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Behavioral and Neurochemical Effects of Orally Administered MDMA in the Rodent and Nonhuman Primate

W. SLIKKER, JR.[1,2,4], R. R. HOLSON[1], S. F. ALI[1,2,3], M. G. KOLTA[1], M. G. PAULE[1,2,4], A. C. SCALLET[1,2], D. E. MCMILLAN[2], J. R. BAILEY[1], J. S. HONG[5] AND F. M. SCALZO[1]

[1]Division of Reproductive and Developmental Toxicology, National Center for Toxicological Research, Jefferson, Arkansas 72079; [2]Department of Pharmacology and Toxicology, [3]Department of Biochemistry and Molecular Biology, [4]Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, Arkansas 72205; [5]Laboratory of Molecular and Integrated Neurobiology, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina 27709

ABSTRACT: *MDMA (methylenedioxymethamphetamine) is a recreational drug of abuse known as "Ecstasy"which markedly decreases regional brain serotonin (5-HT) content and produces 5-HT nerve terminal degeneration in forebrain areas of the rat. In order to determine the acute and chronic behavioral effects of MDMA, adult rats were given MDMA at 0, 5 or 10 mg/kg, po for 4 consecutive days. Alternatively, parachloroamphetamine (PCA) at 5 mg/kg was administered under the same regimen. Within 30 min after the first dose, the MDMA-treated rats exhibited the serotonin motor syndrome consisting of straub tail and splayed hindlimbs comparable to that seen in the PCA-treated rats. This serotonin motor syndrome, with a duration of about 2 hr, was less pronounced after subsequent doses. At 2 - 4 wk after the last dose, no significant differences between control and treated rats were seen in emergence, hot plate response, auditory startle response or complex maze behavior even though a significant dose-related decrease (50%) in 5-HT concentration was observed in the frontal cortex and hippocampus of these rats 4 wks after the last dose. Adult female monkeys dosed po with 5 or 10 mg/kg of MDMA twice/day for 4 consecutive days demonstrated no spontaneous behavioral changes or weight loss compared to controls, but forebrain 5-HT concentration was reduced by 80% 1 mon after dosing. These data indicate that at doses only 2 - 3 times the human dose, MDMA produces significant forebrain 5-HT decreases but does not produce detectable residual behavioral alterations as assessed by these behavioral paradigms.*

Key Words: MDMA (Methylenedioxymethamphetamine), "Ecstasy", Serotonin, Parachloroamphetamine (PCA), Behavioral Effects, Neurochemistry, Rats, Monkeys

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Please send requests for reprints to Dr. William Slikker, Jr., Division of Reproductive and Developmental Toxicology, National Center for Toxicological Research, Jefferson, Arkansas 72079 USA.

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INTRODUCTION

Methylenedioxymethamphetamine (MDMA) administration to rodents or nonhuman primates results in a longlasting decrease in serotonin (5-HT) and its metabolite (5-hydroxyindoleacetic acid, 5-HIAA) concentrations in selective brain regions (primarily the frontal cortex, hippocampus and caudate nucleus) after either sc or oral administration (Slikker *et al.*, 1986; Schmidt, 1987; Finnegan *et al.*, 1988; Slikker *et al.*, 1988). Neuropathological studies with MDMA (Commins *et al.*, 1987; Scallet *et al.*, 1988) have demonstrated serotonin nerve terminal degeneration in these same brain regions. Other studies have demonstrated that MDMA treatment inhibits tryptophan hydroxylase activity (Gibb *et al.*, 1986) and decreases the density of 5-HT uptake sites (Battaglia *et al.*, 1987). These MDMA-induced effects are reminiscent of the effects produced by parachloroamphetamine (PCA, a substituted amphetamine with a chemical structure similar to MDMA) and related analogs (Harvey *et al.*, 1975; Fuller, 1985). Although MDMA drug discrimination (Schechter, 1986) and self-administration (Beardsley *et al.*, 1986) studies have been conducted in animal models, a systematic behavioral assessment of the acute and chronic effects of MDMA has not been reported.

PCA has been assessed for acute and chronic behavioral alterations (Lorens, 1978; Sanders-Bush *et al.*, 1975). Both acutely and at 24 hr post-injection, PCA has been reported to increase pain thresholds, as measured by the paw-compression test (Gorlitz and Frey, 1972). Open-field locomotor activity scores of PCA-treated rats were decreased from 1-30 days after injection (Vorhees *et al.*, 1975). PCA administration has also been reported to facilitate acquisition of a discriminated Y-maze conditioned avoidance response (Vorhees *et al.*, 1975). PCA treatment failed to produce conditioned avoidance response alterations in several other studies, however, prompting Lorens (1978) to conclude that conditioning procedures and apparatus are critical variables in the assessment of PCA-induced behavioral effects.

Because of the reported neurochemical and neuropathological similarities between MDMA and PCA, this study was designed to extend these comparisons through several behavioral assessments in the rat. Based on the known behavioral effects of PCA (Lorens, 1978; Davis and Sheard, 1976; Lints and Harvey, 1969), we selected motor activity, auditory startle habituation, complex maze activity, emergence, analgesia and conditioned punished behavior to evaluate the acute and chronic effects of MDMA administration in the rat. Neurochemical assessments of brain 5-HT, 5-HIAA and catecholamine concentrations were also conducted on MDMA- and PCA-treated rats to compare these endpoints to any behavioral effects produced by these two different serotonergic neurotoxicants. Finally, the overt behavioral and neurochemical effects of MDMA were determined in the rhesus monkey in order to begin the process of species extrapolation.

METHODS

Chemicals

MDMA was provided by the National Institute on Drug Abuse (Rockville, MD). The structure was confirmed by chemical ionization mass spectrometry at NCTR. The purity and stability of the MDMA (> 99%) was determined by high-performance liquid chromatography (HPLC) (Gollamudi *et al.*, 1988). Parachloroamphetamine (PCA) was purchased from Sigma Chemical Co. (St. Louis, MO) and used as received. Chemicals and solvents used were HPLC grade.

Experimental Design (Rodent Studies)

Subjects. Forty adult male Sprague-Dawley rats (body weight 300 ± 20 gm) and forty adult female Sprague-Dawley rats (body weight 250 ± 20 gm) from the NCTR colony were used in the studies. The rats were individually housed and maintained on Purina Rodent Laboratory Chow and filtered water *ad libitum*. Animal rooms were maintained at

23 ± 3°C and 50 ± 10% humidity with a 12 hr light/dark cycle (lights on at 0600 hr).

Dosing. The rats were dosed by oral gavage once each morning for four consecutive days with 5 or 10 mg/kg MDMA, 5 mg/kg PCA or deionized water vehicle (n = 10/group). Assessment of spontaneous behavior was conducted after each of the daily doses. The other behavioral assessments were conducted from 2-4 weeks after MDMA treatment. The rats were sacrificed for neurochemical assessment 4 weeks after treatment.

Assessment of Spontaneous Behavior. Nine rats from each of the four treatment groups were individually observed in a lighted viewing chamber (45 x 45 x 60 cm) with a glass front, opaque sides and a floor inscribed with a 3 x 3 grid (the grid consisted of nine 15 x 15 cm squares). Six such chambers were arranged so that observers could score the spontaneous behavior of six rats sequentially. Each rat was observed over a 60 min period beginning 10 min after dose administration on each of 4 days. For 20, 15-sec observation periods, the incidence of the serotonin motor syndrome (i.e., splayed hindlimbs, straub tail, abdominal contact with the floor, side-to-side motion of the head, urination and/or salivation) was recorded by an observer. The maximal serotonin motor score achievable was 20. During the same 20, 15-sec observation periods, the number of square entries, as designated by the floor grid, was recorded. A repeated measures ANOVA was used to evaluate dose effects on the intensity of the serotonin motor syndrome score and the general motor activity score.

Auditory Startle. Auditory startle habituation was measured in 40 males. During this test, each rat was placed in a wire mesh chamber and was repeatedly presented with 4000 Hz tones delivered at 120 dBA against a 60 dBA background noise level (Adams *et al.*, 1985). An accelerometer attached to the chamber recorded cage movement immediately following each tone presentation. The resultant voltage was amplified and fed through an A to D converter and then to a microprocessor. A total of 50 tones were presented and 50 responses recorded. For each rat, means were taken of each successive 10 trials, resulting in five, 10-trial means/rat. A repeated measures ANOVA was used to evaluate dose effects on startle amplitude and habituation.

Emergence. This test and statistical analysis has been described elsewhere (Holson *et al.*, 1988). Briefly, subjects were placed individually in an open runway for a single 20-min trial. If subjects moved to the end of the runway, they could enter a large, dark chamber. At any time during the 20-min trial, subjects could emerge from the dark chamber into the runway, then re-enter the chamber. Variables included latency to enter the darkened chamber, latency to first emergence, and total number of emergences.

Complex Maze. The apparatus and method are described elsewhere in this volume (Holson *et al.*, 1989). All subjects were tested individually for three consecutive daily 15-min sessions. Twenty-four hr prior to the first trial, food was removed from the home cage. Thereafter, until completion of the third session some 72 hr later, the only food obtainable was in the maze. In the maze, food was always available in arm 15. Placing the subject in arm 4, the start box, automatically started the first trial in a session. Thereafter, behavior was automatically recorded until the rat actually obtained and began eating one of the 10 Purina lab chow pellets placed in arm 15. The rat was allowed to eat for 10 sec, after which the experimenter terminated the trial, captured the rat, and started the next trial by replacing the rat in the start box (arm 4). Total time per session (15 min) did not include the inter-trial time taken to capture the rat and return it to the start box.

The data produced by this apparatus allowed quantitation of a number of variables, including total arm entries, total goal entries, total successful trials, time spent in each maze region or arm, maze arm entries, errors at each arm or choicepoint and latencies to goal, all by session. The statistical analysis is described elsewhere in this volume (Holson *et al.*, 1989).

Hot Plate Test. In this test, each rat was placed inside a plexiglass cylinder (34 cm diameter x 48 cm high) on the top of a heated metal box connected to a circulating water bath where the water temperature and the surface of the box were maintained at 54 - 55°C. The endpoints of the analgesic tests were: 1) licking of the hind paws; 2) tapping on the surface of the heated box; or 3) attempting to jump out of the cylinder cage. The cutoff time was 45 sec. The hot plate test was repeated every four min for 12 consecutive trials over a 44 min period. Ten rats per treatment group (5 males and 5 females) were assessed. Significant differences for the analgesic responses were determined between groups by a repeated measured analysis of variance.

Operant Behavioral Study. Seven additional adult male Sprague-Dawley rats were conditioned to respond under a multiple fixed interval (FI) 90 sec FI 90 sec schedule with punishment. When a sonalert signal was on, the first response after 90 sec elapsed produced food. When the sound stimulus was off, the same food contingency prevailed, but every 5th response during this component also produced a 100 msec, 0.1 mAmp electric shock scrambled through the floor grids. Punishment reduced responding to about 50% of control (unpunished) rates. On test days, rats were dosed by oral gavage with 0, 5, or 10 mg/kg MDMA and assessed for this operant behavior 20 min later.

Experimental Design (Monkey Studies). Adult female rhesus monkeys ranging in age from 8 to 15 years and in weight from 4.2 to 9.5 kg were maintained as previously described (Slikker *et al.*, 1988). A total of nine monkeys (three per group) were dosed with water vehicle or 5 or 10 mg/kg MDMA by gastric intubation twice per day for four days as previously described (Slikker *et al.*, 1988). Body weights were recorded the week before dosing and three days after the last dose. Spontaneous behaviors were assessed with the use of a behavior check list (Brocco and Slikker, 1976; Slikker *et al.*, 1976). The monkeys were observed in their home cage for 15 min before dosing and for up to four hr after dosing on each of the four days after the morning dose. One month after the last dose of MDMA, the monkeys were overdosed with pentobarbital and their brains quickly dissected into different regions including frontal cortex, hippocampus, caudate nucleus and brain stem as previously described (Slikker *et al.*, 1988).

Neurochemistry. Monoamines and their acid metabolites were quantified in selected brain regions of both rats (n = 5 per treatment

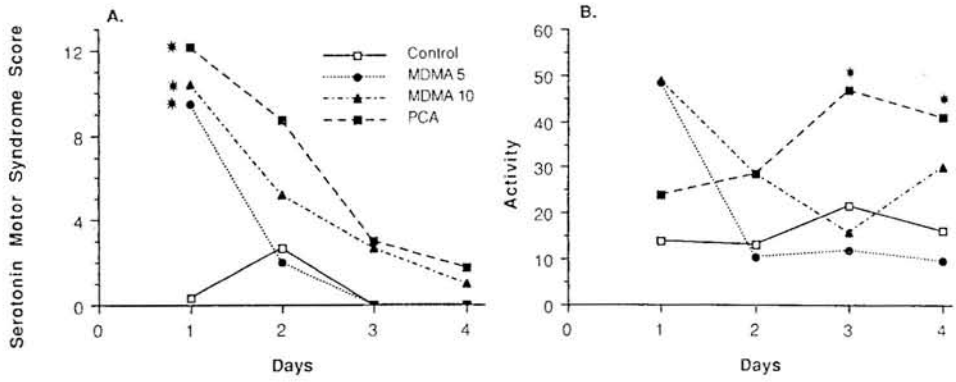

FIG. 1.A. The serotonin motor syndrome score 1 hr after treatment on each of 4 consecutive days. **1B.** General motor activity 1 hr after treatment on each of 4 days (*indicates $P < 0.05$ as compared to control, N = 9).

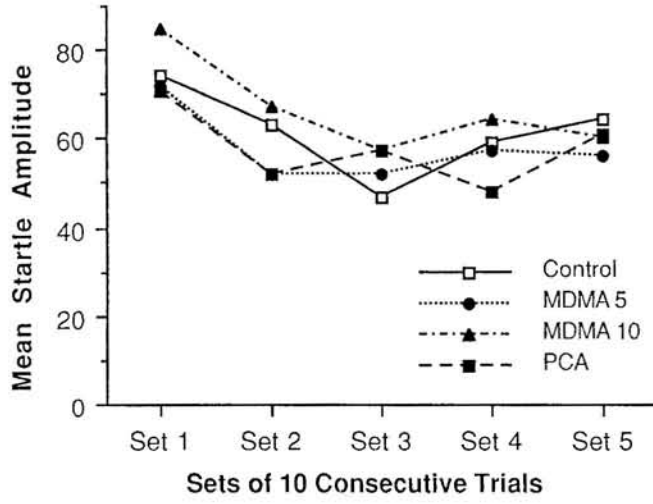

FIG. 2. Mean auditory startle amplitude for 5 sets of 10 consecutive auditory stimulus trials (N = 10, 5 male and 5 female rats).

group per sex) and monkeys (n = 3 per treatment group) with the use of HPLC/EC methods previously described (Ali *et al.*, 1986).

Radioimmunoassay of B-Endorphin. Because of the known serotonergic modulation of B-endorphins (Sapun *et al.*, 1981), we determined the concentration of B-endorphin (B-E) in various regions of monkey brain by radioimmunoassay (RIA) as described previously (Hong *et al.*, 1978; Hong and Ali, 1982). Briefly, tissue was homogenized in 2 M acetic acid and immersed in boiling water for 5 min. After centrifuging at 25,000 x g for 20 min, the supernatant was lyophilized. The residue was then reconstituted in distilled water and aliquots were used for RIA. The specificity of antisera against B-E has been described in previous reports (Hong *et al.*, 1978; Hong *et al.*, 1976; Fratta *et al.*, 1979). The antiserum against B-E used in this study cross-reacts with B-lipotropin and pro-opiocortin. In the hypothalamus the content of B-E is much higher than that of B-lipotropin or pro-opiocortin. Thus, the immunoreactivity measured in this area mainly represents B-E. The pituitary, however, contains large quantities of B-lipotropin and pro-opiocortin in addition to B-E. Therefore, the levels of B-endorphin-like immunoreactivity (BE-LI) obtained from pituitaries represent the total immunoreactivity against B-E, B-lipotropin, and pro-opiocortin (Fratta *et al.*, 1979).

RESULTS

Rodent Studies

Spontaneous Behavior. On the first day of treatment, the serotonin motor syndrome scores after administration of PCA and both doses of MDMA were significantly higher than for vehicle controls. No significant differences between sexes were observed, so all results were pooled across sex for presentation (Fig. 1). The scores were decreased on treatment days 2, 3 and 4 and were not significantly different from concurrent controls on these days. General motor activity in these same rats was not different between groups on days 1 and 2 of treatment. On days 3 and 4, however, the motor activity of the PCA rats was significant-

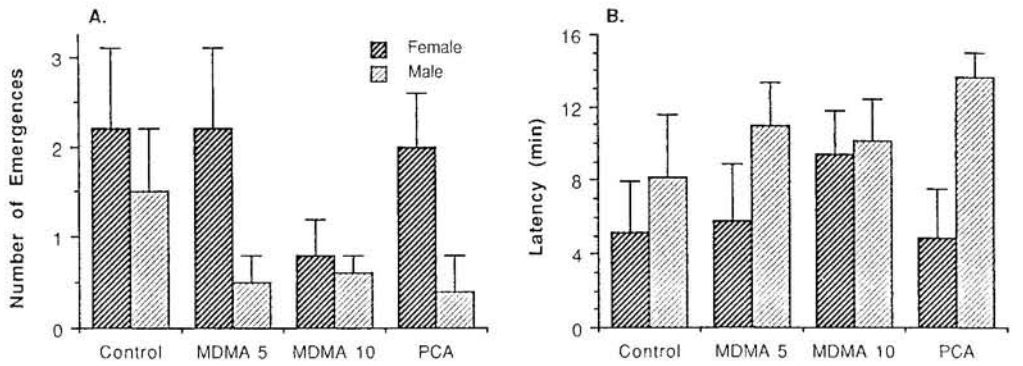

FIG. 3. A. Number of emergences (mean SEM) in 15 min for either male or female rats in each treatment group (N = 5). B. Latency in min (mean SEM) to first emergence for male and female rats of each treatment group (N = 5).

ly higher than that for controls with a similar trend exhibited by the 10 mg/kg MDMA group (Fig. 1).

Startle. Mean auditory startle amplitudes were not different between treatment groups and the habituation occurred as expected with repeated presentation of the auditory stimuli. No sex differences were observed so data were pooled for presentation (Fig. 2).

Emergence. Although the number of emergences per 15 min period tended to decrease with drug treatment, especially in female rats, the large variation between animals precluded any significant findings (Fig. 3). Likewise, no significant differences were observed between treatment groups for latency to first emergence (Fig. 3).

Hot Plate. Latency to respond on the hot plate was not altered by previous treatment

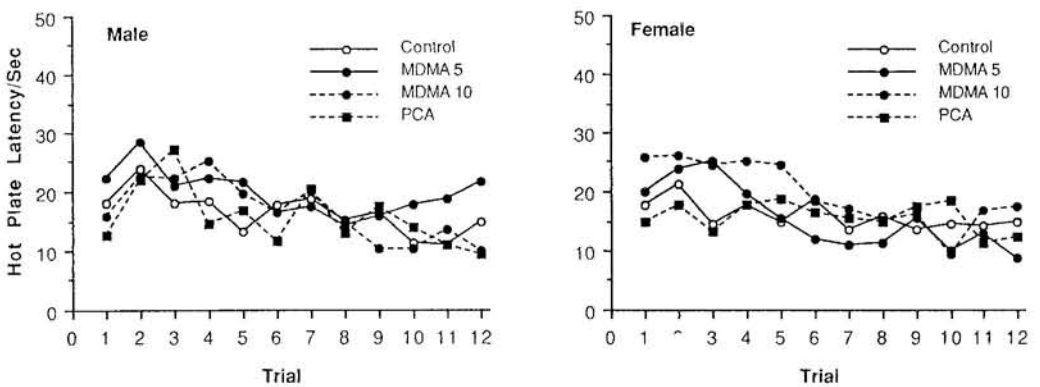

FIG. 4. Latency in sec for hot plate response for 12 consecutive trials for male (left panel) and female (right panel) rats (n = 5 of each sex).

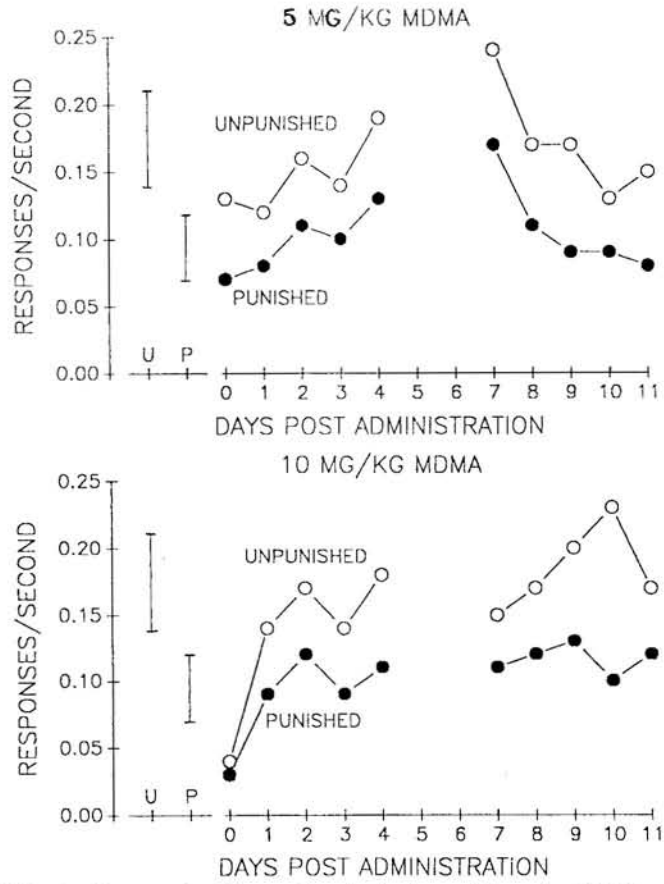

FIG. 5. Top panel. Effects of a single dose of 5 mg/kg MDMA on unpunished (open symbols) and punished (solid symbols) lever responses/second. U represents 2 standard deviations around the mean for control response rates for unpunished behavior. P represents 2 standard deviations around the mean for control response rates for punished behavior. Bottom panel. Effects of a single dose of 10 mg/kg MDMA on unpunished and punished lever responses/second (symbols the same as before, N = 7).

with PCA or either dose of MDMA. Both male and female rats were equally insensitive to treatment (Fig. 4).

Operant Study. The data from a separate group of rats (n = 7) conditioned to respond under a multiple FI 90 sec FI 90 sec with punishment did not show significant changes from controls after a single 5 mg/kg MDMA dose administered 20 min before behavioral assessment (Fig. 5). At the 10 mg/kg dose, however, both unpunished and punished responding was significantly depressed on the day of treatment. Recovery occurred within 24 to 48 hr and no residual effects were observed in these rats.

Neurochemistry. A total of 40 rats, 10 per treatment group (5 per sex) were assessed one month after treatment for 5-HT and 5-HIAA concentrations in four brain areas. As shown in Fig. 6, 5-HT concentrations were decreased significantly by PCA and both doses of MDMA in the frontal cortex, by PCA and 10 mg/kg MDMA in the hippocampus and by only PCA in the caudate nucleus as compared

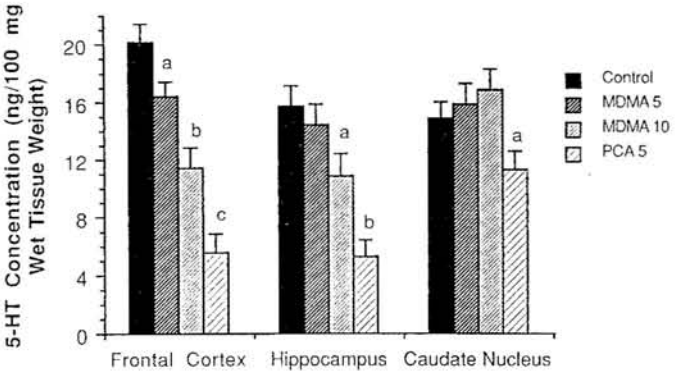

FIG. 6. Effects of MDMA or PCA on 5-HT concentrations in frontal cortex, hippocampus and caudate nucleus (mean SEM, N = 5 of each sex). a = $p < 0.05$; b = $p < 0.01$; c = $p < 0.001$

to controls. Although similar trends were seen in 5-HIAA concentrations (Fig. 7), only PCA significantly decreased this monoamine metabolite in the frontal cortex and caudate, whereas both PCA and 10 mg/kg MDMA decreased 5-HIAA in the hippocampus.

Monkey Studies

The monkeys were weighed before the beginning of treatment and three days after the last of four days of dosing with MDMA or vehicle. As shown in Table 1, no significant treatment-related changes in body weight were observed. The body weights of all monkeys under study decreased by about 0.3 kg but this

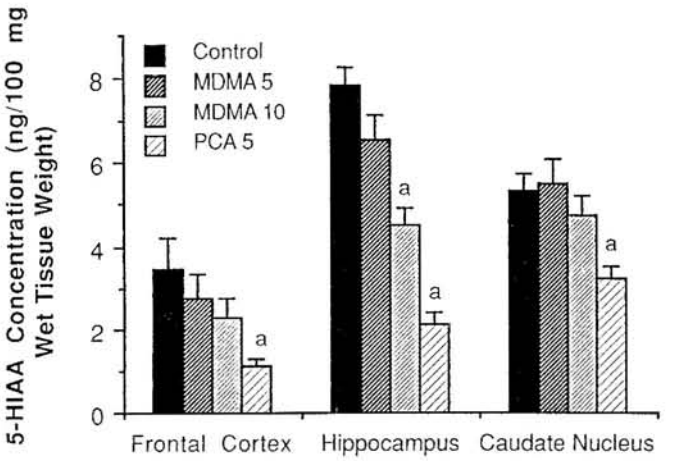

FIG. 7. Effects of MDMA or PCA on 5-HIAA concentrations in frontal cortex, hippocampus and caudate nucleus (mean SEM, N = 10, 5 of each sex). a = $p < 0.01$

was seen in both controls and MDMA treated monkeys and probably reflected the decreased daily food rations that all of the monkeys were given during the 4 days of dosing.

Spontaneous Behavior. The data obtained from the behavioral check-list used to assess spontaneous home-cage behavior in the nine monkey subjects failed to demonstrate any significant behavioral alterations on any of the treatment days (data not shown). In addition, spontaneous behavior observed three days after the last MDMA dose did not differ between treatment groups.

TABLE 1. Monkey Body Weights (Kg)

	Control	5.0 mg/kg	10.0 mg/kg
	5.3	5.3	9.2
Predosing	7.9	8.3	3.9
	6.2	7.3	6.7
(Mean ± SEM)	6.5 ± 0.8	7.0 ± 0.9	6.6 ± 1.5
3 Days After	5.1	5.2	9.0
Last Dose	7.4	8.0	3.6
	6.0	7.0	6.4
(Mean ± SEM)	6.2 ± 0.7	6.7 ± 0.8	6.3 ± 1.6

TABLE 2. Effect of MDMA on Neurotransmitter Concentrations (Mean ± SEM) in Monkeys Four Weeks After Last Treatment.

	Frontal Cortex		
Group	HVA	5-HT	5-HIAA
	(ng/100 mg Wet Tissue Weight)		
Controls	62 ± 19	10 ± 2	13 ± 2
5 mg/kg	28 ± 10	4 ± 0.4*	7 ± 0.6*
10 mg/kg	38 ± 10	3 ± 0.6*	4 ± 1**

*Means significantly different from controls at $p < 0.05$
**Means significantly different from controls at $p < 0.01$

TABLE 3. Effect of MDMA on Neurotransmitter Concentrations (Mean ± SEM) in Monkeys Four Weeks After Last Treatment.

	Hippocampus		
Group	HVA	5-HT	5-HIAA
	(ng/100 mg Wet Tissue Weight)		
Controls	20 ± 3	4 ± 0.5	11 ± 2
5 mg/kg	20 ± 3	1 ± 0.1**	4 ± 0.5*
10 mg/kg	21 ± 3	0.7 ± 0.1**	2 ± 0.4**

*Means significantly different from controls at $p < 0.05$
**Means significantly different from controls at $p < 0.01$

Neurochemistry. Of the 4 brain regions assessed for dopamine (DA), 5-HT and metabolite concentrations one month after treatment, only 5-HT and 5-HIAA showed significant reductions by MDMA treatment and these alterations were exhibited only in the frontal cortex and hippocampus (Tables 2 through 5). No significant changes in DA, DOPAC or HVA were observed in any brain region evaluated. As shown in Table 6, no significant changes in B-endorphin concentrations were seen in the caudate nucleus, frontal cortex, hypothalamus or pituitary.

DISCUSSION

The similarity in the spontaneous behavior exhibited by rats after the initial administration of either PCA or MDMA suggests a similar acute action of these agents. The PCA-induced serotonin motor syndrome observed in these animals may be a result of the acute release of 5-HT by serotonergic neurons; it is followed by a longlasting depletion of 5-HT and 5-HIAA and by decreases in the high-affinity uptake of 5-HT (Sanders-Busch *et al.*, 1975) and in tryptophan hydroxylase activity (Sanders-Bush *et al.*, 1972). These same neurochemical consequences were also observed after MDMA administration (Schmidt *et al.*, 1986; Stone *et al.*, 1987; Battaglia *et al.*, 1987; Slikker *et al.*, 1988). In addition, effects induced by either PCA or MDMA may be blocked by 5-HT uptake inhibitors (Fuller, 1985; Battaglia *et al.*, 1988). Thus, the acute behavioral and neurochemical effects produced by MDMA appear to be similar to those produced by PCA.

The decrease in the serotonin motor syndrome scores on days 2, 3 and 4 of MDMA or PCA administration can be explained on the basis of these acute neurochemical effects.

TABLE 4. Effect of MDMA on Neurotransmitter Concentrations (Mean ± SEM) in Monkeys Four Weeks After Last Treatment.

	Caudate Nucleus				
Group	DA	DOPAC	HVA	5-HT	5-HIAA
	(ng/100 mg Wet Tissue Weight)				
Controls	709 ± 151	131 ± 25	992 ± 159	33 ± 6	40 ± 5
5 mg/kg	423 ± 64	96 ± 12	964 ± 49	27 ± 5	31 ± 5
10 mg/kg	460 ± 106	95 ± 14	893 ± 109	23 ± 6	22 ± 8

TABLE 5. Effect of MDMA on Neurotransmitter Concentrations (Mean ± SEM) in Monkeys Four Weeks After Last Treatment.

	Brain Stem				
Group	DA	DOPAC	HVA	5-HT	5-HIAA
	(ng/100 mg Wet Tissue Weight)				
Controls	26 ± 8	12 ± 1	186 ± 22	75 ± 15	143 ± 23
5 mg/kg	30 ± 5	13 ± 2	203 ± 14	58 ± 19	114 ± 13
10 mg/kg	29 ± 1	13 ± 0.6	228 ± 7	56 ± 6	100 ± 14

TABLE 6. Effect of MDMA on β-Endorphin Concentrations (Mean ± SEM) in Monkeys Four Weeks After Last Treatment.

Group	Caudate Nucleus	Frontal Cortex	Hypothalamus	Pituitary
	(ng/g Wet Tissue Weight)			(ng/mg)
Control	400 ± 257	5.6 ± 0.3	17.8 ± 5.4	42.4 ± 4.9
5 mg/kg	555 ± 196	5.9 ± 0.6	26.0 ± 10	36.5 ± 1.4
10 mg/kg	337 ± 162	6.1 ± 1.3	13.0 ± 4.5	57.7 ± 12.4

Because exposure to these agents results in release of 5-HT and subsequent depletion, 5-HT-induced behaviors would be expected to decline with repeated treatment as observed (Fuller, 1978). An interesting increase in motor activity was observed in the PCA treated rats on days 3 and 4 of dosing. A similar trend was seen on day 4 of MDMA treatment. Although more evidence is necessary, one could speculate that this increased motor activity is due to the interaction of PCA with a responsive dopaminergic system in the presence of a compromised, inhibitory serotonergic system (Messing *et al.*, 1976).

Because of the reported clinical effects of acute MDMA administration (Greer, 1985), we decided to test the hypothesis that MDMA exhibited anxiolytic properties. A behavioral method known to be sensitive to anxiolytic agents, punished responding (McMillan, 1975) was therefore used to measure behavior after acute exposure to MDMA. Although 10 but

not 5 mg/kg MDMA po altered response rates of the punished portion of the paradigm, the nonpunished portion of the behavior was equally affected suggesting that MDMA does not manifest marked anxiolytic properties in rodents.

Subsequent behavioral assessment with a number of techniques reported to be sensitive to serotonergic manipulation failed to demonstrate a significant effect at 2 to 4 weeks after MDMA or PCA treatment. Although PCA treatment has been reported to alter nociception, open-field locomotor activity, acquisition of discriminated Y-maze, conditioned avoidance responses and auditory startle habituation (Gorlitz and Frey, 1972; Vorhees *et al.*, 1975; Davis and Sheard, 1976), neither PCA nor MDMA significantly altered hot plate sensitivity, emergence, startle response or complex maze behavior in the present study. This lack of persistent behavioral effects was observed in the same animals which had demonstrated significant acute behavioral responses on day 1 of dosing and that demonstrated significant depletions of 5-HT and 5-HIAA in selected forebrain regions at sacrifice after behavioral assessment (4 weeks after the last treatment). Several reasons may underlie this apparent discrepancy between these neurochemical and behavioral endpoints: (1) a larger than 50% reduction in 5-HT content is required before behavioral alterations can be detected, (2) an adequately sensitive behavioral test has yet to be used, or (3) the brain areas demonstrating the 5-HT depletion are not subserving the behaviors that were monitored, these areas are being functionally compensated for by less affected brain areas.

The degree of 5-HT depletion produced by medial forebrain bundle electrolytic lesions has been correlated with behavioral indices such as jump thresholds in the rat. These investigators reported that an 18 to 35% decrease in whole brain 5-HT concentrations accompanied a 17 to 54% decrease in jump threshold (Lints and Harvey, 1969). Moreover, lesions lying outside the medial forebrain bundle did not decrease the jump threshold and had no effect on brain 5-HT concentrations (Lints and Harvey, 1969). Thus a 50% or less depletion of brain 5-HT content appears to be sufficient to alter nociception. Our data and that from a recent report by Nencini *et al.*, (1988) fail to demonstrate an alteration of nociception 2-4 weeks after MDMA treatment even though forebrain 5-HT was depleted 50% or more. Therefore, at least part of the explanation as to why 5-HT reductions did not result in behavioral effects may reside in the anatomical selectivity of MDMA-induced toxicity (Lints and Harvey, 1969; Nencini *et al.*, 1988).

Our findings with MDMA and PCA administration may more appropriately be compared with results from other studies employing chemical rather than electroyltic lesions (Lorens, 1978). With the use of parachlorophenylalanine (an inhibitor of 5-HT synthesis), Tenen (1967) demonstrated depletion of whole-brain 5-HT content and an increased sensitivity to electric shock as measured by the flinch-jump method. The whole brain 5-HT depletion was large (88%), however, compared to the decrease in jump threshold (32%). With the use of PCA (5 mg/kg), Messing *et al.*, (1976), demonstrated a decrease of 5-HT concentration in regional brain areas (23 - 49% depletion) and an increase in motor activity at 2 days after treatment. At 7 days after PCA treatment however, brain 5-HT concentrations were less, but significantly depleted (11 - 41%), whereas motor activity was not different from control values. Thus, the degree of 5-HT depletion as well as the anatomical selectivity of the depletion may provide an explanation for the apparent discrepancy between these neurochemical and behavioral endpoints.

The spontaneous behavior assessment checklist used to determine the effect of MDMA in the monkey has been shown to be sensitive to a variety of drug-induced effects including those produced by amphetamine, chlopromazine and diazepam (Slikker *et al.*, 1976; Brocco and Slikker, 1976). No alterations in spontaneous behaviors such as walking, vocalizing, grooming or visual exploration were observed after MDMA treatment. Significant decreases in 5-HT and 5-HIAA (over 70%) were observed in frontal cortex and hippocampus but not brain stem, one month after MDMA treatment at either 5 or 10 mg/kg, po (2 doses per day for 4 consecutive days). DA and HVA concentrations tended to

decrease in the frontal cortex and caudate nucleus, but these effects were not significant.

Therefore, the regional and neurochemical subsystem selectivity of MDMA-induced effects are evidenced in both the rat and nonhuman primate (Slikker *et al.*, 1988). The neurochemical effects of MDMA were observed after multiple oral doses as low as 5 mg/kg in both species, a dose only 2-3 times the human dose (Shulgin and Nichols, 1978). Because these regional neurochemical effects of MDMA have been correlated with serotonergic nerve terminal degeneration in the same brain areas (Scallet *et al.*, 1988; O'Hearn *et al.*, 1988; Commins *et al.*, 1987; Slikker *et al.*, 1988), there is need for concern that MDMA may produce similar neurotoxicity in humans.

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