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Behavioral and Neurochemical Effects of Chronic Methylenedioxymethamphetamine (MDMA) Treatment in Rhesus Monkeys

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FREDERICK, D. L., S. F. ALI, W. SLIKKER, JR., M. P. GILLAM, R. R. ALLEN AND M. G. PAULE. *Behavioral and neurochemical effects of chronic methylenedioxymethamphetamine (MDMA) treatment in rhesus monkeys.* NEUROTOXICOL TERATOL **17**(5) 531–543, 1995.—Effects of chronic treatment with the putative serotonergic neurotoxicant MDMA were assessed in rhesus macaques using behavior in an operant test battery (OTB) designed to model aspects of time estimation, short-term memory, motivation, learning, and color and position discrimination. After an initial acute dose–response assessment, escalating doses of MDMA (0.10–20.0 mg/kg, im, twice daily, for 14 consecutive days at each dose) were administered, followed by three additional acute dose–response assessments. In general, tolerance to MDMA's acute effects was evident in all OTB tasks by the second week of repeated exposure to each individual MDMA dose and as doses escalated. Baseline OTB performance after chronic treatment was not significantly altered. Residual behavioral tolerance to MDMA's acute effects, however, was evident in all OTB tasks but was least pronounced in the motivation task. Monkeys were sacrificed (21 months after chronic treatment) and brains were dissected into several regions for neurochemical analyses. Serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), dopamine (DA), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) were analyzed via HPLC. Although MDMA-treated monkeys tended to have lower 5-HT concentrations in the frontal cortex, chronic MDMA treatment had no significant effects on 5-HT concentrations in any brain area sampled. Hippocampal 5-HIAA concentration, 5-HT uptake sites, and turnover of 5-HT of MDMA-treated monkeys were significantly lower than control values. DA concentrations in the CN of MDMA-treated monkeys were significantly greater than control values. No significant effects on DA concentrations were noted in any other brain area sampled. The absence of significant decreases in 5-HT and the general increase in DA concentrations are dissimilar to neurochemical effects reported after a short course of MDMA treatment at relatively high doses. These data suggest that chronic administration of gradually increasing doses of MDMA results in long-lasting tolerance to the drugs acute effects on the complex brain functions modeled in the OTB. It is uncertain, however, if such tolerance is related to the observed decreases in uptake sites and turnover of 5-HT in the hippocampus of these monkeys.

MDMA Ecstasy Monkey Operant behavior Learning Short-term memory Time estimation Motivation Color and position discrimination Food reinforcement Serotonin Amphetamine Hallucinogen

3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA or "Ecstasy") is a phenylisopropylamine amphetamine analog with both amphetamine-like (dopaminergic) and to a lesser extent, lysergic acid diethylamide (LSD)-like (serotonergic) receptor properties (22,35). Unlike LSD and other psychedelics, MDMA produces "positive" changes in affect that are not

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accompanied by intense sensory distortion or feelings of disassociation from ones self (12,25). Because of MDMA's unique actions, its utility as psychotherapeutic adjunct was quietly explored by some clinicians (11). It has since been established that MDMA produces long-lasting decreases in brain 5-HT and 5-HIAA concentrations, decreases tryptophan hydroxylase activity (the rate-limiting step in 5-HT synthesis), and causes the degeneration of fine serotonergic axon terminals in laboratory animals. These effects have been noted after MDMA has been administered sc (5,7,24) or orally (2,3,4, 8,23,31,32) twice daily at relatively high doses (10–40 mg/kg for rats; 5–10 mg/kg for monkeys) for several consecutive days (typically 4) and are frequently cited as evidence of MDMA's neurotoxicity to the 5-HT system.

Although the effects of MDMA on 5-HT systems in laboratory animals are well documented, few functional deficits have been associated with such changes (30). There are many possible reasons why behavioral alterations might not be easily detected after exposure to a putative serotonergic neurotoxicant such as MDMA. These include: the decreases in regional 5-HT concentrations are not severe enough to alter behavior; other brain areas functionally compensate; the behavior in question was not subserved by the 5-HT systems affected; the behavioral task used was not sensitive to MDMA-induced changes in the 5-HT system (33).

The present experiment was designed to determine if administration of chronic, gradually escalating doses of MDMA to rhesus monkeys would result in serotonergic neurotoxicity and/or persistent changes in specific operant behaviors that might serve as functional evidence of such toxicity. The operant test battery (OTB) used here was developed at the National Center for Toxicological Research (NCTR) and was designed to permit the simultaneous assessment of complex behaviors believed to model several different brain functions. The tasks and the brain functions they are thought to model include: temporal response differentiation (time estimation), delayed matching-to-sample (short-term memory and attention), progressive ratio (motivation to work for food), incremental repeated acquisition (learning), and conditioned position responding (color and position discrimination). Rhesus monkeys were chosen as subjects based on previous experiments from this laboratory which have demonstrated that OTB performance of well-trained rhesus monkeys is generally no different from that of children (19) and that several measures of OTB performance are significantly correlated with traditional IQ measures in children (20). The similarity in OTB performance between monkeys and children serves to reduce the uncertainty in extrapolating to humans the behavioral and neurochemical effects of MDMA exposure in primates. Additionally, previous data from our laboratory (2,3,31,32) and others (23,24) demonstrated persistent depletions in 5-HT in nonhuman primates at doses utilized in the present study.

It was hypothesized that one or more of the functions modelled in the OTB would be subserved to some extent by the 5-HT system and would therefore be sensitive to disruption by MDMA. The 5-HT system has been implicated as a significant component of MDMA's actions in several central nervous system functions. Two such functions, learning and memory, are modelled in the OTB and the involvement of 5-HT in learning and memory processes has received considerable experimental attention [see (13) for a brief review]. To explore aspects of behavioral tolerance or sensitivity that might occur in response to chronic MDMA treatment, dose-response curves were obtained for the acute effects of MDMA once before and several times after the chronic treatment regimen.

METHOD

Subjects

Three adult male rhesus monkeys (*Macaca mulatta*) between 10 and 11 years of age and weighing from 9–10 kg served as subjects. All had previously been trained to perform the tasks in the OTB for several years and had been used as subjects in previous studies on the acute effects of several psychoactive compounds (10,27,28,29). Animal housing, feeding, etc. were as previously described (26). Briefly, each monkey was individually housed and fed its daily allotment of food immediately after each test session. Water was available ad lib. Animal care and use procedures were in accordance with the American Association for Accreditation of Laboratory Animal Care (AAALAC) guidelines and approved by the NCTR Institutional Animal Care and Use Committee.

Apparatus

The apparatus have been previously described in detail (26) and consisted of portable primate restraint chairs, sound-attenuated behavioral chambers, operant panels, and computer consoles. The operant or behavioral panels were equipped with 3 rear-projection press-plates, 4 retractable levers, 6 serial position indicator lights, and correct and incorrect response indicator lights. The press-plates, levers, and indicator lights were aligned horizontally with the press-plates and serial position indicator lights located above the levers. Symbols and colors were projected onto the press-plates from the rear. When operated, both levers and press-plates effected a switch closure. Serial position and correct and incorrect indicator lights were illuminated from behind the panel with various colors. A trough for reinforcer (190 mg banana flavored food pellet) delivery was centered below the levers.

Operant Schedules

The use and description of the tasks contained in the OTB have also been reported in detail elsewhere (18,26) and a diagram of the behavioral test panel is shown in Paule et al. (21). A brief description of each task follows.

Time estimation task (temporal response differentiation; TRD). Only the left lever was extended and active. Subjects were required to hold the lever in the depressed position for a minimum of 10 s but not longer than 14 s. Releasing the lever within the 4-s window resulted in reinforcer delivery. Releasing the lever too early or too late ended the current trial, after which subjects could immediately start another trial.

Short-term memory task (delayed matching-to-sample; DMTS). Only the three press-plate manipulanda were used (levers were retracted). At the start of each trial, 1 of 7 geometric symbols (the "sample") was projected onto the center plate in a random fashion (side press-plates were dark). To continue the trial, each monkey was required to make an "observing" response (a press) to the center plate. After the observing response was made, the center plate was extinguished for 1 of 6 time delays (i.e., 2, 4, 8, 16, 32, and 48 s, presented pseudorandomly) during which all three press plates were dark. After the time delay, all three plates were illuminated, each with a different geometric symbol, only one of which matched the sample. A response to the "match" resulted in reinforcer delivery and initiation of a new trial with another sample stimulus (presented randomly). A nonmatching response was followed by a 10-s time-out period (all plates darkened) and then initiation of a new trial.

Motivation task (progressive ratio; PR). Only the far right

retractable lever was extended and active. Each monkey was required to increase the number of lever presses made for each subsequent reinforcer. Initially, 1 or 2 lever presses (depending on the individual monkey but the same for each subject every test session) resulted in reinforcer delivery. The number of responses required for the next reinforcer was increased by the initial number of lever presses required for the first reinforcer. Thus, if two lever presses were required for the initial reinforcer, four lever presses were required for the next, then 6, 8, etc. The ratio increments were chosen so that marked periods of pausing or cessation of responding generally occurred during each baseline (noninjection) or vehicle control session.

Learning task (incremental repeated acquisition; IRA). All 4 retractable levers were extended and the serial position and correct and incorrect response indicator lights were used. Subjects were required to acquire or learn a new sequence of lever presses each test session. The learning task began with the presentation of a one-lever sequence (IRA1). Each response on the correct 1 of the 4 levers resulted in reinforcer delivery. After 20 correct, but not necessarily consecutive, response sequences (criterion performance), a 1-min time-out period was followed by the presentation of an "incremented" two-lever sequence (IRA2) in which a response on a different lever was required before a response on the original (IRA1) lever produced a reinforcer. After 20 errorless (i.e., no errors were made between the first and last correct lever presses of the required sequence) two-lever sequences, the task was incremented to a three-lever sequence and so on, up to a 6-lever sequence or until the allotted task time had elapsed. The serial position indicator lights signalled position in the response sequence, indicating the remaining number of correct responses necessary for reinforcer delivery. Incorrect responses were followed by a 2-s time-out (illumination of the incorrect response indicator light) but did not reset the response requirement; thus, error correction was permitted. Correct responses were followed by illumination of the appropriate serial position indicator light and a 1-s time-out with illumination of the correct response indicator light.

Color and position discrimination task (conditioned position responding; CPR). Only the 3 press-plates were used (levers were retracted). At the start of each trial, the center plate was illuminated with either a solid red, yellow, blue, or green color (side press-plates were dark). Subjects continued the trial by making an observing response (a press) to the center plate, after which it was extinguished and the 2 side plates were immediately illuminated white. If the center plate color had been either blue or green, a response to the right press-plate (white) resulted in reinforcer delivery and initiation of a new trial. If the center press-plate had been either red or yellow, a response to the left press-plate (white) resulted in reinforcer delivery and initiation of a new trial. Responding to the incorrect position initiated a 10-s time-out period followed by the initiation of a new trial. The sequence of color presentation was random.

Behavioral Testing Procedure

Behavioral test sessions were conducted daily (Monday-Friday) and lasted approximately 50 min. Monkeys were rotated through 9 identical behavioral test chambers so that, in general, no monkey was placed in the same chamber on 2 consecutive test days. Behavioral schedules alternated daily. For example, if the motivation task (10 min), learning task (35 min), and color and position discrimination task (5 min) were presented on 1 test day, the time estimation task (20 min) and short-term memory task (30 min) were presented the next test day.

Drug and Dosing Procedure

MDMA was provided by the National Institute on Drug Abuse (Rockville, MD). The purity was determined to be >99% by high-performance liquid chromatographic analysis at NCTR. MDMA was dissolved in saline (vehicle) so that the final injection volume was 0.1 ml/kg (intramuscular; im). The chronology of MDMA administration is presented in Table 1 and described in detail below.

Acute drug studies. MDMA injections were given on Tuesdays and/or Fridays while vehicle injections were given on Tuesdays, Thursdays, and/or Fridays. Testing without prior injection was conducted on Mondays and Wednesdays. Due to the daily alternation of behavioral tasks, all MDMA doses were given twice to provide dose response data for each operant task. Approximately 30 min after injection, each monkey was placed into an operant chamber and the behavioral session began 1 min later. Inspection of cumulative response records under vehicle and drug conditions indicated that effective doses of MDMA affected responding throughout the 50-min test sessions. The specific doses administered for each

TABLE 1

CHRONOLOGY OF MDMA ADMINISTRATION

Experiment	Time Between Experiments	Duration of Experiments	Dosing Regimen (mg/kg)
Acute 1		≈ 2 Months	0.1, 0.3, 1.0, & 1.75
	2 Weeks		
Chronic		≈ 4 Months	0.1, 0.3, 1.0, 1.75*, 3.0, 5.6, 7.5*, 10.0, 15.0*, & 20.0
	≈ 5 Months		
Acute 2		≈ 2 Months	0.1, 0.3, 1.0, 1.75, 3.0, & 5.6
	≈ 5 Months		
Acute 3		≈ 2 Months	0.1, 0.3, 1.0, 1.75, 3.0, & 5.6
	≈ 5 Months		
Acute 4		≈ 2 Months	0.3, 1.0, 1.75, 3.0, & 5.6

*Denotes additional doses administered to one monkey. MDMA subjects were sacrificed 1 month after Acute 4 for neurochemical evaluation.

dose-response curve (all doses administered im in a randomized order) are as follows: Acute dose-response curve #1 (Acute 1; immediately preceding chronic exposure): MDMA doses were 0.0, 0.10, 0.30, and 1.0 mg/kg [see (9) for details]; Acute 2 (~5 months after the last chronic dose): 0.0, 0.10, 0.30, 1.0, 1.75, 3.0, and 5.6 mg/kg; Acute 3 (~5 months after Acute 2): 0.0, 0.10, 0.30, 1.0, 1.75, 3.0, and 5.6 mg/kg; Acute 4 (~5 months after Acute 3): MDMA doses were 0.0, 0.30, 1.0, 1.75, 3.0, and 5.6 mg/kg.

Chronic administration. Two of the 3 subjects received escalating doses of 0.10, 0.30, 1.0, 3.0, 5.6, 10, and 20 mg/kg and 1 subject received escalating doses of 0.10, 0.30, 1.0, 1.75, 3.0, 5.6, 7.5, 10, 15, and 20 mg/kg. All doses were administered twice daily (7 days/week) for 14 days, once at about 8:00 a.m. and again at about 4:00 p.m., with behavioral sessions conducted Monday–Friday (as previously described), 30 min after the first MDMA injection of the day. Each specific dose began on a Monday (8:00 a.m.) and was administered for 14 consecutive days, with the final administration occurring on a Sunday (4:00 p.m.); treatment with the next highest dose began the following Monday. In general, performance of each OTB task was assessed on five occasions (due to the daily alternation of behavioral schedules) at each dose. Saline injections were administered twice daily for 2 weeks prior to and at least 2 weeks after chronic exposure, with behavioral sessions conducted Monday–Friday, 30 min after the first injection of the day.

Behavioral Endpoints

The endpoints measured in each task have been described in detail elsewhere (26). Three fundamental measures were monitored for most tasks: percent task completed (PTC), response rate or latency, and response accuracy.

PTC. The PTC data are measures of a predetermined performance criteria and are functions of both response rate and response accuracy. The PTC measure is calculated by dividing the total number of reinforcers earned in a given session by the total number of reinforcers possible and multiplying this quotient by 100. The total number of reinforcers possible for a given task was chosen arbitrarily based on the length and difficulty of the task. The PTC endpoint is a convenient and comprehensive measure showing intra-animal stability and it has proven useful for comparing drug effects on performance across tasks.

Response rate and response latency. Response rate for the time estimation and motivation tasks was calculated by dividing the total number of lever presses by the total session time (in seconds). Response rate for the short-term memory, learning, and color and position discrimination tasks was calculated by dividing the total number of responses by the total session time minus time-out and delay periods (in seconds). For the short-term memory and color and position discrimination tasks, mean response latencies were also calculated for both observing and choice responses. If a monkey did not make an observing and/or choice response for 300 s, a maximum response latency of 300 s was used in the analyses. In addition to overall response rate for the learning task (collapsed across sequences of different lengths), response rates were measured for individual response sequence lengths or components within the learning task.

Response accuracy. Response accuracy for the short-term memory and color and position discrimination tasks was calculated by dividing the number of correct responses by the total number of trials in a given session and multiplying this quotient by 100. For the time estimation and learning tasks, response accuracy was calculated by dividing the total number of correct lever presses by the total number of lever presses in a given session and then multiplying this quotient by 100. Response accuracy is not applicable for the motivation task.

Other measures. For the time estimation task, mean duration of lever hold and for the motivation task, the breakpoint (the number of lever presses made to obtain the last reinforcer of a particular test session) were also calculated. Interresponse times (from press initiation to press initiation) were recorded for the motivation and color and position discrimination tasks. For the learning task, within-sequence (recall) errors and between-sequence (acquisition) errors were also recorded. Recall errors occur after the subject has entered into a response sequence (made the first correct lever press for that sequence) but before the last correct lever press for that sequence (completion of that sequence). For example, once the first correct lever of a three lever response chain is pressed, a recall error occurs every time an incorrect lever is pressed prior to reinforcer delivery (i.e., completion of the response chain). A recall error cannot occur during the one-lever sequence. Acquisition errors occur prior to the first correct lever press (entry) of a particular response sequence.

Brain Dissection

One month after determination of the last acute MDMA dose-response curve (i.e., approximately 21 months after the end of chronic treatment), each monkey was sacrificed by a lethal injection (IV) of pentobarbital and their brains were removed, quickly dissected into different regions and frozen on dry ice for later analysis of 5-HT, dopamine (DA) and their metabolites 5-hydroxyindoleacetic acid (5-HIAA), 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) concentrations as described below. Dissections occurred on an ice-cold plate according to a minor modification of the method of Brown et al. (6) using the atlas of Szebenyi (34). The cortex was removed as four large pieces: The prefrontal cortex, the premotor/precentral gyrus, the preoptic/parietal cortex, and the occipital cortex. The basal ganglia, thalamus, hypothalamus, and brain stem were removed as described by Brown et al. (6), except that the basal ganglia were further dissected into the caudate nucleus and putamen and the hippocampi were rolled out from the surrounding temporal gyrus and frozen as two entire, separate pieces, one from each hemisphere. Approximately 450 mg of the ventral caudate nucleus, 600 mg of the rostral brain stem, and the entire right hippocampus were assayed. As each sample was obtained, it was immediately frozen on dry ice. Each brain dissection was completed within 5 min of removal of the brain from the animal (31).

Determination of Neurotransmitter Concentrations

Concentrations of 5-HT, 5-HIAA, DA, HVA, and DOPAC were quantitated using a modified high performance liquid chromatography (HPLC) method combined with electrochemical (EC) detection as described by Ali et al. (1). Briefly, each individual brain region was weighed and a measured volume (20 % W/V) of 0.2 N perchloric acid containing 100 ng/mg of the internal standard 3,4-dihydroxybenzylamine (DHBA) was added. The tissue was then disrupted by ultrasonication, centrifuged (15,000 × g; 7 min), and 150 μl of the supernatant was removed and filtered through 0.2 μM Nylon-66 microfilter [MF-1 centrifugal filter, Bioanalytical System (BAS), W. Lafayette, IN]. Aliquots of 25 μl represent-

ing 2.5 mg of brain tissue were injected directly onto the HPLC/EC system for the separation of 5-HT, DA, and their metabolites 5-HIAA, DOPAC, and HVA.

The analytical system included a Waters Associates Model 510 liquid chromatographic pump (Milford, MA), a Rheodyne Model 7125 injector (Rheodyne, Inc., Cotati, CA), a Supelco Supelcosil LC-18, 3 uM (7.5 cm × 4.6 mm) analytical column, a LC-4B amperometric detector and LC-17 oxidative flow cell (BAS) consisting of a glassy carbon electrode (TL-5) versus Ag-AgCl reference electrode maintained at a potential of 0.75 V. The mobile phase consisted of 0.07 M potassium phosphate, pH 3.0, 8% methanol, and an ion pairing reagent of 1.02 mM 1-Heptane sulfonic acid. Chromatograms were recorded and integrated on a Perkin-Elmer LCI-100 integrator (Perkin-Elmer Corp., Norwalk, CT). The concentrations of 5-HT, 5-HIAA, DA, DOPAC, and HVA were calculated using standard curves generated by determining, in triplicate, the ratio between 3 different known amounts of each amine or metabolite and a constant amount of internal standard.

5-HT Uptake Site Assay

A modified procedure of Marcusson et al. (16) was used for 5-HT uptake site analysis. Tissue samples from each brain region were homogenized in 5.0 ml ice cold 0.32 M sucrose using a motor driven Teflon pestle and glass mortar. The resulting homogenate was centrifuged at 40,000 × g for 12.5 min. The supernatant was discarded and the resulting pellet washed twice by resuspension in 5.0 ml of ice cold 50 mM TRIS buffer, pH 7.4 at 25°C, with 120 mM NaCl and 5 mM KCl, and centrifuged at 40,000 × g for 12.5 min. After washing, the tissue pellet was resuspended in 100 vol of TRIS buffer. Incubations were initiated with the addition of 100 μl of tissue homogenate (40–50 μg protein) to 1.0 mM [^{3}H]paroxetine (22.00 Ci/mmol, Dupont NEN, Boston, MA) in TRIS buffer at a final volume of 2.0 ml and allowed to reach equilibrium for 1 h at 25°C. Nonspecific binding was determined in the presence of 100 μM 5-HT. Incubations were terminated with the addition of 4.0 ml ice cold TRIS buffer and filtration through glass fiber filter paper soaked in 0.1% polyethyleneimine using a 24-well cell harvester. Filters were then washed rapidly three times with 5.0 ml ice cold TRIS buffer. Filters were equilibrated with 7.5 ml scintillation cocktail and counted on a scintillation counter at a counting efficiency of approximately 50%. Protein content was determined by the method of Lowry et al. (15) using bovine serum albumin as a standard.

Statistical Analysis and Graphical Representation

For the acute and chronic behavioral studies, the overall effect of drug treatment on each behavioral endpoint in each task was determined using a one-way repeated-measures analysis of variance (ANOVA). If overall significance was evident ($p < 0.05$), then performance (each significantly affected endpoint) at each dose was compared to saline control performance (of the same endpoints) using a Bonferroni correction (17). For a subject's data to be included in the time estimation and color and position discrimination accuracy analyses, a minimum of three trials must have been completed. For inclusion in the short-term memory and learning accuracy analyses, a minimum of 10 trials must have been completed. For comparison of each escalating MDMA dose during chronic treatment, data were averaged across subjects and treatment ses-

TABLE 2

ACUTE EFFECTS OF MDMA ON OTB PERFORMANCE OF RHESUS MONKEYS PRIOR TO CHRONIC EXPOSURE (ACUTE 1)

		Dose of MDMA in mg/kg			
Task	Endpoint	Vehicle	0.1	0.3	1.0
Time estimation (TRD)	PTC	37.2 ± 11.5	44.7 ± 5.8	42.2 ± 5.6	0*
	RR	.1 ± .01	0.1	0.1	.1 ± .01
	ACC	49.8 ± 13	58.1 ± 5	67.4 ± 9.6	0*
	Avg. hold	8 ± 1	9 ± .2	10.2 ± .6	.5*
Short-term memory and attention (DMTS)	PTC	35.6 ± 7.3	26.7 ± 10.8	24.2 ± 8.7	16.1 ± 9.6
	Overall RR	.2 ± .1	0.1	.1 ± .1	.04 ± .03
	Observ. RL	4.2 ± 1.3	10 ± 5.4	8.6 ± 3.1	109.7 ± 95.2
	Choice RL	2.6 ± .3	11.3 ± 4.8	5.5 ± 2.8	9.6 ± 5.2
	Overall ACC	76.4 ± 9	73.1 ± 7.8	64.5 ± 17	75.2 ± 3.2
Motivation (PR)	PTC	20.6 ± 1.7	20.6 ± 1.7	18.6 ± .6	1.9 ± .9*
	BP	125.4 ± 17.1	129.3 ± 11.1	118.7 ± 13.8	14 ± 1.4*
	RR	2.8 ± .4	3.1 ± .2	2.5 ± .3	.1*
Learning (IRA)	PTC	83.2 ± 4	81.9 ± 13.4	95.3 ± 2.4	25.6 ± 13.1*
	Overall RR	1.5 ± .5	1.4 ± .8	2.1 ± .4	.13 ± .1
	Overall ACC	72.7 ± 3.8	71.3 ± 6.4	78 ± 1.1	51.4 ± 8.1*
Color and position discrimination (CPR)	PTC	99.1 ± .9	72.2 ± 27.8	100	40.6 ± 30.4
	RR	1.1 ± .2	.7 ± .4	1.2 ± .2	.4 ± .3
	Observ. RL	1.8 ± .3	7.1 ± 5	1.6 ± .32	101.9 ± 99
	Choice RL	0.3	.6 ± .4	0.2	1.7 ± 1.5
	ACC	98.7 ± .4	92.3 ± 7.7	99.5 ± .5	77.1 ± 22.9

*Denotes significant differnce from vehicle (saline) performance ($p < 0.05$).

PTC = percent task completed; RR = response rate (resp/sec); ACC = accuracy; RL = response latency (s); BP = breakpoint [adapted from (9)].

sions. For the neurochemical analyses, a one-tailed Student's t test ($p < 0.05$) was used to determine the significance of an effect of chronic MDMA treatment on 5-HT and 5-HIAA concentrations and on the number of 5-HT uptake sites using frozen (−70°C) brain samples from three 10- to 12-year-old female control monkeys analyzed concurrently with the MDMA-treated monkeys. In instances when 5-HT and/or 5-HIAA concentrations of MDMA-treated monkeys were greater than those values obtained from control monkeys, a two-tailed ($p < 0.05$) t test was used. A two-tailed t test ($p < 0.05$) was also used to compare the effects of chronic MDMA on DA, DOPAC, and HVA concentrations and on the turnover of 5-HT (5-HIAA/5-HT) and DA (DOPAC/DA).

RESULTS

Effects of MDMA on OTB Performance

The acute effects of MDMA before chronic treatment (Acute 1) are summarized in Table 2. Baseline (noninjection) data were not significantly different from those for saline vehicle injections for any of the behavioral endpoints monitored (not shown). In Table 2 and for all subsequent references, "overall" refers to data combined across all time delays in the short-term memory task and across all response-sequence lengths (IRA components) in the learning task. Data presented in Table 2, Table 3, and Table 4 are average values for all three subjects, ± the SEM. Note that response accuracy in the time estimation and learning tasks was disrupted at doses that did not significantly affect response rate.

The effects of each escalating MDMA dose during chronic treatment are summarized in Table 3. During the chronic treatment regimen, motivation task PTC and response rate were significantly decreased at ≥3.00 mg/kg MDMA, whereas breakpoint was only affected at the 20.0 mg/kg dose. No endpoints monitored for the time estimation, short-term memory, learning, and color and position discrimination tasks were significantly affected at doses <20.0 mg/kg when averaged over the 2-week treatment periods and response rate was not significantly affected in any of these tasks at any dose tested.

Figure 1 and Figure 2 show the effects of escalating doses of MDMA (in chronological order) on performance of the color and position discrimination and motivation tasks, respectively, and are presented as representative examples of MDMA's chronic effects on performance of all OTB tasks. During the chronic treatment regimen, tolerance was generally evident as the doses of MDMA increased, in that subjects continued to perform the OTB tasks at doses that significantly disrupted performance of most tasks during first acute dose-response assessment (Acute 1). Such an effect was evident for all primary endpoints monitored (PTC, response rate, and accuracy) and was even seen at the highest dose tested (20 mg/kg). In general, tolerance to the effects of repeated exposure to specific MDMA doses also occurred as evidenced by a decrease in task performance during the first week of administration of specific MDMA doses, followed by a recovery of behavior as exposure persisted into the second week. In most instances, vehicle (saline) performance returned to prechronic values after the cessation of chronic treatment. A notable exception was subject 23's performance in the color and position discrimination task (see Fig. 1), in which postchronic MDMA treatment saline response rate remained suppressed for at least 14 sessions; PTC and accuracy for this task, however, returned to near prechronic treatment values within a few sessions (data not shown).

The acute effects of MDMA on OTB performance during the last acute dose-response assessment (Acute 4; approx. 21 months after the end of chronic treatment) are summarized in Table 4. Baseline (noninjection) data were not significantly different from those for saline vehicle injections for any of the behavioral endpoints monitored (not shown). Except for the motivation task, chronic MDMA treatment produced a residual tolerance to MDMA's acute effects in all OTB tasks that persisted for at least 21 months post-treatment. As mentioned previously, response accuracy in the time estimation and learning tasks were differentially affected at relatively low doses prior to chronic treatment (Table 2). However, this selective sensitivity to the effects of acute MDMA exposure disappeared after chronic treatment (Table 4) Figure 3 presents an acute dose-response curve for accuracy in the learning task. Note the stability of the rightward shift in the dose-response function for this task for many months after chronic treatment. Similar, but less pronounced, rightward shifts were also noted in the dose-response curves for endpoints in the time estimation, short-term memory, and color and position discrimination tasks. In contrast to the learning task, the rightward shifts noted in the time estimation, short-term memory, and color and position discrimination tasks were greatest during the second acute dose-response assessment (Acute 2), and appeared to attenuate during the third (Acute 3) and final (Acute 4) dose-response assessments (data not shown).

Similar to the time estimation, short-term memory, and color and position discrimination tasks, a rightward shift in the dose-response curves for the motivation task (Fig. 4) was also evident during the second acute dose-response assessment (Acute 2) and became less evident during the third (Acute 3) and final (Acute 4) acute dose-response assessments. However, the lowest dose required to significantly disrupt performance of at least one major endpoint of the motivation task remained 1.0 mg/kg approximately 21 months after chronic MDMA treatment (see Table 4), while higher doses had to be administered to disrupt performance of the other OTB tasks (Table 2 and Table 4). Figure 5 and Figure 6 show interresponse time (IRT) distributions for the motivation and color and position discrimination tasks during the second acute dose-response assessment (Acute 2), respectively, and illustrate, in terms of residual tolerance, the differential effects of chronic MDMA administration on performance of these two tasks. During Acute 2, the frequency of short IRTs in the motivation task was significantly decreased at ≥1.75 mg/kg MDMA, while the frequency of short IRTs in the color and position discrimination task was not clearly affected at doses <5.6 mg/kg.

Tolerance to MDMA's acute effects after chronic treatment is also illustrated in Fig. 7, which shows the effects of acute MDMA on total errors in the learning task at the three-lever response sequence before (top panel) and after (bottom panel) chronic treatment, respectively. Before chronic treatment (Acute 1), subjects were not able to complete the 20 errorless three-lever sequences necessary to advance to the four-lever sequence learning component after receiving 1.0 of mg/kg MDMA. Failure to reach learning task level 4 indicates that subjects were completing less than 50% of this task at 1.0 mg/kg MDMA prior to chronic exposure (A six-lever sequence being the highest attainable component of the learning task). However, as Fig. 7 shows, subjects were able to complete the necessary 20 errorless three-lever sequences to advance to the four-lever response sequence (greater than 50% PTC) at ≤3.00 mg/kg of MDMA after chronic treatment (acute 4).

As shown in representative Fig. 3 and Fig. 4, post-chronic

TABLE 3

EFFECTS OF CHRONIC TREATMENT WITH ESCALATING DOSES OF MDMA ON OTB PERFORMANCE OF RHESUS MONKEYS

Task	Endpoint	Dose of MDMA in mg/kg							
		Vehicle	0.1	0.3	1.0	3.0	5.6	10.0	20.0
Time estimation (TRD)	PTC	43.6 ± 7	43.1 ± 11	39.8 ± 11.1	16.9 ± 9.5	5.2 ± 3.1	7.7 ± 3.2	6.1 ± 3.3	.2 ± .2*
	RR	.1 ± .01	.1 ± .03	.1 ± .02	.1 ± .03	.04 ± 0.3	.1 ± 0.4	.1 ± .1	.1 ± .1
	ACC	57.8 ± 6.6	56.6 ± 13.1	49 ± 11.3	28.6 ± 16	15.7 ± 3.2	17.5 ± 5.1	7.2 ± 5	.1 ± .1*
	Avg. hold	9.1 ± .2	9 ± .4	8 ± .5	5.6 ± 2.6	5.3 ± .6	6.8 ± 2	3.1 ± 1.4	6.2 ± 5.5
Short-term memory and attention (DMTS)	PTC	34.7 ± 11.3	33.3 ± 7.4	30.5 ± 3.9	19.8 ± 9.3	16 ± 8.5	19.6 ± 8.2	28.6 ± 13.1	2.4 ± 1.3
	Overall RR	.2 ± .04	.2 ± .1	.2 ± .1	.1 ± .1	.1 ± .04	.1 ± .03	.2 ± .1	.02 ± .01
	Observ. RL	14.2 ± 11	4.6 ± .2	6 ± 2.1	52.4 ± 44.5	99.9 ± 65.7	69.4 ± 37.2	44.5 ± 42	185 ± 16.3*
	Choice RL	3.5 ± 1.9	2.6 ± 1.2	3.1 ± 1.3	20.4 ± 11.7	8.5 ± 3.3	10.4 ± 5.7	7.5 ± 5.7	22.2 ± 7.6
	Overall ACC	75.3 ± 11.2	78.5 ± 9.5	73.6 ± 7	66.5 ± 9	77.4 ± 1.1	69.9 ± 9.4	69.7 ± 9.4	39.7 ± 10.8
Motivation (PR)	PTC	19 ± .4	15.7 ± .2	15.4 ± .6	13.4 ± 2.4	6.1 ± 2.5*	10.1 ± 1.3*	11 ± 1.6*	6.9 ± 3*
	BP	122.2 ± 17.2	100.4 ± 12.7	97.9 ± 8.9	82.7 ± 7.7	41.9 ± 19.6	66.4 ± 15.3	72.9 ± 17.8	41.5* 18.7
	RR	2.7 ± .4	2.1 ± .2	1.9 ± .2	1.4 ± .2	.6 ± .4*	1 ± .3*	1.1 ± .4*	.6 ± .3*
Learning (IRA)	PTC	68 ± 11.9	66.8 ± 12.6	74.3 ± 6.9	71.8 ± 6.2	70.5 ± 12.1	61.2 ± 16.7	57.7 ± 3.5	32.8 ± 4.8*
	Overall RR	1 ± .2	1.2 ± .1	1.3 ± .5	1.1 ± .4	.8 ± .2	.9 ± .3	1 ± .1	.5 ± .2
	Overall ACC	69.3 ± 5.7	62.3 ± 12.6	72.4 ± 5	74 ± 5.4	66 ± 7.9	63 ± 8.1	53.4 ± 5.2	44.1 ± 2.5*
Color and position discrimination (CPR)	PTC	94.3 ± 5.7	82 ± 10	93.4 ± 6.6	93.6 ± 6.4	91 ± 9	68.9 ± 15.7	72.7 ± 9.5	40.2 ± 5.2*
	RR	1 ± .1	.9 ± .1	1 ± .1	1 ± .2	.8 ± .1	.8 ± .2	.1 ± .1	.4 ± .1
	Observ. RL	1.8 ± .2	7.7 ± 4.3	11.7 ± 9.9	6.5 ± 5	6.9 ± 4.8	15.5 ± 13.4	26 ± 6	76.8 ± 17.6*
	Choice RL	.3 ± 0.3	.3 ± 0.3	.2 ± 0.2	.3 ± .1	.3 ± 0.4	.6 ± .3	.5 ± .1	1.7 ± .13
	ACC	94.3 ± 3.8	89.7 ± 6	98.7 ± 8	97.5 ± 2.1	96 ± 2.7	85.5 ± 7.7	80.6 ± 5.4	78.5 ± 8.9*

*Denotes significant difference from vehicle (saline) performance ($p < 0.05$).
PTC = percent task completed; RR = response rate; RL = response latency; ACC = accuracy; BP = breakpoint.
Data averaged over 2 weeks for each dose shown.

TABLE 4

ACUTE EFFECTS OF MDMA ON OTB PERFORMANCE OF RHESUS MONKEYS AFTER CHRONIC EXPOSURE (ACUTE 4)

Task	Endpoint	Dose of MDMA in mg/kg					
		Vehicle	0.3	1.0	1.75	3.0	5.6
Time estimation (TRD)	PTC	33.6 ± 2.7	16.5 ± 7.8	23.3 ± 15.4	7.9 ± 6.1	1.4 ± 1.4	.1 ± .1
	RR	.1 ± .01	.1 ± .01	.1 ± 0.3	.1 ± .05	.1 ± .1	.04 ± .03
	ACC	47.2 ± 3	28.2 ± 12	32.7 ± 11.3	18.1 ± 13.6	22 (1)	11.5 (1)
	Avg. hold	7.9 ± .4	6.6 ± 2	7.8 ± .1	5.4 ± 2.1	5.5 ± 2.6	6.5 ± 2.6
Short-term memory and attention (DMTS)	PTC	28.8 ± 5.9	224.6 ± 6.8	21.3 ± 8.3	14.7 ± 1.4	21 ± 5.6	2.4 ± 2.2*
	Overall RR	.2 ± .05	.1 ± 0.4	.1 ± .1	.1 ± .06	.1 ± .1	.03 ± .02
	Observ. RL	13.1 ± 6.4	24.3 ± 15	57.2 ± 50.4	36 ± 9.6	54.6 ± 51.6	175.2 ± 66.1*
	Choice RL	4.9 ± 2.3	10.7 ± .5	5.8 ± 4.3	7.4 ± 1.4	40.6 ± 38.8	15 ± 15.6
	Overall ACC	76.4 ± 8.7	74.5 ± 5.3	72.6 ± 8.1	65.9 ± 8.3	74.3 ± 7.9	55 (1)
Motivation (PR)	PTC	15.4 ± .3	13.9 ± 1.2	8.5 ± 2.9	3.9 ± 2.4*	8.3 ± 2.3	4.3 ± 3*
	BP	122.6 ± 14.4	110 ± 13.9	61.7 ± 13.6	26 ± 14.5*	62 ± 11	28.2 ± 17.4*
	RR	2.3 ± .2	1.9 ± .3	.8 ± .4*	.3 ± .2*	1.1 ± .3	.4 ± .3*
Learning (IRA)	PTC	65.1 ± 2.6	70 ± 12.8	74.4 ± 2.7	59.7 ± 9.9	46.7 ± 11	29 ± 16.5
	Overall RR	1.1 ± .2	.7 ± 0.4	1.5 ± .8	.7 ± .3	.8 ± .5	.7 ± .6
	Overall ACC	64.3 ± 4.9	66.5 ± 13.4	71 ± 3.7	64.9 ± 10.5	65.9 ± 3.4	37.1 ± 15.3
Color and position discrimination (CPR)	PTC	79.7 ± 5.7	81.9 ± 18.1	88.6 ± 11.4	85.8 ± 7.3	71.4 ± 14.9	52.2 ± 26.9
	RR	.8 ± .1	.7 ± .1	1 ± .3	.9 ± .3	.8 ± .3	.5 ± .3
	Observ. RL	3.9 ± .7	2.6 ± .2	2.6 ± 1.1	3 ± 1.1	52 ± 49.5	104 ± 51.3
	Choice RL	.3 ± 0.2	.3 ± 0.2	.2 ± 0.3	.3 ± 0.4	.2 ± 0.4	.7 ± .4
	Overall ACC	88.4 ± 5.3	87.3 ± 12.3	94.8 ± 5.2	93.9 ± 5.3	91.2 ± 8.4	87.3 ± 10.4

*Denotes significant difference from vehicle (saline) performance; PTC = percent task completed; RR = response rate; RL = response latency; ACC = accuracy; BP = breakpoint; $N = 3$ unless otherwise noted.

baseline OTB performance was generally no different than prechronic baseline performance during any of the acute phases of the experiment. Also, an examination of pre- and postchronic learning task errors (Fig. 7) shows that the pre- and postchronic 95% confidence intervals (constructed from vehicle test sessions) were generally not different.

Neurochemical Analysis

5-HT and 5-HIAA concentrations. Figure 8 shows the effect of chronic MDMA administration on regional 5-HT and 5-HIAA concentrations in the frontal cortex, hippocampus, and caudate nucleus of these same monkeys when sacrificed 21 months after the cessation of treatment. While there were no statistically significant effects, there was a trend toward lower 5-HT concentrations in the frontal cortex and caudate nucleus of MDMA-treated monkeys, and 5-HT concentrations in the hippocampus were slightly elevated. 5-HIAA concentrations in the hippocampus were significantly decreased ($p < 0.05$; one-tailed), while concentrations in the frontal cortex and caudate nucleus were only slightly decreased.

DA, DOPAC, AND HVA concentrations. No significant changes in DOPAC or HVA concentrations were noted in any brain area sampled. DA concentrations in the caudate nucleus of MDMA-treated monkeys, however, were significantly elevated ($p < 0.05$; two-tailed) from 522.45 ± 179.2 (control) to 1368.96 ± 113.244, and there was a trend toward higher DA concentrations in the frontal cortex and hippocampus.

5-HT uptake sites. There was a trend toward a decrease in the number of 5-HT uptake sites in all brain areas sampled in MDMA-treated monkeys (Fig. 9). These effects were, however, only significant in the hippocampus ($p < 0.05$; one-tailed).

5-HT and DA turnover. In MDMA treated monkeys, the turnover of 5-HT in the hippocampus was significantly decreased ($p < 0.05$; two-tailed) from 0.92 ± 0.18 (control) to 0.25 ± 0.09, while a slight trend toward increased turnover was noted in the frontal cortex and caudate nucleus. There was also a trend toward lower DA turnover in all brain areas sampled, but these effects were not significant.

DISCUSSION

During chronic treatment with MDMA, behavioral tolerance was evident in all operant tasks as the doses increased, and after repeated exposures to the same dose. A residual tolerance to MDMA's acute effects was also observed and appeared to persist (for some behavioral tasks) for at least 21 months after cessation of chronic treatment. In general, little or no change in baseline levels of operant performance were noted in any task after chronic MDMA exposure. It did appear, however, that a differential tolerance developed to MDMA's acute effects, as evidenced by the observation that the order of OTB task sensitivity to disruption by acute MDMA treatment changed after chronic MDMA exposure. Brain 5-HT concentrations in rhesus monkeys treated with gradually escalating doses of MDMA over an extended period of time were not significantly lower than control values in any brain area sampled, and 5-HIAA concentrations were significantly decreased only in the hippocampus. These neurochemical effects (or lack thereof) were observed even though the doses given in the present experiment were much higher than those reported to produce long lasting depletions in brain 5-HT and 5-HIAA in rhesus monkeys when administered over a short course of exposure (2,3,4,14,31).

All subjects displayed tolerance to MDMA's effects on per-

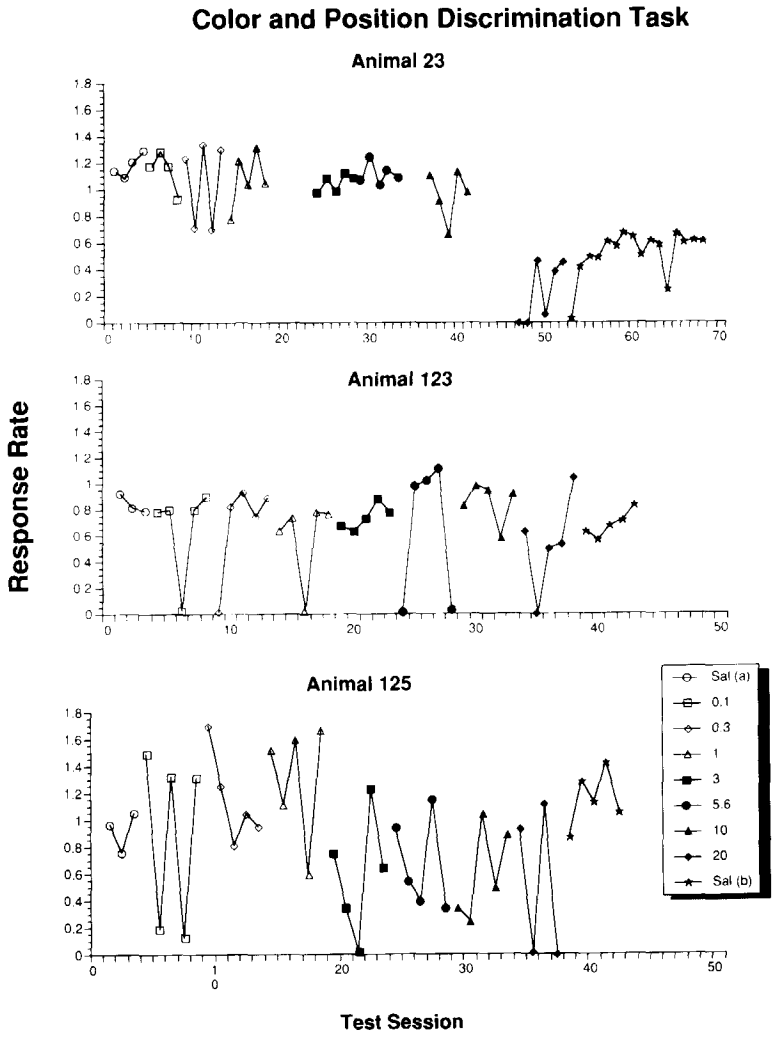

FIG. 1. Effects of escalating doses of MDMA given chronically on response rate of individual monkeys performing the color and position discrimination task. Data are presented in chronological order. Data from vehicle sessions precede [Sal(a)] and immediately follow [Sal(b)] chronic data.

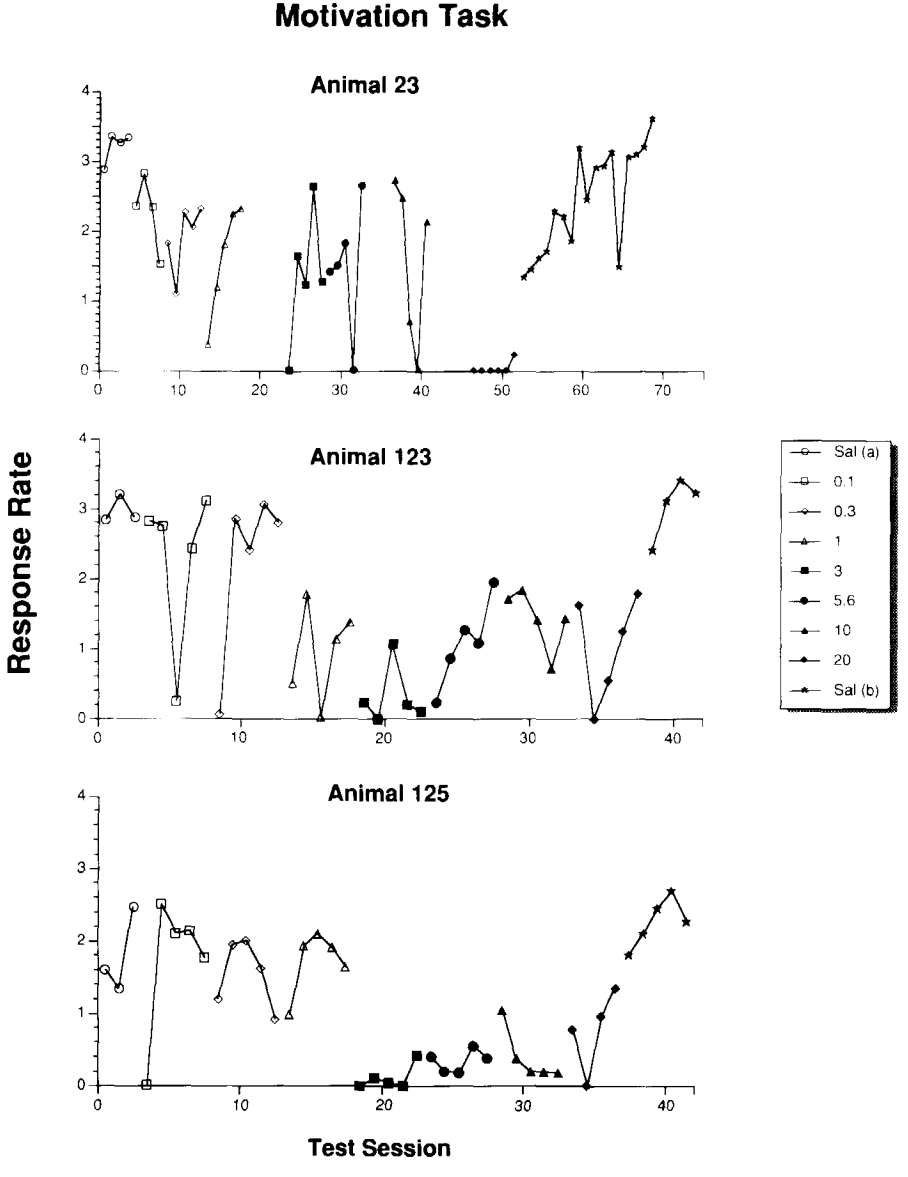

FIG. 2. Effects of escalating doses of MDMA on the response rate of individual monkeys in the motivation task. Data presented as in Fig. 1.

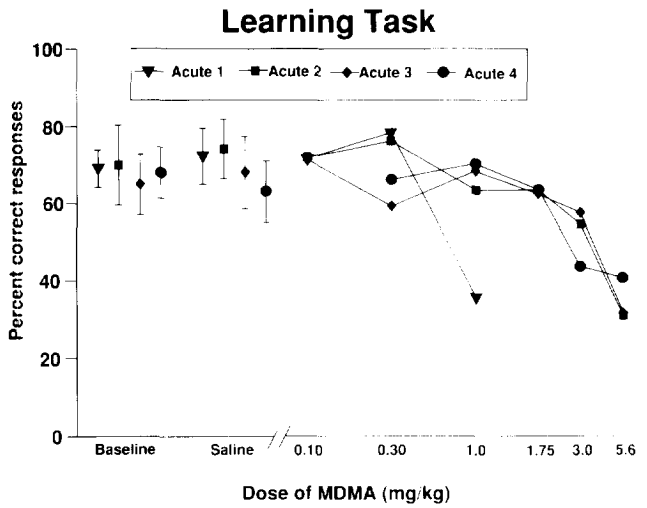

FIG. 3. Acute dose-response curves for learning task accuracy obtained prior to chronic MDMA treatment (acute 1) and at approximately 6 (acute 2), 12 (acute 3), and 18 (acute 4) months after chronic MDMA treatment.

formance of the OTB tasks during the chronic regimen as the doses increased. Prior to chronic treatment, acute exposure to 1.00 mg/kg of MDMA disrupted performance of most OTB tasks. However, during the chronic regimen, monkeys continued to perform all OTB tasks even as the doses increased to 20 mg/kg. In fact, at the highest dose of 20.0 mg/kg MDMA, the only significant effects on response rate were seen in the motivation task. Tolerance was also evident as repeated exposure to the same MDMA dose continued. In general, a trend emerged where disruption of OTB task performance was observed during the first week of exposure to each specific dose, but behavioral tolerance developed as administration of each dose continued into the second week.

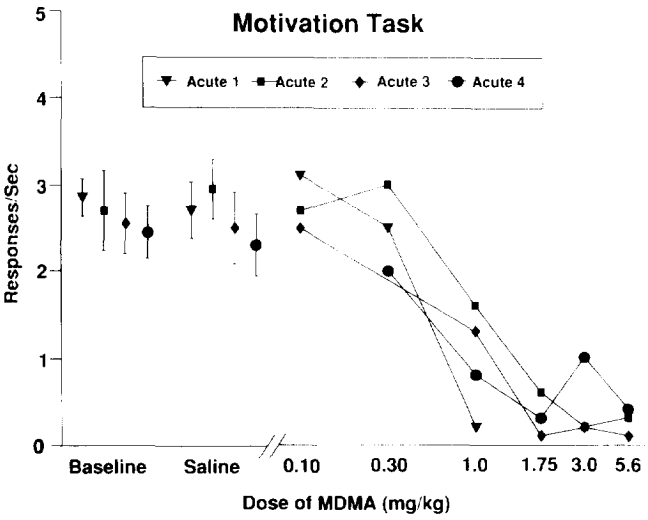

FIG. 4. Acute dose-response curves for motivation task response rate. Data presented as in Fig. 3. Note the minimal residual tolerance to MDMA's acute effects in this task compared to that of the learning task (Fig. 3).

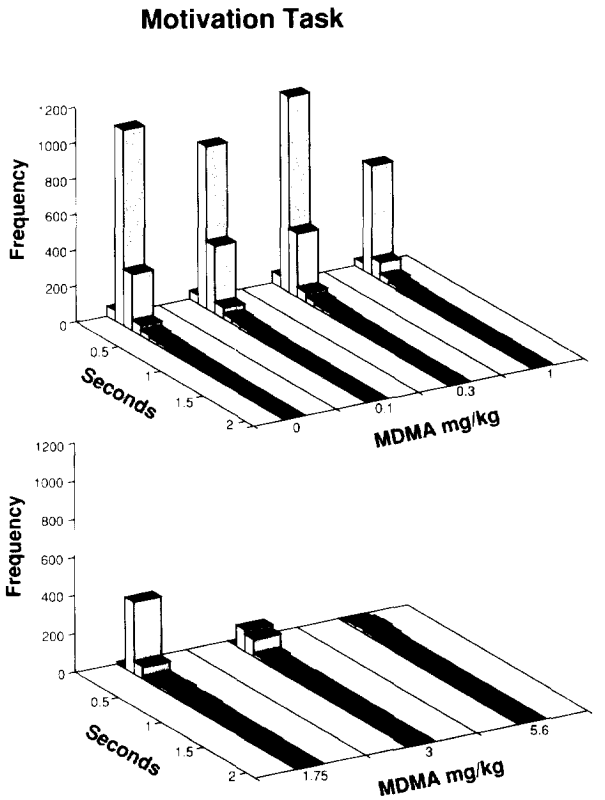

FIG. 5. Inter-response time distributions (IRTs) for the motivation task obtained during Acute 2. Data are means for all three subjects.

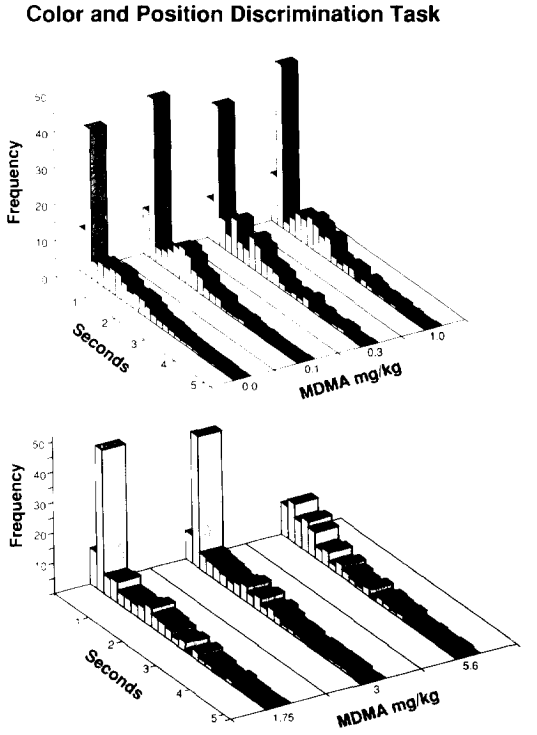

FIG. 6. Inter-response time distributions (IRTs) for the color and position discrimination task obtained during Acute 2. Data are means for all three subjects.

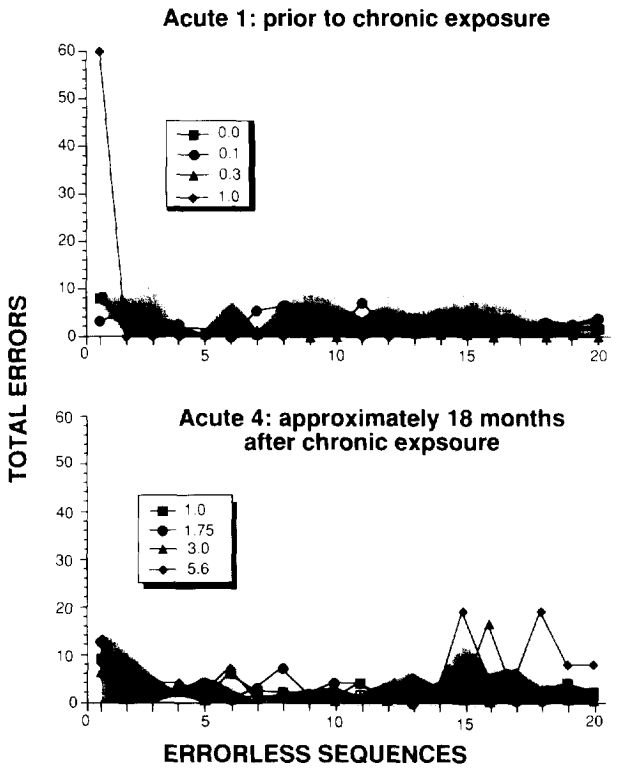

FIG. 7. Acute effects of MDMA on total errors in the learning task for three-lever sequences prior to (Acute 1; top panel) and after (Acute 4; bottom panel) chronic MDMA treatment (the 0.3 mg/kg dose of MDMA produced no significant effect on total errors in this task during Acute 4 and is not presented). Data are means for all three subjects. The shaded area represents the 95% confidence interval constructed from data for vehicle control sessions.

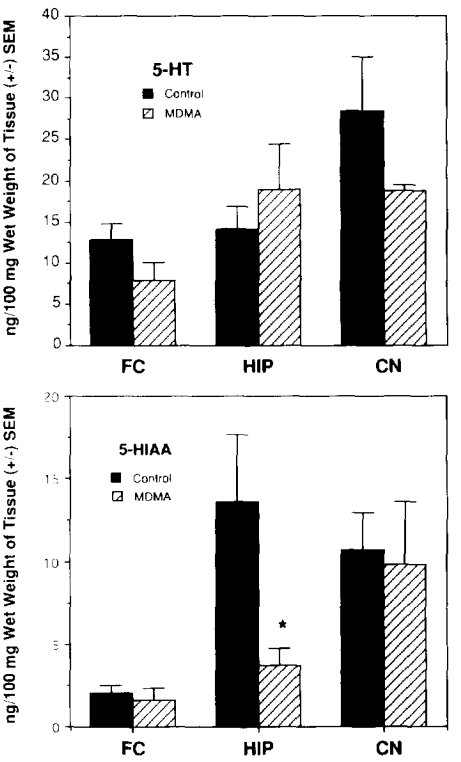

FIG. 8. Effects of chronic MDMA on the concentration of 5-HT (top panel) and 5-HIAA (bottom panel) in the frontal cortex (FC), hippocampus (HIP), and caudate nucleus (CN) of control (n = 3) and MDMA-treated (n = 3) monkeys.

Residual tolerance to MDMA's acute effects on OTB performance was evident in all OTB tasks during the second dose-response assessment (Acute 2), and for at least 21 months after chronic exposure (Acute 4) in the learning task. Little, if any, residual tolerance was evident in the motivation task during the determination of the third (Acute 3) and fourth (Acute 4) dose-response curves. Prior to chronic treatment, response accuracy was generally more sensitive to the acute effects of MDMA than was response rate in the time estimation and learning tasks. During the determination of the final acute dose response-curve, however, accuracy was not significantly affected in any OTB task at any dose tested and response rate was only significantly affected in the motivation task.

In the present experiment, baseline performance of each OTB task before and after chronic MDMA treatment was not significantly different although the relative sensitivity of individual OTB tasks appeared to change after chronic MDMA exposure. Using the occurrence of a significant disruption in task performance at doses lower than those affecting other tasks as a criteria for determining relative task sensitivity, the order of sensitivity prior to chronic treatment (Acute 1) was: time estimation task = motivation task = learning task > short-term memory task = color and position discrimination task. After chronic MDMA (Acute 4), however, the order of sensitivity was motivation task > short-term memory task > time estimation task = learning task = color and position discrimination task. The development of differential tolerance to MDMA's acute effects suggests that the tasks contained in the OTB are subserved by mechanisms that are also differentially sensitive to chronic MDMA exposure.

Decreased concentrations of 5-HT and 5-HIAA, and decreased 5-HT uptake sites are frequently cited as evidence of

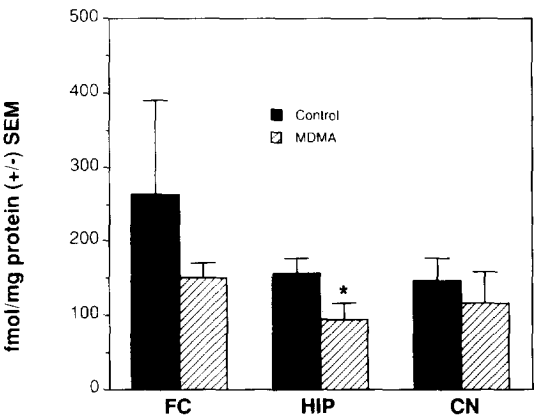

FIG. 9. Effects of chronic MDMA on regional 5-HT uptake sites in the FC, HIP, and CN (abbreviations and n's as in Fig. 8).

MDMA-induced neurotoxicity to the 5-HT system and such decreases have been shown to occur in rhesus monkeys after twice daily oral administration of MDMA at doses ranging from 2.5–20.0 mg/kg for 4 consecutive days (2,3,4,14,31). In the present experiment, there was a trend toward lower 5-HT concentrations in the frontal cortex and caudate nucleus of MDMA-treated monkeys, but these effects were not significant and hippocampal 5-HT concentrations were actually slightly higher. 5-HIAA concentrations were significantly decreased in the hippocampus of MDMA-treated monkeys, but were only slightly lower in the frontal cortex and caudate nucleus. 5-HT uptake sites were decreased in all brain areas, but this effect was only significant in the hippocampus.

In general, few consistent effects on the DA system have been reported after exposure to a short course of MDMA administration at relatively high doses (2,3,14,23,24,32). In the present experiment a significant increase in DA concentration was observed in the caudate nucleus and there was a trend toward higher DA concentrations in the frontal cortex and hippocampus as well. The increases in DA concentrations in the frontal cortex and hippocampus were, however, not significant and the DA concentrations in the caudate nucleus of the control monkeys in the present experiment were somewhat lower than those previously reported for other control animals from this laboratory (3,32)

In the hippocampus, MDMA-treated monkeys had increased 5-HT concentrations and a lower 5-HT turnover as compared to controls. This effect may be related to the significant decrease in hippocampal 5-HT receptor uptake sites, or to the possibility that 5-HT was not being metabolized by monoamine oxidase (MAO) with the same efficiency as in control monkeys. Such an effect would suggest a possible mechanism of MDMA's putative psychotherapeutic action. However, if chronic MDMA had decreased the efficacy of MAO, higher 5-HT levels should also have been observed in the other brain areas sampled. In the present experiment, 5-HT concentrations were lower in the frontal cortex and caudate nucleus of MDMA-treated monkeys, but the 5-HT turnover in these brain areas did not significantly differ from control values. Additionally, neither of these brain regions had significantly lower 5-HT uptake sites as compared to controls.

The present neurochemical data suggest that in rhesus monkeys, twice daily administration of gradually escalating doses of MDMA (0.1–20 mg/kg) over a period of months is not as neurotoxic (in terms of reduction in brain 5-HT and 5-HIAA and decreased 5-HT uptake sites) as exposure to 5–20 mg/kg administered twice daily for 4 consecutive days. Thus, the residual behavioral tolerance noted after chronic MDMA exposure occurred in the absence of many common biomarkers of MDMA-induced neurotoxicity. It must be emphasized, however, that these neurochemical data were collected nearly 2 years after exposure to gradually escalating doses of MDMA and that the control monkeys used in the present experiment were all females, whereas all treated monkeys were males. Additionally, because tryptophan hydroxylase activity was not monitored and no histological evaluations were conducted in the present experiment, the effects of the current treatment regiment on these markers of MDMA-induced serotonergic neurotoxicity remain unknown. Whether the residual behavioral tolerance noted in the present experiment can be attributed to neurochemical changes in the hippocampus and/or alterations in the DA system is not known.

In summary, repeated exposure to each escalating dose of MDMA produced behavioral tolerance to MDMA's disruptive effects on OTB performance and tolerance was also evident as the doses increased. When operant performance was assessed 21 months after chronic treatment, a residual behavioral tolerance to MDMA's acute effects was evident, but no significant difference in baseline operant performance was observed. Although the doses administered in the present experiment were much higher than those previously reported to produce long lasting depletions in 5-HT and 5-HIAA when administered over a short course of exposure, 5-HT concentrations of MDMA-treated were not significantly decreased in any brain area sampled, and 5-HIAA concentrations were significantly decreased only in the hippocampus. Thus, the behavioral tolerance noted in the present experiment appeared to be mediated by mechanisms distinct from those typically associated with MDMA-induced neurotoxicity noted in animals sacrificed shortly (1–4 weeks) after repeated exposure to high doses of MDMA.

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