>
<td>2.6 (1.9)</td>
<td></td>
</tr>
</tbody>
</table>

| **Memory function (total scores)** |             |             |              |                | 0.04* |
| Logical memory immediate | 17.9 (6.1)  | 16.1 (5.2)  | 17.9 (3.8)   | 16.3 (3.5)     |       |
| Logical memory delayed  | 15.3 (5.8)  | 12.7 (5.4)  | 14.4 (3.9)   | 13.8 (6.2)     |       |
| WMS immediate           | 39.4 (1.9)   | 39.2 (1.8)  | 38.4 (2.6)   | 37.7 (3.2)     |       |
| WMS delayed             | 36.4 (5.4)   | 36.2 (5.5)  | 35.6 (5.6)   | 35.9 (4.1)     |       |
| RavLT immediate          | 59.1 (7.4)   | 51.2 (8.6)  | 47.0 (6.8)   | 48.0 (12.5)    |       |
| RavLT delayed            | 13.1 (2.1)   | 10.7 (5.2)  | 9.8 (2.9)    | 10.1 (2.9)     |       |
| Corsi Block Span         | 5.2 (0.7)    | 5.7 (1.1)   | 5.6 (1.3)    | 5.7 (1.3)      |       |
| Corsi Block Span plus one| 5.6 (0.6)    | 5.9 (1.0)   | 6.0 (1.1)    | 6.0 (1.2)      |       |

| **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)    |       |
| Letter fluency (sum score)   | 44.4 (9.3)     | 41.5 (9.8)  | 41.6 (12.6)  | 39.6 (10.4)    |       |
| Stroop color (sec)           | 53.9 (9.0)     | 56.7 (10.5) | 53.2 (9.0)   | 53.5 (7.9)     |       |
| Stroop color-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 (3.2)   | 24.0 (11.6)    |       |
| Trailmaking B (sec)          | 47.9 (17.5)    | 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)  | 35.3 (19.2)    |       |
| WCST perseverations          | 19.3 (15.7)    | 15.8 (8.6)  | 19.7 (14.6)  | 15.1 (11.6)    |       |
| WCST categories              | 4.6 (1.5)      | 4.8 (1.7)   | 4.4 (1.6)    | 4.7 (2.1)      |       |

- Data are expressed in mean ± SD
- Significant group effect (MANOVA: F = 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.
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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).

Table 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).

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’. Our results indicate that higher lifetime doses of MDMA are associated with greater decrements in memory function. Lastly, our data provide no evidence for a role of 5-HTTLPR genotype in MDMA (ab)use or cognitive performance.

The present observations made in heavy users of MDMA are generally consistent with previous reports suggesting that recreational MDMA users display significant memory impairments, whereas performance on other cognitive tests is generally normal (Parrott 2000; Spatt et al., 1997; Morgan 1998; Parrott et al., 1998). Impairments have been demonstrated in immediate and delayed verbal recall (Parrott et al., 1998; Bolla et al., 1998; Morgan 1999) and in verbal working memory (Waring 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 (Vanden 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).

In agreement with previous studies we observed that greater use of MDMA is associated with greater impairment in immediate verbal memory. Interestingly, we observed that individuals who had stopped heavy use of MDMA for more than 6 months performed equally poorly on the word recall test as recent heavy MDMA users. Shum and co-workers (2000) reported on RAVLT scores of patients with similar age and educational level to our subjects who had suffered from severe traumatic brain injury (TBI) 2 years previously. Criteria for severe TBI were: a Glasgow Coma Scale < 9 or a duration of posttraumatic amnesia of more than 7 days. Scores on the immediate RAVLT were 59.4 in controls vs. 47.4 in the TBI patients, and approximately 13.8 vs. 10.8, respectively, on the delayed recall. These scores are comparable to the scores presently observed in heavy recent and ex-MDMA users, which may indicate the severity and clinical significance of the memory disturbances induced by MDMA use. However, it is likely that the memory impairments will not be noticed by the subjects themselves because the deterioration is a gradual one and extends over a long period of time. It is possible that the cognitive impairment becomes apparent only after many years when the effects of normal aging add to the possible 5-HT neurotoxic damage.

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 seven years after MDMA treatment, particularly prominent in the hippocampus (Hatzidimitriou et al., 1999). To our knowledge no study has previously investigated the effects of long-term MDMA abstinence on memory function.

With respect to moderate MDMA users, contrary to findings in previous studies in which 'novice' MDMA users (Parrott et al., 1998; Bhattachary & Powell 2001) demonstrated verbal memory deficits, we did not observe memory deficits in moderate users of this drug. As discussed above, presently only subjects with high MDMA exposure (> 55 tablets, on average 530 tablets lifetime) were found to have memory deficits. 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 (1998). Alternatively, subjects in the previous mentioned studies may have used extremely high doses of MDMA, causing brain 5-HT neurotoxicity 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 line with this, we previously observed dose-related decreases in memory function. 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-HT2A receptors (presumably reflecting lower synaptic 5-HT levels) 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. It was 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 MDMA users and MDMA-naive subjects (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 (Hoeh e 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. 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. However, because of our small sample size, the preliminary findings remain to be proven.

We previously reported gender differences in the neurotoxic effects of MDMA (Reneman et al., 2001a), since greater reductions in 5-HT transporter densities were observed in female than in male MDMA users, suggesting that females are more susceptible than males to the neurotoxic effects of MDMA. In line with this, a recent study suggested a more pronounced subjective response to MDMA in females compared to males (Liechti et al., 2001). In addition, McCann and co-workers (1994) observed greater reductions in 5-HIAA in female than in male MDMA users, suggesting that females may be more susceptible than males to the neurotoxic effects of MDMA. In contrast to this, we presently did not observe differences between males and females in the effects of MDMA on memory function. Previous studies have failed to investigate the effect of gender on memory function in MDMA using subjects, or not observed an effect. However, Bolla and colleagues (1998) reported that females were less susceptible than males to MDMA dose-related decreases 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 (Croft et al., 2001; Rodgers 2000). However, the adverse effects of long-term cannabis use on cognitive skills have not been clearly demonstrated in the literature (Fletcher et al., 1996). For instance, Gouzoulis-Mayfrank and co-workers (2000) did not observe differences in cognitive performance between cannabis users and ecstasy users or controls, whereas Croft and co-workers (2001) observed no difference in cognitive functioning between combined cannabis and MDMA users as compared to sole cannabis users. 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 (Bhattachary & 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 (including MDMA) 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. Recently a longitudinal study was published (Zakzanis & Young 2001) in which memory function was assessed over the period of 1 year in 15 current users of MDMA. Continued use of MDMA was associated with a progressive decline in terms of immediate and delayed recall of a short passage (logical memory).

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 as 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 for more than one year 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.

Acknowledgements

The authors wish to thank Stefanie Jansen for carrying out the neuropsychological assessments.

References


Alpherts W, Aldenkamp A. FEPSY The non Psycho 5 OA. Instituut voor Epileptobestrijding, Heemstede, the Netherlands. 1999.


Bhattachary S, Powell JH. Recreational use of 3,4-methylenedioxymethamphetamine (MDMA) or 'ecstasy': evidence for cognitive impairment. Psychol Med 2001; 31(6): 647-658.


Commins DL, Vosmer G, Virus RM, Woollerton WL, Schuster CR, Selden, LS. Biochemical and histological evidence that methylenedioxymethy-
Part III | Potential functional consequences of MDMA-induced neuronal loss

Lamphetamine (MDMA) is toxic to neurons in the rat brain. J Pharmacol Exp Ther 1987; 241: 338-345.


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

Morgan MJ. Memory deficits associated with recreational use of "ecstasy". (MDMA). Psychopharmacology 1999; 141: 30-36.


Investigating the potential neurotoxicity of Ecstasy (MDMA): An imaging approach


