

Lasting Effects of (±)-3,4-Methylenedioxymethamphetamine (MDMA) on Central Serotonergic Neurons in Nonhuman Primates: Neurochemical Observations¹

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ABSTRACT

The purpose of this study was to assess the duration of (±)-3,4-methylenedioxymethamphetamine's (MDMA's) effects on serotonin containing neurons in nonhuman primates. Fifteen squirrel monkeys were used: three served as controls, 12 received MDMA s.c. at a dose of 5 mg/kg twice daily for 4 consecutive days. Two weeks, 10 weeks, 8 months and 18 months after drug treatment, groups ($n = 3$) of MDMA-treated monkeys, along with controls, were examined for regional brain content of serotonin and 5-hydroxyindoleacetic acid, and for the number of [³H] paroxetine-labeled serotonin uptake sites. Two weeks after MDMA treatment, monkeys showed profound reductions in all three serotonergic presynaptic markers. By 10 weeks, there was evidence of partial recovery in some brain regions (e.g., hippocampus, caudate nucleus, frontal cortex). However, by 18 months, it was evident that recovery did not continue, as sero-

tonergic deficits returned to the level of severity observed 2 weeks after MDMA treatment. This was the case in all brain regions examined except the thalamus and hypothalamus. In the thalamus, the level of serotonin increased to 63% of control, whereas that of 5-hydroxyindoleacetic acid recovered completely. In the hypothalamus, concentrations of serotonin and 5-hydroxyindoleacetic acid were 140 and 187% of control, respectively. These results suggest that MDMA produces lasting effects on serotonergic neurons in nonhuman primates, with most brain regions showing evidence of persistent denervation and some showing signs of reinnervation (thalamus) or possibly even hyperinnervation (hypothalamus). The morphological and functional correlates of these enduring neurochemical changes in the MDMA-treated primate remain to be delineated.

In recent years, MDMA has emerged as a recreational drug of abuse (Bost, 1988). Although the toxicity of MDMA toward 5-HT-containing neurons in rodents (Stone *et al.*, 1986; Schmidt, 1987; Commins *et al.*, 1987; Battaglia *et al.*, 1987; O'Hearn *et al.*, 1988) as well as in nonhuman primates (Ricaurte *et al.*, 1988a; Insel *et al.*, 1989; Kleven *et al.*, 1989; Wilson *et al.*, 1989) is now well established, the fate of damaged serotonergic neurons remains uncertain. In particular, it is not known if all serotonergic neurons damaged by MDMA recover and, if they do, whether they reestablish normal synaptic contacts.

Two reports have suggested that at least in rodents, substantial serotonergic recovery does take place after MDMA injury. The first, by Battaglia *et al.* (1988), shows that although MDMA-treated rats sustain an initial severe loss of 5-HT

content and uptake, 1 year later they have a normal number of [³H]paroxetine-labeled 5-HT uptake sites (though 5-HT remains depleted by approximately 40%). The second, a report based on a series of immunohistochemical studies (Molliver *et al.*, 1990), shows that recovery of presynaptic 5-HT markers is most likely related to regrowth of serotonergic axons. Interestingly, axonal reinnervation in these studies was found to involve fine serotonergic axons (Wilson and Molliver, 1990; Molliver *et al.*, 1989), which are selectively damaged by MDA and MDMA (O'Hearn *et al.*, 1988; Wilson *et al.*, 1989). Thick beaded axons, which are spared by MDMA (O'Hearn *et al.*, 1988; Wilson *et al.*, 1989), showed no evidence of collateral sprouting (Molliver *et al.*, 1990; Wilson and Molliver, 1990). On the basis of these findings, Molliver and colleagues (1990) have concluded that although further studies are needed to determine if normal reinnervation patterns are established, MDMA-treated rats show evidence of 5-HT axonal regeneration.

Whether similar axonal regeneration takes place in the

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ABBREVIATIONS: MDMA, (±)-3,4-methylenedioxymethamphetamine; MDA, (±)-3,4-Methylenedioxyamphetamine; 5-HT, serotonin; 5-HIAA, 5-hydroxyindoleacetic acid; DOPAC, dihydroxyphenylacetic acid; DA, dopamine; NE, norepinephrine; FC, frontal cortex; PFC, prefrontal cortex; OC, occipital cortex; TC, temporal cortex; SC, somatosensory cortex; HYP, hypothalamus; TH, thalamus; HI, hippocampus; CH, caudate nucleus head; CB, caudate nucleus body; PR, putamen rostral; PC, putamen caudal; HPLC, high-performance liquid chromatography; ANOVA, analysis of variance.

MDMA-treated primate is not known, but important to determine, because findings in nonhuman primates could be more indicative of what is likely to occur in humans. In two respects, nonhuman primates are already known to differ from rodents in their response to MDMA: 1) monkeys are more sensitive than rats to the serotonin-depleting effects of the drug (Ricaurte *et al.*, 1988a; Ricaurte, 1989) and 2) neuronal perikarya in the dorsal raphe nucleus of the monkey develop pathological changes (inclusions) (Ricaurte *et al.*, 1988a), whereas those in the rat appear entirely unaffected (Molliver *et al.*, 1990). Based on these differences, the long-term effects of MDMA in rodents and primates might be expected to differ. In particular, it might be that although the effects of MDMA in the rodent are reversible, those in the primate are permanent. To test this hypothesis, the present study was undertaken.

Methods

Animals. Adult squirrel monkeys (*Saimiri sciureus*) of both sexes were used. Their weight ranged from 499 to 906 g. The precise age of the monkeys could not be determined with certainty because the animals were feral reared. However, neither extremely young nor old animals were used. Monkeys were housed individually in standard steel cages with free access to food (Purina Monkey Chow) and water throughout. The colony room was maintained at 22–24°C. Fluorescent lighting in the room was automatically turned on daily at 6:00 A.M. and off at 6:00 P.M.

MDMA treatment. Racemic MDMA, as the hydrochloride salt, was injected at a dose of 5 mg/kg twice daily (at approximately 8:00 A.M. and 5:00 P.M.) for 4 consecutive days. This dosing regimen of MDMA was selected because it produces a degree of axonal damage in the squirrel monkey (Ricaurte *et al.*, 1988; Wilson *et al.*, 1989), which is comparable to that from which rats have been reported to recover (Battaglia *et al.*, 1988a; Molliver *et al.*, 1990). MDMA was dissolved in a sterile 0.9% sodium chloride solution and administered s.c. on an ml/kg basis, with the dose expressed as the salt. Animals tolerated MDMA treatment without apparent difficulty.

Brain dissection. The brain was removed from the skull and placed on its ventral surface on a Petri dish over ice. Tissue samples were then obtained from the following cortical regions bilaterally: FC, OC, TC and SC. In all cases, care was taken to remove white matter underlying the cortical tissue. The HYP was dissected free using the optic chiasm, infundibulum and oculomotor nerves as landmarks. The hypothalamic sample was isolated by trimming away the optic tracts and then removing a diamond-shaped tissue sample that had the tuber cinereum at its center. The brain stem was obtained by making a coronal cut from the rostral border of the basis pedunculi to the anterior margin of the superior colliculi. At this point, the cerebral hemispheres were separated by sectioning the corpus callosum. The HI was removed by peeling it away from the temporal lobe and sectioning its point of attachment (subiculum). The TH was dissected using the hypothalamic sulcus and lateral and third ventricles as landmarks. Coronal sections of each hemisphere were then made with the ventral surface facing upwards. Proceeding caudally from the FC, six coronal slices were made. All slices were approximately 2 to 3 mm in thickness. From the first three coronal slices, FC tissue was obtained and designated FC₁. The fourth slice was obtained by making a coronal cut at the point where the olfactory tracts begin to diverge laterally (approximately 2 to 3 mm anterior to the optic chiasm). From this section, additional FC tissue was collected from that portion of the cortical mantle overlying the CH. This FC tissue was designated FC₂. The CH was also obtained from this section. The fifth coronal slice was made by making a cut anterior to the optic chiasm, and another posterior to the mammillary bodies. From this slice, additional FC₃ tissue overlying the CB was obtained, along with tissue of the caudate nucleus (CB₁) and putamen (PR). From the sixth and final coronal slice, additional tissue from the CB (CB₂) was obtained, along with an additional sample of the putamen

(PC). Once dissected free, tissue samples were immediately wrapped in aluminum foil and stored frozen in liquid nitrogen until assay.

Measurement of brain monoamines and their major metabolites. Concentrations of 5-HT, DA, NE, 5-HIAA and DOPAC in brain tissue were measured by reverse phase liquid chromatography (HPLC) coupled with electrochemical detection using the method of Kotake *et al.* (1985) with minor modification. Briefly, frozen tissue was weighed, placed in 10 parts ice-cold 0.4 N perchloric acid, then homogenized for 15 sec using a Polytron homogenizer (setting = 5). The homogenate was centrifuged for 10 min at 15,000 rpm in a refrigerated Sorvall RC2B centrifuge, and fractions of the supernatant were transferred to polypropylene tubes, which were then returned to liquid nitrogen until assay. Separation of monoamines and their metabolites was accomplished with a Brownlee Spheri-5 RP-18 250 × 4.6 mm column (5-μm particle size). For separating the various monoamines and their metabolites, a mobile phase consisting of 98 parts of an aqueous phase (125 mM citric acid, 125 mM sodium phosphate, 0.27 mM EDTA and 0.12 mM sodium octane sulfate) and 2 parts methanol with a pH of 2.5 was used. For separating NE, a mobile phase consisting of an aqueous phase (0.09 M sodium acetate, 0.035 M citric acid, 0.13 mM EDTA and 0.23 mM sodium octane sulfate) with a pH of 4.6 was used (Kilpatrick *et al.*, 1986). The flow rate of the mobile phase was approximately 1.0 ml/min. The column was maintained in a CTO-6A column oven module (Shimadzu) at 40°C. Detection was achieved by means of an amperometric L-ECD-6A detector (Shimadzu, Columbia, MD), with a glassy carbon working electrode and a silver/silver chloride reference electrode. The fixed potential difference between the reference and working electrodes was +0.70 V. The electrochemical response was quantified using a Shimadzu Chromatopac C-R4A data processor equipped to measure the area under the curve for a given sample and compare it to that of standards processed in an identical manner. The sensitivity of this method is approximately 20 pg per 20 μl of sample. Samples from the various treatment groups were always assayed in parallel.

Measurement of serotonin uptake sites. [³H]Paroxetine-labeled 5-HT uptake sites were measured using the method of Habert *et al.* (1985), as modified by Battaglia *et al.* (1987). Briefly, frozen brain regions were weighed, placed in 50 volumes of ice-cold 50 mM Tris-HCl (pH 7.4) buffer containing 120 mM NaCl and 5 mM KCl and homogenized for 30 sec with a Brinkman Polytron homogenizer. The homogenate was centrifuged at 19,500 rpm for 10 min. The pellet was resuspended in the same buffer and centrifuged once again as above. The final pellet was resuspended in the same buffer at a concentration of 6 mg/ml. Diluted aliquots (0.5 ml) of the tissue suspension containing 3 mg of tissue were then placed in tubes. To these tubes, increasing concentrations (0.013–0.207 nM) of [³H]paroxetine (20.5 Ci/mM, New England Nuclear, Boston, MA) were added. This was done in both the presence and absence of 1 μM citalopram. Tubes were then incubated for 1 hr at 22 ± 1°C. After incubation, the tubes were rapidly filtered through GFB (Whatman) filters. The filters were washed three times with 5 ml of buffer, and transferred to plastic vials containing 10 ml of scintillation cocktail. Radioactivity was counted in a Beckman scintillation spectrometer at an efficiency of 64%. Specific binding was calculated by subtracting the counts obtained in samples containing citalopram (nonspecific binding) from the counts obtained in identical samples not containing citalopram (total binding). Assays were performed in triplicate. Specific [³H]paroxetine binding was expressed as pM [³H]paroxetine bound/mg tissue. Binding data were analyzed with the aid of the computer program entitled EBDA (GraphPAD Software, San Diego, CA). As with HPLC monoamine determinations, samples from the various treatment groups were always assayed in parallel.

Statistical analysis. The significance of differences among group means was evaluated by means of analysis of variance (ANOVA). Once a significant *F* test was obtained, Duncan's multiple range comparisons were performed to assess the significance of differences among various treatment groups. The α level for all *post hoc* comparisons was .05. Version 4.1 of the computer program STATPAK (Northwest Analytical Inc., Portland, Oregon) was used.

Drugs and chemicals. MDMA hydrochloride was obtained from

the National Institute on Drug Abuse. 5-HT creatinine sulfate, DA hydrochloride, NE hydrochloride, 5-HIAA and DOPAC were purchased from the Sigma Chemical Co. (St. Louis, MO). Tritiated paroxetine (specific activity 20.5 Ci/mM) was purchased from New England Nuclear Co.

Results

Serotonergic Neurons

Effects at 2 weeks. Two weeks after MDMA treatment (5 mg/kg, s.c., twice daily for 4 days), there was a marked depletion of 5-HT in all brain regions examined (fig. 1A). MDMA treatment also produced a large depletion of 5-HIAA (fig. 1B). Scatchard analysis of [3 H]paroxetine binding showed that along with depletions of 5-HT and 5-HIAA, MDMA treatment reduced the maximal number of [3 H]paroxetine-labeled serotonin

uptake sites (B_{max}), without causing a significant change in the apparent affinity constant (K_d) of the residual sites (fig. 2). Reductions in 5-HT content, [3 H]paroxetine binding and 5-HIAA concentration were comparable in magnitude (table 1).

Time course. By 10 weeks, a trend toward recovery of 5-HT (table 2), 5-HIAA (table 3) and [3 H]paroxetine binding (fig. 3, A-C) was evident in some (but not all) of the forebrain regions examined. For example, in the CH and HI, there was indication that all three presynaptic markers had partially recovered by 10 weeks. However, in other brain regions (e.g., FC₃), only recovery of 5-HIAA achieved statistical significance (tables 2 and 3 and fig. 3B). Evidence of partial recovery was still apparent 8 months after MDMA treatment in some brain regions (e.g., HI). However, by 18 months, it was clear that recovery did not continue in any of the brain regions examined except for the TH (tables 2 and 3) and HYP (fig. 4).

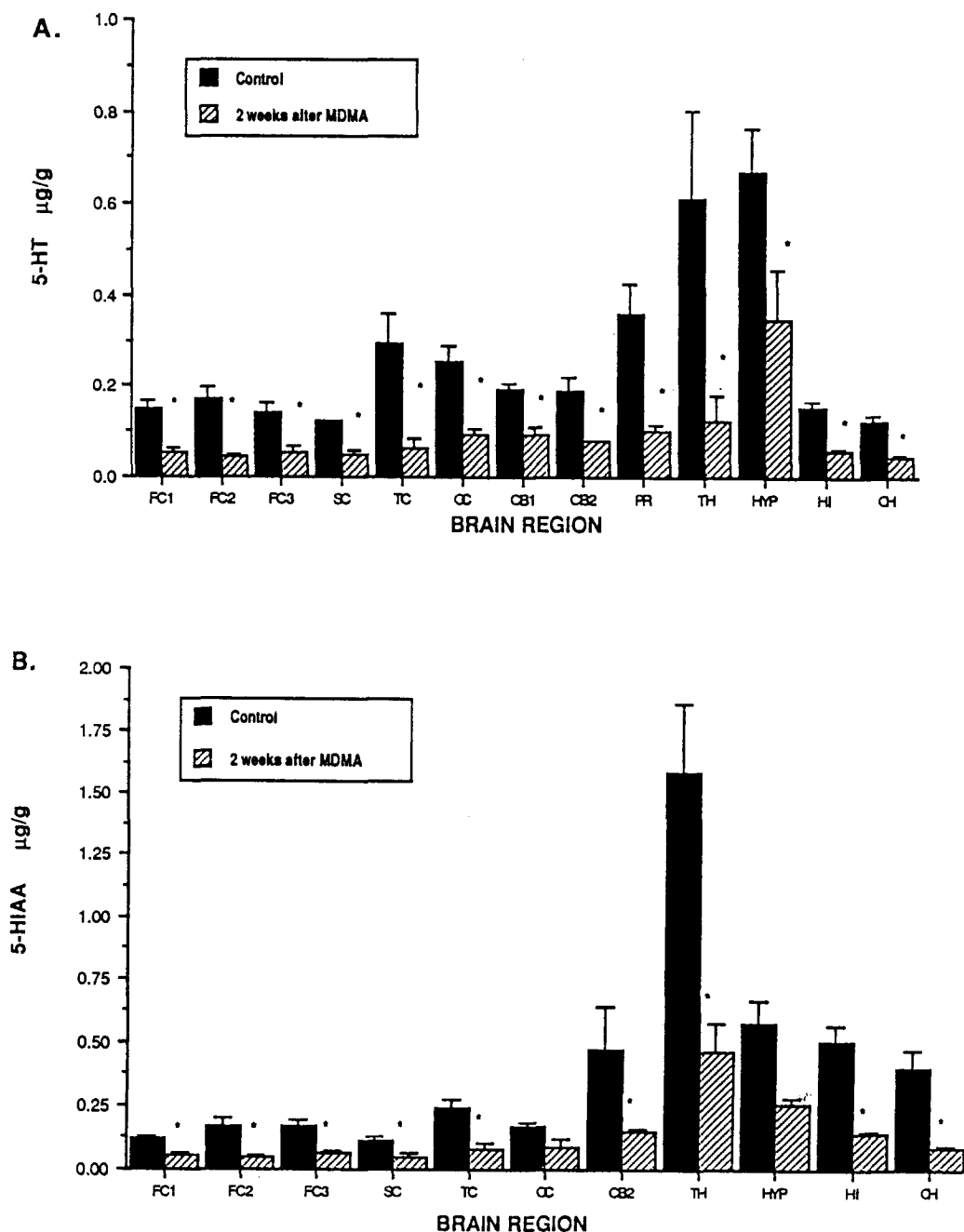


Fig. 1. Depletion of A) 5-HT and B) 5-HIAA in various regions of the monkey forebrain 2 weeks after MDMA treatment. MDMA was given s.c. at a dose of 5 mg/kg twice daily for 4 days. 5-HT and 5-HIAA were measured by means of reversed phase HPLC-EC as detailed under "Materials and Methods." Values graphed represent the mean ($\pm$ S.E.M., $n = 3$) and are expressed in μ g/g of tissue. *Designates significant difference from control group, $P < .05$, ANOVA followed by Duncan's multiple range test.

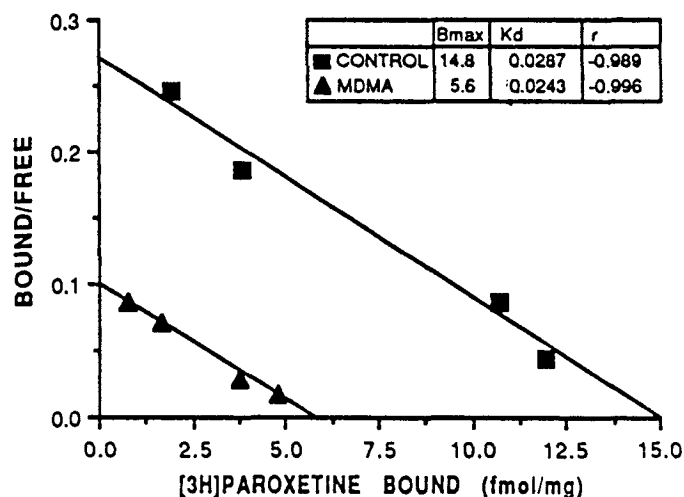


Fig. 2. Scatchard analysis of specific *in vitro* [³H]paroxetine binding to membranes prepared from parietal cortex of (■) control and (▲) MDMA-treated monkeys. MDMA treatment (5 mg/kg twice daily for 4 days) took place 2 weeks previously. Concentrations of [³H]paroxetine used ranged from 0.013 to 0.207 nM. Binding studies were performed as described under "Materials and Methods." Data are from a representative experiment repeated twice with similar results. Kinetic constants (shown in inset) were calculated with the aid of the computer program EBDA.

TABLE 1

Mean (±S.E.M) percent reductions in 5-HT, 5-HIAA and [³H]paroxetine binding 2 weeks after MDMA treatment

5-HT, 5-HIAA and [³H]paroxetine binding were determined as described under "Materials and Methods."

Brain Region	N	5-HT	5-HIAA	[³ H]Paroxetine
FC ₁	3	64.6 ± 5.2	56.2 ± 6.1	65.5 ± 6.5
HI	3	63.9 ± 4.9	72.1 ± 1.9	72.3 ± 4.8
CB ₁	3	59.1 ± 7.3	68.8 ± 1.7	65.3 ± 6.1

In the TH, there was still partial recovery of 5-HT (table 2) with complete recovery of 5-HIAA (table 3). In the HYP, there was near total recovery of both 5-HT and 5-HIAA by 8 months and, by 18 months, 5-HT and 5-HIAA levels had risen to 140 and 187% of control values, respectively (fig. 4). [³H]Paroxetine binding studies could not be performed using HYP samples because of insufficient tissue.

Eighteen months after MDMA treatment, the reductions in 5-HT content, [³H]paroxetine binding and 5-HIAA concentration did not appear to be as concordant as they had been 2 weeks after MDMA treatment (table 4). For example, in the

TABLE 2

Regional content of 5-HT in the monkey forebrain at various time points after MDMA treatment

Values shown represent the mean (±S.E.M., n = 3 per group) and are expressed in µg/mg of tissue. 5-HT was measured as detailed in "Materials and Methods."

Brain Region	Control	2 Weeks	10 Weeks	8 Months	18 Months
FC ₁	0.148 ± 0.017	0.052 ± 0.008*	0.065 ± 0.012*	0.069 ± 0.017*	0.039 ± 0.005*
FC ₂	0.172 ± 0.026	0.044 ± 0.004*	0.086 ± 0.019*	0.073 ± 0.008*	0.070 ± 0.024*
FC ₃	0.141 ± 0.021	0.053 ± 0.013*	0.080 ± 0.008*	0.051 ± 0.012*	0.037 ± 0.002*
SC	0.121 ± 0.002	0.047 ± 0.008*	0.075 ± 0.013	0.096 ± 0.035	0.027 ± 0.002*
TC	0.296 ± 0.065	0.063 ± 0.020*	0.108 ± 0.012*	0.065 ± 0.013*	0.055 ± 0.010*
OC	0.255 ± 0.036	0.090 ± 0.016*	0.127 ± 0.040*	0.115 ± 0.039*	0.048 ± 0.012*
CH	0.123 ± 0.013	0.042 ± 0.008*	0.096 ± 0.010**	0.075 ± 0.003**	0.055 ± 0.002*
CB ₁	0.192 ± 0.013	0.093 ± 0.017*	0.190 ± 0.021**	0.152 ± 0.031	0.087 ± 0.003*
CB ₂	0.189 ± 0.032	0.077 ± 0.003*	0.152 ± 0.011**	0.123 ± 0.020	0.076 ± 0.019*
PR	0.358 ± 0.068	0.101 ± 0.014*	0.224 ± 0.002**	0.201 ± 0.003*	0.130 ± 0.025*
HI	0.152 ± 0.015	0.055 ± 0.008*	0.106 ± 0.014**	0.106 ± 0.025**	0.057 ± 0.005*
TH	0.610 ± 0.192	0.121 ± 0.058*	0.190 ± 0.010**	0.263 ± 0.049**	0.385 ± 0.020*

* Significantly different from control group, P < .05, ANOVA followed by Duncan's multiple range test.

** Significantly different from 2-week group, P < .05, ANOVA followed by Duncan's multiple range test.

HI, [³H]paroxetine binding was reduced to a greater extent than 5-HT or 5-HIAA. Similarly, in the CH, paroxetine binding was reduced to a greater extent than 5-HIAA (table 4).

Dopaminergic Neurons

Two weeks after drug treatment, concentrations of DA and DOPAC were unaltered in the CH, PR and PFC of MDMA-treated monkeys (table 5). Over time, no changes in the levels of either DA or DOPAC were evident in any of the forebrain regions examined except in the CB₂, where DOPAC levels were slightly but significantly reduced (table 5).

Noradrenergic Neurons

Regional brain NE levels were also unaffected 2 weeks after MDMA (table 6). As with DA levels, no changes in regional brain NE levels developed over time in any of the brain regions examined (table 6).

Discussion

The results of the present study indicate that the neurotoxic effects of MDMA in the primate, in contrast to those in the rodent, are long-lasting, possibly permanent. Monkeys examined as long as 18 months after MDMA treatment continue to show marked decrements in 5-HT levels, [³H]paroxetine-labeled 5-HT uptake sites and 5-HIAA concentrations. Because 5-HT, 5-HT uptake and 5-HIAA are the principal presynaptic markers of serotonergic nerve fibers, the present results suggest that serotonergic axons in the primate, unlike those in the rodent, fail to recover from MDMA injury.

Results from two other studies also point to persistent effects of MDMA and related drugs on serotonergic neurons in non-human primates. Insel *et al.* (1989) have shown that that rhesus monkeys given repeated high doses of MDMA show severe 5-HT neurochemical deficits 14 weeks after drug treatment. Similarly, Woolverton *et al.* (1989) have found that methamphetamine-treated rhesus monkeys show large depletions of brain 5-HT and 5-HIAA when examined 4 years later. Together with the present results, these findings suggest that the neurotoxic effects of MDMA and related drugs on 5-HT neurons in the primate may be permanent.

As alluded to above, lack of axonal recovery in the MDMA-treated primate contrasts with the vigorous 5-HT axonal recovery that has been observed in MDMA-treated rats (Battaglia *et al.*, 1988; Molliver *et al.*, 1990) and in rats treated with

TABLE 3

Regional content of 5-HIAA in the monkey forebrain at various time points after MDMA treatment

Values shown represent the mean ($\pm$ S.E.M., $n = 3$ per group) and are expressed in $\mu\text{g/g}$ of tissue. 5-HIAA was measured as detailed under "Materials and Methods."

Brain Region	Control	2 Weeks	10 Weeks	8 Months	18 Months
FC ₁	0.123 $\pm$ 0.010	0.054 $\pm$ 0.008*	0.089 $\pm$ 0.011*	0.057 $\pm$ 0.007*	0.051 $\pm$ 0.010*
FC ₂	0.171 $\pm$ 0.027	0.048 $\pm$ 0.006*	0.104 $\pm$ 0.019*	0.077 $\pm$ 0.010*	0.061 $\pm$ 0.007*
FC ₃	0.169 $\pm$ 0.027	0.064 $\pm$ 0.010*	0.120 $\pm$ 0.017**	0.073 $\pm$ 0.010*	0.067 $\pm$ 0.003*
SC	0.115 $\pm$ 0.013	0.051 $\pm$ 0.014*	0.083 $\pm$ 0.023*	0.059 $\pm$ 0.011*	0.039 $\pm$ 0.004*
TC	0.244 $\pm$ 0.030	0.083 $\pm$ 0.020*	0.093 $\pm$ 0.012*	0.069 $\pm$ 0.018*	0.104 $\pm$ 0.018*
OC	0.167 $\pm$ 0.019	0.090 $\pm$ 0.027	0.111 $\pm$ 0.026	0.100 $\pm$ 0.035*	0.044 $\pm$ 0.009*
CH	0.405 $\pm$ 0.070	0.089 $\pm$ 0.010*	0.224 $\pm$ 0.021**	0.148 $\pm$ 0.014*	0.163 $\pm$ 0.005*
CB ₂	0.476 $\pm$ 0.170	0.149 $\pm$ 0.008*	0.333 $\pm$ 0.082**	0.224 $\pm$ 0.018	0.298 $\pm$ 0.034
HI	0.510 $\pm$ 0.059	0.142 $\pm$ 0.009*	0.218 $\pm$ 0.003**	0.202 $\pm$ 0.022*	0.218 $\pm$ 0.018
TH	1.588 $\pm$ 0.275	0.467 $\pm$ 0.011*	0.699 $\pm$ 0.067**	0.808 $\pm$ 0.080*	1.723 $\pm$ 0.104**

* Significantly different from control group, $P < .05$, ANOVA followed by Duncan's multiple range test.

** Significantly different from 2-week group, $P < .05$, ANOVA followed by Duncan's multiple range test.

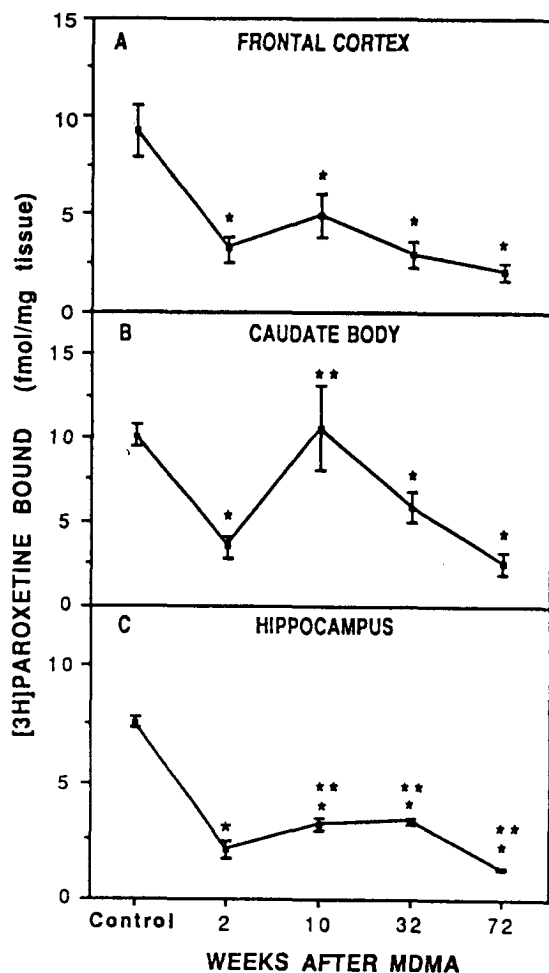


Fig. 3. Serotonin uptake sites labeled with [^3H]paroxetine in various regions of the monkey forebrain 2 weeks, 10 weeks, 8 months and 18 months after MDMA treatment (5 mg/kg twice daily for 4 days). Values were determined from studies in which a saturating concentration (0.207 nM) of [^3H]paroxetine was used. Binding studies were performed as described under "Materials and Methods." Data are expressed in pM of [^3H]paroxetine bound per mg of tissue. *Designates significantly different from control group, $P < .05$, ANOVA followed by Duncan's multiple range test. **Designates significantly different from 2-week group, $P < .05$, ANOVA followed by Duncan's multiple range test.

other serotonergic neurotoxins (Azmitia *et al.*, 1978, Bjorklund *et al.*, 1981). The basis for this apparent species difference is unclear. It could be related to the fact that the effects of MDMA on 5-HT neurons in the primate are initially more extensive

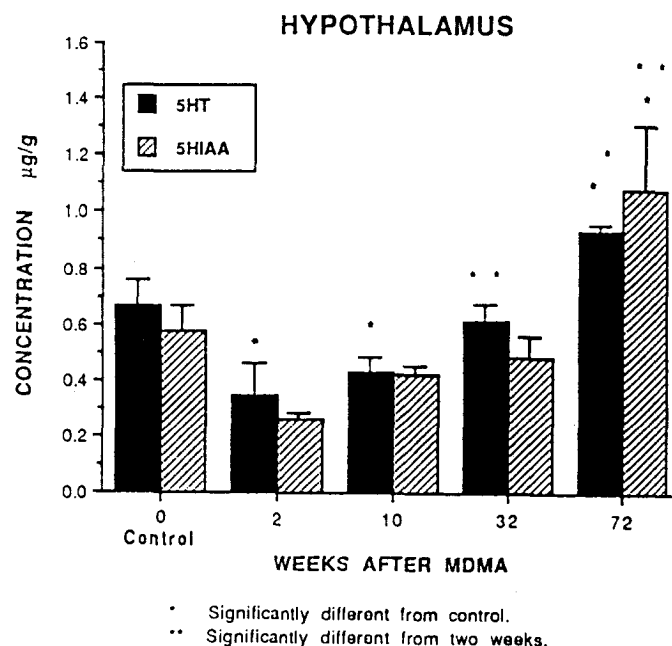


Fig. 4. Hypothalamic 5-HT and 5-HIAA concentrations in control monkeys and in monkeys administered MDMA 2 weeks, 10 weeks, 8 months and 18 months previously. Values shown are the mean ($\pm$ S.E.M., $n = 3$) and are expressed in $\mu\text{g/g}$ of tissue. *Designates significantly different from control group, $P < .05$, ANOVA followed by Duncan's multiple range test. **Designates significantly different 2-week group, $P < .05$, ANOVA followed by Duncan's multiple range test.

TABLE 4

Mean ($\pm$ S.E.M.) percent reductions in 5-HT, 5-HIAA and [^3H]paroxetine binding 18 months after MDMA treatment

Serotonin 5-HIAA and [^3H]paroxetine binding were determined as described under "Materials and Methods."

Brain Region	N	5-HT	5-HIAA	[^3H]Paroxetine
FC ₁	3	73.7 $\pm$ 3.3	58.8 $\pm$ 9.3	77.6 $\pm$ 5.3
HI	3	62.5 $\pm$ 3.1	57.3 $\pm$ 3.4	82.9 $\pm$ 1.0*
CB ₁	3	58.8 $\pm$ 10.2	37.5 $\pm$ 7.1	74.6 $\pm$ 5.9**

* Significantly different from 5-HT and 5-HIAA ($P < .05$).

** Significantly different from 5-HIAA only ($P < .05$).

than those in the rodent (Ricaurte *et al.*, 1988a). Alternatively, it could indicate that 5-HT neurons in the primate have less regenerative potential than those in the rodent. Yet another possibility is that the likelihood of axonal recovery is inversely proportional to the distance that an injured axon has to travel before reestablishing synaptic contact. If this hypothesis is

TABLE 5

Regional brain DA and DOPAC concentrations at various times after MDMA treatmentValues shown represent the mean ($\pm$ S.E.M., $n = 3$ per group) in $\mu\text{g/g}$ of tissue. DA and DOPAC were determined as described under "Materials and Methods."

Drug	Brain Region	Control	2 Weeks	10 Weeks	8 Months	18 Months
DA	PFC	0.04 $\pm$ 0.01	0.05 $\pm$ 0.01	0.02 $\pm$ 0.00	0.04 $\pm$ 0.00	0.05 $\pm$ 0.01
	CH	9.66 $\pm$ 1.59	8.30 $\pm$ 1.52	8.29 $\pm$ 0.30	10.32 $\pm$ 1.40	10.23 $\pm$ 0.56
	CB ₁	10.7 $\pm$ 2.12	11.1 $\pm$ 1.63	8.88 $\pm$ 0.71	11.5 $\pm$ 1.40	14.5 $\pm$ 1.42
	CB ₂	9.84 $\pm$ 0.43	9.49 $\pm$ 1.16	8.40 $\pm$ 0.31	9.49 $\pm$ 0.71	9.36 $\pm$ 0.72
	PR	9.46 $\pm$ 1.36	11.1 $\pm$ 0.64	10.9 $\pm$ 1.81	14.1 $\pm$ 0.80	13.6 $\pm$ 1.85
DOPAC	PFC	0.016 $\pm$ 0.001	0.020 $\pm$ 0.005	0.013 $\pm$ 0.002	0.011 $\pm$ 0.002	0.017 $\pm$ 0.002
	CH	0.412 $\pm$ 0.045	0.348 $\pm$ 0.036	0.319 $\pm$ 0.031	0.435 $\pm$ 0.060	0.306 $\pm$ 0.040
	CB ₁	0.528 $\pm$ 0.107	0.437 $\pm$ 0.046	0.306 $\pm$ 0.028	0.534 $\pm$ 0.103	0.460 $\pm$ 0.015
	CB ₂	0.472 $\pm$ 0.077	0.373 $\pm$ 0.022	0.296 $\pm$ 0.023*	0.314 $\pm$ 0.014*	0.278 $\pm$ 0.034*
	PR	0.525 $\pm$ 0.043	0.483 $\pm$ 0.080	0.504 $\pm$ 0.042	0.575 $\pm$ 0.112	0.427 $\pm$ 0.075

* Significantly different from control group, $P < .05$, ANOVA followed by Duncan's multiple range test.

TABLE 6

Regional brain NE concentrations in the squirrel monkey at various times after MDMA treatmentValues shown represent the mean ($\pm$ S.E.M., $n = 3$ per group) in $\mu\text{g/g}$ of tissue. NE was measured as described under "Materials and Methods."

Drug	Brain Region	Control	2 Weeks	10 Weeks	8 Months	18 Months
NE	SC	0.175 $\pm$ 0.014	0.131 $\pm$ 0.011	0.172 $\pm$ 0.013	0.192 $\pm$ 0.014	0.191 $\pm$ 0.008
	HI	0.258 $\pm$ 0.037	0.192 $\pm$ 0.038	0.184 $\pm$ 0.015	0.203 $\pm$ 0.044	0.213 $\pm$ 0.30
	HYP	3.28 $\pm$.058	2.61 $\pm$ 0.47	2.43 $\pm$ 0.46	3.43 $\pm$ 0.12	2.91 $\pm$ 0.90

correct, lack of recovery in the monkey may be due to the fact that damaged axons in this experimental animal have to travel longer distances than those in the rat before reestablishing synaptic contact (because of the larger brain size of the monkey). Consistent with this proposal is the observation that serotonergic axons in the monkey which have shorter distances to travel (e.g., those innervating the thalamus and hypothalamus) show evidence of recovery, whereas those innervating more distant structures (e.g., cerebral cortex) do not.

The early partial, but unsustained, recovery of presynaptic serotonergic markers noted in some brain regions of the MDMA-treated monkey (HI, CH) deserves comment. First, because a similar partial recovery was observed in an earlier study (Ricaurte *et al.*, 1988b), it would appear that partial recovery is a consistent phenomenon. Second, the fact that in some brain regions there is parallel recovery of all three presynaptic markers (5-HT, 5-HIAA and 5-HT uptake sites) suggests that neurochemical recovery is related to axonal regeneration. Consistent with this view are data in the rodent showing that 5-HT neurons have marked regenerative potential (Molliver *et al.*, 1990; Bjorklund *et al.*, 1981; Azmitia *et al.*, 1978). At seeming odds with this interpretation, however, is the protracted time course of recovery, which in some brain regions (e.g., HI) persists for as long as 8 months after MDMA injury. At first glance, such a protracted time course would seem to argue against the notion that neurochemical recovery is related to axonal regeneration. However, it should be recognized that at present, virtually nothing is known regarding 5-HT axonal regeneration in the primate central nervous system. Hence, it would be premature at this point to dismiss the possibility that partial neurochemical recovery reflects an attempt (albeit an unsuccessful one) at axonal regeneration. Of course, the possibility that the observed neurochemical changes do not have a direct morphological correlate, but are purely biochemical (perhaps compensatory) in nature, also needs to be considered.

If an unsuccessful attempt at axonal regeneration underlies the partial recovery of 5-HT markers observed in some brain regions (e.g., CH, HI), the question arises as to whether axons which fail to recover undergo retrograde degenerative changes,

and whether such changes proceed back to the level of the nerve cell body. If they do, nerve cell body loss could account for the persistent neurotoxic effects of MDMA in the primate. Studies evaluating this possibility are now in progress, along with morphological investigations aimed at elucidating the significance of increased levels of 5-HT and 5-HIAA in the HYP (fig. 4). In particular, the possibility that increased levels of 5-HT and 5-HIAA in the HYP reflect serotonergic hyperinnervation of this brain region is being pursued.

Although findings with presynaptic serotonergic markers point to possible axonal sprouting in some brain regions (e.g., HYP), findings with dopaminergic and noradrenergic markers do not. For example, DA and DOPAC do not increase over time in either the CH or PR, brain regions which are markedly and persistently depleted of their 5-HT content (table 5). Similarly, NE levels fail to increase over time in any of the brain regions examined (table 6). Thus, there is no indication that either DA or NE neurons undergo collateral sprouting into 5-HT-denervated brain areas. Absence of DA or NE collateral sprouting in the MDMA-treated monkey contrasts with the occurrence of 5-HT sprouting in the 6-hydroxydopamine-treated rat (Snyder *et al.*, 1986; Blue and Molliver, 1987). This difference could indicate that 5-HT neurons have greater sprouting potential than catecholaminergic neurons (though this seems unlikely; see Reis *et al.*, 1978). Alternatively, age factors could be involved, because serotonergic sprouting in rats occurs only when dopaminergic innervation is removed during the neonatal period (Stachowiak *et al.*, 1984; Snyder *et al.*, 1986).

For the most part, reasonably good concordance was observed in this study among the various presynaptic markers for serotonergic axons (5-HT, 5-HIAA and [³H]paroxetine binding). For instance, 2 weeks after MDMA treatment, all three markers were comparably reduced (table 1). Such concordance, however, was not observed at all time points examined in this study. For example, at 18 months, [³H]paroxetine-labeled 5-HT uptakes sites appeared more reduced than 5-HT and 5-HIAA levels (table 4). Whether the apparent discordance among markers observed at 18 months (and sometimes at other time points) is

due to the presence of axons which are not entirely normal is not known, but, as suggested by Battaglia *et al.* (1988), important to consider. It is also important to consider the possibility that although some discordance between presynaptic markers may reflect a true alteration in axonal structure or function, some of it may be due to experimental variability.

In conclusion, the results of this study indicate that MDMA, a recreational drug of abuse, produces lasting effects on serotonergic neurons in nonhuman primates, with most brain regions showing signs of persistent 5-HT denervation, and others showing signs of reinnervation (TH) and possibly even hyperinnervation (HYP). Why the long-term effects of MDMA in the primate should contrast with those in the rodent is not known, but of interest to determine. Such studies hold promise for enhancing our understanding of neuronal response to injury, and could help identify factors governing axonal regeneration in the central nervous system of primates. Finally, the usefulness of the MDMA-treated primate for evaluating strategies of neuronal rescue (*e.g.*, neurotrophic factors, transplants) and for elucidating the functional role of 5-HT in the primate brain remains to be explored.

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