

Clinical Investigation

Thomas P. Stossel, MD, Section Editor

(±)3,4-Methylenedioxymethamphetamine Selectively Damages Central Serotonergic Neurons in Nonhuman Primates

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(±)3,4-Methylenedioxymethamphetamine (MDMA) is a popular recreational drug that has been proposed to be useful as an adjunct to psychotherapy. This study assessed the neurotoxic potential of MDMA in nonhuman primates. Monkeys were repeatedly administered doses (2.50, 3.75, and 5.00 mg/kg) of MDMA subcutaneously and analyzed for regional brain content of serotonin and 5-hydroxyindoleacetic acid two weeks later. In all regions of the monkey brain examined, MDMA produced a selective dose-related depletion of serotonin and 5-hydroxyindoleacetic acid. These neurochemical deficits were associated with evidence of structural damage to serotonergic nerve fibers. In addition, MDMA produced pathological changes in nerve cell bodies in the dorsal, but not median, raphe nucleus. These results indicate that MDMA is a selective serotonergic neurotoxin in nonhuman primates and that humans using this drug may be at risk for incurring central serotonergic neuronal damage.

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RECREATIONAL abuse of controlled substance analogues ("designer drugs") potentially poses a major health problem.^{1,2} (±)3,4-Methylenedioxymethamphetamine (MDMA), variously known on the street as "Ecstasy," "Adam," or "XTC,"³ is an analogue of the controlled substance (±)3,4-methylenedioxymphetamine (MDA). Presently, MDMA is one of the more popular recreational drugs in the United States.⁴ It has been estimated that 30 000 capsules of the drug are sold each month (R. K. Siegel, PhD, unpublished data, 1985). It has also been proposed that MDMA may be useful as an adjunct to insight-oriented psychotherapy.^{5,7} This suggestion is based largely on subjective reports that MDMA improves interpersonal com-

munication and enhances emotional awareness.

In 1985, the Drug Enforcement Agency placed MDMA on Schedule I of controlled substances, citing increasing recreational use of this drug and expressing concern that MDMA might cause neurological damage.⁸ This concern arose largely because of evidence that MDA (the *N*-desmethyl derivative of MDMA) destroys central serotonergic nerve terminals in rats.⁹ Recent studies indicate that MDMA, like MDA, is toxic to serotonergic nerve terminals in the rodent brain.¹⁰⁻¹⁵ However, findings in rats appear to have done little to deter recreational use of MDMA. At least in part, this may be because studies in rodents do not always accurately predict drug toxicity in humans. For example, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is relatively inactive in rats^{16,17} but profoundly toxic in primates.^{18,19} Conversely, 1,2,3,6-tetrahydro-1-methyl-4-(methylpyrrol-2-yl)pyridine, an analogue of MPTP, is very toxic in rodents²⁰ but inactive orally in primates.²¹ In addition, differences in the

way rodents and primates metabolize amphetamines²² may alter the neurotoxic effects of these drugs. For these reasons, we thought it critical to assess the neurotoxic activity of MDMA in nonhuman primates.

METHODS

Subjects

Seventeen monkeys were used in this study. Eleven female squirrel monkeys (*Saimiri sciureus*) 6 to 8 years of age and weighing 0.6 to 0.7 kg were used for neurochemical studies and for anatomic studies of the raphe nuclei. Three female rhesus monkeys (*Macaca mulatta*) 1.5 to 4.0 years of age and weighing 2.5 to 3.5 kg and two female and one male cynomolgus monkeys (*Macaca fascicularis*) weighing 2.0 to 4.5 kg were used for immunohistochemical studies. No differences in response to MDMA were noted among the three species.

Drug Treatment

The hydrochloride salt of MDMA was administered subcutaneously twice daily at 0800 and 1700 hours for four consecutive days. This dosing regimen was used to permit comparison of the present results with those previously obtained in rodents.^{12,14} For neurochemical studies, eight of 11 squirrel monkeys were administered the following doses of MDMA according to the above-mentioned schedule of drug administration: 2.50 mg/kg (*n*=2), 3.75 mg/kg (*n*=3), and 5.00 mg/kg (*n*=3). The three remaining squirrel monkeys served as untreated controls. For immunohistochemical studies, three of six macaque monkeys were given the high-dose (5.00 mg/kg) regimen of MDMA; the other three untreated monkeys served as controls.

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Neurochemistry

Two weeks after drug treatment, the monkeys were killed under deep ether anesthesia. The brain was removed from the skull, and the brainstem was dissected away and placed in 10% formol saline for later anatomical study. The forebrain was dissected over ice, and the various brain regions were isolated for analysis of monoamine content. Concentrations of serotonin, 5-hydroxyindoleacetic acid, dopamine, and norepinephrine were measured by reverse-phase high-performance liquid chromatography coupled with electrochemical detection, using the method of Kotake et al²⁴ with minor modification.²⁴

Histology

For routine histological studies of the raphe nuclei, the brainstems of three monkeys that had received the 5-mg/kg regimen of MDMA two weeks previously were immersion-fixed in 10% formol saline for one week prior to paraffin embedding and staining. Sections were stained with hematoxylin-eosin, Luxol fast blue (LFB)-cresyl violet, LFB-periodic acid-Schiff (PAS), or LFB-Bielschowsky. For immunohistochemical studies of serotonergic nerve fibers in the forebrain, three monkeys that had received the 5-mg/kg regimen of MDMA two weeks previously and three controls were administered the monoamine oxidase inhibitor trans-2-phenylcyclopropylamine (10 mg/kg intraperitoneally) one hour prior to being killed by intracardiac perfusion under deep sodium pentobarbital anesthesia. After the vascular tree was cleared with ice-cold phosphate-buffered saline, perfusion was continued with 4% paraformaldehyde, pH 6.5, followed by 4% paraformaldehyde and 0.12% glutaraldehyde (pH 9.5). Tissue blocks were placed in buffered 4% paraformaldehyde for seven hours and then in 10% dimethyl sulfoxide in phosphate-buffered saline overnight. Frozen sections (30 μ m) were incubated in an anti-serotonin antisera (R8) diluted 1:5000 (or in anti-tyrosine hydroxylase antisera diluted 1 U:48 mL) in phosphate-buffered saline with 0.2% octyl phenoxypolyethoxyethanol (Triton X-100) and 1% normal goat serum at 4°C for three days. The antibody was visualized with a peroxidase-labeled avidin-biotin complex (Vector Laboratories Inc, Burlingame, Calif), and staining was enhanced with the osmophilic reaction sequence of Gerfen.²⁵

Statistics

After a simple one-way analysis of variance showed an *F* value of *P* < .05,

Table 1.—Dose-Related Decrease in Serotonin Concentration in the Somatosensory Cortex of the Monkey Two Weeks After Administration of MDMA*

Treatment	N	Serotonin, μ g/g	% Depletion
Control	3	0.167 $\pm$ 0.015	...
MDMA			
2.50 mg/kg	2	0.093 $\pm$ 0.010†	-44
3.75 mg/kg	3	0.037 $\pm$ 0.013†	-78
5.00 mg/kg	3	0.017 $\pm$ 0.003†	-90

*MDMA indicates ($\pm$)3,4-methylenedioxymethamphetamine. Values represent the mean $\pm$ SEM. †*P* < .05, determined by individual comparison with control after a single one-way analysis of variance showed value *P* < .05.

Table 2.—Regional Concentrations of Serotonin in the Monkey Brain Two Weeks After Administration of MDMA (5 mg/kg)*

Serotonin Concentration, μ g/g					
Somatosensory Cortex	Caudate	Putamen	Hippocampus	Hypothalamus	Thalamus
Control (N=3)					
0.14 $\pm$ 0.01	0.21 $\pm$ 0.03	0.28 $\pm$ 0.02	0.13 $\pm$ 0.03	0.90 $\pm$ 0.06	0.73 $\pm$ 0.01
MDMA (N=3)					
0.02 $\pm$ 0.01†	0.03 $\pm$ 0.01†	0.03 $\pm$ 0.01†	0.03 $\pm$ 0.01†	0.21 $\pm$ 0.01†	0.12 $\pm$ 0.01†

*MDMA indicates ($\pm$)3,4-methylenedioxymethamphetamine. Values represent the mean $\pm$ SEM. †*P* < .05, two-tailed Student's *t* test.

Table 3.—Decreased Concentration of 5HIAA in the Monkey Brain Two Weeks After Administration of MDMA (5 mg/kg)*

5HIAA Concentration, μ g/g				
Treatment	N	Neocortex	Caudate	Hypothalamus
Control	3	0.250 $\pm$ 0.010	0.232 $\pm$ 0.005	0.245 $\pm$ 0.008
MDMA	3	0.040 $\pm$ 0.006†	0.056 $\pm$ 0.002†	0.060 $\pm$ 0.001†

*5HIAA indicates 5-hydroxyindoleacetic acid; and MDMA, ($\pm$)3,4-methylenedioxymethamphetamine. Values represent the mean $\pm$ SEM. †*P* < .05, two-tailed Student's *t* test.

individual values were compared with the control using a two-tailed Student's *t* test. Thereafter, regression analysis was performed and the 3 *df* between groups were partitioned into a regression component (1 *df*) and a deviation from regression component (2 *df*).

Materials

Dopamine hydrochloride, norepinephrine hydrochloride, and serotonin creatinine sulfate were purchased from the Sigma Chemical Company, St Louis; MDMA hydrochloride was provided by David Nichols, PhD, Department of Medicinal Chemistry, Purdue University, Lafayette, Ind, and the National Institute of Drug Abuse. Tranlycypromine (tranlyl-2-phenylcyclopropylamine) was purchased from Regis Chemical Company, Morton Grove, Ill. The rabbit antiserotonin was generated by H. Lidov against serotonin conjugated to bovine serum albumin with formaldehyde. Rabbit anti-tyrosine hydroxylase antisera was purchased from Eugene Tech International Inc, Allendale, NJ.

Table 4.—Unchanged Concentrations of Dopamine and Norepinephrine in the Monkey Brain Two Weeks After Administration of MDMA (5 mg/kg)*

Concentration, μ g/g			
Treatment	N	Dopamine	Norepinephrine
Control	3	10.4 $\pm$ 0.5	0.39 $\pm$ 0.03
MDMA	3	9.7 $\pm$ 0.8	0.41 $\pm$ 0.02

*MDMA indicates ($\pm$)3,4-methylenedioxymethamphetamine. Dopamine was measured in the caudate nucleus; and norepinephrine, in the cerebral cortex. Values represent the mean $\pm$ SEM.

RESULTS

Chemistry

Dose Response.—Measurement of serotonin two weeks after drug treatment showed that multiple subcutaneous doses of MDMA (2.50, 3.75, and 5.00 mg/kg) produced a dose-related depletion of serotonin in the somatosensory cortex of the monkey, with the lowest dose (2.50 mg/kg) producing a 44% depletion and the highest dose (5.00 mg/kg) producing a 90% depletion (Table 1). Statistical analysis (simple analysis of variance followed linear regression with partitioning of the

degrees of freedom into a regression component [1 *df*] and a deviation from regression component [2 *df*] revealed that linearity explained virtually all of the variability between doses ($r = .97$). The deviation from regression component was not statistically significant ($F_{2,8} = 2.28$; $P > .05$).

Regional Effects.—Multiple doses of MDMA also produced large depletions of serotonin in the caudate nucleus, putamen, hippocampus, hypothalamus, and thalamus of the monkey (Table 2). One of the most severely affected areas was the cerebral cortex (Table 2), where the lowest dose (2.5 mg/kg) of MDMA produced a 44% depletion of serotonin (Table 1).

Other Markers.—Measurement of 5-hydroxyindoleacetic acid, another chemical marker for serotonergic nerve fibers, showed that multiple doses of MDMA also markedly reduced the concentration of this compound (Table 3). Concentrations of 5-hydroxyindoleacetic acid were reduced by 84% in the neocortex, 76% in the caudate nucleus, 75% in the hippocampus, and 40% in the hypothalamus.

Selectivity.—Measurement of dopamine and norepinephrine concentrations in monkeys receiving the highest dose (5 mg/kg) showed that MDMA produced no depletion of dopamine or norepinephrine (Table 4).

Morphology

Nerve Fibers.—Immunohistochemical studies performed to assess the structural integrity of serotonergic nerve fiber projections to the forebrain demonstrated a marked reduction in the number and density of serotonin-immunoreactive axons throughout the cerebral cortex of three of three monkeys receiving the 5-mg/kg dose of MDMA (Fig 1). In addition, at higher power, some serotonergic axons appeared swollen and misshapen. Staining with an antibody to tyrosine hydroxylase revealed no evidence of damage to catecholamine-containing nerve fibers in the cerebral cortex.

Cell Bodies.—Examination of nerve cell bodies in the raphe nuclei of the monkeys receiving the highest dose of MDMA (5 mg/kg) showed that while MDMA produced no obvious cell loss in either the dorsal or median raphe nuclei, the drug induced striking cytopathological changes in nerve cells of the dorsal raphe nucleus. In three of three of these animals, hematoxylin-eosin-stained paraffin sections of the dorsal raphe nucleus showed numerous, somewhat shrunken nerve cells that contained brownish-red spherical cytoplasmic inclusions that displaced the

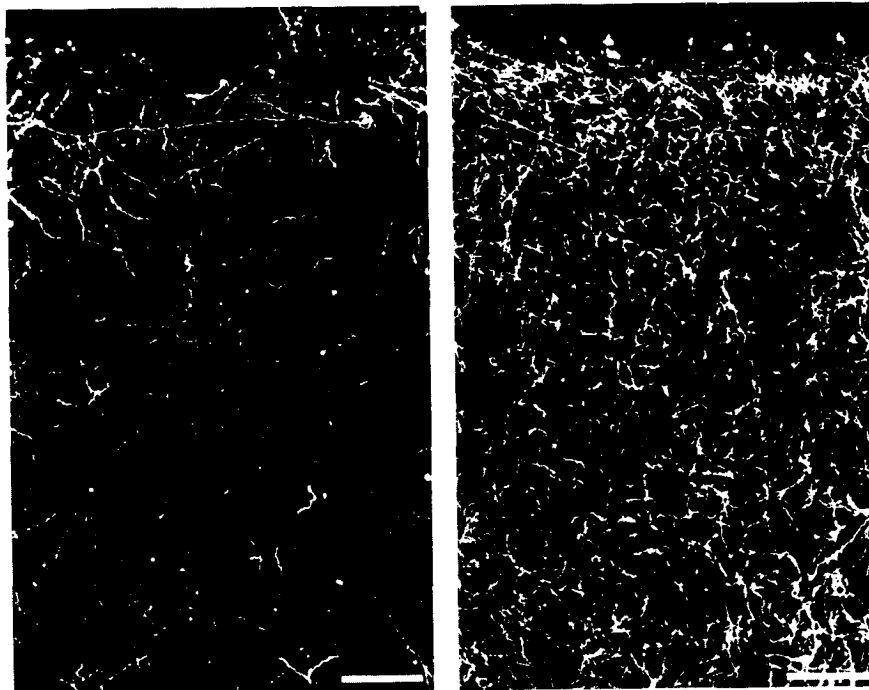


Fig 1.—Serotonin-immunoreactive fibers in somatosensory cortex (area 3) of cynomolgus monkey. Serotonergic axons form dense terminal plexus in control animal (right); in methylenedioxymethamphetamine (MDMA)-treated animal (5 mg/kg) (left), there is marked decrease in density of serotonergic axons after a two-week survival period. Changes in somatosensory cortex are representative of serotonergic denervation caused by MDMA throughout cerebral cortex. Scale bar, 100 μ m.

nucleus to the periphery of the cell (Fig 2, top left). In LFB-PAS-stained sections, the inclusions appeared granular and were vividly PAS positive (Fig 2, bottom right). This staining reaction suggests the presence of an increased amount of ceroid or lipofuscin, possibly due to lipid peroxidation of cell components and subsequent phagolysosomal activity. The presence of lipofuscin within the inclusions was confirmed by a number of staining procedures. Specifically, the granules were autofluorescent in ultraviolet light, acid fast in Ziehl-Nielsen stain for lipofuscin, and positive with Schmorl's reaction and Sudan Black B stain. Glycogen did not account for the staining, as demonstrated in PAS stain with and without diastase.

No abnormal inclusion-bearing cells were found in the median raphe nucleus, in other raphe nuclei, or in nonserotonergic nuclei such as the substantia nigra or locus ceruleus. No similar inclusions were found in ten control monkeys of varying ages (including three 15- to 20-year-old monkeys), although some increased lipofuscin pigment was occasionally found in the older animals. (Seven of these ten animals were not formally part of the present study but had served as controls in other experiments. The brains of these seven animals were fixed by immersion in 10% formol saline.)

COMMENT

The major finding of this study is that central serotonergic neurons in nonhuman primates are highly vulnerable to the toxic effects of MDMA. Compared with the rodent,¹⁰⁻¹⁵ the primate has been found to be approximately four to eight times more sensitive. In the monkey, a dose of 2.5 mg/kg produces a 44% depletion of serotonin in the cerebral cortex (Table 1). By contrast, in the rat a 10- to 20-mg/kg dose is required to produce a comparable effect.¹⁴ Also of note is the fact that in the primate small increments in dose from 2.50 mg/kg to 3.75 and 5.00 mg/kg produced 78% and 90% depletions of serotonin, respectively (Table 1). This indicates that the dose-response curve of MDMA in the monkey is steep, suggesting that the margin of safety of MDMA in humans may be narrow.

The striking loss of serotonin-immunoreactive nerve fibers in the cerebral cortex of the MDMA-treated primate (Fig 1) suggests that MDMA produces a long-term depletion of serotonin by actually damaging serotonergic nerve fibers. Axonal damage is further suggested by the swollen and distorted appearance of some of the remaining fibers. Morphological evidence of nerve fiber damage is important because it suggests that the prolonged depletion of serotonin induced by MDMA is not

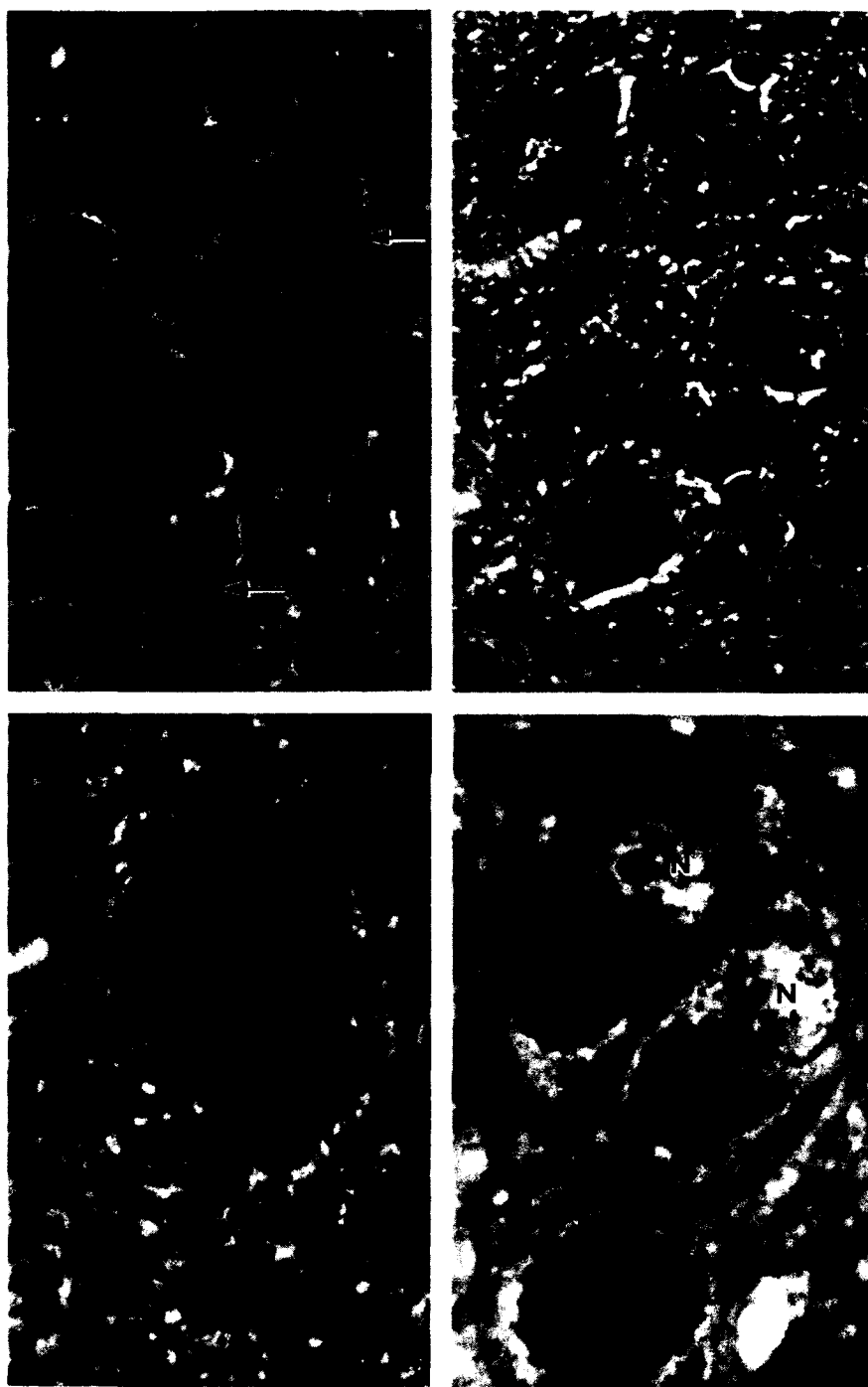


Fig 2.—Top left, Nerve cells in dorsal raphe nucleus of methylenedioxymethamphetamine (MDMA)—treated squirrel monkey. Several of slightly shrunken nerve cells contain intracytoplasmic inclusion (arrows) (hematoxylin-eosin, $\times 550$). Top right, Nerve cells in dorsal raphe nucleus from untreated 11-year-old squirrel monkey, for comparison with Fig 2, top left (hematoxylin-eosin, $\times 550$). Bottom left, Close-up view of one of abnormal inclusion-bearing cells in dorsal raphe nucleus of the MDMA-treated squirrel monkey (hematoxylin-eosin, oil immersion, $\times 1480$). Bottom right, Close-up view of nerve cells in dorsal raphe nucleus to show vividly periodic acid-Schiff-positive granular inclusions in perikarya of several nerve cells (Luxol fast blue-periodic acid-Schiff stain, oil immersion, $\times 1480$). N indicates nucleus.

merely due to a pharmacologic action of the drug, but rather represents a neurotoxic effect. Anatomical studies in rats have led to a similar conclusion.^{12,14}

It is not yet known whether the effects of MDMA on serotonergic neurons in the primate are permanent or reversible. Under some circumstances,

regeneration of serotonergic nerve fibers in the central nervous system can take place.²⁶ However, for axon regrowth to occur, the cell body must be preserved. It remains to be determined if serotonin-containing cell bodies in the dorsal raphe nucleus of the MDMA-treated primate survive beyond two

weeks. If they do, and if regeneration of nerve fibers takes place, it is still not certain that the new fibers would establish normal connections. For functional integrity to be maintained, normal connections would need to be reestablished. It will be important to determine if this occurs in MDMA-treated animals.

This study provides the first direct evidence that serotonergic cell bodies, as well as nerve fibers, are affected by MDMA. As shown in Fig 2, the pathological change in cell bodies involves formation of intracytoplasmic inclusions. These inclusions resemble the more eosinophilic but usually PAS-negative inclusions recently described in monkeys given MPTP,²⁷ a compound that destroys nigral cell bodies.^{18,19} Whether the inclusions in the MDMA-treated primate herald nerve cell death or reflect a metabolic response of the cell body to axonal injury is not yet known but needs to be ascertained because, if cell-body death occurs, the possibility of axonal regeneration would be precluded.

The fact that abnormal inclusions were found in nerve cells of the dorsal, but not median, raphe nucleus is noteworthy because it suggests that MDMA selectively damages a particular subset of serotonergic neurons in the brain (ie, the B7 group of Dahlstrom and Fuxe). That this is the case is also suggested by the recent finding in the rat that serotonergic nerve fibers arising from the dorsal, but not median, raphe nucleus are damaged by MDMA.^{12,28} Taken together, these findings indicate that MDMA is likely to be a valuable new tool for further defining the functional anatomy of different serotonergic cell groups in the mammalian brain.

The mechanism by which MDMA exerts its toxic effects on central serotonergic neurons is at present not well understood. Like a number of other ring-substituted amphetamines (eg, *p*-chloroamphetamine, fenfluramine hydrochloride, MDA), MDMA appears to release serotonin.²⁹⁻³¹ Commins and colleagues³² have proposed that MDMA and related compounds destroy serotonergic neurons by releasing large amounts of serotonin and inducing endogenous formation of 5,6-dihydroxytryptamine, a well-known serotonergic neurotoxin.³³ However, other investigators³⁴ maintain that the degenerative effects of ring-substituted amphetamines may be mediated by a toxic metabolite. It remains to be determined which, if either, of these possibilities proves correct.

The results of this study raise concern that humans presently using MDMA

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may be incurring serotonergic neuronal damage. The fact that monkeys are considerably more sensitive than rats to the toxic effects of MDMA suggests that humans may be even more sensitive. Before extrapolating the present results to humans, however, it should be noted that monkeys were given multiple rather than single doses of MDMA and that the drug was given subcutaneously rather than orally. Humans generally take MDMA via the oral route and use single 1.7- to 2.7-mg/kg doses of the drug, usually weeks apart, although some individuals have used higher and more frequent doses.⁴ It remains to be determined if administration of MDMA to monkeys in a pattern identical to that used by humans produces similar neurotoxicity. In this regard, however, it is important to bear in mind that the sensitivity of human and nonhuman primates to the toxic effects of MDMA may not be the same. In fact, humans are generally regarded as being more sensitive than monkeys to the toxic effects of drugs. For example, humans are fivefold to tenfold more sensitive than monkeys to the toxic effects of MPTP (compare references 19 and 35). In view of these considerations, it would seem prudent for humans to exercise caution in the use of MDMA. Caution may also be warranted in the use of fenfluramine, a ring-substituted amphetamine that is closely related to MDMA and is currently prescribed for obesity³⁶ and autism.³⁷

From an experimental standpoint, MDMA appears to hold promise as a systemically active toxin that can be used to study the functional consequences of altered serotonergic neurotransmission in higher animals. Clinically, it will be important to determine if humans who have taken MDMA show biochemical signs of serotonergic neurotoxicity (eg, decreased 5-hydroxyindoleacetic acid concentration in their cerebrospinal fluid). If they do, it will be critical to ascertain if these individuals have any functional impairment. In particular, such individuals will need to be evaluated for possible disorders of sleep, mood, sexual function, appetite regulation, or pain perception, since central serotonergic neurons have been implicated in all of these functions.^{38,39} These studies could offer the unique opportunity to better delineate the neurobiology of central serotonergic neurons in the human brain, something that until now has not been possible.

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