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MDMA as a Discriminative Stimulus: Isomeric Comparisons

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SCHECHTER, M. D. *MDMA as a discriminative stimulus: Isomeric comparisons.* PHARMACOL BIOCHEM BEHAV 27(1) 41–44, 1987.—Using a two-lever, food-motivated discrimination procedure, eight male rats were trained to discriminate 1.5 mg/kg of racemic 3,4-methylenedioxymethamphetamine (MDMA) from its vehicle, distilled water. Once trained, the rats demonstrated a dose-related decrease in discriminative performance after administration of lower doses of MDMA (ED50=0.27 mg/kg). Racemic MDMA-stimulus generalization occurred with both isomers of MDMA with the ED50 of the (+) isomer calculated as 0.50 mg/kg and for the (−) isomer being 1.07. Time-course data indicate that racemic MDMA has a peak effect from 20–60 min post-injection with a declining effect from 120–240 min. This time-course closely resembles that observed by subjective reports in human abusers and, together with previous data, would indicate that the discriminative paradigm would be useful in investigations as to the neurochemical effects of MDMA.

Drug discrimination Isomers Stimulus properties of drugs MDMA Time-course Rats

THE drug 3,4-methylenedioxymethamphetamine (MDMA, "Ecstasy", XTC) was placed in Schedule I of the Controlled Substances Act by the Drug Enforcement Administration in July of 1985. This would mean that the drug has both a high abuse potential and no accepted medical use; claims recently refuted [8]. This controversy will only be resolved with continued scientific investigations concerning the drug's behavioral and neurochemical effects in laboratory animals and, ultimately, its psychopharmacological and/or toxicological action in man. Indeed, only limited data are available on the effects of MDMA in humans. One report indicates, however, that MDMA possesses similar potency and produces behavioral effects like that of the hallucinogen MDA [13] and a second report, from the same investigators, describes a study in which the (+) isomer was found to be more effective than the (−) isomer when evaluated in normal human subjects [1].

This laboratory was the site of experiments in which rats, trained to discriminate the serotonergically-mediated drugs fenfluramine and tetrahydro-beta-carboline or the dopaminergically mediated drugs apomorphine and cathinone, were given MDMA and tested to investigate the ability of MDMA to mimic the discriminative effect of the particular drug used in training [11]. However, as of this writing, no detailed report has appeared on the use of MDMA as the drug that can control discriminative responding in the rat (but see the Abstract; [3]). Thus, the purpose of the present experimentation was to train rats to discriminate between the stimulus properties of (±)-MDMA and its vehicle in a two-lever food-motivated operant task. In addition, the time-course of this effect, as well as the generalization to the (+) and (−) isomers of MDMA, were tested.

METHOD

Subjects

The subjects were 8 male ARS/Sprague-Dawley rats weighing 252–327 g at the beginning of experimentation. They were individually housed in galvanized cages with free access to tap water except during experimental sessions. Their weights were adjusted, by daily rationing of commercial rat chow, to approximately 80 to 85% of their expected free-feeding weights as determined by daily weighing of 2 control free-feeding rats purchased from the supplier (Zivic-Miller, Allison Park, PA) at the same time as the experimental subjects. Room lights were on from 0600 to 1800 in a room with a constant temperature of 20–22°c.

Apparatus

The experimental space consisted of 8 identical standard rodent operant test cages (Lafayette Instruments Corp., Lafayette, IN) each equipped with 2 levers located 7 cm apart and 7 cm above the gridded floor. A food pellet receptacle was mounted 2 cm above the floor at an equal distance between the levers and food delivered into this cup consisted of a single 45 mg food pellet. The test cage was housed in a sound-attenuating cubicle equipped with an exhaust fan and a 9 W house-light. Solid-state programming equipment (Med Associates, E. Fairfield, VT) was used to control and record the sessions and was located in an adjacent room.

Discrimination Training

Training was based upon procedures described elsewhere

[12]. There were two training phases. In the first phase, the food-deprived rats were trained to lever press on both levers for food reinforcement on a fixed ratio 10 (FR10) schedule. The saline-appropriate lever was activated first for all subjects. The rats were trained, by successive approximations, to press this lever on an FR1 schedule. The fixed ratio requirement was progressively increased, in daily 15 min sessions over 10 days, until an FR10 schedule was achieved. Throughout lever press training, rats received daily intraperitoneal (IP) injections of distilled water 20 min prior to being placed into the two-lever operant box. Immediately following attainment of the FR10 schedule after water administration, the opposite lever was activated and rats were trained on an FR1 schedule 20 min after the IP administration of an equal volume of water (1 ml/kg body weight) containing 1.5 mg/ml (±)-MDMA HCl (calculated as salt). Daily sessions of 15 min were continued over 6 days with MDMA administration until an FR10 schedule was attained. In order to minimize effects due to any possible position preference, the 8 rats were divided into 2 groups. For one group, responding on the left lever was reinforced by delivery of food pellets in every session following drug injection, whereas the other group was reinforced for responding on the right lever following drug injections. Responses on the opposite lever were reinforced with food pellets after water injections and the running order was randomized amongst the 8 chambers.

Phase II discrimination training then began. Subjects were trained 5 days per week with alternation of reinforcement in a pseudo-random sequence. Thus, in each 2 week period, there were 5 days with drug lever (D) correct and 5 days with water lever (W) correct. The pattern was D, W, W, D, D; W, D, D, W, W. Due to the varied sensitivity of individual rats to drug training in the past [10], it was decided to modify previously employed criteria for training to insure that an animal was, in fact, trained to the MDMA-induced discriminative stimulus. This modification in protocol required that an animal select the correct lever, according to the drug condition imposed on a given day, on 8 of 10 consecutive daily sessions twice before it was allowed to be used for data collection. The sessions-to-criterion (STC 1) measurement [7] indicates the first session of 10 consecutive daily sessions in which 8 correct first choice lever selections were made initially. The STC 2 measurement relates to the second set of 8 of 10 correct consecutive lever selections.

Dose-Response Relationships to Other Doses of (±)-MDMA

Once these animals attained the training criterion, they were tested for their sensitivity to various doses of (±)-MDMA. Training sessions of 15 min duration with alternating administrations of 1.5 mg/kg (±)-MDMA and saline were continued on Mondays, Wednesdays, and Fridays. This procedure endeavored to ensure and maintain behavioral discrimination of the trained drug conditions and it was lever selection during these maintenance trials that was employed to generate those table values at 1.5 mg/kg (±)-MDMA and water. On Tuesdays and Thursdays, the rats were injected IP with doses of (±)-MDMA differing from that used for initial training, i.e., 0.125, 0.25, 0.5, and 1.0 mg/kg and, 20 min later, they were placed into the experimental chamber and were allowed to lever press, in extinction, until 10 responses were made on either lever. To preclude training at a (±)-MDMA dose different than the 1.5 mg/kg dose employed to train the animals, the rats were immediately removed from the experimental chamber upon making 10 responses on either lever.

TABLE 1

LEARNING RECORD OF 8 RATS DISCRIMINATING 1.5 mg/kg (±)-MDMA FROM WATER

	Percent Responses on MDMA-Correct Lever After:			
	Water		MDMA	
Weeks	Quantal	Quantitative (S.D.)	Quantal	Quantitative (S.D.)
1 and 2	60.0	63.3 (25.8)	77.5	72.2 (12.7)
3 and 4	35.0	41.9 (19.8)	72.5	71.9 (24.5)
5 and 6	12.5	23.4 (4.7)	80.0	70.6 (11.3)
7 and 8	12.5	21.1 (8.6)	92.5	84.7 (4.3)

Sessions-To-Criterion (STC): Mean number of sessions to the first of ten consecutive sessions in which an accuracy rate of 80% was maintained. 1st: 7.8 ± 5.2; 2nd: 20.4 ± 4.4.

Each of the (±)-MDMA doses was tested in each animal on two occasions with each test preceded both by a 1.5 mg/kg (±)-MDMA and a water maintenance session. The lever first pressed 10 times was designated as the "selected" lever (below).

Generalization to (+)-MDMA and (−)-MDMA

After establishing the dose-response relationship to doses of racemic MDMA, test days (Tuesdays and Thursdays) were used to test the ability of various doses of each of the isomers of MDMA to mimic the discrimination stimulus produced by the racemic MDMA. Doses of the (+) isomer ranged from 0.25–1.5 mg/kg and for the (−) isomer from 1.0–1.5 mg/kg, inclusive. Each dose was administered on two occasions, to each of the eight rats, 20 min prior to testing. The rat was removed immediately upon making 10 responses on either of the two levers.

Time-Course of MDMA Action

The time-course of the discriminative stimulus of racemic MDMA was investigated by administering the 1.5 mg/kg training dose of MDMA of test days and returning the rats to the home cage for 5, 10, 60, 90, 120, 180, 240 min before placing them into the test chamber. After 10 responses on either lever, the rats were immediately removed without receiving reinforcement. Each post-injection time was tested in each of the eight rats on two occasions each preceded by a maintenance session with 1.5 mg/kg MDMA or water; each tested at 20 min post-injection.

Measurements

The lever pressed 10 times first was designated as the "selected" lever. The percentage of rats selecting the lever appropriate for the training drug was the quantal measurement of discrimination and quantal data are presented as percent correct first choice responses on the MDMA-correct lever. In addition, the number of responses on the MDMA-correct lever divided by total responses on both levers made prior to 10 responses (including the ten on the MDMA-correct lever), times 100, constitutes the quantitative measurement. This measurement allows for analysis of counts upon the "unselected" lever and the advantages in using both measurements have been discussed by Stolerman and

TABLE 2

DISCRIMINATIVE RESPONDING IN (±)-MDMA-TRAINED RATS AFTER VARIOUS DOSES OF (±), (+) AND (−)-MDMA

	(±)-MDMA		(+)-MDMA		(+)-MDMA	
Dose (mg/kg)	Quantal	Quantitative (S.D.)	Quantal	Quantitative	Quantal	Quantitative
1.5	97.5	88.4 (5.1)	100.0	98.8 (1.7)	87.5	72.6 (12.7)
1.25	ND		ND		75.0	65.4 (7.7)
1.0	87.5	77.0 (6.4)	81.3	74.1 (4.7)	37.5	45.7 (1.8)
0.5	62.5	56.0 (13.3)	56.3	54.0 (1.9)	ND	
0.25	50.0	48.4 (7.8)	12.5	17.1 (8.4)	ND	
0.125	25.0	30.0 (2.2)	ND		ND	
ED50 (95% conf. limits)	0.27 (0.16–0.47)	0.30 (0.14–0.63)	0.50 (0.27–0.92)	0.48 (0.26–0.86)	1.07 (0.89–1.29)	1.04 (0.76–1.43)

ND: Not determined.

D'Mello [14]. The quantal data for the dose-response experiments were analyzed by the method of Litchfield and Wilcoxon [5] which employs probit vs. log-dose effects and generates ED50s and tests for parallelism.

RESULTS

Discriminative Learning

The learning record of the eight rats is extensively provided in Table 1. The biweekly schedule of testing, i.e., D, W, W, D, D; W, D, D, W, W, (see the Method section) allows for 5 water (W) and 5 MDMA (D: drug) administration tests in each two-week period. The percent responses on the drug-correct lever for each two-week period after administration of water is observed to decrease, over time, both in terms of quantal and quantitative measurements, whereas, the responses upon the drug-correct lever after MDMA generally increases over the 40 days of training. The number of sessions before the beginning of criterion performance (sessions-to-criterion; STC; [7]) was computed for each rat for both the first set of 8 of 10 consecutive sessions and for the second set of 8 of 10 consecutive sessions. The mean of the first of 10 consecutive sessions in which the subjects first selected the correct lever, according to whether they received water or MDMA, was 7.8 sessions; thus, the rats attained the 8 out of 10 correct consecutive session criteria by a mean of 18 sessions. The mean of the first session that constituted the second sessions-to-criterion, of 8 out of 10 correct, was attained after a mean 20.4 sessions; all rats attained the second criterion, and were thus judged able to discriminate MDMA from water, by the 36th session of training.

Dose-Response to (±)-MDMA and Transfer to (+)- and (−)-MDMA

Once trained the rats maintained discriminative performance to 1.5 mg/kg (±)-MDMA (97.5% quantal; Table 2) and water (5.6%; not shown). Decreasing doses of (±)-MDMA, i.e., 1.0, 0.5, 0.25, and 0.125 mg/kg, produced decreased discriminative performance both in terms of quantal and quantitative measurements. Analysis [5] of the dose-response relationship yielded an ED50 (with 95% confidence limits) of 0.27 (0.16–0.47) mg/kg for the quantal data and a similar ED50 (0.30 mg/kg) for the quantitative data.

TABLE 3

TIME-COURSE OF MDMA ACTION

Post-Injection Time (min)	Quantal	Quantitative (S.D.)
5	26.7	36.6 (6.4)
10	87.7	81.5 (1.3)
20	100.0	94.9 (2.6)
60	100.0	98.2 (0.9)
90	93.8	80.2 (5.7)
120	37.5	44.3 (24.4)
180	25.0	35.1 (27.2)
240	18.8	27.5 (10.6)

Administration of various doses (0.25–1.5 mg/kg) of the (+) isomer of MDMA, likewise, produced a similar dose-response relationship in which no dose of the isomer produced a significantly different quantitative measurement when compared to the (±)-MDMA effect at the same dose. The ED50 for the quantal and quantitative measurements were 0.50 and 0.48 mg/kg, respectively. Although the ED50 of (+) MDMA was higher than that of the racemate, there was extensive overlap of the 95% confidence intervals of each ED50.

In contrast, administration of the (−) isomer of MDMA produced a significantly less discriminable cue when doses of 1.0 and 1.5 mg/kg are compared to the same doses of its enantiomer. The calculated ED50 of (−)-MDMA was 1.07 and 1.04 mg/kg for the quantal and quantitative measurements, respectively, and there was no overlap in the 95% confidence intervals between the (±)- and (−)-MDMA ED50s. Test for parallelism [5] between the racemate and the

(+) isomer (calculated $t=0.92$) and between the racemate and the (−) isomer (calculated $t=1.64$) indicate that they are parallel within $p<0.05$ limits (critical $t=2.78$)

Time-Course of (±)-MDMA Action

Maintenance trials, with 1.5 mg/kg (±)-MDMA tested at 20 min post-injection interspersed throughout this study, indicated errorless discrimination. Thus, the continued training/testing of the rats from initial training (Table 1: 92.5%) to the dose-response experiments (Table 2: 97.5%) to the dose-response experiments (Table 3: 100%) would suggest improving discriminative performance over time and/or continued maintenance sessions. In any case, 1.5 mg/kg (±)-MDMA also produced 100% correct (quantal) responses at 60 min post-injection and, thus, the discriminative ability of the rats progressively increased from 5 to 20 min and declined from 90 to 240 min post-injection. At this last time, the quantitative measurement was not significantly different from that of water.

DISCUSSION

MDMA ("Ecstasy"), the N-methyl analogue of (±)-3,4-MDA, can be employed as a drug to control differential responding in the rat. Previous work from this [11] and one other laboratory [4] has indicated the ability of MDMA to substitute for the trained drug in rats discriminating either fenfluramine, l-cathinone or MDA. However, this is the first detailed report indicating that MDMA is discriminable when paired with water. This discriminative stimulus was seen to be dose-responsive in the range of 0.125 to 1.5 mg/kg and analysis of this relationship indicated an ED50=0.27 mg/kg.

Substitution tests with both the (+) and (−) isomers of MDMA resulted in a transfer from the (±)-MDMA-induced stimulus cue and both isomers, likewise, produced decreased discriminative performance with decreasing doses. Similar to previous work with amphetamine [9,15], the discriminative stimulus effects of MDMA are stereoselective with the (+) isomer being more potent than the (−) isomer. This confirms previous *in vitro* findings [6]. In addition, the racemate was observed to be more potent than either of the two isomers. This was, indeed, suggested by work with MDMA in human subjects as: "the racemate is more effective as a CNS agent than would be expected or calculated from the separate activities of the component optical isomers" [1].

Present results indicate that the discriminative stimulus effects of (±)-MDMA increased from 5 to 10 min, are maximal at 20–60 min post-injection and progressively decline from 90 to 240; at this latter time the drug is water-like. This time-course is similar to that reported in humans: "Recreational users say that the Ecstasy experience can be roughly divided into three phases. The first half hour is often referred to as the WP, or Weird Period . . . The WP is followed by the "rush," a wave of tingling sensation . . . For most users, the "high" lasts three to five hours . . ." [2]. After this time, "the intoxication symptoms are largely dissipated except for a mild residual sympathomimetic stimulation which can persist for several additional hours" [13].

This experimentation indicates that MDMA can successfully be used in the drug discrimination paradigm. It is hoped that this sensitive, stable, and specific behavioral task will be employed to elucidate the mechanism of MDMA action by conducting generalization and antagonism studies.

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