Investigating the potential neurotoxicity of ecstasy (MDMA). An imaging approach
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Chapter 9.1

Cortical serotonin transporter density and verbal memory in individuals who stopped using 3,4-methylenedioxymethamphetamine (MDMA or “Ecstasy”) - Preliminary findings

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Abstract

**Background** - Although the popular recreational drug 3,4-methylenedioxyamphetamine (MDMA or “ecstasy”) has been shown to damage brain serotonin (5-HT) neurons in animals, the fate and functional consequences of 5-HT neurons after MDMA injury are not known in humans. We investigated the long-term effects of MDMA use on cortical 5-HT neurons in humans and memory function, because brain 5-HT emission tomography and single-photon emission computed tomography (SPECT), it is now possible to measure SERT densities in human brain. Recent imaging studies have shown decreases in central SERTs in MDMA-treated primates and human MDMA users (Scheffel et al., 1998; McCann et al., 1998; Semple et al., 1999).

Few functional consequences of MDMA-induced neurotoxicity have been identified, however, either in animals or in humans. Since MDMA-induced 5-HT neurotoxic damage may lead to impairment of functions in which 5-HT is involved (eg, memory function), it is important to study the effects of MDMA not only on 5-HT neurons but on memory function as well. Memory function is of particular interest because 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).

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 nonhuman 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. Therefore, it is of particular interest to establish the long-term fate of cortical 5-HT neurons after MDMA injury in the human brain.

The development of iodine-123-2β-carbomethoxy-3β-(4-iodophenyl)tropane ([^123]Iβ-CIT), a radioligand that binds with high affinity to SERTs, has made it possible to assess the density of SERT in the living human brain, by means of SPECT (Pirker et al., 1995; Scheffel et al., 1992).

The purpose of the present study was to investigate the density of cortical[^123]Iβ-CIT-labeled SERTS and memory function in recent MDMA users who were abstaining from use, and MDMA users who had not been using MDMA for more than 1 year. Also, this study examined whether possible memory deficits in MDMA users correlate with decrements in the density of[^123]Iβ-CIT labeled SERTs, and whether memory deficits in MDMA users are dose related.

**Methods**

Twenty-two recent MDMA users, 16 ex-MDMA users who had stopped using MDMA for more than 1 year, and 13 controls were enrolled. The effects of MDMA use on cortical 5-HT neurons was studied by means of single-photon emission computed tomography with iodine-123-labeled 2β-carbomethoxy-3β-(4-iodophenyl)tropane ([^123]Iβ-CIT) by quantification of brain 5-HT densities. Verbal memory performance was assessed with the Rey Auditory Verbal Learning Test. Results - Mean cortical[^123]Iβ-CIT-labeled 5-HT transporter density was significantly lower in recent MDMA users than in controls (1.17 vs. 1.28 [-9%]) but not in ex-MDMA users (1.24 vs. 1.28 [-3%]). Recent and ex-MDMA users recalled significantly fewer words than did controls on the immediate recall (47.0 and 48.0 vs. 60.0, respectively; p = 0.001) as well as the delayed recall (9.8 and 10.1 vs. 13.1, respectively; p = 0.003). Greater use of MDMA was associated with greater impairment in immediate verbal memory. However, memory performance was not associated with[^123]Iβ-CIT binding to cortical 5-HT transporters or duration of abstinence from MDMA.

**Conclusion** - The present study suggests that, while the neurotoxic effects of MDMA on 5-HT neurons in the human cortex may be reversible, the effects of MDMA on memory function may be long-lasting.

**Introduction**

Although generally regarded as relatively safe, the popular recreational drug 3,4-methylenedioxyamphetamine MDMA ("Ecstasy") has increasingly been shown to lead to toxic effects on brain serotonin (5-HT) neurons in animals and possibly in humans. 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 (SERTs) (Schmidt et al., 1986; 1987; Stone et al., 1986; Commins et al., 1987; Battaglia et al., 1988). Since the SERT is located on the pre-synaptic axons and axon terminals of 5-HT neurons, it is considered to be a reliable marker of 5-HT neurotoxic changes. With the development of imaging techniques such as positron emission tomography and single-photon emission computed tomography (SPECT), it is now possible to measure SERT densities in human brain. Recent imaging studies have shown decreases in central SERTs in MDMA-treated primates and human MDMA users (Scheffel et al., 1998; McCann et al., 1998; Semple et al., 1999).

Few functional consequences of MDMA-induced neurotoxicity have been identified, however, either in animals or in humans. Since MDMA-induced 5-HT neurotoxic damage may lead to impairment of functions in which 5-HT is involved (eg, memory function), it is important to study the effects of MDMA not only on 5-HT neurons but on memory function as well. Memory function is of particular interest because 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).

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 nonhuman 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. Therefore, it is of particular interest to establish the long-term fate of cortical 5-HT neurons after MDMA injury in the human brain.

The development of iodine-123-2β-carbomethoxy-3β-(4-iodophenyl)tropane ([^123]Iβ-CIT), a radioligand that binds with high affinity to SERTs, has made it possible to assess the density of SERT in the living human brain, by means of SPECT (Pirker et al., 1995; Scheffel et al., 1992).

The purpose of the present study was to investigate the density of cortical[^123]Iβ-CIT-labeled SERTS and memory function in recent MDMA users who were abstaining from use, and MDMA users who had not been using MDMA for more than 1 year. Also, this study examined whether possible memory deficits in MDMA users correlate with decrements in the density of[^123]Iβ-CIT labeled SERTs, and whether memory deficits in MDMA users are dose related.
Investigating the potential neurotoxicity of Ecstasy (MDMA): An imaging approach

reduction information and advice. Experimental and control groups were thus recruited from the same community sources. Subjects selected were group-matched for sex and age (between 18 and 45 years), otherwise healthy, and with no psychiatric history.

Twenty-two recent but abstinent ecstasy users (mean ±SD) time since last dose before study: 2.4 ± 2.4; "MDMA group"), and 16 ex-ecstasy users (29.0 ± 20.4 months; "ex-MDMA group") were recruited. The eligibility criterion for the MDMA group was lifetime previous use of a minimum of 50 tablets of ecstasy. The ex-MDMA group had to have taken a minimum of 50 tablets but stopped using ecstasy for at least 1 year before the study. The 13 controls were healthy subjects with no self-reported previous use of ecstasy.

All participants agreed to abstain from use of psychoactive drugs (including MDMA) for at least 3 weeks before the study and were asked to undergo urine drug screening to assess current exposure to psychoactive drugs (with an enzyme-multiplied immunoassay for amphetamines, barbiturates, benzodiazepine metabolites, cocaine and metabolite, opiates, and marijuana) before enrollment. After urine samples were tested, exclusion criteria were as follows: a positive drug screen, pregnancy, a severe medical or neuropsychiatric illness that precluded informed consent, and a lifetime psychiatric disorder. Use of prescribed psychotropic medications, such as 5-HT reuptake inhibitors, had to be stopped for at least 3 weeks before the study. Subjects were interviewed with the computer-assisted 2.1. version of the Composite International Diagnostic Interview (Core version 2.1. 1997; World Health Organization, Geneva, Switzerland) to screen for current DSM-IV Axis I diagnoses.

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. All participants provided written informed consent after the study was completely described to them.

Imaging

Subjects underwent SPECT imaging (810X tomographic equipment; Strichman Medical Equipment Inc, Medfield, Mass.). This 12-detector single-slice scanner has a full-width at half-maximum resolution of approximately 7.5 mm. Each acquisition consisted of approximately 15 slices (acquired in a 64 x 64 matrix) at 3 minutes per slice (interslice distance, 5 mm). The energy window was set at 135-190 keV. Subjects lay supine with the head parallel to the orbito-meatal line. Acquisition was commenced 4 hours after intravenous injection of approximately 3.8 mCi (140 MBq) of [11]beta-CIT (specific activity, >5 mCi/nmol [>185 MBq/nmol]; radiochemical purity, >98%), a time when specific binding to SERTs is stable (Laruelle et al., 1994). Reconstruction and attenuation correction of all images were performed as earlier described (Booj et al., 1999).

For binding analysis, a standard template with regions of interest was constructed manually from magnetic resonance images. For positioning we used these images as a guide. A template, including regions of interest for the frontal, temporal, parieto-occipital- and occipital cortex, was placed on 3 consecutive SPECT slices, demonstrating best visualization of the striatum (typically 30 mm above the orbito-meatal line), by an investigator unaware of the participant's history. An additional template was constructed with a region of interest for the cerebellum. The binding in the cerebellum, presumed free from SERTs, was used as a reference for background radioactivity (non-specific binding + free ligand). Since no differences in [11]beta-CIT uptake ratios between cortical brain regions were observed in all groups under study, we calculated mean cortical SERT densities (mean counts per pixel of frontal, temporal, parieto-occipital, and occipital cortex). Cortical binding ratios were calculated as cortical binding divided binding in the cerebellum.

Memory testing

The Dutch Adult Reading Test (DART) (Schmand et al., 1992; Bouma et al., 1996) was administered as an estimate of verbal intelligence. The DART is the Dutch adaptation of the National Adult Reading Test (NART; Nelson 1991), a short reading test for the estimation of premorbid verbal IQ.

Memory was assessed within 1 day from SPECT imaging by means of the Rey Auditory Verbal Learning Test (RAVLT; Van den Burg et al., 1985). The subject memorizes a series of 15 words in 5 learning trials (RAVLT immediate recall). After a 20-minute delay, the subject is asked to recall the words (RAVLT delayed recall), followed by recognition of the 15 items between 15 distractor words (RAVLT recognition). Raw scores are used.

Statistical Analyses

Differences in mean cortical [11]beta-CIT binding ratio and RAVLT scores (RAVLT immediate recall, RAVLT delayed recall, and RAVLT recognition) were analyzed by analysis of covariance, with 1 between-group factor (group) and 3 covariants (age, sex and DART IQ). When a significant main group effect was observed,
Bonferroni post hoc tests were performed to analyze differences between groups. Differences between the 3 groups with regard to demographic variables and other drug exposure were analyzed by analysis of variance. Differences in characteristics of MDMA use between both MDMA-using groups were studied with the t test.

Pearson correlation analyses were performed between RAVLT scores and mean cortical [123]β-CIT binding ratio, between RAVLT scores and duration of abstinence from MDMA, between RAVLT scores and extent of previous MDMA, and between RAVLT scores and extent of previous cannabis, amphetamine, and cocaine use. Because age, sex and vocabulary have been shown to be highly associated with most memory tests, we also performed partial correlations to control for age, sex and DART IQ on those tests for which the correlations were significant. The chance of a type I error (α) was set at 0.05 by 2-tailed tests of significance. In cases Bonferroni corrections were made, statistical significance within the text is reported as a corrected P (corrected p = 0.017 [0.05 +3] [3 paired comparisons]). All data were analyzed using SPSS version 9.0 (SPSS Inc, Chicago, Ill) and are presented as mean±SD unless otherwise indicated.

Results

Characteristics of the sample

The 3 groups were similar for age and sex distribution. The level of education was significantly lower in MDMA users. However, MDMA users did not differ from controls in verbal intelligence (DART IQ) (Table).

Apart from the anticipated differences between groups caused by inclusion criteria, no significant differences between MDMA groups were observed. Whereas the MDMA group had on average not used MDMA for months, the ex-MDMA group had on average not used MDMA for nearly 2.5 years (Table). Recreational drug use of alcohol and tobacco was comparable between the different groups. MDMA users indicated having used more cannabis in the year

### Table 1. Characteristics of Subjects

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>MDMA</th>
<th>Ex-MDMA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 13</td>
<td>n = 22</td>
<td>n = 16</td>
</tr>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>25.0 (3.6)</td>
<td>26.2 (5.3)</td>
<td>25.3 (5.4)</td>
</tr>
<tr>
<td>Men/women</td>
<td>7/6</td>
<td>11/11</td>
<td>8/8</td>
</tr>
<tr>
<td>Years of education</td>
<td>14.5 (1.3)</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>105.2 (8.5)</td>
<td>103.9 (9.8)</td>
</tr>
<tr>
<td>MDMA use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of use (years)</td>
<td>NA</td>
<td>5.5 (2.7)</td>
<td>4.6 (2.6)</td>
</tr>
<tr>
<td>Usual dose (no. tablets/occasion)</td>
<td>NA</td>
<td>2.2 (0.7)</td>
<td>2.1 (1.0)</td>
</tr>
<tr>
<td>Lifetime dose (tablets)</td>
<td>NA</td>
<td>485 (598)</td>
<td>268 (614)</td>
</tr>
<tr>
<td>Time since last tablet (months)</td>
<td>NA</td>
<td>2.4 (2.4)</td>
<td>29.0 (20.4)</td>
</tr>
<tr>
<td>Use of other drugs in the past year</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol (no. alcoholic consumptions)</td>
<td>478.8 (452.0)</td>
<td>490.5 (372.9)</td>
<td>323.7 (256.4)</td>
</tr>
<tr>
<td>Tobacco (no. cigarettes)</td>
<td>3590.4 (1927.7)</td>
<td>3302.4 (3857.6)</td>
<td>4572.5 (2996.3)</td>
</tr>
<tr>
<td>Cannabis (no. joints)</td>
<td>15.3 (16.0)</td>
<td>326.9 (514.9)</td>
<td>456.7 (881.9)</td>
</tr>
<tr>
<td>Amphetamine (no. times used)</td>
<td>0</td>
<td>12.8 (18.7)*</td>
<td>–</td>
</tr>
<tr>
<td>Cocaine (no. times used)</td>
<td>0.07 (0.28)</td>
<td>7.2 (6.4)*</td>
<td>1.5 (3.3)</td>
</tr>
<tr>
<td>LSD (no. times used)</td>
<td>0</td>
<td>1.9 (1.5)</td>
<td>3.3 (12.9)</td>
</tr>
<tr>
<td>Psilocybin (no. times used)</td>
<td>0.08 (0.28)</td>
<td>1.5 (2.3)</td>
<td>2.9 (9.2)</td>
</tr>
</tbody>
</table>

1 Data are expressed in mean ± SD values

2 Significant lower level of education in MDMA users compared to controls (ANOVA: F = 6.5, df = 10, p = 0.003, post hoc analysis MDMA group vs control group: Bonferroni corrected p value = 0.034, ex-MDMA group vs control group p = 0.003)

3 Significant longer time since last dose in ex-MDMA group compared to MDMA group (Student t test: t = -6.1, df = 36, p < 0.000)

4 Significant more amphetamine consumption in the past year in MDMA users compared to controls and ex-MDMA users (ANOVA: F = 5.9, df = 50, p = 0.003, post hoc analysis MDMA group vs control group: Bonferroni corrected p value = 0.023, MDMA group vs ex-MDMA group p = 0.0014)

5 Significant more cocaine consumption in the past year in MDMA users compared to controls and ex-MDMA users (ANOVA: F = 11.8, df = 50, p = 0.003, post hoc analysis MDMA group vs control group: Bonferroni corrected p value = 0.000, MDMA group vs ex-MDMA group p = 0.002)
before this investigation than had controls, although this difference did not reach statistical significance. The use of amphetamine and cocaine was significantly higher in the MDMA group compared to the control and ex-MDMA group (Table). None of the subjects under study reported using drugs other than the ones listed in the Table, such as for instance phencyclidine or opiates, in the year before this study.

**Imaging**

No differences in $[^{11}]\beta$-CIT uptake between cortical brain regions were observed in all groups under study. Analysis of variance showed a significant main effect of group ($F_{2.5} = 4.63, p = 0.02$), with current MDMA having lower (-5%) mean cortical $[^{11}]\beta$-CIT binding ratios than controls (Figure 1). No significant differences in mean cortical binding ratios of ex-MDMA users were observed when compared with controls subjects (-3%; Figure 1).

**Memory performance**

As with mean cortical $[^{11}]\beta$-CIT binding ratios, analysis of covariance showed a significant main effect of group on RAVLT immediate recall scores ($F_{2.5} = 8.31, p = 0.001$). Both current and ex-MDMA users recalled significantly fewer words on the immediate RAVLT compared with controls ($47.0 \pm 8.6$ and $48.0 \pm 12.5$ vs $60.0 \pm 6.8$, respectively; Figure 2). Similar findings were observed for the RAVLT delayed recall ($F_{2.5} = 6.53, p = 0.003; 9.8 \pm 2.9$ and $10.1 \pm 2.9$ vs $13.1 \pm 2.1$, respectively, Figure 2), but not for RAVLT recognition. Correlation analysis demonstrated no specific relationships between RAVLT scores and cortical binding ratio, duration of abstinence from MDMA, or extent of previous cannabis, amphetamine, or cocaine use. However, partial correlation analysis with RAVLT immediate recall scores was significant for extent of previous MDMA use ($r_{1.6} = -0.29, p = 0.049$).

**Comment**

The present study indicates that, in individuals who stopped using MDMA more than 1 year ago, cortical SERT densities did not differ from those of control subjects, whereas recent MDMA users showed global decreases in SERTs. Our findings also indicate that individuals who stopped using MDMA had a deficit in verbal memory, similar to that of current MDMA users, and that higher lifetime doses of MDMA are associated with greater decrements in immediate verbal memory function.

The observed decreases in cortical SERT densities in recent MDMA users most likely reflects MDMA-induced brain 5-HT neurotoxic effects, since reductions in SERT densities have been documented in animals with known MDMA-induced 5-HT injury (Commins et al., 1987; Scheffel et al., 1998; O'Hearn et al., 1988; Hatzidimitriou et al., 1999; Fischer et al., 1995; Battaglia et al., 1987; Ricaurte et al., 1988; 1992; Insel et al., 1989). For instance, reductions in 5-HT axons in MDMA-treated monkeys vary from approximately -95% in the temporal cortex to -83% in the pyriform cortex (Hatzidimitriou et al., 1999). This is a much stronger effect than on human 5-HT axons as observed in the present study (on the order of 9%). Interestingly, Semple and coworkers also reported a 10% reduction in SERT densities in the occipital cortex of recent MDMA users by means of $[^{11}]\beta$-CIT SPECT (Semple et al., 1999). Even though cortical $\beta$-CIT uptake is low, displacement studies in rats and monkeys have shown that cortical uptake of $\beta$-CIT is associated with SERTs (Scheffel et al., 1992; Farde et al., 1994). Furthermore, $[^{11}]\beta$-CIT has been
shown to adequately detect changes in cortical as well as subcortical SERT densities secondary to 5-HT neurotoxic effects (Scheffel et al., 1992; Lew et al., 1996). However, it is an assumption that a decrease in SERT density directly reflects axonal loss. Several factors, such as allosteric changes in the actual binding unit of the protein, also could result in decreased binding. Nevertheless, it has been shown that central 5-HT levels also are reduced after MDMA treatment (Sabol et al., 1996). Furthermore, correlating post-mortem studies indicate that loss of pre-synaptic SERTs in MDMA-treated animals is related to damage of 5-HT axons and axon terminals (Commens et al., 1987; Fischer et al., 1995; Battaglia et al., 1987).

The absence of decreases in SERT densities in ex-MDMA users suggests reversibility of MDMA-induced changes in brain SERTs in MDMA users. In line with this, autoradiographic studies have shown a partial recovery of [125I]-β-CIT binding 16 weeks after lesion induction in the frontal cortex of rats, and complete recovery by 32 weeks (Lew et al., 1996). In nonhuman primates, cortical 5-HT terminal markers remain decreased up to 7 years after MDMA treatment, although significant recovery occurs compared with 2 weeks after the lesion induction (Hatzidimitriou et al., 1999).

Our findings of memory impairment in recent MDMA users are consistent with those of previous reports (Krystal et al., 1992; Parrott et al., 1998; Parrott 2000; Bolla et al., 1998; Curran & Travill, 1997; Gouzoulis-Mayfrank et al., 2000; Morgan 1999). In agreement with these studies, we observed that greater use of MDMA is associated with greater impairment in immediate verbal memory. Interestingly, Shum and coworkers (2000) reported on RAVLT scores of patients with age and educational level similar to those of our subjects who had suffered from severe traumatic brain injury 2 years previously. Criteria for severe traumatic brain injury were a Glasgow Coma Scale less than 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 patients with traumatic brain injury, and approximately 13.8 vs 10.8, respectively, on the delayed recall. These scores are comparable to the scores observed in this study in recent and ex-MDMA users (Figure 2), which may indicate the severity and clinical significance of the memory disturbances induced by MDMA use.

Studies in rats and monkeys have shown that MDMA produces serotonergic neurodegeneration in various brain areas important for memory function, including the hippocampus (Hatzidimitriou et al., 1999).
1999). Unfortunately, because of the relative low resolution of the SPECT imaging technique, it was not possible to study all brain regions implicated in learning and memory.

Several studies suggest that SERTs may play an important role in cognitive processes such as memory function (Meneses 1999). It has been shown that selective 5-HT reuptake inhibitors in nondoned elderly depressed patients improved both mood and cognitive function (Meltzer et al., 1998). However, we did not observe a correlation between memory function and cortical SERT densities. It could be argued that memory testing is more sensitive to MDMA’s neurotoxic effects than are SERT densities are. Furthermore, the observed memory deficits in ex-MDMA users may not be attributable to MDMA-induced 5-HT deficits at all. However, we did observe a negative correlation between extent of previous MDMA use and immediate verbal memory recall, suggesting that immediate verbal memory deficits may be at least partially attributable to MDMA use. Another explanation may be that SERT densities in brain areas implicated in learning and memory of former MDMA users are still decreased but could, unfortunately, not be visualized using SPECT (eg, hippocampus or hypothalamus). Finally, although loss of SERTs is indicative of neuronal degeneration, their restoration does not necessarily imply normal axonal or neuronal regeneration and therefore normal behavioral recovery. For instance, after 5-HT axonal degeneration induced by 5,6-dihydroxytryptamine, abnormal reinnervation patterns of 5-HT axons coincide with the return of tritiated 5-HT uptake (Bjorklund et al., 1973). Since it was previously observed by our group that mean (postsynaptic) cortical 5-HT2A receptor binding positively correlated with RAVLT recall in MDMA users (Reneman et al., 2001), it could be hypothesized that functional consequences of MDMA-induced brain 5-HT neurotoxic lesions may be related to postsynaptic rather than presynaptic 5-HT neurons.

The implications of our findings are relevant to people who use MDMA. In the present study we identified that MDMA use is not only associated with short-term consequences (5-HT neurotoxicity and memory impairment), but long-term consequences as well (memory impairment). These findings will provide a cogent argument for consumers to make informed decisions about recreational drug use.

In addition, since the consequences of loss of the “serotonergic” reserve in later life is difficult to predict but could be clinically significant, the present study indicates the necessity, and would probably jus-

ify, prospective studies of psychiatric morbidity in MDMA users to foresee future demands on healthcare. Furthermore, the present study of MDMA-exposed individuals with highly selective brain SERT deficits, adds to our knowledge about a neurotransmission system thought to be involved in the etiology and treatment of very common psychiatric illnesses, such as depression.

Several potential limitations of the current study should be mentioned. First, as with all retrospective studies, there is a possibility that preexisting differences between MDMA users and controls underlie differences in SERT densities. People with low SERT densities may be predisposed to use MDMA and to have low SERT densities and/or lower performance on memory tests. Future studies taking the recently described functional polymorphism in the promoter for the SERT gene into account could of interest (Lesch et al., 1996). Second, observed decreases in brain [3H]β-CIT labeled SERT densities and memory performance are unlikely to be caused by immediate pharmacological effects of MDMA or other drugs, since MDMA-using participants reported that they had abstained from use of MDMA or other psychoactive drugs for at least 3 weeks before the study. Unfortunately, we were not able to ensure abstinence from MDMA for more than 1 year in the ex-MDMA users. In future studies, hair-sample analysis may be useful to ascertain long periods of abstinence from MDMA. Third, follow-up studies in human subjects with known MDMA-induced neurotoxic effects need to be conducted to allow definite conclusions on reversibility or permanence of MDMA-induced changes in the human brain. Finally, although the MDMA users in our study had more experience with other recreational drugs than did control subjects, none of the drugs is a known 5-HT neurotoxin in human beings, and they were therefore not likely to account for changes in SERTs or memory performance. In addition, since recent MDMA users had used significantly more amphetamine and cocaine compared than controls and ex-MDMA users, but memory impairments were observed in both recent and ex-MDMA users, amphetamine and cocaine are not likely to account for changes in SERTs and/or memory performance. In support of this, we did not observe an association between RAVLT scores and extent of previous amphetamine or cocaine use. Furthermore, since no statistical differences in the use of LSD (lysergic acid diethylamide) and psilocybin were observed between the three groups under study, it seems unlikely that the findings of the present study
should be attributed to substances other than MDMA. We cannot, however, completely rule out the possibility that the observed memory impairment in the MDMA-using subjects is unrelated to cannabis use. However, no association between RAVLT scores and extent of previous cannabis use was observed. In addition, the adverse effects of long-term cannabis use on cognitive skills have not been clearly demonstrated in the literature and seem to contradict each other (Fletcher et al., 1996). For instance, Gouzoulis-Mayfrank and coworkers did not observe differences in cognitive performance between cannabis users and ecstasy users or control subjects (Gouzoulis-Mayfrank et al., 2000).

In summary, our data suggest that MDMA use can lead to neurotoxic changes in human cortical 5-HT brain neurons and that these changes may be reversible. However, our data also suggest that the functional consequences of MDMA on cortical brain 5-HT neurons may not be reversible because individuals who had stopped using MDMA more than 1 year earlier had impaired memory function, similar to that of recent MDMA users.

References

Battaglia G, Yeh SY, O’Hearn E, Molliver ME, Kuchar MJ, De Souza EB. 3,4-Methylenedioxymethylamphetamine and 3,4-methylenedioxymethamphetamine destroy serotonin terminals in rat brain: quantification of neurodegeneration by measurement of [H]paroxetine-labeled serotonin uptake sites. J Pharmacol Exp Ther 1987; 241: 33s.


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

Morgan MJ. Memory deficits associated with recreational use of 'Ecstasy' (MDMA). Psychopharmacology 1993; 141: 30-36.


Chapter 9.2

Prefrontal N-acetylaspartate is strongly associated with memory performance in (abstinent) Ecstasy users: Preliminary report

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Abstract

Background – 3,4-methylenedioxymethamphetamine (MDMA or “Ecstasy”) is known to damage brain serotonin neurons in animals and possibly in humans. Because serotonergic damage may adversely affect memory, we compared verbal memory function between MDMA users and MDMA-naïve control subjects, and evaluated the relationship between verbal memory function and neuronal dysfunction in the MDMA users.

Methods – An auditory verbal memory task (Rey Auditory Verbal Learning Test) was used to study eight abstinent MDMA users and seven control subjects. In addition, MR’s was used in different brain regions of all MDMA users to measure N-acetyl aspartate/creatinine (NAA/Cr) ratios, a marker for neuronal viability. Results – The MDMA users recalled significantly fewer words than control subjects on delayed (p = 0.03) but not immediate recall (p = 0.08). In MDMA users, delayed memory function was strongly associated with NAA/Cr only in the prefrontal cortex (R² = 0.76, p = 0.01). Conclusions – Greater decrements in memory function predicted lower NAA/Cr levels and by inference greater neuronal dysfunction in the prefrontal cortex of MDMA users.

Introduction

Although generally perceived by the public as 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 (Boote et al., 2000) at doses that approach or overlap those used by humans. Recent studies suggest that MDMA may also be neurotoxic to the human brain. For instance, recent positron emission tomography (PET) studies have shown decreases in a structural component of 5-HT neurons, similar to those observed in MDMA-treated monkeys (McCann et al., 1998; Scheffel et al., 1998).

5-HT neurotoxic damage induced by MDMA may lead to impairment of functions in which 5-HT is involved (such as memory and other cognitive functions); however, few functional consequences of MDMA-induced neurotoxicity have been identified. Memory function is of particular interest because several studies have found that recreational MDMA users display significant memory impairments (Bolla et al., 1998) and because the hippocampal formation may be particularly vulnerable to MDMA’s neurotoxic effects (Hatzidimitriou et al., 1999).

The reduction of the amino acid N-acetylaspartate (NAA) detected by proton magnetic resonance spectroscopy (¹H MRS) represents a robust but unspecific marker for neuronal loss or dysfunction (Urenjak et al., 1993). In addition to NAA, creatine/phosphocreatine (Cr) can be assessed. Determining NAA changes in relation to Cr is commonly employed and apparently valid, because Cr remains stable in a variety of brain diseases.

The purpose of our study was to determine whether memory deficits exist in MDMA users and, if they do, whether memory deficits correlate with regionally specific NAA/Cr ratios. We obtained NAA/Cr ratios in the prefrontal cortex, occipital gray matter and tempo-parietal white matter, because PET studies have shown that the bilateral prefrontal cortex is the site of maximal increase in cerebral blood flow during execution of auditory verbal memory tasks (Grasby et al., 1993), such as those used in the present study. Furthermore, although MDMA is known to induce 5-HT neurotoxicity in cortical gray matter, cortical white matter remains relatively unaffected (Scheffel et al., 1998).

With this in mind, we hypothesized that MDMA users would perform worse on a memory test than MDMA-naïve subjects, and that memory impairments in MDMA users would only predict NAA/Cr ratios in brain regions known to be affected by MDMA and involved in auditory verbal memory tasks. Thus, we expected that memory deficits in MDMA users would only be associated with decrements in NAA/Cr of the prefrontal cortex.

Methods and Materials

Subjects

Eight male subjects with a history of MDMA use participated in the study. Subjects were recruited with flyers distributed at venues associated with the “rave scene” in Amsterdam. For a comparison group, we used our Rey Auditory Verbal Learning Test (RAVLT) database of seven male MDMA-naïve control subjects matched for age, gender and IQ. The same inclusion and exclusion criteria were applied to all control subjects. A detailed drug history questionnaire was obtained from all subjects. All participants agreed to abstain from use of psychoactive drugs for at least 1 week before the study and were asked to undergo urine drug screening with an enzyme-multiplied immunoassay before enrollment. Exclusion criteria were a positive drug screen and a lifetime psychiatric disorder (obtained with Composite International Diagnostic Interview, version 2.1). The institutional medical ethics committee approved the study. After a complete description of the study to the subjects,
Investigating the potential neurotoxicity of Ecstasy (MDMA): An imaging approach

written informed consent was obtained from all.

Assessments

Brain $^1$H spectroscopy was performed on a 1.5 T Signa Echo Speed (General Electric Medical Systems, Milwaukee, WI). Multislice coronal fast spin-echo T2-weighted images (TE/TR, 97/4000 msec; 5 mm slice thickness; 23 cm field of view; 256 x 256 matrix) were obtained. Voxel size was 4.5 cm$^3$ and chosen carefully to ensure that each voxel primarily contained gray or white matter and placed consistently across subjects. Data were acquired using a fully automated execution of PROBE (Proton Brain examination), developed to automatically and reliably acquire and process single-voxel proton spectra, as described elsewhere (Webb et al., 1994). The PRESS sequence was optimized for the chosen echotimes and locations (TE/TR, 35/3000 msec; 128 averages). We computed NAA/Cr ratios from the fitted peak integrals.

Memory was assessed in the MDMA users within 1 day from the $^1$H MRS study using the RAVLT, a verbal memory test. It comprised immediate and delayed recall. In addition, the Dutch adaptation of the National Adult Reading Test (DART) was administered as an estimate of verbal intelligence (Schmand et al., 1992).

Statistical analysis

Differences between both groups with regard to demographic variables and other drug exposure were analyzed using Student $t$-test. Differences in RAVLT scores were analyzed using ANCOVA, with one between group factor (Group) and two covariants (age and DART-IQ). To examine the relation between NAA/Cr ratios and memory performance, we used linear regression analysis with both immediate and delayed recall as independent variables and NAA/Cr ratios obtained in the three different brain regions as dependent variables. In addition, the relation between RAVLT scores and extent of previous MDMA and cannabis use was assessed using linear regression. To correct for multiple comparisons in the multiple regression analysis, $p < 0.017$ was taken to be significant [0.05 $\div$ 3: 3 brain regions studied].

Results

The two groups were similar in age. The level of education was significantly lower in MDMA users; however, verbal intelligence (DART-IQ) was similar between MDMA users and control subjects (Table 1). The use of drugs other than MDMA was higher in MDMA users compared with control subjects, although not statistically different (Table 1). Three MDMA users reported incidental use of cocaine and psilocybin in the 3 months prior to this study.

The MDMA users recalled a significantly lower number of words compared with the MDMA-naive control subjects on the delayed (10.6 [SD: 2.0] vs. 12.8 [1.9], respectively; $F = 6.67$, df = 1, $p = 0.03$), but not on the immediate recall (45.8 [SD: 9.3] vs. 53.8 [6.6], respectively; $F = 3.71$, df = 1, $p = 0.08$).

Within the group of MDMA users, NAA/Cr ratios in the prefrontal cortex were highly associated with delayed recall ($B = 0.16$, $SE = 0.04$, $R^2 = 0.26$, $p = 0.013$; Figure 1) but not with immediate recall ($B = 0.01$, $SE = 0.009$, $R^2 = 0.06$, $p = 0.237$). In contrast, no associations were observed between RAVLT scores and NAA/Cr ratios in midoccipital gray matter (delayed recall: $B = 0.08$, $SE = 0.06$, $R^2 = 0.25$, $p = 0.238$; immediate recall: $B = 0.01$, $SE = 0.013$, $R^2 = 0.25$, $p = 0.573$), or parietal white matter (delayed recall: $B = 0.02$, $SE = 0.06$, $R^2 = 0.30$, $p = 0.748$; immediate recall: $B = 0.01$, $SE = 0.012$, $R^2 = 0.30$, $p = 0.402$).

Extent of previous MDMA use was significantly associated with delayed recall ($B = -0.01$, $SE = 0.001$, $p = 0.047$), but not immediate recall ($B = -0.01$, $SE = 0.003$, $p = 0.208$). In contrast, the extent of previous cannabis use was not significantly associated with either immediate or delayed verbal memory function.

### Table 1. Demographics, characteristics of MDMA use and other recreational drug exposure in control subjects and MDMA users

<table>
<thead>
<tr>
<th></th>
<th>Control subjects</th>
<th>MDMA users</th>
</tr>
</thead>
<tbody>
<tr>
<td>$n$</td>
<td>$7$</td>
<td>$8$</td>
</tr>
<tr>
<td>Age</td>
<td>29.3 (6.9)</td>
<td>28.3 (7.0)</td>
</tr>
<tr>
<td>Education (years)</td>
<td>15.0 (1.1)</td>
<td>11.5 (2.1)*</td>
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<tr>
<td>DART-IQ</td>
<td>92.4 (4.8)</td>
<td>92.6 (5.6)</td>
</tr>
<tr>
<td>MDMA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of use (years)</td>
<td></td>
<td>6.6 (3.3)</td>
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<tr>
<td>Usual dose (no. tablets)</td>
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<td>2.6 (0.7)</td>
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<tr>
<td>Lifetime exposure (no. tablets)</td>
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<td>902.0 (801.2)</td>
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<tr>
<td>Time since last dose (months)</td>
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<td>Last 3 months use of:</td>
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<tr>
<td>Alcohol (no. consumptions)</td>
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<td>158.6 (140.7)</td>
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<td>Tobacco (no. cig)</td>
<td>516.1 (517.1)</td>
<td>1044.6 (1312.0)</td>
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<tr>
<td>Cannabis (no. joints)</td>
<td>1.3 (1.3)</td>
<td>138.5 (174.8)</td>
</tr>
<tr>
<td>Amphetamine (exposure g)</td>
<td></td>
<td>4.4 (5.2)</td>
</tr>
</tbody>
</table>

Control subjects are obtained from a RAVLT database

*Data are expressed in mean ± SD values

*Significantly different from controls ($p < 0.01$; Student $t$-test)
Part IV | Linking biological markers of neuronal loss with memory function

Figure 1. Association between memory function (number of words remembered on RAVLT delayed recall trial) and the ratio of N-Acetylaspartate to creatine (NAA/Cr) in the prefrontal cortex, mid-occipital gray matter and temporo-parietal white matter. Adjusted R² values, obtained with linear regression analysis, are shown for each brain region studied.

Discussion

We found significant differences in delayed recall between MDMA users and MDMA-naïve control subjects. In addition, a strong association was observed between impaired memory function in MDMA users and neuronal pathology of the prefrontal cortex. This relationship was regionally specific, involving only the prefrontal cortex.

In line with our findings, a number of studies have reported verbal memory impairments in MDMA users (Bolla et al., 1998), whereas their performance on other cognitive tests is generally normal. Similarly, it has been shown that 5-HT appears to play a role in mnemonic function and that MDMA severely damages 5-HT axons in brain regions implicated in learning and memory in MDMA-treated animals (Hatzidimitriou et al., 1999).

We observed that poorer performance on a verbal memory test predicted lower prefrontal NAA/Cr ratios - and by inference greater neuronal loss or dysfunction - in MDMA users. This finding was in agreement with our hypothesis and confirms earlier studies that detected an association between indicators of MDMA-induced 5-HT neuronal damage and memory impairment (Bolla et al., 1998; Renema et al., 2000).

Taken in conjunction with reports of reduced CSF 5-HIAA (McCann et al., 1994) and density of 5-HT transporters (McCann et al., 1998) in recreational MDMA users and similar observations in animals with documented neurotoxic lesions, our findings suggest that the association between memory function and prefrontal NAA/Cr ratios and may be attributed to MDMA-induced neuronal pathology or dysfunction.

In view of the small brain mass occupied by 5-HT axon terminals in the prefrontal cortex (eg, much less than 1%) it is not likely that the presently observed association between reduced NAA levels and poor memory function can be fully ascribed to MDMA-induced gross loss of 5-HT neurons in the prefrontal cortex. It could also be hypothesized that our findings reflect a low abundance of 5-HT axon terminals from other regions (eg, the thalamus). In line with this, it has been shown that neonatal mesial-temporal limbic lesions can induce NAA deficits in the dorsolateral prefrontal cortex (Bertolin et al., 1997), perhaps reflecting a loss of inputs from the lesioned areas.

Frontal lobe lesions, although not generally recognized as causing memory deficits, are reported to impair the free recall of word lists similar to the one
used in this study. Furthermore, PET measurements of regional cerebral blood flow show that the prefrontal cortex is involved in auditory-verbal long-term memory (Grasby et al., 1993). It has been suggested that the free recall of words requires extensive use of retrieval strategies and planning. A neuropsychological explanation for the observed association may relate to the retrieval strategies necessary for dealing with an amount of information which exceeds the span of delayed verbal memory such as present in the RAVLT.

Because most of the MDMA users had experimented with other recreational drugs, primarily cannabis, we cannot completely rule out the possibility that the present findings are related to other drugs of abuse; however, no significant differences in the use of recreational drugs other than MDMA were observed between both groups. Also, we did not observe an association between RAVLT scores and extent of previous cannabis use, whereas the extent of previous MDMA use was significantly associated with delayed memory performance. Because recreational cannabis use, as reported by our subjects, has not been shown to produce neurotoxic effects in animals (Scallet 1991) and drug tests indicated that no participant used cannabis in the week before the study, it is unlikely that the findings of the present study should be attributed to substances other than MDMA. In addition, the adverse effects of long-term cannabis use on cognitive skills compared with MDMA use have not been clearly demonstrated in the literature. For instance, Gouzoulis-Mayfrank et al (2000) did not observe differences in cognitive performance between cannabis users, ecstasy users, and control subjects, whereas Rodgers (2000) did. As with all retrospective studies, there is a possibility that individuals engaged in the "rave" scene have preexisting risk factors (e.g., familial history or psychiatric disorder) for impaired memory function and reduced prefrontal NAA; however, by excluding MDMA users with a lifetime psychiatric disorder, this was not likely to account for changes in NAA and memory performance. Clearly, to definitively establish whether the presently observed relationship between neuronal function and performance in the prefrontal cortex is caused by MDMA-induced neuronal loss or dysfunction, a prospective study design would be needed; however, because studies of MDMA in humans are subject to ethical and methodological constraints it is difficult, if not impossible, to perform such a study. Another limitation of our study is that other than self-report, we have no confirmation that the MDMA-using subjects did in fact take MDMA. A recent survey in the Netherlands investigated the validity of the drug history questionnaire that was used in this study. It was found that in 93% of the cases (n = 594) the reported use of MDMA was in agreement with the drug urine test (van de Wijngaard et al., 1997).

In future studies, hair sample analysis would be a useful way to ascertain previous use of MDMA.

In sum, our preliminary data, which must be confirmed in a larger number of subjects, suggest that verbal memory impairment is associated with prefrontal cortex neuronal loss or dysfunction (as indicated by low NAA measures) in MDMA users. Our study shows a potentially unique (regionally specific) relationship between function of cortical neurons and cognitive performance.

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References


Rennenberg L, Boon J, Schmand B, van den Brink W, Gunnig B. Memory disturbances in "Ecstasy" users are correlated with an altered brain serotonin neurotransmission.
Part IV | Linking biological markers of neuronal loss with memory function


Chapter 9.3

Memory disturbances in “Ecstasy” users are correlated with an altered brain serotonin neurotransmission

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Part IV | Linking biological markers of neuronal loss with memory function

Rationale - Methylenedioxymethamphetamine (MDMA) is known to damage brain pre-synaptic serotonin (5-HT) neurons. Since loss of 5-HT neurons has been implicated in memory loss, it is important to establish whether MDMA use may produce changes in post-synaptic 5-HT receptors and memory function in humans. Objectives - To investigate whether MDMA use leads to compensative alterations in post-synaptic 5-HT2A receptors and whether there is a relation with memory disturbances. Methods - Brain cortical 5-HT2A receptor densities were studied with [3H]-5-HT1150 SPECT in 5 abstinent MDMA-users and 9 healthy controls. Memory performance was assessed using RAVLT. Results - [3H]-5-HT1150 binding ratios were significantly higher in the occipital cortex of MDMA users than in controls, indicating up-regulation. Mean cortical 5-HT2A receptor binding correlated positively with RAVLT-recall in MDMA users. Conclusion - Our preliminary results may indicate altered 5-HT neuronal function with correlated memory impairment in abstinent MDMA users.

Use of the popular recreational drug (±)3,4-methylenedioxyamphetamine (MDMA, "Ecstasy") leads to toxic effects on brain serotonin (5-HT) pre-synaptic neurons in humans, as recently reported (McCann et al., 1998, Semple et al., 1999). While neurotoxic effects of MDMA on 5-HT neurons lead to 5-HT depletion, little is known about the effects of this depletion on postsynaptic 5-HT2 receptors. Furthermore, since MDMA-induced 5-HT depletion may lead to impairments in which 5-HT and 5-HT2 receptors are involved (such as learning and mnemonic function), it is important to study the effects of MDMA on 5-HT2 receptors and memory (Buhot et al., 1997). This is of particular interest since several studies have found that recreational MDMA users display significant memory impairments, whereas their performance on other cognitive tests is generally normal (Krystal et al., 1992, Parrott et al., 1998).

Recent development of [3H]-5-I-R91150, a radioligand with high affinity and selectivity for the 5-HT2A receptor subtype, has made it possible to assess the density of post-synaptic 5-HT2A receptors in the living human brain, using single photon emission computed tomography (SPECT). Cortical binding of [3H]-5-I-R91150 for 5-HT2A receptors is specific and reversible, as shown by inhibition of binding by ritanserin and displacement by ketanserin. Furthermore, the cortico-cerebellar ratios at pseudo-equilibrium reflect a distribution similar to that expected from post-mortem studies (Busatto et al., 1997). The pilot present study was designed to investigate whether MDMA use leads to quantitative alterations in [3H]-5-I-R91150 labelled post-synaptic 5-HT2A receptors and related memory functions.

Five individuals with a history of MDMA use ("MDMA group"); mean age: 23.6 years [SD 3.3], men/women: 4/1, time since last dose: 4.6 months [range 2-11], lifetime number of tablets: 218 [50-500], mean education: 13 yrs [6], and nine age-, and education matched control subjects (mean age: 22.8 years [2.9], men/women: 4/5, mean education: 15 yrs [SD 5]) participated in the study. All MDMA subjects had used at least 50 tablets. The controls were healthy subjects with no self-reported use of psychoactive drugs, including MDMA. Recruitment was through advertisements (local newspapers). Participants agreed to abstain from use of psychoactive drugs for at least 2 months before the study, and were asked to undergo urine drug screening (with an enzyme-multiplied immunoassay for amphetamines, barbiturates, benzodiazepine metabolites, cocaine and metabolite, opiates, and marijuana) before enrolment. After testing urine samples, exclusion criteria were: a positive drug screen; pregnancy; a severe medical or neuropsychiatric illness that precluded informed consent; claustrophobia; and neuropsychiatric disease in which 5-HT has been implicated. We obtained written informed consent from all participants. The procedures used were approved by the local ethics board and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

For SPECT scanning the Strichmann Medical Equipment 810X tomographic system was used. The transaxial resolution of this camera is 7.6 mm full-width at half-maximum of a line source in air, while the axial resolution is 13.5 mm. Each acquisition consisted of at least 15 slices, (acquired in a 128 x 128 matrix), 3 min per slice, and with a slice distance of 5 mm. The energy window was set at 135-190 keV. Subjects lay in the supine position with the head aligned in a parallel to the orbitomeatal line, and were positioned such that the scanning volume initially included the cerebellum. Acquisition was commenced two hours after i.v. injection of approximately 140 MBq [3H]-5-I-R91150 (radiolabeling as described by Busatto and co-workers, 1997), a time when specific binding is maximal and stable for up to 8 h following injection. Analysis of scans was performed blind to subject status. For analysis of [3H]-5-I-R91150 binding, a standard template with regions of interest (ROIs) was constructed manually from co-registered MR images. For positioning we used these MR images as a guide. The template, including ROIs for the frontal-, parietal-, and occipital cortex, was
Investigating the potential neurotoxicity of Ecstasy (MDMA): An imaging approach

In the MDMA group, a significant lower number of recalled words in the RAVLT-recall was observed than in controls (8.14 [3.4] vs 12.3 [1.8], p < 0.001; Figure 2). In the MDMA group, but not in controls, mean cortical 5-HT2A receptor binding highly correlated (Spearman’s rho) with recall (p = -0.98 [p = 0.005], p = -0.29 [p = 0.46], respectively; Figure 2). Age, sex, extent of previous MDMA use, and education had no significant effect on this correlation (p = 0.28, 0.17, 0.25, and 0.16, respectively).

The high 5-HT2A receptor binding in the occipital cortex in the MDMA group may be caused by 5-HT depletion. It is known that severe 5-HT depletion causes up-regulation of 5-HT2 receptors (Heal et al., 1985). Moreover, MDMA-treated monkeys showed most severe 5-HT depletion in the occipital cortex. In these monkeys, fourteen months after MDMA administration 5-HT levels were still reduced in the occipital cortex by 97% (Scheffel et al., 1998). In a recent SPECT study, MDMA users showed a significant reduction only in occipital 5-HT neurons (Semple et al., 1999). Thus, the presently observed up-regulation of 5-HT2A receptors in the occipital cortex may reflect MDMA-induced brain 5-HT neurotoxicity.

In the present study, MDMA users showed significant deficits in delayed memory tasks, consistent with reports of memory problems in previous studies (Bolla et al., 1998, Parrott et al., 1998). Numerous laboratory studies with rats and monkeys have shown that MDMA produces serotonergic neurodegeneration. This has been demonstrated in various brain areas including the hippocampus, which is important for memory functioning (Hatzidimitriou et al., 1999). There is also clinical evidence for 5-HT brain damage in humans (Squier et al., 1995, McCann et al., 1998, Semple et al., 1999). There is therefore consistent evidence that memory deficits found in the present study may at least be attributed to MDMA-induced 5-HT deficits. Particularly since high densities of 5-HT2A receptors (an indirect measure of 5-HT depletion) were associated with lower performance on the delayed memory tests. This provisional finding is in agreement with a recent study which showed that the extent of memory impairment correlated with the reduction of brain 5-HT, as indexed by CSF 5-HIAA (Bolla et al., 1998). We presently observed that only individuals with apparent higher densities of occipital 5-HT2A receptors (presumably reflecting a greater extent of 5-HT injury) demonstrated detectable difficulties with memory function.

All participants in the MDMA group in our study reported that they had abstained from use of MDMA placed on three consecutive SPECT slices. Additional templates were constructed with ROIs for the cerebellum and temporal cortex. Mean cortical signal densities were calculated (mean counts/pixel of frontal-, parietal-, temporal-, occipital cortex). An investigator unaware of the participant’s history performed ROI analysis. The uptake in the cerebellum, presumed free from 5-HT2A receptors, was used as a reference for background radioactivity (non-specific binding + free ligand) (Busatto et al., 1997). Relative indices of “specific” binding are calculated as: “mean” ROI binding / cerebellar binding = 5-HT2A binding ratio.

Memory was assessed the day prior to SPECT imaging using the Rey Auditory Verbal Learning Test (RAVLT). The RAVLT is a verbal memory test. The immediate verbal memory comprised RAVLT logical memory, the delayed verbal memory incorporated RAVLT-recall, and RAVLT-recognition.

Overall, [11C]5-I-R91150 binding ratios were higher in the MDMA group than in controls, and reached statistical significance in the occipital cortex (mean 2.04 [SD 0.20] vs 1.74 [0.19], p < 0.05 Mann-Whitney U test Figure 1), indicating an up-regulation of post-synaptic 5-HT2A receptors.

![Figure 1](image)
or other psychoactive drugs for at least 2 months before the study. Although most of the MDMA users had experimented with other recreational drugs (mainly alcohol and cannabis), none was a known 5-HT neurotoxin in human beings, and was therefore not likely to account for changes in $[^{11}I]-5-I$-RI1150 binding to 5-HT$_2_A$ receptors.

This pilot study was performed using small samples. Nevertheless, this study at least suggests an intriguing relationship between 5-HT$_2_A$ receptor densities and memory performance in MDMA users. Because of the small sample size, the observed correlation cannot be said to be a definitive finding, and future studies investigating 5-HT neurotransmission and memory performance in MDMA users need to be conducted.

In conclusion, we provide additional evidence suggesting that human MDMA users are susceptible to MDMA-induced brain 5-HT neuronal injury and related functional disturbances, by showing a correlation between 5-HT2 receptor densities and memory disturbances. Thus, reductions in 5-HT, as indexed by elevated cortical 5-HT2 receptor densities, may be responsible for decrements in abstinent MDMA users.

References


Hatzidimitrou G, McCann UD, Ricaurte GA. Altered serotonin innervation patterns in the forebrain of monkeys treated with 3,4-methylenedioxymethamphetamine seven years previously; factors influencing abnormal recovery. J Neurosci 1999; 19: 3506-3517.

Heal DJ, Philpot K, Molyneux SG, Metz. Intracerebroventricular administration of 5,7-dihydroxytryptamine to mice increases both head-twist response and the number of cortical 5-HT2 receptors. Neuropharmacology 1985; 24: 1201-1205.
Investigating the potential neurotoxicity of Ecstasy (MDMA): An imaging approach


