

(±)3,4-Methylenedioxymethamphetamine ('Ecstasy')-Induced Serotonin Neurotoxicity: Studies in Animals

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Key Words

MDMA · Amphetamines · Neurotoxicity · Monkeys · Serotonin

Abstract

The popular recreational drug, (±)3,4-methylenedioxy-methamphetamine (MDMA; 'Ecstasy') is a potent and selective brain serotonin (5-HT) neurotoxin in animals. MDMA-induced 5-HT neurotoxicity can be demonstrated using a variety of neurochemical, neuroanatomical and, more recently, functional measures of 5-HT neurons. Although the neurotoxic effects of MDMA in animals are widely accepted, the relevance of the animal data to human MDMA users has been questioned, largely because dosages of drugs used in animals are perceived as being much higher than those used by humans. In the present paper, we review the extensive body of data demonstrating that MDMA produced toxic effects on brain 5-HT neurons in animals and present new data indicating that levels of the type 2 vesicular monoamine transporter are reduced in MDMA-treated animals, providing further indication of MDMA's 5-HT neurotoxic potential. Further, we demonstrate, using principles of interspecies scaling, that dosages of MDMA known to be neurotoxic in animals fall squarely in the range of dosages used typically by recreational MDMA users.

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Introduction

More than a decade ago, the first evidence emerged indicating that ring-substituted amphetamine derivatives such as (±)3,4-methylenedioxyamphetamine (MDA) and (±)3,4-methylenedioxy-methamphetamine (MDMA) produced selective toxic effects on brain serotonin (5-HT) neurons [1–5]. These early studies indicated that rats treated with MDA or MDMA developed long-lasting decreases in brain 5-HT, without evidence of long-term effects on other brain monoaminergic systems. Further, these early reports indicated that the doses of MDMA required to produce long-term effects on brain 5-HT neurons were lower than those required to produce comparable effects with another amphetamine derivative, methamphetamine [1, 4, 5]. After these reports appeared, the neurotoxic effects of MDMA on brain 5-HT neurons were confirmed and demonstrated using a variety of experimental techniques in numerous animal species, including primates [6–11]. Despite the large body of preclinical data demonstrating MDMA's neurotoxic potential toward brain 5-HT neurons, the relevance of these findings to humans continues to be questioned [12]. The purpose of this paper is twofold: (1) to review the extensive preclinical literature indicating that MDMA is a brain 5-HT neurotoxin in animals, presenting data using a new method that extends previous results, and (2) to dispel the notion

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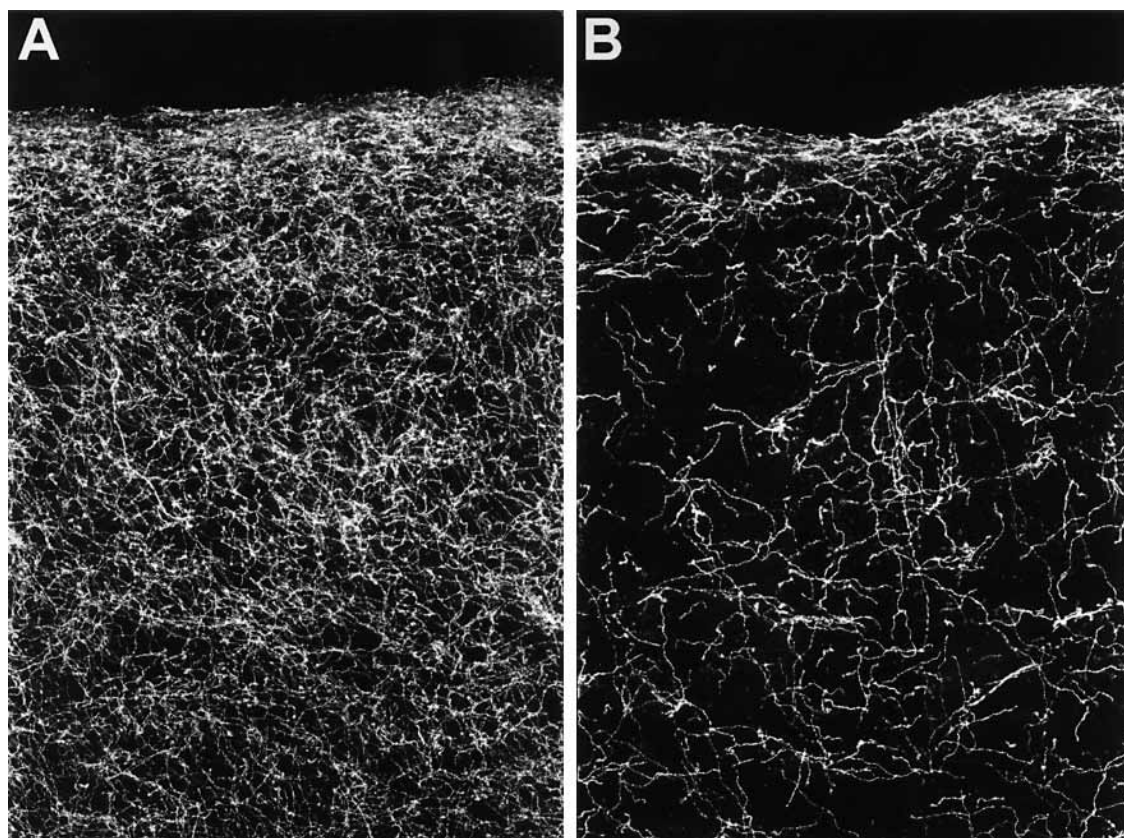


Fig. 1. Serotonin transporter immunoreactive axons in the neocortex of a control rat (**A**) and a rat treated with MDMA (**B**) 2 weeks previously. MDMA was given at a dose of 20 mg/kg, subcutaneously, twice daily for 4 consecutive days.

that doses and drug regimens of MDMA that produce 5-HT neurotoxicity in animals are not in the order of those typically used by humans.

Neurotoxic Effects of MDMA in Animals

Lasting Decreases in Axon Markers Unique to 5-HT Neurons

MDMA produces reductions in 5-HT axon terminal markers which last for months or years after cessation of drug exposure [13–19]. In particular MDMA produces dose-related reductions in the levels of 5-HT and 5-hydroxyindoleacetic acid (5-HIAA, the major metabolite of 5-HT) [2–5], the density of 5-HT uptake sites or transporters [4, 20], and the activity of tryptophan hydroxylase (TPH) [3, 21], the rate-limiting enzyme in the 5-HT synthetic pathway. These lasting effects on brain 5-HT neurons can also be demonstrated anatomically, using immu-

nocytochemical methods for visualizing 5-HT-containing axons [22–26]. Recently, we have also obtained evidence of 5-HT axon loss after MDMA using an antibody directed at the 5-HT transporter, a structural component of the 5-HT axon terminal (fig. 1). These findings, along with results of silver degeneration studies [4], strongly suggest that the loss of 5-HT axonal markers after MDMA is due to the destruction of 5-HT axon terminals. Notably, the long-term effects of MDMA are highly selective for brain 5-HT neurons [6, 8, 19], and are not reproduced by psychostimulant drugs such as cocaine and other uptake inhibitors [27].

Lasting Decreases in Vesicular Monoamine Transporters

Another marker that has recently been used to assess the status of monoaminergic neurons after neurotoxic drug exposure is the vesicular monoamine transporters (VMAT; type 2). Findings with the VMAT2 ligand,

[³H]dihydrotetrabenazine (DTBZ) indicate that it is useful for documenting monoaminergic neuronal degeneration in animals, and that VMAT2 ligands may have the advantage of being less susceptible to pharmacologic influence than ligands that bind to other elements of monoaminergic neurons [28–30]. Frey et al. [31] have validated the utility of VMAT in humans by demonstrating that decreases in [¹¹C]DTBZ binding provide a highly reliable measure of decreased monoaminergic innervation in the striatum of patients with Parkinson's disease, known to involve degeneration of striatal monoaminergic nerve terminals.

Encouraged by these reports, we recently used [³H]DTBZ to measure the density of striatal VMAT2 transporters in baboons previously treated with MDMA. In all MDMA-treated animals, the density of striatal VMAT2 sites was reduced, although decreases in VMAT2 were less severe than those in other 5-HT nerve terminal markers (fig. 2). Since striatal dopamine terminal markers were unaffected by MDMA in these and other studies in nonhuman primates [6, 8, 10], the observed reductions in VMAT2 are not likely to be secondary to dopamine axonal loss. Rather, they most likely reflect 5-HT axonal loss, since other 5-HT axonal markers are reduced, and since the only other major neuron system which contains the VMAT2, the noradrenergic system, is unaffected by MDMA [6, 8]. These findings constitute another line of evidence that MDMA has neurotoxic potential toward brain 5-HT neurons in animals.

Reduced Anterograde Transport

Anterograde transport has been used to trace various neural projections in the central nervous system. For example, Halaris et al. [32] used anterograde transport of [³H]proline from the raphe nuclei to trace ascending 5-HT axonal pathways in the rat forebrain. We recently employed this technique to assess the functional status of ascending 5-HT axonal projections following MDMA exposure [33]. Rats were treated with MDMA, and, 2 weeks later, [³H]proline was injected into the rostral raphe nuclei. Compared to controls, MDMA-treated animals showed marked reductions in the anterograde transport of labeled material to various forebrain regions. Reductions in axoplasmic transport paralleled long-term decreases in regional 5-HT axonal markers. Virtually identical results were obtained in animals previously lesioned with 5,7-dihydroxytryptamine (DHT), a documented 5-HT neurotoxin [34]. These findings further attest to MDMA's 5-HT neurotoxic potential in laboratory animals.

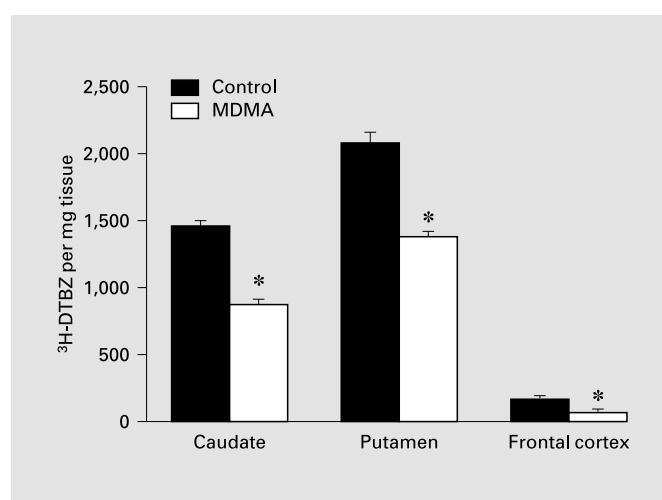


Fig. 2. Decreased density of VMAT2 in baboons treated with MDMA 2 weeks previously. MDMA was given at a dose of 5 mg/kg, subcutaneously, twice daily for 4 consecutive days. * $p < 5\%$.

'Pruning' Effect

Studies investigating the fate of 5-HT neurons in MDMA-treated monkeys lend additional support to the view that MDMA produces toxic effects on brain 5-HT neurons [16]. In particular, MDMA-treated monkeys show evidence of a 'reorganization' of ascending 5-HT projections that is remarkably similar to the 'pruning effect' observed after a variety of neuron lesioning techniques [35–39], including some using 5,6- and 5,7-DHT [40–43]. The term pruning effect was originally coined by Schneider [35] to describe an apparent tendency of neurons to conserve the quantity of their axon terminal fields, so that loss of synaptic contacts in distant brain regions was associated with increased synaptic contacts in more proximal brain areas. The fact that evidence of pruning is evident after MDMA lends additional support to the view that MDMA is a 5-HT neurotoxin.

Relevance of Animal Studies to Humans

There is a popular misconception that doses of MDMA used in animal studies are exceedingly high, and that findings of neurotoxic injury in animals have no bearing on what might occur in humans. Clearly, when attempting to relate animal data to humans, several critical factors must be considered, including: (1) potential species differences that might make humans resistant to MDMA-induced injury; (2) route and schedule of drug administration, and (3) drug dosages. Each of these points is addressed below in turn.

Table 1. Evidence for MDMA-induced 5-HT neurotoxicity in animals

Long-term decreases in levels of 5-HT ¹
Long-term decreases in levels of 5-HIAA ¹
Long-term decreases in 5-HT transporters ¹
Long-term decreases in activity of TPH ¹
Histological evidence of 5-HT axon degeneration ¹
Long-term decrease in anterograde transport ²
Long-term decreases in VMAT transporters ³
Evidence of pruning effect ⁴

¹ See Steele et al. [50] and text for primary references.
² Callahan et al. [33].
³ See figure 1.
⁴ Fischer et al. [16] and Hatzidimitriou et al. [19].

Species Differences in the Neurotoxic Potential of MDMA

Neurotoxic effects after MDMA have been demonstrated in a number of animal species, including rats, guinea pigs, squirrel monkeys, cynomolgus monkeys, rhesus monkeys and, more recently, in baboons [27]. In all species tested, the neurotoxicity of MDMA is highly selective, affecting 5-HT-containing neurons almost exclusively (the only exception is a small population of non-monoaminergic neurons in the rat neocortex) [4]. The mouse is the one animal species that is relatively resistant to MDMA-induced 5-HT injury, but at high doses also develops a persistent loss in brain 5-HT markers [44]. Relative to rodents, primates are more sensitive to the neurotoxic effects of MDMA and, as indicated above, may not recover from MDMA-induced 5-HT injury, at least after severe lesions [19]. Thus, unless some, as yet unidentified, factor renders humans uniquely insensitive to the toxic effects of MDMA, species differences are not likely to be associated with protection from MDMA-induced brain 5-HT injury.

Route and Schedule of Drug Administration

Most animal studies of MDMA-induced 5-HT neurotoxicity involve systemic administration of the drug twice daily for 4 days. Humans typically use MDMA orally, and although they may use up to 10 dosages per night, usually do not use it on 4 consecutive days. Two lines of evidence indicate that these differences between animal studies and human usage patterns do not proffer protection to human MDMA users. First, the level of neurotoxicity produced by oral versus parenteral MDMA does not differ [45]. Second, even a single 5-mg dose of MDMA in non-

human primates has been found to produce 5-HT neurotoxicity [46]. In sum, studies on the route and schedule of MDMA administration in animals suggest that human MDMA users may well be at risk for incurring brain 5-HT neural injury.

Dosage of MDMA Used in Animal Studies

Dosages (on a mg/kg basis) of MDMA that produce neurotoxicity in rodents are higher than those that produce neurotoxicity in primates [3, 6, 20, 22, 46]. The observation that smaller animal species require higher doses of drug to achieve equivalent drug effects is predicted by the principles of interspecies scaling. This method utilizes known relations between body mass/surface area and accounts for differences in drug clearance. For example, using this method, it is possible to accurately predict a neurotoxic dose of MDMA in monkeys using a known neurotoxic dose of MDMA in rats (20 mg/kg) [5]. Specifically, using an accepted adjustment for body mass/surface area and drug clearance [47, 48], the equivalent dose in monkeys is found to be 5 mg/kg, a dose that, indeed, has been shown to be neurotoxic in monkeys [46]. Similarly, using the same technique, it is possible to predict dosages of MDMA that would be neurotoxic in humans based on those that are neurotoxic for rats or monkeys. Specifically, using the known neurotoxic dose of 5 mg/kg in a 1-kg squirrel monkey, the equivalent dose in humans is found to be 1.28 mg/kg or approximately 96 mg in a 75-kg individual. Human MDMA users typically use single dosages of MDMA of 75–125 mg, falling squarely in the neurotoxic range predicted by the interspecies scaling method. The fact that some individuals report using up to 10 individual dosages of MDMA in a given night, further suggests that there is little or no margin of safety between the recreationally used and neurotoxic dosages of MDMA. Notably, O’Shea et al. [49] have reached similar conclusions based on their studies of MDMA in rodents.

Conclusions

The findings discussed in this article, which are summarized in table 1, provide compelling evidence that MDMA has neurotoxic potential toward brain 5-HT neurons in animals. Following MDMA exposure, there are lasting decrements in every 5-HT axonal marker that has been measured, including VMAT2. Further, morphologic studies indicate that the loss of 5-HT axonal markers is due to degeneration of 5-HT nerve terminals, and func-

tional studies indicate that anterograde transport along 5-HT axons is markedly impaired. Taken together, these preclinical findings strongly suggest that MDMA produces toxic effects on brain 5-HT neurons.

The neurotoxic effects of MDMA have broad species generality. Evidence of MDMA-induced 5-HT neurotoxicity has been found in every species that has been tested, including various nonhuman primate species (squirrel monkeys, cynomolgus monkeys, rhesus monkeys, baboons). Clearly, when evaluating the relevance of animal toxicity studies to human MDMA users, it is important to consider the dose, as well as the route and frequency of drug administration required to produce neurotoxic effects in animals. When each of these factors is considered for MDMA, the conclusion that emerges is that doses of MDMA which produce neurotoxic effects in animals are squarely in the range of those used by humans. Thus, findings in animals raise concern that human MDMA users are at risk of incurring brain 5-HT neural injury.

Future animal studies should be directed at better characterizing the functional consequences of MDMA neurotoxicity, and toward determining if functional alterations progress as animals age. In addition, mechanisms underlying MDMA's 5-HT neurotoxic action need to be elucidated, as an understanding of these mechanisms could shed important new light on neurodegenerative diseases involving 5-HT brain neurons in humans. Finally, factors that govern regrowth of injured 5-HT neurons after MDMA injury should be identified, with the long-term goal of developing methods for reversing brain 5-HT injury or diminishing its functional consequences.

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