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Locomotor effects of 3,4-methylenedioxymethamphetamine (MDMA) and its deuterated form in mice: psychostimulant effects, stereotypy, and sensitization

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Abstract

Rationale—There is a renewed interest in the use of 3,4-methylenedioxymethamphetamine (MDMA) for treating psychiatric conditions. Although MDMA has entered phase II clinical trials and shows promise as an adjunct treatment, there is an extensive literature detailing the potential neurotoxicity and adverse neurobehavioral effects associated with MDMA use. Previous research indicates that the adverse effects of MDMA may be due to its metabolism into reactive catechols that can enter the brain and serve directly as neurotoxicants. One approach to mitigate MDMA's potential for adverse effects is to reduce *O*-demethylation by deuterating the methylenedioxy ring of MDMA. There are no studies that have evaluated the effects of deuterating MDMA on behavioral outcomes.

Objectives—The purpose of the present study was to assess the motor-stimulant effects of deuterated MDMA (d2-MDMA) and compare them to MDMA in male mice.

Methods—Two experiments were performed to quantify mouse locomotor activity and to vary the drug administration regimen (single bolus administration or cumulative administration).

Results—The results of Experiments 1 and 2 indicate that d2-MDMA is less effective at eliciting horizontal locomotion than MDMA, however the differences between the compounds diminish as the number of cumulative administrations increase. Both d2-MDMA and MDMA can elicit

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Conflict of interest

MDB, SLP, JLK, and WEF declare that they have no conflict of interest.

Conflict of interest:

None

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sensitized responses and these effects cross-sensitize to the prototypical drug of abuse methamphetamine. Thus, d2-MDMA functions as a locomotor stimulant similar to MDMA, but, depending on the dosing regimen, may be less susceptible to inducing sensitization to stereotyped movements.

Conclusions—These findings indicate that d2-MDMA is behaviorally active and produces locomotor effects that are similar to MDMA, which warrant additional assessments of d2-MDMA's behavioral and physiological effects to determine the conditions under which this compound may serve as a relatively safer alternative to MDMA for clinical use.

Keywords

3,4-Methylenedioxymethamphetamine; MDMA; Locomotor activity; Sensitization; Stereotypy; Deuterium substitution; Cumulative administration; Cross-sensitization; Methamphetamine

Introduction

There is a renewed interest in the use of 3,4-methylenedioxymethamphetamine (MDMA) for the treatment of psychiatric conditions, especially posttraumatic stress disorder (PTSD) for which MDMA has entered phase 2 clinical trials (reviewed in Thal and Lommen, 2018). MDMA-assisted therapy sessions typically include intense psychotherapy between therapists and clients during which the clients receive 50–125 mg MDMA—amounts which have been shown to be within the range for recreational consumption (Schifano, 1991; Henry, 1992). Although current therapy sessions utilize relatively few MDMA administration sessions (i.e., 2–3) (Mithoefer et al. 2011; 2018; Ot'alora et al. 2018), future treatment strategies with MDMA may involve larger doses, more frequent administration, and prolonged exposure in treatment-resistant individuals. Based on an extensive literature documenting long-term effects of MDMA consumption in recreational users, chronic exposure and escalating doses of MDMA may be associated with some adverse neurobehavioral effects, such as persistent visual hallucinations (Creighton et al. 1991), pronounced cognitive deficits (Wareing et al. 2000; reviewed in Morgan, 2000), and lasting increases in serotonin 5-HT_{2A} receptor densities (Reneman et al. 2002; Di Iorio et al. 2012). Moreover, preclinical studies in laboratory animals have documented numerous persistent neurochemical alterations which may be consistent with neurotoxicity following some repeated MDMA administration regimens (Stone et al. 1987a; 1987b; Commins et al. 1987; Ricaurte et al. 1988). As such, given the current focus on MDMA as a viable adjunct treatment, it is important to identify strategies that may reduce the probability of developing neurotoxicity and minimize adverse effects, but retain MDMA's desirable therapeutic effects. One approach which may achieve these goals is to alter the metabolism of MDMA.

MDMA metabolism is complex and has been extensively reviewed elsewhere (de la Torre et al. 2004a). Briefly, MDMA metabolism involves *N*-demethylation by cytochrome P450 (CYP) enzymes to the active metabolite 3,4-methylenedioxymphetamine (MDA). MDA is then *O*-demethylated by CYP enzymes to form 3,4-dihydroxyamphetamine (HHA). MDMA is also *O*-demethylated by CYP enzymes to 3,4-dihydroxymethamphetamine (HHMA). It is noteworthy that *N*-demethylation of MDMA to MDA is a major metabolic pathway in rats (Chu et al. 1996), but *O*-demethylation of MDMA to HHMA is more

common in humans (Segura et al. 2001; reviewed in de la Torre and Farre, 2004b). HHA and HHMA can then undergo *O*-methylenation by catechol-*O*-methyltransferase (COMT) to form 4-hydroxy-3-methoxyamphetamine (HMA) and 4-hydroxy-3-methoxymethamphetamine (HMMA), respectively, and conjugate with glucuronides or sulphates; or, HHA and HHMA can rapidly undergo autoxidation to *ortho*-quinones and conjugate with glutathione to form glutathionyl adducts (Carvalho et al. 2004). Accumulations of glutathionyl adducts may be especially problematic as they can cross the blood-brain-barrier via glutathione transporters (Bai et al. 2001), and, once in the brain, glutathionyl adducts are metabolized to *N*-acetylcysteine adducts, which may directly serve as serotonergic neurotoxicants (Miller et al. 1996; Bai et al. 1999) and generate reactive oxygen species. In sum, accumulations of HHA and HHMA may be a major contributing factor toward neurotoxicity observed with MDMA use. As such, reducing levels of HHA and HHMA may reduce the risk of MDMA-elicited toxicity. Substituting deuterium for hydrogen in the methylenedioxy ring (see Figure 1A; top left graph) is one strategy which may reduce the rate of *O*-demethylenation of MDMA and MDA.

Deuterium is a naturally occurring, stable isotope of hydrogen that contains a neutron and proton in its nucleus. Because of its greater atomic mass, deuterium-carbon covalent bonds are generally more stable and require more energy to break compared to hydrogen-carbon bonds. Therefore, replacing hydrogen with deuterium in a drug molecule may lead to an altered pharmacokinetic profile of the host molecule, but pharmacodynamics are typically unaffected by this minor structural alteration. As such, deuterated compounds may potentially delay drug metabolism, increase tolerability, and enhance clinical effectiveness compared to non-deuterated analogues (reviewed in Zhang and Tang, 2018). In April 2017 the United States Food and Drug Administration (FDA) approved a deuterated form of tetrabenazine (Xenozine), deutetabenazine (Austedo), for the treatment of choreic movements associated with Huntington's disease. Deutetabenazine, known prior to FDA approval as SD-809 or tetrabenazine 6D, showed similar effectiveness in reducing the severity of choreic symptoms in people with Huntington's disease compared to placebo (Huntington Study Group et al. 2016), but with greater tolerability and fewer adverse effects compared to tetrabenazine (Claassen et al. 2017; reviewed in Dean and Sung, 2018). Given the promising findings with deutetabenazine, it is likely that more preclinical efforts will be devoted to improving a compound's tolerability and toxicity profile through deuteration (e.g., Mullard, 2016).

There are no studies that have characterized the pharmacological effects of deuterated MDMA. As such, the present studies were undertaken to assess and compare the locomotor stimulant effects of MDMA to deuterated MDMA in mice. The purpose of the studies is to confirm that d2-MDMA is behaviorally active, which sets the stage for later studies that will assess pharmacological effects that are thought to relate to the effective treatment of PTSD. Assessments of motor activity serve as efficient procedures for gauging *in vivo* activity of psychostimulants, including MDMA (Fantegrossi et al. 2003; Young and Glennon, 2008), and are considered major endpoints for comparing the abuse liability of novel compounds to established drugs of abuse (FDA, 2017). In addition, it is known that MDMA has the capacity to produce a relatively long-lasting increased motor-stimulant response to MDMA administration (i.e., locomotor sensitization) following a prolonged drug-free break (Spanos

and Yamamoto, 1989; Dafters, 1995a; Kalivas et al. 1998; Itzhak et al. 2003; Ball et al. 2011). Inducing locomotor sensitization with psychostimulants involves neural circuits that are implicated in the development and maintenance of drug addiction, which supports the utility of sensitization procedures in preclinical research (Steketee and Kalivas, 2011). As such, the present studies included assessments of the capacities of deuterated MDMA and MDMA to induce locomotor sensitization. The first experiment of the present study comprised single bolus administrations of deuterated MDMA or MDMA to compare their motor-stimulant effects and determine time course-activity curves in mice. Following acute administration, mice were challenged with an intermediate dose of deuterated MDMA or MDMA after a drug-free break. Last, mice were injected with the well-characterized motor-stimulant *S*(+)-methamphetamine to assess deuterated MDMA's capacity to cross-sensitize motor-stimulant responses to another psychostimulant in comparison to MDMA. In the second experiment, mice received cumulative doses of deuterated MDMA or MDMA and their locomotor activity was evaluated. The purpose of the second experiment was to evaluate if deuterated MDMA's locomotor stimulant, stereotypy, and sensitization effects varied when multiple doses are administered in a relatively limited time frame.

Materials and Methods

Experiment 1: single bolus administration and time course

Subjects—Twelve male NIH Swiss mice (Charles River Laboratories, Inc., Wilmington MA, USA) weighing 20–25g on delivery were housed three per cage in polycarbonate cages (15.24 × 25.40 × 12.70 cm) in corn cob bedding in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility. Mice were given one week of *ad libitum* feeding (Laboratory Rodent Diet no. 5001, PMI Feeds, St. Louis, MO, USA) and tap water prior to any handling. Colony room conditions were maintained at 22 ± 2 °C and 45–50% humidity, with lights set to a 12:12-h light-dark cycle (lights on at 0600 am). All test conditions used groups of six mice, and all mice were drug naïve (with the exception of surgical anesthetics) before testing. All experimental protocols were conducted in accordance with the Guide for the Care and Use of Laboratory Animals (2013) and approved by the Institutional Animal Care and Use Committee at the University of Arkansas for Medical Sciences.

Drugs—Racemic deuterated MDMA hydrochloride (hereafter referred to as “d2-MDMA”) was prepared by Sebastian Leth-Petersen (Copenhagen, Denmark) from commercially available CD₂Cl₂ and 3,4-dihydroxybenzaldehyde, following the procedures outlined in WO2008016677A2 with minor modifications (see Supplemental File “Online Resource S2” for details and H¹-NMR and C¹³-NMR spectra). Racemic MDMA hydrochloride (hereafter referred to as “MDMA”) and *S*(+)-methamphetamine hydrochloride (hereafter referred to as “methamphetamine”) were provided by the National Institute on Drug Abuse (NIDA). All doses are expressed as weight of the salt and were delivered via intraperitoneal injection (ip) in a 10 ml/kg injection volume.

Apparatus—Locomotor activity was monitored within the home cage environment by 6 custom-built activity frames equipped with Hall effect sensors activated by magnetic fields.

Frames were housed within light- and sound-attenuating chambers equipped with ventilation fans. Full details of this locomotor monitoring system have been previously described by Fantegrossi et al. (2013), but the present locomotor studies all occurred in the absence of any lights, tones, or other environmental stimuli.

Surgical procedures—Briefly, all mice were surgically implanted with disc-shaped neodymium magnets (1 cm diameter; 0.3 cm thick) (K&J Magnetics, Inc., Jamison, PA, USA) for the duration of the experiment. The magnets were enclosed in Parafilm and sterilized in 70% alcohol prior to implantation. Following appropriate anesthesia using 5% isoflurane + 1.5% oxygen, mice were moved to a clean Plexiglas surface, restrained using silastic tubing around the limbs, and continued to receive anesthesia via a snout cone delivering a continuous flow of isoflurane and oxygen. The abdominal area of each mouse was shaved and sanitized with iodine swabs. A rostral-caudal cut of the skin approximately 1.5 cm in length was made approximately 1.5 cm off the midline using scissors. Another cut of the muscle tissue approximately 1 cm in length was made using scissors, which exposed the peritoneal cavity. The magnet wrapped in Parafilm was then inserted into the peritoneal cavity using non-magnetic forceps and the muscle and skin were each closed using absorbable suture material. Immediately following surgery, mice were singly housed in Lab Products, Inc. Super Mouse 750 Micro-Isolator cages (32.7 × 19.0 × 14.3 cm) placed on top of plastic platforms that were situated inside light- and sound-attenuating cabinets. Mice had ad libitum access to food and water in their home cages and were given at least 7 days to recover before starting experiments.

Experimental Procedures

Locomotor activity and sensitization

Mice ($N=12$) were randomly assigned to MDMA ($n=6$) or d2-MDMA ($n=6$) treatment groups and groups were counterbalanced across locomotor activity chambers. The intermittent dosing regimen for the acute injection/induction phase of the experiment comprised a single daily injection of saline, MDMA (3, 10, 30 mg/kg; ip), or d2-MDMA (3, 10, 30 mg/kg; ip). Doses of MDMA and d2-MDMA were selected from previous research demonstrating increases in horizontal movements within the dose range of 10–32 mg/kg MDMA in male Swiss mice (Fantegrossi et al. 2003). Larger doses were not tested due to a limited supply of d2-MDMA. Following each injection, locomotor activity was recorded for 24 hours. Mice were administered doses of MDMA or d2-MDMA in ascending order and each injection was spaced 48 hours apart (i.e., mice received injections on experiment days 1, 3, 5, and 7). Immediately following injection of 30 mg/kg, a four-day drug-free break was imposed wherein mice received no injections. After this break (i.e., experiment day 12), all mice were injected with 10 mg/kg MDMA or d2-MDMA and ambulatory activity was recorded to assess the expression of sensitization. Forty-eight hours after the expression phase (i.e., experiment day 14), mice were again injected with saline to assess conditioned activation that may have resulted from repeated drug exposure in their home cages.

Cross-sensitization

To evaluate if locomotor activity of mice previously injected with MDMA or d2-MDMA would cross-sensitize to a different locomotor stimulant, mice were injected with various doses of methamphetamine in an ascending order (1, 3, 10 mg/kg; ip). Methamphetamine-elicited activity was recorded for 24 hours and injections were spaced 48 hours apart as described above.

Data analysis

Data are presented as mean $\pm$ standard error (*SE*). Three locomotor activity measures were collected for the present study: counts, ambulations, and computed stereotypy scores. Counts refer to the total number of Hall Effect switch activations (including repetitive switch activations); ambulations refer to successive switch activations (i.e., horizontal movements about the cage); and, stereotypy scores were determined as number of counts - number of ambulations, which results in repetitive switch activations. As such, repetitive switch activations were considered as stereotypy scores. Analysis of variance (ANOVA) procedures or *t*-tests were used to analyze time course-activity data, summary data, and dose-effect curves. ANOVAs were followed by posthoc tests with a Holm-Šidák correction or Dunnett's multiple comparisons tests. Linear regression analyses were used to compute potency estimates (ED_{50} values) and create 95% confidence intervals. For purposes of clarification, locomotor sensitization in the present study is defined as 1) an increase in the effectiveness of a drug as a result of a previous drug history (i.e., an upward shift in a dose-effect curve; main effect of treatment), or 2) a decrease in an ED_{50} value as a result of a previous drug history (i.e., a leftward shift in a dose-effect curve). In addition, activity data were summated over the first two hours (experiment 1) or four hours (experiment 2) for graphing and statistical analysis of dose-effect curves. Creation of figures and statistical analysis was performed using GraphPad Prism version 7.05 (La Jolla, CA, USA). Unless otherwise stated, statistical significance was declared at $p < .05$.

Experiment 2: cumulative dosing

Subjects

Sixteen male NIH Swiss mice (Charles River Laboratories, Inc., Wilmington MA, USA) weighing 20–25g on delivery were housed two per cage in polycarbonate cages (15.24 $\times$ 25.40 $\times$ 12.70 cm) in corncob bedding in an Association for Assessment and Accreditation of Laboratory Animal Care-accredited facility. All remaining housing and animal husbandry conditions were identical to those described in the Subjects section of Experiment 1.

Apparatus

Locomotor activity was monitored by 8 clear acrylic chambers (43.2 $\times$ 43.2 $\times$ 29.8 cm; Med Associates Inc., St. Albans, VT, USA) equipped with photocell beam detectors sensitive to infrared light that were spaced 2.5 cm apart along two perpendicular walls and an acrylic lid. Photocell beam detectors were mounted on opposing walls of the chambers. One activity count was recorded each time a subject interrupted a single light beam. One subject was tested in each testing chamber at a time. All data collection was accomplished with Activity

Monitor 7 Software (Med Associates Inc., St. Albans, VT, USA). Each acrylic chamber was housed within a wooden cabinet. The interior of each cabinet was equipped with two 10V light bulbs situated near the ceiling, and a fan for ventilation and masking noise. All locomotor studies were conducted in the dark.

Experimental Procedures

Locomotor activity and sensitization

Cages of pair-housed mice ($N=16$) were randomly assigned to MDMA ($n=8$) or d2-MDMA ($n=8$) treatment groups and were counterbalanced across locomotor activity chambers. The timeline for the cumulative dosing regimen of the experiment was similar to that used in previous locomotor sensitization experiments with other psychostimulants (McGuire et al. 2011; Baladi et al. 2012; Li et al. 2013; Thorn et al. 2014). Briefly, mice experienced two 60 minute habituation sessions in the locomotor activity chambers before receiving injections. On day 1, a 180 minute session was conducted during which mice received six saline injections at session times 0, 30, 60, 90, 120, and 150 minutes. The purpose of the repeated saline injections was to acclimate mice to the injection regimen used for the remainder of the experiment. On day 2, a 180 minute session was conducted during which d2-MDMA or MDMA dose-effect curves were determined by a cumulative dosing procedure; d2-MDMA or MDMA was administered to separate groups of 8 mice. Saline was administered immediately before the start of the session (session time 0) and ascending doses of d2-MDMA or MDMA (cumulative doses of 3.2, 10, 32, 56 mg/kg; ip) were given at times 30, 60, 90, 120, and 150 minutes. Data collected in the first 5 minutes following each injection were discarded to remove the effects of experimenter handling. The day 2 dose-effect curve determination was followed by a 6 day conditioning period of daily drug treatment. That is, on experimental days 3–7, mice received either 10 mg/kg d2-MDMA or 10 mg/kg MDMA, which is a dose of MDMA previously demonstrated to produce statistically significant increases in locomotor activity compared to saline in male NIH Swiss mice (Fantegrossi et al. 2003), according to the grouping assignments established in the day 1 test session, and the mice were placed in the locomotor activity chambers. Conditioning sessions were 60 minutes long. On day 8, d2-MDMA and MDMA dose-effect curves were redetermined using procedures identical to day 2, followed by a 6 day drug-free break during which mice remained in their home cages with no handling. On day 15, cumulative d2-MDMA or MDMA dose-effect curves were again redetermined as described above. On day 16, d2-MDMA and MDMA dose-effect curves were determined according to a crossover design, in which the d2-MDMA-conditioned mice were tested with MDMA, and the MDMA-conditioned mice were tested with d2-MDMA.

Data Analysis

Data are presented as mean $\pm$ standard error (*SE*). Two locomotor activity measures were collected for the present experiment: horizontal movements computed as distance traveled in centimeters, and stereotypy counts, which were recorded as repetitive beam breaks occurring within a 2×2 cell beam box around the animal's center mass. Time course-activity curves of distance traveled and stereotypy counts were averaged into 5 minute bins. Twenty-five minute sums of distance traveled (cm) and stereotypy counts were computed to generate

dose-effect curves for d2-MDMA and MDMA. Data were analyzed using procedures described in Data Analysis for Experiment 1 except Emax values were determined for each subject (at whatever dose the maximal effect occurred at), and 2) ED50s and Emaxes were analyzed using a simultaneous confidence interval approach with a Šidák adjustment.

Results of Experiment 1

Injections of d2-MDMA or MDMA impacted locomotor activity in mice within the dose range of 3–30 mg/kg. Separate two-way ANOVAs on number of ambulations revealed statistically significant interactions between dose and time in d2-MDMA-treated mice ($F[33, 165] = 4.19, p < .0001$) and in MDMA-treated mice ($F[33, 165] = 6.04, p < .0001$). 30 mg/kg d2-MDMA and 30 mg/kg MDMA each produced a statistically significant increase in ambulations compared to saline from 30 minutes to 2 hours post-injection (Supplemental Figures S1–S2 in “Online Resource S1”). In separate analyses of time course-activity curves comparing MDMA to d2-MDMA within each dose, a statistically significant interaction was observed between treatment group and dose ($F[11, 110] = 2.63, p = .0051$) following administration of 30 mg/kg. MDMA was more effective than d2-MDMA at increasing ambulations following administration of 30 mg/kg, but there were no differences in stereotypy scores at any dose (Figure 1A). Because 30 mg/kg d2-MDMA and 30 mg/kg MDMA were each statistically different from saline for 2 hours post-injection, 2 hour sums of ambulations and stereotypy scores were computed to generate dose-effect curves (Figure 1B). Two-way mixed-measures ANOVAs revealed a statistically significant interaction between treatment and dose ($F[3, 30] = 3.58, p = .025$) for 2 hour sums of ambulations. In addition, a linear regression analysis and associated 95% confidence interval estimation revealed no potency difference between MDMA ($ED_{50} = 16.76$ mg/kg; 95% CI: 14.83, 18.83) and d2-MDMA ($ED_{50} = 15.79$ mg/kg; 95% CI: 14.06, 17.73) in ambulation sums. A two-way mixed-measures ANOVA revealed a statistically significant interaction between treatment and dose ($F[3, 30] = 4.24, p = .01$) for 2 hour sums of stereotypy scores. Similar to ambulation sums, there were no potency differences between MDMA ($ED_{50} = 13.37$ mg/kg; 95% CI: 12.09, 14.71) and d2-MDMA ($ED_{50} = 13.31$ mg/kg; 95% CI: 8.94, 19.84) in stereotypy score sums. In general, d2-MDMA produced time-dependent increases in activity similar to MDMA, but MDMA was more effective at increasing horizontal movements and repetitive motions at higher doses.

Following a 4 day drug-free break, mice received injections of 10 mg/kg d2-MDMA or 10 mg/kg MDMA according to their treatment history (Figure 2). Mice conditioned with d2-MDMA showed a sensitized locomotor response to 10 mg/kg d2-MDMA (Figure 2; top left panel), while MDMA-conditioned mice showed a sensitized locomotor responses and stereotyped movements to 10 mg/kg MDMA (Figure 2; center column). Sensitization to stereotyped movements in the MDMA-conditioned mice is recapitulated in the lower right bar graph of Figure 2, which displays the stereotypy score data summated over the first two hours of the experimental session. Full statistical results for Figure 2 can be found in the Online Resource S1 file. In addition, the number of ambulations between the first and second injections of saline did not differ in the d2-MDMA-conditioned mice ($t[5] = 0.74, p = .49$) or in the MDMA-conditioned mice ($t[5] = 1.73, p = .14$) (data not shown).

Injections of methamphetamine impacted locomotor activity in d2-MDMA- or MDMA-treated mice within the dose range of 1–10 mg/kg (Figure 3). Separate two-way ANOVAs on number of ambulations revealed statistically significant interactions between dose and time in d2-MDMA-treated mice ($F[24, 120] = 2.69, p = .0002$) and in MDMA-treated mice ($F[24, 120] = 4.08, p < .0001$) (Supplemental figures S3–S4 in “Online Resource S1”). 3 mg/kg methamphetamine increased ambulations compared to saline from 30 minutes to 4 hours post-injection in d2-MDMA-conditioned mice, and 1 mg/kg methamphetamine increased ambulations compared to saline from 30 minutes to 3 hours post-injection in MDMA-conditioned mice. (Supplemental figures S3–S4 in “Online Resource S1”). In separate analyses of time course-activity curves comparing MDMA to d2-MDMA within each methamphetamine dose, a statistically significant main effect of treatment was observed at 3 mg/kg methamphetamine ($F[1, 10] = 2.21, p = .027$) in stereotypy scores (Figure 3A). Because statistically significant differences between saline and 3 mg/kg methamphetamine in d2-MDMA-treated mice occurred up to 4 hours post-injection (i.e., Supplemental figure S3), 4 hour sums of ambulations and stereotypy scores were computed to generate dose-effect curves (Figure 3B). A mixed-measures ANOVA revealed a statistically significant interaction between treatment group and dose for 4 hour sums of ambulations ($F[3, 30] = 8.50, p = .0003$), however, post-hoc simple main effects tests failed to reveal where the significant interactions occurred between treatment groups. Peak number of ambulations occurred following administration of 1 mg/kg methamphetamine in MDMA-conditioned mice, whereas 3 mg/kg produced peak number of ambulations in d2-MDMA-conditioned mice, which indicates a possible difference in methamphetamine potency between d2-MDMA and MDMA groups. However, ED₅₀ values were not determined for sums of ambulations because an ascending limb in the dose-effect curve was not acquired from the MDMA-conditioned mice. A mixed-measures ANOVA revealed a statistically significant interaction between treatment group and dose for 4 hour sums of stereotypy scores ($F[3, 30] = 6.60, p = .0015$). ED₅₀ values were not determined for sums of stereotypy scores because an ascending limb in the dose-effect curve was not acquired from the MDMA-conditioned mice.

Results of experiment 2

Two mice in the MDMA treatment group died during the first eight days of cumulative administration and their data are excluded from all analyses and graphs. To visualize the capacities of d2-MDMA and MDMA to produce sensitization over the course of the experiment, Figure 4A (distance traveled) and 4D (stereotypy counts) show activity after injections of 10 mg/kg either as part of a cumulative administration session (i.e., dose-effect curves; DEC 1, 2, 3, and C) or during the 6-day conditioning period (i.e., induction days 1–6). A mixed-measures ANOVA on total distance traveled revealed a statistically significant main effect of conditioning group [$F(1, 12) = 8.54, p = .01$], a statistically significant main effect of experimental phase (i.e., DEC 1, 2, 3, and C) [$F(9, 108) = 19.64, p < .0001$], and a statistically significant interaction between conditioning group and experimental phase [$F(9, 108) = 2.42, p = .02$]. Dunnett’s multiple comparisons tests revealed a statistically significant increase in distance traveled after 10 mg/kg in all sessions compared to distance traveled counts observed during DEC 1. A mixed-measures ANOVA on stereotypy counts

revealed a statistically significant main effect of experimental phase [$F(9, 108) = 27.3$, $p < .0001$]. Indeed, Dunnett's multiple comparisons tests revealed a statistically significant difference between induction day 1 and DEC 1, DEC 3, and the cross challenge (C) day ($p < .05$).

Cumulative dosing of d2-MDMA or MDMA impacted locomotor activity in mice within the dose range of 3.2–56 mg/kg (Figures 4B,C,E,F). Dose-effect curves for MDMA (Figure 4B) and d2-MDMA (Figure 4C) in distance traveled; and, for MDMA (Figure 4E) and d2-MDMA (Figure 4F) in stereotypy counts are presented for visual display. Potency estimates (ED_{50} values) for all dose-effect curves can be found in Table 1. Notably, ED_{50} values progressively decreased in the d2-MDMA treatment group, but only decreased up to the third dose-effect curve in the MDMA treatment group.

Discussion

This is the first report to demonstrate that d2-MDMA is behaviorally active and produces locomotor stimulant effects similar to MDMA. Using two distinct methods for quantifying mouse motor activity, it was observed that d2-MDMA and MDMA impacted locomotor activity within the dose range of 3–56 mg/kg, which is consistent with a previous report that assessed the locomotor effects of MDMA in male NIH Swiss mice (Fantegrossi et al. 2003). Comparisons of time course-activity curves between acute administration of d2-MDMA and MDMA revealed no major differences in onset or duration of action, but MDMA produced greater maximal effects on horizontal activity and stereotyped movements than d2-MDMA with no apparent difference in potency; although, differences between d2-MDMA and MDMA in motor activity appeared to be dose-specific. In addition, potency estimates must be interpreted with caution because descending limbs of activity were not determined in MDMA- or d2-MDMA-treated animals. These results are somewhat surprising given that the major metabolite of MDMA in mice is MDA (Ortuno et al. 1999); which, in rats, appears to increase motor activity (Yeh and Hsu, 1991; Bexis and Docherty, 2006). Because of deuteration of the methylenedioxy ring of d2-MDMA, it is hypothesized that *N*-demethylation, instead of *O*-demethylation, will occur preferentially in d2-MDMA. As such, mice would be expected to show greater accumulations of MDA following administration of d2-MDMA compared to MDMA with perhaps *greater* levels of locomotor stimulation. However, the present experiment revealed reduced effectiveness observed with d2-MDMA at specific doses. Regardless of the mechanism(s) by which MDMA produces greater maximal effects on horizontal locomotion and stereotyped movements than d2-MDMA at equivalent doses, d2-MDMA functioned as a locomotor stimulant in a manner similar to MDMA, but with less stereotyped movements.

Locomotor sensitization is typically understood to involve neural circuitries implicated in drug addiction (Steketee and Kalivas, 2011), and may be associated with drug-seeking and relapse behavior observed in users with a substance use disorder. In the present study, single, acute administration of d2-MDMA or MDMA led to an increased motor response to each drug following a brief drug-free break (i.e., sensitized motor responses). Thus, similar to MDMA (Spanos and Yamamoto, 1989; Dafters, 1995b; Kalivas et al. 1998; Itzhak et al. 2003; Ball et al. 2011), d2-MDMA has the capacity to sensitize responses to itself. Itzhak et

al. (2003) found that a single injection of 10 mg/kg MDMA in male mice was sufficient to induce sensitized motor activity following administration of 10 mg/kg MDMA five days after the first injection, and 10 mg/kg MDMA was sufficient to induce cross-sensitization to cocaine. In Experiment 1 of the present study, an intermittent injection regimen wherein mice received ascending doses of MDMA or d2-MDMA led to a significant enhancement of motor response to 10 mg/kg of MDMA or d2-MDMA when tested after a four-day drug-free break. It is noteworthy that, unlike MDMA, d2-MDMA did not elicit sensitized stereotyped movements. Indeed, peak effects and duration of action on horizontal movements appeared to be preferentially affected by single bolus administration of d2-MDMA. As a final comparison in Experiment 1 of the present study, d2-MDMA- and MDMA-conditioned mice were injected with doses of the prototypical locomotor stimulant methamphetamine to assess any differences between groups of mice conditioned to these compounds in their response to methamphetamine. Unfortunately, ascending limbs in the ambulations and stereotypy scores dose-effect curves were not determined in the MDMA-conditioned mice, which precluded computation of potency estimates in the treatment groups. Nevertheless, it is possible that methamphetamine showed a rightward shift in d2-MDMA-conditioned mice as an approximate half-log difference in dose between groups was required to produce peak horizontal locomotion, however further work will be needed to confirm and quantify the shift. In addition, although it was unexpected that stereotypy scores would decrease with ascending doses of methamphetamine, doses of methamphetamine within the range of 1–10 mg/kg dose-dependently increased jumping behavior in NIH Swiss mice (unpublished observations). Neither rearing nor jumping behavior would activate the Hall Effect switches in the locomotor chambers used in Experiment 1, perhaps resulting in missing data relevant to assessments of motor stereotypy. Nonetheless, the results of the cross-sensitization experiment with methamphetamine indicate that repeated exposure to ascending doses of d2-MDMA may lead to a heightened response to other drugs of abuse. Thus, similar to MDMA, d2-MDMA may have potential for abuse and addiction by modifying neural circuitries that mediate the rewarding effects of drugs of abuse.

Importantly, the results of Experiment 1 indicate that d2-MDMA may be less susceptible to some adverse effects (i.e., stereotyped movements and less sensitization) associated with its non-deuterated analog; although, it is important to emphasize that hyperprexia and cardiovascular effects are the major health concerns following acute MDMA administration. Nevertheless, in clinical settings a unit amount of MDMA is often provided to clients and, depending on an individual client's response, an additional amount of drug can be given (Mithoefer et al. 2011;2018; Ot'alora et al. 2018). As such, cumulative administration occurs in MDMA-assisted psychotherapy sessions. The major results of the cumulative administration experiment in the present study indicate that d2-MDMA less potently elicits motor activity compared to MDMA, but the differences between groups become less apparent as the number of cumulative administration sessions increase. Moreover, d2-MDMA- and MDMA-treated mice showed an upward shift in their dose-effect functions after the first cumulative administration session. These results indicate that both compounds may rapidly elicit the development of sensitization following cumulative administration. Although cumulative administration appeared to attenuate some of the differences between MDMA and d2-MDMA in terms of locomotor stimulant effects, future studies investigating

the effects of cumulative administration with these substances on more therapeutic-like effects of MDMA (e.g., prosocial behavior, facilitation of fear extinction) is necessary.

Similar to the results of Experiment 1 in the present study, differences in motor activity between MDMA and d2-MDMA were not apparent until doses reached or exceeded 30 mg/kg. MDMA is suggested to auto-inhibit its own metabolism in mice (Fantegrossi et al. 2009), which may account for the differences between d2-MDMA and MDMA following administration of 30 mg/kg. However, if the rate of *O*-demethylation is reduced in d2-MDMA, increased accumulations of the parent drug (i.e., d2-MDMA) would be expected and thus *greater* levels of motor activity would be produced following administration of d2-MDMA. Without knowing the exact concentrations of MDMA and MDA produced by these dosing regimens used in the present study, it is uncertain if greater accumulations of the parent compound can account for the observed differences at 30 or 32 mg/kg. It is noteworthy that d2-MDMA produced greater levels of stereotyped movements than MDMA following administration of 32 mg/kg, which may also support the possibility that greater accumulations of the parent compound occurred following cumulative administration of d2-MDMA. Furthermore, in the present study, a potency difference between d2-MDMA and MDMA occurred following the first cumulative administration session, but, as in Experiment 1, potency estimates must be interpreted with caution because a descending limb for the d2-MDMA dose-effect function was not observed. Higher doses were not tested in the present study because 2 of 8 animals in the MDMA-conditioning group died during the experiment. MDMA doses approaching or exceeding 56 mg/kg may thus lead to the development of serotonin syndrome and ultimately a loss of experimental subjects. It is noteworthy that no subjects were lost in the d2-MDMA treatment group, which indicates that factors related to MDMA-induced death (e.g., hyperthermia, serotonin syndrome) may be mitigated somewhat by the process of deuteration.

In sum, the present study revealed that d2-MDMA is behaviorally active and functions as a motor stimulant in a manner similar to MDMA using two methods for quantifying mouse locomotor activity. Acute administration of d2-MDMA appears to be less likely to produce stereotyped movements compared to MDMA in mice, but d2-MDMA retains the capacity to induce the development of locomotor sensitization. Moreover, the relative advantage of d2-MDMA to MDMA may diminish following cumulative administration, which is possibly due to a complex interaction between an accumulated dose and metabolism of the parent compound. Nevertheless, the positive results demonstrating that d2-MDMA is behaviorally active warrants additional investigation into its capacity to alter body temperature, to elicit MDMA-like discriminative stimulus effects, and into its potentially distinct pharmacokinetic profile. Considering recent evidence demonstrating that MDMA may be an effective adjunct treatment for PTSD and social anxiety disorders, it is worthwhile to consider modifications to its chemical structure that may maximize its therapeutic benefits while diminishing its potential to produce adverse effects. Future studies will help determine if deuteration is useful for achieving this goal.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- Bai F, Lau SS, Monks TJ (1999) Glutathione and N-acetylcysteine conjugates of alpha-methyldopamine produce serotonergic neurotoxicity: possible role in methylenedioxymphetamine-mediated neurotoxicity. *Chem Res Toxicol* 12:1150–1157. DOI: tx990084t [PubMed: 10604863]
- Bai F, Jones DC, Lau SS, Monks TJ (2001) Serotonergic neurotoxicity of 3,4-(+/-)-methylenedioxymphetamine and 3,4-(+/-)-methylenedioxymphetamine (ecstasy) is potentiated by inhibition of gamma-glutamyl transpeptidase. *Chem Res Toxicol* 14:863–870. DOI: tx010011l [PubMed: 11453733]
- Baladi MG, Koek W, Aumann M, Velasco F, France CP (2012) Eating high fat chow enhances the locomotor-stimulating effects of cocaine in adolescent and adult female rats. *Psychopharmacology (Berl)* 222:447–457. DOI: 10.1007/s00213-012-2663-7 [PubMed: 22418731]
- Ball KT, Klein JE, Plocinski JA, Slack R (2011) Behavioral sensitization to 3,4-methylenedioxymphetamine is long-lasting and modulated by the context of drug administration. *Behav Pharmacol* 22:847–850. DOI: 10.1097/FBP.0b013e32834d13b4 [PubMed: 21979834]
- Bexis S, Docherty JR (2006) Effects of MDMA, MDA and MDEA on blood pressure, heart rate, locomotor activity and body temperature in the rat involve alpha-adrenoceptors. *Br J Pharmacol* 147:926–934. DOI: 0706688 [PubMed: 16491100]
- Carvalho M, Milhazes N, Remião F, Borges F, Fernandes E, Amado F, Monks TJ, Carvalho F, Bastos ML (2004) Hepatotoxicity of 3,4-methylenedioxymphetamine and alpha-methyldopamine in isolated rat hepatocytes: formation of glutathione conjugates. *Arch Toxicol* 78:16–24. DOI: 10.1007/s00204-003-0510-7 [PubMed: 14586543]
- Chu T, Kumagai Y, DiStefano EW, Cho AK (1996) Disposition of methylenedioxymphetamine and three metabolites in the brains of different rat strains and their possible roles in acute serotonin depletion. *Biochem Pharmacol* 51:789–796. DOI: 0006-2952(95)02397-6 [PubMed: 8602874]
- Claassen DO, Carroll B, De Boer LM, Wu E, Ayyagari R, Gandhi S, Stamler D (2017) Indirect tolerability comparison of Deutetrabenazine and Tetrabenazine for Huntington disease. *J Clin Mov Disord* 4:3–017-0051–5. eCollection 2017. DOI: 10.1186/s40734-017-0051-5
- Commings DL, Vosmer G, Virus RM, Woolverton WL, Schuster CR, Seiden LS (1987) Biochemical and histological evidence that methylenedioxymphetamine (MDMA) is toxic to neurons in the rat brain. *J Pharmacol Exp Ther* 241:338–345. PMID: 2883295 [PubMed: 2883295]
- Creighton FJ, Black DL, Hyde CE (1991) ‘Ecstasy’ psychosis and flashbacks. *Br J Psychiatry* 159:713–715. DOI: S0007125000031147 [PubMed: 1684523]
- Dafters RI (1995a) Hyperthermia following MDMA administration in rats: effects of ambient temperature, water consumption, and chronic dosing. *Physiol Behav* 58:877–882. DOI: 0031-9384(95)00136-7 [PubMed: 8577883]
- Dafters RI (1995b) Hyperthermia following MDMA administration in rats: effects of ambient temperature, water consumption, and chronic dosing. *Physiol Behav* 58:877–882. DOI: 0031-9384(95)00136-7 [PubMed: 8577883]

- de la Torre R, Farre M, Roset P, Pizarro N, Abanades S, Segura M, Segura J, Cami J (2004a) Human pharmacology of MDMA: pharmacokinetics, metabolism, and disposition. *Ther Drug Monit* 26:137–44. PMID: 15228154 [PubMed: 15228154]
- de la Torre R, Farre M (2004b) Neurotoxicity of MDMA (ecstasy): the limitations of scaling from animals to humans. *Trends Pharmacol Sci* 25:505–508. DOI: 10.1016/j.tips.2004.08.001 [PubMed: 15380932]
- Dean M, Sung VW (2018) Review of deutetrabenazine: a novel treatment for chorea associated with Huntington's disease. *Drug Des Devel Ther* 12:313–319. DOI: 10.2147/DDDT.S138828
- Di Iorio CR, Watkins TJ, Dietrich MS, Cao A, Blackford JU, Rogers B, Ansari MS, Baldwin RM, Li R, Kessler RM, Salomon RM, Benningfield M, Cowan RL (2012) Evidence for chronically altered serotonin function in the cerebral cortex of female 3,4-methylenedioxymethamphetamine polydrug users. *Arch Gen Psychiatry* 69:399–409. DOI: 10.1001/archgenpsychiatry.2011.156 [PubMed: 22147810]
- Fantegrossi WE, Godlewski T, Karabenick RL, Stephens JM, Ullrich T, Rice KC, Woods JH (2003) Pharmacological characterization of the effects of 3,4-methylenedioxymethamphetamine ("ecstasy") and its enantiomers on lethality, core temperature, and locomotor activity in singly housed and crowded mice. *Psychopharmacology (Berl)* 166:202–211. DOI: 10.1007/s00213-002-1261-5 [PubMed: 12563544]
- Fantegrossi WE, Murai N, Mathuna BO, Pizarro N, de la Torre R (2009) Discriminative stimulus effects of 3,4-methylenedioxymethamphetamine and its enantiomers in mice: pharmacokinetic considerations. *J Pharmacol Exp Ther* 329:1006–1015. DOI: 10.1124/jpet.109.150573 [PubMed: 19276400]
- Fantegrossi WE, Xiao WR, Zimmerman SM (2013) Novel technology for modulating locomotor activity as an operant response in the mouse: implications for neuroscience studies involving "exercise" in rodents. *J Neurosci Methods* 212:338–343. DOI: 10.1016/j.jneumeth.2012.10.020 [PubMed: 23164960]
- Food and Drug Administration (FDA) (2017) Assessment of Abuse Potential of Drugs: Guidance for Industry. Accessed 12/6/18 from <https://www.fda.gov/drugs/guidance-compliance-regulatory-information/guidances-drugs>
- Henry JA (1992) Ecstasy and the dance of death. *BMJ* 305:5–6. [PubMed: 1353390]
- Huntington Study Group, Frank S, Testa CM, Stamler D, Kayson E, Davis C, Edmondson MC, et al. (2016) Effect of Deutetrabenazine on Chorea Among Patients With Huntington Disease: A Randomized Clinical Trial. *JAMA* 316:40–50. DOI: 10.1001/jama.2016.8655 [PubMed: 27380342]
- Itzhak Y, Ali SF, Achat CN, Anderson KL (2003) Relevance of MDMA ("ecstasy")-induced neurotoxicity to long-lasting psychomotor stimulation in mice. *Psychopharmacology (Berl)* 166:241–248. DOI: 10.1007/s00213-002-1320-y [PubMed: 12590354]
- Kalivas PW, Duffy P, White SR (1998) MDMA elicits behavioral and neurochemical sensitization in rats. *Neuropsychopharmacology* 18:469–479. DOI: S0893133X97001954 [PubMed: 9571655]
- Li JX, Shah AP, Patel SK, Rice KC, France CP (2013) Modification of the behavioral effects of morphine in rats by serotonin 5-HT(1)A and 5-HT(2)A receptor agonists: antinociception, drug discrimination, and locomotor activity. *Psychopharmacology (Berl)* 225:791–801. DOI: 10.1007/s00213-012-2870-2 [PubMed: 22993050]
- McGuire BA, Baladi MG, France CP (2011) Eating high-fat chow enhances sensitization to the effects of methamphetamine on locomotion in rats. *Eur J Pharmacol* 658:156–159. DOI: 10.1016/j.ejphar.2011.02.027 [PubMed: 21371470]
- Miller RT, Lau SS, Monks TJ (1996) Effects of intracerebroventricular administration of 5-(glutathione-S-yl)-alpha-methyldopamine on brain dopamine, serotonin, and norepinephrine concentrations in male Sprague-Dawley rats. *Chem Res Toxicol* 9:457–465. DOI: 10.1021/tx9501546 [PubMed: 8839050]
- Mithoefer MC, Mithoefer AT, Feduccia AA, Jerome L, Wagner M, Wymer J, Holland J, Hamilton S, Yazar-Klosinski B, Emerson A, Doblin R (2018) 3,4-methylenedioxymethamphetamine (MDMA)-assisted psychotherapy for post-traumatic stress disorder in military veterans, firefighters, and police officers: a randomised, double-blind, dose-response, phase 2 clinical trial. *Lancet Psychiatry* 5:486–497. DOI: S2215-0366(18)30135-4 [PubMed: 29728331]

- Mithoefer MC, Wagner MT, Mithoefer AT, Jerome L, Doblin R (2011) The safety and efficacy of {+/-}3,4-methylenedioxymethamphetamine-assisted psychotherapy in subjects with chronic, treatment-resistant posttraumatic stress disorder: the first randomized controlled pilot study. *J Psychopharmacol* 25:439–452. DOI: 10.1177/0269881110378371 [PubMed: 20643699]
- Morgan MJ (2000) Ecstasy (MDMA): a review of its possible persistent psychological effects. *Psychopharmacology (Berl)* 152:230–248. [PubMed: 11105933]
- Mullard A (2016) Deuterated drugs draw heavier backing. *Nat Rev Drug Discov* 15:219–221. DOI: 10.1038/nrd.2016.63 [PubMed: 27032821]
- Ortuno J, Pizarro N, Farre M, Mas M, Segura J, Cami J, Brenneisen R, de la Torre R (1999) Quantification of 3,4-methylenedioxymetamphetamine and its metabolites in plasma and urine by gas chromatography with nitrogen-phosphorus detection. *J Chromatogr B Biomed Sci Appl* 723:221–232. [PubMed: 10080649]
- Ot'alora GM, Grigsby J, Poulter B, Van Derveer JW 3rd, Giron SG, Jerome L, Feduccia AA, Hamilton S, Yazar-Klosinski B, Emerson A, Mithoefer MC, Doblin R (2018) 3,4-Methylenedioxymethamphetamine-assisted psychotherapy for treatment of chronic posttraumatic stress disorder: A randomized phase 2 controlled trial. *J Psychopharmacol* 32:1295–1307. DOI: 10.1177/0269881118806297 [PubMed: 30371148]
- Reneman L, Endert E, de Bruin K, Lavalaye J, Feenstra MG, de Wolff FA, Booij J (2002) The acute and chronic effects of MDMA ("ecstasy") on cortical 5-HT_{2A} receptors in rat and human brain. *Neuropsychopharmacology* 26(3):387–96. DOI: 10.1016/S0893-133X(01)00366-9 [PubMed: 11850153]
- Ricaurte GA, DeLanney LE, Irwin I, Langston JW (1988) Toxic effects of MDMA on central serotonergic neurons in the primate: importance of route and frequency of drug administration. *Brain Res* 446:165–168. DOI: 10.1016/0006-8993(88)91309-1 [PubMed: 2897228]
- Schifano F (1991) Chronic atypical psychosis associated with MDMA ("ecstasy") abuse. *The Lancet* 338:1335.
- Segura M, Ortuno J, Farre M, McLure JA, Pujadas M, Pizarro N, Llebaria A, Joglar J, Roset PN, Segura J, de La Torre R (2001) 3,4-Dihydroxymethamphetamine (HHMA). A major in vivo 3,4-methylenedioxymethamphetamine (MDMA) metabolite in humans. *Chem Res Toxicol* 14:1203–1208. DOI: tx010051p [PubMed: 11559034]
- Spanos LJ, Yamamoto BK (1989) Acute and subchronic effects of methylenedioxymethamphetamine [(+/-)MDMA] on locomotion and serotonin syndrome behavior in the rat. *Pharmacol Biochem Behav* 32:835–840. DOI: 0091–3057(89)90044–0 [PubMed: 2572003]
- Stekete JD, Kalivas PW (2011) Drug wanting: behavioral sensitization and relapse to drug-seeking behavior. *Pharmacol Rev* 63:348–365. DOI: 10.1124/pr.109.001933 [PubMed: 21490129]
- Stone DM, Hanson GR, Gibb JW (1987a) Differences in the central serotonergic effects of methylenedioxymethamphetamine (MDMA) in mice and rats. *Neuropharmacology* 26:1657–1661. [PubMed: 2448703]
- Stone DM, Merchant KM, Hanson GR, Gibb JW (1987b) Immediate and long-term effects of 3,4-methylenedioxymethamphetamine on serotonin pathways in brain of rat. *Neuropharmacology* 26:1677–1683. [PubMed: 2893986]
- Thal SB, Lommen MJJ (2018) Current Perspective on MDMA-Assisted Psychotherapy for Posttraumatic Stress Disorder. *J Contemp Psychother* 48:99–108. DOI: 10.1007/s10879-017-9379-2 [PubMed: 29720767]
- Thorn DA, Jing L, Qiu Y, Gancarz-Kausch AM, Galuska CM, Dietz DM, Zhang Y, Li JX (2014) Effects of the trace amine-associated receptor 1 agonist RO5263397 on abuse-related effects of cocaine in rats. *Neuropsychopharmacology* 39:2309–2316. DOI: 10.1038/npp.2014.91 [PubMed: 24743376]
- Wareing M, Fisk JE, Murphy PN (2000) Working memory deficits in current and previous users of MDMA ('ecstasy'). *Br J Psychol* 91 (Pt 2):181–188. [PubMed: 10832513]
- Yeh SY, Hsu FL (1991) The neurochemical and stimulatory effects of putative metabolites of 3,4-methylenedioxymethamphetamine and 3,4-methylenedioxymethamphetamine in rats. *Pharmacol Biochem Behav* 39:787–790. DOI: 0091–3057(91)90165-X [PubMed: 1723801]

- Young R, Glennon RA (2008) MDMA (N-methyl-3,4-methylenedioxyamphetamine) and its stereoisomers: Similarities and differences in behavioral effects in an automated activity apparatus in mice. *Pharmacol Biochem Behav* 88:318–331. DOI: S0091–3057(07)00281-X [PubMed: 17904622]
- Zhang Z, Tang W (2018) Drug metabolism in drug discovery and development. *Acta Pharm Sin B* 8:721–732. DOI: 10.1016/j.apsb.2018.04.003 [PubMed: 30245961]

Fig. 1.

a Time course-activity curves of number of ambulations $\pm SE$ (top row) and stereotypy scores $\pm SE$ (bottom row) in male NIH Swiss mice administered ascending doses (indicated in column titles) of d2-MDMA (filled symbols) or MDMA (open symbols) ($n=6$ /treatment group). Data were collected for 24 hours after drug administration, but only the first 6 hours are shown. Error bars are contained within the symbol for some data. Injections occurred at time point 0. Asterisks indicate a statistically significant difference between d2-MDMA and MDMA treatment groups ($p < .01$). **b.** Dose-effect curves depicting two-hour sums of number of ambulations $\pm SE$ (left graph) and stereotypy scores $\pm SE$ (right graph) in male NIH Swiss mice administered ascending doses of d2-MDMA (filled symbols) or MDMA (open symbols) ($n=6$ /treatment group). Error bars are contained within the symbol for some data. V = activity after saline injection. Octothorpes (multiple comparisons tests with Holm-Šidák adjustment) indicate a statistically significant difference between d2-MDMA and MDMA treatment groups ($p < .05$) and asterisks (Dunnett's multiple comparisons tests) indicate a statistically significant difference between dose of drug and vehicle within a treatment group ($p < .05$).

Expression (10 mg/kg)

Fig. 2.

Time course-activity curves of number of ambulations $\pm SE$ (top row; scatterplots) and stereotypy scores $\pm SE$ (bottom row; scatterplots) in male NIH Swiss mice administered 10 mg/kg d2-MDMA (left column) or MDMA (center column). Filled symbols indicate the first injection of 10 mg/kg and open symbols indicate the challenge injection of 10 mg/kg following a four-day drug-free break. Error bars are contained within the symbol for some data. Injections occurred at time point 0. Bar graphs display two-hour sums of ambulations (top) and stereotypy scores (bottom) in mice injected with d2-MDMA or MDMA for the first time (filled bars) or after a four-day drug-free break (open bars). Asterisks indicate a statistically significant difference between d2-MDMA or MDMA treatment group (scatterplots; $p < .05$) or between first injection and challenge within a treatment group (bar graphs; $p < .05$).

Fig. 3.

a Time course-activity curves of number of ambulations $\pm SE$ (top row) and stereotypy scores $\pm SE$ (bottom row) in male NIH Swiss mice administered ascending doses (indicated in column titles) of methamphetamine. d2-MDMA-conditioned mice are presented as filled symbols, and MDMA-conditioned mice are presented as open symbols ($n=6$ /treatment group). Error bars are contained within the symbol for some data. Injections occurred at time point 0. **b.** Dose-effect curves depicting four-hour sums of number of ambulations $\pm SE$ (left graph) and stereotypy scores $\pm SE$ (right graph) in male NIH Swiss mice administered ascending doses of methamphetamine (d2-MDMA; filled symbols) (MDMA; open symbols) ($n=6$ /treatment group). Error bars are contained within the symbol for some data. V2 = activity after second saline injection. Octothorpes (multiple comparisons tests with Holm-Šidák adjustment) indicate a statistically significant difference between d2-MDMA and MDMA treatment groups ($p<.05$) and asterisks (Dunnnett's multiple comparisons tests) indicate a statistically significant difference between dose of drug and vehicle within a treatment group ($p<.05$).

Fig. 4.

a Scatterplots displaying 25 minute sums of distance traveled in mice injected with 10 mg/kg as part of a cumulative administration session (DECs 1, 2, 3, and C) or as a single dose administration that occurred during a 6-day conditioning period (Induction days 1–6). DEC = dose-effect curve; C = crossover, wherein d2-MDMA-conditioned mice received cumulative administration of MDMA, and MDMA-conditioned mice received cumulative administration of d2-MDMA. MDMA-conditioned mice are indicated by open symbols and d2-MDMA-conditioned mice are indicated by filled symbols. Dose-effect curves displaying 25 minute sums of distance traveled in MDMA-conditioned mice (**b**) and d2-MDMA-conditioned mice (**c**). Symbols for dose-effect curves can be found in the legend in Figure 4b. **d** displays 25 minute sums of stereotypy counts in mice injected with 10 mg/kg (same descriptors in graph as those described in 4a above). Dose-effect curves displaying 25 minute sums of stereotypy counts in MDMA-conditioned mice (**e**) and d2-MDMA-conditioned mice (**f**).

Table 1.

Summary table of ED₅₀ and E_{max} values from Experiment 2

Distance traveled (cm)	d2-MDMA	MDMA	d2-IMDMA	MDMA
Dose-effect curve	ED ₅₀ (mg/kg)	ED ₅₀ (mg/kg)	E _{max}	E _{max}
1	28.97 [*]	21.74	14771	18215
2	23.46 [*]	19.38	14972	17031
3	18.02	15.93	12596	13905
Crossover ^a	16.99	17.44	13720	14818
Stereotypy counts				
Dose-effect curve				
1	22.91	19.34	6773	6257
2	16.50	12.34	7842	7565
3	12.95	10.35	7945	8293
Crossover ^a	9.53	12.44	7794	8204

Data were compared using a simultaneous confidence interval approach with Šidák adjustment.

^{*} indicates a potency difference between d2-MDMA and MDMA (i.e., non-overlapping 95% SCIs).

^a indicates that d2-MDMA-conditioned mice were tested with cumulative administration of MDMA, and MDMA-conditioned mice were tested with cumulative administration of d2-MDMA.

Confidence intervals and additional comparisons can be found in supplemental file S1