Investigating the potential neurotoxicity of ecstasy (MDMA). An imaging approach
Reneman, L.

Citation for published version (APA):

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Chapter 7.1

MDMA ("Ecstasy") and its predisposition to cerebrovascular accidents – preliminary findings

Liesbeth Reneman¹, Jan B.A. Habraken¹,², Charles B.L.M. Majoie², Jan Booij¹ and Gerard J. den Heeten¹,²

¹Graduate School of Neurosciences, Department of Nuclear Medicine, Academic Medical Center, 1105 AZ Amsterdam, the Netherlands
²Department of Radiology, Academic Medical Center, 1105 AZ Amsterdam, the Netherlands

Abstract

Background and purpose - Abuse of the popular recreational drug "Ecstasy" (MDMA) has been linked to the occurrence of cerebrovascular accidents. It is known that MDMA alters brain serotonin (5-HT) concentrations, and that brain post-synaptic 5-HT2 receptors play a role in the regulation of brain microcirculation. Therefore, we used brain imaging to find out whether MDMA use predisposes to cerebrovascular accidents by means of alterations in brain 5-HT neurotransmission. Methods: The effects of MDMA on brain cortical 5-HT2A receptor densities were studied using [3H]R91150 single-photon emission CT in 10 abstinent recent MDMA users, 5 former MDMA users, and 10 healthy control subjects. Furthermore, to examine whether changes in brain 5-HT2A receptor densities are associated with alterations in blood vessel volumes, we calculated relative cerebral blood volume (rCBV) maps from dynamic MR image sets in 5 MDMA users and 6 healthy control subjects. 

Results: An analysis of variance revealed that mean cortical [3H]R91150 binding ratios were significantly lower in recent MDMA users than in former MDMA users and control subjects. This finding suggests down-regulation of 5-HT2 receptors caused by MDMA-induced 5-HT release. Furthermore, in MDMA users, low cortical 5-HT2 receptor densities were significantly associated with low cerebral blood vessel volumes (implicating vasoconstriction) and high cortical 5-HT2 receptor densities with high cerebral blood vessel volumes (implicating vasodilation) in specific brain regions. Conclusions: These findings suggest a relationship between the serotonergic system and an altered regulation of 5-HT2 receptors in human MDMA users. MDMA users may therefore be at risk for cerebrovascular accidents resulting from alterations in the 5-HT neurotransmission system.

Introduction

Recently, several case reports have linked the abuse of the popular recreational party drug 3,4-methylenedioxyamphetamine (MDMA, or "Ecstasy") to the occurrence of cerebrovascular accidents (De Silva et al., 1992; Gledhill et al., 1993; Hanyu et al., 1995; Harries et al., 1992; Henry 1992a; 1992b; Hughes et al., 1993; Teggin 1992). The brain area most vulnerable to the vascular effects of MDMA is the globus pallidus, a region rich in serotonin (5-HT) nerve terminals (Spatt et al., 1997; Squier et al., 1995). Considerable evidence has accumulated over the years strongly pointing to the involvement of 5-HT and 5-HT2 receptors in the regulation of brain microcirculation (Cohen et al., 1996; Parsons 1991). MDMA induces release of 5-HT from serotonergic neurons. However, abuse of this drug eventually leads to loss of serotonergic neurons, causing 5-HT depletion and a compensatory up-regulation of post-synaptic 5-HT2 receptors. It has therefore been suggested in several reports that MDMA abuse may predispose to cerebrovascular disease as a result of MDMA-induced effects on brain 5-HT concentrations and 5-HT2 receptors (Green et al., 1995; Henry 1992b; Spatt et al., 1995; Squier et al., 1995). Advances in neuroimaging techniques such as single photon emission CT (SPECT), have made it possible to study 5-HT2 receptors in the living human brain, using iodine-123 labelled R91150. [3H]R91150 binds selectively and with high affinity to the 5-HT2A receptor subtype. Cortical binding of [3H]-5-HT1-R91150 for 5-HT2A receptors is specific and reversible, as shown by inhibition of binding by ritanserin and displacement by ketanserin (Busatto et al., 1997). Moreover, by using cerebral blood volume (CBV) maps calculated from dynamic MR imaging sets, it is now possible to study relative CBV (rCBV) (Belliveau et al., 1990; Rosen et al., 1991) in the brain, in which regional vasospasm will decrease rCBV values, and vasodilatation will increase CBV values (Kaufman et al., 1998).

The aim of the present preliminary study was to use [3H]R91150 SPECT to investigate the effects of MDMA use on brain 5-HT2A receptor density, and, with the use of MR imaging sets, to ascertain whether these effects are associated with alterations in rCBV in abstinent recent MDMA users, former MDMA users, and healthy control subjects.

Methods

Participants

Fifteen participants who reported previous heavy use of MDMA (mean age, 26 years) and 10 age-matched control subjects (mean age, 23 years) (Table 1) were enrolled in the SPECT study. The eligibility criterion for the MDMA group was previous use of at least 50 tablets of MDMA. Ten participants had recently used this drug (MDMA group) and five former MDMA users had abstained from using MDMA (ex-MDMA group; Table 1). The eligibility criterion for the MDMA group was a drug-free interval of 1 week to 2 months prior to the study. Because animal studies have shown that down-regulation of 5-HT2 receptors persists for at least 1 month after the last intake of MDMA (Scheffel et al., 1992), the cut-off point of the drug-free interval for the ex-MDMA group was established at 2 months. The control group consisted of healthy sub-

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jects with no self-reported prior use of psychoactive drugs, including MDMA. Recruitment was through advertisements in local newspapers. 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 for amphetamines, barbiturates, benzodiazepine metabolites, cocaine and metabolite, opiates, and marijuana) before enrollment. After the testing of urine samples, exclusion criteria were a positive drug screen, pregnancy, severe medical or neuropsychiatric illness that precluded informed consent, claustrophobia, a cardiac pacemaker or surgical clip, and neuropsychiatric disease in which 5-HT has been implicated. All participants gave written informed consent.

5-HT2A receptor imaging
For SPECT studies, the Strichman Medical Equipment 810X tomographic system was used (Strichman Medical Equipment, Inc., Medfield, MA). The transaxial resolution of this camera is 7.6 mm full-width at half-maximum of a line source in air, and the axial resolution is 13.5 mm. Each acquisition consisted of 15 slices acquired in a 128 x 128 matrix with a slice distance of 5 mm and a scanning time of 3 minutes per slice. The energy window was set at 135 to 190 keV. Subjects lay in the supine position with the head aligned parallel to the orbitomeatal line, and were positioned such that the scanning volume initially included the cerebellum.

Acquisition of images began 2 hours after intravenous injection of approximately 140 MBq [111]R91150 (radiolabeling as described by Busatto and co-workers; Busatto et al., 1997), a time at which specific binding is maximal and stable for up to 8 hours after injection. For assessment of the scans, reviewers were blinded to subject status. For analysis of [111]R91150 binding, a standard template with regions of interest (ROIs) was constructed manually from coregistered MR images. For positioning, we used these MR images as a guide. Coregistration of MR images and SPECT scans was performed using the Hermes Multi Modality software package (Nuclear Diagnostics, Stockholm, Sweden). The template, including ROIs for the frontal, parietal, and occipital cortices, was placed on the three highest consecutive SPECT slices. An additional template was constructed with an ROI for the cerebellum. Mean signal density of the left and right cortices (mean counts per pixel of frontal, parietal, and occipital cortices) and of the cerebellum was determined. ROI analysis was performed by an investigator unaware of the participant’s history. The uptake in the cerebellum, presumed free from 5-HT2A receptors, was used as a reference for background radioactivity (nonspecific binding plus free ligand). ROI/cerebellum activity ratios were calculated as a relative measure of specific binding to 5-HT2A receptors for a given brain region (Busatto et al., 1997; Travis et al., 1998).

Calculating rCBV values
rCBV maps were calculated from dynamic MR image sets acquired with echo-planar spin-echo imaging after intravenous injection of gadolinium-based contrast material. MR images were obtained at 1.5 T. MR imaging was performed, on average, 6 hours before SPECT studies were obtained. An 18-gauge catheter was inserted into a large peripheral vein before MR imaging was performed. A saline drip was used to maintain the vein’s patency. Gadopentetate dimeglumine (0.2 mmol/kg) was power-injected at a rate of 5 mL/s through the angiocatheter. A series of images (46 series of 12 slices in 64 seconds) was obtained at intervals of 1202 milliseconds using a lipid-suppressed spin-echo planar pulse sequence (TR/TE = 0.8/54) before, during, and after injection of the contrast agent. Lipid suppression was used to suppress subcutaneous fat. We used a 128 x 128 x 12 matrix with a voxel size of 1.8 x 1.8 x 6.0 mm.

After data collection, rCBV maps were derived on a voxel-by-voxel basis from the dynamic image sets (using software developed at MGH-NMR Center, Charlestown, MA) (Ostergaard et al., 1996; Rosen et al., 1989). Since susceptibility contrast rCBV mapping method yields relative rather than absolute value of rCBV, comparison among subjects is facilitated by reference to an internal standard. In analogy to previous studies (Aronen et al., 1994; 1995), normal white matter was used as this reference. To calculate rCBV/white matter, the ROI’s of various brain regions (left and right frontal-, and occipital cortex, white matter, putamen and globus pallidus) were defined on rCBV maps by a radiologist unaware of the participant’s history. Ratios were calculated by dividing the mean rCBV of the brain region by that of unilateral mean white matter. Because rCBV maps enable quantification of vasculatization in relative terms (Aronen et al., 1995), a high rCBV ratio implies high regional blood volume, or vasodilatation, whereas a low rCBV ratio implies vasoconstriction (Kaufman et al., 1998).

Statistics
Differences in mean cortical [111]R91150 radioligand binding among groups were tested by one-way analy-
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Table 1. 5-HT₂ receptor imaging. Characteristics of participants, mean cortical [¹¹⁹]R91150 binding ratios

<table>
<thead>
<tr>
<th></th>
<th>Control subjects</th>
<th>MDMA Group</th>
<th>ex-MDMA Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 10</td>
<td>n = 10</td>
<td>n = 5</td>
</tr>
<tr>
<td>Mean age (y)</td>
<td>23 (3)</td>
<td>27 (5)</td>
<td>24 (5)</td>
</tr>
<tr>
<td>Men/women</td>
<td>4/6</td>
<td>7/5</td>
<td>4/1</td>
</tr>
<tr>
<td>Time since last dose (wk)</td>
<td></td>
<td>7 (5)</td>
<td>18 (19)</td>
</tr>
<tr>
<td>Lifetime no. of tablets</td>
<td></td>
<td>139 (129)</td>
<td>218 (201)</td>
</tr>
<tr>
<td>Last 3 months use of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDMA (no. of tablets)</td>
<td></td>
<td>1 (0.8)</td>
<td>0.4 (0.6)</td>
</tr>
<tr>
<td>Alcohol (units)</td>
<td></td>
<td>27 (31)</td>
<td>38 (33)</td>
</tr>
<tr>
<td>Tobacco (no. of cigarettes)</td>
<td></td>
<td>57 (45)</td>
<td>54 (44)</td>
</tr>
<tr>
<td>Cannabis (no. joints)</td>
<td>3 (6)</td>
<td>20 (29)</td>
<td>45 (19)</td>
</tr>
<tr>
<td>Cocaine (no. of lines)</td>
<td></td>
<td>6 (15)</td>
<td>0.8 (2)</td>
</tr>
<tr>
<td>LSD (no. of times used)</td>
<td></td>
<td>1.0 (1.7)</td>
<td></td>
</tr>
<tr>
<td>Cortical [¹¹⁹]R91150 binding ratio</td>
<td>1.78 (0.15)</td>
<td>1.58 (0.17)</td>
<td>1.91 (0.11)</td>
</tr>
</tbody>
</table>

Data are expressed as mean values (± SD).
*Statistically significant difference in binding in the MDMA group as compared with control group and ex-MDMA group (p = 0.02, and 0.001, respectively; ANOVA, Tukey Post Hoc test).

m | of variance. Differences in rCBV values among groups were analyzed using an unpaired Student's t-test. The relationship between mean cortical [¹¹⁹]R91150 radioligand binding and rCBV values in specific brain regions was investigated with Spearman’s rank correlation, since it has the advantage that it does not specifically assess a linear association but a more general one. A p value less than 0.05 was taken to be significant with a two-tailed test. We analyzed all data with SPSS version 9.0 software (Statistical Package for the Social Sciences, Chicago, Ill).

Results

5-HT₂ receptor imaging
Participants in the MDMA and ex-MDMA group had used, on average, 139 ± 129 tablets and 218 ± 201 tablets of MDMA, respectively (Table 1). Participants in the MDMA and ex-MDMA group had not used MDMA, on average, 7 ± 5 and 18 ± 15 weeks, respectively, before this investigation. All participants were right-handed.

Left and right cortical [¹¹⁹]R91150 binding did not differ significantly between control subjects and MDMA users. Therefore, mean cortical 5-HT₂A receptor-binding ratios were calculated (average of left and right frontal, parietal, and occipital [¹¹⁹]R91150 binding). Mean cortical 5-HT₂A receptor binding ratios in the MDMA group were significantly lower than in those in the ex-MDMA and control group (p = 0.001, and 0.02, respectively). Mean cortical 5-HT₂A receptor binding ratios were higher in the ex-MDMA group than in the control group, although this difference was not statistically significant (Table 1, Figures 1 and 2).

rCBV values
In addition to the SPECT studies, we performed dynamic MR imaging in a random sample of participants of the SPECT study to calculate rCBV values.
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Eventually, a random sample of 5 MDMA users (3 recent users and 2 ex-MDMA users) and 6 healthy control subjects were enrolled in the MR imaging study (Table 2).

No significant difference in left and right rCBV

Table 2. CBV values: characteristics of participants and mean rCBV ratio in brain areas studied

<table>
<thead>
<tr>
<th></th>
<th>Control subjects</th>
<th>MDMA users</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( n = 6 )</td>
<td>( n = 5 )</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>22 (1)</td>
<td>25 (5)</td>
</tr>
<tr>
<td>Men/women</td>
<td>3/3</td>
<td>4/1</td>
</tr>
<tr>
<td>Time since last dose (wk)</td>
<td>–</td>
<td>7 (5)</td>
</tr>
<tr>
<td>Lifetime no. of tablets</td>
<td>–</td>
<td>310 (247)</td>
</tr>
<tr>
<td>Last 3 months' use of:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MDMA (no. tablets)</td>
<td>–</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Alcohol (units)</td>
<td>36 (24)</td>
<td>56 (36)</td>
</tr>
<tr>
<td>Tobacco (no. cigarettes)</td>
<td>18 (21)</td>
<td>72 (40)</td>
</tr>
<tr>
<td>Cannabis (no. joints)</td>
<td>6 (7)</td>
<td>40 (45)</td>
</tr>
<tr>
<td>Cocaine (no. lines)</td>
<td>–</td>
<td>14 (20)</td>
</tr>
<tr>
<td>LSD (no. of times)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>rCBV ratios</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>1.99 (0.45)</td>
<td>2.02 (0.35)</td>
</tr>
<tr>
<td>Occipital cortex</td>
<td>3.06 (1.05)</td>
<td>3.04 (1.10)</td>
</tr>
<tr>
<td>Putamen</td>
<td>1.54 (0.30)</td>
<td>1.72 (0.42)</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>1.06 (0.27)</td>
<td>1.29 (0.26)</td>
</tr>
</tbody>
</table>

1 Data are expressed as mean values (± SD)

values was found between the control subjects and the MDMA users. Therefore, a mean of left and right cerebral rCBV values was calculated for the brain regions studied. Mean rCBV values for the MDMA users did not differ significantly from those obtained in control subjects in the brain regions studied (Table 2). The subgroup of 2 ex-MDMA users had higher rCBV values in the brain regions studied than did recent MDMA users. Compared with control subjects, the ex-MDMA users had higher rCBV in some specific brain regions (Figure 3).

Figure 2. \(^{[23]}\)R91150 SPECT images of a control subject, a recent MDMA user and an ex-MDMA user. Transverse slices from the brain at the level of the basal ganglia, approximately 3 cm above the orbitomeatal line. In the three images, the level of \(^{[23]}\)R91150 activity is color-coded from low (black) to high (white) and scaled to the maximum in the slice obtained in the control subject. The three images are representative for the three groups: in the control subject, there is normal \(^{[23]}\)R91150 binding, in the recent MDMA user, low \(^{[23]}\)R91150 binding, and in the ex-MDMA user, high \(^{[23]}\)R91150 binding.

Figure 3. Higher rCBV values are observed in the left globus pallidus and right thalamus in the ex-MDMA users than in control subjects. After registration in the same orientation (in six control subjects and two ex-MDMA users), an unpaired Student's t test was performed on each voxel of generated rCBV maps, revealing significant differences in the colored regions (yellow, \( p < 0.025 \); red, \( p < 0.005 \)). Images were obtained at intervals of 1202 milliseconds (800/54) before, during, and after injection of the contrast agent.

Correlations between 5-HT2A receptor densities and rCBV values

In MDMA users, but not in control subjects, a significant positive correlation was found between cortical 5-HT2A receptor binding ratios and rCBV values in the globus pallidus and occipital cortex (in control subjects, \( [\rho] = -0.12 \) and \( -0.06 \), respectively, and \( p = 0.74 \) and \( 0.91 \), respectively; in MDMA users, \( [\rho] = +0.90 \), and \( \rho = -0.90 \), \( p = 0.04 \) and \( 0.04 \), respectively) (Figure 4). The covariance effects of age, sex, and extent of previous MDMA use were not significant in the globus pallidus (\( p = 0.89 \), \( p = 0.74 \), and \( p = 0.18 \), respectively).
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respectively) or in the occipital cortex ($p = 0.15$, $p = 0.11$, and $p = 0.08$, respectively).

Discussion
Data obtained in MDMA-treated rats have shown down-regulation of 5-HT2 receptors until several weeks after treatment, owing to high levels of synaptic 5-HT (Scheffel et al., 1992). Other studies have also shown that 5-HT release leads to a compensatory down-regulation of post-synaptic 5-HT2A receptors (Peroutka et al., 1980), whereas 5-HT depletion leads to an up-regulation of 5-HT2A receptors (Heal et al., 1985). Interestingly, in this study, we observed a significant lower cortical $[^{111}]R91150$ binding to 5-HT2A receptors in the MDMA group, compared to controls and ex-MDMA users. This finding suggests down-regulation of 5-HT2A receptors. MDMA is an amphetamine derivative, which induces release of 5-HT from serotonergic neurons (Green et al., 1996; White et al., 1996). The presently observed low cortical 5-HT2A receptor density in the recent MDMA group therefore suggests down-regulation due to MDMA-induced 5-HT release.

In contrast, the high binding of $[^{111}]R91150$ in the ex-MDMA group (though not statistically significant) suggests an up-regulation of post-synaptic 5-HT2A receptors due to MDMA-induced 5-HT depletion. It is known that abuse of MDMA leads eventually to loss of serotonergic neurons. For example, cortical 5-HT levels in MDMA-treated monkeys were still signifi-

![Figure 4](image-url)

*Figure 4. Cortical $[^{111}]R91150$ binding versus rCBV values in specific brain regions. Open circles: controls; closed circles: MDMA users.*
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cantly reduced 13 months after treatment (Scheffel et al., 1998). Thus, the presently observed high
[^3H]R115150 binding may be the result of low synaptic 5-HT, possibly caused by loss of 5-HT neurons due to previous MDMA use. In a recent study it was demonstrated that MDMA causes loss of 5-HT neurons not only in animals, but in human beings as well (McCann et al., 1998). Therefore, it could be hypothesized that in the ex-MDMA group loss of 5-HT neurons resulted in low synaptic 5-HT levels, leading to up-regulation of 5-HT2 receptors. The SPECT results obtained in the present study indicate the necessity, and would probably justify, repeated 5-HT2 receptor studies within MDMA users.

In MDMA users, but not in controls, we found a significant positive correlation between cortical 5-HT2A receptor densities (measured with[^3H]R115150 SPECT) and rCBV values (measured with dynamic MR) in the occipital cortex and globus pallidus. Interestingly, several studies have pointed out that necrosis of the globus pallidus was the most striking neuropathological change in post-mortem material of MDMA users (Spatt et al., 1995). The globus pallidus is an area rich in 5-HT terminals. It is thought that local release of 5-HT, as induced after recent intake of MDMA, led to prolonged vasospasm and necrosis of the globus pallidus (Henry et al., 1997; Squier et al., 1995), possibly via stimulation of 5-HT receptors situated on microvessels by 5-HT. In addition, several studies have described cortical cerebral vascular accidents after MDMA use (De Silva et al., 1992; Gledhill et al., 1993; Hanu et al., 1995; Harries & De Silva 1992; Henry 1992a; 1992b; Hughes et al., 1993; Teggin 1992). The occipital cortex is also a brain area rich in 5-HT releasing neurons and 5-HT2 receptors (Aronen et al., 1995). It has been shown that the occipital cortex is particularly sensitive to 5-HT neuronal injury, since MDMA-treated monkeys showed the most severe 5-HT depletion in the occipital cortex (Scheffel et al., 1998).

The presently observed correlation between cortical 5-HT2A receptor availability and rCBV values in the occipital cortex and globus pallidus in MDMA users, but not in controls, suggests that 5-HT2A receptors are involved in the pathogenesis of MDMA-induced abnormal vascular reactions, possibly leading to cerebrovascular accidents. It is known that 5-HT2A receptors play a key role in the regulation of brain microcirculation, since they are located on brain microvessels (Cohen et al., 1996; Parsons 1991). For years, 5-HT2 antagonists were proven effective in preventing migraine headache (Mylecharane 1991). It is thought that stimulation of 5-HT2A receptors by 5-HT mediate cerebral vasoconstriction. However, vasodilatations have also been observed (Cohen et al., 1996). The short-term effect of MDMA involves excessive 5-HT release and stimulation of 5-HT2A receptors, leading to vasoconstriction. In line with this, we found that recent MDMA users had a significantly lower density of cortical 5-HT2A receptors (down-regulation due to high synaptic 5-HT levels), and a low rCBV (vasoconstriction) in the occipital cortex and globus pallidus of this group. On the other hand, former (ex-)MDMA users had a high density of cortical 5-HT2A receptors (up-regulation due to 5-HT depletion), and a high rCBV (vasodilatation) (as illustrated in Figure 3). In such a 5-HT deprived system, 5-HT2A receptors are not sufficiently stimulated, thus leading to vasodilatation instead of vasoconstriction. These findings suggest that MDMA users are susceptible to cerebrovascular accidents, due to vasoconstriction in recent MDMA users, and vasodilatation in ex-MDMA users.

The ratio obtained in this study between cortical gray and white matter rCBV in controls, approximately 2.5, correlates well other MR rCBV mapping studies (Aronen et al., 1994; Lambers et al., 1985). In addition, the ratios of[^3H]R115150 binding in controls are wholly consistent with other studies (Busatto et al., 1997; Travis et al., 1998).

Several potential limitations of the current study should be mentioned. First, as with all retrospective studies there is a possibility that pre-existing differences between MDMA users and nonusers underlie differences in 5-HT2A receptor densities and rCBV. Thus, people with low 5-HT2A receptor densities may be predisposed to use MDMA and to have low occipital and pallidal rCBV values. Second, this study was performed using small samples. Nevertheless, 5-HT2A receptor densities are unequivocal and these data do provide useful preliminary evidence of the relationship between 5-HT2A receptor densities and rCBV as revealed by SPECT and MR. Furthermore, despite the known presence of 5-HT2A receptors in the globus pallidus, as demonstrated in in vitro and in vivo studies (Pazos et al., 1987; Schotte et al., 1983), it is not possible to visualize 5-HT2A receptors in the globus pallidus or basal ganglia, using SPECT. In cortical regions, 5-HT2A receptor densities are about 10 times higher than in the basal ganglia (Schotte et al., 1983). Therefore, reliable quantification of 5-HT2A receptor is difficult in the basal ganglia using SPECT. In a recent study it was shown that in MDMA users brain 5-HT transporter densities were globally decreased (McCann et al., 1998). It can be expected, therefore, that the extent of alterations in cortical 5-HT2A recep-
tor densities, observed in the present study, reflect those in the globus pallidus. Third, in the present study, a larger number of females than males was included in the control group. Therefore, there is evidence from animal experiments that 5-HT2 receptor density in brain is higher in females than in male animals, the observed difference between controls and MDMA users may be an artifact of the larger number of women in the control group. However, a recent [28] study, performed in healthy human subjects, showed no influence of gender on [28] binding (Baeken et al., 1998). Finally, all participants in the MDMA group in our study reported that they had abstained from use of MDMA or other psychoactive drugs for at least one week 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 [28]-5-I-R91150 binding to 5-HT2A receptors.

**Conclusion**

We now provide new support for the hypothesis that a relation between 5-HT2 receptor density and rCBV in specific regions of the brain exists. Taken in conjunction with the clinical data from other published studies and historical findings of cerebrovascular accidents in MDMA users, one may infer that a relation between the serotonergic system and MDMA-induced cerebrovascular accidents exists. Our data suggest a trend in which MDMA users may be susceptible to abnormal vascular reactions, induced by alterations in the 5-HT system, eventually predisposing to cerebrovascular accidents. Additional studies and converging lines of evidence are needed to better delineate the potential of MDMA to induce cerebrovascular accidents in humans by alterations in the 5-HT system. Our observations, in accord with other (case) reports, indicate that people who use MDMA are not only unwittingly putting themselves at risk of developing neuronal 5-HT brain injury, but cerebrovascular accidents as well. Furthermore, this study indicates that the putative relation between cortical 5-HT receptors density and rCBV values in the occipital cortex and globus pallidus may implicate a target for prevention and treatment in the form of selective 5-HT receptor agents, in patients suffering from abnormal vascular reactions after MDMA use.

**Acknowledgements**

We thank Ms Kora de Bruin and Mr Ruud Smit for their technical assistance with the SPECT and MR experiments.

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Peterska SJ, Snyder SH. Long-term antidepres-


Chapter 7.2

Effects of Ecstasy (MDMA) on the brain in abstinent users: Initial observations with diffusion and perfusion MR imaging

Liesbeth Reneman\textsuperscript{1,2}, Charles B. L.M. Majoie\textsuperscript{2}, Jan B.A. Habraken\textsuperscript{1,2} and Gerard J. den Heeten\textsuperscript{2}

\textsuperscript{1}Graduate School of Neurosciences, Department of Nuclear Medicine, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands
\textsuperscript{2}Department of Radiology, Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, the Netherlands

Radiology 2001; 220: 617-617
Abstract

**Purpose** - To evaluate the effects of 3,4-methylenedioxyamphetamine (MDMA, ecstasy) on the human brain by using diffusion and perfusion magnetic resonance (MR) imaging.

**Materials and Methods** - Eight abstinent ecstasy users and six ecstasy nonusers underwent diffusion and perfusion MR imaging. Apparent diffusion coefficient and relative cerebral volume ratios between the groups were analyzed using the Mann-Whitney-Wilcoxon test. The relationship between apparent diffusion coefficient values and relative cerebral blood volume ratios between the groups was investigated with the Spearman rank correlation.

**Results** - Apparent diffusion coefficient values (0.84 vs 0.65 ± 0.109 cm²/sec, p < 0.025) and relative cerebral blood volume ratios (1.22 vs 1.01, p < 0.025) were significantly higher in the globus pallidus of ecstasy users compared with nonusers, respectively. Increases in pallidal relative cerebral blood volume were positively correlated with the extent of previous ecstasy use (p = 0.73, p < 0.04). **Conclusion** - Ecstasy may be associated with tissue changes in the globus pallidus. These findings are in agreement with case reports, suggesting that the globus pallidus is particularly sensitive to the effects of ecstasy.

Introduction

3,4-Methylenedioxyamphetamine MDMA ("Ecstasy") is an amphetamine congener that has gained marked popularity as a recreational drug. MDMA induces release of serotonin (serotonin) from serotonergic neurons. However, it has become increasingly apparent that MDMA use eventually can lead to toxic effects on brain serotonin neurons in animals as well as humans. In animals, damage to serotonin neurons has been demonstrated by reductions in various markers unique to serotonin axons, including brain serotonin, 5-hydroxyindoleacetic acid (5-HIAA), and the density of serotonin transporters (Battaglia et al., 1987; Ricaurte et al., 1988a; 1992; Schmidt, 1987; Stone et al., 1986). Anatomic studies in MDMA-treated animals indicate that these neurochemical changes are secondary to a distal axonotomy of serotonin neurons (O'Hearn et al., 1988; Wilson et al., 1989). Findings in recent positron emission tomography, or PET, and single photon emission computed tomography, or SPECT, studies have shown decreases in the number of central serotonin transporters in human MDMA users, findings which are similar to those observed in MDMA-treated primates (McCann et al., 1998; Scheffel et al., 1998; Semple et al., 1999).

Few functional consequences of MDMA-induced neurotoxicity have been identified, however, in either animals or humans (Boot et al., 2000). Because MDMA-induced serotonergic damage may lead to impairment of functions in which serotonin is involved, it is important to study the potential consequences of MDMA-induced neurotoxicity. The brain microcirculation is of particular interest since considerable evidence (Cohen et al., 1996; Parsons 1991) has been accumulated in past years that strongly suggests that serotonin is involved in the regulation of brain microcirculation. It is, therefore, of interest to note that findings in several case reports have linked the abuse of MDMA to the occurrence of cerebrovascular accidents (Gledhill et al., 1993; Henry 1992a; Henry et al., 1992b; Spatt et al., 1997; Squier et al., 1995), as a result of MDMA-induced effects on brain serotonin concentrations.

Diffusion-weighted magnetic resonance (MR) imaging provides a form of contrast that enables the quantitative measurement of diffusional motion of water molecules in biological tissue, especially axons (Le Bihan et al., 1992). Cellular structures, such as highly organized myelinated axons in white matter, restrict water molecular motion, and the apparent diffusion coefficient (ADC) is reduced compared with diffusion in bulk water (Basser et al., 1996; Conturo et al., 1995; Moseley et al., 1990; Pierpaoli et al., 1996; Van Gelderen et al., 1994). Any process that results in changes in structural elements of tissue, such as removal of some of the restrictive barriers, can result in increased ADC values. It is, therefore, thought that diffusion-weighted MR imaging is a promising approach for the evaluation of tissue changes in degenerating brain and nerve matter (Horsfield et al., 1998; Kinoshita et al., 1999; Larsson et al., 1992). Moreover, the use dynamic contrast-enhanced perfusion-weighted MR imaging has made it possible to study the brain vasculature by means of calculating relative cerebral blood volume (rCBV) maps (Rosen et al., 1989).

The purpose of our study was to evaluate the effects of ecstasy on the human brain with diffusion and perfusion MR imaging techniques.

**Materials and Methods**

**Participants**

The study was carried out at the Academic Medical Center in Amsterdam, the Netherlands, from October through December 1998. Eight ecstasy users (seven
men, one woman; mean age, 27.6 years ± 4.9 [SD]; age range, 22-35 years) were compared with six ecstasy nonusers (three men, three women; mean age, 22.3 years ± 0.8; age range, 22-23 years) who were using drugs. Recruitment was through advertisements in local newspapers.

Subjects selected were group-matched for age and sex, were otherwise healthy, and had no history of psychiatric illness. The six ecstasy nonusers reported no prior use of ecstasy. Participants agreed to abstain from use of psychoactive drugs for at least 3 weeks before the study and were asked to undergo a urine drug screening (with an enzyme-multiplied immunonassay for amphetamines, barbiturates, benzodiazepine metabolites, cocaine metabolite, opiates, and cannabis [marijuana]) before enrollment in the study. After urine samples were tested, subjects were excluded on the basis of the following criteria: positive results of the urine test for drug screening, pregnancy, or severe medical illness.

Subjects were interviewed with a structured automated diagnostic psychiatric interview, or Composite International Diagnostic Interview (CORE, version 2.1; World Health Organization, Geneva, Switzerland) to screen for current axis I psychiatric diagnoses. A detailed drug-history questionnaire was obtained, and in addition, subjects were screened for left- or right handedness. Written informed consent was obtained from all participants. The institutional Medical Ethics Committee approved the study.

**Table 1. Demographics, characteristics of ecstasy users and ecstasy non-users**

<table>
<thead>
<tr>
<th></th>
<th>Ecstasy non-users</th>
<th>Ecstasy users</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>n = 6</td>
<td>n = 8</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>22.3 ± 0.8</td>
<td>27.6 ± 4.9</td>
</tr>
<tr>
<td>Men/women</td>
<td>3/3</td>
<td>7/1</td>
</tr>
<tr>
<td>DART IQ</td>
<td>112 ± 5.8</td>
<td>98.3 ± 14.3</td>
</tr>
<tr>
<td>Ecstasy Duration</td>
<td>4.3 (1.4 - 6.4)</td>
<td></td>
</tr>
<tr>
<td>Usual dose (tablets)</td>
<td>2.4 (0.5 - 5.0)</td>
<td></td>
</tr>
<tr>
<td>Lifetime dose (tablets)</td>
<td>154 (30 - 500)</td>
<td></td>
</tr>
<tr>
<td>Time since last dose (weeks)</td>
<td>14.6 (3.0 - 52.0)</td>
<td></td>
</tr>
<tr>
<td>Alcohol (no. consumptions/week)</td>
<td>7.1 ± 5.9</td>
<td>10.5 ± 17.6</td>
</tr>
<tr>
<td>Tobacco (cig./week)</td>
<td>1.8 ± 2.4</td>
<td>42.9 ± 58.2</td>
</tr>
<tr>
<td>Cannabis (no.joints/week)</td>
<td>0.3 ± 0.3</td>
<td>4.4 ± 7.6</td>
</tr>
<tr>
<td>Amphetamine</td>
<td></td>
<td></td>
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<tr>
<td>(no times used/3 months)</td>
<td>--</td>
<td>2.9 ± 4.5</td>
</tr>
</tbody>
</table>

Data are expressed in mean ± SD values.
1 DART = Dutch Adult Reading Test
2 Statistical significant difference (p < 0.025)

magnet. Nine diffusion-weighted images were obtained along each axis (b = 0 sec/mm²). Then, a second diffusion-weighted image was acquired with extended diffusion gradients to obtain a larger b value (b = 1,000 sec/mm²). Although signal intensity in the diffusion-weighted imaging is affected by T₁ and T₂, the ADC map is not. It is obtained by calculating the logarithmic ratio of the signal intensity at each pixel according to the following equation: ADC = ln(S₁/S₂)/(b₂-b₁), where S₁ and S₂ are the signal intensity of the baseline and diffusion-weighted images, respectively, and b₁ and b₂ are the b values for the corresponding pulse sequences.

The ADC value is also dependent on the direction in which diffusion is measured, which makes a comparison of ADC values without taking into account the measurement direction, meaningless. By measuring the ADC value in three orthogonal directions and then averaging the results, with the equation ADC = (ADCₓ + ADCᵧ + ADC₂)/3, we are able to measure diffusion that is independent of the orientation of structures.

**Echo-planar T₂<sup>−</sup>-weighted dynamic contrast-
enhanced images (0.8/54, one signal acquired, 23 cm FOV, 128 x 128 matrix) were obtained in 12 transverse sections at 1.2-second intervals for 64 seconds immediately after intravenous bolus injection of gadopentate dimeglumine (Magnevist; Schering, Berlin, Germany) at 0.1 mmol/kg of body weight. The contrast agent was power-injected intravenously at a rate of 5 mL/sec through an 18-gauge antecubital needle with an MR-compatible power injector (Spectris MR injector; Medrad, Indianola, Pa).

Quantitative ADC and rCBV maps were automatically derived on a voxel-by-voxel basis by using software (Massachusetts General Hospital, NMR Center, Charlestown, Mass.) (Ostergaard et al., 1996; Rosen et al., 1989). Regions of interest of various brain regions (left and right frontal cortex, occipital cortex, white matter, putamen, and globus pallidus) were defined on ADC and rCBV maps by a radiologist who was unaware of the participant's history. Mean signal intensities were measured in the ROI on each ADC (expressed in $10^{-3} \times \text{cm}^2$/sec) and rCBV (expressed in arbitrary units) map. Since susceptibility-contrast rCBV mapping method yields a relative rather than an absolute rCBV value, comparison among subjects is facilitated by reference to an internal standard. Analogous to previous studies (Aronen et al., 1994; 1995), normal white matter was used as this reference. Ratios were calculated by dividing the mean rCBV of the brain region by the mean rCBV of white matter.

**Verbal intelligence**

<table>
<thead>
<tr>
<th></th>
<th>Ecstasy non-users</th>
<th>Ecstasy users</th>
</tr>
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<tbody>
<tr>
<td><strong>rCBV ratios</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>1.90 ± 0.24</td>
<td>1.89 ± 0.20</td>
</tr>
<tr>
<td>Occipital cortex</td>
<td>2.93 ± 0.84</td>
<td>2.67 ± 1.28</td>
</tr>
<tr>
<td>Putamen</td>
<td>1.48 ± 0.17</td>
<td>1.50 ± 0.27</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>1.01 ± 0.15</td>
<td>1.22 ± 0.14*</td>
</tr>
<tr>
<td><strong>ADC values ($10^{-3} \times \text{cm}^2$/sec)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Frontal cortex</td>
<td>1.10 ± 0.13</td>
<td>1.23 ± 0.09</td>
</tr>
<tr>
<td>Occipital cortex</td>
<td>1.03 ± 0.15</td>
<td>1.01 ± 0.13</td>
</tr>
<tr>
<td>White matter</td>
<td>0.81 ± 0.06</td>
<td>0.89 ± 0.15</td>
</tr>
<tr>
<td>Putamen</td>
<td>0.72 ± 0.02</td>
<td>0.78 ± 0.12</td>
</tr>
<tr>
<td>Globus pallidus</td>
<td>0.65 ± 0.03</td>
<td>0.84 ± 0.22*</td>
</tr>
</tbody>
</table>

- Data are expressed in mean ± SD values
- **rCBV** = relative cerebral blood volume
- **ADC** = apparent diffusion coefficient
- Statistical significant difference (p < 0.05)

The Dutch Adult Reading Test (DART) (Schmand et al., 1992) was administered to obtain 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 intelligence quotient (IQ) (population mean IQ, 100; SD, 15). Results of this test were used to describe the sample.

**Statistical analysis**

Differences between the two groups with regard to demographic variables and exposure to other drugs were analyzed using the non-parametric Mann-Whitney-Wilcoxon test. Differences in ADC values and rCBV ratios between both groups were also analyzed using the Mann-Whitney-Wilcoxon test. The relationship between ADC values and rCBV ratios in specific brain regions and the extent (lifetime number of ecstasy tablets taken) of previous ecstasy and amphetamine use was investigated with the Spearman rank correlation, since it has the advantage that it is not used to specifically assess a linear association but a

![Figure 1. Graph shows the mean (bar) and individual ADC values in the globus pallidus in ecstasy nonusers and ecstasy users. Data show significantly higher ADC values in ecstasy users. * = statistically significant difference in mean binding ratio between ecstasy nonusers and ecstasy users.](image-url)
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more general association. To explore the effects age, sex, and DART-IQ test results may have on rCBV ratios and ADC values, a correlation analysis was performed between these variables and ADC values and rCBV ratios. Because of the small sample size, the chance of a type I error was set at a 0.10, with the use of two-tailed tests of significance. To correct for multiple comparisons, p values less than 0.025 (0.10 ÷ 4, for four different brain regions) were considered significant. All data were analyzed by using computer software (SPSS version 9.0; SPSS Software, Chicago, Ill).

Results

No statistical differences between any of the demographic variables were found between both groups. Ecstasy users consumed more alcohol and used more tobacco and cannabis than ecstasy nonusers before this investigation (Table 1), though this difference did not reach statistical relevance. Ecstasy users ingested more amphetamine compared with ecstasy nonusers, and the difference was statistically significant. All participants were right-handed.

In the ecstasy user group, participants had generally ingested more than 150 tablets of ecstasy over a 2-3-year period. Most of the ecstasy users had not ingested ecstasy for several weeks, and some indicated that they had not ingested ecstasy for several months (Table 1).

Imaging

The conventional T1- and T2-weighted images showed no edematous changes in the brain of ecstasy users and ecstasy nonusers. In both groups, the differences between left and right ADC values and rCBV ratios were not statistically significant. Because of this, and because we did not expect left-right differences in the effects of ecstasy, a mean of left and right cerebral ADC values and rCBV ratios was calculated for brain regions studied. Overall, mean ADC values were higher in ecstasy users compared with ecstasy nonusers. This difference was statistically significant only with regard to the globus pallidus (0.650 x 10^-5 x cm^2/sec in ecstasy nonusers vs 0.84 x 10^-5 x cm^2/sec in ecstasy users) (Table 2) (Figures 1 and 2).

Similar observations were made for rCBV ratios. Overall, mean rCBV values were higher in ecstasy users compared with ecstasy nonusers, although this difference reached statistical significance only in the
Figure 4. Correlation between rCBV in the globus pallidus and extent of previous ecstasy use, suggesting that the effects on rCBV values are dosage-related in this brain region.

globus pallidus of ecstasy users (mean, 1.22) compared with nonusers (mean, 1.01) (Table 2) (Figure 3).

Correlations of findings
No significant correlations were observed between ADC values and extent of previous ecstasy or amphetamine use. Age, sex, and DART-IQ test results were not significantly associated with rCBV ratios or ADC values. However, a significant association was observed between extent of previous ecstasy use and rCBV ratio in the globus pallidus ($p = 0.73$, $p < 0.04$; Figure 5). The higher the ecstasy exposure, the higher the rCBV ratio in the globus pallidus.

Discussion
In the present study, we observed increased ADC values and rCBV ratios in the globus pallidus of ecstasy users. As we have previously discussed, diffusion weighted MR imaging enables evaluation of the random motion of water on a molecular level. ADC values are a rotationally invariant measurement of the amount of total diffusion within a tissue (Basser & Pierpaoli 1996; Pierpaoli et al., 1996; Warach et al., 1992). The in vivo cellular environment contains cell membranes that form a restrictive barrier to water diffusion. Findings in experimental models have shown that the axonal cell membrane is sufficient to account for most of the restriction of water diffusion in white matter (Beaulieu et al., 1994; Le Bihan et al., 1992). Diffusion is much more restricted in a direction perpendicular to the axis of the axon, than in a direction parallel to the axon (Basser & Pierpaoli 1996; Conturo et al., 1995; Moseley et al., 1990; Pierpaoli et al., 1996; Van Gelderen et al., 1994). It is, therefore, not surprising that any process that disrupts the integrity of the axon or results in axonal loss would change the diffusion of water in this tissue.

The increase in ADC value in the globus pallidus of ecstasy users may be due to axonal injury or loss or increased extracellular fluid (vasogenic edema). T2-weighted MR images are more sensitive to brain edema than are other measurements. However, local brain edema was not detected on the T2-weighted images obtained in the ecstasy users. These results indicate that changes in the globus pallidus of ecstasy users at diffusion weighted MR imaging are not due to an increased water content in the extracellular space but may reflect axonal loss.

In support of this idea, it is known that extensive serotonergic axonal loss occurs in various brain regions of animals treated with MDMA. These results have been demonstrated anatomically in numerous studies with the use of immunocytochemical methods for visualization of axons that contain serotonin (Molliver et al., 1990; O’Hearn et al., 1988; Wilson et al., 1989). In MDMA-treated monkeys, serotonergic axons have shown to be reduced by 80-90% in cortical brain areas, striatum and thalamus and by 60% in the globus pallidus 2 weeks after MDMA administration. In time, some axonal sprouting also seems to take place, but reinnervation patterns up to 7 years after treatment are abnormal, with some brain regions remaining denervated and others showing evidence of reinnervation (Hatzidimitriou et al., 1999).

There is suggestive evidence that MDMA may also be neurotoxic to serotonin neurons in humans (McCann et al., 1998; Semple et al., 1999), as well as to serotonin neurons in animals and non-human primates. These observations support the suggestion that the increased ADC values which we observed in the globus pallidus of ecstasy users, compared to ecstasy nonusers, reflect a distal axotomy or axonal injury of ascending serotonergic projections to the globus pallidus. In keeping with this idea, it has been shown that a disturbance in the axonal integrity, produced by the toxic action of methylmercury (methyl-mercury), resulted in increased ADC values in rats treated methylmercury (Kinoshita et al., 1999). Findings in studies in patients with the demyelinating disease multiple sclerosis have shown increased ADC values...
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in multiple sclerosis lesions and increased ADC values sometimes in normal white matter (Christiansen et al., 1993; Horsfield et al., 1998; Larsson et al., 1992). It is thought that the pronounced increase in diffusion in the chronic stage of multiple sclerosis may represent axonal loss and tissue destruction (Scanderberg et al., 2000).

In addition to increased ADC values, we observed increased rCBV ratios in the globus pallidus of ecstasy users. Findings in studies have shown that administration of the potent cerebral vasodilator acetazolamide results in large increases in the rCBV ratio (de Crespigny et al., 1998; Nighoghossian et al., 1997). Furthermore, it has been shown that following administration of cocaine, rCBV ratios in brain pial arterioles were consistent with the arteriolar diameter reduction. Thus, a high rCBV ratio implies high regional blood volume, or vasodilation, whereas a low rCBV ratio implies vasoconstriction (Kaufman et al., 1998). Therefore, the increased rCBV ratios in the globus pallidus of ecstasy in this study most probably reflects vasodilation.

Considerable evidence has accumulated over the years that strongly points to a vasoconstrictor role of serotonin in the control of brain microcirculation (Cohen et al., 1996). The remarkable sensitivity of brain vessels for serotonin has been paralleled by visualization, as evidenced by autographical, biochemical and immunocytochemical methods, of a rich network of nerve fibers around major cerebral arteries and pial vessels that contain serotonin. In keeping with this idea, it has been shown that induction of serotonin lesions with systemic administration of 5-hydroxy-2-(di-n-propylamino)tetrazen (8-OH-DPAT) mediates a vasodilatory response in the cerebrovasculature (de Crespigny et al., 1998). It is thought that, because of reduced serotonin content, vasodilation occurs due to removal of serotonergic constrictor effects. The increases in rCBV ratios in the globus pallidus of ecstasy users in this study may result from a similar mechanism.

As discussed previously, numerous studies have shown that in animals and nonhuman primates MDMA administration results in loss of serotonergic axons and terminals, which leads to persistent losses in serotonin (Battaglia et al., 1988; Hatzidimitriou et al., 1999; Molliver et al., 1990; O’Hearn et al., 1988; Scanzello et al., 1993; Wilson et al., 1989). Findings in studies in human ecstasy users have shown selective neurotoxic effects on the serotonergic system, as indicated by decreases in 5-hydroxyindoleacetic acid in cerebrospinal fluid (McCann et al., 1994; Peroutka et al., 1987) and in the number of amount of serotonin transporters (McCann et al., 1998; Semple et al., 1999). These findings in human ecstasy users are similar to findings in MDMA-treated animals with documented serotonergic neurotoxic lesions (Scheffel et al., 1998; Ricart et al., 1998b). There is, therefore, consistent evidence that the observed increased rCBV values in the globus pallidus of ecstasy users in this study may at least be attributed to MDMA-induced serotonin deficits. In the present study, the positive association between ecstasy exposure and rCBV ratios further supports this finding.

Interestingly, the finding of increased rCBV ratios in the globus pallidus of ecstasy users in this study is in agreement with the finding in a recent study by Reneman and co-workers (2000). Findings in that study show that in specific brain regions (particularly the globus pallidus) high cortical serotonin, receptor densities, which are suggestive of low synaptic serotonin levels, were correlated with high rCBV ratios and implicated vasodilation. On the other hand, low cortical serotonin 2 receptor densities, which are suggestive of high synaptic serotonin levels, were correlated with low rCBV ratios, implicating vasoconstriction.

Furthermore, findings in a recent study by Chang and co-workers (2000) show that in subjects who received MDMA in a controlled setting, larger decreases in cerebral blood flow, which implicated vasoconstriction, were observed in subjects who received MDMA more recently (on average, 2-3 weeks before the examination). In addition, the authors observed increased cerebral blood flow values, which implicated vasodilatation, in several subjects who underwent imaging after 2-3 months. The short-term effect of MDMA involves excessive release of serotonin. It, therefore, was suggested that, with normalization of the excess of serotonin or depletion of serotonin in some regions at later time, cerebral blood flow may return to normal or increase to greater than normal values, due to removal of serotonergic constrictive effects. In this study, we observed significant increases in rCBV ratios in the globus pallidus in the ecstasy users with a long period of abstinence from ecstasy use, which was at least 3 weeks but on average was 3.5 months.

The ratio obtained in this study between cortical gray and white matter rCBV in ecstasy nonusers, which was approximately 2.5, correlates well with results in other MR rCBV mapping studies (Aronen et al., 1994). The observed ADC values of approximately 1.1 x 10^−5 x cm^2/sec in gray matter in this study are in good agreement with those in the literature (1.0 x 10^−5 x cm^2/sec) (Chien et al., 1992).

As with all retrospective studies, there is a possibi-
lity that preexisting differences between ecstasy users and nonusers underlie differences in rCBV ratios and ADC values. Thus, people with high palidal rCBV ratios and ADC values may be predisposed to use ecstasy. Another potential limitation of the present study may that the samples were small. Nevertheless, to our knowledge, there have been few studies in which the effects of ecstasy on the central nervous system have been investigated with MR imaging and no studies with diffusion-weighted MR imaging. Age, sex, and results of the DART-1Q test are not likely to have influenced our findings, since they were not significantly related to rCBV ratios or ADC values. Finally, although most of the ecstasy users in our study had more experience with other recreational drugs than ecstasy nonusers, no statistically significant differences in the use of drugs other than amphetamine and ecstasy were observed between the two groups in this study and were, therefore, not likely to account for changes in rCBV ratios or ADC values.

We cannot, however, completely rule out the possibility that the observed changes in the globus pallidus of ecstasy users were unrelated to amphetamine use. Since amphetamine and the other drugs that ecstasy users reported having used are unknown serotonin neurotoxins in human beings, it seems unlikely that the findings in this study should be attributed to substances other than MDMA.

In conclusion, we provide suggestive evidence that ecstasy use is associated with changes in rCBV ratios and ADC values in the globus pallidus of human ecstasy users. These findings are consistent with findings of serotonergic axonal loss and serotonin depletion in animals treated with MDMA, data in humans from other published reports, and findings of cerebrovascular accidents in medical histories of ecstasy users. Future studies with larger samples of ecstasy users will help in a further evaluation of the association between findings of cerebrovascular accidents in medical histories and findings of MDMA-induced cerebrovascular accidents. Our data indicate that MR imaging may be a valuable tool in the investigation of the consequences of MDMA use in brain tissue and the microvasculature.

Acknowledgements
We thank Ruud G. Smit, Department of Radiology, Academic Medical Center, Amsterdam, the Netherlands, for his technical assistance with the MR imaging experiments.

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


