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Title Page

Metabolism and Disposition of 3,4-Methylenedioxymethamphetamine (MDMA, "Ecstasy") in Baboons After Oral Administration: Comparison to Humans Reveals Marked Differences

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Running Title Page

Running Title: MDMA Pharmacokinetics in baboons

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List of nonstandard abbreviations:
MDMA – 3,4-methylenedioxymethamphetamine
HHMA – 3,4-dihydroxymethamphetamine
HMMA – 4-hydroxy-3-methoxymethamphetamine
MDA – 3,4-methylenedioxyamphetamine
C_{max} – peak plasma concentration
T_{max} – time of peak plasma concentration
$T_{1/2}$ – elimination half life
AUC – area under the concentration-time curve
COMT – catechol *O*-methyltransferase
CYP2D6 – cytochrome P460 2D6
LC/MS – liquid chromatography coupled with mass spectrometry

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Abstract

The baboon is potentially an attractive animal for modeling 3,4-methylenedioxymethamphetamine (MDMA) effects in humans. Baboons self-administer MDMA, are susceptible to MDMA neurotoxicity, and are suitable for positron emission tomography, the method most often used to probe for MDMA neurotoxicity in humans. Because pharmacokinetic equivalence is a key feature of a good predictive animal model, we compared the pharmacokinetics of MDMA in baboons and humans. Baboons were trained to orally consume MDMA. Subsequently, pharmacokinetic profiles of MDMA and its major metabolites were determined following various oral MDMA doses using the same analytical method recently employed to carry out similar studies in humans. Results indicate that the MDMA pharmacokinetics after oral ingestion differ markedly between baboons and humans. Baboons had little or no MDMA in their plasma, but had high plasma concentrations of 3,4-dihydroxymethamphetamine (HHMA), pointing to much more extensive first-pass metabolism of MDMA in baboons compared to humans. Other less prominent differences included less *O*-methylation of HHMA to 4-hydroxy-3-methoxymethamphetamine, greater *N*-demethylation of MDMA to 3,4-methylenedioxyamphetamine and a shorter half life of HHMA in the baboon. To our knowledge, this is the first study to characterize MDMA metabolism and disposition in the baboon. Differences in MDMA pharmacokinetics between baboons and humans suggest that the baboon may not be ideal for modeling human MDMA exposure. The unusually rapid conversion of MDMA to HHMA in the baboon, however, may render this animal uniquely useful for clarifying the relative role of the parent compound (MDMA) versus metabolites (particularly HHMA) in the biological actions of MDMA.

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Introduction

(±) 3,4-Methylenedioxymethamphetamine (MDMA, Ecstasy) is a psychoactive drug with significant abuse liability and neurotoxic potential (Capela et al., 2009; Green et al., 2003; Kalant, 2001). A recent national survey indicates that recreational MDMA use may be once again on the rise (Substance Abuse and Mental Health Services Administration., 2010). In an effort to better understand effects of MDMA in humans, various laboratory animal models have been used to study the behavioral pharmacology and neurotoxicology of MDMA. Intravenous self-administration of MDMA (a measure of abuse liability) has been demonstrated in baboons (Lamb and Griffiths, 1987), rhesus macaques (Beardsley et al., 1986; Fantegrossi et al., 2002; Lile et al., 2005) and rats (Schenk et al., 2007). Likewise, neurotoxic effects of MDMA have been reported in baboons (Scheffel et al., 1998), rhesus monkeys (Kleven et al., 1989) and rats (Commins et al., 1987; Schmidt, 1987).

Translating MDMA findings from laboratory animals to human MDMA users has proved challenging, for a number of reasons. In most laboratory animal studies, MDMA has been administered parenterally (intravenously, intramuscularly, and subcutaneously) rather than orally, even though MDMA is nearly always ingested orally by people. In addition, doses used in animal studies have generally been higher than those used by humans (Green et al., 2003). Although interspecies dose-scaling principles dictate that small laboratory animals will require higher mg/kg doses than humans to achieve the same pharmacologic effect (Mordenti, 1986), dose-scaling methods have been the subject of debate (de la Torre and Farre, 2004; Fantegrossi, 2007). A third factor complicating generalization of preclinical MDMA findings to humans has been the difference in MDMA metabolism across species. In particular, MDMA is metabolized

via two main pathways that are co-existent, but operate at different rates in different species. In rodents (rats, mice) *N*-demethylation of MDMA predominates, whereas in primates (human and nonhuman) ring demethylenation is more prevalent (Meyer et al., 2008) (Fig. 1).

In order to circumvent uncertainties related to dose and metabolism, there has been recent interest in relating MDMA effects to plasma drug (and metabolite) concentrations, rather than only to MDMA dose (Mechan et al., 2006; Mueller et al., 2009). With respect to neurotoxicity, studies taking this approach have shown that plasma MDMA concentrations that are associated with neurotoxicity in squirrel monkeys overlap those found in some recreational 'ecstasy' consumers (Morefield et al., 2011; Samyn et al., 2002), and are only two to three times higher than those produced by a single 100–150 mg dose of MDMA in humans (Kolbrich et al., 2008b; Mechan et al., 2006; Mueller et al., 2009a). Related studies have also shown that the metabolism and disposition of MDMA in squirrel monkeys closely parallel those in humans (Mueller et al., 2009a). Given these findings, there is concern that the margin of safety of MDMA in humans may be narrow (at least with respect to neurotoxicity).

To determine if the safety margin of MDMA in humans is, in fact, narrow, it would be ideal if a large primate animal model could be developed, one that metabolized MDMA in a manner similar to humans and one that could be studied with the same methods used to study the neurotoxic potential of MDMA in humans [e.g., positron emission tomography (PET)] (Kish et al., 2010; McCann et al., 2005). In this regard, baboons offer significant promise because they are known to be susceptible to MDMA neurotoxicity (Scheffel et al., 1998),and because PET

imaging identical to that employed in recreational MDMA users has already been shown to reliably detect MDMA neurotoxicity in baboons (Scheffel et al., 1998; Szabo et al., 2002).

Because pharmacokinetic equivalence is a key feature of a good predictive laboratory animal model (Mahmood and Balian, 1999), the present studies were undertaken to characterize the metabolism and disposition of MDMA in baboons and compare them to those in humans. Plasma MDMA and metabolite concentrations in baboons were determined using the same liquid chromatographic/mass spectrometric (LC/MS) procedure recently employed to measure MDMA and metabolites in humans (Mueller et al., 2009b). MDMA was administered orally at doses selected to be equivalent to those used by humans. To our knowledge, this is the first study to characterize the pharmacokinetic profile of MDMA and its metabolites [3,4-dihydroxymethamphetamine (HHMA), 4-hydroxy-3-methoxymethamphetamine (HMMA), and 3,4-methylenedioxyamphetamine (MDA)] in the baboon.

Methods

Animals: Four (MO, BR, BO, and BS) adult male baboons (*Papio hamadryas anubis)* served as subjects. Body weights of the animals were as follows: MO: 35.4 kg; BR: 32.3 kg; BO: 46.7 kg; BS: 27.3 kg. Three of the baboons (MO, BR, BO) had a history of participation in previous behavioural pharmacology experiments which, for some of the animals, included exposure to cocaine, alcohol, gamma-hydroxybutyric acid, tryptamine derivatives, benzodiazepines or various non-benzodiazepine hypnotic sedatives (e.g., zolpidem). The other baboon (BS) was drug-naive at the beginning of the present study. For the animals with a drug history, at least four weeks had elapsed between participation in prior studies and the start of the

present study. Animals were housed singly, with free access to food and water. Animal care and use were in accordance with the US Public Health Service Policy on Humane Care and Use of Laboratory Animals and the US Animal Welfare Act Regulations.

Drugs and Reagents: Racemic MDMA hydrochloride was obtained from Research Triangle Institute (Research Triangle Park, NC, USA) through the National Institute on Drug Abuse (Rockville, MD, USA). Racemic HHMA hydrochloride and methanolic solutions (1000 mg/l) of racemic MDMA hydrochloride and racemic MDA hydrochloride were purchased from Lipomed (Cambridge, MA, USA). Methanolic solutions (1000 mg/l) of racemic HMMA and methanolic solutions (100 mg/l) of racemic MDMA-d_5 and MDA-d_5 were obtained from Cerilliant (Round Rock, TX, USA). 4-Hydroxymethamphetamine (pholedrine), 4-methylcatechol, and ethylenediaminetetraacetic acid disodium salt dihydrate were obtained from Sigma-Aldrich (Saint Louis, MO, USA). Sodium metabisulfite was obtained from E. Merck (Darmstadt, Germany). Perchloric acid was obtained from J.T. Baker (Phillipsburg, NJ, USA). The authenticity of the MDMA, HHMA, HMMA, and MDA samples was confirmed using LC/MS methods to determine the corresponding pseudomolecular ions and at least one fragment ion for each compound. Analysis was performed in full scan (mass range from 100 – 1000) to check for presence of possible impurities.

Drug treatment: MDMA was administered orally. To facilitate comparison to doses administered to humans, interspecies dose scaling (Mordenti and Chappell, 1989) was used to calculate doses for each baboon estimated to be equivalent doses of 0.5, 1.6, 3.2, and 5.0 mg/kg in humans. Actual doses in each baboon depended on the weight of each animal and were

approximately 0.6, 2.0, 3.9, and 6.1 mg/kg for MO, 0.6, 2.0, 4.0, and 6.3 mg/kg for BR, 0.6, 1.6, 3.2 and 5.7 mg/kg for BO and 0.7, 2.1, 4.3, and 6.5 mg/kg for BS. These doses were selected because they bracket doses previously tested in humans (de la Torre et al., 2000a; de la Torre et al., 2000b; Kolbrich et al., 2008a; Kolbrich et al., 2008b). For interspecies dose scaling, the allometric equation, shown below, was used where *D* is the dose in mg, *W* is the weight of the animal in kg, and 0.7 is a commonly used (empirically derived) exponent: $D_{human} = D_{animal} (W_{human}/W_{animal})^{0.7}$. All doses were calculated as the salt form. To achieve reliable oral dosing, baboons were habituated to the taste of quinine sulfate dissolved in the readily consumed orange drink TANG® by gradually increasing the concentration in 60 ml of TANG® presented at approximately the same time each morning (training phase) (Turkkan et al., 1989). The solution was delivered to the baboon from the end of a 60 mL syringe held at the bars of the cage by a research technician familiar to the animal. The quinine solution was followed by delivery of 10 mL of unadulterated TANG®. Once a baboon was reliably consuming 60 ml of 0.325 mg/ml of the quinine solution in less than 15 min (usually 5 min), MDMA dosing began (testing phase). At least 24 hours lapsed between the end of the training phase and the beginning of the testing phase. MDMA was dissolved in 60 mL of TANG® (without quinine). Doses were administered in a mixed order within and across baboons; at least 8 and typically >14 days elapsed between doses.

Blood Sampling: To obtain blood samples, baboons were anesthetized with intramuscular injections of either ketamine hydrochloride or the commercially available solution of 50:50 tiletamine hydrochloride and zolazepam hydrochloride (Telazol®); atropine sulfate was given to control secretions. Preliminary studies showed no difference of MDMA metabolism after use of

either anesthetic. In particular, a baboon was administered 1.3 mg/kg MDMA orally on two occasions, with an interval of two weeks between treatments each treatment. Blood was collected as described below, the first time using ketamine hydrochloride as anaesthetic and the second time using Telazol®. No differences were observed (data not shown). After all doses tested, blood samples were taken at 0.125, 0.25, 0.5, 1, 2, 4, 8, and 12 h after MDMA administration. The first 4 blood draws (i.e., those within the first hour) were collected after the first anesthetic dose; all subsequent blood draws took place after separate anesthetic dosages. At each time point, at least 1.5 mL of blood was collected, via either the saphenous or cephalic vein into a tube containing EDTA as an anticoagulant. Plasma was processed and stored as previously described (Mueller et al., 2009).

Measurement of plasma MDMA and metabolite concentrations: Plasma MDMA, MDA, HHMA and HMMA concentrations were determined as recently described (Mueller et al., 2009b). The linear range for the method used in the present study was 20 – 1000 ng/mL for HHMA, HMMA, and MDMA and 10 – 500 ng/mL for MDA. If values were found, after initial plasma analysis, to be above the calibration range, the corresponding plasma samples were diluted in the same way as samples for the determination of the above-calibration-range quality control samples (ACR QC) during the method validation procedure (Mueller et al., 2007), and were re-analyzed. Values below the limit of quantification [LOQ, 20 ng/mL (HHMA, HMMA, and MDMA) or 10 ng/mL (MDA)] were assumed to be *zero* and treated as such for calculation of pharmacokinetics.

Calculation of pharmacokinetic parameters: Peak plasma concentration (C_{max}), time of peak plasma concentration (T_{max}), elimination half life ($T_{1/2}$), and area under the concentration-time curve (AUC) were calculated using the pharmacokinetic functions for Microsoft Excel (developed by Usansky et al., http://www.boomer.org/pkin/xcel/pkf/pkf.doc). AUC was calculated using the linear trapezoidal rule starting at time zero and finishing at the last quantifiable point.

Results

MDMA: Plasma time-concentration profiles of orally administered MDMA and its metabolites in baboons and humans are shown in Fig. 2. The human data, shown for comparative purposes, are from recently published studies (de la Torre et al., 2000a; Kolbrich et al., 2008b; Mueller et al., 2009a). As indicated above, doses in baboons are expressed as human equivalent doses (calculated using interspecies dose scaling methods – see Methods). Surprisingly, after the two lower MDMA doses (0.5 and 1.6 mg/kg), no MDMA was detected in baboon plasma (Fig. 2). After the two higher doses (3.2 and 5.0 mg/kg), some MDMA was detected but levels were much lower than those found in humans given lower doses (Fig. 2, Tab. 1). For example, after administration of 3.2 mg/kg of MDMA to baboons, the C_{max} of MDMA was approximately 100 ng/mL, whereas the C_{max} of MDMA in humans receiving half the dose of MDMA (1.6 mg/kg) was more than twice as high (approximately 250 ng/mL). In addition to these differences in C_{max}, there were marked differences between AUC values of MDMA in baboons and humans. This is illustrated by the fact that a five-fold higher dose of MDMA was required to generate comparable AUC values in baboons (1201 ng/mL after 5.0 mg/kg of MDMA) and humans (1389 ng/mL after 1.0 mg/kg of MDMA; see Tab. 1). The time required to

reach peak MDMA concentrations (T_{max}) also differed between the two species: 2.3 hours in humans compared to 7 hours in baboons (Tab. 1).

HHMA: After all MDMA doses, including the two doses that gave rise to no detectable MDMA in baboon, HHMA was readily detected in plasma of baboons (Fig. 2, Tab. 1). Levels of HHMA in baboons were substantially higher than those in humans given an identical MDMA dose. For example, after administration of the 1.6 mg/kg dose, the C_{max} of HHMA in the baboon was nearly threefold higher than that in human (Tab. 1). As shown in Fig. 2, HHMA levels in baboons rose as the dose of MDMA was increased from 0.5 to 3.2 mg/kg. However, despite a dose increase of approximately 60% (from 3.2 to 5.0 mg/kg), the C_{max} of HHMA remained relatively constant (Fig. 2, Tab. 1). Similar to C_{max}, after administration of equivalent doses (namely, 1.6 mg/kg), the AUC of HHMA in baboons was twice as high as in humans (Tab. 1). The $T_{1/2}$ of HHMA in baboons was substantially shorter than that in humans (approximately 3 hours versus 10 hours). The T_{max} of HHMA was approximately two-fold longer in baboons than in humans (Tab. 1).

HMMA: The C_{max} of HMMA increased as the dose was increased from 0.5 to 3.2 mg/kg but then remained constant despite a further increase in MDMA dose from 3.2 to 5.0 mg/kg (Fig. 2, Tab. 1). After administration of equivalent doses (namely 1.6 mg/kg), the C_{max} of HMMA in baboons was only approximately half of that in humans, while its AUC was one fourth of that in humans (Fig. 2, Tab. 1). The relative proportion of HMMA to HHMA also differed between baboons and humans, with plasma levels of HMMA in baboons being only approximately one-tenth of those of HHMA across the dose range tested (Fig. 2, Tab. 1). Similar to the T_{max} of

HHMA, the T_{max} of HMMA was approximately two-fold longer in baboons than in humans (Tab. 1).

MDA: Like MDMA, its *N*-demethylated metabolite, MDA, was only detected in baboon plasma after administration of the two higher MDMA doses (3.2 and 5.0 mg/kg) (Fig. 3, Tab. 1). As in humans, levels of MDA in the baboon were lower than those of MDMA. However, the relative proportion of MDA to MDMA in baboons was greater than in humans (approximately 20-60% versus 3-5 %) (Fig. 3, Tab. 1). Because MDA was not detected in baboons after treatment with 1.6 mg/kg of MDMA, C_{max} and AUC could not be directly compared between the two species. In baboons, the T_{max} of MDA was approximately 2 hours longer than that of MDMA (9-10 hours versus 7 hours, respectively) (Tab. 1). As already seen with the parent compound and the other metabolites, the T_{max} of MDA was longer in baboons, when compared to that of humans (Tab. 1).

Extensive first-pass metabolism severely limiting the systemic bioavailability of MDMA was evident in all 4 baboons used in this study, including the animal (BS) without a prior drug history (Fig. 4).

Discussion

The major finding of the present study is that MDMA metabolism in baboons differs markedly from what has been reported in humans. In particular, after oral ingestion of MDMA, first pass metabolism of MDMA to HHMA in the baboon appears to be much more rapid than in humans. In baboons, MDMA doses that engender MDMA plasma levels ranging from 100 to

300 ng/ml in humans [0.5 to 1.6 mg/kg, (de la Torre et al., 2000a; Kolbrich et al., 2008b; Mueller et al., 2009a)] give rise to no detectable MDMA, but high HHMA levels. An interesting and noteworthy consequence of the rapid first-pass *O*-demethylenation of MDMA in baboons is that, despite receiving MDMA, this animal is exposed to little or no MDMA, at least not after doses that engender substantial amounts of MDMA in humans. Extensive first-pass metabolism, occurring after oral administration, that limits the systemic bioavailability of MDMA in baboons may render this animal ideal for determining which MDMA actions are related to the parent compound (MDMA) and which are related to its major metabolite, HHMA and other downstream metabolites (e.g., HMMA).

As alluded to above, the present data indicate that HHMA is the major metabolite of MDMA in baboons. HHMA levels in baboons far exceeded those of other MDMA metabolites (HMMA and MDA) (Fig. 2). Of note, HHMA levels in baboons were 4- to 10-fold higher than those of MDMA (Fig. 2, Tab. 1). This differs from what is seen in humans, where HHMA levels are typically lower than those of MDMA (Fig. 2). One potential reason for the far more rapid conversion of MDMA to HHMA in baboons could be greater activity of the cytochrome P450 2D6 (CYP2D6) ortholog in baboons, relative to humans. Other possible explanations include higher amounts of the enzyme and/or the involvement of other CYP subtypes in catalyzing the *O*-demethylenation of MDMA in baboons.

The pharmacokinetic profile of HMMA, the *O*-methylated product of HHMA, also differed between baboons and humans. In particular, in baboons, HMMA plasma levels are substantially lower than those that have been reported in humans exposed to similar dosages of

MDMA (de la Torre et al., 2000a; Kolbrich et al., 2008b; Mueller et al., 2009a), possibly due to the decreased conversion of HHMA to HMMA in baboons. Over the dose range tested in the current study, approximately 10% of HHMA was converted to HMMA in baboons, whereas in humans approximately 50% of HHMA undergoes metabolism to HMMA (resulting in a 1:1 ratio of HHMA to HMMA in humans) (Mueller et al., 2009a). These observations suggest that catechol-*O*-methyltransferase (COMT)-mediated metabolism of HHMA to HMMA is less efficient in baboons than in humans (Fig. 2). One possible explanation for the reduced formation of HMMA in baboons could be that the very high levels of HHMA saturate COMT (Meyer and Maurer, 2009). However, in the present circumstance, this seems unlikely because HMMA levels would be expected to remain constant across the MDMA dose range tested, and this was not observed. Rather, as the dose of MDMA was increased from 0.5 to 3.2 mg/kg, levels of HMMA also increased (Fig. 2), suggesting that overall decreased COMT activity, rather than COMT saturation, is the basis for the lower levels of HMMA in baboons when compared to humans.

As in humans, MDA appears to be a relatively minor metabolite of MDMA in baboons. In fact, MDA was only detected in baboons after the two higher doses of MDMA tested (3.2 and 5.0 mg/kg) (Fig. 3). Of note, however, *N*-demethylation appears to be more efficient in baboon than in humans because, in baboons, approximately 20-60% of MDMA was converted to MDA, whereas in humans it is only 3-5% (Kolbrich et al., 2008b) (Fig. 3). Higher enzyme activity and/or a greater amount of the enzyme system responsible for the formation of MDA in baboons may be the basis for this species difference.

Notably, as in other species, the pharmacokinetics of MDMA in baboons appears to be nonlinear. That is, MDMA plasma levels rose to a higher degree than predicted by the increase in dose. Specifically, when the dose of MDMA was further increased from 3.2 to 5 mg/kg, plasma MDMA levels rose by a factor of 2.56 rather than by the dose predicted factor of 1.56. This observation, coupled with the fact that HHMA levels remained constant (despite approximately a 60% increase in MDMA dose), point to nonlinear MDMA pharmacokinetics in baboons. CYP2D6, the enzyme responsible for *O*-demethylenation of MDMA to HHMA in humans, has been shown to be susceptible to saturation and/or inhibition by MDMA (Heydari et al., 2004; Ramamoorthy et al., 2002), and this saturation/inhibition is thought to be responsible for the nonlinear MDMA pharmacokinetics observed in humans (de la Torre et al., 2000a; Kolbrich et al., 2008b; Mueller et al., 2009a) as well as in rats (Chu et al., 1996) and squirrel monkeys (Mueller et al., 2008). The present observations indicate that the CYP2D6 ortholog in baboons is also susceptible to MDMA saturation and/or inhibition.

Of note, the plasma concentration of MDMA at which MDMA metabolism to HHMA becomes saturated and/or inhibited (and MDMA pharmacokinetics become nonlinear) is approximately the same plasma concentration at which nonlinear MDMA pharmacokinetics becomes evident in humans (de la Torre et al., 2000a; Kolbrich et al., 2008b; Mueller et al., 2009a), rats (Chu et al., 1996), and squirrel monkeys (Mueller et al., 2008). An important consequence of MDMA pharmacokinetics becoming nonlinear at plasma levels engendered by typical pharmacological doses of MDMA is that high doses are not required to produce unexpectedly high MDMA plasma concentrations. That is, seemingly small increases in dose

may result in unexpectedly high plasma MDMA concentration. Such disproportionate increases in plasma MDMA concentration may give rise to unexpected toxicities.

Differences in pharmacokinetics between baboons and humans do not generalize to other non-human primates. In particular, squirrel monkeys metabolize MDMA in a manner that is highly similar to that in humans (Mueller et al., 2009a). One of the few differences between humans and squirrel monkeys is that the $T_{1/2}$ of MDMA in the squirrel monkey is shorter than in humans (2-3 hours versus 6-9 hours). However, this difference is expected given the substantial difference in body mass between the two species (approximately 1 kg versus 70 kg), and the known fact that smaller animals metabolize drugs faster than larger animals (Mordenti, 1986). The different pharmacokinetics of MDMA in the baboon are unlikely to be related to the fact that it is an "Old World" rather than a "New World" primate (like the squirrel monkey) because rhesus monkeys, which are also "Old World" primates, display MDMA pharmacokinetics that appear to resemble those in humans (Banks et al., 2007).

Potential limitations of the study should be acknowledged. Three of the four baboons used in the present study had previous drug exposure. In theory, prior drug exposure could have induced or altered hepatic enzyme activity in such a way as to contribute to the observed differences between baboons versus humans. Mitigating against this concern, however, is the fact that the animals had been drug-free for at least 4 weeks. Further, one baboon had no drug history and showed a similar profile of MDMA metabolism as the 3 baboons that had had prior drug exposure (Fig. 4). Another potential limitation is that use of anesthesia in the baboon (to facilitate collection of blood samples) could have altered the metabolism and disposition of

MDMA. However, there is no evidence that either of the anesthetic agents used in this study (Telazol®, ketamine) alters MDMA metabolism, and preliminary studies with Telazol® and ketamine yielded similar findings with regard to the metabolic pattern of MDMA (unpublished observation).

It remains to be determined how the extensive first-pass metabolism that limits systemic bioavailability of MDMA in baboons relates to previously reported biological effects of MDMA in this animal, namely, self-administration (Lamb and Griffiths, 1987) and neurotoxicity (Scheffel et al., 1998). In this regard, it is important to note that both of the aforementioned studies (Lamb and Griffiths, 1987; Scheffel et al., 1998) involved *repeated* MDMA dosing. With repeated dosing, we suspect that there is inhibition/saturation of CYP2D6-ortholog-mediated *O*-demethylenation of MDMA to HHMA, and that this leads to MDMA accumulation. Further, Lamb and Griffiths (1987) employed the intravenous route for their self-administration studies, thereby bypassing hepatic first-pass metabolism that occurs after oral administration. Thus, baboons in the above mentioned studies were in all likelihood exposed to a combination of MDMA and metabolites. This makes it impossible to know if the observed effects were primarily related to the parent compound or one of its metabolites.

In sum, the present results suggest that baboons may not be ideal for modeling human exposure to MDMA and its metabolites because the metabolism and disposition of MDMA in the baboon differ markedly from those in humans. Nevertheless, the distinct pharmacokinetic profile of MDMA in baboons suggests that this laboratory animal may be extremely useful for

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clarifying the relative role of parent compound versus metabolites in pharmacology and toxicology of MDMA.

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Authorship Contributions

Participated in research design: Mueller, Goodwin, McCann, Ator, and Ricaurte.

Conducted experiments: Mueller, Goodwin.

Contributed analytic tools: Mueller, Goodwin.

Performed data analysis: Mueller.

Wrote or contributed to the writing of the manuscript: Mueller, Goodwin, Ator, McCann, and Ricaurte.

Other: Ator, Ricaurte, McCann acquired funding for the research.

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Footnotes

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Legend for Figures

Fig. 1: Metabolic pathways of MDMA, along with the associated microsomal enzymes.

Fig. 2: Relative proportions of MDMA and its *O*-demethylenated metabolites (HHMA and HMMA) in baboons and humans at various times after administration of different oral doses of MDMA. Doses given to baboons were calculated to be equivalent to 0.5, 1.6, 3.2 and 5.0 mg/kg in humans (see Methods). Human data are from Mueller et al. 2009a (1.0 mg/kg and 1.6 mg/kg; same analytic method) and de la Torre et al. 2000a (2.0 mg/kg; different analytic method). Values shown are the mean. For sake of clarity error bars are not shown. *Subjects in the study performed by de la Torre et al. received 150 mg of MDMA, which equals a 2.0 mg/kg dose, assuming an average body weight of 70 kg.

Fig. 3: Relative proportion of MDMA to its *N*-demethylenated metabolite, MDA, in baboons and humans. Data shown are from baboons that received MDMA at a dose of 3.2 mg/kg and humans that received MDMA at a dose of 1.0 mg/kg. These doses were compared because they produced comparable MDMA levels. Human data are from Kolbrich et al. 2008a. Values shown are the mean. For sake of clarity error bars are not shown.

Fig. 4: Individual plasma-concentration-time profiles of *O*-demethylenated MDMA metabolites (HHMA and HMMA) after administration of equivalent doses of MDMA (1.6 mg/kg) to different baboons. Listed along with the individual plasma profiles is the history of prior drug exposure of each animal. Three of the animals had drug histories (subjects 1-3); one had had no

prior drug exposure (subject 4). Note that MDMA and MDA were not detected in the plasma of any of the baboons after the 1.6 mg/kg dose of MDMA. Values shown are the mean. For sake of clarity error bars are not shown.

Tab. 1 Comparison of pharmacokinetic parameters C_{max}, AUC, T_{max}, and $T_{1/2}$ for MDMA and its metabolites in baboons and humans after administration of different oral doses of MDMA. Doses consumed orally by baboons were equivalent to 0.5, 1.6, 3.2 and 5.0 mg/kg in humans, which were, respectively, approximately 0.6, 2.0, 4.0, and 6.0 mg/kg for each baboon. Values shown are the mean ± SEM; except for the 2.0 mg/kg dose, where individual pharmacokinetic data are presented. Human data are from Mueller et al. 2009 and Kolbrich et al. 2009 (1.0 mg/kg and 1.6 mg/kg) and from de la Torre et al. 2000 (2.0 mg/kg). *Note: Subjects in the study performed by de la Torre et al. received 150 mg of MDMA, which equals a 2.0 mg/kg dose assuming an average body weight of 75 kg.

Human						Baboon				
Dose (mg/kg)	**C_{max} (ng/mL)**	**AUC (ng·h/mL)**	**T_{max} (h)**	**$T_{1/2}$ (h)**	**Analyte**	**C_{max} (ng/mL)**	**AUC (ng·h/mL)**	**T_{max} (h)**	**$T_{1/2}$ (h)**	**Dose (mg/kg)**
1.0	147 ±10	1389 ±119	2.3 ±0.2	7.2 ±0.6	**MDMA**	0	0	0	0	**0.5**
	146 ±12	1471 ±146	1.3 ±0.1	10.7±0.9	**HHMA**	331 ±91	1589 ±575	3.1 ±0.9	2.5 ±0.2	
	180 ±18	1759 ±92	1.4 ±0.1	9.8 ±1.1	**HMMA**	28 ±11	123 ±53	3.0 ±1.0	N/A	
	8 ±0.5	188 ±13	7.5 ±0.4	11 ±1.1	**MDA**	0	0	0	0	
1.6	255 ±20	3071 ±225	2.4 ±0.2	8.4 ±0.5	**MDMA**	0	0	0	0	**1.6**
	152 ±11	1801 ±130	1.6 ±0.2	11.9 ±0.9	**HHMA**	694 ±104	4000 ±644	3.0 ±0.6	2.6 ±0.3	
	168 ±14	2060 ±109	1.7 ±0.1	13.7 ±1.3	**HMMA**	85 ±17	505 ±95	3.0 ±0.6	4.0 ±0.6	
	14 ±1.0	352 ±27	7.6 ±0.6	12 ±0.9	**MDA**	0	0	0	0	
2.0*	442 - 487	5133 - 5232	1.5 – 2.0	6.9 – 7.2	**MDMA**	97 ±49	631 ±357	7.0 ±1.0	N/A	**3.2**
		Not Analyzed			**HHMA**	1059 ±87	6896 ±465	4.5 ±1.3	4.6 ±1.6	
		Values Not Reported			**HMMA**	135 ±22	859 ±109	5.5 ±1.5	2.9 ±0.5	
	34 - 31	590 - 374	4 - 10	37.3 – 23.2	**MDA**	59 ±16	334 ±96	9.0 ±1.9	N/A	
					MDMA	249 ±86	1201 ±458	7.1 ±2.5	N/A	**5.0**
					HHMA	988 ±233	6081 ±1211	6.5 ±2.2	2.6 ±0.9	
					HMMA	131 ±31	723 ±143	7.0 ±1.9	2.8 ±0.3	
					MDA	58 ±27	256 ±147	10.7 ±1.3	N/A	

Figure 1

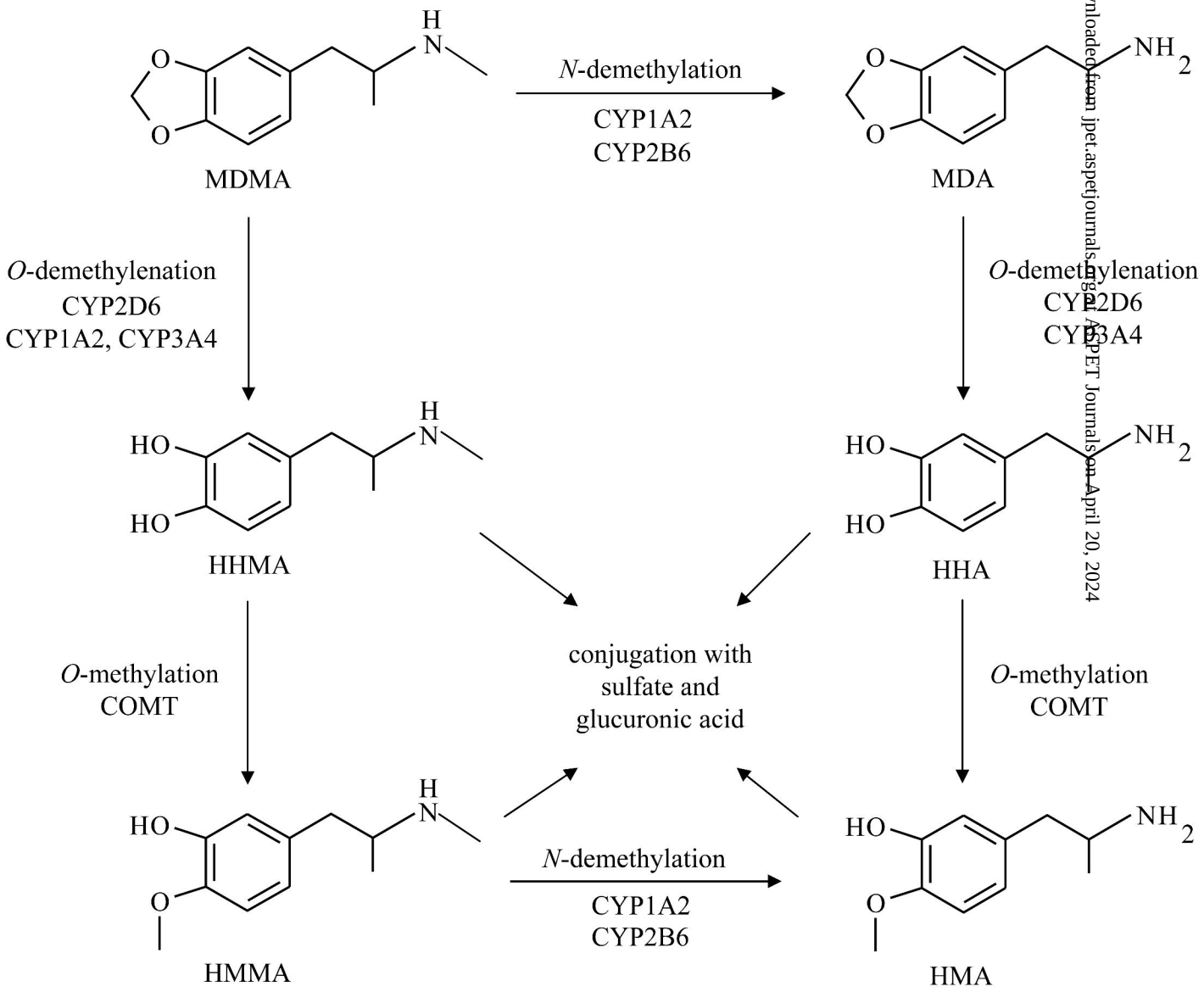

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Figure 2

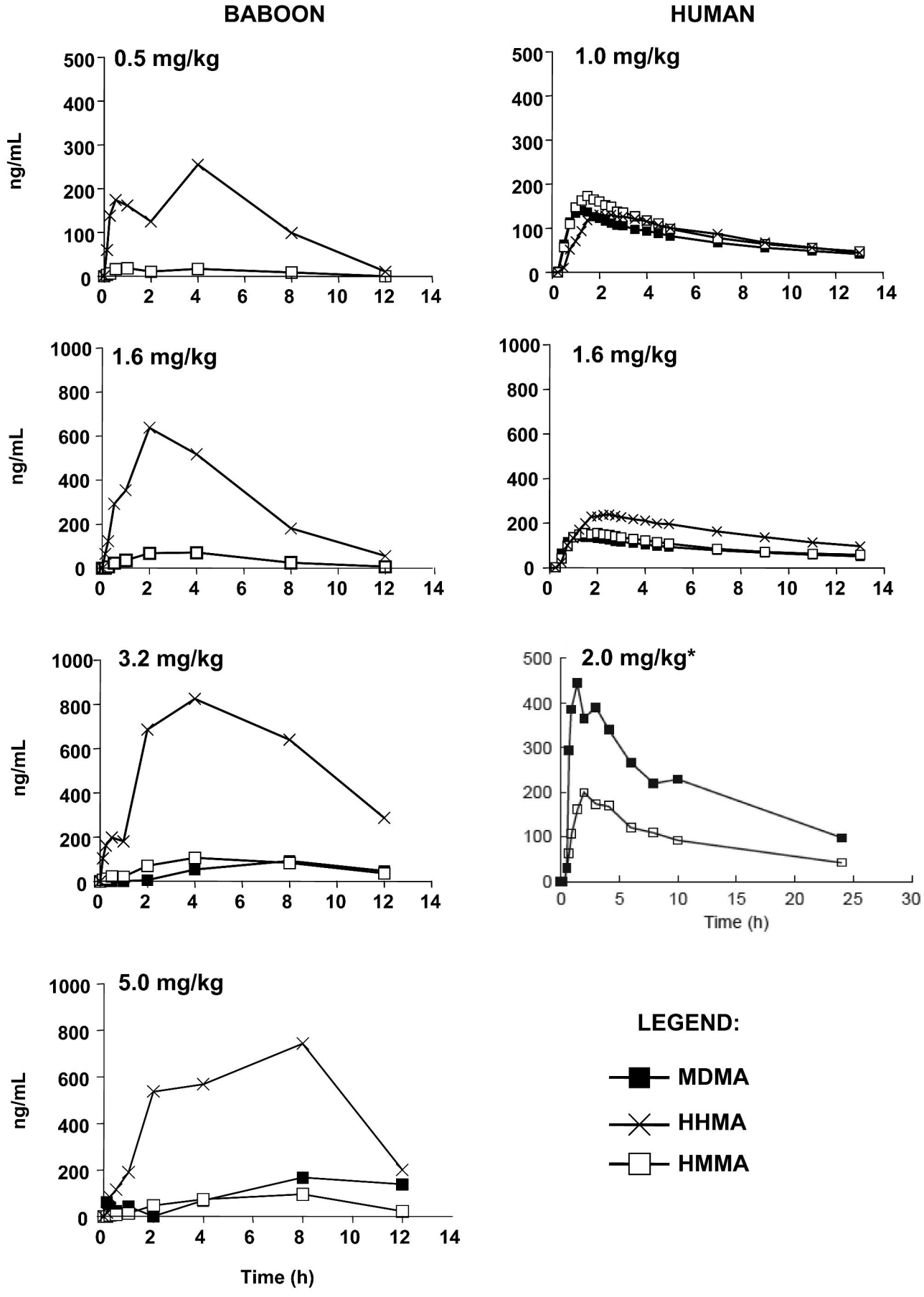


Figure 3

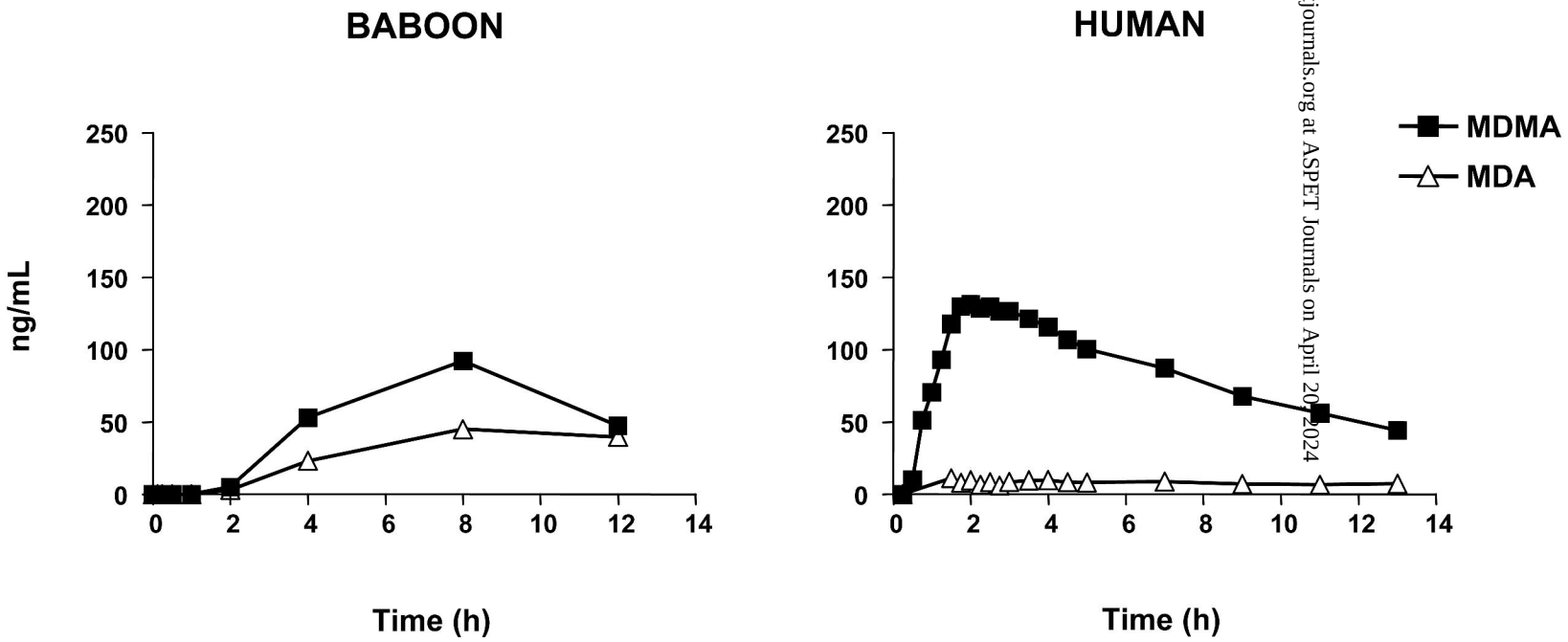
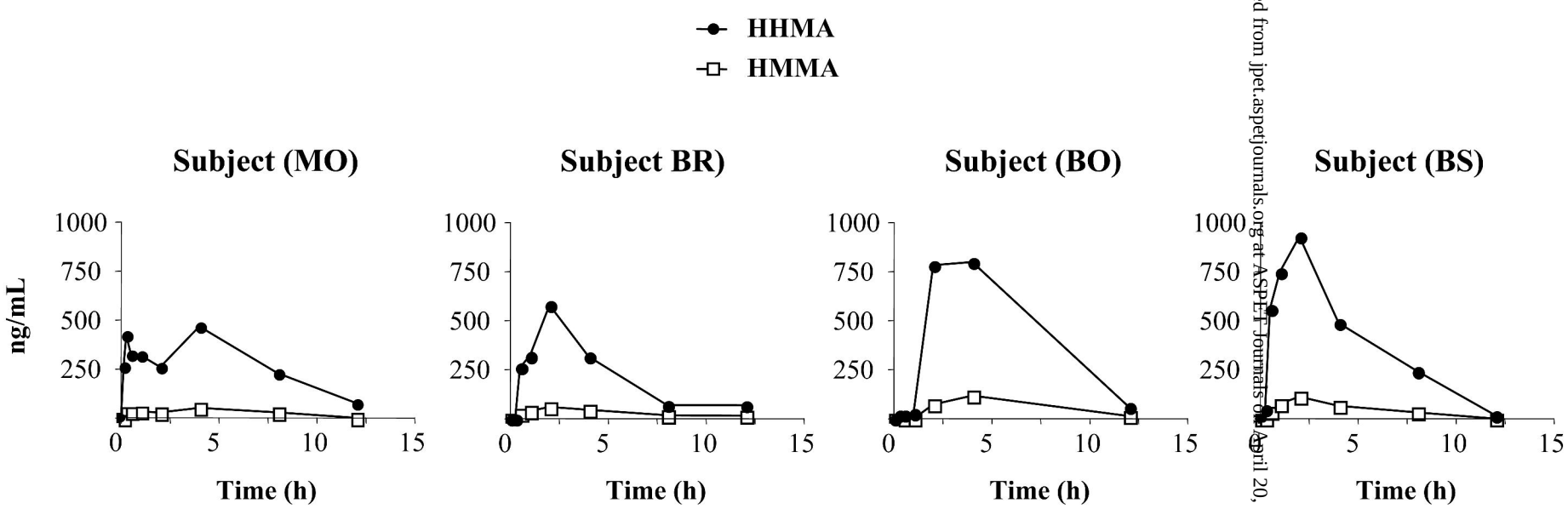


Figure 4



Prior Drug Exposure

MO	BR	BO	BS
Cocaine	Ethanol	Cocaine	None
Tryptamine derivatives		Tryptamine derivatives	
		Benzodiazepines	
		NBZ hypnotic sedativa	
		Gamma-hydroxybutyric acid	