

Neurochemical and Neurohistological Alterations in the Rat and Monkey Produced by Orally Administered Methylenedioxymethamphetamine (MDMA)¹

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Neurochemical and Neurohistological Alterations in the Rat and Monkey Produced by Orally Administered Methylenedioxymethamphetamine (MDMA). SLIKKER, W., JR., ALI, S. F., SCALLET, A. C., FRITH, C. H., NEWPORT, G. D., AND BAILEY, J. R. *Toxicol. Appl. Pharmacol.* 94, 448-457. MDMA is an amphetamine analog prescribed by some health professionals in the field of psychotherapy and used as a recreational drug by the general public. In recent reports, investigators have suggested that MDMA produces acute neurotoxicity when administered by subcutaneous injection. In order to determine if MDMA produces lasting neurochemical alterations after oral administration, groups of six rats (adult male Sprague-Dawley) were dosed by gavage with either 40 or 80 mg/kg of MDMA or saline vehicle once every 12 hr for 4 days. These rats were terminated 2 weeks after the first dose along with an additional group of rats (80 mg/kg) terminated 4 weeks after the first dose. Brain regions including the hippocampus (H), caudate nucleus (CN), hypothalamus (HY), frontal cortex (FC), and brain stem (BS) were analyzed by HPLC with electrochemical detection for concentrations of dopamine (DA), dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA), serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), and norepinephrine (NE). In the CN, 40 mg/kg MDMA produced no change in DA, DOPAC, or HVA, but a 50-60% decrease in 5-HT and 5-HIAA concentrations was observed at 2 weeks. Similar effects were observed at 80 mg/kg at both 2 weeks and 4 weeks. A temporary decrease was also seen in DA (21%) and in HVA (34%) 2 weeks but not 4 weeks after the 80 mg/kg dose regimen. In the H, MDMA (40 or 80 mg/kg) produced no change in NE, but a 50-60% decrease was seen in 5-HT and 5-HIAA concentrations at 2 weeks. Concentrations of 5-HT and 5-HIAA were significantly decreased in the HY and FC by all MDMA treatments, but DA and DOPAC concentrations were not altered as compared to vehicle controls. BS was least affected by treatment with no change in DA, DOPAC, or 5-HIAA concentrations and only a slight decrease in 5-HT (19-33%) concentrations at 2 weeks but not at 4 weeks. To determine the sensitivity of the nonhuman primate to MDMA, a total of nine rhesus monkeys were dosed with vehicle or 5 or 10 mg/kg MDMA ($n = 3$) by gastric intubation twice per day for 4 days. One month after MDMA dosing, a dose-related reduction from vehicle control values for 5-HT and 5-HIAA was observed. These results indicate that the monkey may be more sensitive than the rat to the persistent serotonergic neurotoxicity of MDMA. Histological assessment in the rat with a stain specific for degenerating fibers and terminals 18 hr after a single treatment with MDMA (40 and 80 mg/kg, po) indicated a significant enhancement of the density of silver-impregnated terminals in the CN as compared to vehicle controls. Selective reductions in brain 5-HT and 5-HIAA concentrations, along with histologically observed neuronal degeneration, suggest that orally administered MDMA produces selective destruction of serotonergic nerve terminals.

Methylenedioxymethamphetamine (MDMA) is an amphetamine analog and has been used by some health professionals in the field of psychotherapy and by the general public as a recreational drug (Greer, 1985; Frith *et al.*, 1987). Psychotherapists suggest that the drug enhances communication between therapist and patient by apparently imparting a profound sense of peacefulness and interpersonal trust to the user. MDMA is known as "Ecstasy" on the street and users testify that it intensifies emotional feelings without distorting the senses. MDMA has recently been reported to be a potential neurotoxicant in the rat after subcutaneous administration (Gibb *et al.*, 1986; Schmidt, 1987). MDMA decreased tryptophan hydroxylase (the rate-limiting enzyme for serotonin synthesis) but not tyrosine hydroxylase activity in the hippocampus, frontal cortex, and striatum of the rat (Gibb *et al.*, 1986). MDMA also reduced the content of 5-HT itself as well as its metabolite (5-HIAA) in several brain regions in a biphasic manner; an initial early decrement returned to control values by 24 hr while a subsequent decrease lasted at least 7 days (Schmidt, 1987). Neuropathological studies have recently been reported for MDMA (Commins *et al.*, 1986) and a closely related structural analog, methylenedioxyamphetamine, MDA, (Ricaurte *et al.*, 1985) after subcutaneous administration.

The use of findings of MDMA-induced neurotoxicity in the rat to predict human risk is difficult. The parenteral route of MDMA administration described in previous studies (Gibb *et al.*, 1986; Schmidt, 1987; Commins *et al.*, 1987) is one source of uncertainty since MDMA is usually administered orally in humans (Greer, 1985; Shulgin and Nichols, 1978) and MDMA and its congeners undergo hepatic metabolism (Glennon, 1984; Brady *et al.*, 1986; Gollamudi *et al.*, 1988). Another source of uncertainty involves extrapolation of the finding in the rodent to the human

population. With the knowledge that certain neurotoxicants, such as MPTP, are much more potent in the human and nonhuman primate than the rodent (D'Amato *et al.*, 1985), it became warranted to investigate the sensitivity of the monkey to the effects of MDMA after oral administration.

The present study was designed to determine if the decrease in 5-HT and metabolite concentration observed after subcutaneous administration of MDMA occurs after oral administration. The time course and selectivity of the MDMA-induced alterations as well as associated neurodegeneration as quantified by silver staining were also assessed. Finally, the effects of orally administered MDMA in the monkey were compared to those observed in the rat.

MATERIALS AND METHODS

Chemical. MDMA was kindly supplied by Dr. David Nichols, Department of Medicinal Chemistry, Purdue University, and National Institute on Drug Abuse, Washington, D.C. The structure was confirmed by chemical ionization mass spectrometry at NCTR. The purity and stability of the MDMA (>99%) has been previously described (Frith *et al.*, 1987). Other chemicals and solvents used were HPLC grade.

Experimental design (rodent studies). Adult male Sprague-Dawley rats (Charles River, Portage, MI) were used in the studies. Animal quarantine, caging, and feeding provisions have been previously described (Frith *et al.*, 1987). A total of 24 rats (four groups of $n = 6$) were terminated for neurochemical evaluation on the same experimental day in balanced alternation between groups in order to control for any effects of circadian variation. Based on the data of Ricaurte *et al.* (1985) a regimen of MDMA treatment (eight repeated oral doses of vehicle (saline) or 40 or 80 mg/kg once every 12 hr for 4 days at 2 ml/kg) was employed to evaluate the presence, dose-relatedness, and persistence of neurochemical alterations following repeated MDMA administration.

Experimental design (monkey studies). Adult female rhesus monkeys ranging in weight from 4.2 to 9.5 kg and in age from 8 to 15 years were maintained as previously described (Slikker *et al.*, 1983). A total of nine monkeys (three per group) were dosed with vehicle (water) or 5 or 10 mg/kg MDMA by gastric intubation twice per day (9 AM and 4 PM) for 4 days. Monkey body weights were recorded the week before dosing and 3 days after the last dose. One month after the last MDMA dose, the monkeys were overdosed with pentobarbital and their brains removed, quickly dissected into different regions, and

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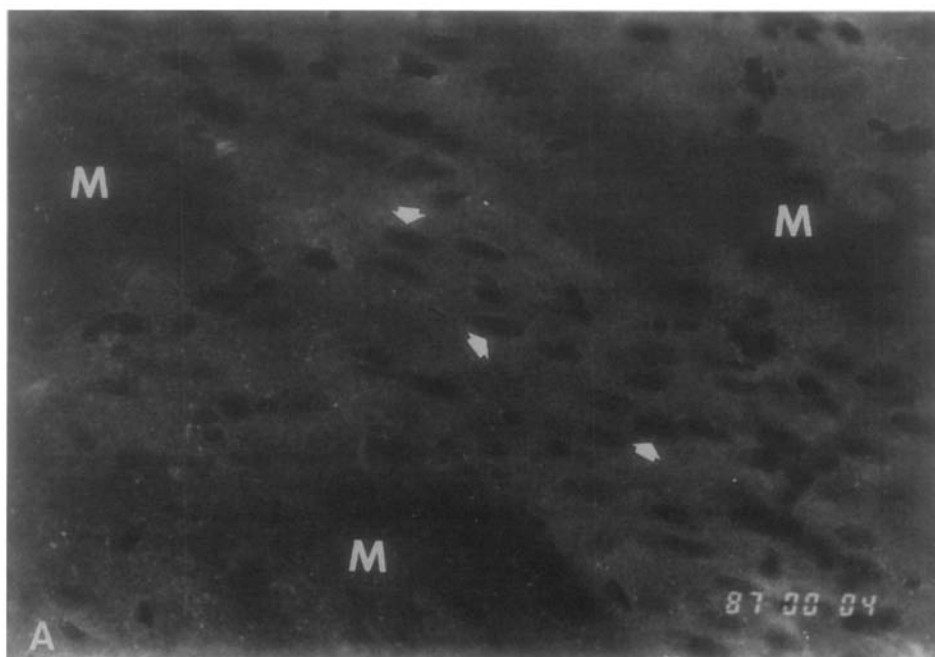


FIG. 1A. Darkfield photomicrograph of a section through the caudate nucleus of a control rat. Note the absence of any silver-impregnated axons which would appear as white fibers due to scattered light from the silver deposits contained in the axons as in Fig. 1B. Figs. 1A and 1B: M indicates patches of myelinated fibers characteristic of the caudate nucleus. Small arrows indicate silver-impregnated fibers, whereas large arrows indicate intrinsic neurons of the caudate nucleus. Both micrographs $\times 420$.

frozen on dry ice and analyzed for 5-HT and 5-HIAA concentrations as described below.

Brain dissection. Six rats, from each group, at each time point were randomly selected and killed by decapitation, and their brains were removed quickly and dissected midsagittally into two halves. Each half was dissected into frontal cortex (FC), caudate nucleus (CN), hippocampus (H), hypothalamus (HY), frontal cortex (FC), and brain stem (BS) following the guidelines of Glowinski and Iverson (1966); tissues were placed on dry ice and stored at -70°C until processed.

The monkey brains were dissected on an ice-cold plate according to a minor modification of the method of Brown *et al.* (1979) using the atlas of Szebenyi (1970). The cortex was removed as four large pieces: The prefrontal cortex, the premotor/precentral gyrus cortex, the preoptic/parietal cortex, and the occipital cortex. Approximately 600 mg of tissue of the ventral frontal cortex was assayed. The basal ganglia, thalamus, hypothalamus, and brain stem were removed as described by Brown *et al.* (1979), except the basal ganglia was 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 removed, it was immediately frozen on dry ice and each dissection was completed within 5 min of removal of the brain from the animal.

Neurotransmitter and metabolite assay. Neurotransmitters and their acid metabolites were resolved by high-performance liquid chromatography (HPLC) and quantified by electrochemical detection (Ali *et al.*, 1986). Briefly, each individual region of the brain was weighed and placed in a measured volume (10:1, v/w) of 0.2 N perchloric acid containing 250 ng/ml of the internal standard 3,4-dihydroxybenzylamine (DHBA). Brain tissue was then disrupted by ultrasonication and centrifuged (1000g, 5 min), and 150 μl of the supernatant was removed and filtered through a 0.2- μm microfilter (MF-1 microcentrifuge filter, Bioanalytic System (BAS), W. Lafayette, IN). Aliquots of 25 μl representing 10 mg of brain tissue were injected directly onto the HPLC system for separation of the neurotransmitters and their metabolites.

Neuropathology. In order to determine the presence of neuronal toxicity or death and its neurochemical basis,

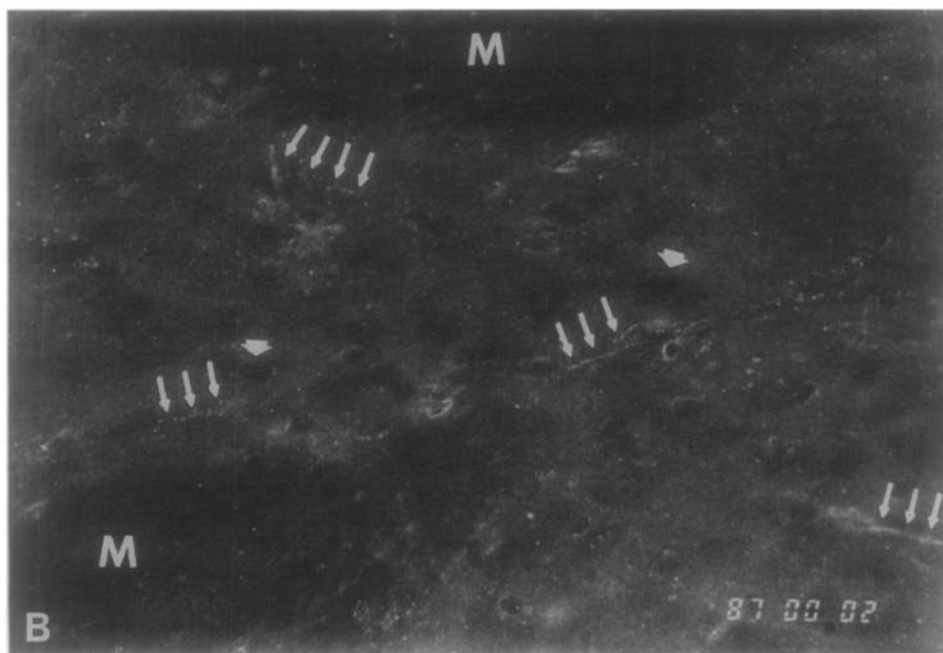


FIG. 1B. Similar section through the caudate of an animal treated 2 weeks previously with 80 mg/kg po of MDMA. Small arrows indicate several small fibers presumed to be degenerating argyrophilic axons.

11 rats were terminated for silver impregnation of degenerating neuronal elements. The caudate nucleus was selected for analysis because the neurochemical data indicated a 50% reduction of 5-HT concentration in this brain region after MDMA treatment and earlier studies by Ricaurte *et al.* (1985) demonstrated the caudate sensitive to neurodegeneration by a MDMA congener. Four groups were perfused on a single experimental day. One group ($n = 3$) was terminated 18 hr after a single 80 mg/kg dose of MDMA and another group was terminated 18 hr after 40 mg/kg in order to evaluate the earliest signs of dose-related toxicity from our MDMA regimen. Two control rats were intubated with an equal volume of saline 18 hr prior to termination. An additional group of three animals were terminated at 2 weeks following the eight-dose regimen in order to determine the correspondence of histological alterations to the neurochemistry results.

Rats were anesthetized with Nembutal (Abbott Laboratories) and perfused through the left ventricle of the heart with a needle clamped into the ascending aorta. An initial flush of 20–30 ml of 0.9% saline buffered to pH 7 with 0.1 M potassium phosphate was followed by 100–200 ml of pH 7 phosphate-buffered 10% formalin. The whole brains were removed and stored overnight in the same buffered formalin. On the following day, all brains were blocked and a series of 30 μ m sections cut with a

vibratome (Lancer Instruments Co.) through the caudate/putamen of each rat at the level of bregma (Paxinos and Watson, 1982). Two nonadjacent sections were stained with cresyl violet for neuronal Nissl substance, and according to a modification of the silver impregnation method of Fink and Heimer (1967) for degenerating fibers and cell bodies (Nadler and Evenson, 1983). To confirm that the method was sensitive to and specific for degenerating neuronal elements, sections from rats treated 1 week previously with 9 mg/kg of the hippocampal neurotoxin trimethyltin (Paule *et al.*, 1986) were included with the MDMA sections in each batch that was processed for silver degeneration.

Silver-impregnated terminals could be observed in the caudate sections as spidery, multiprocess varicose fibers generally found in proximity to cell bodies (Figs. 1A and 1B). Quantification of the silver-impregnated terminals in the caudate regions was performed by an observer blind to the group identification of the sections. Four randomly chosen, non-overlapping 20-mm-diameter fields-of-view were examined at a magnification of 400 $\times$ from each of the two caudate sections stained per brain. The total number of terminals observed in all fields was then divided by 0.0157 mm² (the total area of 30- μ m-thick tissue comprising the eight fields counted) in order to derive an estimate of the density of stained terminals in each MDMA or control rat.

Statistical analysis. Main effects of MDMA treatment on both neurochemistry and neuropathology were determined by one-way analyses of variance. The null hypothesis that the treated and control groups had the same mean was rejected when F ratios exceeded the 95% confidence levels. Subsequent individual comparisons between the groups with a $p < 0.05$ by the Fisher's least significant difference approach (Winer, 1971) were considered statistically reliable.

RESULTS

Neurochemistry (rodent studies). In the CN, 40 mg/kg MDMA produced no change in DA, DOPAC, or HVA concentrations but reductions in 5-HT (51%, $p < 0.01$) and 5-HIAA (63%, $p < 0.01$) concentrations were observed 2 weeks after the first dose ($p < 0.01$). Similar effects (48–58% decreases, $p < 0.01$ from vehicle control) were observed at 80 mg/kg both at 2 and 4 weeks after the first dose. A transient decrease from control levels was also seen in DA (21%, $p < 0.05$) and HVA (34%, $p < 0.05$) 2 weeks but not 4 weeks after the 80 mg/kg dose regimen. DA and HVA were not altered by the 40 mg/kg treatments (Table 1).

In H, MDMA (40 mg/kg) induced no change in NE (data not shown) but 5-HT and 5-HIAA concentrations were about equally decreased (55–58%) 2 weeks after the first dose ($p < 0.01$). Similar effects (51–60% decrease, p values < 0.01 from vehicle) were seen at 2 and 4 weeks after the 80 mg/kg doses (Table 2).

In HY and FC, DA and DOPAC concentrations were unchanged by treatment (data not shown) whereas 5-HT and 5-HIAA exhibited a 23–60% reduction in concentration with treatment (p values < 0.05 ; Table 2).

The BS was least affected by MDMA treatment with no changes in DA, DOPAC, or 5-HIAA concentrations and reductions of only 23 and 19% in 5-HT concentrations 2 weeks after the 40 or 80 mg/kg doses, respectively (p values < 0.05 , Table 2). At four weeks, although the 80 mg/kg dosed rats had lower BS concentrations of 5-HT than those of con-

trols, the differences were not statistically significant.

Neurochemistry (monkey studies). The four brain areas examined in the monkey all tended to show a dose-related decrease in 5-HT and 5-HIAA concentrations as the MDMA dose was increased from 0 to 10 mg/kg po (Table 3). Both the FC and H concentrations of 5-HT exhibited a 70–80% reduction from control values after the 10 mg/kg po dose and the H values were significantly different at the $p < 0.01$ level. Serotonin and 5-HIAA content of the H and FC was less markedly affected but still significantly reduced by the lower 5 mg/kg dose as well ($p < 0.05$). Smaller MDMA-induced reductions in CN 5-HT and 5-HIAA concentrations (45%) did not achieve statistical significance. As in the rat, MDMA produced lesser reductions in 5-HT and 5-HIAA concentrations in the brain stem which were not significantly different from controls. No significant body weight alterations occurred during or within 3 days after dosing.

Neuropathology. Positive control (trimethyltin treated) sections included in the batch processed for silver staining showed numerous large degenerating fibers and terminals in the hippocampus as well as degenerating cell bodies in layer 5 of cerebral cortex (not shown). Quantitation of degenerating terminals in MDMA rats revealed a 90% increase in the density of affected terminals 18 hr after a single 40 mg/kg dose ($p < 0.05$) and a 105% increase after 80 mg/kg, ($p < 0.01$), (see Fig. 2). An additional group of rats that had been treated 2 weeks before termination with four doses of 80 mg/kg MDMA also exhibited a significantly enhanced number of silver-stained terminal processes in the CN ($p < 0.05$, see Figs. 1 and 2).

DISCUSSION

These data demonstrate that MDMA induces neurochemical alterations in rats after oral administration, the most common route

TABLE 1
EFFECTS OF MDMA ON NEUROTRANSMITTER CONCENTRATIONS IN THE RAT

Group	Caudate nucleus				
	DA	DOPAC	HVA (ng/100 mg wet tissue wt)	5-HT	5-HIAA
Controls					
Mean	1267	123	66.3	57.7	23.1
±SE	67	8	6.9	4.9	1.9
40 mg/kg					
SAC—2 weeks					
Mean	1265	116	70.3	28.5 ^a	8.6 ^a
±SE	69	8	4.4	2.0	1.1
80 mg/kg					
SAC—2 weeks					
Mean	1001 ^b	98	43.6 ^a	36.0 ^a	13.3 ^a
±SE	33	5	8.4	3.5	1.4
80 mg/kg					
SAC—4 weeks					
Mean	1227	119	70.4	31.4 ^a	9.6 ^a
±SE	110	14	4.8	2.3	1.3

^a Mean different from control at $p < 0.01$.

^b Mean different from control at $p < 0.05$.

TABLE 2
EFFECTS OF MDMA ON NEUROTRANSMITTER CONCENTRATIONS IN THE RAT

Group	Hippocampus		Hypothalamus		Frontal cortex		Brain stem	
	5-HT	5-HIAA	5-HT	5-HIAA	5-HT	5-HIAA	5-HT	5-HIAA
	(ng/100 mg wet tissue weight)							
Controls								
Mean	12.8	7.3	97	38	40	15	64	25
±SE	2.1	0.9	4	4	4	1	3	0.5
40 mg/kg								
SAC—2 weeks								
Mean	5.7 ^b	3.1 ^b	60 ^b	22 ^b	16 ^b	9 ^b	49 ^a	21
±SE	0.6	0.4	2	2	2	0.6	4	3
80 mg/kg								
Sac—2 weeks								
Mean	6.3 ^b	3.1 ^b	63 ^b	21 ^b	18 ^b	9 ^b	52 ^a	25
±SE	0.5	0.4	3	3	2	0.3	3	5
80 mg/kg								
Sac—4 weeks								
Mean	6.2 ^b	2.9 ^b	75 ^a	27 ^b	18 ^b	9 ^b	54	22
±SE	0.3	0.3	5	2	2	0.8	4	0.2

^a Mean different from control at $p < 0.05$.

^b Mean different from control at $p < 0.01$.

TABLE 3
EFFECTS OF MDMA ON NEUROTRANSMITTER CONCENTRATIONS IN THE MONKEY

Group	Frontal cortex		Hippocampus		Caudate nucleus		Brain stem	
	5-HT	5-HIAA	5-HT	5-HIAA (ng/100 mg wet tissue wt)	5-HT	5-HIAA	5-HT	5-HIAA
Control								
Mean	9.5	13.1	4.2	10.7	32.8	39.8	75.3	143.0
±SE	2.3	2.4	0.5	2.1	5.8	4.5	14.7	23.3
5 mg/kg								
Sac—4 weeks								
Mean	3.6 ^a	6.9 ^a	1.1 ^b	4.0 ^a	27.3	30.7	58.5	113.9
±SE	0.4	0.6	0.1	0.5	5.2	5.1	18.7	13.2
10 mg/kg								
Sac—4 weeks								
Mean	2.7 ^a	4.0 ^b	0.7 ^b	2.2 ^b	22.7	21.9	56.2	100.3
±SE	0.6	1.3	0.1	0.4	6.1	8.1	5.5	14.3

^a Mean different from control at $p < 0.05$.

^b Mean different from control at $p < 0.01$.

of administration in the human. The effects of MDMA were quite marked (55% reduction in frontal cortex 5-HT concentration) as

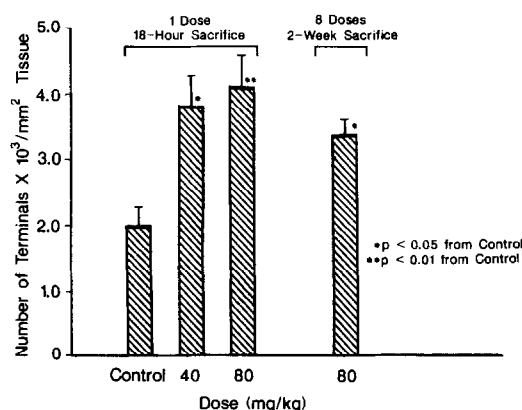


FIG. 2. The means ± SE are shown for the density of silver-impregnated terminals in caudate nucleus sections counted by an observer unaware of treatment identifications. Two sections per rat from two to three rats per treatment group were scored as described in the text. All MDMA-treated groups had significantly more stained terminals per square millimeter of tissue area than controls (* $p < 0.05$, ** $p < 0.01$).

long as 4 weeks after treatment. This indicates that MDMA effects on the serotonergic system are not transient but long-lasting. Recent preliminary studies from our laboratory (Ali *et al.*, 1987) confirm the long-lasting effects of MDMA in that a significant reduction of 5-HT concentration was observed in the hippocampus of female rats 4 months after eight successive oral doses of MDMA (40 mg/kg). In contrast, MDMA produced only transient (2 weeks) alterations of the DA and NE systems which were no different from control levels by 4 weeks. This transient effect of MDMA on the catecholaminergic system is consistent with the lack of effect of MDMA on tyrosine hydroxylase (Gibb *et al.*, 1986) and dopamine receptors (Ali *et al.*, 1986; Scallet *et al.*, 1988).

In the monkey, oral doses of MDMA (5 or 10 mg/kg) resulted in a dramatic dose-related decrease (71–83% reduction) in 5-HT concentration in the frontal cortex and hippocampus. As in the rat, the brain stem was least affected, exhibiting only a 25% reduction of 5-HT as compared to vehicle control values.

TABLE 4

REGIONAL SELECTIVITY OF ORALLY ADMINISTERED MDMA ON 5-HT AND 5-HIAA CONCENTRATIONS
(PERCENT OF REDUCTION FROM VEHICLE CONTROL VALUES 1 MONTH AFTER DOSING)

Brain region	5-HT		5-HIAA	
	Rat (80 mg/kg)	Monkey (10 mg/kg)	Rat (80 mg/kg)	Monkey (10 mg/kg)
Frontal cortex	55 ^a	71 ^a	40 ^a	69 ^a
Hippocampus	52 ^a	83 ^a	60 ^a	80 ^a
Caudate nucleus	46 ^a	30	58 ^a	45
Brain stem	16	25	12	30

^a These values were significantly different from their respective controls ($p < 0.05$).

These lasting changes were observed 1 month after MDMA dosing and occurred in the absence of body weight loss.

The regional effects of MDMA on the serotonergic system are summarized in Table 4 for both the rat and monkey. It is interesting to note that the greatest losses of serotonin content occurred in those brain areas containing only serotonergic axons and nerve terminals (Cooper *et al.*, 1984). Those areas supporting serotonergic cell bodies (brain stem) were less severely affected by MDMA treatment. These neurochemical data suggest that the effects of MDMA on the serotonergic system are predominantly directed toward the terminals rather than the soma of the neuron.

The increase in the density of silver-staining caudate axons of MDMA rats compared to vehicle controls also supports the concept of a nerve terminal site of MDMA toxicity. These neuropathological signs of terminal degeneration were observed as early as 18 hr after MDMA administration and lasted for at least 2 weeks thereafter. These findings with MDMA are consistent with those reported by Ricaurte *et al.* (1985) for MDA, a structurally related amphetamine derivative, and raise concern about the neurotoxic potential of this chemical class of psychedelic stimulants in humans. In the rats studied here, the tryptophan or monoamine oxidase pretreatments

typically used to enhance serotonergic immunohistochemical staining (Cooper *et al.*, 1984) were avoided because of their potential interaction with the MDMA treatment of interest. However, in a separate set of tryptophan pretreated rats studied 4 months following treatment (Scallet *et al.*, 1988), the dorsal raphe-neurons visualized with an anti-serotonin antisera were not obviously different in numeric density between MDMA and control rats. Although these findings further support a nerve-terminal site of action for MDMA, direct studies of the dorsal raphe-neuron response to acute MDMA treatment will be required to establish whether actual death of certain brain stem cell-bodies might occur in response to this serotonergic toxicant.

The concern over the route of MDMA administration is heightened because Molliver *et al.* (1986) reported that direct intracerebral injection of MDMA did not effect the appearance of axons revealed by a specific stain for their content of serotonin. These data suggest that a peripheral metabolite of MDMA may be responsible for the neurotoxicity. Our rodent data clearly indicate the profound effects of MDMA on the serotonergic system (over 50% depletion of 5-HT and 5-HIAA) in selected brain regions after oral administration.

The repeated doses of MDMA (40 and 80 mg/kg) used in these rodent studies are approximately 15 to 30 times the usual human oral MDMA dose (Shulgin and Nichols, 1978) although oral doses of MDMA of 50 and 100 mg/kg administered to rats once daily for 28 days have been reported to produce reduced weight gain and minor alteration of clinical chemistries but no histopathologic changes or deaths (Frith *et al.*, 1987).

The significant effect of orally administered MDMA at doses as low as 5 mg/kg in the monkey suggests that the nonhuman primate is more sensitive than the rodent. At 1 month after MDMA treatment, the percentage of frontal cortex and hippocampal 5-HT depletion in the monkey was greater than that observed in the rat 1 month after 80 mg/kg MDMA or 2 weeks after 40 mg/kg MDMA administered under the same regimen (Table 4). These data enhance the concern for human risk since the 5 mg/kg oral MDMA dose is approximately twice the usual human dose (Shulgin and Nichols, 1978) albeit eight such doses were administered over a 4-day period in the case of the monkey. Although the lowest effective oral dose of MDMA is yet to be determined, the present data in the rat and especially in the monkey clearly indicate that orally administered MDMA is an effective serotonin depletor and potential neurotoxicant at doses which do not produce morbidity and are relevant to human exposure.

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