Behavioral effects and pharmacokinetics of (±)-3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) after intragastric administration to baboons

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Running Title Page

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d. Non-standard abbreviations: FR, fixed ratio; HCl, hydrochloride; HHMA, 3,4-dihydroxymethamphetamine; HMMA, 4-hydroxy-3-methoxymethamphetamine; i.g., intragastric; i.v., intravenous; LOQ, limit of quantification; MDA, 3,4-methylenedioxyamphetamine; MDMA, 3,4-methylenedioxymethamphetamine; PCA, perchloric acid; RO, reverse osmosis; SMBS, sodium metabisulfite; SO4, sulfate

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Abstract

(±)-3,4-Methylenedioxymethamphetamine HCl (MDMA, “Ecstasy”) is a popular drug of abuse. We aimed to characterize the behavioral effects of intragastric MDMA in a species closely related to humans and to relate behavioral effects to plasma MDMA and metabolite concentrations. Single doses of MDMA (0.32 – 7.8 mg/kg) were administered via an intragastric catheter to adult male baboons (N=4). Effects of MDMA on food-maintained responding were assessed over a 20-h period, while untrained behaviors and fine-motor coordination were characterized every 30 min until 3 h post-administration. Levels of MDMA and metabolites in plasma were measured in the same animals (N=3) following dosing on a separate occasion. MDMA decreased food-maintained responding over the 20-h period, and systematic behavioral observations revealed increased frequency of bruxism as the dose of MDMA was increased. Drug blood level determinations showed no MDMA after the lower doses of MDMA tested (0.32-1.0 mg/kg) and modest levels following higher MDMA doses (3.2-7.8 mg/kg). High levels of 3,4-dihydroxymethamphetamine (HHMA) were detected after all doses of MDMA, suggesting extensive first-pass metabolism of MDMA in the baboon. The present results demonstrate that MDMA administered via an intragastric catheter produced behavioral effects that have also been reported in humans. Similar to humans, blood levels of MDMA following oral administration may not be predictive of the behavioral effects of MDMA. Metabolites, particularly HHMA, may play a significant role in the behavioral effects of MDMA.
Introduction

(±)-3,4-Methylenedioxymethamphetamine HCl (MDMA; Ecstasy) is a popular drug of abuse. Structurally, MDMA is related to both amphetamine (Gold et al., 1989; Verrico et al., 2008) and the hallucinogen mescaline (Kovar, 1998). According to the National Survey on Drug Use and Health, lifetime MDMA use in the U.S. has increased significantly from 4.3% in 2002 to 6.3% in 2010 among people aged 12 and older.

Behavioral effects following MDMA administration are undoubtedly functional consequences of MDMA-induced changes in brain neurochemistry. Thus, it is important to note that MDMA is a potent indirect monoaminergic agonist and reuptake inhibitor that also inhibits tryptophan hydroxylase, an essential enzyme in the synthesis of serotonin (Malberg and Bonson, 2001), for reviews see Cole and Sumnall, 2003; Green et al., 2003. Biologically relevant differences in the serotonin-1 (5-HT1) and 5-HT2 receptor systems of rodent and primate species (Weerts et al., 2007), and an overall >98% gene homology between monkeys and humans in the case of monoamine transporters (Hacia et al., 1998), makes baboons an attractive species for characterizing the behavioral effects of MDMA. In addition, a characterization of the relationship between behavioral and pharmacokinetic effects of MDMA in baboons would be useful for comparison of similar effects in humans.

In addition to possible species differences in relevant physiology, there are also species differences in the metabolism of MDMA. For example, while 3,4-methylenedioxymphetamine (MDA) is a minor metabolite of MDMA in humans (3-5%) whereas, in rats, the percentage of MDMA N-demethylated to form MDA was 23-34% following oral MDMA administration (Baumann et al., 2009; Mueller et al., 2009c). In comparison, 3-5% of MDMA is metabolized to MDA in humans (Farre et al., 2004; Kolbrich et al., 2008) and squirrel monkeys (de la Torre et al., 2000b; Mueller et al., 2008). In baboons, as reported in humans, MDA appears to be a relatively minor metabolite of MDMA, which is only detected after higher MDMA doses. Initially MDMA to MDA ratios in baboons seem higher when
compared to humans, however, ratios tend to decrease with increases in body weight (Mueller et al., 2011).

There is a surprising lack of research characterizing the behavioral effects of orally administered MDMA in non-human subjects given that the oral route is the one most commonly used by recreational MDMA users (Green et al., 2003). Moreover, differential behavioral and pharmacological profiles as a function of the route of administration for abused drugs are a focus of clinical research (e.g., see Kuramoto et al., 2011), and the pharmacokinetics of MDMA do differ across routes of administration in rats (Baumann et al., 2009). In non-human primates, oral MDMA administration increased body temperature while suppressing locomotion in rhesus monkeys (Crean et al., 2007) and increased error rates in a cognitive task in cynomolgus monkeys (Verrico et al., 2008). In human laboratory studies, oral administration of MDMA produced increases in heart rate and blood pressure as well as increases in subjective responses such as energy level, empathy (Kolbrich et al., 2008; Peiro et al., 2012), and feelings associated with increased social behavior (Bedi et al., 2010).

The pharmacokinetics of orally administered MDMA are non-linear across species (de la Torre et al., 2000a; Pacifici et al., 2001; Farre et al., 2004; Mechan et al., 2006; Mueller et al., 2008; Mueller et al., 2009a; Mueller et al., 2011). Hence, clinically relevant comparisons of the behavioral and pharmacokinetic effects of MDMA must include a consideration of the dose range and dosing schedules used. For example, human studies of the behavioral and pharmacokinetic effects of MDMA have been limited to the lower portion of the abused MDMA dose range (for review see Parrott, 2005) and this is likely due to the neurotoxic effects of MDMA reported in rodents and non-human primates (Sarkar and Schmued, 2010).

We sought to follow up on our previous work with oral MDMA in the baboon (Mueller et al., 2011) to characterize the behavioral effects of MDMA across a wide dose range (0.32-7.8 mg/kg) as well as to determine the plasma concentration-over-time profiles of MDMA and its major metabolites in the same subjects. To assure reliable oral delivery of the dose throughout the assessments, we used a
route of administration that mimics oral administration (i.e., via chronically indwelling intragastric, i.e., catheters).
Methods

Subjects

Four adult male baboons (*Papio hamadryas anubis*) designated as GD, PY, SHA, and YO served as subjects and ranged in weight from 18.4-33.2 kg at the start of the study (see table 1). Each of the subjects had previously served in experimental conditions that involved chronic i.g. administration of a single dose of a test drug for approximately a month, followed by evaluation of the effects of drug withdrawal. All had experience with one (PY) to five (YO) such studies of novel GABA-A allosteric modulators (e.g., Ator et al., 2010). Two baboons (GD, PY) also had served in an intravenous self-administration procedure in which they had experience with cocaine and five or six sedative/anxiolytic compounds (e.g., Ator et al., 2010), and PY had experience with oral ethanol self-administration. Otherwise, GD, SHA, and YO had experience with bolus i.g. dosing with three compounds to characterize effects on food-maintained behavior under the procedure used in the present paper. The baboons had not served in experimental conditions for 6 months prior to the present investigation. They had patent i.g. catheters, which had been implanted at the time of previous studies. The catheter was protected using a tether/vest system that allowed for free movement within the cage. The procedures for catheter implantation surgery, as well as a complete description of the tether/vest system, have been described previously (Lukas et al., 1982).

The baboons had continuous access to tap water via a drinking spout located at the front of the cage. In addition to 20-h/day access to food pellets (see below), diets were supplemented with once-daily feeding of two pieces of fresh fruit or vegetables and a multivitamin delivered late morning each day (i.e., between hours 3 and 4 in the food-maintained behavior measure; see below). Overhead lights were on a 13/11 h schedule, with the overhead lights being illuminated for 13 h/day, and dim lighting 11 h/day; natural light also was provided via windows in the lab area. Physical examinations occurred every 2-3 weeks using atropine sulfate (SO4,) to control secretions, and ketamine hydrochloride (HCl) to permit handling of the baboons. During these examinations, the baboon was
weighed, the catheter exit site was shaved and scrubbed, nails were clipped as needed, and teeth were brushed. The program of environmental enrichment included providing two or more toys or other objects to manipulate (e.g., stainless steel mirrors), which were changed periodically.

**Apparatus**

Baboons were housed in individual stainless steel cages that also served as the experimental chamber. Cages were equipped with a bench along the length of one of the side walls, and a custom-made intelligence panel formed the back wall of the cage. The intelligence panel contained a plunger (Lindsley operandum) over which a cue light was mounted, a food hopper, and a speaker for the delivery of a tone. The Lindsley operandum and food hopper were within easy reach of the baboon when seated on the bench. A strain relief mount (model SR-750B, Instech-Soloman, Plymouth Meeting, PA) was attached at the top of the cage and connected to a peristaltic pump (Harvard Model 1201 or 1203, Harvard Apparatus, S. Natick, MA) and to the catheter via a custom 18-gauge liquid swivel. Reverse osmosis (RO) water was continuously infused (approximately 0.3 ml/min) via the peristaltic pump to maintain catheter patency. Personal computers with MED-PC software and instrumentation (Med Associates, Inc., St. Albans, VT), located in an adjacent room, were used to collect operant data.

**Drugs**

(±)-3,4-methylenedioxymethamphetamine HCl (MDMA) was acquired through the National Institute on Drug Abuse Drug Supply Program (Research Triangle Institute International; Research Triangle, NC). Doses were chosen based on interspecies dose calculations (Dews, 1976; Mordenti and Chappell, 1989) to include the range of doses used recreationally by humans. More specifically, the US Drug Enforcement Administration reported in 2009 that seized tablets generally contained between 50-150 mg of MDMA, and similar ranges have been reported from regions such as the United Kingdom (range of 20-131 mg/tablet) (Wood et al., 2010) and Australia (range of 0-124 mg/tab with an outlier at 249 mg/tab) (Morefield et al., 2011). Self-report studies suggest regular users of MDMA ingest 2-3 tablets at
a time, while highly experienced users may ingest 10-25 tablets at a time (for review see Parrott, 2005). In addition, the majority of users escalate the number of tablets ingested over time (Parrott, 2005). The results of a retrospective self-report study conducted in the UK found that men took an average of 3.55 tablets an average of 6.33 days a month, while women averaged 2.26 tablets an average of 4 days a month (Verheyden et al., 2002). Thus, the dose of MDMA for recreational users may be described as generally falling between 50-450 mg of MDMA (0.7-6.4 mg/kg; baboon equivalent of 0.8-9.1 mg/kg using interspecies dose calculations; (Dews, 1976; Mordenti and Chappell, 1989), depending on how many tablets are ingested.

MDMA (0.32-7.8 mg/kg) was dissolved in 60 ml of reverse osmosis (RO) water and administered within one hour of mixing. MDMA or an equal volume of vehicle alone was administered via the i.g. catheter over 5-6 min, followed by a flush of 10 mls of RO water. MDMA deliveries were separated by at least 5 days, and assessment of vehicle administration never occurred in the 3 days following administration of MDMA. Because total dose can be a relevant variable when individual subjects vary significantly in weight, Table 1 provides the average individual body weights during each phase of the study as well as the order in which doses were studied for each baboon.

**Behavioral Procedures**

**Food Maintained Behavior.** Baboons had daily access (20 h/day) to 1-g banana-flavored food pellets (Bio-SERV, Inc., Frenchtown, NJ) under a fixed-ratio (FR) schedule of reinforcement, in which a pellet was delivered after 10 responses on the Lindsley operandum. Access generally began at approximately 9:00 AM. On test days, MDMA or vehicle was administered between 8:45 and 9:15 AM, and pellet availability began 15 min after the infusion was completed. A cue light illuminated above the Lindsley operandum was correlated with the availability of food pellets under the FR-10 schedule of reinforcement. After 20 h elapsed, the cue light was extinguished and responses had no programmed consequences.
Behavioral Observations. One of two trained observers completed a 17-item behavioral checklist noting the frequency and occurrence of the listed behaviors and postures for 2 min, beginning 30 min after MDMA or vehicle infusion and repeated 60-, 90-, 120-, and 150-min post infusion. Behaviors and postures on the checklist were precisely defined and included those indicative of activity (locomotion, standing), motor coordination (ataxia), muscle tensor/convulsant effects (tremor/jerk), gastro-intestinal symptoms (head-below-torso posture, gag/retch/vomit), social behaviors (lip-smacking, grunting), aggression (yawn), and other behaviors of interest (bruxism, scratching/grooming, rubbing nose, tactile-seeking, shaking cage, posture changes, responsiveness, and stereotypy). The observers had memorized the definitions of the behaviors in advance of the study. The behaviors included were based on those used to characterize the acute effects of other psychoactive drugs (Goodwin et al., 2009) and have been described in detail previously (Weerts et al., 1998). Baboons had habituated to the presence of observers, and reliability between trained observers was 95% prior to the start of the present study. The observer was aware of whether the baboon had received vehicle or an MDMA dose, but the specific demands of the 2-min observation task focus attention on presence or absence of defined behaviors/changes in appearance and reduce ambiguity/subjectivity in recording.

Fine Motor Task. The effects of MDMA on fine-motor coordination were characterized immediately after the behavioral observation described above. In this procedure, a food item (raisin, or plain M&M® if the baboon did not readily perform the task for raisins) was placed in each of 6 small cups on a Plexiglas tray, and the tray was then presented to the baboon at the front of the cage for a maximum duration of 2 min. The positioning of the cups was such that the baboon had easy access to each through the bars of the cage. The number of items retrieved and the duration to retrieve all 6 was recorded, as well as whether there was evidence of manual incoordination (e.g., dropping retrieved items, difficulty grasping), hand tremor, ataxia in movement toward the task and whether there was a failure to engage in the task itself (i.e., failure to approach the front of the cage).
Behavioral Data Analysis. A single-subject design was used in which each baboon served as its own control. Multiple observations after infusion of vehicle were completed in each baboon to characterize the range of behaviors to serve as a baseline for comparison of drug effects for each baboon. Vehicle test results were used to calculate z-scores for each behavioral measure for comparison to results after i.g. MDMA administration. Total food pellets obtained, duration to complete the fine-motor task, and some behaviors recorded in observation sessions (i.e., locomotion, standing, lip-smacking, grunting, yawning, bruxism, scratching/grooming, rubbing nose, tactile-seeking, shaking cage, posture changes, responsiveness, and stereotypy) were judged as significant if the value was outside the 95% confidence limits of the calculated z-scores that delineated the top 2.5% and bottom 2.5% of the distribution (i.e., a two-tailed test). For behaviors that were predicted to increase under drug (ataxia, tremor/jerk, head-below-torso posture, gag/retch/vomit), a change was judged as significant when the frequency of the behavior exceeded the vehicle control z-score above which only 5% of the scores would fall (i.e., a one-tailed test).

Blood Collection Procedures and Analysis

Plasma concentration-over-time profiles of MDMA and its major metabolites were determined after completion of study of the effects of MDMA on behavior because sedation of the baboon was required to be able to draw blood. Using the same administration procedures described above, MDMA or vehicle was administered in mixed order via the i.g. catheter in three (GD, PY, and YO) of the four subjects (see Table 1). The fourth subject (SHA) no longer had a functioning catheter during the blood collection phase of the study. In addition, due to the loss of the catheter in baboon GD, blood collection after the highest dose of MDMA (7.8 mg/kg) occurred in only two subjects (PY and YO).

To allow handling and blood collection, baboons were sedated with a commercially available solution of 50:50 tiletamine HCl and zolazepam HCl (Telazol® Fort Dodge Animal Health, Fort Dodge, IA). Atropine SO4 (Penn Vet, Lancaster, PA) was given to control secretions. Approximately 1.5-2.0 ml of blood was collected from a saphenous vein into a vacutainer containing EDTA as an
anticoagulant at the following timepoints post-administration: 30-min, 1-, 2-, 2.5-, 3-, 3.5-, 6-, and 24-hr.

Blood for all timepoints was collected sequentially in the same 24-hr period. After collection, samples were centrifuged at 3200 rpm for 12 min, and serum was drawn off and transferred to polypropylene tubes that were kept frozen at -80°C until analysis.

Plasma concentrations of MDMA and its metabolites 3,4-dihydroxymethamphetamine (HHMA), 4-hydroxy-3-methoxymethamphetamine (HMMA), and MDA were determined using liquid chromatographic/mass spectrometric (LC/MS) methods as described and validated previously (Mueller et al., 2009b). Briefly, aliquots (100 µL) of plasma were preserved with 20 µL of sodium metabisulfite (SMBS; 250 mM) and 10 µL EDTA (250 mM). 100 µL of the corresponding analytical standard solution was added to the calibrator samples. Accordingly, 100 µL of vehicle was added to each non-calibrator sample to adjust the volume. After addition of 100 µL of an aqueous solution of the racemic internal standards (IS) MDMA-d5, MDA-d5, and pholedrine (1.0 µg/mL, each) and 300 µL 0.5 M of HCl, the samples were mixed (15 s) on a rotary shaker and left at 100°C for 80 min to perform conjugate cleavage. After cooling to room temperature 20 µL of 4-methylcatechol was added to the samples and then briefly mixed. 10 µL of perchloric acid (PCA) was then added and the samples were mixed again on a rotary shaker for 15 s to perform protein precipitation. The samples were centrifuged (16,000 rpm for 5 min), and the supernatant transferred to autosampler vials. Aliquots (5 µL) were injected into the LC/MS system. Analysis was performed using an Agilent Technologies (Waldbronn, Germany) AT Series 1100 LC/MSD, VL version, using electrospray ionization in positive ionization mode, and including an AT 1100 Series HPLC system which consisted of a degasser, a quaternary pump, a column thermostat, and an autosampler. Isocratic elution was performed on a Zorbax 300-SCX column (Narrow-Bore 2.1 x 150 mm, 5-Micron) and a Zorbax SCX guard column (4.6 x 12.5 mm, 5-Micron). The limit of quantification (LOQ) of the method for each analyte was as follows: 20 ng/mL for MDMA, HHMA, and HMMA, each, and 10 ng/mL for MDA.
Results

Behavioral Data

The numbers of 1-g food pellets obtained across the 20 h of availability were recorded in 30-min bins, and the cumulative numbers earned per hour by individual baboons are shown in Figure 1. Three baboons (GD, PY, and YO) typically obtained 50–100 pellets within the first hour or two of pellet availability after vehicle infusion, while the fourth baboon (SHA) did not do so until after the second hour of pellet availability. Two baboons (PY and YO) generally did not continue earning pellets beyond hour 7, while the other two baboons tended to earn at least small numbers of pellets beyond that timepoint. MDMA changed the timing and/or patterning of feeding bouts, and did so at most doses in the largest baboon (YO, Table 1). Although lower doses shifted the curves upward in some cases, MDMA doses of 1.0 mg/kg and above generally produced dose-related decreases in food-maintained behavior, compared to vehicle, during the 6 h following administration (Fig. 1), and feeding for the day was severely disrupted. At doses of 3.2 mg/kg and higher, feeding was almost completely eliminated for the entire 20 h. Because of unprogrammed restricted access to pellets (i.e., feeder malfunction) on the day preceding 3.2 mg/kg MDMA, data for this dose are not included for baboon GD. Figure 2 shows the mean number of pellets earned after administration of MDMA for all baboons. When considered as a group, MDMA doses of 3.2 mg/kg and higher produced decreases in food-maintained behavior when compared to vehicle.

Performance of the fine-motor task is shown in Figure 3. Three baboons typically retrieved all 6 raisins or M&Ms within 20 s or less, without dropping any, regardless of the time at which the task was offered following vehicle administration; the fourth (SHA) took longer to approach completing the task when it was offered for the third, fourth, and fifth time. After MDMA infusion, there was a generally dose-related increase in time taken to complete the task such that it often was not completed when it was offered more than 30 min following the higher MDMA doses. Ataxia (defined as slow, uncertain movements, swaying, or falling over; see Adams and Vistor, 1993; Weerts et al., 1998) was not
observed during presentations of the fine-motor task. Performance on the task was not disrupted by motor incoordination (e.g., dropping the items); rather, baboons failed to engage in the task on those occasions when it was not completed in the allotted time. Dose order (Table 1) may explain occasions when significantly longer durations were required to complete the task (or subjects failed to engage in the task) after administration of the lower MDMA doses (0.32-1.0 mg/kg). For example, baboon PY received 0.56 mg/kg MDMA following the 3.2 mg/kg dose. Observer comments repeatedly included “never approached task” and “sniffed food items but never picked any up.” Thus, MDMA (3.2-7.8 mg/kg) decreased performance of the fine-motor task in all subjects but not as a result of altering motor coordination.

The occurrence of bruxism (defined as gritting or grinding the teeth; see Adams and Vistor, 1993; Weerts et al., 1998) and tremor (defined as more or less regular, rhythmic movement of the limbs, head, or trunk produced by synchronous contractions of antagonistic muscles; see Adams and Vistor, 1993; Weerts et al., 1998) or jerks (defined as muscle contraction and/or relaxation leading to a quick jerking movement or twitch; see Adams and Vistor, 1993; Weerts et al., 1998) during the observation periods are shown in Table 2. After vehicle, all baboons exhibited very low rates of bruxism. After MDMA administration, dose-related increases in bruxism occurred in all four baboons, with the effect generally appearing 30-90-min after administration and persisting at the 150-min observation. Although tremor and jerks were distinctively defined, they were recorded in a single category (tremor/jerk) on the behavioral checklist as indicators of muscle tensor/convulsant effects, and cannot be separated for analysis (notes permitted differentiation on some occasions). Neither tremor nor jerks were observed following vehicle administration. One baboon (SHA) exhibited only one limb tremor (30 min after 5.6 mg/kg MDMA) and a second baboon (GD) exhibited one limb tremor (30 min post-MDMA 1.0 mg/kg and 30 min after 3.2 mg/kg). The remaining two baboons exhibited tremor/jerks after multiple doses of MDMA (see Table 2). Only one baboon exhibited signs of gastro-intestinal distress (data not shown). More specifically, two episodes of vomiting were observed in baboon (YO)
2.5 h following the 7.8 mg/kg dose of MDMA. One baboon (PY) exhibited ataxia following 1.0 mg/kg MDMA 2.5 hr post-administration. Locomotion occurred at relatively low levels following vehicle administration, and the frequency of locomotion did not significantly change following MDMA administration (data not shown).

**Pharmacokinetic Data**

Figure 4 shows plasma concentration levels (ng/mL) of MDMA in the three subjects (GD, PY, and YO) for which the blood study could be conducted and for the group. Levels of the metabolites HHMA, HMMA, and MDA are shown, respectively, in Figures 5, 6, and 7. Neither MDMA, nor the psychoactive metabolite MDA, was detected in plasma at any timepoint for any subject after i.g., administration of the lower MDMA doses (0.32-1.0 mg/kg). After the higher doses (3.2-7.8 mg/kg), MDMA was detected in all subjects (except one after the 3.2 mg/kg) during at least one timepoint post-administration. In contrast, HHMA (Figure 5) was detected in all subjects after administration of all MDMA doses. HMMA (Figure 6) was detected in all subjects at MDMA doses above 0.32 mg/kg. There were striking individual differences in the metabolism of MDMA across subjects. For example, much lower levels of MDMA were generally detected for GD when compared to PY and YO, lower levels of HHMA were generally observed for YO when compared to GD and PY, and MDA was detected only after the two highest MDMA doses.

For the group, mean levels of MDMA, HMMA, and MDA generally did not exceed 150 ng/mL, whereas mean levels of HHMA were greater than 500 ng/mL at all MDMA doses above 0.56 mg/kg. The highest mean level (±SEM) of MDMA (103.8 ± 60.6 ng/mL; n=3), HHMA (1860.2 ± 1000.6 ng/mL; n=3), and HMMA (116.4 ng/mL ± 64.6; n=3) were measured 1 h after administration of the 5.6 mg/kg MDMA dose. In contrast, the highest mean (±SEM) of MDA (26.9 ± 16.4 ng/mL) in plasma following that 5.6 mg/kg MDMA dose was measured at the 24 h timepoint. After administration of 7.8 mg/kg MDMA, albeit in only two baboons, the highest mean level of MDA was also at the 24-h timepoint, but was considerably higher (97.9± 60.0 ng/mL; n=2).
Relation between Behavior and Pharmacokinetic Data

There are limitations on relating the behavioral effects observed to the plasma levels achieved at the same doses in the same animals because the pharmacokinetic data were collected in the context of a second dose-effect study after the behavioral data had been collected and only three of the four baboons could be studied. To the extent that some generalizations can be made, the behavioral effects observed in the present study seemed to be most clearly associated with the detection of high levels of HHMA and/or HMMA in the plasma. For example, feeding behavior measured under the FR schedule was completely suppressed within 30 min after a high (5.6 mg/kg) MDMA dose in baboons (GD and PY; Fig. 1) that later showed high levels of those metabolites 30 min after administration of that dose (Figs. 5 and 6). Feeding behavior in the third baboon (YO) did not differ from vehicle levels until 5 hours after that dose (Fig. 1), which was paralleled by the peaking of HHMA and HMMA measured 6 hours after that dose (Figs. 5 and 6). By contrast, performance on the fine motor task, involving retrieval of food treats, was hardly affected in baboons GD and PY when tested 30 min after the 5.6 mg/kg dose even though feeding behavior per se had been completely suppressed. For YO, for which MDMA, but not metabolite levels, were high 30 min after the 5.6 mg/kg dose, performance of the fine motor task was eliminated, despite feeding behavior showing little change.
Discussion

The present study demonstrates, for the first time, that MDMA is behaviorally active in an Old World Monkey, the baboon, when administered via an i.g. catheter. Moreover, some of the behavioral effects reported for the baboons in this study have also been reported in humans, suggesting that the baboon is a useful animal model for study of oral MDMA. All four baboons exhibited significant amounts of bruxism and decreases in food-maintained behavior, and human MDMA users also experience bruxism and consistently report decreases in appetite (Baylen and Rosenberg, 2006). Overt behavioral changes in the baboons were observed beginning 30-60 min post-MDMA administration and continued to occur at the last observation 2.5 hr post-administration. Decreases in food-maintained responding following MDMA were generally observed across the 20-h experimental sessions at the higher doses. In humans, many of the behavioral effects of MDMA began occurring by 1 h post-administration and continued for several hours (Farre et al., 2004; Kolbrich et al., 2008; Bedi et al., 2010; Peiro et al., 2012). In addition, significant increases in movements defined as tremor and/or limb or torso jerking movements were observed in baboons, and the latter resembled myoclonic jerks. These types of movements have also been described in humans (Baylen and Rosenberg, 2006; Rodgers et al., 2006) and are considered to be one result of altered serotonin activity (Ronditzky, 2005; Dvir and Smallwood, 2008). Indeed, myoclonus may be an early indicator of serotonin syndrome, an adverse effect of MDMA reported in humans (Sarkar and Schmued, 2010).

Consistent with studies in humans (Kolbrich et al., 2008; Mueller et al., 2009a; Peiro et al., 2012), other primate species (Mechan et al., 2006; Mueller et al., 2008; Mueller et al., 2009a), as well as rodents (Baumann et al., 2009; Scheidweiler et al., 2011), the pharmacokinetics of MDMA were nonlinear in baboons. Similar to our previous results with oral administration (Mueller et al., 2011), MDMA was absent from baboon plasma following administration of the lower MDMA doses (0.32-1.0 mg/kg; human equivalent of approximately 0.26-1.42 mg/kg; \( D_{\text{human}} = D_{\text{animal}} \left( \frac{W_{\text{human}}}{W_{\text{animal}}} \right)^{0.7} \) where \( D \) = dose in mg, \( W \) = weight of the animal in kg, and 0.7 is a commonly used and empirically derived
Higher doses of MDMA (3.2-7.8 mg/kg; human equivalent of approximately 2.26-5.5 mg/kg) resulted in a mean maximum concentration of 135 ng/mL MDMA in plasma in the present study and 249 ng/mL (after 5.0 mg/kg) in our previous study (Mueller et al., 2011). In humans, lower doses of MDMA (0.75-1.6 mg/kg) produced somewhat similar ranges of maximum MDMA concentrations in blood (ranging from 161 ng/mL to 306 ng/mL; Farre et al., 2004; Kolbrich et al., 2008; Dumont et al., 2009). While the dose range of MDMA investigated in baboons (0.32-7.8 mg/kg) can be considered to encompass the range of abused doses in humans (0.7-6.4 mg/kg; baboon equivalent of 0.8-9.1 mg/kg using interspecies dose calculations; Sidman, 1960; Dews, 1976; Mordenti and Chappell, 1989), such a range has not been examined in humans under laboratory conditions.

Despite the absence of MDMA in baboon plasma after the lower MDMA doses, the HHMA and HMMA metabolites of MDMA were detected within the first 30 min following every MDMA dose (present study; Mueller et al. 2011). In addition, vastly higher levels of the metabolite HHMA were observed in baboons when compared to levels reported in humans. Since MDMA is metabolized to HHMA mainly via O-demethylation and subsequent O-methylation to HMMA in humans and other primate species, the absence of MDMA after low doses may be related to a remarkably efficient rate of O-demethylation during first-pass metabolism of MDMA to HHMA in baboons. There is no evidence that Telazol® (tiletamine HCl and zolazepam HCl) alters MDMA metabolism, and preliminary studies in our laboratory comparing Telazol® with ketamine, as well as ketamine with saline, have yielded similar findings with regard to the metabolic pattern of MDMA (unpublished observation). We cannot, however, totally rule out the possible influence of Telazol® in the detection of MDMA and its metabolites in plasma. Ideally, behavioral and pharmacological effects following peripheral administration of the HHMA and HMMA should be examined. Additionally, in order to better understand the pharmacokinetics of MDMA in baboons after intragastric administration, analysis of plasma at additional timepoints post-MDMA administration (e.g., 15-min, 7-, 9-, 11-hr, etc.) is needed.
The lack of a strict correspondence between doses and subjects included in both the behavioral and pharmacokinetic studies, as well as the variability in metabolism found in the present study, precluded useful statistical analysis of the correlation between plasma levels of MDMA and its metabolites. However, study of the correspondences for individual subjects permits certain generalizations to be made. First, there did not appear to be a clear correlation between MDMA-induced onset of behavioral changes and baboon plasma levels of MDMA in the present study. The detection of MDMA in baboon plasma sometimes corresponded with the onset of a specific behavioral effect. For example, the appearance of bruxism and tremor/jerks, as well as disruption of the fine-motor task, generally occurred in the first 60 min post-MDMA administration at higher doses, as did peak MDMA levels in plasma at these doses. However, these same behavioral effects were also observed after lower MDMA doses, at which no MDMA was detected in baboon plasma. Indeed, the behavioral effects observed in the present study seem to be most clearly associated with the detection of high levels of HHMA and/or HMMA in baboon plasma.

There are few studies in humans that have examined behavioral changes and pharmacokinetics following oral MDMA administration. The earliest study reported that peak levels of MDMA in plasma occurred around the same time peak subjective effects were reported (1-2 h post administration of 100 mg MDMA; Farre et al., 2004). The next study found that the behavioral effects of MDMA (1.6 mg/kg; total dose of 111-150 mg) were correlated with MDMA plasma concentrations but correlation coefficients were low and clinically insignificant (Kolbrich et al., 2008). A third study reported that the correlation between oxytocin blood levels and the subjective effects of MDMA following MDMA administration (100 mg) was significantly stronger than the correlation between the subjective effects and MDMA blood levels (Dumont et al., 2009). The most recent study in humans (Peiro et al., 2012), reported that the subjective effects of MDMA (100 mg, orally administered) peaked between 1-2 h and returned to baseline by 4 h following administration. Plasma MDMA levels, however, peaked at 3.75 h
(Peiro et al., 2012). Thus, blood levels of MDMA following peripheral administration may not be the best predictor of the behavioral effects of MDMA.

Similar to data with human subjects (Farre et al., 2004; Kolbrich et al., 2008), we observed high intersubject variability in the maximum concentration of MDMA and its metabolites in baboon plasma, as well individual differences in some behavioral effects. Such individual differences in humans, combined with the identification of polymorphisms in the cathechol-O-methyltransferase (COMT) and CYP2D6 genes in humans, has led some to conclude there may be significant individual differences in genetic vulnerability to the effects of MDMA (Carmo et al., 2006; Schilt et al., 2009; Fagundo et al., 2010). Taken together, genetic vulnerability to the effects of MDMA may explain at least some of the individual differences observed in the present study. Indeed, the notion of significant individual differences in response to MDMA being mediated via genetic polymorphisms is reminiscent of current findings in drug toxicity (Spanagel et al., 2010; Johansson and Ingelman-Sundberg, 2011). The role of genetics in mediating the magnitude of individual responses following MDMA administration will be more readily addressed as further models (e.g., genetic knock-outs, etc.) become available for use in this area of study.

In conclusion, MDMA administered via an i.g. catheter produced observable behavioral effects in baboons that were similar to those reported following oral MDMA administration in humans. Behavioral effects in baboons were generally evident 30-60 min following MDMA administration, similar to the onset of behavioral effects in humans. Overall, the baboon appears to be a suitable model for investigations of the behavioral effects of MDMA administered via an i.g. catheter (or orally), although first pass effects are more pronounced in baboons. Studies in humans confirm the notion that the behavioral effects of MDMA may not readily be correlated with detection of MDMA in plasma. HHMA levels, however, were vastly higher in baboons when compared to humans. This species difference in metabolism may render the baboon an ideal model for examining the relative contributions of MDMA versus HHMA and other downstream metabolites following peripheral MDMA administration.
Moreover, a characterization of species differences in the pharmacokinetics of orally administered MDMA will be useful for understanding relationships between behavior and pharmacokinetic effects.
Acknowledgments

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Authorship Contributions

Participated in research design: Goodwin, Ator, Mueller, Ricaurte

Conducted experiments: Goodwin, Mueller, Shell

Performed data analysis: Goodwin, Mueller

Wrote or contributed to the writing of the manuscript: Goodwin, Mueller, Ator, Ricaurte, Shell
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Mueller M, Peters FT, Huestis MA, Ricaurte GA and Maurer HH (2009b) Simultaneous liquid chromatographic-electrospray ionization mass spectrometric quantification of 3,4-methylenedioxymethamphetamine (MDMA, Ecstasy) and its metabolites 3,4-dihydroxymethamphetamine, 4-hydroxy-3-methoxymethamphetamine and 3,4-methylenedioxymethamphetamine in squirrel monkey and human plasma after acidic conjugate cleavage. Forensic Sci Int 184:64-68.


Footnotes

a) This work was supported by the National Institutes of Health [National Institute on Drug Abuse grants DA-05707 (GAR), DA-01796401 (GAR), and DA-021616 (NAA)]

b) This work has not been previously presented.

c) Nancy A. Ator

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Legends for Figures

Figure 1. Food-maintained behavior after i.g. MDMA (0.32-7.8 mg/kg) or vehicle administration in four baboons during the daily 20-h period of food pellet availability. The solid line presents the mean (±SD) cumulative total of 1 g food pellets earned from 15 min to 20 h after vehicle was infused in control sessions (n=5 for GD, n=6 for PY and YO, and n=4 for SHA). Open and closed symbols present the cumulative total food pellets earned from 15 min to 20 h after the MDMA dose was infused. Note difference in scale of Y axis in panels on the left compared to those on the right. MDMA doses were studied once in each baboon (see table 1 for dose order; note that 0.32 was not studied in SHA and 7.8 mg/kg was not studied in GD and SHA). For GD, unprogrammed restricted access to pellets (i.e., feeder malfunction) on the day preceding 3.2 mg/kg MDMA preclude including data for this dose.

*Indicates the total number of food pellets earned over the 20-h period was significantly lower when compared to vehicle (value fell outside the 95% confidence limits of the calculated z-scores for vehicle).

Figure 2. Food-maintained behavior following i.g. MDMA (0.32-7.8 mg/kg) or vehicle administration for the group (n=4 baboons except n=3 for 0.32 mg/kg and n=2 for 7.8 mg/kg). Data for vehicle are the mean (±SEM) total number of food pellets earned and data for MDMA are the mean total of food pellets earned. Other details same as for Fig. 1.

Figure 3. Performance on the fine motor task at multiple timepoints following i.g. MDMA (0.32-7.8 mg/kg) or vehicle administration in four baboons. Data for vehicle are the mean (±SD) durations to complete the task within 120 s at each timepoint post-administration (number of days on which vehicle was administered are the same as in Fig. 1), and data for MDMA are the durations to complete the task at each timepoint following administration. A value of 120 s indicates the task was not completed. The task was not presented to baboon YO at 30 and 60 min following 3.2 mg/kg MDMA administration.

*Indicates the duration to complete the task was significant higher when compared to vehicle at the same timepoint (value was outside the 95% confidence limits of the calculated z-scores for vehicle).
Figure 4. Plasma levels (ng/mL) of MDMA at consecutive sampling intervals after administration of i.g. MDMA (0.32-7.8 mg/kg) to baboons (n = 3, except for 7.8 mg/kg n=2) as well as mean plasma levels of MDMA for the group. The limit of quantification was 20 ng/ml and is indicated with a dashed line.

Figure 5. Plasma levels (ng/mL) of HHMA at consecutive sampling intervals after administration of i.g. MDMA (0.32-7.8 mg/kg) to baboons (n=3 except for 7.8 mg/kg n=2), as well as mean plasma levels of HHMA in for the group. The limit of quantification was 20 ng/ml and is indicated with a dashed line.

Figure 6. Plasma levels (ng/mL) of HMMA at consecutive sampling intervals after administration of i.g. MDMA (0.32-7.8 mg/kg) to baboons (n=3 except for 7.8 mg/kg n=2), as well as mean plasma levels for the group. The limit of quantification was 20 ng/ml and is indicated with a dashed line.

Figure 7. Plasma levels (ng/mL) of MDA at consecutive sampling intervals after administration of i.g. MDMA (0.32-7.8 mg/kg) to baboons (n=3 except for 7.8 mg/kg n=2), as well as mean plasma levels for the group (n=3 except for 7.8 mg/kg n=2). The limit of quantification was 10 ng/ml and is indicated with a dashed line.
## Tables

Table 1. Mean body weights (BW±SD), and order of study of MDMA doses (mg/kg) for the behavioral and pharmacokinetic portions of the experiments. Vehicle sessions occurred prior to MDMA administration and between MDMA sessions during the behavioral assessment.

<table>
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<th>Subject</th>
<th>Average BW (kg±SD)</th>
<th>Order of MDMA (mg/kg) Doses: Behavioral Assessment</th>
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<td>YO</td>
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Order of MDMA (mg/kg) Doses: Plasma Assessment

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Table 2. Mean frequency (±SD) after vehicle administration and frequency after MDMA (0.32-7.8 mg/kg) of "bruxism" and "tremor/jerk" during 2-min observations that began 30 min after MDMA or vehicle infusion and repeated 60-, 90-, 120-, and 150-min post infusion.

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<td>4</td>
<td>0</td>
<td>5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>12&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>1.0 (2.1)</td>
<td>0.0 (0.0)</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>20&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>value was outside the 95% confidence limits of the calculated z-scores for vehicle

<sup>b</sup>limb tremor specifically noted

"n/a" indicates the dose was not administered
Figure 1.

Cumulative Number of 1 g Food Pellets

Hours post-MDMA Administration
Figure 2.
Figure 3.
Figure 4.

Plasma Concentration (ng/mL) vs. Hour Post-MDMA Administration for Baboon GD, Baboon PY, Baboon YO, and Group.

Key:
- ▲: 0.32
- ▼: 3.20
- □: 0.56
- △: 5.60
- ▽: 1.00
- ▼: 7.80
Figure 5.

The graphs represent plasma concentration (ng/mL) over the hours post-MDMA administration for three different baboons: GD, PY, and YO, and for different groups with plasma concentrations of 0.32, 0.56, 1.00, 3.20, and 5.60 ng/mL.
Figure 6.

Baboon GD

Baboon PY

Baboon YO

Group

Plasma Concentration (ng/mL)

Hour Post-MDMA Administration

0.32, 3.20
0.56, 5.60
1.00, 7.80
Figure 7.

**Baboon GD**

**Baboon PY**

**Baboon YO**

**Group**

Plasma Concentration (ng/mL) vs. Hour Post-MDMA Administration

- **Baboon GD**: Plasma concentrations range from 0 to 250 ng/mL with peaks observed at 12 and 20 hours post-administration.
- **Baboon PY**: Shows similar trends with peaks at 12 and 20 hours.
- **Baboon YO**: Displays lower concentrations compared to GD and PY, with peaks at 5.60 and 7.80 ng/mL.
- **Group**: Shows a trend with increasing plasma concentrations over time, with peaks at 12 and 20 hours.

Legend:
- ○ 0.56
- ▲ 3.20
- △ 0.32
- ● 5.60
- □ 1.00
- ■ 7.80