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Serotonergic–dopaminergic mediation of MDMA's discriminative stimulus effects in a three-choice discrimination

Amy K. Goodwin*, Dori M. Pynnonen, Lisa E. Baker

Western Michigan University, Kalamazoo, MI, USA

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Abstract

(±)3,4-Methylenedioxymethamphetamine (MDMA; "Ecstasy") is a common drug of abuse that is often described as both a psychostimulant and a hallucinogen. Two-choice drug discriminations (i.e. drug vs. nondrug) in nonhumans comparing the discriminative stimulus properties of MDMA to psychostimulants or hallucinogens have produced somewhat inconsistent findings. The relative contribution of serotonergic versus dopaminergic actions to MDMA's discriminative stimulus effects may depend on the training stimulus conditions employed. We have previously demonstrated that rats can learn to discriminate the effects of MDMA and D-amphetamine in a three-choice drug discrimination procedure, and that LSD produced nearly complete substitution for MDMA under these conditions, and fenfluramine fully substituted for MDMA. In the present study, 12 rats were trained to discriminate LSD (0.08 mg/kg) and MDMA (1.5 mg/kg) from saline in a three-choice drug discrimination procedure under a fixed-ratio (FR) 10 schedule of food reinforcement. D-Amphetamine produced only partial substitution for MDMA while fenfluramine produced complete stimulus generalization. Low doses of D-amphetamine and fenfluramine produced greater stimulus generalization when administered in combination than when given alone. The serontonin$_2$ antagonist MDL-100,907 only partially blocked the MDMA cue, but completely antagonized LSD discrimination. The dopamine antagonist haloperidol also failed to block MDMA discrimination. These results indicate that 5-HT release is a salient feature to MDMA's discriminative stimulus effects but that MDMA produces a compound discriminative stimulus.

Keywords: MDMA; LSD; D-Amphetamine; Fenfluramine; MDL-100,907; Haloperidol; Drug discrimination; Rats

1. Introduction

(±)-3,4-Methylenedioxymethamphetamine (MDMA) is a commonly abused drug known as "ecstasy." MDMA was originally produced by Merck in the early 1900s as an intermediate product in the development of a vasoconstrictive drug (Beck, 1997; Holland, 2001; Pentney, 2001). During the 1970s, MDMA was used as an adjunct to psychotherapy (Greer and Tolbert, 1986; Holland, 2001; McDowell and Kleber, 1994), reportedly to enhance communication and "self-examination." In 1978, Shulgin and Nichols published the first report about the usefulness of MDMA in psychotherapy. They reported that the subjective effects of MDMA included altered states of consciousness with emotional components such as empathy, acceptance, and insight. In the early 1980s, MDMA became a popular recreational drug and was sold legally, typically through mail order (Eisner, 1989; Ray and Ksir, 1999). Citing nationwide abuse and the potential health problems of MDMA, the Drug Enforcement Agency (DEA) succeeded in making MDMA a Schedule I substance in 1988. Despite increased public awareness of the health risks associated with MDMA, its use has continued to rise in recent years, particularly among young people.

MDMA is a phenylethylamine, a structural analog of D-amphetamine which reportedly possesses both hallucinogenic and stimulant properties (Callahan and Appel, 1988; Evans and Johanson, 1986; Schechter, 1986). MDMA users have consistently reported the subjective effects include elevated mood, feelings of closeness and intimacy, increased empathy, insightfulness, mild alterations in per-

* Corresponding author. Division of Behavioral Biology, Johns Hopkins Medical Institutions, Hopkins Bayview Medical Center, 5510 Nathan Shock Drive, Suite 3000, Baltimore, MD 21224, USA. Tel.: +1-410-550-2775; fax: +1-410-550-2780.
E-mail address: Goodwin@jhu.edu (A.K. Goodwin).

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ception, accelerated thinking, jaw clenching, and appetite suppression (Cami et al., 2000; Greer and Tolbert, 1986; Grinspoon and Bakalar, 1986; Peroutka et al., 1988; Shulgin and Nichols, 1978; Siegal, 1986; Solowij et al., 1992). MDMA is currently classified into the traditional drug classes as both a stimulant and a hallucinogen. However, some investigators advocate a distinct classification for MDMA, such as "entactogen" proposed by Nichols (1986) in order to illustrate that MDMA is distinctly different from traditional stimulants and hallucinations.

The drug discrimination procedure is a popular assay used to classify the stimulus properties of drugs and to examine neurochemical mechanisms of drug action. MDMA has been investigated extensively using traditional two-choice drug discrimination methods. However, these reports have yielded conflicting results (see Table 1). Using D-amphetamine (2.0 mg/kg) as the training drug, Evans and Johanson (1986) reported stimulus generalization to MDMA (3.0 mg/kg) in pigeons. Glennon and Young (1984) also reported that MDMA (2.25 mg/kg) substituted for 1.0 mg/kg D-amphetamine in three rats. However, Oberlander and Nichols (1988) reported that MDMA (2.63 mg/kg) did not substitute for D-amphetamine (1.0 mg/kg) in rats ($n=14$). Oberlander and Nichols also reported that D-amphetamine (1.2 mg/kg) *did* substitute for MDMA (1.75 mg/kg) when MDMA was the training stimulus, but was disruptive in 7 of the 13 rats tested. Schechter (1989) reported that D-amphetamine (0.8 mg/kg) only partially substituted for MDMA (1.5 mg/kg) in rats. Glennon and Misenheimer (1989) also reported that D-amphetamine (1.0 mg/kg) only partially substituted for MDMA (1.5 mg/kg) in rats. Furthermore, Baker et al. (1995) reported that D-amphetamine did not substitute for either of the optical isomers of MDMA.

Table 1
Two-lever drug discrimination and MDMA

Training compound	Test compound	Substitution?	Authors
AMPH (1.0 mg/kg)	MDMA	Yes	Glennon and Young (1984)
AMPH (1.0 mg/kg)	MDMA	No	Oberlander and Nichols (1988)
MDMA (1.75 mg/kg)	AMPH LSD	Yes Partial	Oberlander and Nichols (1988)
MDMA (1.5 mg/kg)	AMPH Norfenfluramine	Partial Yes	Schechter (1989)
MDMA (1.5 mg/kg)	AMPH	Partial	Glennon and Missenheimer (1989)
(+)-MDMA (1.25 mg/kg)	AMPH Fenfluramine	Partial Yes	Baker et al. (1995)
(−)-MDMA (3.5 mg/kg)	AMPH Fenfluramine	No Yes	Baker et al. (1995)
MDMA (1.5 mg/kg)	LSD	Yes	Schechter (1998)
Fenfluramine (2.0 mg/kg)	MDMA	Yes	Schechter (1986)
LSD (0.08 mg/kg)	MDMA	No	Callahan and Appel (1988)

Reports regarding the substitution of MDMA to serotonin (5-HT) agonists are somewhat more consistent. It has been reported that MDMA (2.0 mg/kg) substitutes for the 5-HT releaser, fenfluramine (2.0 mg/kg) (Schechter, 1986). Fenfluramine also substitutes for both isomers of MDMA (Baker et al., 1995), and its metabolite, norfenfluramine (1.4 mg/kg) substitutes for MDMA (1.5 mg/kg) (Schechter, 1989).

While symmetrical stimulus generalization occurs between MDMA and fenfluramine, stimulus generalization between MDMA and LSD appears to be asymmetrical. Oberlander and Nichols (1988) reported nearly complete (78%) substitution of LSD (0.16 mg/kg) for MDMA (1.75 mg/kg). A more recent study by Schechter (1998) demonstrated that LSD (0.12 mg/kg) substitutes for MDMA (1.5 mg/kg) in fawn-hooded rats (Schechter, 1998). However, Callahan and Appel (1988) reported that MDMA did not substitute for LSD.

The three-lever drug discrimination procedure is reported to be a more sensitive tool with which to investigate the stimulus properties of psychoactive drugs (Stolerman, 1993), particularly those with multiple pharmacological actions (Baker and Taylor, 1997). Relatively few studies have examined the stimulus properties of MDMA in three-choice drug discriminations. Evans et al. (1990) trained five pigeons to discriminate D-amphetamine (1.7 or 3.0 mg/kg), fenfluramine (5.6 or 10 mg/kg), and saline using a three-choice procedure. In the three subjects tested, they reported that MDMA substituted for D-amphetamine in two of the subjects and for fenfluramine in the third subject. Baker and Taylor (1997) also examined the stimulus properties of MDMA in two separate, three-lever drug discrimination experiments. In the first experiment, rats were trained to discriminate D-amphetamine (1.0 mg/kg), mescaline (12.5 mg/kg), and saline under a fixed-ratio (FR) 20 schedule of reinforcement. Stimulus generalization tests with (+)-MDMA resulted in mostly saline-appropriate responding, with some responding on the mescaline-appropriate lever. Administration of (−)-MDMA produced 78% mescaline-appropriate responding. In the second experiment, Baker and Taylor trained rats to discriminate D-amphetamine (1.0 mg/kg), LSD (0.08 mg/kg), and saline. Neither isomer of MDMA substituted for D-amphetamine, but produced significant responding on the LSD-appropriate lever.

In an attempt to further investigate the compound stimulus properties of MDMA, Goodwin and Baker (2000) trained rats to discriminate between D-amphetamine (1.0 mg/kg), MDMA (1.5 mg/kg), and saline. The administration of LSD resulted in almost complete substitution for MDMA (i.e. 78% MDMA-appropriate responding) at the two highest doses tested (0.08 and 0.16 mg/kg). Additionally, fenfluramine substituted for MDMA, as did both isomers of MDA. However, the 5-HT_2 antagonist pirenperone only partially blocked the discrimination of MDMA. These results indicate that the serotonergic actions of MDMA were more salient in maintaining stimulus control when

animals were trained to discriminate both D-amphetamine and MDMA.

Since MDMA is classified as both a stimulant and a hallucinogen, and it has been established that rats can discriminate between D-amphetamine and MDMA in a three-choice procedure, the present study sought to determine if rats could be trained to discriminate between an LSD (0.08 mg/kg) and an MDMA (1.5 mg/kg) in a similar three-lever procedure, and to determine what pharmacological actions of MDMA were most salient in maintaining stimulus control. Some of the data (acquisition and terminal accuracy) from the present study have been published previously (Goodwin and Baker, 2002).

2. Methods

2.1. Subjects

Subjects consisted of 12 experimentally naïve male Sprague–Dawley rats (Harlan Breeding Laboratories, Indianapolis, IN) that were approximately 60 days old and weighed between 250 and 300 g at the beginning of the study. Subjects were individually housed in plastic shoebox cages in a colony maintained on a 12-h light (0700 to 1900)/12-h dark cycle, at relatively constant temperature and humidity. In the home cages, subjects were allowed free access to water while food intake was restricted to maintain body weights between 85% and 90% of free feeding weights for the duration of the study. The experimental protocol was reviewed by the Institutional Animal Care and Use Committee of Western Michigan University and subjects were maintained according to the general principles of animal husbandry outlined by the National Institutes of Health Guide for Care and Use of Laboratory Animals.

2.2. Materials

All training and testing procedures were conducted in eight standard operant test chambers (MED Associates, Georgia, VT) measuring $30 \times 31 \times 24$ cm, maintained in sound-and light-attenuating cubicles. The chambers were equipped with three retractable levers on the front panel, a 28-V house light located on the rear panel, and a food pellet delivery mechanism located above the center lever.

(±)-MDMA-hydrochloride, (+)-LSD-tartrate, D-amphetamine sulfate, and (+)-fenfluramine-hydrochloride were provided by the National Institute on Drug Abuse (Bethesda, MD). MDL-100,907 was provided by Aventis (Bridgewater, NJ) and haloperidol was obtained from Sigma (St. Louis, MO). Doses were based on the weight of the salt form of each compound. (±)-MDMA, (+)-LSD, D-amphetamine, and fenfluramine were dissolved in 0.9% bacteriostatic sodium chloride and administered intraperitoneally 15 min prior to training and testing sessions. MDL-100,907 was dissolved in 0.1 N HCl and then adjusted to pH ≈ 5.0 with NaOH and administered 30 min prior to testing. Haloperidol was dissolved in a few drops of lactic acid and adjusted to pH ≈ 4.5 with NaOH and administered 45 min prior to testing. All drugs were administered in an injection volume of 1 ml/kg.

2.3. Training procedures

An autoshaping procedure was used for the first week of training. Subjects received between five and six 1-h sessions where no substances were administered, no levers were present in the chamber, and food pellets were delivered on a fixed-time 60 s (FT 60) schedule. Subsequently, errorless discrimination training was employed where only the condition-appropriate lever was present for alternate 20-min training sessions of saline and each drug condition. This was continued until each subject was exposed to at least four errorless training sessions for each of the three conditions. During the errorless training sessions an FR 1 schedule of reinforcement was used.

Following the errorless training procedure, all three levers were presented and discrimination training began with an FR 1 schedule of reinforcement during daily 20-min training sessions. The ratio was gradually increased to 10 as responding became stable. The terminal schedule of reinforcement was a resetting FR 10. That is, reinforcement was contingent on 10 consecutive responses on the condition-appropriate lever, responses on any other lever reset the response counter and reinforcement was not delivered until 10 consecutive responses were made on the condition-appropriate lever. Subjects were able to obtain an unlimited number of reinforcers during the 20-min training sessions. Following MDMA (1.5 mg/kg) administration, half of the subjects were reinforced for responses on the left lever and half were reinforced for responses on the right lever. The conditions were reversed following LSD (0.08 mg/kg) administration. Under saline conditions, all subjects were reinforced for responses on the center lever. In order to reduce the effects of olfactory cues between animals in the operant chambers, all levers were wiped with isopropyl alcohol between training sessions (Extance and Goudie, 1981). Additionally, the order in which subjects were run during the daily sessions was altered randomly. Training sessions were conducted 6 days a week at approximately the same time each day and treatment conditions (i.e., MDMA, LSD, or saline) were presented in variable order.

2.4. Testing procedures

Once subjects met a predetermined criterion for discrimination (80% of responses on the condition-appropriate lever prior to the delivery of the first reinforcer for at least 8 out of 10 consecutive training sessions), testing procedures were implemented. Test sessions were similar to training sessions except that no reinforcers were delivered and the animals were removed from the chambers immediately upon com-

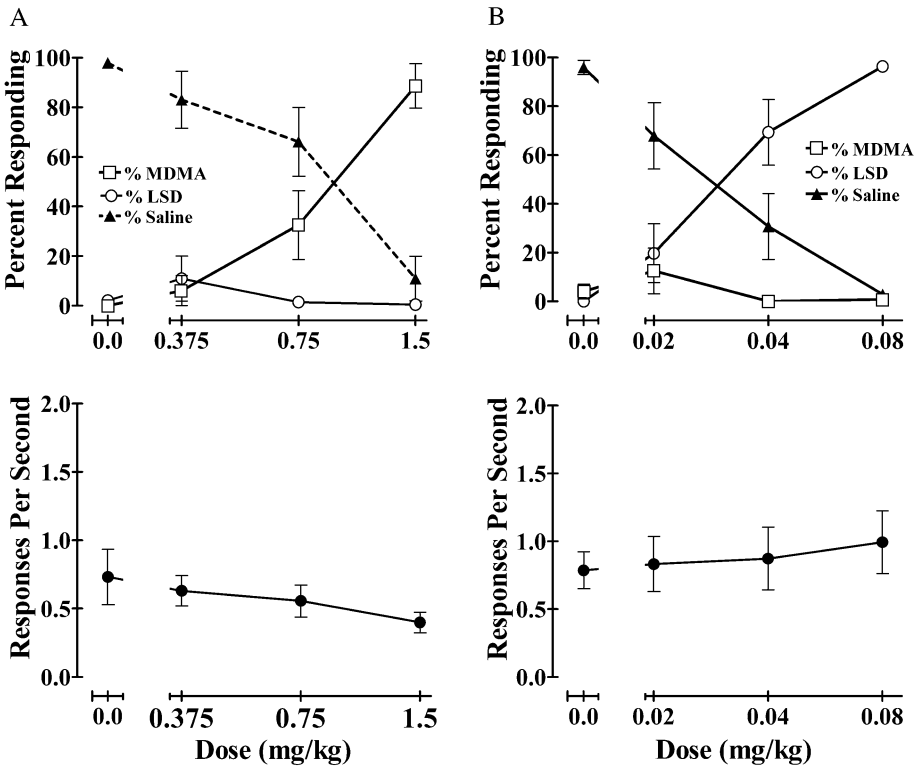

Fig. 1. Results of stimulus generalization tests with (A) MDMA (0.375–1.5 mg/kg, $n = 11$) and (B) LSD (0.02–0.08 mg/kg, $n = 11$). The mean percentage of responses on each lever are presented in the top graphs and the overall response rate is presented in the bottom graphs.

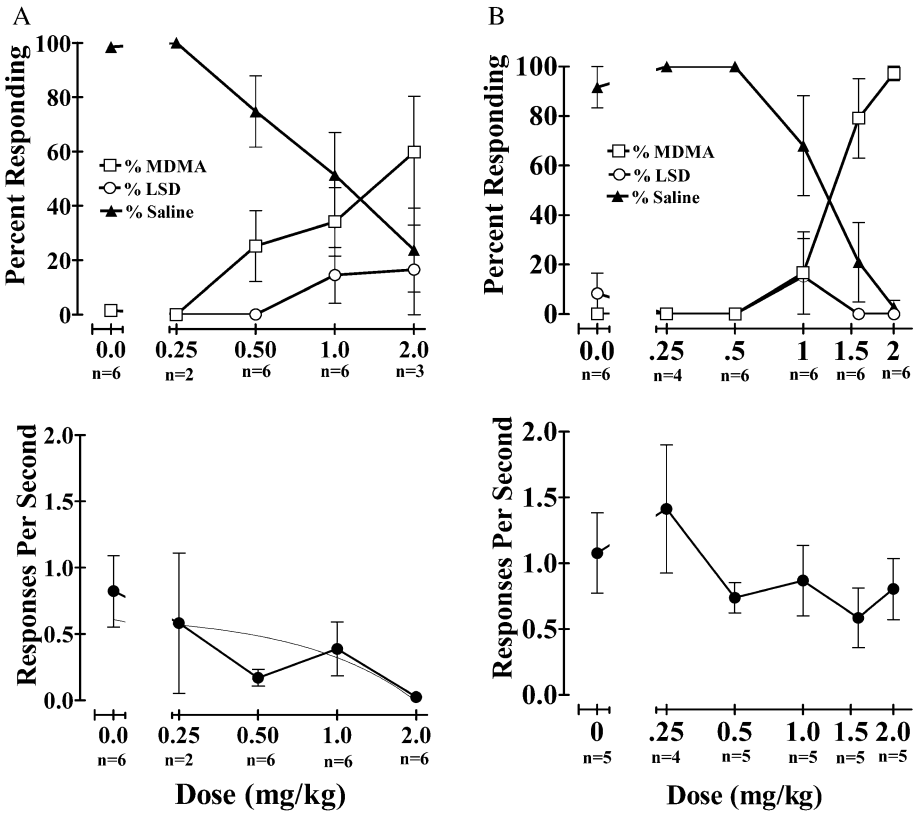

Fig. 2. Results of stimulus generalization tests with (A) D-amphetamine (0.25–2.0 mg/kg) and (B) fenfluramine (0.25–2.0 mg/kg). The mean percentage of responses on each lever are presented in the top graphs and the overall response rate is presented in the bottom graphs.

pletion of 10 consecutive responses on any lever. Test sessions were conducted once per week in place of training sessions, provided that during training sessions the animals maintained 80% or better condition-appropriate responding prior to the delivery of any reinforcers under each stimulus condition.

Stimulus generalization tests were conducted with three doses of each training drug (MDMA 0.375–1.5 mg/kg; LSD 0.02–0.08 mg/kg), D-amphetamine (0.50–2.0 mg/kg), fenfluramine (0.50–2.0 mg/kg), and the combination of fenfluramine (0.25–0.50 mg/kg) and D-amphetamine (0.25–1.0 mg/kg). Antagonist tests were conducted with the 5-HT_{2A} antagonist MDL-100,907 (0.0325–0.50 mg/kg) in combination with the training dose of MDMA (1.5 mg/kg) and in combination with the training dose of LSD (0.08 mg/kg), and with the dopamine antagonist, haloperidol (0.1–0.4 mg/kg) in combination with the training dose of MDMA. The number of subjects tested is indicated in the figure legends.

2.5. Data analysis

Initially, all subjects received the same stimulus during training sessions until the criterion for discrimination was met. However, following the initiation of dose–response tests with the training drugs (i.e., MDMA and LSD), it appeared that stimulus control was not reliable in all 12 animals. Despite methods used to reduce olfactory cues, it is possible that some subjects were using residual olfactory cues, or some other cue, during training sessions. Therefore, rather than continuing to administer the same training stimulus to all subjects, the three stimulus conditions were varied across subjects starting at training session number 137. In this way, any residual olfactory cues were not reliable prompts for identification of the lever correlated with the presentation of reinforcement during any given training session. Following this change, all subjects were required to again meet the discrimination criterion. The mean number of sessions to criterion was calculated both before and after this procedural change.

A dose–response curve was generated for each compound tested in order to depict the percentage of total responses on each lever for each dose tested, as well as the overall response rate at each dose. A group mean was calculated for each measure at each dose. Only the data from subjects that emitted at least 10 responses during test sessions were included to calculate the percentage of responses on each lever. The data from all subjects were used to calculate response rates. For compounds where one or more subjects did not complete the test session, the number of subjects that completed the session and the number included in calculating response rate are indicated in the graph.

A one-way analysis of variance (ANOVA) was used to analyze response rate for each compound tested. Complete stimulus generalization was defined as at least 80% responding on either the (±)-MDMA- or (+)-LSD-appropriate lever. Complete stimulus blockade was defined as at least 80% responding on the saline-appropriate lever. For compounds that produced stimulus generalization or stimulus blockade, nonlinear regression analyses were calculated to determine ED_{50} values.

3. Results

All 12 subjects initially acquired the discrimination of LSD and MDMA. The mean number of sessions to criterion was 55 (±5). However, when initial dose–response tests with the training compounds were conducted, stimulus control was not reliably maintained with the training dose in all subjects. Therefore, the training stimulus conditions during the daily training sessions were varied across sub-

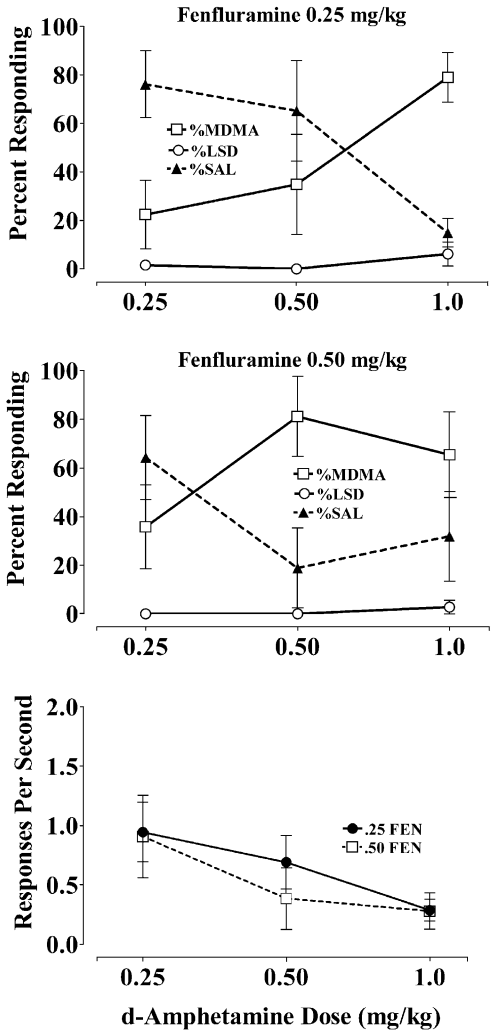

Fig. 3. Results of stimulus generalization tests following combinations of D-amphetamine (0.25, 0.50, 1.0 mg/kg) and fenfluramine (0.25, 0.50 mg/kg) ($n=6$). The mean percentage of responses on each lever are presented in the top graph and the overall response rate is presented in the bottom graph.

jects as described above, and all subjects were required to again meet the discrimination criterion prior to conducting dose response tests. Following this manipulation, the mean number of sessions to meet the discrimination criterion a second time was 153 (±3).

Fig. 1A illustrates the results of stimulus generalization tests with MDMA (0.375–1.5 mg/kg). There were dose-dependent increases in MDMA-appropriate responding with virtually no LSD-appropriate responding across doses. The ED_{50} for MDMA was 0.97 mg/kg (95% confidence intervals: 0.2488–1.694). There were no differences in response rates across doses [$F(3,43)=1.09$, $P>.05$].

The dose–response data for LSD (0.02–0.08 mg/kg) are presented in Fig. 1B. The ED_{50} for LSD was 0.038 mg/kg (95% confidence intervals: 0.006–0.223). There were dose-dependent increases in LSD-appropriate responding with no MDMA-appropriate responding at either 0.04 mg/kg or the training dose, 0.08 mg/kg. Response rates did not differ across doses [$F(3,43)=0.19$, $P>.05$].

Fig. 2 shows the results of stimulus generalization tests with D-amphetamine (0.25–2.0 mg/kg) and fenfluramine (0.25–2.0 mg/kg). D-Amphetamine (Fig. 2A) produced dose-dependent increases in MDMA-appropriate responding and dose-dependent decreases in saline-appropriate responding. However, this compound did not produce complete stimulus generalization at any of the doses tested. Significant MDMA-appropriate responding occurred following both 1.0 and 2.0 mg/kg D-amphetamine [$F(4,29)=3.028$, $P<.05$], with 60% MDMA-appropriate responding following 2.0 mg/kg. There were significant dose-dependent decreases in response rate, with severe rate suppression following 2.0 mg/kg [$F(1,24)=6.55$, $P<.05$], which precluded the testing of higher doses of D-amphetamine.

Fenfluramine produced dose-dependent increases in MDMA-appropriate responding, with complete substitution at the highest dose tested, 2.0 mg/kg (Fig. 2B). The ED_{50} for fenfluramine was 1.42 mg/kg (95% confidence intervals: 0.927–1.91). There was no significant LSD-appropriate responding at any of the doses tested, although 1.0 mg/kg fenfluramine produced 20% LSD-appropriate responding. There were no significant differences across doses with respect to response rate [$F(5,33)=1.01$, $P>.05$].

To investigate the possibility that these compounds may have synergistic effects, combinations of D-amphetamine (0.25, 0.50, 1.0 mg/kg) and fenfluramine (0.25, 0.50 mg/kg) were also examined for stimulus generalization (Fig. 3). The combination that resulted in the highest percentage of MDMA-appropriate responding, with nearly complete substitution (79%), was 1.0 mg/kg D-amphetamine and 0.25 mg/kg fenfluramine. When 1.0 mg/kg of D-amphetamine was administered with 0.50 mg/kg fenfluramine, the percentage of MDMA-appropriate responding dropped to 65%. There was virtually no LSD-appropriate responding at any combination of doses tested. There were dose-dependent decreases in response rate, with the greatest suppression following 1.0 mg/kg D-amphetamine in combination with 0.25 and 0.50 mg/kg fenfluramine. Although the response

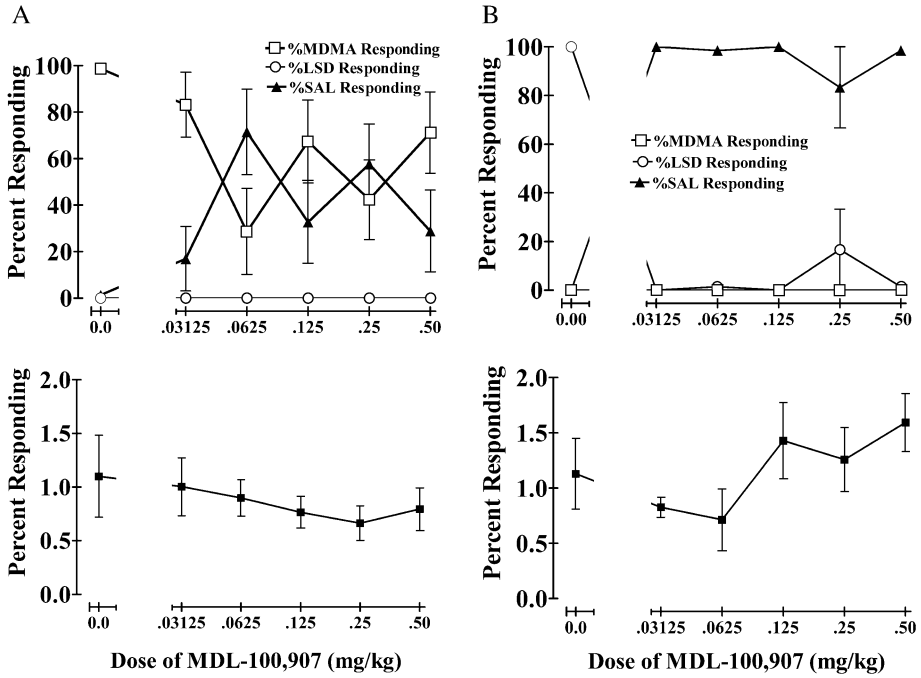

Fig. 4. Results of stimulus antagonism tests with (A) MDL-100,907 (0.03125–0.50 mg/kg) and MDMA (1.5 mg/kg) ($n=7$) and (B) MDL-100,907 (0.04125–0.50 mg/kg) and LSD (0.08 mg/kg) ($n=7$). The mean percentage of responses on each lever are presented in the top graphs and the overall response rate is presented in the bottom graphs.

rate was significantly suppressed [$F(5,17) = 3.86$, $P > .05$], all subjects completed the test sessions at all the dose combinations tested.

The administration of MDL-100,907 (0.03125–0.50 mg/kg) prior to the training dose of MDMA (1.5 mg/kg) did not produce dose-dependent decreases in MDMA-appropriate responding (Fig. 4A). In fact, this 5-HT_{2A} antagonist produced nearly complete blockade of MDMA (21% MDMA-appropriate responding) following 0.0625 mg/kg, but higher doses failed to antagonize MDMA discrimination. Although the rate of responding decreased in a dose-dependent fashion, the difference across doses was not significant [$F(5,41) = 0.47$, $P > .05$]. In contrast, administration of MDL-100,907 (0.03125–0.50 mg/kg) produced complete blockade of the LSD (0.08 mg/kg) stimulus at all of the doses tested (Fig. 4B). LSD-appropriate responding occurred in only one subject at one dose (0.25 mg/kg). The differences in response rate across doses were not significant [$F(5,30) = 1.08$, $P > .05$].

Haloperidol (0.1–0.4 mg/kg) was also tested in combination with MDMA (1.5 mg/kg) to examine the importance of dopaminergic mediation of MDMA's discriminative stimulus effects. As noted in Fig. 5, haloperidol did not block the discriminative stimulus effects of MDMA at any of the doses tested, but produced a significant decrease in response rate [$F(3,11) = 4.85$, $P < .05$].

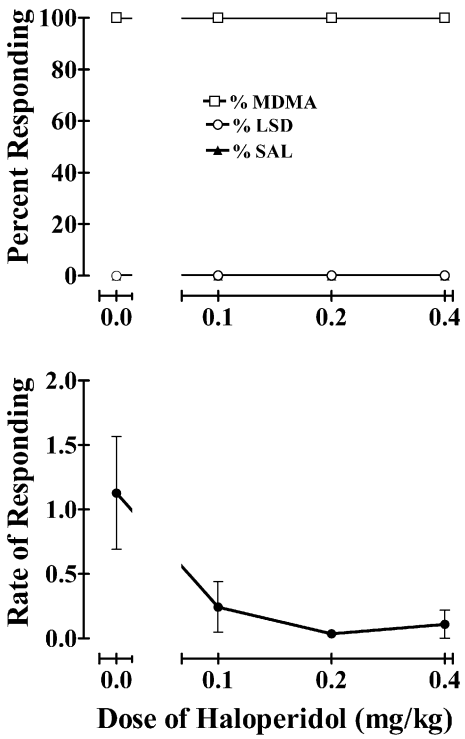

Fig. 5. Results of stimulus antagonism tests with haloperidol (0.1–0.4 mg/kg) and MDMA (1.5 mg/kg) ($n = 3$). The mean percentage of responses on each lever are presented in the top graph and the overall response rate is presented in the bottom graph.

4. Discussion

The present results support the notion that, despite its classification as both a stimulant and a hallucinogen, MDMA produces complex discriminative stimulus effects that are distinctly different from those of either psychostimulants or hallucinogens. Indeed, it has been proposed that MDMA and similar amphetamine analogs belong to a separate drug class called "entactogens" (Nichols, 1986). Previous studies have concluded that the discriminative stimulus properties of MDMA are mediated through both serotonergic and dopaminergic actions (Glennon et al., 1992; Malberg and Bonson, 2001; Schechter, 1989), though the relationship between these actions and the resulting discriminative stimulus effects is not well understood. The relative importance of dopaminergic versus serotonergic actions in maintaining stimulus control by MDMA appears to depend on the drug discrimination methods employed. Moreover, conflicting results from previous drug discrimination studies with MDMA are likely due to methodological differences among laboratories.

It is well established that the stimulus properties of D-amphetamine are primarily mediated via changes in dopamine (Goudie, 1991; Ho and Huang, 1975; Nielsen and Jepsen, 1985; Woolverton, 1984; Yokel and Wise, 1976). It is also well documented that the stimulus effects of LSD are primarily mediated through actions on serotonin (Cameron and Appel, 1973; Glennon et al., 1982; Sadzot et al., 1989). Goodwin and Baker (2000) recently demonstrated that rats could be trained to dissociate the effects of D-amphetamine from those of MDMA in a three-choice drug discrimination procedure. One may conclude that, in that procedure, serotonergic actions were a more salient feature of MDMA's discriminative stimulus effects compared to the dopaminergic actions. This is further supported by the observation that the administration of other serotonin agonists (i.e., LSD and fenfluramine) resulted in dose-dependent increases in MDMA-appropriate responding, while cocaine, a dopamine agonist, produced full substitution for D-amphetamine. Following this logic, the present study investigated the possibility that the dopaminergic effects of MDMA would become a more salient feature of the MDMA cue in rats trained to discriminate between MDMA and a 5-HT agonist, LSD. This hypothesis was not supported by the three main results of this study. First, D-amphetamine failed to produce complete substitution for MDMA. Second, haloperidol did not block the discrimination of MDMA. Finally, the 5-HT releaser fenfluramine did produce full substitution for MDMA. Thus, it appears that 5-HT release is a critical component to MDMA's discriminative stimulus effects, even in rats trained to discriminate MDMA from a 5-HT agonist.

Stimulus generalization between fenfluramine and MDMA is a fairly consistent finding (Schechter, 1986, 1989; Baker et al., 1995; Goodwin and Baker, 2000; present study) indicating that 5-HT release is a major

component of MDMA's compound discriminative stimulus effects. Nevertheless, because D-amphetamine produced partial substitution for MDMA (≈60%), it was hypothesized that low doses of fenfluramine might potentiate the effects of D-amphetamine. This hypothesis is supported by the present data. When administered alone, 0.25 mg/kg fenfluramine produced saline-appropriate responding (see Fig. 2B) and 1.0 mg/kg D-amphetamine produced a mean of 34.5% MDMA-appropriate responding. When these doses were administered in combination, MDMA-appropriate responding increased to 79% (see Fig. 3). The combination of 0.50 mg/kg D-amphetamine and 0.50 mg/kg fenfluramine also produced nearly complete substitution for MDMA, while neither of these doses alone produced significant MDMA-lever responding. Although none of the D-amphetamine–fenfluramine dose combinations yielded full stimulus generalization to MDMA, the present results clearly indicate synergistic actions between the two compounds. These results support previous conclusions that MDMA's discriminative stimulus effects are mediated by a combination of dopaminergic and serotonergic actions (Schechter, 1989; Glennon et al., 1992; Malberg and Bonson, 2001).

The interpretation of the present results is further complicated because both the positive and negative isomers of MDMA are behaviorally active (Baker et al., 1995, 1997). Because the present study employed the MDMA racemate, interpretation of the results is limited to this form of MDMA. Previous investigations have indicated some differences in the discriminative stimulus effects of (+)-MDMA and (−)-MDMA. For example, greater stimulus generalization to LSD was observed in rats trained to discriminate (−)-MDMA than in rats trained to discriminate (+)-MDMA (Baker et al., 1995). Thus, it is possible that a discrimination between LSD and (+)-MDMA would be more readily established than between LSD and (−)-MDMA or between LSD and (±)-MDMA.

The discrimination of drugs with multiple components have been classified as either 'redundant' stimuli, requiring only one component for stimulus generalization, or as 'conditional' stimuli, requiring the presence of all composite stimuli for generalization to occur (Grant, 1999). In a review of drug discrimination research on ethanol, Grant (1999) proposed that it is possible to transfer the basis of the ethanol cue from a redundant cue to a conditional cue with specific training procedures. For example, in two-choice discriminations between ethanol and water, ethanol appears to produce a redundant stimulus complex, but in a three-choice (e.g., ethanol vs. water vs. pentobarbital) discrimination, the ethanol discrimination shifts to a conditional basis. MDMA, on the other hand, appears to produce a conditional stimulus complex in both two-choice (MDMA vs. saline) and three-choice (MDMA vs. D-amphetamine vs. saline; MDMA vs. LSD vs. saline) discriminations.

MDL-100,907, a 5-HT_{2A} antagonist, has been reported to block both MDMA stimulated dopamine release and long-term 5-HT deficits produced by MDMA (Schmidt et al., 1992). However, in the present study, MDL-100,907 had differential effects when administered in combination with MDMA. No clear linear relationship between an increase or a decrease in MDL-100,907 dose can be attributed to an increase or a decrease in MDMA-appropriate responding. Indeed, as is evident in Fig. 4A, the range of responding at all of the MDL-100,907 doses was highly variable across subjects. These findings suggest that the discriminative stimulus properties of MDMA are highly complex and likely involve multiple 5-HT receptor subtypes. In contrast, MDL-100,907 completely blocked the LSD cue at all of the doses tested, which supports previous reports that the stimulus properties of LSD are mediated primarily through its actions on 5-HT_2 receptors (Glennon et al., 1982; Sadzot et al., 1989).

In summary, three major conclusions may be drawn from present study. First, the discriminative stimulus effects of MDMA are clearly distinguished from those of LSD in this procedure. This conclusion is supported by the fact that animals learned to discriminate MDMA and LSD and by the fact that LSD discrimination is completely blocked by MDL-100,907, while MDMA discrimination is not. Second, whether animals are trained to discriminate MDMA from D-amphetamine (Goodwin and Baker, 2000) or from LSD (present study), 5-HT release remains a salient feature of MDMA discrimination. Third, although DA release appears to be a less salient feature of the MDMA cue compared to 5-HT release, the combination of DA and 5-HT release may have synergistic actions. Moreover, while neither DA receptor antagonism nor 5-HT_{2A} receptor antagonism appears to completely block the MDMA cue, combinations of 5-HT and DA antagonists are likely to produce greater blockade MDMA discrimination. Unfortunately, the advanced age of the subjects precluded additional antagonism testing in the present study. Thus, although three-lever drug discrimination procedures provide a useful tool to examine drugs with compound stimulus properties, the time required to train such a discrimination limits the number of substances that can be assessed for stimulus generalization and antagonism. Future studies on the combination of 5-HT and DA antagonists on MDMA discrimination are clearly warranted.

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