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Acute effects of 3,4-methylenedioxymethamphetamine on brain serotonin synthesis in the dog studied by positron emission tomography

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Abstract

The influence of an acute dose (2 mg/kg; i.v.; infused over 10 min) of 3,4-methylenedioxymethamphetamine (MDMA; Ecstasy) on the brain serotonin synthesis in the dog was assessed using α[^{11}C]methyl-L-tryptophan and positron emission tomography. The rate of serotonin synthesis measured 1 h after injection of MDMA was six times greater than the base line (before MDMA) synthesis. Five hours after the MDMA injection, serotonin synthesis was about one half that at the base line, and about one thirteenth of the synthesis at 1 h after MDMA. A large increase seen 1 h after MDMA probably relates to the large release of serotonin by MDMA and reflects an attempt of the serotonergic system to replenish released serotonin. This probably correlates with the mood changes reported by humans after MDMA intake. Decrease observed 5 h after MDMA, in part, probably relates to the inhibitory effects of the released serotonin, which could act on the activity of tryptophan hydroxylase directly or indirectly via other monoaminergic systems (e.g. dopaminergic).

1. Introduction

MDMA (3,4-methylenedioxymethamphetamine, Ecstasy) has been shown to deplete brain serotonin (5-HT) and its metabolite 5-hydroxyindolacetic acid (5-HIAA) in a dose related manner (Stone et al., 1996), and reduces the number of 5-HT transporter sites (De Souza et al., 1990). Some of these effects occur soon after the drug is taken and could last for a rather long period of time. It has also been shown that MDMA increases dopamine synthesis (Nash, 1990), releases dopamine from its storage (Koch and Gallaway, 1997), and after depletion of dopamine in the rat brain before MDMA injection, there is a reduction in the MDMA serotonergic neurotoxicity (Brodkin et al., 1993). In the neuronal tissue culture of human serotonergic cells MDMA produced programmed death (Simantov and Tauber, 1997). In addition, it was shown that neurotoxicity in rats where only terminals are destroyed, is somewhat different from that in non-human primates since in the latter cell bodies are also affected (Ricaurte et al., 1988). These discrepancies are probably, at least in part, related to a different metabolic disposition of this drug in different species (Caldwell et al., 1976).

MDMA is an amphetamine analog often used as a recreational drug (Peroutka, 1990), despite substantial evidence of its neurotoxicity in rodents (Ricaurte et al., 1985; Commins et al., 1987; O'Hearn et al., 1988) and non-human primates (Ricaurte et al., 1988; Insel et al., 1989), and recently an indirect evidence showing possible toxicity in humans (McCann and Ricaurte, 1993). The exact mechanism(s) of this neurotoxicity has(have) not been established as yet, but the possible involvement of several monoaminergic systems has been proposed. MDMA acutely induces release of serotonin from its vesicular and cytosolic storage sites, possibly by a direct interaction with vesicular and neuronal uptake sites (Gudelsky and Nash, 1996; Rudnick and Wall, 1992). It seems that the release of dopamine is somehow linked to the neurotoxicity of MDMA (Nash et al., 1990; Lew et al., 1996). Methylamphetamine (MDA), an in vivo metabolite of MDMA (Lim and Foltz, 1989, 1991), has been shown to produce in rat caudate nucleus 6-hydroxydopamine (Seiden and Vosmer, 1984), a well known neurotoxic dopamine derivative.

The main aim of the present study was to investigate the effects of MDMA on brain 5-HT synthesis 1 and 5 h

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after i.v. injection of MDMA. The importance of the synthesis stems from the fact that altered synthesis would result in different amounts of 5-HT available for release. To obtain proper dynamic information of the MDMA effects, measurements were done at different times after MDMA injection in the same dog utilizing positron emission tomography (PET), and ^{11}C-labelled analog of tryptophan, α-methyl=L-tryptophan (α-MTrp). Labelled α-MTrp was recently shown to be a good tracer for the brain serotonergic system (Diksic et al., 1990, 1991; Cohen et al., 1995; Mück-Šeler et al., 1996) as well as the measurements of the regional brian 5-HT synthesis in humans (Nishizawa et al., 1997, 1998; Muzik et al., 1997; Chugani et al., 1998; Okazawa and Diksic, 1998).

2. Materials and methods

Experiments described here were approved by the Institutional Animal Care Committee and conformed to the Guide to the Care and Use of Experimental Animals (Canadian Council on Animal Care, 1993).

MDMA used in the study was obtained from the Drug Directorate of the Government of Canada as a racemic mixture. The drug was administered at a dose of 2 mg/kg (i.v.). The dog was obtained through the McGill University Animal Resources Centre. The dog was selected as an experimental animal because it is probably the smallest animal for which we can obtain good PET images of the brain with the scanner used. The dog was intubated about 1 h before surgical implantation of arterial (femoral) and two venous (femoral vein on each side) catheters, and pretreated with atropine (0.04 mg/kg; i.m. and 1 ml/9 kg; i.m.) and was on an animal respirator using halothane anesthesia with periodic adjustments of the rate of breathing, assessment of the heart beat, and periodic analysis of blood gases. If needed anesthesia was supplemented with Nembutal (60 mg). At the end of the experiment the dog was killed by an i.v. injection of 200 ml of potassium chloride solution.

The dog was positioned in the PET scanner (Scanditronix) to insure imaging of the entire brain. The dog was positioned in such a way that the top slice was imaged in the front of the dog brain. The body temperature was monitored with a rectal probe connected to an automatically controlled heating blanket. Respiration rate was adjusted, when needed, to keep blood gases within the physiological range. α-[^{11}C]Methyl-L-tryptophan (α-[^{11}C]MTrp) was prepared by the method described previously (Mzengeza et al., 1995), and had a specific activity above 100 Ci/mmol at the time of injection. About 10 mCi of α-[^{11}C]MTrp was injected and the scanning was started at the beginning of tracer injection. The tracer was injected over a 2-min period and the arterial blood samples were taken at progressively increasing time periods (Diksic et al., 1991). The blood samples were centrifuged as soon as possible at 12,500 g for 2 min in a refrigerated centrifuge (5°C), and the radioactivity of the plasma was measured with a NaI(Tl) well detector connected to a multichannel analyzer. After correction for decay and the scanner-NaI(Tl) detector cross calibration efficiency coefficient, the plasma samples were used as the plasma input function in the calculation of the 5-HT synthesis rates (see below). Additional plasma samples were taken for the measurement of plasma concentrations of total and free tryptophan (Trp). Free Trp was measured in the ultrafiltrate of plasma obtained by filtering plasma through a Millipore membrane with 10,000 MW cut-off. Plasma total Trp was measured in the deproteinized plasma [plasma-trichloroacetic acid (20%) 1 : 1]. Plasma amino acids were measured by HPLC using reverse-phase column and a post-column derivatization with o-phthalaldehyde (Takada et al., 1993).

Images were reconstructed using 8 mm filter, and were corrected for attenuation by using transmission scan obtained by a rotating ^{68}Ge–^{68}Ga source. Image acquisition time was 0.5 min for the first 3 min, then 1 min until 10 min, 2 min until 20 min, and 5 min after to the end of scanning which lasted 60 min. The MDMA (2 mg/kg) was administered at the end of the first (base line) scan by i.v. injection over 10 min. The second scan was done 1 h after the MDMA injection and lasted 60 min. The third scan was done 5 h after the MDMA injection, and also lasted 60 min. The brain time radioactivity curves were obtained from the serial images on at least three different levels and were used for the rate of serotonin synthesis calculation. The time radioactivity curves were fitted to the theoretical tissue time radioactivity curve (Eq. 1) represented by an equation derived from the three-compartment model (Diksic et al., 1991).

$$C_T^*(T) = K^* \cdot \int_0^T C_P^*(t)\,dt + V \int_0^T e^{V2} C_P^*(t) \cdot dt \quad (1)$$

Here K^* (ml/g/min) is the plasma to brain clearance constant which represents the rate at which the trace is trapped in the unit volume of the brain irreversible compartment, $C_P^*(t)$ [nCi/ml] is the plasma tracer radioactivity concentration as a function of time from the time of the tracer injection, $V2$ is a variable representing the sum of the first order rate constants for the transfer of tracer from tissue back to plasma (k_2^*; min^{-1}) and from the tissue precursor pool to the tissue irreversible compartment (k_3^*; min^{-1}), and V is variable consisting of the model rate constants (Diksic et al., 1991). Using SAAM II (SAAM Institute, University of Washington, Seattle, WA) the tissue time radioactivity curves were fitted to obtain blood to tissue net clearance K^* (ml/g/min), which was used in the calculation of brain serotonin synthesis. Brain 5-HT synthesis rates were calculated as: $R = K^* C_p^{Trp}/LC$ (nmol/g/min). C_p^{Trp} is the plasma free Trp concentration (nmol/ml) and LC is the lumped constant.

We used $LC = 0.42$, the value measured in vivo in the rat brain (Vanier et al., 1995). The use of the plasma free Trp in this equation has been justified before (Diksic et al., 1990, 1991, 1995; Nagahiro et al., 1990). Images of the synthesis rates shown in Fig. 4 were calculated as the slope of the linear portion of the tissue volume of distribution as a function of exposure time (Nishizawa et al., 1997; Okazawa and Diksic, 1998) by using images acquired after 20 min on the pixel-by-pixel bases. Using these values of K^* and the intercepts obtained in the linear fit, the synthesis images were calculated according to the above-mentioned equation. This approximation was used to avoid instability of the exponential fits done pixel-by-pixel using Eq. 1 (Okazawa and Diksic, 1998). Using this approximation rather than full operational equation is also justified by reports that there is no large difference between K^*-s calculated by this approach and those obtained by the fit to Eq. 1 (Nishizawa et al., 1998; Okazawa and Diksic, 1998).

Statistical comparison was done using ANOVA with Bonferroni correction when applicable.

3. Results

There was no significant ($P > 0.05$; ANOVA) change in the plasma concentration of amino acids known to compete with Trp for blood-brain barrier transport, and the values were within physiological levels. There was no significant ($P > 0.05$; ANOVA with Bonferroni correction) change in the plasma total Trp (Table 1), between three experiments, or plasma free Trp between the first and second experiment, but there was a significant ($P < 0.05$; ANOVA with Bonferroni correction) increase in the plasma free Trp in the study done 5 h after MDMA (third study).

The plasma time-radioactivity curves obtained in the dog before MDMA, and 1 and 5 h after the MDMA injection are shown in Fig. 1A. It should be noted that the plasma time radioactivity curves after the MDMA injection are very similar in shape to those after the MDMA injection. It seems that there was no influence of the MDMA on the total body clearance and/or the rate of the body α-[^{11}C]MTrp distribution. In contrast, a visual examination of the brain time radioactivity curves (Fig. 1B) suggests a major influence of MDMA on the shape of the brain time radioactivity curves. A small plasma time radioactivity curve time shift of 0.15 min was used to correct for a difference in the arrival of the bolus to the brain and the point of blood sampling. Fitting of the experimental data to the model operational equation (Eqn 1) to obtain the brain uptake constant K^* (ml/g/min) produced excellent agreement between fitted and experimental curves (Fig. 1B). A set of PET images obtained in dog brain between 1 and 2 h after the injection of MDMA is shown in Fig. 2. In the insert in Fig. 2, a plot of the volume of distribution (y-axis) as a function of exposure time (x-axis) is given. From the shape of the graph in the insert it seems that an apparent steady state has been achieved approximately 25–30 min (clock time) after tracer injection. The PET images representing the rates of serotonin synthesis obtained in the dog brain before, 1 and 5 h after the MDMA injection are presented in Fig. 3. Since all the images are shown with the same scale, visual examination provides a feeling for the synthesis range in the different experiments.

The calculated brain serotonin synthesis rates at different stages after the MDMA injection are given in Table 1. The rate of 5-HT synthesis was about six times greater 1 h after the MDMA injection as compared to the rate before the MDMA injection. Five hours after MDMA injection, the 5-HT synthesis rate was about two times lower than at the baseline (before MDMA injection) or 13 times lower than at 1 h after MDMA injection. MDMA did not have any influence on the plasma total Trp (Table 1), but it seems to have had some

Table 1
Rates of serotonin synthesis measured in dog brain and the plasma tryptophan concentrations, before and at different times after MDMA (2 mg/kg; i.v.; over 10 min) injection. The rates are given as mean ± standard deviation

Experiment (time after MDMA) Experiment	Plasma Trp[1] (nmol/ml)		
	Free	Total[4]	Serotonin synthesis rate (pmol/g/min)[2]
1 (pre-MDMA)	16.0 ± 1.8	46 ± 1.5	42 ± 3
2 (1 h after MDMA)	12.7 ± 0.3	48 ± 1.8	248 ± 5
3 (5 h after MDMA)	20.2 ± 0.2[3]	45 ± 2.7	18.7 ± 4.9

[1] The mean of at least three measurements.
[2] The mean of at least three slices taken through dog brain at different levels.
[3] Significantly ($P < 0.05$; ANOVA with Bonferroni correction) different from the first (pre-MDMA) and second (1 h after MDMA) studies.
[4] No significant difference in the concentration between different studies (ANOVA).

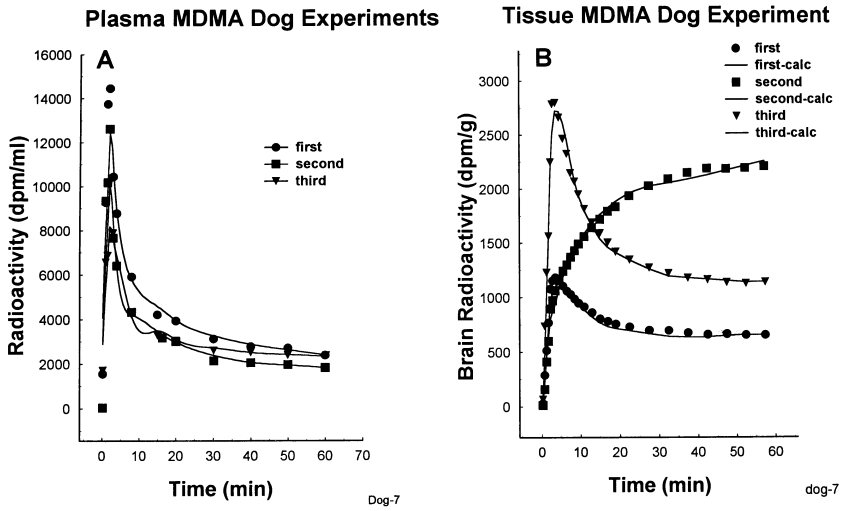

Fig. 1. (A) Plasma time radioactivity curves in a dog before the MDMA injection, and 1 and 5 h after the MDMA injection. Individual plasma radioactivities are shown as symbols (see graph legend for identification), and lines represent a least-squares fit to the experimental points; (B) a set of brain tissue time radioactivity curves obtained after an injection of about 10 mCi of α-[^{11}C]methyl-L-tryptophan. The tissue time radioactivity curves are obtained before (●), 1 (■), and 5 (▼) h after the MDMA injection. The model fitted curves are shown as solid lines.

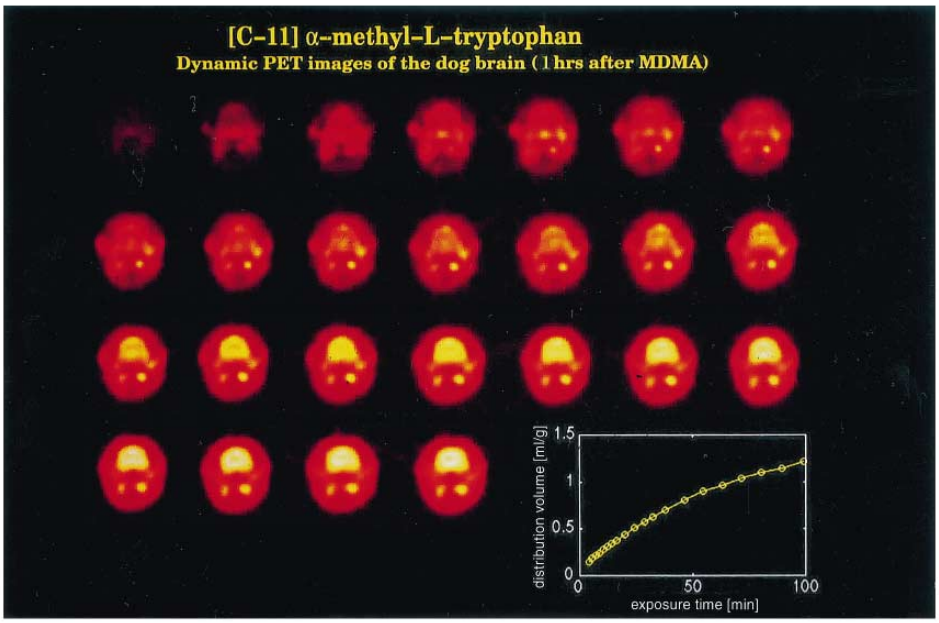

Fig. 2. A set of PET images obtained at different times after tracer injection. Images were obtained with different time durations (see Material and methods) for 60 min from the time of tracer injection. Tracer was injected 1 h after MDMA injection. In the insert a plot of the tissue distribution volume (ml/g; *y*-axis) is plotted as a function of the exposure time (min; *x*-axis). These images were used to obtain time-radioactivity curve shown in Fig. 1B.

effect on plasma free Trp. Plasma free Trp was decreased 1 h after MDMA, and increased 5 h after MDMA (Table 1), as compared to the baseline.

4. Discussion

This report represents the first study of MDMA on brain 5-HT synthesis in a living brain. PET measurements have great advantages in comparison to the measurements by autoradiography or immunocytochemistry, because they permit repeated measurements in the same animal reducing subject-to-subject variability. The present results demonstrate a direct influence of MDMA on brain 5-HT synthesis rates in a living dog using PET and α-[^{11}C]MTrp as the tracer. Because MDMA produces effects soon after it is taken in humans, it is important to understand drug effect(s) on the brain serotonergic system soon after

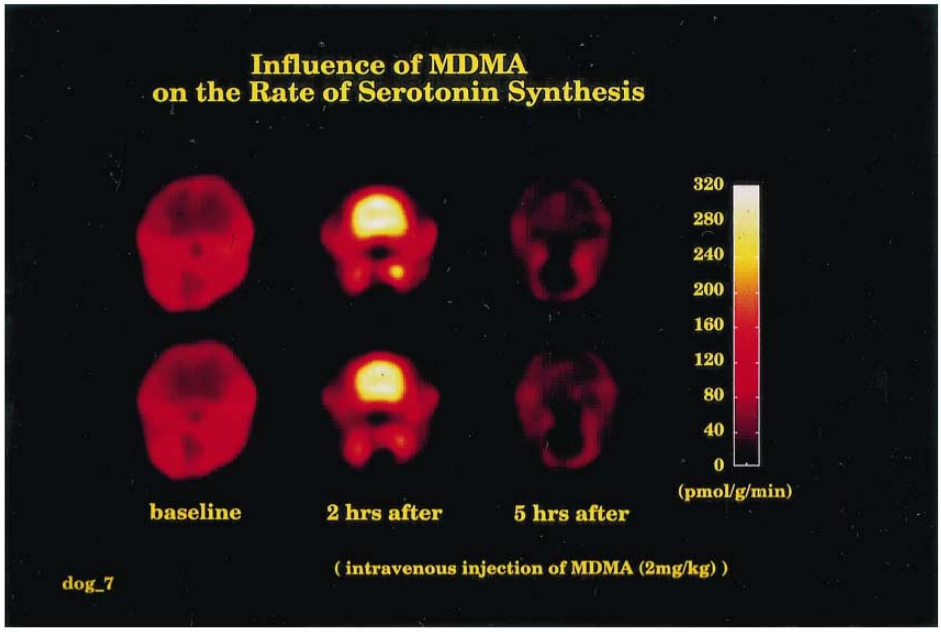

Fig. 3. Images of the serotonin synthesis rates obtained in dog brain before, 1 and 5 h after MDMA injection shown at two different levels. Note a large increase in 5-HT synthesis at an early time after MDMA, and a large decrease at a later time, in comparison to the synthesis before MDMA injection.

MDMA is taken, as well as several hours later. It has been reported that humans require a 'booster' dose approximately 4–6 h after the first dose (Downing, 1986; Green and Tolbert, 1986; Leister et al., 1992; Eisner, 1989; Steel et al., 1994). The results presented here clearly show that MDMA produces a large increase in brain 5-HT synthesis 1 h after treatment, which is probably related to the euphoric feelings reported by human abusers. Elevation of 5-HT synthesis are likely to result in large amounts of 5-HT release because MDMA releases and also prevents reuptake of 5-HT into vesicular storage. The high concentration of 5-HT present in the extravesicular pool (both intra- and extra-neuronal) seems to result in a profound inhibitory effect on 5-HT synthesis observed 5 h later. However, there is also the possibility that MDMA, and/or its metabolite, acts as an inhibitor of TPH; this would be in agreement with a reduction of TPH activity measured ex vivo (Schmidt and Taylor, 1987).

Five hours after the MDMA injection, 5-HT synthesis is reduced to about one half of the synthesis before the MDMA injection, and to one thirteenth of the synthesis 1 h after the MDMA injection. Since in the third study (5 h after MDMA), the plasma concentration of free Trp was significantly greater than those measured at the base line and 1 h after MDMA, the decrease in the 5-HT synthesis observed in the third study is probably attenuated by a significant increase in the plasma free Trp (Table 1). This notion stems from the fact that the plasma free Trp has been related to brain 5-HT synthesis (Salter et al., 1989; Bloxam and Curzon, 1978; Takada et al., 1993). The reason for this increase in the plasma free Trp is not obvious, but it could be due to the inhibition of the liver tryptophan pyrrolase, an enzyme known to regulate the plasma balance between free and bound Trp (Salter et al., 1989; Badawy et al., 1996). An alternative explanation could be that a release of corticosterone and other steroid hormones by 5-HT could displace Trp from the albumin binding sites. Actions of MDMA have been shown to occur via the release of 5-HT from vesicular storage, and prevention of its uptake by binding to the 5-HT transporter sites, as well as by replacing 5-HT inside vesicles (Rudnick and Wall, 1992; Schuldiner et al., 1993). A large increase in the synthesis observed 1 h after MDMA is probably related to an acute attempt of neurons to replenish vesicular 5-HT, especially that of the 5-HT readily releasable pool. A similar observation was obtained in acute experiments in rats given fenfluramine (Mück-Šeler and Diksic, 1996) and fluoxetine (Mück-Šeler et al., 1996). Even though the mechanism(s) of these drugs are not exactly the same as those of MDMA, 5-HT release and interference with 5-HT uptake are common modes of action of all these drugs. Since the activities of the brain serotonergic system have been related to the mood and depression, the reduction in the 5-HT synthesis several hours after MDMA could correlate with the observation in abusers of this drug that express

a need for a 'booster' dose 5–6 h after the first dose of MDMA (Downing, 1986; Green and Tolbert, 1986; Leister et al., 1992; Eisner, 1989; Steele et al., 1994). The reduction in 5-HT synthesis found 5 h after MDMA to about half of that determined at baseline is in agreement with a reduction of about the same magnitude found by in vitro measurements of TPH activity from rat brain treated with MDMA (20 mg/kg; Schmidt and Taylor, 1987). However, the increase seen in our experiments 1 h after MDMA, was missed because of the experimental design of Schmidt and Taylor (1987). In reality it would be hard to design ex vivo experiments which could mimic the in vivo design used in the present investigation.

Many investigators have described interactions between serotonergic and dopaminergic systems in the brain (Stone et al., 1988; Yamamoto et al., 1995; Simontov and Tomhen, 1997). Results have also been presented suggesting an increase in dopamine by MDMA (Stone et al., 1988; Koch and Galloway, 1997) and formation of 5,6-dihydroxytryptamine (Commins et al., 1987) and 6-hydroxydopamine (Seiden and Vosmer, 1984) known neurotoxins for serotonergic and dopaminergic neurons, respectively. On the bases of these investigations and results reported here, it is possible that MDMA induced neurotoxicity, is at least in part, induced by acutely increased 5-HT synthesis.

Since the most common route of the MDMA administration in humans is oral, and intraperitoneal or subcutaneous in laboratory animals, the use of intravenous injection needs to be emphasized. We used a dose of 2 mg/kg of the racemic mixture of MDMA, which corresponds to about 1 mg/kg of the more potent stereoisomer (S)-MDMA. Doses used in other laboratory animals and non-human primates of the racemic MDMA varied between 2.5 and 40 mg/kg given s.c. or i.p. The biological effects of MDMA are related to the integral of the plasma concentration of the drug, which could be influenced differently by the liver enzymes in different species (Steele et al., 1994; Colado et al., 1995; Tucker et al., 1994). We injected MDMA over a 10 min period (0.2 mg/min) into the femoral vein. Assuming a blood flow in the femoral vein of about 250 ml/min, the concentration of MDMA in the plasma was about 0.8 μg/ml, and this is approximately one half (Chu et al., 1996) of the concentration of MDMA in the plasma of a rat after injection of 20 mg/kg (s.c.).

In summary, we have shown for the first time in a living brain that shortly after MDMA injection there is a large increase in brain 5-HT synthesis, which could be related to the feelings described by the recreational uses of this drug. In addition, we have shown that several hours after MDMA, the rate of 5-HT synthesis falls far below the synthesis rate pre-MDMA (baseline). The latter finding can explain reports of abusers needing a 'booster' dose. The findings reported here could help to better understand the MDMA influence on behavior, and possibly provide some biological information regarding the euphoric effects reported by users.

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