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Serotonergic-Dopaminergic Mediation of 3,4-Methylenedioxymethamphetamine (MDMA, "Ecstasy")

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SCHECHTER, M. D. *Serotonergic-dopaminergic mediation of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy")*. PHARMACOL BIOCHEM BEHAV 31(4) 817–824, 1988.—A series of three experiments were conducted to investigate the possible serotonergic and dopaminergic mediation of the discriminative stimulus properties of the "designer" drug MDMA. In Experiment 1, rats trained to discriminate 1.5 mg/kg (±)-MDMA from its vehicle at 20 min postadministration were shown to generalize to another drug of abuse, N-ethyl-3,4-methylenedioxyamphetamine (MDE) and to the serotonergically-active agents norfenfluramine and TFMPP. In contrast, testing of various dopaminergically-active agonists did not result in MDMA-like responding. In Experiment 2, dopaminergic and serotonergic antagonist were employed to observe their effect upon MDMA discrimination at 20 min postinjection. The serotonin antagonist pirenperone significantly decreased MDMA discrimination, whereas the dopamine decreasing drugs CGS 10746B and haloperidol had no effect. In Experiment 3, another group of rats were trained to discriminate MDMA at 105 min postadministration to investigate if, at this (later) time, the dopaminergic properties of MDMA may be more salient. Indeed, the dopaminergically-active drugs had a heightened effect upon MDMA at this later time, although the serotonergic component of the MDMA discriminative stimulus was predominant. The results suggest that the effects of MDMA at 20 min postadministration are solely serotonergic in nature. At 105 min postinjection there appears to be the presence of a weak dopaminergic component. This biphasic serotonergic-then-dopaminergic action of MDMA may explain the reported human experience with the drug, as well as the often controversial results in the literature.

MDMA MDE Drug discrimination Norfenfluramine TMFPP Serotonin Rats Dopamine

MDMA (3,4-methylenedioxymethamphetamine) is a psychoactive drug that was first synthesized in 1914 by chemists who mistakenly thought that, as a chemical similar to amphetamine, it could be marketed as an anorexiant (50). It has recently received much attention in the lay press [e.g., (10, 13, 36)] as a result of its July 1, 1985 assignment by the Drug Enforcement Administration (DEA) as a highly restricted Schedule I drug. The drug, popularly known as "ecstasy" (XTC) or "Adam," is chemically related to both amphetamine and mescaline and users maintain that it intensifies emotional feelings without sensory distortion, as well as increasing perception of self-insight, empathy, and esthetic awareness. Indeed, MDMA's apparent ability to relax inhibitions and enhance communication has been well-recognized by an estimated 35–200 physicians who were using it in their practice, as a psychotherapeutic adjunct, prior to the DEA ban (2). Nonmedical MDMA use has, in addition, been estimated to have reached 400,000 doses in 1985 (2) and a recent survey of a college population indicated that 39% of the students have used this drug (33). The N-ethyl derivative of MDMA (popularly known as "Eve" or MDE) has also been produced by clandestine laboratories (3) and appears to produce pharmacological effects similar to those of MDMA (6).

A recent and intense research effort has been mounted to determine the acute and chronic effects of MDMA upon the central nervous system. The results of neurochemical investigations have evidence the fact that MDMA not only increases the release of serotonin (21,44), but also stimulates dopamine release (21). It, thus, seems possible that the action of this drug may, like amphetamine, involve dopaminergic neuronal systems and, like LSD, may also involve serotonergic neurons. The purpose of this series of experiments was, therefore, to extend our knowledge as to possible serotonergic and dopaminergic mediation of the interoceptive cue produced by MDMA to allow rats to make differential responses in a discrimination task.

EXPERIMENT 1

This laboratory was the site for an investigation that indicated that 1.5 mg/kg (±)-MDMA can serve as a discriminative stimulus in animals trained in a two-lever, food-motivated operant procedure (39). Once trained, these animals demonstrated a dose-related decrease in discriminative performance after administration of lower doses of MDMA and the ED_{50} was shown to be 0.27 mg/kg. The purpose of Experiment 1 was to extend this study by investigat-

ing if the MDMA-produced discriminative performance would generalize (transfer) to other drugs thought to act upon either dopaminergic or serotonergic neurons in the central nervous system.

METHOD

Subjects and Discriminative Training

The animals used in this study were 8 male ARS/Sprague-Dawley rats that had been previously trained to discriminate 1.5 mg/kg (±)-MDMA from saline under a fixed ratio 10 (FR10) schedule of reinforcement for food (45 mg Noyes pellet) reward using standard two-lever operant chambers (Med Associates, E. Fairfield, VT). The discrimination training procedure for these animals has already been described (39).

Stimulus Generalization Studies

Once the rats met the training criterion, tests of stimulus generalization were conducted, i.e., the MDMA-trained rats were challenged with various doses of other agents in order to determine whether or not the rats would recognize the challenge agent as producing stimulus effects similar to those produced by MDMA. Maintenance of the MDMA/saline discrimination was ensured by continuation of training sessions throughout this phase of the study. Thus, training sessions were conducted with MDMA (1.5 mg/kg) and saline (1.0 ml/kg) every second day, i.e., each animal was administered MDMA and 20 min later was placed into the two-lever operant box and required to press the MDMA-appropriate lever in order to receive reinforcement. The lever pressed 10 times first was considered the "selected" lever and the animal was allowed to continue lever pressing for 10 min. On the next maintenance session, the animal was administered saline and 20 min later was required to press the (opposite) saline-appropriate lever to receive reinforcement and training was, likewise, continued for 10 min. Interspersed between these maintenance sessions were days used to test the effects of other drugs and, by employing this pattern, each novel test drug/dose was preceded by one maintenance MDMA and one maintenance saline session. It was the first 10 presses ("selected" lever) on these maintenance sessions which were used to judge if the animal was maintaining its discriminative performance to the training conditions. It was planned that if any rat fell below the 80% criterion, originally set (39), that rat would be dropped from subsequent data collection. This, however, did not occur. On days that novel drugs/doses were tested, the rats were immediately removed from the test chamber upon making 10 responses on either of the two levers. This precluded any continued training with a drug that was not used for initial training, i.e., a drug different than MDMA. Stimulus generalization (transfer) from MDMA to a test drug was said to occur when the rats, after being administered a given dose of a novel drug, made 80% or more of their first choice responses on the MDMA-correct lever. This seemed appropriate as the original criterion to judge MDMA-appropriate responding was, indeed, 80% correct responding.

Each test drug was administered in a random order, in at least three doses, with the initial dose chosen from the literature available on that agent. Doses higher than those used were precluded by the appearance of behavioral disruption, i.e., long onset to lever pressing, at the highest dose reported. Drugs chosen for use, and the rationale behind that choice, were: N-ethyl-3,4-methylenedioxyamphetamine (MDE), a drug of abuse chemically related to MDMA and currently unscheduled with the DEA (6); both direct-acting (apomorphine) and indirect-acting (l-cathinone, d-amphetamine) dopaminergic agents (56); and indirect- (norfenfluramine) and direct- (putatively specific receptor) serotonin agonists [TFMPP acting upon $5HT_{1B}$; 8-OHDPAT acting upon $5HT_{1A}$; DOI acting upon $5HT_2$ receptors; (16)].

Measurements and Statistics

The lever pressed 10 times first was designated as the "selected" lever. The percentage of rats selecting the lever appropriate for MDMA 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 the total responses on both levers made prior to 10 responses (including the 10 on the MDMA-correct lever) times 100, constitutes the quantitative measurement. This latter measurement was used to analyze data on both levers and to incorporate counts on the "unselected" lever in the statistical analysis. The advantages of using both measurements have been previously discussed (48). The quantal data for the dose-response experiments were analyzed by the method of Litchfield and Wilcoxon (23) which employs probit vs. log-dose effects and generates ED_{50}'s and tests for parallelism. When a drug was observed to produce 80% or greater quantal response on the MDMA lever, the drug was considered to generalize from MDMA and its dose-response curve was analyzed both for ED_{50} and for parallelism to a previously (39) generated MDMA dose-response curve.

RESULTS

The ability of rats trained to discriminate 1.5 mg/kg MDMA from saline to generalize this discrimination to novel drugs is represented by data in Table 1. Administration of 2.0 mg/kg MDE produced 93.8% quantal responding upon the MDMA-correct lever and decreasing doses of MDE were shown to produce decreased discriminative performance. Analysis (23) of the MDE dose-response curve yields an ED_{50} (with 95% confidence limits) of 1.29 (1.06–1.57) mg/kg for the quantal measurement and a similar 1.19 (0.86–1.65) mg/kg for the quantitative measurements. Analyses of the slopes of this MDE-produced dose-response curve and the dose-response curve previously produced by various doses of MDMA in these rats (39) indicate that these curves are parallel within statistical limitations and MDMA appears to be 4.7 times more potent in this behavioral paradigm then does MDE. In contrast, none of the doses of the dopaminergically-active drugs, viz., *l*-cathinone, *d*-amphetamine or apomorphine, produced an MDMA-appropriate (80% quantal) discrimination and, thus, none of these can be said to have generalized. However, it was observed that the 0.6 mg/kg dose of *l*-cathinone, as well as the 0.8 mg/kg dose of *d*-amphetamine, produced 62.5% quantal responding in these animals. No dose of apomorphine produced greater than 56.3% quantal responding on the MDMA lever.

The indirect-acting serotonergic agent norfenfluramine, at 1.4 mg/kg, produced generalization in the MDMA-trained rats and decreasing doses of norfenfluramine produced decreased discriminative performance. Analysis of the dose-response relationship indicated an ED_{50} of 0.78 (0.55–1.13) mg/kg and parallelism to the MDMA dose-response curve.

TABLE 1

EFFECT OF VARIOUS DOPAMINERGICALLY- AND SEROTONERGICALLY-MEDICATED DRUGS IN RATS (n=8) TRAINED TO DISCRIMINATE 1.5 mg/kg MDMA FROM SALINE AT 20 MIN POSTINJECTION

Treatment	Dose (mg/kg)	Quantal	(SD) Quantitative
MDE	2.0	93.8	83.0 (0.5)
	1.5	68.8	62.3 (5.7)
	1.0	18.8	39.7 (0.7)
l-cathinone	1.2	37.5	46.1 (19.8)
	0.6	62.5	57.9 (7.9)
	0.3	6.3	19.6 (12.0)
d-amphetamine	1.2	43.8	49.7 (5.9)
	1.0	25.0	42.0 (10.8)
	0.8	62.5	60.9 (19.0)
	0.4	37.5	42.2 (0.1)
Apomorphine	0.32	56.3	52.8 (16.2)
	0.16	43.8	43.1 (15.7)
	0.08	5.0	18.8 (12.6)
Norfenfluramine	1.4	87.5	67.2 (7.2)
	1.0	62.5	61.1 (5.8)
	0.7	43.8	42.8 (4.5)
TFMPP	0.8	81.3	76.9 (0.1)
	0.6	75.0	70.3 (0.4)
	0.3	31.3	40.2 (3.9)
8-OHDPAT	0.8	35.0	39.3 (1.1)
	0.6	55.0	50.8 (6.7)
	0.4	35.0	42.8 (2.9)
DOI	0.75	50.0	52.8 (6.7)
	0.50	43.8	51.9 (2.1)
	0.25	18.8	27.1 (13.9)

Likewise, 0.8 mg/kg TFMPP produced 81.3% quantal responding and decreasing doses produced decreased discrimination. In contrast, the $5HT_{1A}$ specific 8-OHDPAT and the $5HT_2$ specific DOI did not produce generalization.

EXPERIMENT 2

In order to replicate and extend the results obtained in Experiment 1, a new group of rats was trained to discriminate 1.5 mg/kg (±)-MDMA from its vehicle in the two-lever discrimination paradigm. After testing (for purposes of replication) of some of the agonists observed to produce generalization, this group of rats was to be used to study the effect of pretreatment with specific dopaminergic and serotonergic antagonists upon the MDMA-produced discriminative cue.

METHOD

Subjects and Discriminative Training

Eight male ARS/Sprague-Dawley rats purchased from the same supplier as those used in Experiment 1 (Zivic-Miller Labs., Allison Park, PA) were trained by the same methodology. One of these rats died of unrelated causes and the data, thus, reflect an n=7.

Dose-Response and Generalization Studies

Once the rats attained the training criterion, every second day was employed to test for generalization to lower MDMA doses (dose-response study) or to various doses of agonists (generalization study). Replication of the generalization experiments with MDE and norfenfluramine were conducted and the drug quipazine, at four doses, was employed to test for generalization. Alternate days were used to maintain and ensure discrimination to 1.5 mg/kg (±)-MDMA and its vehicle.

Stimulus Antagonism Studies

Subsequent to the agonist generalization studies, and with the MDMA/vehicle discrimination maintained throughout, stimulus antagonism tests were conducted to evaluate the effect of a specific serotonin or dopaminergic antagonists upon MDMA-appropriate responding. Thus, the serotonergic antagonist pirenperone (16), at doses of 0.08–0.32 kg, was administered 15 min prior to MDMA or saline and animals were tested 20 min after the second injection. Likewise, the dopaminergic antagonist haloperidol (56), at doses of 0.15–0.25 mg/kg, was administered 15 min prior to either the training dose of MDMA or saline and the rats tested 20 min later. In a similar manner, the recently synthesized benzothiadiazepine CGS 10746B was tested to evaluate its effect upon MDMA discrimination. This agent has been reported to decrease dopamine release without either changing dopamine metabolism or occupying dopaminergic receptors (1) and it has been observed to block the discriminative stimulus properties of *d*-amphetamine in a similar behavior paradigm (41).

RESULTS

The dose-response results of treating rats trained to discriminate 1.5 mg/kg (±)-MDMA from its vehicle with two lower doses of MDMA appear in the first part of Table 2. The ED_{50} generated by analysis (23) of the quantal data is 0.73 mg/kg and a similar 0.76 mg/kg for the quantitative measurement. As in Experiment 1, the highest dose of MDE and norfenfluramine tested produced generalization. Decreasing doses of each of these agonists produced decreasing MDMA-appropriate lever selections and the quantal ED_{50} for MDE was 1.59 mg/kg and for norfenfluramine the ED_{50} was 0.72 mg/kg. These ED_{50}'s are similar to those of 1.29 and 0.78 mg/kg for MDE and norfenfluramine, respectively, derived in a different group of rats used in Experiment 1. The potency ratio of MDMA:MDE in this experiment was 2.2. In contrast, quipazine at doses of 0.5 to 1.5 mg/kg did not generalize. However, the 1.0 mg/kg dose of quipazine did result in 71.3% of first choice responses (quantal) on the MDMA lever.

The results of the pretreatment experiments with antagonists appear in section A of Table 3. The dopamine release inhibitor CGS 10746B, at the highest dose (30 mg/kg), reduced MDMA discrimination to 71.4%, whereas no dose of haloperidol had an effect on discrimination. When these drugs were administered prior to saline, the rats maintained saline-appropriate responding (data not shown). Higher doses of antagonists were precluded because of the appearance of behavioral disruption. The serotonin-specific antagonist pirenperone produced a dose-responsive decrease in MDMA-appropriate discriminative performance with 0.32 mg/kg pirenperone pretreatment reducing MDMA quantal responding to 28.6%.

TABLE 2

DOSE-RESPONSE AND GENERALIZATION RESULTS IN RATS (n=7) TRAINED TO DISCRIMINATE 1.5 mg/kg (±)-MDMA AT 20 MIN POSTINJECTION

Treatment	Dose	Quantal	Quantitative (SD)
(±)-MDMA	1.5	100.0	92.1 (0.0)
	1.0	85.7	77.2 (10.1)
	0.5	14.3	16.5 (13.9)
	0.0 veh.	0.0	8.4 (4.4)
	ED_{50} mg/kg	0.73	0.76
	(95% conf. lim.)	(0.55–0.96)	(0.55–1.07)
MDE	2.0	85.7	74.4 (18.0)
	1.5	42.9	50.8 (19.9)
	1.0	0.0	17.9 (4.9)
	ED_{50}	1.59	1.50
	(95% conf. lim.)	(1.37–1.84)	(1.15–1.95)
Norfenfluramine	1.5	92.9	75.1 (0.8)
	1.0	78.6	75.4 (12.9)
	0.5	21.4	24.5 (6.3)
	ED_{50}	0.72	0.77
	(95% conf. lim.)	(0.51–1.02)	(0.48–1.21)
Quipazine	1.5	64.3	63.1 (6.8)
	1.0	71.3	62.5 (3.5)
	0.75	64.3	62.6 (8.6)
	0.5	7.1	15.5 (10.6)

EXPERIMENT 3

Recent work from this site (25,55) indicates that brain dopamine content is greatly increased in the rat caudate and nucleus accumbens between 60 and 120 min following intraperitoneal administration of MDMA. If rats were trained to discriminate the stimulus effects of MDMA at a time later than employed in Experiments 1 and 2, i.e., at 20 min postinjection, then perhaps the dopaminergic component of the MDMA effect might be enhanced.

METHOD

Subjects and Discriminative Training

Eight experimentally-naive Sprague-Dawley male rats were trained to discriminate (±)-MDMA from its vehicle in a manner exactly like that used in Experiments 1 and 2 except for two differences: 1) All phases of shaping and training with MDMA and its vehicle, as well as subsequent dose-response and generalization experiments, were conducted at 105 min after IP administration of (±)-MDMA; 2) Previous time-course studies (39) indicated that 1.5 mg/kg MDMA produced 93.8% MDMA-appropriate responding at 90 min postinjection, whereas this quantal measurement dropped to 37.5% at 120 min postinjection. Since that dose of MDMA would be poorly discriminable at 105 min (as later evidenced, see Table 4A), the training and maintenance dose of MDMA chosen for this group of rats was 2.5 mg/kg.

Dose-Response and Antagonism Experiments

Once the rats trained to discriminate 2.5 mg/kg (±)-MDMA from its vehicle at 105 min post-IP injection (referred to as the "105′ rats") attained criterion performance, alternate days were employed to determine dose-response relationships. After doses of 1.0–2.5 mg/kg MDMA were tested at 105 min postadministration, MDMA in doses of 0.125 to 1.5 mg/kg were tested, in the 105′ rats, at 20 min postinjection. In addition, the rats trained to 1.5 mg/kg MDMA at 20 min post-IP injection (referred to as the "20′ rats" and detailed in Experiment 2) were given another series of dose-response sessions with an expanded MDMA dosage range of 0.5–2.5 mg/kg. After these doses were tested at 20 min postinjection, doses of 0.75–2.5 kg MDMA were tested at 105 min postinjection. Thus, each of the two groups of rats were tested with various doses of MDMA at both 20 and 105 min postinjection.

The 105′ rats were subsequently pretreated with the same doses of antagonists as used in the 20′ rats (Experiment 2). To ensure consistency, the antagonists were administered at the same time prior to testing as used in Experiment 2. Thus, e.g., in the 20′ rats, haloperidol was injected 30 min prior to testing and 1.5 mg/kg MDMA administered 10 min later or 20 min prior to testing. In the case of the 105′ rats, 2.5 mg/kg was administered and 75 min later haloperidol was injected. Thus, the time between administration of the training dose of MDMA and testing was always 105 min and the effects of antagonists were tested at the same pretreatment (or co-treatment) times as in Experiment 2.

RESULTS

The dose-response effects of various doses of (±)-MDMA in the 105′ rats appears in Table 4B (right side). The training dose maintained MDMA discrimination in 92.9% of all trials, whereas the testing of the vehicle resulted in 8.9% quantal responses on the MDMA-lever (or 91.1% of responses on the saline-correct lever). Decreasing doses of MDMA generally produced decreased discrimination and the ED_{50} generated from this data was quantal ED_{50}=1.46 mg/kg and the quantitative ED_{50}=1.23 mg/kg. When the 105′ rats were tested with MDMA at 20 min postinjection (Table 4B, left side) the quantal ED_{50} decreased to 0.27 mg/kg.

The 20′ rats also displayed decreased discrimination with decreasing MDMA doses and a quantal ED_{50}=0.71 mg/kg (Part A of Table 4, left side). This ED_{50} is similar to that of 0.73 mg/kg generated by dose-response experiments in this group of rats in Experiment 2. When the 20′ rats were tested with various doses of MDMA at 105 min postinjection, their discriminative performance decreased at each dose resulting in an (increased) ED_{50}=1.48 mg/kg.

The results of cotreatment with antagonists in rats trained to discriminate 2.5 mg/kg (±)-MDMA at 105 min after its administration is presented in Part B of Table 3. The dopamine-release inhibitor CGS 10746B reduced MDMA discrimination to 62.5% at the lowest dose (20 mg/kg) used. However, increasing doses produced no greater decrease in MDMA discrimination. Likewise, all doses of haloperidol reduced MDMA discrimination at 105′ with the highest dose (0.25 mg/kg) resulting in 46.2% quantal responding. Higher doses of each could not be used because of the appearance of behavioral disruption. As with the 20′ rats, pirenperone co-treatment produced a dose-responsive decrease in the rats' ability to discriminate 2.5 mg/kg MDMA at 105′.

TABLE 3

EFFECT OF DOPAMINERGIC AND SEROTONERGIC ANTAGONISTS UPON (±)-MDMA DISCRIMINATION IN RATS TRAINED AT EITHER 20 MIN (n=7) OR 105 MIN (n=8) POSTADMINISTRATION

		A. Trained at 20' With 1.5 mg/kg		B. Trained at 105' With 2.5 mg/kg	
Pretreatment	Dose (mg/kg)	Quantal	Quantitative	Quantal	Quantitative
CGS 10746B	20	100.0	90.3 (0.9)	62.5	58.4 (11.8)
	25	85.4	66.1 (1.3)	68.5	59.6 (6.8)
	30	71.4	69.1 (13.4)	62.5	60.2 (8.1)
Haloperidol	0.15	85.7	68.0 (0.4)	56.3	58.7 (7.6)
	0.2	92.9	76.5 (6.8)	66.7	66.6 (10.5)
	0.25	85.7	79.4 (3.7)	46.2	62.7 (6.2)
Pirenperone	0.08	100.0	89.2 (2.4)	62.5	53.3 (3.9)
	0.16	50.0	45.2 (42.6)	56.3	58.4 (1.3)
	0.32	28.6	40.4 (24.2)	27.8	39.9 (2.7)

TABLE 4

DOSE-RESPONSE RELATIONSHIP OF RATS TRAINED AT 20 AND 105 MIN AFTER INJECTION OF MDMA

MDMA Dose (mg/kg)	Quantal		Quantitative	Quantal		Quantitative
A. Trained at 20 Min Postinjection (n=7)						
		Tested at 20 Min			Tested at 105 Min	
2.5	100.0		95.9 (3.7)	85.7		75.2 (13.2)
2.0		ND		85.7		69.4 (5.0)
1.5	93.7		88.6 (10.0)	64.3		57.1 (7.4)
1.0	85.7		78.5 (14.4)	14.3		19.4 (19.4)
0.75	35.7		42.9 (10.0)	0.0		7.3 (4.2)
0.5	14.3		22.4 (1.0)		ND	
0.0 (veh.)	7.1		10.4 (9.4)		ND	
ED_{50} (mg/kg)	0.71		0.75	1.48		1.57
B. Trained at 105 Min Postinjection (n=8)						
MDMA Dose (mg/kg)		Tested at 20 Min			Tested at 105 Min	
2.5		ND		92.9		89.7 (11.3)
2.0		ND		87.5		75.2 (8.1)
1.5	97.5		88.4 (5.1)	50.0		51.9 (1.1)
1.25		ND		18.8		27.2 (12.2)
1.0	87.5		77.0 (6.4)	25.0		31.2 (11.7)
0.5	62.5		56.0 (13.3)		ND	
0.25	50.0		48.4 (7.8)		ND	
0.125	25.0		30.0 (2.2)		ND	
0.0 (veh.) (8.6)		ND		8.9		16.6
ED_{50} (mg/kg)	0.27		0.30	1.46		1.23

ND: not determined.

GENERAL DISCUSSION

As a previous study (39) indicated, MDMA was capable of continued control of discriminative responding in the rat. Subsequently, the pharmacological nature of the MDMA-produced interoceptive cue was evaluated in substitution and antagonism tests. N-ethyl-3,4-methylenedioxy-amphetamine (MDE), the N-ethyl derivative of MDMA, generalized from MDMA in both Experiment 1 and 2. This effect was observed to be dose-responsive and the ED_{50} of MDE in both Experiment 1 and 2 would indicate that MDMA is more potent than MDE. This difference in potency has been reported to occur in both rats (6) and humans (10). Furthermore, the dose-response curve for MDE is parallel to that generated by MDMA; this suggests a commonality of site/mechanism of action (22). This evidence may be important in any future considerations as to the DEA scheduling of MDE, as previously discussed (6).

The psychostimulants *d*-amphetamine and *l*-cathinone were, likewise, tested for generalization from MDMA. The *d*-isomer of amphetamine has been shown to be discriminable by many laboratories [see review (56)] and its discriminative cue has been evidenced to be dopaminergically-mediated (41,43). Likewise, *l*-cathinone is discriminable and its stimulus properties are also mediated by dopaminergic mechanisms (37). In the present study, neither drug, when tested 20 min postadministration, was capable of producing an MDMA-appropriate response with the maximum effect of each being 62.5% quantal MDMA-like responding. In previous studies, MDMA had been shown to generalize in *l*-cathinone- (38) and *d*-amphetamine-trained (18) rats. This type of asymmetrical, or one-way, generalization has been shown to occur in other studies [e.g., (40)] and it has been suggested (54) that rats trained to a drug state attend to only the major component of the compound discriminative stimuli produced by the training drug. Rats trained to another, more selective component of this complex cue may generalize only partially to the minor component inherent in the multi-component pathway mediating the larger cue. In this case, the cathinone- and/or amphetamine-induced discriminative stimulus may be, in large part, dopaminergically-mediated, whereas the dopaminergic component of the MDMA discriminative properties would be weaker. Therefore, when MDMA-trained rats are tested with the more dopaminergically-mediated drugs only a partial interoceptive cue is available to allow for differential responding, i.e., discrimination. Furthermore, the direct-acting dopaminergic agent apomorphine did not generalize to MDMA at any dose tested. Previously, MDMA had been shown to be incapable of transfer when tested in apomorphine-trained rats (38).

Generalization testing with serotonergically-mediated drugs produced more positive results. Norefenfluramine, the first active metabolite of the anorexiant fenfluramine, produced generalization and, with administration of decreasing doses, this effect was shown to be dose-responsive. Recently, norfenfluramine was reported to be discriminable at 1.4 mg/kg (7) and its mechanism of action appears to be similar to that proposed for fenfluramine, i.e., it releases presynaptic serotonin (11). This released serotonin would, in turn, be available to any and all putative serotonin receptors.

Serotonergic (5HT) receptors in mammalian brain have been differentiated on the basis of their affinities for spiperone and tritiated 5HT (34). These heterogenous receptor "subtypes" have been labeled as $5HT_1$ and $5HT_2$ and are hypothesized to be differentially located in the brain (27). In addition, the $5HT_1$ receptor has been further subdivided into $5HT_{1A}$ and $5HT_{1B}$ (32,47) and more recently $5HT_{1C}$, $5HT_{1D}$ (31) and $5HT_3$ sites have been disclosed (16). Indeed, specific ligands for these putative receptor sites have been discovered, e.g., 8-OHDPAT acting at $5HT_{1A}$ sites (28), TFMPP acting at $5HT_{1B}$ sites (26) and DOI being $5HT_2$-specific (46). In a series of ingenious experiments, Glennon has successfully trained rats to discriminate each of these agents (14, 15, 17) and evidenced the specificity of each by the lack of generalization to any of the other receptor (subtype) agonists.

Each of these specific receptor agonists was administered to the MDMA-trained rats and, although 8-OHDPAT and DOI produced intermediate results, only TFMPP produced a generalization from MDMA. This may be suggestive evidence that the $5HT_{1B}$ receptor is somehow involved in the stimulus recognition of MDMA. However, it must be kept in mind that TFMPP is as potent a 5HT-releaser as fenfluramine (35). In regards to the $5HT_2$ specific agent DOI, Glennon *et al.* (19) had reported that it will substitute for the hallucinogen DOM in DOM-trained rats in contrast to MDMA which produced no generalization. Although there are two reports that indicate that MDMA is a potent releaser of 5HT (30,45), only one laboratory has reported differential affinity for MDMA at the receptor level (24) and MDMA has slightly greater affinity for $5HT_1$ than for $5HT_2$ receptors. The $5HT_2$ receptor has recently been evidenced to be extensively involved in the discrimination of the hallucinogen LSD (8) and coupled with the lack of DOM or DOI effects (cited above) the apparent lack of hallucinatory activity in humans of MDMA can be explained. Furthermore, a recent report (29) indicates that MDMA does not generalize from LSD in rats trained to discriminate 0.08 mg/kg LSD tartrate. The administration of quipazine (0.5–1.5 mg/kg) in the present study produced only intermediate results (71.3% at best) on the MDMA-appropriate lever. Previous work (52,53) indicated that LSD and quipazine share a common discriminative stimulus, one that is mediated by an action at central $5HT_2$ sites (12). Other work indicated a dopaminergic, as well as a serotonergic component, to the behavioral effects of quipazine (42), as has been found to occur with mescaline (51).

An explanation for the results of the present experimentation in which at least two of serotonergically-mediated agents, but not the dopaminergically-mediated drugs, produced MDMA-appropriate responding when tested at 20 min postadministration may reside in the possibility that the effects of MDMA are temporally biphasic. That would suggest that the first temporal effect, occurring within the first 30 min after MDMA administration, does not involve dopaminergic systems. Preliminary data from this laboratory, using in vivo voltammetry, indicates that the maximum release of 5HT occurs before 30 min, whereas dopamine release peaks at 90 min in the nucleus accumbens after IP administration of 5 mg/kg (±)-MDMA to freely-moving rats (Yamamato, personal communication). In addition, in an investigation of the time-course of (±)-MDMA in rats trained to discriminate between norfenfluramine and *d*-amphetamine, the rats were observed to select the norfenfluramine-appropriate lever to a greater extent at 15–30 min postadministration and the *d*-amphetamine lever more at 90–120 min after the injection of 1.5 mg/kg MDMA (Boja, personal communication). Lastly, human abusers report a "weird period" within 30 min of MDMA ingestion and a

euphoriant phase at times later than 30 min (10). To further investigate this hypothesis, rats were trained to discriminate 2.5 mg/kg MDMA at 105 min postadministration; at a time of presumed heightened dopaminergic effect (Experiment 3). The results represented in Table 3 indicate that, although the specific serotonergic antagonist pirenperone was equally effective in reducing the MDMA-induced discriminative stimulus cue at both 20 and 105 min postinjection, the dopamine lowering (CGS 10746B) or blocking (haloperidol) agents were slightly more effective at 105 min postinjection. It must, however, be kept in mind that pirenperone may possess dopamine antagonist properties as evidenced by both behavioral (9) and in vitro receptor ligand binding studies (20), as well as the absence of evidence regarding any possible serotonergic activity of CGS 10746B.

In conclusion, recent studies regarding the biochemical actions of MDMA have indicated that it is a potent releaser of both central 5HT (30,49) and dopamine (49). The present behavioral evidence would indicate that at 20 min postinjection MDMA produces a nonselective serotonergic stimulus effect without a dopaminergic component. A later (105 min postadministration) aspect of MDMA's action indicates a greater dopamine component to its behavioral effect. This biphasic serotonergic-dopaminergic activity may account for the recent controversial evidence that reports that MDMA actions are either solely serotonergic (4) or solely dopaminergic (5).

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REFERENCES

1. Altar, C. A.; Wesley, A. M.; Liebman, J.; Gerhardt, S.; Kim, H.; Welsh, J. J.; Wood, P. L. CGS 10746B: An atypical antipsychotic candidate that selectively decreases dopamine release at behaviorally effective doses. Life Sci. 39:699–705; 1987.
2. American Medical News, DEA to outlaw hallucinogenic "ecstasy," June 14, 1985; 18.
3. Antony, J. C.; Trinkoff, A. M. Epidemiologic issues pertinent to international regulation of 28 stimulant-hallucinogen drugs. Drug Alcohol Depend. 17:193–211; 1986.
4. Battaglia, G.; DeSouza, E. B. New perspectives on MDMA (3,4-methylenedioxymethamphetamine). Subst. Abuse 8:31–42; 1987.
5. Bird, M. P.; Svendsen, C. N.; Knapp, C.; Hrbek, C. C.; Bird, E. D.; Kornetsky, C. Evidence for dopaminergic and not serotonergic mediation of the threshold lowering effects of MDMA on rewarding brain stimulation. Soc. Neurosci. Abstr. 365.13:1323; 1987.
6. Boja, J. W.; Schechter, M. D. Behavioral effects of N-ethyl-3,4-methylenedioxyamphetamine (MDE; "Eve"). Pharmacol. Biochem. Behav. 28:153–156; 1987.
7. Boja, J. W.; Schechter, M. D. Norfenfluramine, the fenfluramine metabolite provides stimulus control: Evidence for serotonergic mediation. Pharmacol. Biochem. Behav. 31:305–311; 1988.
8. Cunningham, K. A.; Appel, J. A. Neuropharmacological reassessment of the discriminative stimulus properties of *d*-lysergic acid diethylamide (LSD). Psychopharmacology (Berlin) 91:67–73; 1987.
9. Cunningham, K. A.; Callahan, P. M.; Appel, J. B. Discriminative stimulus properties of lisuride revisted. Involvement of dopamine D_2 receptors. J. Pharmacol. Exp. Ther. 241:147–151; 1987.
10. Dowling, C. G. Ecstasy. Life Aug:88–91; 1985.
11. Duhault, J.; Malen, Ch.; Boulanger, M.; Voisin, C.; Beregi, L.; Schmitt, H. Fenfluramine and 5-hydroxytryptamine. Arzneimittelforshung 25:1755–1758; 1975.
12. Friedman, R. L.; Barrett, R. J.; Sanders-Bush, E. Discriminative stimulus properties of quipazine. Mediation by serotonin$_2$ binding sites. J. Pharmacol. Exp. Ther. 228:628–635 1984.
13. Gertz, K. R. Hug drug alert: The agony of ecstasy. Harper's Bazaar 263:55–56; 1985.
14. Glennon, R. A. Discriminative stimulus properties of the 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OHDPAT). Pharmacol. Biochem. Behav. 25:135–139; 1986.
15. Glennon, R. A. Discriminative stimulus properties of the serotonergic agent 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). Life Sci. 39:825–830; 1986.
16. Glennon, R. A. Central serotonin receptors as targets for drug research. J. Med. Chem. 30:1–12; 1987.
17. Glennon, R. A.; McKenney, J. D.; Young, R. Discriminative stimulus properties of the serotonin agonist 1-(3-tri-fluoromethylphenyl)piperazine (TFMPP). Life Sci. 35:1475–1480; 1984.
18. Glennon, R. A.; Young, R. Further investigations of the discriminative stimulus properties of MDA. Pharmacol. Biochem. Behav. 20:501–504; 1984.
19. Glennon, R. A.; Young, R.; Rosecrans, J. A.; Anderson, G. M. Discriminative stimulus properties of MDA analogs. Biol. Psychiatry 17:807–814; 1982.
20. Janssen, P. A. J. 5-HT_2 receptor blockade to study serotonin-induced pathology. Trends Pharmacol. Sci. 4:198–206; 1988.
21. Johnson, M. P.; Hoffman, A. J.; Nichols, D. E. Effects of the enantiomers of MDA, MDMA and related analogues of ^{3}H serotonin and ^{3}H dopamine release from superfused rat brain slices. Eur. J. Pharmacol. 132:269–276; 1986.
22. Levine, R. R. Pharmacology: Drug actions and reactions. 2nd ed. New York: Little, Brown and Co.; 1978:169–209.
23. Litchfield, J. T.; Wilcoxon, F. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther. 96:99–106; 1949.
24. Lyon, R. A.; Glennon, R. A.; Titeler, M. 3,4-methylenedioxymethamphetamine (MDMA): Stereoselective interactions at brain 5-HT_1 and 5-HT_2 receptors. Psychopharmacology (Berlin) 88:525–526; 1986.
25. Maloney, R. E.; Schechter, M. D.; Yamamoto, B. K. Effect of low dose regimen of 3,4-methylenedioxymethamphetamine upon regional brain neurotransmitters. Soc. Neurosci. Abstr. 27.14:86; 1987.
26. Martin, L. L.; Sanders-Bush, E. Comparison of the pharmacological characteristic of 5HT_1 and 5HT_2 with those of serotonin autoreceptors which modulate release. Naunyn Schmiedebergs Arch. Pharmacol. 321:165–170; 1986.
27. Maura, G.; Roccatagliata, E.; Raiteri, M. Serotonin autoreceptor in rat hippocampus: Pharmacological characterization as a subtype of the 5-HT_1 receptor. Naunyn Schmiedebergs Arch. Pharmacol. 334:323–326; 1986.
28. Middlemiss, D. N.; Fozard, J. R. 8-hydroxy-2-(di-n-propylamino)tetralin discriminates between subtypes of the serotonin-1 recognition site. Eur. J. Pharmacol. 90:151–153; 1983.

29. Nichols, D. E.; Hoffman, A. J.; Oberlander, R. A.; Jacob, P., III; Shulgin, A. T. Derivatives of 1-(1,3-benzodioxol-5-yl)-2-butanamine: Representative of a novel therapeutic class. J. Med. Chem. 29:2009–2015; 1986.
30. Nichols, D. E.; Lloyd, D. H.; Hoffman, A. J.; Nichols, M. B.; Yim, G. K. W. Effects of certain hallucinogenic amphetamine analogues on the release of (H^3) serotonin from rat brain synaptosomes. J. Med. Chem. 25:530–535; 1982.
31. Pazos, A.; Hoyer, D.; Palacios, J. M. The binding of serotonergic ligands to the porcine choroid plexus characterization of a new type of serotonin recognition site. Eur. J. Pharmacol. 106:539–546; 1984.
32. Pedigo, N. W.; Yamamura, H. I.; Nelson, D. L. Discrimination of multiple ^{3}H-5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain. J. Neurochem. 36:220–226; 1981.
33. Peroutka, S. J. Incidence of recreational use of 3,4-methylenedimethoxymethamphetamine (MDMA, "Ecstasy") on an undergraduate campus. N. Engl. J. Med. 317:1542–1543; 1987.
34. Peroutka, S. J.; Snyder, S. H. Two distinct serotonin receptors: Regional variation in receptor binding in mammalian brain. Brain Res. 208:339–347; 1981.
35. Pettibone, D. J.; Williams, M. Serotonin-releasing effects of substituted piperazines *in vitro*. Biochem. Pharmacol. 33:1531–1533; 1984.
36. Rolbein, S. In search of ecstasy. Boston Magazine Nov:210–213, 296–297; 1985.
37. Schechter, M. D. Dopaminergic mediation of a behavioral effect of cathinone. Pharmacol. Biochem. Behav. 25:337–340; 1986.
38. Schechter, M. D. Discriminative profile of MDMA. Pharmacol. Biochem. Behav. 24:1533–1534; 1986.
39. Schechter, M. D. MDMA as a discriminative stimulus: Isomeric comparisons. Pharmacol. Biochem. Behav. 27:41–44; 1987.
40. Schechter, M. D. Use of TFMPP stimulus properties as a model of $5HT_{1B}$ receptor activation. Pharmacol. Biochem. Behav. 31:53–57; 1988.
41. Schechter, M. D.; Boja, J. W. CGS 10746B is able to attenuate the effects of amphetamine: Further evidence for dopaminergic mediation. Pharmacol. Biochem. Behav. 30:1089–1092; 1988.
42. Schechter, M. D.; Concannon, J. T. Dopaminergic activity of quipazine. Pharmacol. Biochem. Behav. 17:393–397; 1982.
43. Schechter, M. D.; Cooke, P. G. Dopaminergic mediation of the interoceptive cue produced by *d*-amphetamine in the rat. Psychopharmacologia 42:185–193; 1975.
44. Schmidt, C. J.; Levin, J. A.; Lovenberg, W. In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain. Biochem. Pharmacol. 36:747–755; 1987.
45. Schmidt, C. J.; Wu, L.; Lovenberg, W. Methylenedioxymethamphetamine: a potentially neurotoxic amphetamine analogue. Eur. J. Pharmacol. 124:175–178; 1986.
46. Shannon, M.; Battaglia, G.; Glennon, R. A.; Titeler, M. 5-HT_1 and 5-HT_2 binding properties of derivatives of the hallucinogen 1-(2,5-dimethoxyphenyl)-2-aminopropane (2,5-DMA). Eur. J. Pharmacol. 102:23–29; 1984.
47. Sills, M. A.; Wolfe, B. B.; Frazer, A. Determination of selective and non-selective compounds for the 5-HT_{1A} and 5-HT_{1B} receptor subtypes in rat frontal cortex. J. Pharmacol. Exp. Ther. 231:480–487; 1984.
48. Stolerman, I. P.; D'Mello, G. D. Role of training condition in discrimination of central nervous system stimulants. Psychopharmacology (Berlin) 73:295–303; 1981.
49. Stone, D. M.; Stahl, D. C.; Hanson, G. R.; Gibb, J. W. The effects of 3,4-methylenedioxymethamphetamine (MDMA) on monoaminergic systems in the rat brain. Eur. J. Pharmacol. 128:41–48; 1986.
50. Toufexis, A. A crackdown on ecstasy. Time June 10:64; 1985.
51. Trulson, M. E.; Crisp, T.; Henderson, L. J. Mescaline elicits behavioral effects in cats by an action at both serotonin and dopamine receptors. Eur. J. Pharmacol. 96:151–154; 1983.
52. White, F. J.; Kuhn, D. M.; Appel, J. B. Discriminative stimulus properties of quipazine. Neuropharmacology 16:827–832; 1977.
53. Winter, J. C. Quipazine-induced stimulus control in the rat. Psychopharmacology (Berlin) 60:265–269; 1979.
54. Wood, P. M.; Lal, H.; Yaden, S.; Emmett-Oglesby, M. W. One-way generalization of clonidine to the discriminative stimulus produced by cocaine. Pharmacol. Biochem. Behav. 23:529–533; 1985.
55. Yamamoto, B. K.; Spanos, L. J. The acute effects of methylenedioxymethamphetamine on dopamine release in the awake-behaving rat. Eur. J. Pharmacol. 148:195–203; 1988.
56. Young, R.; Glennon, R. A. Discriminative stimulus properties of amphetamine and structurally related phenalkylamines. Med. Res. Rev. 6:99–130; 1986.