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ARTICLE

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# Single oral doses of (±) 3,4-methylenedioxymethamphetamine ('*Ecstasy*') produce lasting serotonergic deficits in non-human primates: relationship to plasma drug and metabolite concentrations

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**Abstract**

Repeated doses of the popular recreational drug methylenedioxymethamphetamine (MDMA, '*Ecstasy*') are known to produce neurotoxic effects on brain serotonin (5-HT) neurons but it is widely believed that typical single oral doses of MDMA are free of neurotoxic risk. Experimental and therapeutic trials with MDMA in humans are underway. The mechanisms by which MDMA produces neurotoxic effects are not understood but drug metabolites have been implicated. The aim of the present study was to assess the neurotoxic potential of a range of clinically relevant single oral doses of MDMA in a non-human primate species that metabolizes MDMA in a manner similar to humans, the squirrel monkey. A secondary objective was to explore the relationship between plasma MDMA and metabolite concentrations and lasting serotonergic deficits. Single oral doses of MDMA produced lasting dose-related serotonergic neurochemical deficits in the brains of squirrel monkeys. Notably, even the lowest dose of MDMA tested (5.7 mg/kg, estimated to be equivalent to 1.6 mg/kg in humans) produced significant effects in some brain regions. Plasma levels of MDMA engendered by neurotoxic doses of MDMA were on the order of those found in humans. Serotonergic neurochemical markers were inversely correlated with plasma concentrations of MDMA, but not with those of its major metabolites, 3,4-dihydroxymethamphetamine and 4-hydroxy-3-methoxymethamphetamine. These results suggest that single oral doses of MDMA in the range of those used by humans pose a neurotoxic risk and implicate the parent compound (MDMA), rather than one of its metabolites, in MDMA-induced 5-HT neural injury.

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## Introduction

(±) 3,4-Methylenedioxymethamphetamine (MDMA, '*Ecstasy*') is a recreational drug of abuse with toxic potential toward brain serotonin (5-HT) neurons (Green *et al.* 2003; Sarkar & Schmued, 2010*c*; Steele *et al.* 1994). Animals treated with repeated doses of MDMA develop lasting reductions in various presynaptic serotonergic neuronal markers including 5-HT, 5-hydroxyindoleacetic acid (5-HIAA, 5-HT's major metabolite), tryptophan hydroxylase (5-HT's rate-limiting biosynthetic enzyme) and membrane 5-HT transporters (SERTs). Accompanying these lasting 5-HT neurochemical deficits are morphological changes that are pathognomonic of 5-HT nerve fibre damage (Callahan *et al.* 2001; Molliver *et al.* 1990; Sarkar & Schmued, 2010). There are also data indicating that humans are susceptible to MDMA-induced 5-HT neural injury. For example, abstinent MDMA users have lasting reductions in cerebrospinal fluid (CSF) 5-HIAA (McCann *et al.* 1994) and regional brain densities of the SERT (Kish *et al.* 2010; McCann *et al.* 1998; Reneman *et al.* 2001), both of which are validated

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measures of MDMA-induced 5-HT neurotoxicity (Ricaurte *et al.* 1988*b*; Szabo *et al.* 2002).

The question of whether single typical oral MDMA doses have the potential to damage brain 5-HT neurons is not solely of interest to the drug abuse community. In particular, studies evaluating the therapeutic potential of MDMA in psychiatric patients are now underway (Mithoefer *et al.* 2011) and various experimental studies involving MDMA administration to humans have been carried out (Cami *et al.* 2000; Freedman *et al.* 2005; Grob *et al.* 1996; Kolbrich *et al.* 2008; Liechti *et al.* 2001; Tancer & Johanson, 2001; Vollenweider *et al.* 1998). The justification for administering a neurotoxic drug to humans is based upon the assumption that single oral doses are unlikely to produce neurotoxic effects. This assumption appears to derive from three lines of reasoning: (1) most animal studies have demonstrated the neurotoxic effects of MDMA using repeated parenteral doses; (2) studies in abstinent MDMA users demonstrating reductions of CSF 5-HIAA and/or SERT have involved individuals who have taken multiple MDMA doses over time; (3) differences between the metabolism and disposition of MDMA in animals and humans may render data obtained in animals irrelevant to humans (Green *et al.* 2011; Vollenweider *et al.* 2001).

Although most preclinical studies of MDMA neurotoxicity have involved repeated parenteral MDMA doses, there are isolated studies demonstrating that single oral dosages of MDMA also produce lasting 5-HT neurochemical deficits. For example, Mueller *et al.* (2009) showed that a single 20 mg/kg oral dose of MDMA produces lasting 5-HT neurochemical deficits in rats. Notably, 5-HT neurochemical deficits after this oral dose were comparable to those seen after a s.c. dose of 20 mg/kg (Schmidt, 1987), suggesting that the oral route of administration does not proffer significant protection. Nevertheless, differences in MDMA metabolism between rodents (rats and mice) and humans (Meyer & Maurer, 2010) may limit the relevance of rodent data to humans.

To our knowledge, only one study has evaluated the neurotoxic potential of single oral doses of MDMA in non-human primates (Ricaurte *et al.* 1988*a*). This early study compared single *vs.* multiple and oral *vs.* subcutaneous dosages of MDMA in small groups ($n=3$ per group) of squirrel monkeys. Results demonstrated that a single oral dose of MDMA produced persistent reductions in 5-HT and 5-HIAA in some brain regions. Although these findings suggest that clinically relevant single oral dosages of MDMA can lead to 5-HT neurotoxicity, only three animals were examined. Further, although the single dose employed was estimated to be equivalent to a typical human dose based upon interspecies dose scaling methods (McCann & Ricaurte, 2001), plasma concentrations of MDMA were not determined, making it difficult to state with certainty that plasma concentrations of MDMA generated were relevant to those that develop in humans after typical single oral doses.

The main objective of the present study was to assess the neurotoxic potential of a range of clinically relevant single oral doses of MDMA in non-human primate species known to metabolize MDMA in a manner similar to humans (Mueller *et al.* 2009*a*) and to determine whether plasma MDMA and metabolite concentrations engendered by each of these doses were on the order of those that develop in humans. A secondary objective of the study was to explore the relationship between plasma drug concentrations and lasting 5-HT deficits produced by MDMA in non-human primates.

## Method

### *Animals*

Adult squirrel monkeys (*Saimiri sciureus*) of both genders, ranging in weight (0.7–1.4 kg), were used. Animals were housed in pairs in standard steel cages at an ambient temperature of $26\pm2$ °C and 20–40% humidity, with free access to food and water. During drug treatment, animals were housed individually. The colony room was maintained on a 14–10 h light–dark cycle, lights on 07:00 hours. The facilities for housing and care of the animals were accredited by the American Association for the Assessment and Accreditation of Laboratory Animal Care. Animal care and experimental manipulations were approved by the Institutional Animal Care and Use Committee at the Johns Hopkins University School of Medicine and were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

### *Drugs and chemicals*

(±) 3,4-MDMA hydrochloride was obtained through the Drug Supply Program of the National Institute on Drug Abuse (USA). For analytic studies, racemic 3,4-dihydroxymethamphetamine (HHMA) hydrochloride and methanolic solutions of racemic MDMA hydrochloride and racemic 3,4-methylenedioxyamphetamine (MDA) hydrochloride were purchased from Lipomed (USA). Methanolic solutions (1000 mg/l) of racemic 4-hydroxy-3-methoxymethamphetamine (HMMA) and methanolic solutions (100 mg/l) of

racemic MDMA-$d_5$ and MDA-$d_5$ were obtained from Cerilliant (USA). 4-Hydroxymethamphetamine (pholedrine), 4-methylcatechol, ethylenediaminetetraacetic acid disodium salt dihydrate, 5-HT creatinine sulphate complex, 5-HIAA dicyclohexylammonium salt and sodium octyl sulphate were obtained from Sigma-Aldrich (USA). Sodium metabisulfite was obtained from E. Merck (Germany). Sodium phosphate, citric acid, phosphoric acid, perchloric acid and sodium chloride were purchased from J. T. Baker, USA. Tris was purchased from Fisher Scientific (USA). The authenticity of the MDMA, MDA, HHMA and HMMA samples used in the present studies was confirmed using liquid chromatographic/mass spectroscopic (LC/MS) methods.

### *Drug administration*

MDMA hydrochloride was dissolved in sterile normal saline (NaCl; 0.9% w/v) and given by gavage. Animals were placed in a Plexiglas restraining chair and a number 8 French feeding tube was inserted while the conscious animal was gently restrained. MDMA, dissolved in saline at the appropriate concentration, was administered on a mg/kg basis, via the tube. The tube was then immediately flushed with additional saline (1 ml). Doses are expressed as the salt.

### *Experimental design*

Two different experiments using different groups of animals were performed. The first examined the dose–response relationship between single oral doses of MDMA and lasting regional brain serotonergic neurochemical deficits in the squirrel monkey. The second examined the pharmacokinetic profile of each of the MDMA doses tested in the first experiment. Each of these experiments is described, in turn, below. Different animals were used for these two experiments because of the need to anaesthetize animals during blood collection for pharmacokinetic studies. As anaesthesia invariably lowers body temperature and hypothermia is known to afford neuroprotection against MDMA neurotoxicity (see Green *et al.* 2003), using the same animals for neurotoxicity and pharmacokinetic studies was not appropriate.

### *Dose–response study*

Three different single oral doses of MDMA (5.7, 10.0 or 14.3 mg/kg) were tested, each in a separate group of drug-naive squirrel monkeys ($n=4$–8 per group). Doses were selected because they were calculated to be equivalent to 1.6, 2.8 and 4.0 mg/kg in a 70 kg human, respectively, using a standard interspecies dose-scaling equation:

$$D_{\text{human}} = D_{\text{animal}}(W_{\text{human}}/W_{\text{animal}})^{0.7},$$

where $D$ = dose (mg), $W$ = weight of the animal (kg) and 0.7 is a commonly used and empirically derived exponent (Mordenti & Chappell, 1989). The sample size for each dose ($n=8$ for the 5.7 mg/kg dose, $n=6$ for the 10.0 mg/kg dose and $n=4$ for the 14.3 mg/kg) was adjusted so as to minimize the likelihood of a type 2 error, to minimize the number of animals employed and to take into account the fact that the anticipated effect was likely to be dose-related. One week after MDMA administration, the animals' brains were analysed for regional 5-HT neuronal marker content and compared to those in vehicle-treated controls ($n=8$). Tissues from control and experimental animals were assayed in parallel, as described below.

### *Pharmacokinetic study*

For this experiment, a separate group of squirrel monkeys was used. In these animals, three different doses of MDMA (5.7, 9.6 and 14.3 mg/kg) were tested. As plasma concentrations generated by two of the three doses tested in this study (5.7 and 10 mg/kg) had been previously determined (Mueller *et al.* 2008, 2009*a*), only plasma MDMA and metabolite levels generated by the third dose herein tested (14.3 mg/kg) were determined for the present study. At least 6 wk elapsed between the testing of each dose in each animal, in random order. As above, the absolute doses (5.7, 9.6 and 14.3 mg/kg) were calculated to be equivalent to 1.6, 2.8 and 4.0 mg/kg doses in a 70 kg human, a dose range that brackets single oral doses used by humans (Irvine *et al.* 2006; Kolbrich *et al.* 2008; Morefield *et al.* 2011). Forty five min and 1.5, 3.0, 4.5, 6, 7, 9, 11, 23 and 25 h after oral MDMA administration, approximately 0.7 ml blood was obtained and plasma concentrations of MDMA and metabolites were determined, as below. Peak plasma concentrations ($C_{\max}$), times of peak plasma concentration ($T_{\max}$), area under the concentration-time curve (AUC) and the elimination half-lives ($T_{1/2}$) were obtained using the pharmacokinetic functions for Microsoft Excel (developed by Usansky *et al.*, http://www.boomer.org/pkin/xcel/pkf/pkf.doc).

### *Analysis of plasma MDMA and metabolite concentrations*

Plasma concentrations of MDMA, HHMA, HMMA and MDA were determined by LC/MS as recently described (Mueller *et al.* 2009*c*). Briefly, analytes were

separated by LC/MS using a Zorbax 300-SCX column (Agilent Technologies, USA) and quantification was performed using ESI-MS in positive selected ion monitoring mode. The protonated molecular ions of MDMA, MDA, HHMA, HMMA, MDMA-$d_5$, MDA-$d_5$, and pholedrine were used as target ions. Quantification of the analytes was carried out by comparison of their peak area ratio (analyte *vs.* internal standard) to calibration curves in which the peak area ratios of spiked calibrators had been plotted *vs.* their concentration. Values for HHMA and HMMA represent total free amounts (i.e. amounts after cleavage of sulfate and glucuronic acid conjugates using a previously described procedure; Mueller *et al.* 2009*b*).

### *High performance liquid chromatography determination of regional brain 5-HT and 5-HIAA content*

Regional brain concentrations of 5-HT and 5-HIAA were determined by means of high performance liquid chromatography with electrochemical detection, as described previously (Mechan *et al.* 2006).

### *Measurement of regional brain SERT density*

Regional brain density of the SERT was determined in tissue homogenates with tritiated paroxetine employing previously described methods (Ricaurte *et al.* 1992).

### *Quantitative autoradiographic determination of SERT and dopamine transporter (DAT)*

Half-hemisphere coronal sections (20 $\mu$m) at the level of the hippocampus were thaw-mounted onto gelatine-coated microscope slides and labelled with 50 pM [$^{125}$I] RTI-55 for estimation of SERT and DAT densities as previously described (Mechan *et al.* 2006).

### *Statistics*

The significance of differences among means was determined using one-way analyses of variance (ANOVA) followed by Tukey's multiple comparison tests. Correlations were explored using Pearson's product moment correlations. Statistical analyses were performed using Prism, Version 3.02 (GraphPad Software Inc., USA). Differences and correlations were considered significant when $p < 0.05$.

## Results

Single oral doses of MDMA produced significant dose-related depletions of 5-HT, 5-HIAA and/or the SERT in multiple regions of the squirrel monkey brain 1 wk later (Figs. 1, 2). Reductions of one or more regional brain serotonergic axonal markers were observed even after the lowest oral dose tested (5.7 mg/kg, equivalent to 1.6 mg/kg in humans; Fig. 1). Markers of serotonergic axons innervating portions of the cerebral cortex (e.g. parietal cortex, temporal cortex) were particularly vulnerable to the effects of MDMA, as were those innervating the hippocampus and thalamus (Figs. 1, 2).

Dopaminergic neuronal markers in the caudate and putamen were unaffected by any dose of MDMA (Table 1, Fig. 2).

Dose-related peak plasma concentrations of MDMA ($C_{max}$) occurred within 1–2 h after oral MDMA administration (Fig. 3, top panel). By 4 h, concentrations of MDMA had dropped by >50% and, by 12–25 h, MDMA concentrations were close to zero.

As expected, concentrations of MDMA's *O*-demethylenated metabolites lagged slightly, relative to those of MDMA. The highest concentrations of *O*-demethylenated metabolites appeared in the form of HMMA, followed by HHMA. A very small percentage of MDMA (<5% of the maximal MDMA concentration) was metabolized to MDA (Fig. 3, top panel). Peak HHMA levels decreased as the dose of MDMA increased (Fig. 3, top and middle panels).

Serotonergic axonal markers were significantly or near-significantly inversely correlated with the $C_{max}$ and AUC of MDMA and MDA, but not with the $C_{max}$ and AUC of MDMA's *O*-demethylenated metabolites HHMA or HMMA (Fig. 3, bottom panel). In contrast, HHMA concentrations were directly, rather than inversely, correlated with 5-HT axonal marker concentrations. Although the $C_{max}$ and AUC of MDA were near-significantly inversely correlated with 5-HT neuronal markers, concentrations of MDA were quite low at all times post-MDMA ingestion (Fig. 3, top panel).

Neither MDMA nor any of its metabolites (HHMA, HMMA, and MDA) could be detected in the brain of squirrel monkeys given MDMA 1 wk previously (data not shown). Limit of detection for each of the analytes was 2 $\mu$g/g for MDMA, 1 $\mu$g/g for MDA and 0.1 $\mu$g/g each for HHMA and HMMA.

## Discussion

The results of the present study are the first to demonstrate that single oral doses of MDMA that generate plasma drug levels on the order of those seen in humans (de la Torre *et al.* 2000; Helmlin *et al.* 1996; Kolbrich *et al.* 2008; Mueller *et al.* 2009*a*; Peters *et al.*

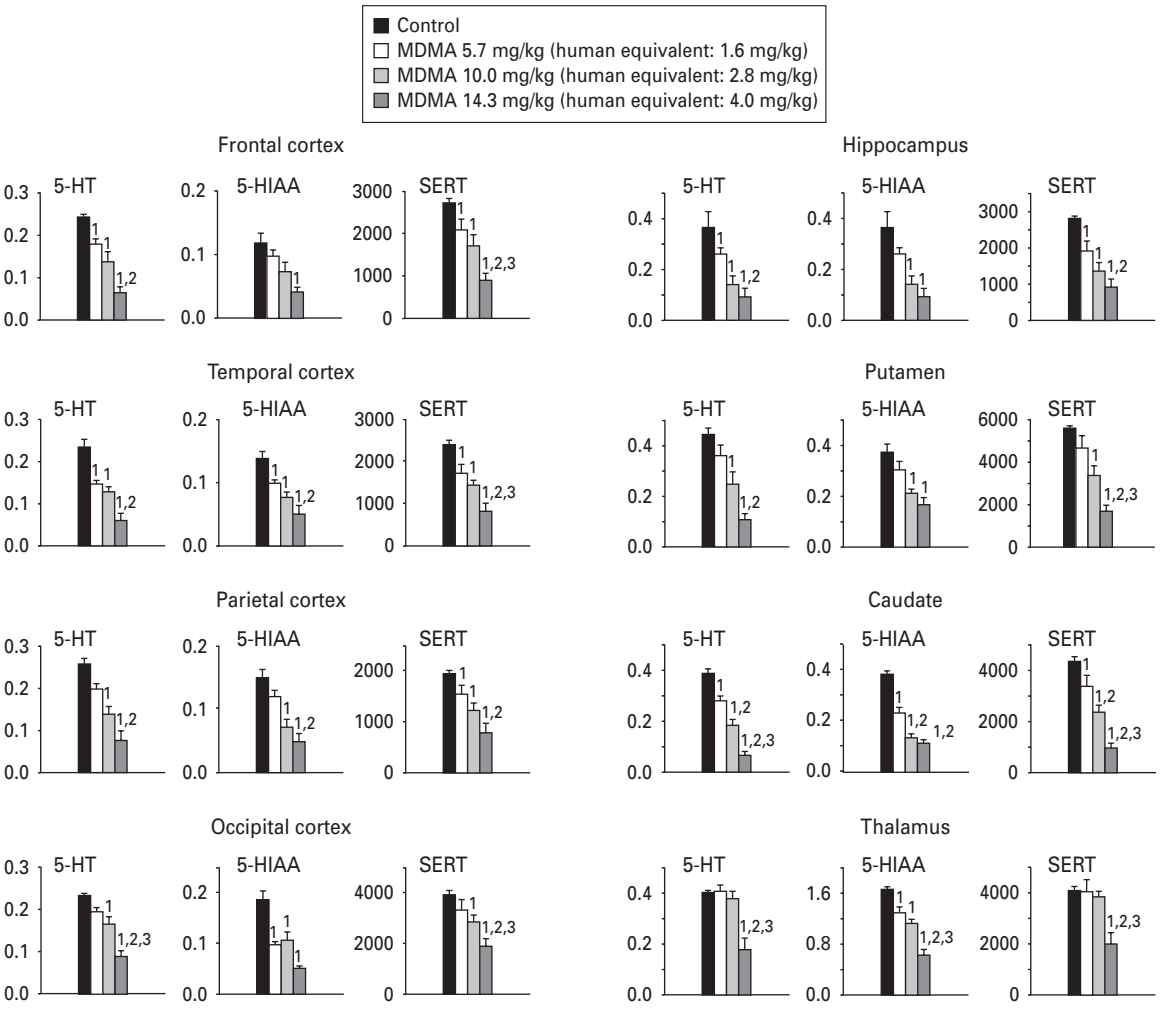



**Fig. 1.** Dose-related effects of single oral doses of 3,4-methylenedioxymethamphetamine (MDMA) on serotonergic markers [serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA) and 5-HT transporter (SERT)] in various regions of the squirrel monkey brain. Animals were treated with either saline ($n=8$) or 5.7 mg/kg ($n=8$), 10.0 mg/kg ($n=6$) or 14.3 mg/kg ($n=4$) MDMA. Brain tissue was analysed 1 wk after treatment for content of 5-HT, 5-HIAA and SERT. Values represent the mean (±S.D.). [1]Indicates significant difference from control group; [2]Indicates significant difference from group treated with 5.7 mg/kg MDMA; [3]Indicates significant difference from group treated with 10.0 mg/kg MDMA (one-way analysis of variance followed by Tukey's multiple comparison test). Significance was assumed when $p<0.05$.

2003) produce lasting dose-related 5-HT neurochemical deficits in various regions of the squirrel monkey brain. The serotonergic deficits presently observed were documented 1 wk after MDMA exposure, suggesting that they are unrelated to residual drug or metabolites. Indeed, efforts to identify MDMA and/or its various metabolites (HHMA, HMMA and MDA) in the brain of squirrel monkeys given MDMA 1 wk previously were unsuccessful. This is in keeping with the fact that, by 1 wk, >45 half-lives of MDMA had elapsed (together with >13 half-lives of HHMA and 20 half-lives of MDA and HMMA). The present results also show that even the lowest single oral dose tested (5.7 mg/kg, equivalent to 1.6 mg/kg in humans) produced lasting losses of some, but not all, regional brain 5-HT neuronal markers, suggesting that this dose represents a threshold neurotoxic dose. As the doses tested in this study were calculated to be equivalent to those used by humans, and plasma concentrations of MDMA were on the order of those seen in humans (Fig. 3, top and middle panels), the present results indicate that single doses of MDMA used recreationally and/or experimentally may not be entirely free of neurotoxic risk, as is widely assumed.

Interspecies dose scaling was used to calculate doses in squirrel monkeys that were 'equivalent' to

**Table 1.** Tissue content of DA and DOPAC in caudate and putamen of squirrel monkeys 1 wk after treatment with saline or various doses of MDMA (5.7, 10.0 or 14.3 mg/kg)

| Dose (mg/kg) | DA (ng/mg) | | | | DOPAC (ng/mg) | | | |
|---|---|---|---|---|---|---|---|---|
| | Control | 5.7 | 10.0 | 14.3 | Control | 5.7 | 10.0 | 14.3 |
| Caudate | 9.09 ± 0.22 | 9.67 ± 0.42 | 9.71 ± 0.11 | 9.53 ± 0.18 | 0.89 ± 0.10 | 0.82 ± 0.05 | 1.01 ± 0.06 | 0.90 ± 0.10 |
| Putamen | 11.77 ± 0.62 | 9.84 ± 0.37 | 11.45 ± 0.39 | 11.28 ± 0.79 | 0.67 ± 0.14 | 0.50 ± 0.06 | 0.55 ± 0.09 | 0.51 ± 0.07 |

DA, Dopamine; DOPAC, 3,4-dihydroxy-phenylacetic acid; MDMA, 3,4-methylenedioxymethamphetamine.
Values represent group means ± S.D.
No significant differences in DA markers were observed between the treatment groups following one-way analysis of variance followed by Tukey's multiple comparison test.

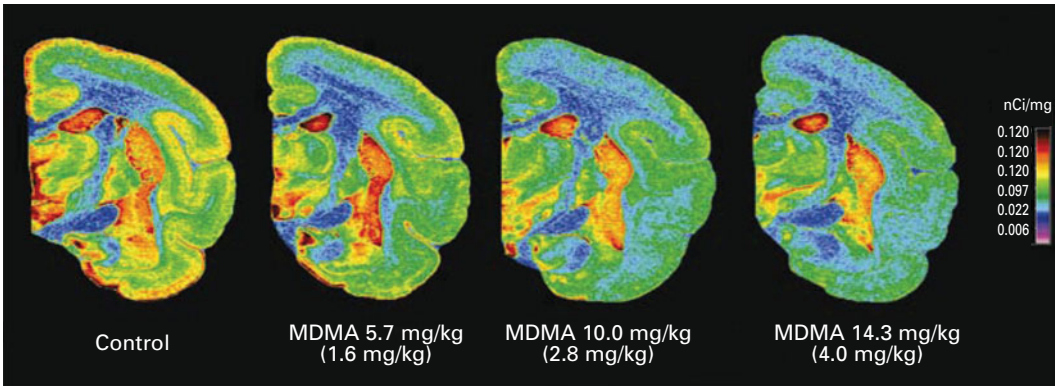
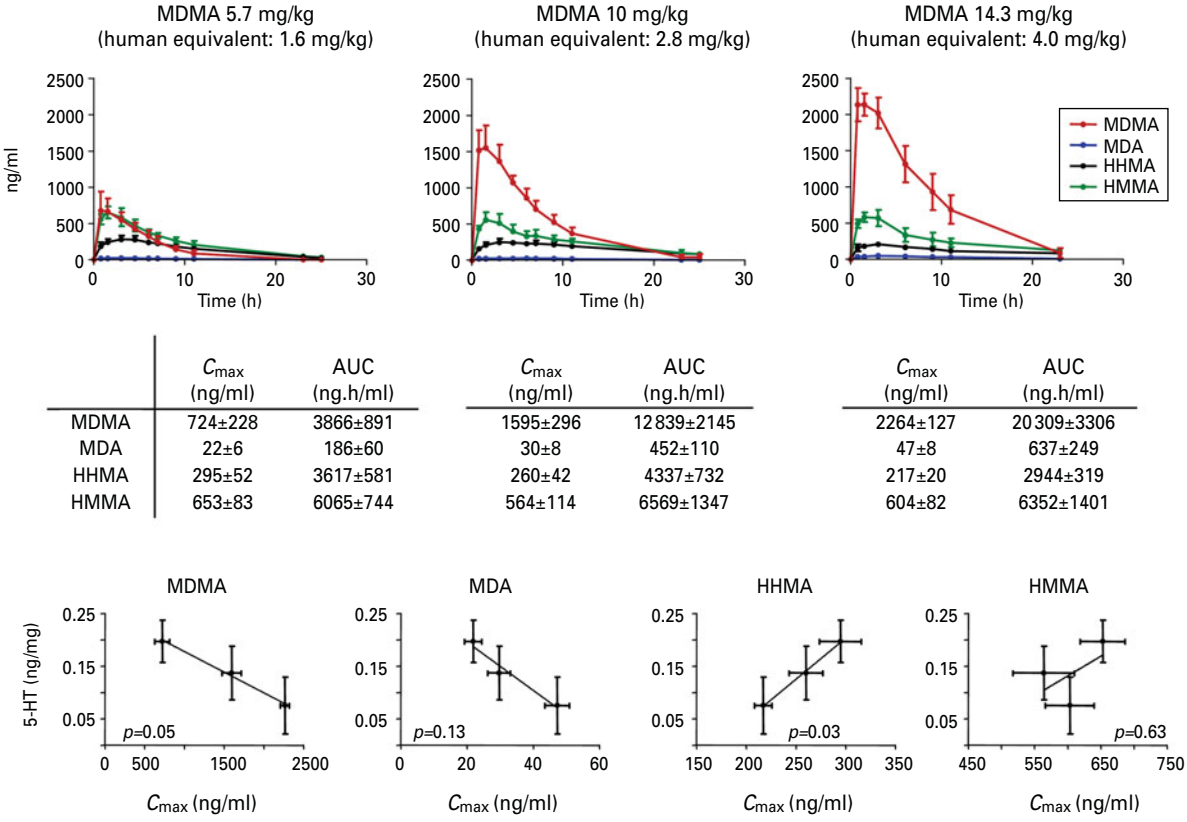



**Fig. 2.** [$^{125}$I] RTI-55-labelled dopamine transporter (DAT) and serotonin transporter (SERT) sites in coronal brain sections at the level of the 8.0 rostro-caudal coordinate in the Gergen & McLean (1962) atlas of the squirrel monkey brain. Most [$^{125}$I] RTI-55 binding in the caudate and putamen represents binding to DATs, while most [$^{125}$I] RTI-55 binding in extra-striatal regions represents binding to SERTs (Laruelle *et al.* 1993). Sections shown are from a control animal and animals treated 1 wk previously with single oral doses of 5.7, 10 or 14.3 mg/kg methylenedioxymethamphetamine (MDMA). Human equivalent doses, namely, 1.6, 2.8 and 4.0 mg/kg, respectively, are shown in parentheses. Note reductions in [$^{125}$I]RTI-55 binding in serotonin (5-HT) innervated regions such as hippocampus, temporal cortex and dorsal neocortex. Also note lack of change in [$^{125}$I]RTI-55 binding in the caudate and putamen, where [$^{125}$I]RTI-55 binding is predominately to DATs. The scale at the right side of the figure shows density of binding sites designated by colour expressed in nCi/mg tissue.

those used by humans but, as we (McCann & Ricaurte, 2001) and others (de la Torre & Farre, 2004) have pointed out, there may be circumstances under which interspecies dose scaling may not be entirely accurate. We therefore measured plasma concentrations of MDMA and its metabolites generated by each of the doses tested, so that they could be directly compared to plasma drug levels generated by the equivalent doses in humans. As shown in Fig. 3 (middle panel), plasma concentrations of MDMA and metabolites that produced lasting 5-HT neurochemical deficits in squirrel monkeys are similar, but not identical to those reported in humans (de la Torre *et al.* 2000; Helmlin *et al.* 1996; Kolbrich *et al.* 2008; Mueller *et al.* 2009*a*; Peters *et al.* 2003). In particular, after the lowest dose of MDMA tested (5.7 mg/kg, estimated to be equivalent to 1.6 mg/kg in humans), AUC values of MDMA were comparable (3866 ± 891 ng/ml.h in squirrel monkeys and 3071 ± 673 ng/ml.h in humans (Mueller *et al.* 2009*a*; non-significant difference), but $C_{max}$ values were different (724 ± 255 ng/ml in squirrel monkeys *vs.* 254 ± 60 ng/ml in humans; Mueller *et al.* 2009*a*). Further, as might be expected given the differences in body mass between squirrel monkeys and humans, the $T_{\frac{1}{2}}$ of MDMA was shorter in the squirrel monkey (2.6 ± 0.7 h *vs.* 8.4 ± 1.6 h). Thus, as previously discussed (Mueller *et al.* 2009*a*), direct comparison of 'exposure to MDMA' between the two species is not facile and the accuracy of the interspecies dose scaling method for such comparison across species is dependent upon which pharmacokinetic parameter is considered (AUC or $C_{max}$). This




| | $C_{max}$ (ng/ml) | AUC (ng.h/ml) | $C_{max}$ (ng/ml) | AUC (ng.h/ml) | $C_{max}$ (ng/ml) | AUC (ng.h/ml) |
|---|---|---|---|---|---|---|
| MDMA | 724±228 | 3866±891 | 1595±296 | 12839±2145 | 2264±127 | 20309±3306 |
| MDA | 22±6 | 186±60 | 30±8 | 452±110 | 47±8 | 637±249 |
| HHMA | 295±52 | 3617±581 | 260±42 | 4337±732 | 217±20 | 2944±319 |
| HMMA | 653±83 | 6065±744 | 564±114 | 6569±1347 | 604±82 | 6352±1401 |




**Fig. 3.** Plasma concentration profiles of 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyamphetamine (MDA), 3,4-dihydroxymethamphetamine (HHMA) and 4-hydroxy-3-methoxymethamphetamine (HMMA) in squirrel monkeys administered three different oral MDMA doses (5.7 mg/kg, 10 mg/kg and 14.3 mg/kg; top panels). All three doses were tested in the same six animals, except for the highest dose (14.3 mg/kg), which was tested in only five of the animals. At least 6 wk elapsed between the testing of each dose. Portions of the pharmacokinetic results (middle panels) have been previously reported (Mueller *et al.* 2008, 2009*a*). The bottom panels show the relationship between lasting serotonin (5-HT) deficits in the parietal cortex and peak concentrations of MDMA, MDA, HHMA and HMMA in plasma. Values represent the mean ± S.D. (vertical error bars represent S.D. of 5-HT values; horizontal error bars represent S.D. of the plasma levels). Correlations between plasma drug levels and 5-HT neuronal markers were explored using Pearson's product monument correlations. *p* values and regression lines are shown. $C_{max}$, peak plasma concentrations; AUC, area under the concentration-time curve.

issue notwithstanding, the present results indicate that single doses of MDMA that produce lasting 5-HT neurochemical deficits in squirrel monkeys generate plasma concentrations of MDMA on the order of those seen in humans (de la Torre *et al.* 2000; Helmlin *et al.* 1996; Kolbrich *et al.* 2008; Mueller *et al.* 2009*a*; Peters *et al.* 2003), with the estimated margin of safety depending upon which pharmacokinetic parameter is most closely related to the neurotoxic process (AUC or $C_{max}$).

The present results are consistent with, but not identical to, results from a previous study that assessed the neurotoxic potential of single oral doses of MDMA in squirrel monkeys (Ricaurte *et al.* 1988*a*). In particular, the earlier study found that an oral dose of 5.0 mg/kg led to significant reductions in 5-HT and 5-HIAA in only two brain regions (thalamus and hypothalamus). In contrast, in the present study, the lowest dose tested (5.7 mg/kg) produced significant reductions in 5-HT neuronal markers in a variety of cortical and subcortical regions. Two factors likely account for the observed differences. First, although the difference in drug dosage (5.0 mg/kg in the earlier study *vs.* 5.7 mg/kg in the current study) might seem trivial, small differences in dose can lead to substantial differences in plasma (and brain) concentrations of MDMA, due to its nonlinear pharmacokinetics (see below). Second, the paucity of significant regional effects in the previous study may have been due to lack of power, because only three animals were used in that study (compared to eight animals in the present investigation). The lack of an effect in the thalamus in the

present study is noteworthy and may be related to the fact that different thalamic nuclei are differentially affected (as is evident in Fig. 2) and that, depending on how the thalamus is dissected and what particular subregion of the thalamus is analysed, results may vary. Unfortunately, we cannot be sure that the subregions of the thalamus analysed in the present study are identical to those analysed in the earlier study.

Data from the present study provide evidence that the parent compound (MDMA), rather than one of its metabolites (HHMA, HMMA or MDA), is likely responsible for the production of lasting 5-HT neurochemical deficits. Specifically, brain 5-HT markers decreased with dose and were inversely correlated with $C_{max}$ and AUC values of MDMA, but not with those of either of its *O*-demethylenated metabolite, HHMA or HMMA (Fig. 3, bottom panel). These observations are in keeping with previous findings in rodents, indicating that lasting 5-HT neurochemical deficits correlated with MDMA but not HHMA or HMMA (Mueller *et al.* 2009). Notably, in rodents, a role for another MDMA metabolite, MDA, could not be excluded because rodents (rats and mice) *N*-demethylate substantial amounts of MDMA to MDA (Meyer & Maurer, 2010) and MDA is known to have 5-HT neurotoxic potential (O'Hearn *et al.* 1988; Ricaurte *et al.* 1985). In contrast, squirrel monkeys (Mueller *et al.* 2009*a*), like humans (de la Torre *et al.* 2000; Kolbrich *et al.* 2008), convert only a small fraction of MDMA to MDA (<5%), such that plasma ratios of MDA:MDMA in squirrel monkeys and humans are only 3–5/100 (compared to 50/100 in rodents) and absolute levels of MDA here do not exceed 40–50 ng/ml (Fig. 3, top panel). At these low concentrations, MDA is unlikely to produce 5-HT neurotoxic effects because MDA and MDMA have roughly equal neurotoxic potency on a mg/kg basis (Battaglia *et al.* 1987; O'Hearn *et al.* 1988) and because plasma concentrations of MDMA and MDA on the order of 300–600 ng/ml are required to produce lasting 5-HT neurochemical deficits in both rodents (Mueller *et al.* 2009) and non-human primates (Mechan *et al.* 2006). Thus, it is unlikely that MDA is an active toxic metabolite in the squirrel monkey.

MDMA pharmacokinetics in squirrel monkeys, as in humans (de la Torre *et al.* 2000; Kolbrich *et al.* 2008; Mueller *et al.* 2009*a*), are nonlinear, with increases in MDMA dosage leading to disproportionate increases in plasma concentrations of MDMA, but little or no change in levels of HHMA or HMMA (Fig. 3, top and middle panels). The nonlinearity of MDMA pharmacokinetics, which has been demonstrated in all species tested (Baumann *et al.* 2009; Chu *et al.* 1996; de la Torre *et al.* 2000; Kolbrich *et al.* 2008; Mueller *et al.* 2008; Scheidweiler *et al.* 2011), has implications for mechanisms underlying the MDMA-induced 5-HT neurotoxicity. In particular, if the primary mediator of MDMA neurotoxicity is the parent compound, then once the enzymatic pathway that is chiefly responsible for MDMA metabolism in primates [cytochrome P450 2D6 (CYP2D6)] has been saturated, further increases in dose would be anticipated to increase MDMA levels and the severity of MDMA-induced injury, possibly in a non-proportional fashion. In contrast, if the primary mediator of MDMA-induced neurotoxicity is one of its *O*-demethylenated metabolites (e.g. HHMA), increases in MDMA dose beyond those required to saturate relevant metabolic enzymes (CYP2D6) would not be anticipated to be associated with increased neurotoxicity (because no increase in plasma or brain HHMA is seen with the increase in dose). Given that the neurotoxic effects of MDMA increase with dose, and that CYP2D6 inhibition occurs after relatively low doses (de la Torre *et al.* 2000; Kolbrich *et al.* 2008; Mueller *et al.* 2008), the effects of MDMA presently observed favour the view that the parent compound (MDMA), rather than an *O*-demethylenated metabolite, is chiefly responsible for MDMA-induced 5-HT neurotoxicity in primates.

In the present study, a decrease of HHMA $C_{max}$ values was observed as the dose of MDMA was increased (Fig. 3). MDMA is known to inhibit CYP2D6 activity in a dose-dependent manner (Yang *et al.* 2006). Higher doses of MDMA may, therefore, cause a more rapid and/or extensive inhibition of the CYP2D6 homologue in the squirrel monkey, leading to dose-related decreases in peak HHMA levels. Alternatively, the degradation or excretion of HHMA may increase as the dose of MDMA is increased.

The nonlinear pharmacokinetics of MDMA observed within the dose range presently examined in primates may have implications for studies employing 'booster' MDMA doses in humans (Mithoefer *et al.* 2011). In particular, ongoing studies involve the administration of an optional second smaller dose of MDMA several hours after the first dose, as the psychoactive effects of the initial dose wane. Given that the first dose of approximately 1.6 mg/kg is sufficient to saturate and/or inhibit enzyme systems that metabolize MDMA (de la Torre *et al.* 2000; Kolbrich *et al.* 2008; Mueller *et al.* 2008), the second 'booster' dose will have the effect of disproportionately increasing plasma (and brain) MDMA concentrations, thereby increasing the intensity and duration of MDMA exposure (by increasing both the $C_{max}$ and AUC of MDMA). Regardless of whether the $C_{max}$ or AUC

of MDMA is the pharmacokinetic parameter most closely linked to MDMA-induced 5-HT neurotoxicity, the practice of 'booster dosing' may not be advisable because it likely disproportionately increases MDMA concentrations and thereby increases the risk for brain 5-HT neural injury. Monitoring of plasma MDMA concentrations in such studies would seem indicated.

In conclusion, the present results demonstrate that single oral doses of MDMA that generate plasma drug levels on the order of those seen in humans produce lasting, dose-related 5-HT neurochemical deficits in the brain of squirrel monkeys, non-human primates that metabolize MDMA in a manner similar to humans. The present results also demonstrate that even the lowest dose of MDMA tested (5.7 mg/kg) produced significant 5-HT deficits in some brain regions. Taken together, these findings suggest that the margin of safety of MDMA, at least with regard to brain 5-HT neurotoxicity, may be narrower than is generally accepted. Finally, the low levels of MDA observed in the squirrel monkey, coupled with the fact that lasting 5-HT neurochemical deficits in the squirrel monkey are more closely related to plasma concentrations of MDMA than those of its *O*-demethylenated metabolites (HHMA or HMMA), suggest that MDMA-induced 5-HT neural injury is more likely produced by the parent compound than a metabolite.

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### Statement of Interest

None.

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