0022-3565/09/3312-717–723$20.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
JPET 331:717–723, 2009
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Vol. 331, No. 2
156174/3525473
Printed in U.S.A.

JPET
PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS
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Discriminative Stimulus Effects of Psychostimulants and Hallucinogens in *S*(+)-3,4-Methylenedioxymethamphetamine (MDMA) and *R*(−)-MDMA Trained Mice

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Received May 12, 2009; accepted August 11, 2009

ABSTRACT

3,4-Methylenedioxymethamphetamine (MDMA) is a substituted phenethylamine more commonly known as the drug of abuse "ecstasy." The acute and persistent neurochemical effects of MDMA in the mice are distinct from those in other species. MDMA shares biological effects with both amphetamine-type stimulants and mescaline-type hallucinogens, which may be attributable to distinct effects of its two enantiomers, both of which are active in vivo. In this regard, among the substituted phenethylamines, *R*(−)-enantiomers tend to have hallucinogen-like effects, whereas *S*(+)-enantiomers tend to have stimulant-like effects. In the present study, mice were trained to discriminate *S*(+)- or *R*(−)-MDMA from vehicle. Drug substitution tests were then undertaken with the structurally similar phenethylamine dopamine/norepinephrine releaser *S*(+)-amphetamine, the structurally dissimilar tropane nonselective monoamine reuptake inhibitor cocaine, the structurally similar phenethylamine 5-hydroxytryptamine (5-HT)$_{2A}$ agonist 2,5-dimethoxy-4-(*n*)-propylthiophenethylamine (2C-T-7), and the structurally dissimilar mixed action tryptamine 5-HT$_{2A}$ agonist/monoamine reuptake inhibitor *N,N*-dipropyltryptamine (DPT). *S*(+)-amphetamine fully substituted in the *S*(+)-MDMA-treated animals but did not substitute for the *R*(−)-MDMA cue. 2C-T-7 fully substituted in the *R*(−)-MDMA-trained animals but did not substitute for the *S*(+)-MDMA cue. Cocaine and DPT substituted for both training drugs, but whereas cocaine was more potent in *S*(+)-MDMA-trained mice, DPT was more potent in *R*(−)-MDMA-trained mice. These data suggest that qualitative differences in the discriminative stimulus effects of each stereoisomer of MDMA exist in mice and further our understanding of the complex nature of the interoceptive effects of MDMA.

Racemic 3,4-methylenedioxymethamphetamine (MDMA) is a substituted phenethylamine that is widely abused as the street drug "ecstasy." MDMA has pharmacological and chemical similarities (Fig. 1) to both phenethylamine stimulants and hallucinogens. MDMA has been shown to produce a complex mixture of subjective effects in humans (Vollenweider et al., 1998; Liechti et al., 2000a,b). In particular, subjects report subjective effects such as "increased activation" and "heightened mood" (typical of psychomotor stimulants), as well as "anxious ego-dissolution" and "oceanic boundlessness" (typical of hallucinogenic compounds). The precise mechanisms for these complex and unusual interoceptive properties of MDMA remain to be determined.

Drugs with chiral centers typically give rise to stereoisomers that engender similar biological effects, but the potency with which they produce these effects is different. However, several studies have shown that the isomers of MDMA tend to induce qualitatively different effects (i.e., apparent efficacy differences), which is suggestive of a mechanism for its complex subjective effects. In this regard, *S*(+)-MDMA has a "stimulant-like" profile, with an EC_{50} for the dopamine transporter that is approximately 30 times greater than *R*(−)-MDMA (Setola et al., 2003). However, *R*(−)-MDMA is

This work was supported by the National Institutes of Health National Institute on Drug Abuse [Grant DA020645] (to W.E.F.); the National Institutes of Health National Center for Research Resources [Grant RR020146] (to W.E.F.); and the National Institutes of Health National Center for Research Resources [Grant RR00165] (to Yerkes National Primate Research Center) (to K.S.M., N.M., L.L.H., W.E.F.).

Preliminary findings from these experiments were previously presented as follows: Murnane KS (2008) *2008 Experimental Biology Meeting*; San Diego, CA.

Parts of this work will be presented as a doctoral dissertation for K.S.M. at Emory University.

Article, publication date, and citation information can be found at http://jpet.aspetjournals.org.

doi:10.1124/jpet.109.156174.

ABBREVIATIONS: MDMA, 3,4-methylenedioxymethamphetamine; 5-HT, 5-hydroxytryptamine; DOM, 2,5-dimethoxy-4-methylamphetamine; LSD, lysergic acid diethylamine; 2C-T-7, 2,5-dimethoxy-4-(*n*)-propylthiophenethylamine; DPT, *N,N*-dipropyltryptamine; FR, fixed ratio; TO, timeout; ANOVA, analysis of variance.

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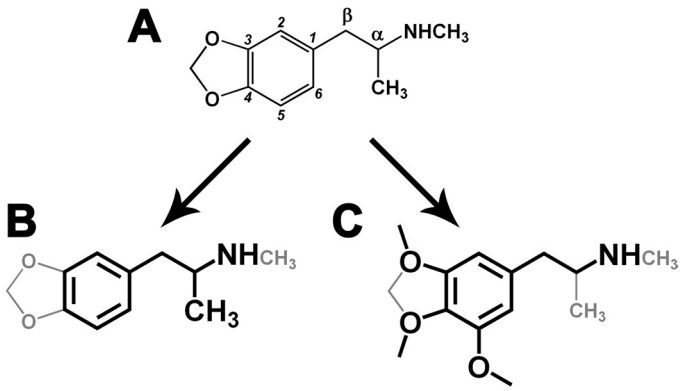


Fig. 1. Chemical similarities among MDMA, the amphetamine-type phenethylamine stimulants, and the mescaline-type phenethylamine hallucinogens. A, MDMA, with ring and side chain carbon position labels. B, amphetamine, bolded and overlaid on the structure of MDMA. C, mescaline, bolded and overlaid on the structure of MDMA.

"hallucinogen-like" in its effects, possessing measurable affinity for the 5-hydroxytryptamine $(5\text{-HT})_{2A}$ receptor (Lyon et al., 1986) and stimulating phosphatidyl inositol hydrolysis upon binding (Nash et al., 1994). At the systems level, only *S*(+)-MDMA increases dopamine neurotransmission in the striatum of Sprague-Dawley rats (Acquas et al., 2007) or rhesus macaques (Murnane et al., 2009). On a behavioral level, *S*(+)-MDMA, but not *R*(−)-MDMA, elicits hyperthermia and locomotor activity in mice (Fantegrossi et al., 2003). Furthermore, only *R*(−)-MDMA induces head-twitch behavior in mice through direct agonism of the 5-HT_{2A} receptor (Fantegrossi et al., 2005a).

This work is buttressed by studies using drug discrimination—the preclinical analog of subjective effects (Schuster and Johanson 1988; Brauer et al., 1997)—in rats. For example, Glennon et al. (1988) reported that, in rats trained to discriminate either *S*(+)-amphetamine or *SR*(±)-2,5-dimethoxy-4-methylamphetamine (DOM) from saline, *S*(+)-MDMA fully substituted for the interoceptive cue produced by amphetamine but not for the interoceptive cue elicited by DOM. Furthermore, Baker et al. (1995) found that the *S*(+)-MDMA cue partially generalized to *S*(+)-amphetamine and cocaine. It is noteworthy that other results in this study were not supportive of *S*(+)-MDMA being a pure psychomotor stimulant, because it also partially or fully generalized to mescaline, lysergic acid diethylamine (LSD), and *SR*(±)-DOM (Baker et al., 1995). However, the preponderance of evidence across studies was supportive of distinct differences in the interoceptive effects of the isomers.

The aim of the present work was to extend these finding by examining the nature of the stimulus effects of the enantiomers of MDMA in mice. Because previous studies have shown that the persistent neurochemical effects of MDMA in mice are distinct from those in rats, monkeys, and perhaps humans (Stone et al., 1987; Logan et al., 1988; O'Shea et al., 2001; Green et al., 2003; Easton and Marsden, 2006), it was of interest to determine whether the interoceptive effects of MDMA were also susceptible to species differences. In mice, the discriminative stimulus effects of MDMA and its enantiomers have been infrequently studied, but in one such report, mice were trained to discriminate 3.0 mg/kg *SR*(±)-MDMA, 1.5 mg/kg *S*(+)-MDMA, or *R*(−)-MDMA from saline, and substitution trials were undertaken among all three compounds. With the exception of *R*(−)-MDMA in mice trained to discriminate *SR*(±)-MDMA, all compounds fully substituted for one another (Fantegrossi et al., 2009). To further examine the nature of the interoceptive cue engendered by *S*(+)- and *R*(−)-MDMA in a parametric fashion in mice, subjects were trained to discriminate *S*(+)-MDMA or *R*(−)-MDMA (1.5 mg/kg) from saline by using a two-lever, liquid food reinforced procedure. The generalization of each discriminative cue was then evaluated by full dose-effect determinations with substitution compounds that parametrically varied in their structural and pharmacological similarity to MDMA (Fig. 2), including the phenethylamine dopamine/norepinephrine releaser *S*(+)-amphetamine (Davids et al., 2002), the nonselective tropane monoamine reuptake inhibitor cocaine (Kuhar et al., 1999), the phenethylamine 5-HT_{2A} agonist 2,5-dimethoxy-4-(*n*)-propylthiophenethylamine (2C-T-7) (Fantegrossi et al., 2005b), and the mixed action tryptamine 5-HT_{2A} agonist/serotonin reuptake inhibitor *N*,*N*-dipropyltryptamine (DPT) (Blough et al., 2007). The specific hypothesis tested was that phenethylamine compounds that selectively share pharmacological effects with an isomer of MDMA would be more likely to substitute for the discriminative stimulus effects of that MDMA isomer, and only of that isomer, in mice.

Materials and Methods

Animals. Twelve (six per group) male Swiss-Webster mice (Charles River Laboratories, Inc., Wilmington, MA) weighing ap-

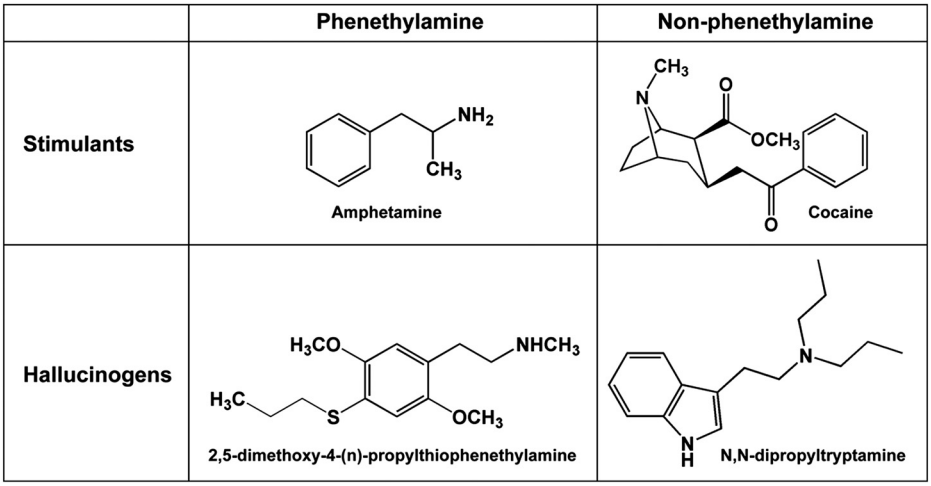


Fig. 2. Chemical structures of all substitution compounds tested in MDMA discrimination experiments.

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proximately 30 g were housed three animals per 44.5 × 22.3 × 12.7 cm Plexiglas cage in a temperature-controlled room within the Yerkes National Primate Research Center. The rodent vivarium was maintained at an ambient temperature of 22 ± 2°C at 45 to 50% humidity, and lights were set to a 12-h light/dark cycle. Animals were fed Lab Diet rodent chow (Laboratory Rodent Diet 5001; PMI Feeds, Inc., St. Louis, MO) and water ad libitum until immediately before testing. Mice were not used in experiments until at least 5 days after arrival in the laboratory. All of the studies were carried out in accordance with the Declaration of Helsinki and with the Guide for Care and Use of Laboratory animals as adopted and promulgated by the National Institutes of Health, and experimental protocols were approved by the Animal Care and Use Committee at Emory University.

Procedure. Acquisition of food-maintained lever-pressing behavior and MDMA discrimination training were carried out as described in previous studies (Yarosh et al., 2007; Fantegrossi et al., 2009). In brief, studies were conducted in operant-conditioning chambers (model ENV-008; MED Associates, St. Albans, VT) that were individually enclosed in larger lightproof Malaguard sound-attenuating cubicles (model ENV-022M; MED Associates) and modified to accommodate murine subjects. The side wall of each chamber compartment used in these studies was equipped with a spout through which liquid reinforcement was delivered, driven by an infusion pump mounted outside the chamber but within the cubicle. The spout was centered between two retractable levers and positioned just beneath a red stimulus light, which was illuminated during reinforcer delivery. Mice were trained 5 days per week under a fixed-ratio (FR) schedule of reinforcement wherein completion of the response requirement on either lever was reinforced by 2 s of access to a palatable liquid reinforcer (approximately 0.02 ml of vanilla-flavored coffee creamer diluted 1:1 with water) followed by a 10-s timeout (TO) before programmed consequences were reinitiated. Once a response requirement was met on either lever, that lever was retracted and subjects were required to meet the response requirement on the other lever. When the response requirement was met on each of the levers, both levers were reintroduced after the TO. In this manner, mice received equivalent reinforcement from each lever, and no subsequent biases for one lever or the other were noted. Animals acquired lever-pressing behavior on a FR1 schedule of reinforcement in sessions lasting 60 min or until 60 reinforcers had been earned (whichever came first). The FR value increased by one for every 20th reinforcer earned within a given session, and the FR value achieved was carried over between sessions until mice were responding under an FR10. This segment of the training was complete when mice performed stably over five consecutive FR10 sessions.

Next, each group of mice was trained in 30-min sessions 5 days per week to discriminate their respective drug [1.5 mg/kg *S*(+)-MDMA or *R*(−)-MDMA administered intraperitoneally] from saline vehicle. Injections were administered 10 min before extension of the response levers, signaling the start of the behavioral session. During discrimination training, a single response on the injection inappropriate lever resulted in retraction of that lever and extinction of the house light for a 30-s TO. During this TO, the injection-appropriate lever remained extended into the chamber, but responses on it had no programmed consequences. After the elapse of the TO, completion of the ratio on the remaining, injection-appropriate lever was reinforced. Percentage of drug-appropriate responding was calculated as the number of reinforcers earned divided by the total number of opportunities to make a choice between the two levers, multiplied by 100. Training was composed of an alternating schedule or drug or saline injection. Subjects were switched from saline to drug or vice versa for the next day of training if they achieved a criterion of greater than 80% correct choices or after three consecutive training days where performance was below criterion. In the latter case, a single day of FR10 responding (as in the lever training condition) was imposed to reestablish contact with the reinforcement contingencies and to increase behavioral output before discrimination training was resumed.

Drug-induced stimulus control was assumed to be present when, in five consecutive sessions, animals made 80% or more correct choices. After stimulus control was established with the training drugs, tests were conducted once per week in each animal as long as performance did not fall below the criterion level of 80% correct responding in any one of the previous three training sessions. Approximately half of the test sessions were conducted the day after saline training sessions with the remainder following drug training sessions. During test sessions, a multiple component cumulative dosing procedure was used, and no responses were reinforced. Each component was terminated after the emission of 10 responses on either lever. Mice were then removed from the chamber, administered the next cumulative dose, and returned to the chamber. Ten minutes later, levers were re-extended into the experimental space. In this manner, four doses of drug could be tested in a single session, over approximately 40 min. The distribution of responses between the two levers was expressed as a percentage of total responses emitted on the drug-appropriate lever. Response rate was calculated for each session by dividing the total number of responses emitted on both levers by the elapsed time before 10 responses on either lever.

Complete generalization of a training drug to a test drug is said to be present when 1) a mean of 80% or more of all test responses occurs on the drug-appropriate lever and 2) there is a statistically significant difference between the response distributions of the test drug and saline control sessions. An intermediate degree of generalization is defined as being present when response distributions after a test drug are less than 80% drug-appropriate and are significantly different from saline control sessions. Finally, when the response distribution after a test drug is not statistically significantly different from that in saline control sessions, an absence of generalization of the training drug to the test drug is assumed. Failure to complete an FR10 on either lever within 2 min terminated the sessions and indicated disruption of schedule-controlled behavior.

Data Analysis. Graphical presentation of all data depicts mean ± S.E.M. Drug discrimination data are expressed as percentage of drug-appropriate responding, which is the number of responses emitted on the drug-appropriate lever as a percentage of the total number of responses emitted. Response rates are expressed as the number of responses per second, calculated for each session by dividing the total number of responses emitted (before the emission of 10 responses on either lever) by elapsed time. Data for any subjects failing to emit 10 responses within 2 min of lever extension were deemed to be behaviorally disrupted and were not considered in the calculation of the percentage of drug-appropriate responding or response rates. Generalization was said to occur if 80% or more of the responses were on the drug-appropriate lever. The statistical significance of the generalization of a training drug was determined using one-way repeated measure analysis of variance (ANOVA) to compare the two training conditions with the test drug. Subsequent multiple comparisons to saline control were made by the method of Dunnett. Control data were repeated for each comparison, and statistical analyses were applied by using the appropriate control sessions. However, for purposes of clarity, mean values for control data are shown in all figures. Nonlinear regression analysis with a variable slope sigmoidal dose-response curve was used to calculate the dose that was 50% effective (ED_{50}; with a set range of 0–100%) and the Hill slope of the dose-effect curve when the test compound partially or fully substituted for the training drug. The equation used for this analysis was Y = 0 + (100)/(1 + 10^((LogED$_{50}$ − X) * Hill Slope)), where X is equal to the logarithm of the dose and Y is equal to the response. All graphical data presentations were created by using GraphPad Prism 4 (GraphPad Software Inc., San Diego, CA), all statistical tests were performed by using SigmaStat 3 (Systat Software, Inc., San Jose, CA), and significance was judged at $P < 0.05$.

Drugs. *S*(+)-MDMA, *R*(−)-MDMA, 2C-T-7, and amphetamine were supplied by the National Institute on Drug Abuse (Research

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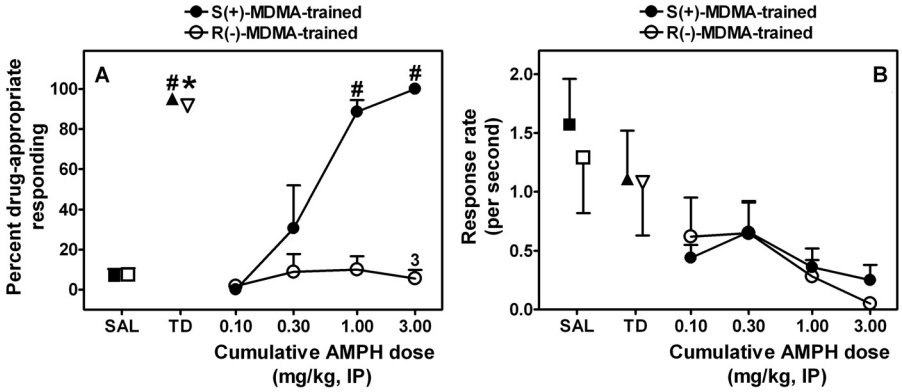


Fig. 3. Effects of amphetamine in mice trained with 1.5 mg/kg *S*(+)-MDMA (●) or 1.5 mg/kg *R*(−)-MDMA (○) as a discriminative stimulus ($n = 6$ per group). All points represent the mean ± S.E.M., and any points without error bars indicate instances in which the S.E.M. is encompassed by the data point. Abscissae, dose of drug expressed as milligram per kilogram and plotted on a logarithmic scale. The points at SAL and TD represent saline and MDMA training dose sessions, respectively. Filled symbols represent data from *S*(+)-MDMA-trained mice, whereas open symbols represent data from *R*(−)-MDMA-trained mice. Ordinates, percentage of MDMA-appropriate responding (A) or response rate (B). A numeral adjacent to a symbol indicates the number of animals completing the test, if less than 6. A # indicates a significant difference for saline in the *S*(+)-MDMA-trained group, whereas an ∗ indicates the same relationship in the *R*(−)-MDMA-trained groups. Significant differences were assessed by one-way repeated measures ANOVA with post hoc analysis carried out using Dunnett's test.

Technology Branch, Research Triangle Park, NC). DPT was synthesized at the Laboratory of Medicinal Chemistry at the National Institutes of Diabetes, Digestive and Kidney Disorders at the National Institutes of Health (Bethesda, MD) and was a generous gift from Dr. Kenner C. Rice. All compounds were weighed as salts, dissolved in 0.9% physiological saline, and all injections were administered intraperitoneally at a volume of 1.0 ml/100 g.

Results

Discrimination. Under the procedures used, all animals learned to reliably discriminate the training dose of the respective training drug from saline. Once subjects were fully trained, group means of each group for drug-appropriate lever responding were greater than 90% subsequent to administration of the training dose and less than 10% subsequent to administration of saline. One-way repeated measures ANOVA was carried out for all drug substitutions in each respective group. A significant main effect of condition (drug and dose) was found for both the *S*(+)-MDMA ($F_{5,17} = 12.706$; $P < 0.001$) and the *R*(−)-MDMA groups ($F_{5,18} = 7.141$; $P < 0.001$).

Amphetamine Substitution. Cumulative administration of *S*(+)-amphetamine engendered dose-dependent and full substitution (100 ± 0%) in *S*(+)-MDMA-trained subjects. However, *S*(+)-amphetamine did not substitute for the *R*(−)-MDMA cue up to doses that suppressed responding (Fig. 3). Post hoc analysis by means of the Dunnett's test revealed that the 1.0 and 3.0 mg/kg doses of *S*(+)-amphetamine were significantly different from saline administration ($P < 0.05$) in the *S*(+)-MDMA-trained animals. No dose of amphetamine was significantly different from saline in the *R*(−)-MDMA-trained subjects. Nonlinear curve fitting determined an ED_{50} of 0.42 mg/kg with a Hill slope of 2.48 ($R^2 = 0.79$) in the *S*(+)-MDMA-trained animals. This analysis was not possible in *R*(−)-MDMA-trained mice due to the failure of amphetamine to substitute in these subjects.

Cocaine Substitution. Cumulative administration of cocaine engendered dose-dependent and full substitution (100 ± 0%) in *S*(+)-MDMA-trained subjects. In the *R*(−)-MDMA-trained subjects, cocaine dose-dependently and partially substituted (66.67 ± 33%) for the training dose (Fig. 4). Post hoc analysis by means of the Dunnett's test revealed that the 1.0 and 3.0 mg/kg doses of cocaine were significantly different from saline administration ($P < 0.05$) in the *S*(+)-MDMA-trained animals, and that 3.0 mg/kg cocaine was significantly different from saline ($P < 0.05$) in the *R*(−)-MDMA-trained animals. Nonlinear curve fitting determined an ED_{50} of 0.36 mg/kg with a Hill slope of 1.63 ($R^2 = 0.60$) in the *S*(+)-MDMA-trained animals. Cocaine was approximately five times less potent in the *R*(−)-MDMA-trained animals with an ED_{50} of 1.54 mg/kg and a Hill slope of 0.95 ($R^2 = 0.60$).

2C-T-7 Substitution. Cumulative administration of 2C-T-7 engendered dose-dependent and full substitution

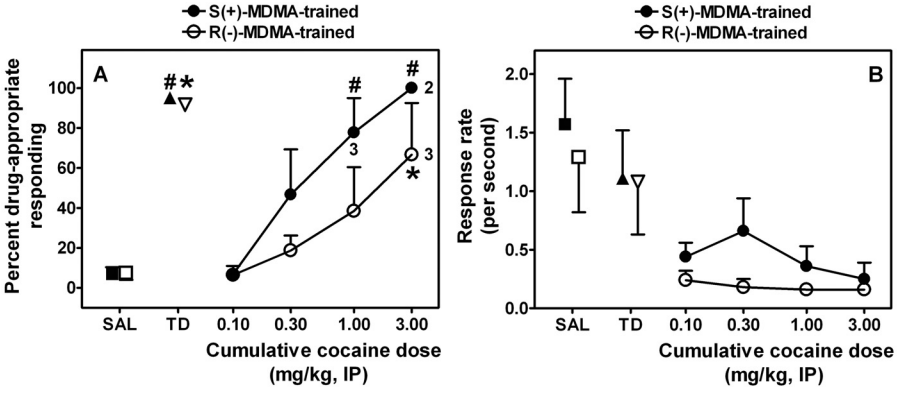


Fig. 4. Effects of cocaine in mice trained with 1.5 mg/kg *S*(+)-MDMA (●) or 1.5 mg/kg *R*(−)-MDMA (○) as a discriminative stimulus ($n = 6$ per group). All points represent the mean ± S.E.M., and any points without error bars indicate instances in which the S.E.M. is encompassed by the data point. Abscissae, dose of drug expressed as milligram per kilogram and plotted on a logarithmic scale. Ordinates, percentage of MDMA-appropriate responding (A) or response rate (B).

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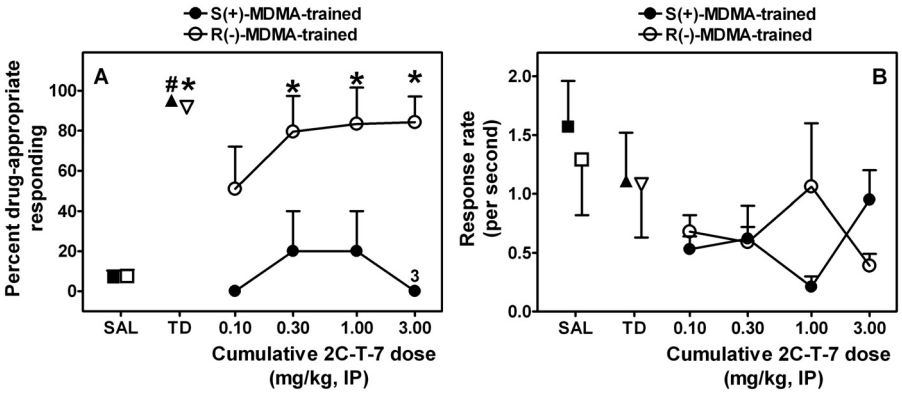


Fig. 5. Effects of 2C-T-7 in mice trained with 1.5 mg/kg *S*(+)-MDMA (●) or 1.5 mg/kg *R*(−)-MDMA (○) as a discriminative stimulus (n = 6 per group). All points represent the mean ± S.E.M., and any points without error bars indicate instances in which the S.E.M. is encompassed by the data point. Abscissae, dose of drug expressed as milligram per kilogram and plotted on a logarithmic scale. Ordinates, percentage of MDMA-appropriate responding (A) or response rate (B).

(84.25 ± 11.76%) in *R*(−)-MDMA-trained subjects; however, 2C-T-7 did not substitute for the *S*(+)-MDMA cue (20.0 ± 20.0%) (Fig. 5). Post hoc analysis by means of the Dunnett's test revealed that the 0.3, 1.0, and 3.0 mg/kg doses of 2C-T-7 were significantly different from saline administration ($P < 0.05$) in the *R*(−)-MDMA-trained animals. No dose of 2C-T-7 was significantly different from saline in the *S*(+)-MDMA-trained subjects. Nonlinear regression analysis could not accurately fit the data for 2C-T-7 in the *R*(−)-MDMA-trained animals. This result was probably due to the lack of multiple intermediate substitution data points at the doses used. Likewise, the failure of 2C-T-7 to substitute for the *S*(+)-MDMA training dose precluded this level of analysis in these subjects as well.

DPT Substitution. Cumulative administration of DPT engendered dose-dependent and full substitution in subjects trained with *R*(−)-MDMA (100 ± 0%) and in subjects trained with *S*(+)-MDMA (96.15 ± 3.847) (Fig. 6). Post hoc analysis by means of the Dunnett's test revealed that the 1.0 and 3.0 mg/kg doses of cocaine were significantly different from saline administration ($P < 0.05$) in the *R*(−)-MDMA-trained animals, whereas the 1.0, 3.0, and 10.0 mg/kg doses of DPT were significantly different from saline ($P < 0.05$) in the *S*(+)-MDMA-trained animals. Nonlinear curve fitting determined an ED_{50} of 0.14 mg/kg with a Hill slope of 1.09 (R^2 = 0.58) for DPT in the *R*(−)-MDMA-trained animals. DPT was approximately six times less potent in the *S*(+)-MDMA-trained animals with an ED_{50} of 0.91 mg/kg and a Hill slope of 2.2 (R^2 = 0.75).

Discussion

The aim of the present study was to examine the nature of the interoceptive cue engendered by *S*(+)- and *R*(−)-MDMA in mice, under conditions that parametrically varied both the chemical similarity of the test drugs to MDMA and the pharmacological selectivity of the test compounds. It is important to note that in the case of both the stimulants (amphetamine and cocaine) and hallucinogens (2C-T-7 and DPT) tested, the pharmacological effects of the phenethylamine-based drugs (amphetamine and 2C-T-7) were more selective than were the effects of the drugs not structurally related to MDMA (cocaine and DPT). Under the procedures used, all animals learned to reliably discriminate the training dose of *S*(+)-MDMA or *R*(−)-MDMA from saline. In combination with previous reports comparing the discriminative stimulus effects of each isomer of MDMA to *N*-substituted piperazines (Yarosh et al., 2007) or to each other (Fantegrossi et al., 2009), these data indicate that mice can be reliably trained to discriminate each isomer of MDMA from saline.

S(+)-amphetamine is structurally similar to MDMA, and both compounds stimulate impulse-independent release of monoamines (Acquas et al., 2007; Fleckenstein et al., 2007). Based on previous reports describing the neurochemical effects of these compounds, and based on the discriminative profiles of the MDMA isomers established in rats, we hypothesized that the discriminative stimulus effects of amphetamine should be more similar to those of *S*(+)-MDMA than those of *R*(−)-MDMA in the mouse. This hypothesis was confirmed by the full substitution of *S*(+)-amphetamine for the discriminative cue of *S*(+)-MDMA and the failure of amphetamine to engender significant *R*(−)-MDMA-like responding in mice. 2C-T-7 is also structurally similar to MDMA, and both compounds are substituted phenethylamines with agonist affinity for the 5-HT_{2A} receptor (Lyon et al., 1986; Fantegrossi et al., 2005b, 2008). Based upon this pharmacological profile and previous work on the discrimi-

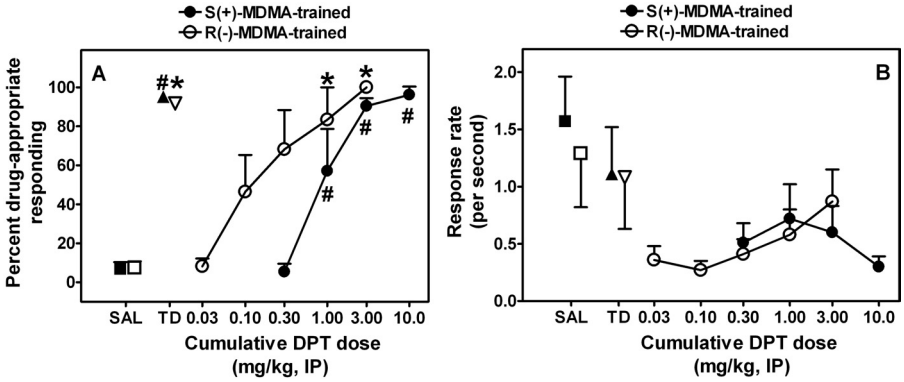


Fig. 6. Effects of DPT in mice trained with 1.5 mg/kg *S*(+)-MDMA (●) or 1.5 mg/kg *R*(−)-MDMA (○) as a discriminative stimulus (n = 6 per group). All points represent the mean ± S.E.M., and any points without error bars indicate instances in which the S.E.M. is encompassed by the data point. Abscissae, dose of drug expressed as milligram per kilogram and plotted on a logarithmic scale. Ordinates, percentage of MDMA-appropriate responding (A) or response rate (B).

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native stimulus effects of the MDMA enantiomers in rats, we hypothesized that the discriminative stimulus effects of 2C-T-7 should be more similar to those of *R*(−)-MDMA than to those of *S*(+)-MDMA in mice. This hypothesis was also confirmed by the full substitution of 2C-T-7 in the *R*(−)-MDMA-trained animals and by the lack of *S*(+)-MDMA-like responding elicited by 2C-T-7 in *S*(+)-MDMA-trained animals. The sum of these two data sets indicates a profound qualitative difference in the discriminative cue engendered by each stereoisomer of MDMA. These data support previous studies that contrast the stimulus properties of the two isomers in rats (Glennon et al., 1988; Baker et al., 1995). Furthermore, the distinct interoceptive effects of the two isomers of MDMA have now been demonstrated across different operant schedules, training procedures, training doses, training drugs (generalization versus substitution), and species. However, it remains difficult to explain the capacity of each MDMA enantiomer to substitute for one another in mice, using procedures identical to those herein described previously (Fantegrossi et al., 2009). Nevertheless, these present findings suggesting qualitatively distinct discriminative stimulus effects in the mouse are supported not only by earlier drug discrimination experiments in the rat, but also by previous studies where *S*(+)-MDMA elicited stimulant-like effects, while *R*(−)-MDMA induced hallucinogen-like effects, on multiple behavioral and physiological endpoints in mice (Fantegrossi et al., 2003, 2005a).

It is also clear from the present studies that, when the structural and pharmacological similarity of the test compounds to MDMA was reduced, the previously observed qualitative differences between the interoceptive effects of the MDMA enantiomers were replaced by simple potency differences. Cocaine has no notable structural features in common with MDMA, and although both compounds alter synaptic monoamine levels, MDMA does so through transporter-mediated release (Setola et al., 2003) while cocaine passively blocks monoamine reuptake (Kuhar et al., 1999). Nevertheless, cocaine fully substituted for the training dose of *S*(+)-MDMA and partially substituted for the interoceptive cue induced by *R*(−)-MDMA. In mice trained with *R*(−)-MDMA, the Hill slope of the cocaine dose-effect curve was 1.63, and only three of the six animals reached the response criterion at the highest dose of cocaine tested, suggesting that full substitution might have been achieved were it not for the rate suppressant effects of cocaine under these conditions in these subjects. Although it is not clear why the rate-suppressant effects of cocaine were more pronounced in mice trained with *R*(−)-MDMA than in mice trained with *S*(+)-MDMA, it is important to note that the interoceptive effects of cocaine were approximately five times more potent in the *S*(+)-MDMA group than in the *R*(−)-MDMA-trained animals. Thus, although cocaine substituted for each MDMA isomer, a conspicuous potency difference remained. Likewise, the chemical structures of MDMA and DPT are not particularly congruent. In addition to its agonist affinity for 5-HT_{2A} receptors, DPT functionally inhibits reuptake of monoamines without stimulating release (Nagai et al., 2007). DPT fully substituted for the training dose of each stereoisomer but was 6-fold more potent in mice trained with *R*(−)-MDMA. These data are in general agreement with previous studies showing that LSD and cocaine, drugs with promiscuous pharmacological profiles that are structurally dissimilar to MDMA, partially or fully substituted for each isomer (Baker et al., 1995 and Bondareva et al., 2005, respectively). Taken together, these data reveal that of the compounds tested in this study, *R*(−)-MDMA shares stimulus properties with direct 5-HT_{2A} receptor agonists and indirect serotonin agonists, whereas *S*(+)-MDMA shares stimulus properties with only indirect agonists in mice. Furthermore, *S*(+)-MDMA shares stimulus properties with selective dopamine substrate releasers and nonselective monoamine reuptake inhibitors, whereas *R*(−)-MDMA shares stimulus properties with only nonselective monoamine reuptake inhibitors.

It is important to note that many procedural variables can profoundly affect the results of drug discrimination studies. In particular, the training dose chosen to establish discriminative control affects both the rate of acquisition of the discrimination task and the "sensitivity" of the animals to subsequent test compounds. For example, rats trained to discriminate 40 mg/kg of the μ-opioid agonist fentanyl from saline acquired the discrimination more rapidly than did animals trained with lower doses, but the dose-effect functions for fentanyl discrimination in these animals were shifted to the right compared with rats trained with lower fentanyl doses (Colpaert et al., 1980). The role of various procedural variables, including training dose, in drug discrimination experiments involving serotonergic compounds (Winter et al., 1999) has been reviewed previously. Thus, whereas the presently reported data are in accordance with previous experiments conducted in rats (Glennon et al., 1988; Baker et al., 1995), further drug discrimination experiments with the MDMA enantiomers in mice trained with both higher and lower doses and maintained under different operant schedules are warranted.

In conclusion, these data indicate that the discriminative cues mediated by each enantiomer of MDMA are distinct, yet overlapping, and further suggest that, as has been demonstrated in the rat, the interoceptive effects of *S*(+)-MDMA are primarily stimulant-like, whereas those of *R*(−)-MDMA are predominantly hallucinogen-like in the mouse. As research into the effects of MDMA in wild-type and genetically modified mice continues to proliferate, this demonstration of a similarity between the interoceptive effects of the MDMA enantiomers in rats and mice—despite the pronounced species differences in both persistent and acute neurochemical effects (Stone et al., 1987; Logan et al., 1988; O'Shea et al., 2001; Green et al., 2003; Easton and Marsden, 2006)—becomes all the more important. The stimulus properties of amphetamine, a relatively dopamine-selective stimulant, or 2C-T-7, a relatively selective serotonergic hallucinogen, completely dissociate the MDMA enantiomers from one another. In contrast, the interoceptive effects of cocaine, a stimulant with approximately equivalent effects on dopamine, norepinephrine, and serotonin, or DPT, a hallucinogen that also inhibits reuptake of serotonin, generalize to both MDMA enantiomers, but do so more potently in the more stimulant-like *S*(+)-MDMA or the more hallucinogen-like *R*(−)-MDMA, respectively. This pattern of findings would seem to suggest that the interoceptive effects of the MDMA enantiomers in mice are mediated by a mixture of dopaminergic and serotonergic components. In the case of *S*(+)-MDMA, the dopaminergic component is a more salient cue than the serotonergic component, whereas the reverse is true for *R*(−)-MDMA. This notion may explain not only the present results, but also

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the capacity for each enantiomer to substitute for one another in mice (Fantegrossi et al., 2009). In so far as drug discrimination experiments can be generalized to human subjective effects, it seems likely that isomers possessing distinct but overlapping interoceptive effects could produce a racemic mixture with complex subjective effects, as is the case with MDMA. Further research into the intriguing subjective effects of MDMA, particularly as they may relate to subtle differences in the interoceptive effects induced by the component enantiomers in man, would be informative.

Acknowledgments

We thank the Emory University International Research Experience for Science Students program for travel and research support for N. Murai. We also thank the animal care staff at the Yerkes National Primate Research Center for their expert animal husbandry services.

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