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Chapter 6

The acute and chronic effects of MDMA ("Ecstasy") on cortical 5-HT$_{2A}$ receptors in rat and human brain

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Abstract
While the pre-synaptic effects of 3,4-methylenedioxymethamphetamine (MDMA) on serotonin (5-HT) neurons have been studied extensively, little is known about its effects on post-synaptic 5-HT2 receptors. Therefore, cortical 5-HT2A receptor densities and 5-HT concentration were studied in MDMA treated rats (10 mg/kg s.c.). Furthermore, 5-HT2A post-synaptic receptor densities in the cerebral cortex of recent as well as ex-MDMA users was studied using \(^{123}\text{I}R_{91150}\) SPECT. In rats we observed a decrease followed by a time-dependent recovery of cortical 5-HT2A receptor densities, which was strongly and positively associated with the degree of 5-HT depletion. In recent MDMA users, post-synaptic 5-HT2A receptor densities were significantly lower in all cortical areas studied, while 5-HT2A receptor densities were significantly higher in the occipital cortex of ex-MDMA users. The combined results of this study suggest a compensatory up-regulation of post-synaptic 5-HT2A receptors in the occipital cortex of ex-MDMA users due to low synaptic 5-HT levels.

Introduction
3,4-Methylenedioxymethamphetamine MDMA ("Ecstasy") is an amphetamine congener that has gained significant popularity as a recreational drug. The neurochemical mechanism of action of MDMA is well studied in animals. MDMA releases serotonin (5-HT) from pre-synaptic 5-HT neurons short after MDMA administration (Nichols et al., 1982; Nishisawa et al., 1999; Schmidt et al., 1986). It is thought that these acute effects of MDMA (increase in extracellular 5-HT levels) are responsible for the psychological effects of MDMA in humans (Liechti et al., 2000). Also, it has become increasingly apparent that MDMA use can lead to toxic effects on brain 5-HT neurons in animals, as demonstrated by reductions in various markers unique to 5-HT axons, including brain 5-HT, 5-hydroxyindoleacetic acid, and the density of 5-HT transporters (Battaglia et al., 1987; Ricaurte et al., 1988; 1992; Schmidt 1987; Stone et al., 1986). Anatomical studies in MDMA-treated rodents and non-human primates indicate that these neurochemical changes are secondary to a distal axonotomy of 5-HT neurons. Although there is good evidence for a neurotoxic potential of MDMA in animals, there is a lack of studies investigating the potential neurotoxic effects of MDMA in humans (Boot et al., 2000).

While the effects of MDMA on 5-HT nerve fibers and terminals have been studied extensively, little is known about its effects on post-synaptic 5-HT receptors. Only one study has evaluated post-synaptic 5-HT2 receptor densities in MDMA-treated rats (Scheffel et al., 1992). There is considerable evidence from the literature that post-synaptic 5-HT2 receptors manifest a down-regulation in situations with high levels of synaptic 5-HT, while 5-HT depletion has been associated with a compensatory up-regulation of 5-HT2 receptors (Kellar et al., 1981; Peroutka & Snyder 1980a; 1980b; Rice et al., 1998; Sharif et al., 1989; Stockmeier & Kellar 1989). The fate of post-synaptic 5-HT2 receptors after MDMA-induced pre-synaptic 5-HT lesions is of considerable interest since there is evidence that 5-HT2 receptors play an important role in the regulation of a wide range of central mechanisms including cognition and emotion, and since abnormalities of 5-HT2 receptors have been proposed in several neuropsychiatric conditions such as major depression and dementia (Busatto 1996). In addition, recent PET studies have shown that the in vivo occupancy of 5-HT2 receptors play an important role in the modulation of action of antipsychotics (Offord et al., 1999; Talvik-Lofft et al., 2000).

The development of iodine-123-4-amino-N-[3-[(4-fluorophenoxo)propyl]-4-methyl-piperidinyl]-5-iodo-2-methoxybenzamide ([123]I R_{91150}), a radiogand which binds with high affinity and selectivity to 5-HT2A receptors (Teriere et al., 1999), has made it possible to assess the density of cortical HT2A receptors in the living human brain, using single photon emission computed tomography (SPECT) (Busatto et al., 1997). Post-synaptic 5-HT2A receptors are present in high densities in cortical brain regions, whereas moderate densities are localized in the hippocampus, globus pallidus, and thalamus (Pazos et al., 1987).

In the present study we investigated the acute and chronic effects of MDMA on post-synaptic 5-HT2A receptors in the cortical brain regions of rats with known MDMA-induced 5-HT neurotoxic lesions. In parallel we investigated 5-HT2A receptor densities in the cerebral cortex of human MDMA users with \(^{123}\text{I}R_{91150}\) SPECT. In addition, the relation between synaptic 5-HT levels and 5-HT2A receptor densities was studied in rat brain. We hypothesized that acute effects of MDMA are associated with decreased 5-HT2A receptor densities (reflecting high synaptic 5-HT levels), whereas the long-term effects involve increased 5-HT2A receptor densities (reflecting low synaptic 5-HT levels).

Methods
Participants
Two groups of ecstasy users were compared with ecstasy-naive controls. Recruitment was through advertisements (local newspapers). Seventeen recent
ecstasy users ("MDMA group"), and 7 ex ecstasy users ("ex-MDMA group") were recruited. The eligibility criterion for both groups of MDMA users was a lifetime previous use of minimum 50 tablets. Since animal studies showed that down-regulation of 5-HT2 receptors may persist for at least 1 month after the last MDMA administration (Scheffel et al., 1992), the cut-off point of the drug-free interval in the ex-MDMA group was chosen at 2 months. Subjects selected were group-matched for gender and age, between 18 and 45 years, otherwise healthy, and with no psychiatric history.

All participants agreed to abstain from use of psychoactive drugs (including MDMA) for at least 1 week prior to the study, since MDMA has some affinity for post-synaptic 5-HT2A receptors (McKenna & Peroutka 1990), 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 enrolment. After testing urine samples, exclusion criteria were: a positive drug screen; pregnancy; a severe medical or neuropsychiatric illness that precluded informed consent, and a lifetime psychiatric disorder. Subjects were interviewed with the computer assisted 2.1. version of the Composite International Diagnostic Interview (CIDI) 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. After complete description of the study to the subjects, written informed consent was obtained from all participants.


Radiolabeling of R91150 is described elsewhere by Busatto and co-workers (Busatto et al., 1997). Briefly, radiiodination by electrophilic substitution was performed on the 5-position of the methoxybenzamid e group of R91150, followed by high-performance liquid chromatographic (HPLC) and mini-column recovery to ensure high purity (Radionucleide Center, Vrije University, Amsterdam, the Netherlands). [11]R91150 had a specific activity > 185 MBq/nmol and a radiochemical purity of > 97%.

SPECT imaging

For SPECT scanning the Strichman Medical Equipment BioX 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 15 slices, (acquired in a 128 x 128 matrix), 3 minutes scanning time 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 [11]R91150, a time when specific binding is maximal and stable for up to 8 h following injection (Busatto et al., 1997).

For binding analysis of [11]R91150, 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. Co-registration of MR and SPECT images was performed using the Hermes Multi Modality software package (Nuclear Diagnostics, Stockholm, Sweden). The template, including ROIs for the frontal-, parietal-, and occipital cortex, was placed on the three highest consecutive SPECT slices, by an investigator unaware of the participant's history. An additional template was constructed with a ROI for the cerebellum.

Radioactivity estimates in the cortical ROIs were assumed to represent 'total' ligand binding (specific + non-specific binding) + free ligand. The uptake in the cerebellum, presumed free from 5-HT2A receptors (Pazos et al., 1987), was used as reference for background activity (non-specific binding + free ligand). ROI/cerebellum activity ratios were calculated as a relative measure of specific binding to 5-HT2A receptors for a given brain region.

Experiments in rodents

Animals and drug treatment

Male Wistar rats (obtained from Broekman Institute B.V., Someren, the Netherlands) weighing 200-250 g were used in these experiments. The animals were housed in a temperature- and humidity controlled environment with food and water available ad lib. Groups of rats (n = 4-5) were given either a vehicle or a neurotoxic regimen of MDMA which consisted of a subcutaneous dose of 10 mg/kg (in 0.5 ml saline) MDMA given twice daily for 4 consecutive days. (±)Methylenedioxymethamphetamine hydrochloride (certified reference compound, purity > 98.9%) was obtained from the Netherlands Forensic Institute (Rijswijk, the Netherlands). All experiments involving procedures using animals were approved by the local Animal Care Committee.
5-HT2A receptor binding studies
MDMA treated rats were injected intravenously with approximately 1.85 MBq \[^{11}R91150\] at 6 h, 3 and 30 days after last treatment. Control rats were injected 30 days after treatment with a vehicle. One hour after injection of \[^{11}R91150\], animals were killed by bleeding via heart puncture under ether anesthesia. The brains were quickly removed, dissected into the following ROIs: frontal cortex, parietal cortex, occipital cortex, cerebellum, and weighed. ROIs located in the left hemisphere were used for \[^{11}R91150\] binding analysis, those in the right hemisphere for biochemical analysis (described below). \[^{11}R91150\] in each region was assayed in a gamma counter. The data were corrected for radioactivity decay back to the time of preparation of the injection syringes in order to compare relative concentrations in the tissues taken and to relate the results to the injected dose. The amount of radioactivity was expressed as a percentage of the injected dose, multiplied by the body weight per gram tissue weight (\%/ID \times \text{kg/g tissue}) as earlier described (Rijks et al., 1996). The cerebellum was used as a reference region for the estimation of free and non-specifically bound radioligand. ROI/cerebellum activity ratios were calculated as a relative measure of specific binding to 5-HT2A receptors for a given brain region by subtraction radioactivity in cerebellum from total radioactivity in the ROI.

Biochemical measurements
Immediately after dissection tissue samples were frozen in liquid nitrogen and stored at -80°C until analyzed. The tissue samples were homogenized in 0.2N perchloric acid and centrifuged at 10000 g for 10 minutes. An aliquot of the supernatant was analyzed with electrochemical detection using an Antec-Decade electrochemical detector equipped with a Hyref electrochemical flow cell at 0.6 Volt. Separation of 5-HT and 5-HIAA from other electrochemical active compounds was achieved by injecting samples onto a Waters Symmetry C18 HPLC column (4.6 x 250mm) protected by a Symmetry C18 guard column (4.6 x 10 mm). The mobile phase consisted of 0.1M sodium acetate, 0.18M ethylenediaminetetraacetic acid, 1 mMol/l sodium octanesulfonic acid, 160mM l methanol, pH = 4.55, pumped at a flow rate of 0.8 ml/min. Peak area's following injection of 50 ml samples were collected within Waters Millennium 32 chromatographic software. The concentration of 5-HT and 5-HIAA was calculated using dihydroxybenzylamine as an internal standard and then expressed as pg/mg protein.

Statistics
Differences between the three groups in the SPECT study with regard to demographic variables and other drug exposure were analyzed using ANOVA with Bonferroni post hoc analysis. Differences in characteristics of MDMA use between both MDMA using groups were studied with the Student t-test. We tested the main effect of MDMA on \[^{11}R91150\] binding ratios and biochemical measurements in the 3 cortical brain regions by general linear model-based multivariate ANOVA (MANOVA), taking possible correlations between brain regions studied and multiple comparisons into account. In the SPECT study age and gender were used as covariates. If MANOVA revealed a significant group effect, we investigated differences in regional \[^{11}R91150\] binding ratios and biochemical measurements by one-way ANOVA and Dunnet's post hoc analysis.

The relationship between mean cortical \[^{11}R91150\] binding ratios and extent of 5-HT depletion (expressed as % of control level) obtained in MDMA-treated rats was investigated with Spearman's rank correlation, since it has the advantage that is does not specifically assess a linear association but a more general association. The extent of 5-HT depletion at which up-regulation of 5-HT2A receptors was observed (control mean + 2 SD) was determined from a scatter plot. In the SPECT study Spearman's rank correlation analysis was also performed between overall cortical \[^{11}R91150\] binding ratios and extent of previous MDMA use and duration of abstinence from MDMA. The chance of a type I error was set at 0.05 using two-tailed tests of significance. All data were analyzed using SPSS version 9.0.

Results
Human subjects
Demographic variables are listed in Table 1. MDMA users were slightly but significantly older than controls and ex-MDMA users. Recreational use of drugs other than MDMA was comparable between the different groups. Apart from the anticipated differences between the MDMA subgroups due to inclusion criteria, no significant differences were observed. In the MDMA and ex-MDMA group participants had used on average 224 and 271 tablets of MDMA, respectively (Table 1).

Participants had not used MDMA on average 3.3 and 19.6 weeks prior to this investigation, respectively.

SPECT imaging
A significant group effect was observed (F = 4.8, df = 6, p < 0.01). In all brain regions studied, \[^{11}R91150\]
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Table 1. Demographics, characteristics of MDMA use and other recreational drug exposure

<table>
<thead>
<tr>
<th></th>
<th>Controls n = 11</th>
<th>MDMA n = 17</th>
<th>ex-MDMA n = 7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>5/6</td>
<td>12/5</td>
<td>5/2</td>
</tr>
<tr>
<td>Age</td>
<td>22.5 (2.7)</td>
<td>27.9 (3.8)</td>
<td>22.7 (4.6)</td>
</tr>
<tr>
<td>Education (years)</td>
<td>14.8 (4.4)</td>
<td>17.7 (3.5)</td>
<td>14.0 (5.5)</td>
</tr>
<tr>
<td>DART-IQ</td>
<td>96.4 (5.6)</td>
<td>92.8 (8.2)</td>
<td>86.2 (11.5)</td>
</tr>
<tr>
<td>MDMA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration of use (years)</td>
<td></td>
<td>5.9 (2.9)</td>
<td>4.6 (2.4)</td>
</tr>
<tr>
<td>Usual dose (tablets)</td>
<td></td>
<td>2.2 (1.7)</td>
<td>1.1 (2.1)</td>
</tr>
<tr>
<td>Lifetime exposure (tablets)</td>
<td></td>
<td>224 (311)</td>
<td>274 (238)</td>
</tr>
<tr>
<td>Time since last dose (weeks)</td>
<td></td>
<td>3.3 (2.4)</td>
<td>19.6 (11.6)</td>
</tr>
<tr>
<td>Last 3 months use of:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alcohol (no. consumptions)</td>
<td>43.1 (36.2)</td>
<td>30.1 (31.4)</td>
<td>30.3 (33.0)</td>
</tr>
<tr>
<td>Tobacco (no. cig)</td>
<td>16.5 (20.2)</td>
<td>52.0 (59.3)</td>
<td>78.5 (34.8)</td>
</tr>
<tr>
<td>Cannabis (no. joints)</td>
<td>3.0 (5.6)</td>
<td>20.5 (38.3)</td>
<td>43.1 (36.6)</td>
</tr>
<tr>
<td>Cocaine (no. times used)</td>
<td>1.7 (0.2)</td>
<td>0.8 (0.4)</td>
<td>1.1 (0.9)</td>
</tr>
<tr>
<td>Amphetamine (no. times used)</td>
<td>-</td>
<td>1.5 (2.2)</td>
<td>0.3 (0.5)</td>
</tr>
</tbody>
</table>

Data are expressed in mean ± SD values

Significantly different from control and ex-MDMA group (p < 0.01 and p < 0.02, respectively; ANOVA, Bonferroni post hoc test)

Significantly different from MDMA group (p < 0.01; Student t-test)

binding ratios were lower in recent MDMA users compared to control subjects, and higher in ex-MDMA users as compared to the control and MDMA-group (Figure 1).

Figure 1. Specific [123I]R91150 binding ratios in controls, recent MDMA users and ex-MDMA users as measured in the three cortical brain regions studied (control: ■; MDMA: ■; ex-MDMA: ■). The results are shown as mean ± S.E.M.

* Statistical significant difference in binding ratio compared to controls.

An ANOVA analysis demonstrated that [123I]R91150 binding ratios were significantly lower in all cortical brain regions studied of recent MDMA users as compared to controls (frontal- and parietal cortex: p < 0.01; occipital cortex: p = 0.04). Interestingly, [123I]R91150 binding ratios were significantly higher in the occipital cortex of ex-MDMA users as compared to controls (p = 0.04). The covariance effects of age and gender were not significant (p = 0.21 and p = 0.66, respectively).

A significant positive correlation was observed between cortical 5-HT2A receptor binding (mean binding ratios of the three brain regions studied) and duration of abstinence from MDMA (p = 0.57, p < 0.01) but not between extent of previous MDMA (p = 0.10, p = 0.66).

Experiments in rats

5-HT2A receptor binding studies

MANOVA demonstrated a significant group effect (F = 16.2, df = 3, p < 0.01). An ANOVA analysis demonstrated that in rats with a survival time of 6 h, [123I]R91150 binding ratios were significantly lower compared to control rats in all cortical brain regions studied (Figure 2). [123I]R91150 binding ratios in rats that were sacrificed 3 days after last MDMA treatment were lower compared to binding ratios in control rats,
which reached statistical significance in the occipital cortex (p = 0.04) only. Binding ratios of rats with a 30 day survival period were comparable or higher compared to control rats. In this group of rats, [3H]Rg1150 binding ratios reached statistical significance in the frontal cortex (p = 0.04; Figure 2), a time point when 5-HT and 5-HIAA levels were shown to be reduced by approximately 90% (Figure 3).

Biochemical measurements

MANOVA revealed a significant main effect when 5-HT and 5-HIAA levels were studied (F = 53.9, df = 3, p < 0.01; F = 16.6, df = 3, p < 0.01, respectively). Data are presented in Figure 3. An ANOVA analysis demonstrated that in rats that were sacrificed 6 h after their last treatment with MDMA, 5-HT levels were significantly lower (-29%) in the frontal cortex and occipital cortex (-60%) when compared to control rats. 5-HIAA depletions paralleled those of 5-HT in all brain regions studied (on average -46%) of rats with a 6 h survival period. In rats with a 3 day survival period, 5-HT and 5-HIAA levels were significantly reduced when compared to control rats (approximately -50%) with the exception of 5-HT levels measured in the parietal cortex.

In rats with a 30 day survival period, 5-HT and 5-HIAA levels were significantly lower in all cortical brain regions studied when compared to controls: both 5-HT and 5-HIAA levels were reduced by on average -90%. The 5-HT level at which statistically higher cortical 5-HT2A receptor densities were observed was determined from a scatter plot (Figure 4).

It was calculated that reductions of at least 80% are needed to induce a statistical significant increase in post-synaptic 5-HT2 receptors.

Discussion

In the present study we observed significantly higher 5-HT2A receptor densities in the occipital cortex of ex-MDMA users, while 5-HT2A receptor densities were significantly reduced in all cortical brain regions studied in recent MDMA users when compared to control subjects. These findings are in line with our hypo-
the most characteristic acute effects of MDMA from presynaptic vesicles. For instance, it has been shown that in dialysates of rat frontal cortex 5-HT levels are increased 8-fold 20-40 minutes after administration of 3 mg/kg MDMA i.v. (Gartsid et al., 1997). 5-HT levels were still increased 3-fold 2 h after administration. A recent [14C]methyl-tryptophan positron emission tomography (PET) study, which assess brain 5-HT synthesis, showed that the rate of 5-HT synthesis in dog brain was six times greater 1 h after MDMA administration than the baseline (before MDMA synthesis (Nishisawa et al., 1999).

Following the acute 5-HT releasing effect of MDMA is a subchronic down-regulating effect of 5-HT2A receptors. Our data suggest that this process is present in human and rat brain although in rats the reduction is less than that after 6 h and only significant in the occipital cortex. In another study, 5-HT2 receptor densities in MDMA-treated rats were reduced for at least 7 days in the prefrontal cortex (Scheffel et al., 1992). It seems likely that the presently observed low cortical 5-HT2A receptor densities in recent MDMA users and rats studied at 3 days reflect down-regulation of these receptors.

Studying the chronic effects, we observed significant higher binding of [3H]R11510 in the occipital cortex of ex-MDMA users, and frontal cortex of rats treated with MDMA 30 days previously, possibly reflecting a compensatory up-regulation due to low synaptic 5-HT levels. We previously reported a trend of increased cortical 5-HT2A receptor densities in MDMA users with a long abstention period (Reneman et al., 2000b). We speculated that in these subjects loss of 5-HT neurons resulted in low synaptic 5-HT levels, leading to up-regulation of 5-HT2 receptors. The present data obtained in rats further support this hypothesis. A strong and positive association between extent of cortical 5-HT depletion and [3H]R11510 binding ratios was observed. It is well known that abuse of MDMA eventually leads to loss of serotonergic axons and axon terminals. For example, cortical 5-HT levels in MDMA-treated monkeys were still significantly reduced 13 months after treatment (Hatzidimitriou et al., 1999). There is also considerable evidence from the literature that post-synaptic 5-HT receptors manifest a compensatory up-regulation after depletion of synaptic 5-HT. In rat brain, 5-HT receptor densities are elevated by pretreatment with 5,7-dihydroxytryptamine (5,7-DHT, a toxin which selectively destroys 5-HT neurons), p-chlorophenylalanine (PCPA, an inhibitor of TRP hydroxylase, the rate-limiting enzyme in 5-HT synthesis), or reserpine (a pre-synaptic monoamine depleting agent) (Sharif et al., 1980; Stockmeier & Kellar 1980). Also, in human subjects a compensatory up-regulation of 5-HT receptors is thought to occur after acute tryptophan depletion (Price et al., 1997).

However, there are several older studies investigating post-synaptic 5-HT2 receptor densities in animals with 5-HT lesions induced by MDMA, 5,7-dihydroxytryptamine (5,7-DHT) or bisection of the cortex, that
do not report up-regulations of 5-HT2 receptors (Eison et al., 1989; Fischette et al., 1987). This is probably due to the relative short survival time of animals that were studied in these reports: generally 1 week. Our findings suggest that at these early time points 5-HT levels are not depleted to the extent that up-regulation occurs.

We are aware that the presently observed increased binding of [125I]Nog50 in specific cortical regions in rat and human brain may not only reflect a compensatory up-regulation of post-synaptic 5-HT2A receptors due to low 5-HT levels, but may also result from an increased availability of the radiotracer to bind to 5-HT2A receptors, due to removal of endogenous 5-HT and lower synaptic 5-HT concentration. Therefore, we conclude that the increased binding possibly reflects a combination of a compensatory up-regulation and increased availability to bind. Either way, these mechanisms are likely to occur only in situations of synaptic 5-HT depletion, such as caused by MDMA-induced 5-HT neurotoxicity. In future studies, it would be of interest to examine the relative contribution of each mechanism.

In the present study we calculated that reductions in cortical 5-HT concentrations of at least 80% are needed to induce up-regulation of post-synaptic cortical 5-HT2A receptors. In line with this, up-regulation of D2 receptors is rather consistently observed following treatment with the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which has been attributed to post-synaptic compensatory mechanisms that occur in response to deficiencies in synaptic dopamine concentrations. Also in human brain, PET or autopsy studies have shown an up-regulation of D2 receptors in the striatum of patients with early Parkinson's disease (Brooks et al., 1992; Rinne et al., 1990). Studies in MPTP-treated monkeys have shown that post-synaptic up-regulation of striatal D2 receptors does not occur until loss of striatal DA levels exceeds 90% (Elsworth et al., 1998; Falardeau et al., 1998). To the extent that our animal data can be extrapolated to humans, one may speculate that the observed up-regulation of 5-HT2A receptors in the occipital cortex of ex-MDMA users reflects a 5-HT depletion of approximately 80%. However, interspecies differences in the MDMA metabolism have been described and should be kept in mind when extrapolating effects from rats to humans. The dose of MDMA (10 mg/kg) used in the present work is closer to the doses used by humans for recreational purpose, but the multiple injections protocols applied here might not represent well the patterns used by humans. However, humans usually take a booster dose several hours after the first one, which could be related to the multiple injections in the present protocol. These data do indicate the need to perform similar studies, as the present one, in non-human primates, since primates have been shown to be more sensitive to MDMA than rats, and are thought to approach the effects of MDMA in humans more closely.

We observed regional differences in the effects of MDMA on rat and human brain. With respect to the effects of MDMA in rat brain, Battaglia and co-workers (1991) have shown that the cingulate, entorhinal, and parietal cortex showed the most extensive depletion in 5-HT uptake sites (i.e., > 90%) 2 weeks after MDMA treatment. It has been postulated that 5-HT neurons may exhibit differential neurotoxic sensitivities to MDMA based on the morphologic differences between 5-HT fibers originating from dorsal versus median raphe nuclei (Mamounas et al., 1991). 'Fine' 5-HT neurons arising from dorsal raphe cell bodies have been reported to be vulnerable to MDMA, although 'beaded' 5-HT neurons from median raphe cell bodies are insensitive to the neurotoxic effects of amphetamine analogs (O'Hearn et al., 1988). The distribution of fine and beaded 5-HT neurons in various brain regions is discrete. Accordingly, innervation of cerebral cortex by fine 5-HT neurons is greatest in frontal cortex and is least in posterior cortical regions such as occipital cortex. In line with this, we observed an increased density of 5-HT2A receptors in the frontal cortex, but not in the occipital or parietal cortex of rats treated with MDMA 30 days previously. Although little regional differences were observed in 5-HT and 5-HIAA levels (in all three cortical brain regions depletions of approximately -90% were observed), the observed up-regulation of 5-HT2A receptors in the occipital-, but not frontal- cortex of ex-MDMA users seems to contradict the contention of the existence of MDMA-sensitive and -insensitive 5-HT neurons. At present there is no apparent explanation for the discrepancy. Species differences have been shown to play an important role in MDMA-induced 5-HT neurotoxicity. Also, the ex-MDMA users were studied after a relatively long MDMA-free period (on average 18 weeks) compared to the MDMA-treated rats (after 30 days). In line with the present findings in ex-MDMA users, the occipital cortex has been shown to be particularly sensitive to MDMA's neurotoxic effects in non-human primates, since the most severe 5-HT depletion was observed in this brain region 13 months after MDMA treatment (Scheffel et al., 1998). These 5-HT neurotoxic changes have been shown to persist for up to seven years after MDMA administration, and were particularly evident in the pyriform and visual cor-
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text, whereas brain regions proximal to the rostral raphe nuclei show evidence of complete recovery. It is thought that the distance of the 5-HT terminal field to the rostral raphe nuclei influences recovery of 5-HT axons after MDMA injury.

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 non-users underlie differences in 5-HT<sub>A</sub> receptor densities. For instance, people with low 5-HT<sub>A</sub> receptor densities may be predisposed to use MDMA. Second, this study was performed using small samples. Nevertheless, 5-HT<sub>A</sub> receptor densities are unequivocal and these data do provide useful preliminary evidence of the relationship between 5-HT<sub>A</sub> receptor densities and 5-HT levels in animals with known 5-HT neurotoxic lesions and their relevance to findings in human MDMA users as revealed by SPECT. Third, as with all retrospective studies, it is impossible to determine exactly what drug at what dose was taken, and to ensure abstention from MDMA, other than self-report. Furthermore, the composition of tablets sold as ecstasy do often not contain MDMA, but other compounds as well, such as amphetamine, cocaine, opiates, caffeine etc. Interestingly, 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 ecstasy was in agreement with the drug-urine test (Van de Wijngaarde et al., 1997). In future studies, hair-sample analysis would be a useful way to assess more appropriately what drug was taken at what time and to ascertain previous use of MDMA. Fourth, we presently observed reduced 5-HT<sub>A</sub> receptor binding in recent MDMA users and MDMA-treated rats, while increased binding in ex-MDMA users and rats with a long survival time. We suggest that the reduced and increased binding represents down and up-regulation of 5-HT<sub>A</sub> receptors, respectively. However, as opposed to the currently used in vivo and ex-vivo binding assays, traditional homogenate studies must be conducted to provide more compelling evidence that the presently findings indeed represent down and up-regulation of 5-HT<sub>A</sub> receptors. Nevertheless, there is considerable evidence from the literature showing that post-synaptic 5-HT<sub>A</sub> receptors manifest a down-regulation in situations with high levels of synaptic 5-HT, while 5-HT<sub>A</sub> depletion has been associated with a compensatory up-regulation of 5-HT<sub>A</sub> receptors. Kellar et al., 1981; Peroutka & Snyder 1980a; 1980b; Price et al., 1998; Sharif et al., 1989; Stockmeier & Kellar 1983). Fifth, since we did not simultaneously perform cerebral blood flow studies in our subjects, the possibility that the changed<sup>[11]R</sup>91150 binding observed in MDMA users partly reflects flow changes (and consequently changes in non-specific binding) cannot be totally excluded, especially since alterations in cerebral blood flow (Chang et al., 2000) and cerebral blood vessel volume (Reneman et al., 2000) have recently been observed in users of MDMA. Sixth, the present findings may be partially explained by differences in metabolism of the radiotracer by MDMA users compared to controls. If indeed MDMA users metabolize<sup>[11]R</sup>91150 differently than controls we would have observed similar binding ratios in the recent and ex-MDMA users, which was not the case. Another potential explanation for the present findings may be related to age. PET and SPECT studies have demonstrated an age related reduction in 5-HT<sub>A</sub> receptors (Ito et al., 1998; Kakuichi et al., 2000; Baeken et al., 1998). To that purpose, we analyzed our results with age as a covariate. Therefore, it seems unlikely that the present findings are related to age. However, future studies should be conducted to investigate to what extent these potential confounders account for the present findings. Finally, although most of the MDMA users in our study had more experience with other recreational drugs than control subjects, this was statistically not significant. Also, since a strong and positive association was observed between cortical 5-HT<sub>A</sub> receptor densities and extent of previous MDMA use, it seems likely that the findings of the present study should be attributed to MDMA.

In conclusion, we have demonstrated that MDMA induced 5-HT neurotoxic lesions induced in rat brain are associated with a time-dependent reduction and recovery of 5-HT<sub>A</sub> receptors. We observed higher densities of 5-HT<sub>A</sub> receptors in the frontal cortex of rats in which 5-HT concentrations were reduced by more than 90%. The density of 5-HT<sub>A</sub> receptors was strongly associated with the degree of 5-HT depletion. Therefore we suggest that the higher 5-HT<sub>A</sub> receptor densities observed in the occipital cortex of human ex-MDMA users are likely to reflect a compensatory up-regulation due to 5-HT depletion, possibly caused by MDMA induced 5-HT neurotoxic lesions.

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