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Long-Term Central 5-HT Depletions Resulting From Repeated Administration of MDMA Enhances the Effects of Single Administration of MDMA on Schedule-Controlled Behavior of Rats[1]

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LI, A. A., G. J. MAREK, G. VOSMER AND L. S. SEIDEN. *Long-term central 5-HT depletions resulting from repeated administration of MDMA enhances the effects of single administration of MDMA on schedule-controlled behavior of rats.* PHARMACOL BIOCHEM BEHAV **33**(3) 641–648, 1989.—The behavioral effect of single administration of ±3,4-methylenedioxymethamphetamine (MDMA) on rats performing on the differential-reinforcement-of-low-rate 72-second schedule (DRL 72-sec) was compared before and after a period of repeated administration of MDMA known to deplete 5-hydroxytryptamine (5-HT) levels in the brain. Single administration of MDMA decreased reinforcement rate (1, 2, 4, 6 mg/kg) and increased response rate (4,6 mg/kg) of rats performing on the DRL 72-sec schedule. This effect is typical of amphetamines and other psychomotor stimulants. Four weeks after repeated administration of MDMA (6 mg/kg twice daily for 4 days) there was an increase in sensitivity to the effect of single administration of MDMA. Doses of 2, 4 and 6 mg/kg of MDMA resulted in increases in response rate that were significantly greater after repeated MDMA administration than before. Doses of 0.5, 2, and 6 mg/kg of MDMA resulted in decreases of reinforcement rate that were significantly greater after repeated MDMA administration than before. Repeated administration of MDMA resulted in long-term depletion of serotonin levels by 30–50% in the amygdala, neostriatum, hippocampus and the frontal cortex. Norepinephrine and dopamine (DA) levels were not significantly different from control in any of the brain regions analyzed. The behavioral and neurochemical results suggest that serotonergic neurons normally exert an inhibitory action upon the psychomotor stimulant effects of MDMA. Since the psychomotor stimulant effects of amphetamines appear to be mediated primarily by the dopamine system, these results provide evidence that 5-HT and DA may represent opposing systems in the DRL schedule-controlled behavior.

±3,4-Methylenedioxymethamphetamine MDMA-induced serotonin depletion Psychomotor stimulants
DRL 72-sec schedule of reinforcement

AMPHETAMINE (AMPH) and its derivatives, methamphetamine (MA) and 3,4-methylenedioxyamphetamine (MDA), have been found to induce neurotoxicity in the CNS of a number of species including rats, cats and monkeys. Repeated administration of AMPH or MA produce long-term depletions of striatal DA levels (29, 38, 45, 48, 49), a reduction in tyrosine hydroxylase activity (10,20), and a loss of DA high-affinity uptake sites (29, 45, 48). In addition to the toxic effects on the DA neurons, repeated administration of MA also produces long-term depletions of 5-HT, decreases tryptophan hydroxylase activity and reduces the number of 5-HT high-affinity uptake sites in rats and other species. (20, 29, 47). MDA is selectively toxic to 5-HT neurons. MDA produces long-term decreases in hippocampal and neostriatal serotonin levels, hippocampal 5-hydroxyindole acetic acid (5-HIAA) levels, hippocampal 5-HT uptake sites along with degenerating axons and terminals in the hippocampus and neostriatum as

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TABLE 1

DRUG HISTORY FOR MDMA-TREATED RATS PERFORMING ON THE DRL 72-SEC SCHEDULE

Day	Time Interval (Days)	Drug Treatment
1–27	27	Dose-response determination for MDMA
28–32	5	No drugs administered
33–36	4	Repeated administration of MDMA (6 mg/kg, twice daily for four days)
37–64	28	No drugs administered
65–83	19	Dose-response redetermination for MDMA
83–87	5	No drugs administered
88	1	Sacrificed rats and removed their brains for neurochemical analysis. The control group of 6 rats was also sacrificed.

revealed by the Fink-Heimer method (27).

No obvious behavioral effects have been observed as a result of the neurotoxicity induced by repeated MA or AMPH injections. Pharmacological challenge, though, has proven to be sensitive in detecting behavioral changes resulting from MA- or AMPH-induced neurotoxicity. For example, long-term AMPH or MA administration results in tolerance to many of the effects of single AMPH or MA administration. Decreases in the effects of single injections of MA have been demonstrated for operant behavior of rhesus monkeys and locomotor activity of rats after repeated administration of MA that led to a 30–50% loss of DA terminals in the caudate (12, 13, 23, 38).

Recently, ±3,4-methylenedioxymethamphetamine (MDMA), an amphetamine analog, has been found to be a serotonergic neurotoxin in rats and other species. Repeated administration of MDMA produces depletions of 5-HT and its metabolite, 5-hydroxyindole acetic acid (5-HIAA), in the hippocampus and neostriatum, reduces the number of 5-HT uptake sites, and causes degeneration of neurons in the hippocampus and neostriatum (8,33). Repeated administration of MDMA also reduces DA and NE levels, but to a lesser extent than 5-HT levels (8).

The purpose of the present report is to study the behavioral effects of single injections of MDMA on rats performing under the differential-reinforcement-of-low-rate 72-second (DRL 72-sec) schedule of water reinforcement, and to determine if these behavioral effects are altered by MDMA-induced 5-HT neurotoxicity. Hence, dose-response determinations for the effects of single MDMA injections on DRL schedule-controlled behavior were made before and 4 weeks after repeated administration of MDMA (6 mg/kg, twice daily for four days). The long-term effects of the repeated MDMA injections on monoamine levels in different brain regions were determined. In vitro studies measuring the effect of MDMA on DA and 5-HT uptake into neostriatal and hippocampal synaptosomes, respectively, were also performed in order to better understand the biochemical effects of the single administrations of MDMA.

The results of the current study indicate that single administrations of MDMA act like a typical psychomotor stimulant to increase response rate (4,6 mg/kg) and decrease reinforcement rate (1, 2, 4, 6 mg/kg) of rats performing on the differential-reinforcement-of-low-rate schedule of water reinforcement. This psychomotor stimulant-like effect is enhanced after the administration of repeated MDMA injections that produces long-term depletions of central 5-HT levels. Evidence is provided to support the hypothesis that DA and 5-HT may represent opposing systems in DRL schedule-controlled behavior, and that the potentiation of the psychomotor stimulant-like effects of single MDMA injections (mediated by DA) seen after repeated administration of MDMA is due to a reduction in the inhibitory influence of 5-HT.

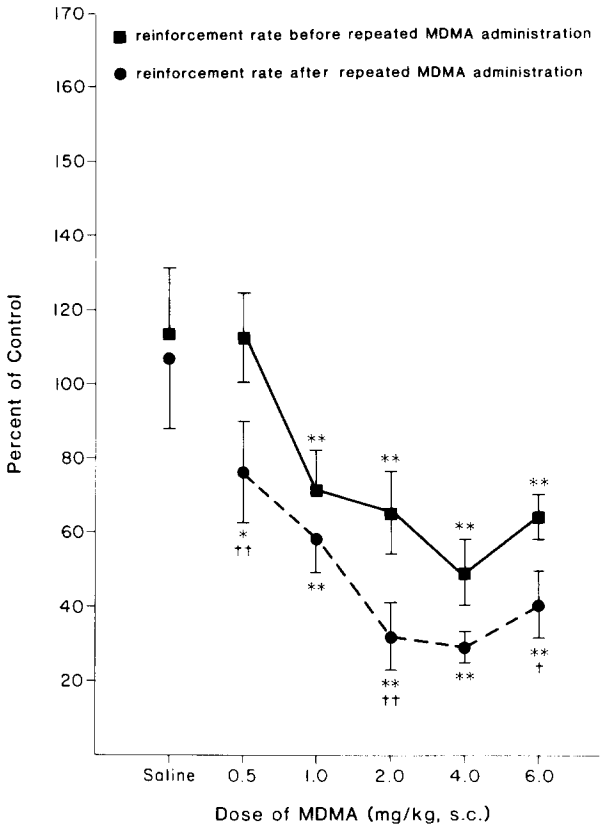

FIG. 1. Effects of MDMA on reinforcement rate of rats responding under a DRL 72-sec schedule before (closed squares) and after (closed circles) repeated administration of MDMA. Each point is the mean reinforcement rate ±S.E.M. for 7 rats expressed as percentage of the mean control reinforcement rate (16.14 reinforcements/session). Significantly different from control reinforcement rate $*p<0.05$, $**p<0.01$. Significantly different from corresponding MDMA dose, $\dagger p<0.05$, $\dagger\dagger p<0.01$.

METHOD

Subjects

Fourteen male Sprague-Dawley rats (Holtzman Co., Madison, WI) weighing an average of 384 g (range 288–454 g) at the beginning of this study were divided into two groups. Group 1 (n = 8) was used to study the effects of single injections of MDMA on DRL schedule-controlled behavior before and after repeated administration of MDMA. These rats were also used to determine the levels of monoamines seven weeks after the repeated administration of MDMA. Group 2 (n = 6) was used to determine control levels of monoamines in different brain regions. Groups 1 and 2 were housed two to a cage in suspended stainless steel wire cages (36.8 × 25.4 × 19.1 cm) and were permitted free access to laboratory chow (Teklad 4% Rat Diet). Both groups were provided access to water for 20 minutes each day.

Eight male Sprague-Dawley rats weighing an average of 502 g (range 388–565 g) and similar in age to Group 1 (1-year-old) were used to control for the effects of the passage of time on the

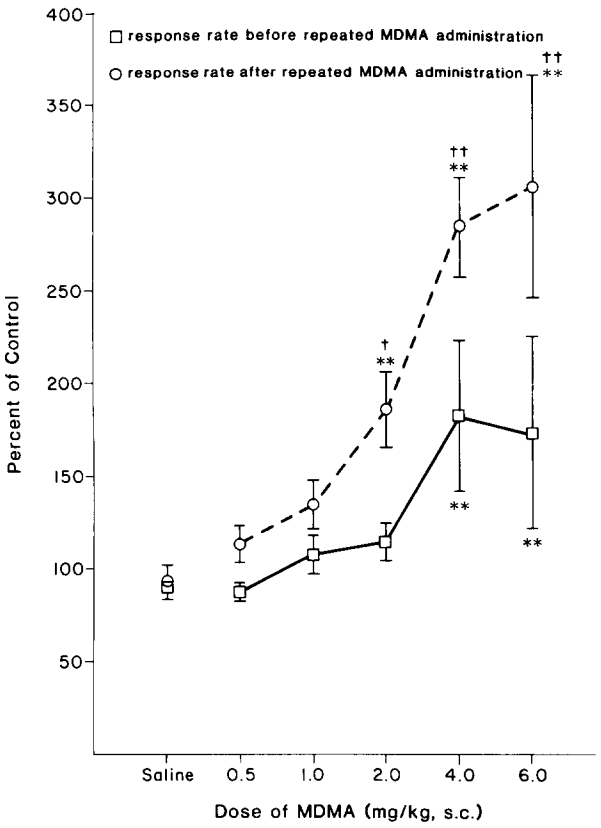

FIG. 2. Effects of MDMA on response rate of rats responding under a DRL 72-sec schedule before (open squares) and after (open circles) repeated administration of MDMA. Each point is the mean ± S.E.M. response rate for 7 rats expressed as percentage of the mean control response rate (79.14 responses/session). *Significantly different from control response, $p<0.05$, **$p<0.01$. Significantly different from corresponding MDMA dose, †$p<0.05$, ††$p<0.01$.

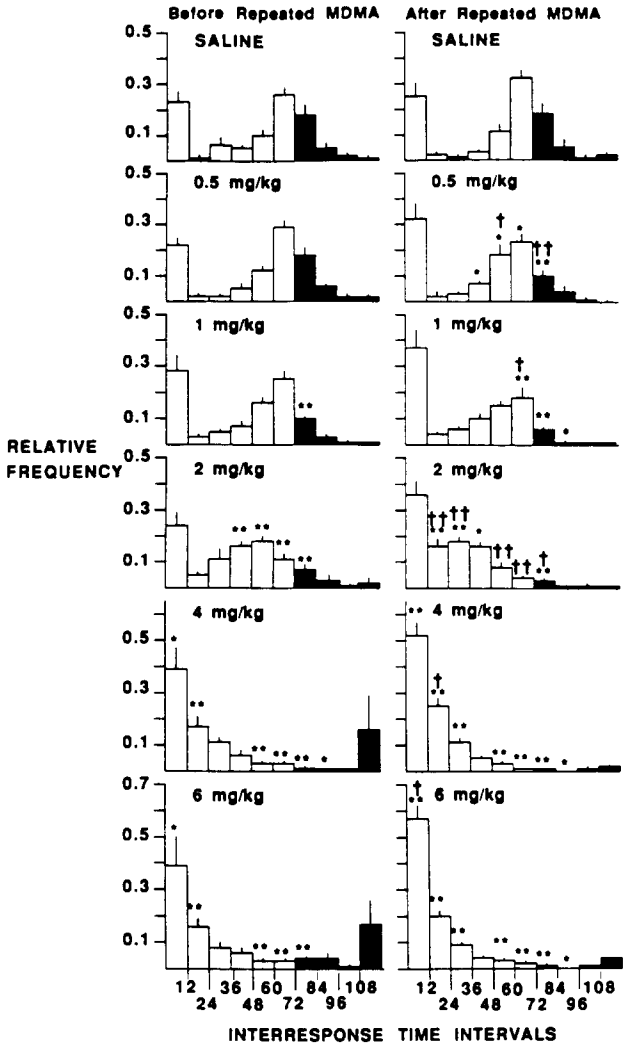

FIG. 3. IRT distributions for rats treated with MDMA and performing under a DRL 72-sec schedule. The bar graphs on the left side of this figure represent IRT distributions for different doses of MDMA before the rats were repeatedly injected with MDMA (6 mg/kg twice daily for 4 days). The bar graphs on the right represent IRT distributions after the rats were repeatedly administered MDMA. The bar heights represent the mean ± S.E.M. proportion of responses for 7 rats that occurred in each IRT class. Filled bars indicate IRTs of reinforced responses (i.e., IRTs greater than 72 sec). The last IRT bin includes all responses with IRTs greater than 108 sec. Significantly different from control, *$p<0.05$, **$p<0.01$. Significantly different from corresponding MDMA dose, †$p<0.05$, ††$p<0.01$.

behavioral response to MDMA. This third group (Group 3) was approximately the same age (1-year-old) as Group 1 and was housed and trained as described for Group 1. A fourth group of rats (Group 4) comprised of twelve male Sprague-Dawley rats (Holtzman Co., Madison, WI) was used to study the effect of MDMA on DA and 5-HT uptake into neostriatal and hippocampal synaptosomes, respectively. They were housed 6 to a cage in plastic bins (38.1 × 48.3 × 20.3 cm) with free access to laboratory chow and water.

Apparatus

Eight Gerbrands Model C operant chambers (21.5 × 23 × 19 cm) were used to test Groups 1, 2 and 3 during experimental sessions. A lever that operated a microswitch was mounted on one wall 3 cm from the side, 2.5 cm above the floor, and 6.5 cm from the dipper access port. A downward force equivalent to approximately 15 g (0.15 N) operated the lever, constituting a response. When a response fulfilled the schedule requirements, the dipper containing 0.025 ml water was lifted from a water trough to an opening in the bottom of the access port for 4 sec, constituting a reinforcement. A houselight was situated on the wall opposite the lever. This houselight remained on thoughout the entire session and turned off at the end of the session. Each experimental chamber was enclosed in a sound-attenuating chamber equipped with a fan to provide ventilation and a masking noise.

Training

Rats were water deprived for 22.5 hr before each session. Each rat was trained under an alternative fixed-ratio 1, fixed-time 1-min schedule for water reinforcement followed by a DRL 18-sec schedule and then a DRL 72-sec schedule as described by Seiden *et al.* (37). On the DRL 72-sec schedule, responses that occurred at least 72-sec after the previous response were reinforced. Responses that occurred less than 72-sec after the previous response were not reinforced and a new 72-sec interval without

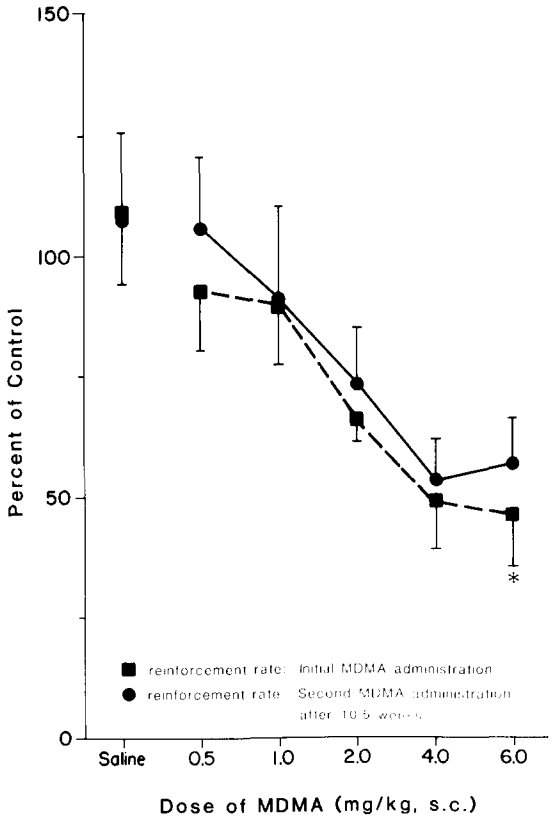

FIG. 4. Effects of MDMA on reinforcement rate of rats responding under a DRL 72-sec schedule with an initial dose-response determination (closed squares) and a second dose-response determination 10½ weeks following the initial one (closed circles). Each point is the mean reinforcement rate ± S.E.M. for 8 rats expressed as percentage of the mean control reinforcement rate (18.12 reinforcements/session). Significantly different from control reinforcement rate, $*p<0.05$, $**p<0.01$.

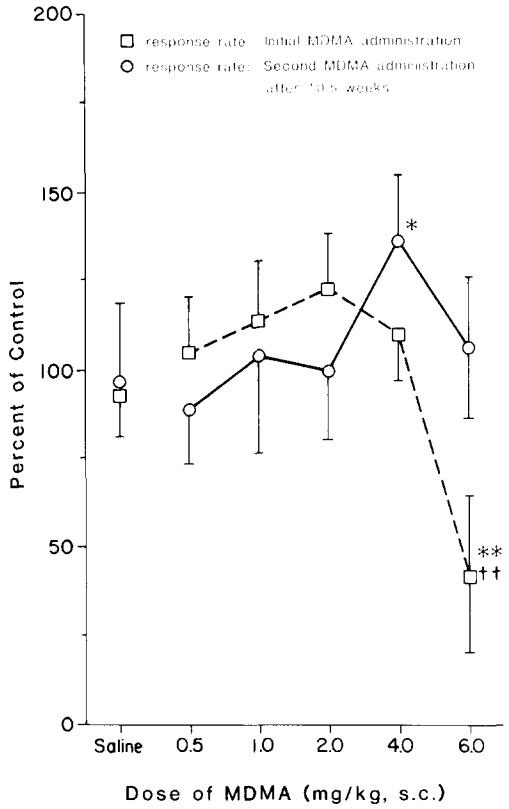

FIG. 5. Effects of MDMA on response rate of rats responding under a DRL 72-sec schedule with an initial dose-response determination (open squares) and a second dose-response determination 10½ weeks following the initial one (open circles). Each point is the mean ± S.E.M. response rate for 8 rats expressed as percentage of the mean control response rate (91.00 responses/session). Significantly different from control response, $*p<0.05$, $**p<0.01$. Significantly different from corresponding MDMA dose, $\dagger p<0.05$, $\dagger\dagger p<0.01$.

any responses was required for the next response to be reinforced. Responding on the DRL 72-sec schedule was considered stable when the standard error of the mean total response rate over 5 consecutive sessions was less than 10% of its own mean response rate. Experimental sessions lasted for 1 hr and were conducted 6 days/week during light hours.

Experimental Protocol for MDMA Injections

MDMA HCl, provided by the National Institute of Drug Abuse, was administered to Group 1. Table 1 shows the sequence of dose-response determinations and repeated administration for this group. A dose-response curve for MDMA was established for the following six doses: 0.0 (saline), 0.5, 1.0, 2.0, 4.0, and 6.0 mg/kg of MDMA (HCl salt). MDMA was dissolved in 0.9% saline and injected subcutaneously in a volume of 1 ml/kg. MDMA injections were separated by a period of at least 2 days. Five days after the last injection for the dose-response determination, 6 mg/kg of MDMA was administered every 12 hours (8:00 a.m. and 8:00 p.m.) for 4 days. Four weeks after the repeated administration of MDMA a second dose-response curve to MDMA was established using the same doses described above. Hence, two dose-response curves for MDMA were initiated 9 weeks apart from each other.

Two dose-response curves for MDMA were also established for Group 3 as described above for Group 1 with the exception that there was no intervening repeated MDMA treatment. The two dose-response curves were initiated 10½ weeks apart from each other. The rats in Group 3 were similar in age to Groups 1 and 2 and also had comparable control reinforcement and response rates.

Group 2 served as controls for the neurochemical assays performed at the end of the DRL study. They were the same age as the MDMA-treated rats, deprived of water, and trained on the DRL 72-sec schedule, but had not received injections of either saline or drug during this study.

Data Acquisition and Analysis

The experimental operant chambers were connected to a PDP-11/73 microcomputer via a Coulbourn lablinc computer interface. The schedule contingencies were programmed, and responses, reinforcements, and sequential interresponse times were recorded using a SKED 11 Software System (41).

The control values for all the drugs were the mean response and reinforcement rate which were averaged over several days: the five days (including the vehicle injection) immediately preceding the first dose and the day preceding each drug injection. Therefore, the number of control days would be five plus the number of drug injections.

TABLE 2

EFFECT OF REPEATED MDMA ADMINISTRATION (6 mg/kg TWICE DAILY FOR 4 DAYS) ON MONOAMINE LEVELS IN THE RAT BRAIN

Brain Region	Treatment	n	5-HT	5-HIAA	NE	HVA	DA	DOPAC
N. Accumbens	control[a]	6	0.91 ± 0.11	0.75 ± 0.14	N.D.[d]	0.67 ± 0.05	5.82 ± 0.83	2.60 ± 0.31
	MDMA[b]	7	0.63 ± 0.08	0.69 ± 0.07	N.D.	0.83 ± 1.7	6.76 ± 0.67	2.43 ± 0.27
	% difference[c]		−31	−8		+23	+16	−7
Amygdala	control	6	0.51 ± 0.05	0.37 ± 0.03	0.58 ± 0.05	N.D.	0.38 ± 0.05	0.12 ± 0.01
	MDMA	7	0.34 ± 0.03*	0.25 ± 0.02**	0.51 ± 0.04	N.D.	0.39 ± 0.04	0.13 ± 0.02
	% difference		−34*	−32**	−13		+4	+10
Hypothalamus	control	6	0.46 ± 0.09	0.40 ± 0.09	1.95 ± 0.10	N.D.	0.23 ± 0.02	N.D.
	MDMA	7	0.34 ± 0.05	0.36 ± 0.04	2.08 ± 0.09	N.D.	0.28 ± 0.02	N.D.
	% difference		−26	−10	+7		+18	
Midbrain	control	5	0.43 ± 0.03	0.35 ± 0.06	0.33 ± 0.01	N.D.	0.11 ± 0.01	0.02 ± 0.003
	MDMA	7	0.35 ± 0.02	0.25 ± 0.03	0.34 ± 0.02	N.D.	0.11 ± 0.01	0.02 ± 0.001
	% difference		−19	−27	+5		+3	−5
Pons-medulla	control	5	0.32 ± 0.01	0.24 ± 0.01	0.36 ± 0.04	N.D.	0.05 ± 0.01	0.02 ± 0.01
	MDMA	7	0.26 ± 0.02	0.19 ± 0.02*	0.33 ± 0.02	N.D.	0.05 ± 0.01	0.02 ± 0.01
	% difference		−18	−24*	−8		+2	+16
Neostriatum	control	6	0.30 ± 0.01	0.42 ± 0.04	N.D.	0.51 ± 0.03	10.98 ± 0.58	1.64 ± 0.13
	MDMA	7	0.22 ± 0.03*	0.29 ± 0.03*	N.D.	0.54 ± 0.04	10.38 ± 0.53	1.47 ± 0.10
	% difference		−27*	−30*		+7	−6	−10
Hippocampus	control	5	0.25 ± 0.04	0.22 ± 0.03	0.37 ± 0.02	N.D.	N.D.	N.D.
	MDMA	7	0.13 ± 0.02*	0.11 ± 0.02**	0.29 ± 0.05	N.D.	N.D.	N.D.
	% difference		−45*	−49**	−23			
Frontal Cortex	control	6	0.22 ± 0.02	0.13 ± 0.02	0.32 ± 0.04	N.D.	0.09 ± 0.02	0.05 ± 0.01
	MDMA	6	0.12 ± 0.02**	0.08 ± 0.01*	0.32 ± 0.04	N.D.	0.12 ± 0.02	0.04 ± 0.01
	% difference		−46**	−42*	+1		+23	−26

[a]Control treatment indicates levels of monoamines in rats that were not treated with repeated injections of MDMA.
[b]MDMA treatment indicates levels of monoamines in rats that were treated with repeated MDMA injections.
[c]% Difference = ((MDMA levels/control levels) − 1)/100.
[d]N.D. indicates that levels were not determined.
Shown are the mean values ± S.E.M. (for 5 to 7 rats) for the levels of monoamines expressed in ng/mg tissue.

The response rates and reinforcement rates from the first dose-response determination (before repeated administration of MDMA) were analyzed by a one-factor repeated measures analysis of variance. The effect of repeated administration of MDMA on the dose-response curve for MDMA was determined by two-factor repeated measures analysis of variance. The effect of the passage of time on the dose-response curve for MDMA was also analyzed by two-factor repeated measures analysis of variance. Multiple comparisons were performed by using the Newman-Keuls test (16). Since one rat died during the repeated administration of MDMA, the analysis for Group 1 was done on the response and reinforcement rates for the remaining 7 rats.

The shortest interresponse interval measured by the computer was 0.01 seconds. The interresponse intervals for a group of rats performing on a DRL-72 sec session were analyzed by first dividing the interresponse intervals into ten 12-sec time bins with the last time bin including all IRTs greater than 120 sec. The effect of repeated administration of MDMA on the interresponse intervals was determined by two-factor repeated measures analysis of variance for each of the 10 time bins. Multiple comparisons were performed by using the Duncan test (16,51).

Dissection and Neurochemical Assays

Groups 1 and 2 were decapitated five days after the second dose-response determination (see Table 1). This corresponds to 7.5 weeks after MDMA was repeatedly administered to Group 1. The brains were dissected on ice according to the procedure of Heffner *et al.* (17) to yield the following eight regions: nucleus accumbens, neostriatum, frontal cortex, hypothalamus, amygdala, hippocampus, midbrain, and pons-medulla. These brain samples were wrapped in aluminum foil and immediately stored in liquid nitrogen until assayed. Concentrations of 5-HT, 5-HIAA, DA, dihydroxyphenylacetic acid (DOPAC), norepinephrine (NE), and homovanillic acid (HVA) were determined in these brain samples by reverse-phase ion-pair high performance liquid chromatography with electrochemical detection as described by Kotake *et al.* (21).

The levels of monoamines in brain regions of rats treated with MDMA were tested for statistically significant differences from control values with paired *t*-tests using a two-tailed criterion of statistical significance.

Measurement of Inhibition of DA and 5-HT Uptake by MDMA Into Synaptosomes in Brain Homogenates

Group 4 was used for the in vitro uptake studies. The neostriatal tissue from 2 rats (approximately 40 mg tissue) was pooled and homogenized in 100 vol. (w/v) of ice-cold 0.32 M sucrose. A crude synaptosomal preparation was obtained by centrifuging the homogenate at 1000 × g for 10 min. Supernatant (0.2 ml aliquots) was added to tubes containing 3.8 ml of Krebs-Ringer phosphate buffer (pH 7.4) that contained an equimolar mixture of cold and tritiated dopamine (0.05 μM), as well

as different concentrations of MDMA (2×10^{-8}, 8×10^{-8}, 2×10^{-7}, 8×10^{-7}, 2×10^{-6}, 8×10^{-6}, and 8×10^{-5} M). DA uptake was measured using a modification of the method of Snyder and Coyle (42) described by Wagner *et al.* (48). [^{3}H]DA (NEN Research Products, Boston, MA) had a specific activity of 27 Ci/mmol.

The hippocampal tissue from 10 rats (approximately 150 mg tissue) was pooled and homogenized in 3.3 vol. of 0.32 M sucrose and used immediately for the determination of [^{3}H]5-HT accumulation by rat brain homogenates in the presence of the same concentrations of MDMA described above. 5-HT uptake was measured the same way DA uptake was measured with the exception that the synaptosomal preparation was incubated with an equimolar mixture of labelled and unlabelled 0.05 μM 5-HT instead of DA. The specific activity for [^{3}H]-5-HT (NEN Research Products, Boston, MA) was 24.3 Ci/mmol.

The assays for both DA and 5-HT uptake were performed in quadruplicate at each drug concentration with half of the tubes serving as blanks (48). The percent inhibition of monoamine accumulation was converted into logit-log plots and the IC_{50} was determined using linear regression analysis over the linear portion of the data.

RESULTS

Effect of Single Administration of MDMA on DRL Scheduled-Controlled Behavior

Single administration of MDMA were found to increase response rates and decrease reinforcement rates of rats performing under the DRL 72-sec schedule in a dose-dependent manner (Figs. 1 and 2). Repeated measures analyses of variance showed that there was a significant overall effect of dose on response rates, $F(5,30)=8.360$, $p<0.001$, and reinforcement rates, $F(5,30)=2.714$, $p<0.05$. Post hoc tests showed that the 4 and 6 mg/kg doses of MDMA significantly increased response rate, and that the 1, 2, 4, and 6 mg/kg doses significantly decreased reinforcement rate.

MDMA increased the number of short interresponse times (IRTs) and decreased the number of long IRTs (see Fig. 3). One-factor repeated analysis of variance revealed that there was a significant overall effect of MDMA to increase the number of IRTs in the 24-sec, $F(5,30)=14.464$, $p<0.001$, and 36-sec, $F(5,30)=2.860$, $p<0.05$, bins. MDMA also had a significant overall effect on the number of IRTs in the 48-sec, $F(5,30)=6.142$, $p<0.001$, and 60-sec bins, $F(5,30)=13.153$, $p<0.001$. MDMA increased the number of IRTs in the 48-sec and 60-sec bins at the lower doses of 0.5, 1 and 2 mg/kg and decreased them at the higher doses of 4 and 6 mg/kg. MDMA significantly decreased the number of IRTs in the 60-sec, $F(5,30)=13.153$, $p<0.001$, 72-sec $F(5,30)=32.453$, $p<0.001$, 84-sec, $F(5,30)=15.444$, $p<0.001$, and 96-sec, $F(5,30)=4.386$, $p<0.005$. These results indicate that the IRT distribution shifted towards shorter IRTs as the dose of MDMA increased.

Effect of Repeated Administration of MDMA

Repeated administration of MDMA did not significantly change control performance (reinforcement and response rates for the 5 days immediately before each dose response determination) of rats performing under the DRL 72-sec schedule. However, two-factor repeated measures analysis of variance revealed a significant drug vs. pre-/postinteraction for the response rate, $F(5,30)=4.849$, $p<0.005$, and the reinforcement rate, $F(5,30)=3.253$, $p<0.05$, indicating that repeated MDMA administration significantly changes the behavioral effects of single injections of MDMA. The mean reinforcement and response rates for each dose of MDMA are graphed as percent of control in Fig. 1 and 2. Post hoc analysis revealed that repeated administration of MDMA significantly potentiated the effect of single injections of MDMA on reinforcement rate at doses of 0.5, 2, and 6 mg/kg and on response rate at doses of 2, 4, and 6 mg/kg.

As in the first dose-response determination, the IRT distribution shifted towards shorter IRTs as the dose of MDMA increased. This shift was greater than that obtained during the first dose-response determination especially at the 2.0 mg/kg dose of MDMA (see Fig. 3). Two-factor repeated analysis of variance showed that there was a significant drug vs. pre-/postinteraction on the number of IRTs in the 24-sec, $F(5,30)=3.374$, $p<0.05$, 48-sec, $F(5,30)=2.546$, $p<0.05$, 60-sec, $F(5,30)=5.009$, $p<0.005$, 72-sec, $F(5,30)=2.678$, $p<0.05$, and 84-sec, $F(5,30)=2.895$, $p<0.05$, time bins. Post hoc analysis revealed that repeated administration of MDMA potentiated the effect of single injections of MDMA to increase short IRTs and/or decrease long IRTs at doses of 0.5, 1, 2, 4, and 6 mg/kg.

Effect of Passage of Time on Dose-Response Curve for MDMA.

The passage of time did not alter the effect of single injections of MDMA on reinforcement rate (Fig. 4), but did attenuate the effect of MDMA on response rate [time is pre-/postinteraction, $F(5,35)=4.474$, $p=0.003$, Fig. 5]. Post hoc analysis revealed that the passage of a 10.5-week interval significantly attenuated the effect of a single injection of MDMA on response rate only at the 6 mg/kg dose.

Effect of Repeated MDMA Administration on Regional Monoamine Levels

Repeated administration of MDMA significantly depleted 5-HT (Table 2) levels in the amygdala ($p<0.05$), neostriatum ($p<0.05$), hippocampus ($p<0.05$), and frontal cortex ($p<0.05$). 5-HIAA levels were also significantly depleted in the amygdala ($p<0.01$), pons-medulla ($p<0.05$), neostriatum ($p<0.05$), hippocampus ($p<0.01$), and frontal cortex ($p<0.05$). In contrast, there were no significant differences in DA, NE, DOPAC, or HVA levels between rats repeatedly treated with MDMA and control rats (see Table 2).

Effect of MDMA on DA and 5-HT Accumulation in Brain Homogenates

The effect of MDMA on DA and 5-HT accumulation in brain homogenates was determined using a different group of rats (Group 4) from those given repeated administrations of MDMA. MDMA was potent at inhibiting accumulation of [^{3}H]-DA into neostriatal synaptosomes (IC_{50} of 1.5×10^{-6} M) and of [^{3}H]-5-HT into hippocampal synaptosomes (IC_{50} of 9×10^{-7} M).

DISCUSSION

Single administration of MDMA had the same effect as AMPH, MA and other psychomotor stimulants on rats responding under a DRL 72-sec schedule by increasing response rate, decreasing reinforcement rates, and shifting the IRT distribution to the left toward shorter IRT's (4, 32, 40). Repeated injections of MDMA that caused long-term central depletions of 5-HT and 5-HIAA potentiated the psychomotor stimulant-like effects of MDMA. This potentiation cannot be attributed simply to the passage of time since it was shown in this study that time did not alter the effects of MDMA on reinforcement rate and did not enhance the effect of MDMA on response rate. These results support our hypothesis that repeated administration, and not the passage of time, enhanced the psychomotor stimulant-like effect of MDMA.

These results and the studies discussed below suggest that DA mediates the psychomotor stimulant-like effects of MDMA on

DRL behavior, and that 5-HT normally exerts an inhibitory action upon the psychomotor stimulant-like effects of MDMA on DRL behavior. Thus, the depletion of serotonin by repeated administration of MDMA reduces this inhibitory influence and allows MDMA to exert a stronger psychomotor stimulant-like effect on DRL scheduled-controlled behavior.

Previous studies indicate that the psychomotor stimulant-like effect of AMPH, MA, and MDMA on the DRL schedule are mediated primarily through the DA system. Pretreatment with alpha-methyltyrosine, an inhibitor of catecholamine biosynthesis, antagonizes the response rate-increasing effects of amphetamine in rats performing on a DRL schedule (36). Compounds that potently inhibit DA uptake such as bupropion and nomifensine have a psychomotor stimulant-like effect on DRL 72-sec behavior (26,39). In contrast, antidepressant drugs that inhibit NE and 5-HT uptake have effects on the DRL 72-sec schedule that are opposite in direction; they decrease response rate and increase reinforcement rate. Other psychomotor stimulant actions of the amphetamines such as increased locomotor activity and stereotypic behavior are also mediated primarily through DA (5, 9, 11, 18, 46).

The present study demonstrates that MDMA inhibits the accumulation of DA and 5-HT into neostriatal and hippocampal synaptosomes, respectively. It has also been shown that MDMA releases 5-HT from whole brain and DA from neostriatal tissue in superfusion preparations (25,34). This suggests that single administrations of MDMA increases both 5-HT and DA transmission in the brain by releasing 5-HT and DA and/or blocking their reuptake. After repeated administration of MDMA, the ability of single MDMA injections to increase 5-HT transmission was attenuated because of the depletion of 5-HT levels in the brain. This results in a reduction of the inhibitory influence of 5-HT and, hence, the potentiation of the psychomotor stimulant-like effect of MDMA.

There are other examples in the literature of the inhibitory influence of 5-HT on the effects of amphetamine and its derivatives on schedule-controlled behavior. Green and Harvey (15) demonstrated that interruption of serotonergic fibers in the medial forebrain bundle (MFB) and the consequent depletion of telencephalic 5-HT enhanced the rate-increasing effects of d-amphetamine on rats performing under a variable interval (VI) 60-sec schedule of reinforcement. This effect of the MFB lesion could not be attributed to changes in catecholamine levels. Green and Harvey argued that amphetamine exerts its primary action on the catecholaminergic system, but that the latter is normally under the inhibitory control of the serotonergic system. Steranka and colleagues (43,44) showed that p-chloroamphetamine increased Sidman (free operant or nondiscriminated) avoidance responding. This increased responding was mediated through catecholamines and under inhibitory control of the serotonergic system.

Consistent with studies involving schedule-controlled behavior, numerous other studies have demonstrated an inhibitory influence of 5-HT on increased locomotor activity induced by amphetamine, apomorphine and DA. Decreased 5-HT neurotransmission caused by 5-HT receptor blockade, serotonin synthesis inhibition, electrolytic lesions of the raphe nuclei or 5-HT axons, intraventricular injection of a 5-HT neurotoxin, and tryptophan-free diets all potentiated the activity-increasing effect of d-amphetamine, apomorphine or DA (2, 3, 6, 7, 14, 15, 18, 22, 24, 35). Conversely, increases in serotonergic activity caused by pargyline and intraventricular 5-HT infusion antagonize the increase in locomotor activity induced by d-amphetamine and intracerebral DA infusion (2, 6, 50).

In summary, MDMA acted as a typical psychomotor stimulant on DRL 72-sec schedule by increasing response rate and decreasing reinforcement rate. These psychomotor stimulant effects of MDMA were enhanced four weeks after repeated administration of MDMA (6 mg/kg, twice daily for four days) which resulted in the selective long-term depletion of 5-HT. MDMA acted potently to inhibit 5-HT and DA uptake into hippocampal and neostriatal synaptosomes, respectively. Since inhibitors of DA uptake, but not NE or 5-HT uptake, have a psychomotor stimulant effect on DRL 72-sec behavior, these results suggest that 5-HT normally has an inhibitory effect on the DA-mediated effects of MDMA.

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