Effects of MDMA and MDA on Brain Serotonin Neurons: Evidence from Neurochemical and Autoradiographic Studies

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INTRODUCTION

The drugs 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) are ring-substituted derivatives of methamphetamine and amphetamine, respectively. These methylenedioxy-substituted amphetamines have been reported to exhibit both stimulant and psychotomimetic properties (Anderson et al. 1978; Braun et al. 1980; Shulgin 1986). MDMA has received a great deal of attention recently, since it represents one of a number of "designer drugs" that have been increasingly abused among certain segments of the population, especially college students. MDMA has been the subject of a recent scientific and legal debate, as several psychiatrists have reported that MDMA may "enhance emotions" and "feelings of empathy" and thus serve as an adjunct in psychotherapy (Greer and Tolbert 1986). Recent data demonstrating that MDMA is self-administered in nonhuman primates (Beardsley et al. 1986; Lamb and Griffiths 1987) suggest that the drug may have high abuse potential in man. These reports are particularly disturbing, as the authors and others have recently demonstrated that MDMA is a potent neurotoxin that appears to cause selective degeneration of brain serotonin neurons (Battaglia et al. 1987; Battaglia et al. 1988; Commins et al. 1987; Schmidt 1986; O'Hearn et al. 1988) comparable to that reported for its structural analog MDA (Battaglia et al. 1987; Ricaurte et al. 1985; Stone et al. 1986).

This chapter describes data on the neurotoxic effects of MDMA on brain monoamine systems in rodents. Specifically, studies are described examining the effects of *in vivo* administration of MDMA on brain monoamine systems with respect to:

(1) the selective neurodegenerative effects on brain serotonin systems;

(2) the effects of dose and frequency of drug administration;

(3) the potential neurochemical mechanisms involved in the neurotoxic effects of the drug;

(4) the characteristics and timecourse of recovery following destruction of serotonin neurons;

(5) the relative sensitivity of various animal species; and

(6) the neuroanatomical and morphological specificity of MDMA- and MDA-induced neurotoxicity.

MARKERS OF NEUROTOXICITY

Typically, neurotoxic effects of drugs on monoamine neurons have been assessed from reductions in brain levels of monoamines and their metabolites, decreases in the maximal activity of synthetic enzymes activity, and decreases in the active uptake carrier. In the present study, the traditional markers described above have been used, including the measurement of the content of monoamines and their metabolites in brain at several different timepoints following drug administration. Since reports in the literature have documented that MDMA and MDA can inhibit the activity of tryptophan hydroxylase (TPH), the rate-limiting enzyme in serotonin synthesis (Stone et al. 1986; Stone et al. 1987). it is unclear whether MDMA-induced reductions in the content of serotonin and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) may be due to suppressed neurotransmission in otherwise structurally intact serotonin neurons or may represent the consequence of the destruction of serotonin neurons and terminals.

Since monoamine uptake sites are highly concentrated on their respective nerve terminals (Kuhar and Aghajanian 1973), the authors' approach has been to use selective radioligands to directly label these uptake sites in brain and to assess the neurodegeneration of specific monoamine neurons, by measuring the reductions in the density of their respective uptake sites. For example, the authors have recently reported the feasibility of using the measurement of [^{3}H]paroxetine-labeled serotonin uptake sites to quantify the neurotoxic effects of MDA (Battaglia et al. 1987) and MDMA (Battaglia et al. 1987; Battaglia et al. 1988) on serotonin neurons in homogenates of various regions of rat brain. Visualization of MDMA- and MDA-induced destruction of serotonin axons and terminals using antibodies directed against serotonin (O'Hearn et al. 1988) and autoradiographic studies demonstrating corresponding decreases in [^{3}H]paroxetine-labeled scrotonin uptake sites in slide-mounted brain sections of MDA-treated rats (De Souza and Kuyatt 1987) further validate use of changes in the density of serotonin uptake sites as an appropriate index of serotonin neurodegeneration.

IN VIVO EFFECTS OF MDMA: NEUROCHEMICAL STUDIES

The following studies were designed to assess and quantify both the neuro chemical and neurodegenerative effects of short-term administration of MDMA on monoamine neurons in rat brain.

Dose Dependence

A repetitive treatment regimen (sc injections twice daily for 4 consecutive days) of MDMA at various doses up to 20 mg/kg resulted in dose-dependent decreases in a variety of serotonergic markers in rat frontal cerebral cortex, including serotonin, 5-HIAA, and the density of serotonin uptake sites at 18 hours following the last injection (figure 1). At the lowest dose of MDMA tested (5 mg/kg), serotonin content was markedly reduced (45 percent), while only a small (14 percent), but statistically significant, decrease in the density of serotonin uptake sites was observed; a small decrease in 5-HIAA content was also observed at this dose, although this change was not statistically significant. Higher doses of MDMA (10 and 20 mg/kg) resulted in comparable reductions in 5-HIAA levels (60 to 70 percent), while the decrease in serotonin was significantly greater at 20 mg/kg (90 percent) than at 10 mg/kg (80 percent). The density of serotonin uptake sites decreased progressively as the dose of MDMA was increased, with a maximal reduction of 90 percent observed at 18 hours following administration of 20 mg/kg MDMA.

In contrast, following a treatment regimen of 20 mg/kg MDMA, there were no significant differences in the density of [3H]mazindol-labeled norepine-phrine (NE) uptake sites (fmol/mg protein) in the frontal cerebral cortex between saline-treated (159±17) and MDMA-treated (152±5) animals. With respect to the dose of MDMA, serotonin levels appeared to be more readily decreased (45 percent reduction at 5 mg/kg), while comparable reductions in 5-HIAA levels and serotonin uptake sites were noted only at 10 or 20 mg/kg MDMA. This apparent discrepancy among the three serotonergic markers measured in the present study may relate to effects of lower doses of MDMA on synthetic enzyme activity (i.e., TPH), whereas the effects of higher doses of MDMA in reducing all three markers may relate in part to effects on TPH activity and in part to destruction of serotonin neurons as evidenced by decreases in serotonin uptake sites.

The Effects of Single Versus Multiple Injections of MDMA

Since repeated systemic administration of 10 mg/kg MDMA caused marked neurodegeneration of frontal cerebral cortex serotonin neurons, the authors chose to investigate the neurodegenerative effects of single versus multiple injections of MDMA at this dose. As shown in figure 2, increasing the number of injections of MDMA (10 mg/kg, sc) resulted in significant and progressively greater reductions in serotonin and 5-HIAA content. While

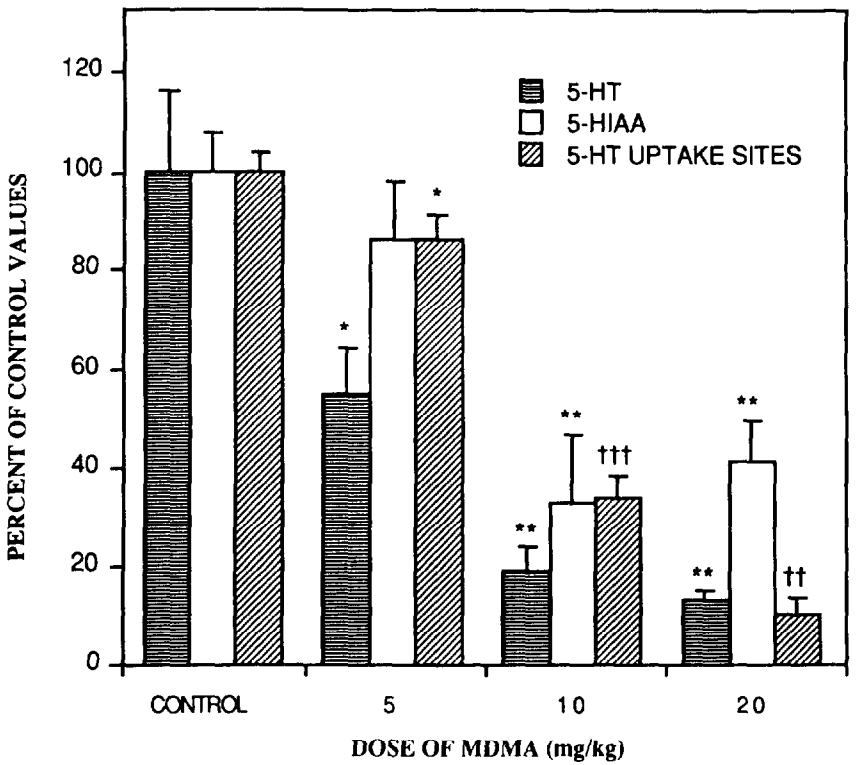


FIGURE 1. *The effect of repeated systemic administration of various doses of MDMA on the content of serotonin (5-HT) and S-HIAA and the density of 5-HT uptake sites in rat frontal cerebral cortex*

*Significantly different from control. $p<0.05$.

**Significantly different from control, $p<0.01$.

††Significantly different from all other MDMA-treated groups. $p<0.01$.

†††Significantly different from all other MDMA-treated groups, $p<0.001$.

NOTE: Rats were administered either saline or MDMA twice a day for 4 consecutive days and sacrificed 18 hours after the last injection. Data represent the mean SEM from three to live rats, expressed as a percent of values in control, saline-injected rats. Control values for 5-HT and 5-HIAA levels were 387±61 and 251±20 pg/mg tissue, respectively. The density of 5-HT uptake sites in the frontal cerebral cortex in controls was 396±15 fmol/mg protein. Data were analyzed by one-way ANOVA and Duncan's multiple range test.

SOURCE: Battaglia et al. 1988.

one injection of MDMA was without effect on any of the serotonergic parameters examined, two doses were sufficient to elicit a significant reduction in serotonin content. As described above, these early effects of MDMA on serotonin content may relate to MDMA suppression of TPH activity. A significant reduction in 5-HIAA content (approximately 34 percent) was observed only after four injections of MDMA. Marked reductions of 84 percent and 75 percent in serotonin and 5-HIAA,

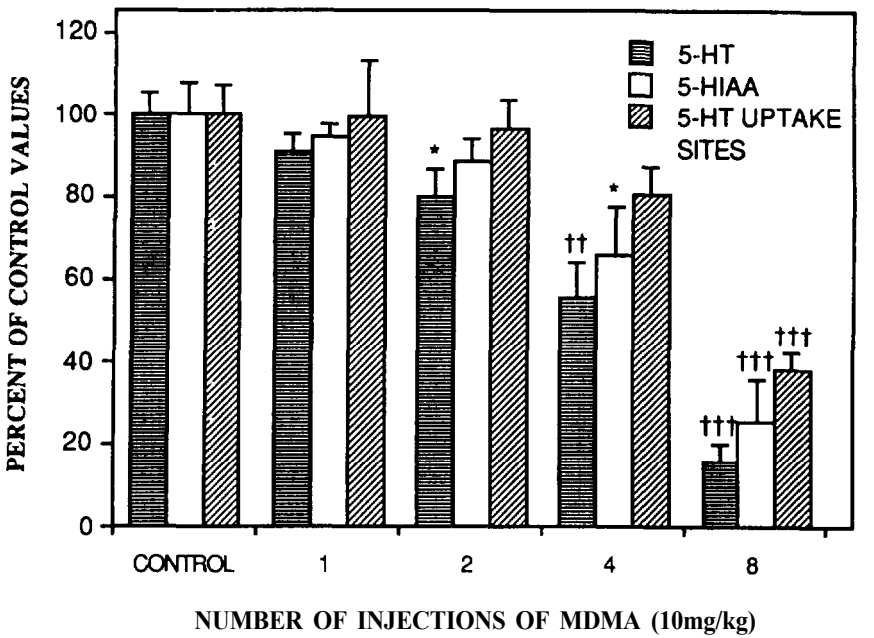


FIGURE 2. *The effects of single and multiple injections of MDMA on the content of serotonin (5-HT) and 5-HIAA and the density of 5-HT uptake sites in rat frontal cerebral cortex*

*Significantly different from control, $p<0.05$.

††Significantly different from all other groups, $p<0.01$.

†††Signiftcantly different from all other groups, $p<0.001$.

NOTE: Rats were injected the specified number of times with either saline or 10 mg/kg MDMA and sacrificed 18 hours after the last injection. Data represent the mean and SEN from three to five animals, plotted as percent of respective values for each marker in control, saline-injected rats. Control levels of 5-HT and 5-HIAA were 475±24 and 332±24 pg/mg tissue, respectively. The density of 5-HT uptake sites was 349±24 fmol/mg protein in control. Data were analyzed by one-way ANOVA and Duncan's multiple range test.

SOURCE: Battaglia et al. 1988.

respectively, were observed following an eight-injection regimen of 10 mg/kg MDMA; the density of [^{3}H]paroxetine-labeled serotonin uptake sites was also significantly decreased (approximately 64 percent) following eight injections of MDMA at this dose. These data suggest that a longer treatment regimen may be required for destruction of serotonin neurons, while effects on the content of serotonin and 5-HIAA may occur following fewer injections.

There was no change in the content of dopamine (DA) in any of the experimental groups; however, a small, inconsistent decrease in NE content (approximately 20 percent) was observed in all MDMA-treated rats. This small change in NE following MDMA treatment was not accompanied by a reduction in the density of [^{3}H]mazindol-labeled NE uptake sites.

Species Differences

Since amphetamines have been shown to be metabolized by different pathways in rat, mouse, and guinea pig (Caldwell 1980). studies were carried out to investigate whether MDMA induced neurotoxicity could be demonstrated in other species such as mouse and guinea pig. Animals in these studies were treated twice daily for 4 consecutive days with 20 mg/kg MDMA. and levels of serotonin, 5-HIAA, and serotonin uptake sites were measured at 7 days following the last injection, to assess the long-term effects of the treatment. As shown in figure 3, MDMA caused comparable and marked decreases in serotonin and 5-HIAA content and in the density of serotonin uptake sites in rat and guinea pig cerebral cortex, but appeared to be without effect on any of these serotonergic markers in the mouse. Other studies (Stone et al. 1987) have also suggested that mice are less susceptible to the neurotoxic effects of MDMA. Similar differences in the sensitivity to the neurotoxic effects of parachloroamphetamine on serotonin neurons have been observed in mouse when compared to its effects in rat and guinea pig (Fuller 1978; Kohler et al. 1978; Sanders-Bush and Steranka 1978). The differential sensitivity may be due, in part, to species-dependent differences in the half-life of the drug (Steranka and Sanders-Bush 1978). Active neurotoxic metabolites or metabolic intermediates of parachloro-amphetamine have been postulated previously as being responsible for its neurotoxic effects on serotonin neurons (Gal and Sherman 1978; Sanders-Bush and Steranka 1978), although to date no active neurotoxic species has been identified. Although there has been no direct demonstration of a neurotoxic metabolite of MDMA, some preliminary data suggest that an active metabolite of MDMA may be responsible for eliciting its ncurotoxic effects. The authors have previously reported that, in contrast to the marked neurodegenerative effects on brain serotonin neurons following systemic administration of MDMA or MDA, single, direct intracerebral injections of MDMA or MDA were without effect on cerebral cortical serotonin neurons, as visualized using immunohistochemistry (Molliver et al. 1986). This observation of marked species differences and sensitivity

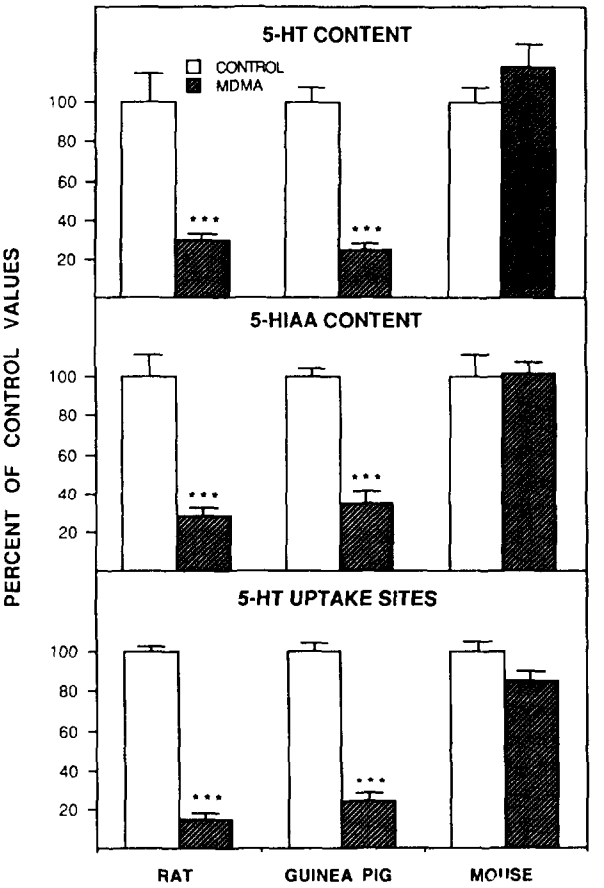


FIGURE 3. *The effects of repealed systemic adminisration of MDMA on content of serotonin {5-HT) and 5-HIAA, and density of 5-HT uptake sites in rat, guinea pig, and mouse frontal cerebral cortex*

**Signifiantly different from control, p<0.001.

NOTE: Animals were treated with saline or 20 mg/kg MDMA twice a day for 4 consecutive days and sacrificed 7 days after the last injection. Data represent ± SEM of five animals per group, expressed as percent of saline-injected control values in respective species. In rat, guinea pig, and mouse, control values of 5-HT were 275±41, 296±14, and 449±36 pg/mg tissue, respectively; control values of 5-HIAA were 345±40, 92±4. and 319±34 pg/mg tissue, respectively; control values of 5-HT uptake site were 397±10, 216±6, and 233±12 fmol/mg protein, respectively. Data were analyzed by Student's *t*-test.

SOURCE: Battaglia et al. 1988.

to MDMA-induced serotonin neurotoxicity would be consistent with the hypothesis of a peripherally produced neurotoxic metabolite of MDMA.

In more recent studies, it has also demonstrated that administration of 2.5 or 10 mg/kg MDMA twice daily for 4 consecutive days resulted in neurotoxic effects in rhesus monkeys, with decreases in the density of scrotonin uptake sites occurring at the higher dose (Johannessen et al. 1988). The neurotoxic effects of MDMA observed in primates included reductions in the content of serotonin and 5-HIAA and marked reductions in the ccrebrospinal (CSF) concentrations of 5-HIAA levels that were observed following drug administration. These fmdings and other reports of neurotoxic effects of MDMA in primates (Ricaurte et al. 1988) raise serious concerns for its potential hazard in humans.

Potential Mechanisms

Since the neurotoxic effects of drugs such as parachloroamphetamine on serotonin neurons can be prevented by serotonin uptake blockers (Ross 1976; Sanders-Bush and Steranka 1978). the possibility that serotonin uptake carrier protein was likewise involved in the neurotoxic effects of MDMA was investigated. As shown in figure 4, pretreatment of rats with the selective serotonin uptake blocker citalopram (10 ml/kg), prior to each injection of 10 mg/kg MDMA, resulted in nearly complete protection against the neurotoxic effects of MDMA. Citalopram-pretreated rats exhibited only a 15 percent decrease in serotonin uptake sites. No significant alterations in the content of serotonin and 5-HIAA were observed following MDMA treatment, in comparison with 60 to 80 percent reductions in the serotonergic parameters observed in rats treated with an identical dose of MDMA alone.

The data described above demonstrate that destruction of serotonin axons by MDMA involves the serotonin active uptake carrier and that administration of citalopram, a selective serotonin uptake blocker, prior to administration of MDMA, can prevent the decreases in serotonin markers elicited by MDMA alone. These data are consistent with previous reports for other potent serotonin neurotoxins, demonstrating that pretreatment with serotonin uptake blockers can prevent the neurotoxic effects of parachloroamphetamine (Ross 1976; Sanders-Bush and Steranka 1978). Furthermore, it has been shown that MDMA-induced neurotoxicity can be prevented or reversed if a serotonin uptake blocker such as fluoxetine is administered no later than 12 hours after MDMA treatment (Schmidt 1986).

In previous studies, it has been observed that, in contrast to the marked serotonin neurodegenerative effects following systemic administration of MDMA or MDA, single, direct intracerebral injections of MDMA or MDA are without effect on cerebral cortical serotonin neurons, as visualized using serotonin immunocytochemistry (Molliver et al. 1986). In addition, as

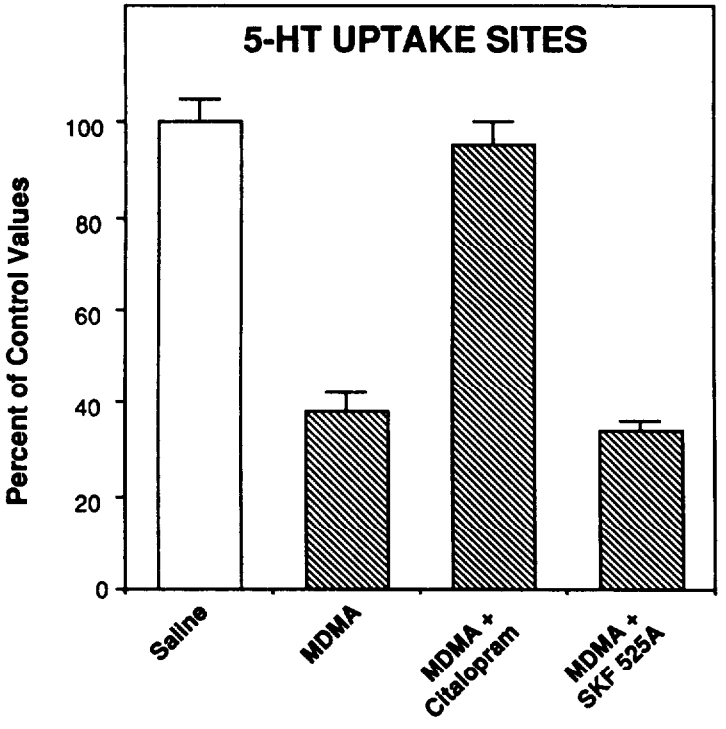


FIGURE 4. *The effect of repeated systemic administration of 10 mg/kg MDMA, MDMA plus 10 mg/kg citalopram, and MDMA plus 25 mg/kg SKF 525A on the density of serotonin (5-HT) uptake sites in homogenates of rat frontal cerebral cortex*

NOTE: Data are expressed as percent of values in control saline-treated rats and represent the mean ± SEM from four to six animals. Control levels of 5-HT uptake sites were 356±15 fmol/mg protein.

described above, there appears to be a differential sensitivity to the neurotoxic effects of MDMA in different species, which metabolize amphetamines by different pathways. These observations of marked differences in serotonin neurotoxicity to-centrally versus systemically administered MDMA and differences in various species would be consistent with the hypothesis of a peripherally produced neurotoxic metabolite of MDMA or MDA. Since MDMA has been reported to interact with the hepatic microsomal enzyme cytochrome P450 (Brady et al. 1986), the authors investigated whether inhibition of this enzyme would alter the neurotoxic effects of MDMA was investigated. In preliminary studies, it was found that, in rats pretreated with the cytochrome P450 enzyme inhibitor SKF 525A, 45 minutes prior to

each administration of MDMA, there was neither potentiation nor attenuation of the neurodegeneration found following repeated administration of 10 mg/kg MDMA. As shown in figure 4, no changes in the density of serotonin uptake sites were observed between rats treated with MDMA alone and those treated with MDMA plus SKF 525A. Although these data suggest that it is unlikely that the putative neurotoxic species is a cytochrome P450-dependent metabolite of MDMA, the involvement of some other peripheral and/or central metabolite of MDMA or the formation of an MDMA-induced endogenous neurotoxin cannot be ruled out. Additional studies are required to identify the mechanisms responsible for MDMA-induced neurotoxicity.

Regeneration of Serotonin Neurons

A detailed timecourse of recovery of affected serotonin neurons was carried out to investigate whether serotonin neurons regenerate subsequent to their destruction following MDMA treatment. As shown in figure 5, the timecourse of neuronal regeneration was investigated by measuring the recovery of serotonin uptake sites and serotonin levels in rat frontal cerebral cortex at various timepoints up to 12 months following repeated systemic administration (i.e., twice daily sc injections for 4 days) of 20 mg/kg MDMA. At all timepoints up to 6 months during the recovery timecourse, the density of serotonin uptake sites was significantly below the corresponding values in age-matched, saline-treated control rats. At the 6-month timepoint, the density of serotonin uptake sites was only 75 percent of the values of saline-treated controls, whereas by 12 months after MDMA treatment, the density of serotonin uptake sites returned to control levels. The shape of the recovery curve suggests that there may be a faster initial rate of recovery of serotonin uptake sites occurring between 18 hours and 4 weeks, which is followed by a slower rate of recovery between 4 weeks and 12 months. These data indicate that more than 6 months are required for a complete recovery of serotonin uptake sites to control levels.

It was of interest that, in spite of the recovery of serotonin uptake sites to control levels, the content of serotonin in the same brain region remained markedly (40 to 50 percent) below age-matched controls for as long as 1 year after MDMA administration. It is unclear from these data whether there is a regeneration of axons that have previously undergone degeneration or whether the increased density of uptake sites is a consequence of increased collateral sprouting of neurons unaffected by the drug treatment. It is also possible that axonal regeneration and collateral sprouting arc associated with considerably greater densities of uptake sites per neuron, thereby making it more difficult to assess neuronal recovery from this index. The fact that serotonin levels remain 40 to 50 percent below age-matched controls for up to 1 year in spite of normal levels of serotonin uptake sites indicates that, following lesion by MDMA, the serotonin

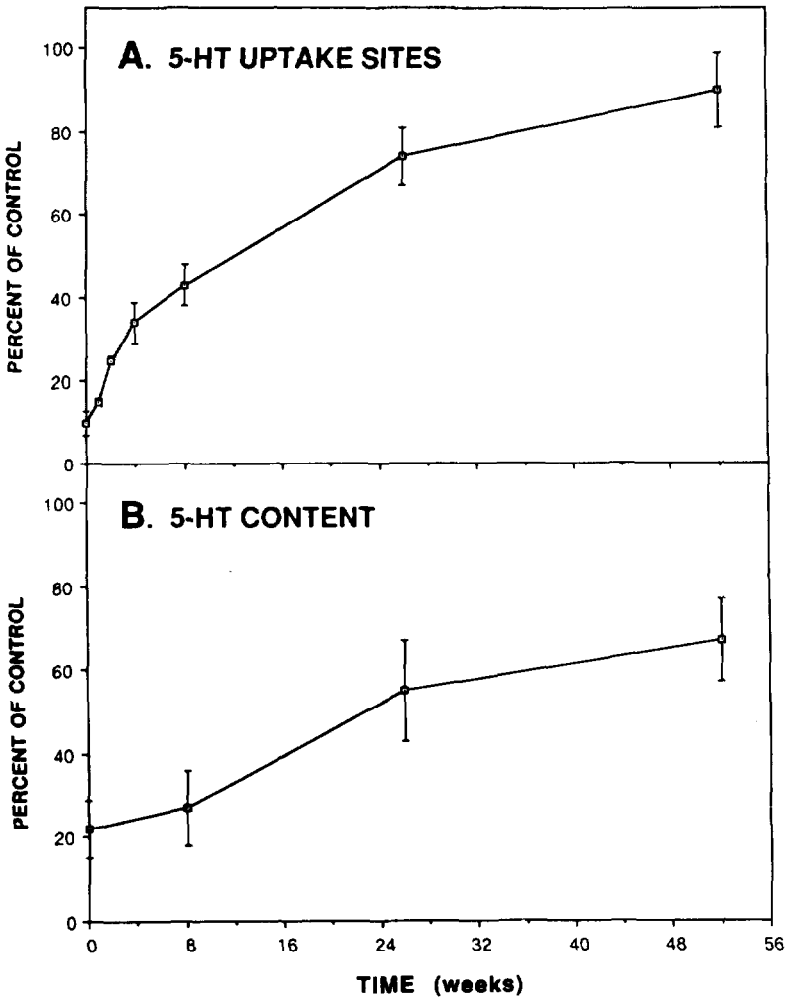


FIGURE 5. *Timecourse of recovery of (A.) serotonin (5-HT) uptake sites and (B.) 5-HT conten in rat cerebral cortex following repeated systemic administration of MDMA*

NOTE: Rats were treated with either saline or 20 mg/kg MDMA twice a day for 4 consecutive days, then sacrificed at various times up to 12 months after the last injection of the drug. Saline-injected control rats were killed at each of the timepoints; data represent the mean ± SEM of five rats per group, plotted as percent of the value of age-matched saline-injected control rats.

SOURCE: Adapted from Battaglia et al. 1988.

neurons that "recover" may not be functionally identical to those present in age-matched control brains.

The persistent neurotoxic effects of MDMA on serotonin neurons is similar to that observed following parachloroamphetamine administration, in which marked reductions in serotonin have been observed up to 4 months after a single injection of parachloroamphetamine (Fuller 1978; Kohler et al. 1978; Sanders-Bush and Steranka 1978). Since neurochemical recovery of serotonin uptake sites and serotonin content have been used, rather than neuroanatomical indices of neuronal regeneration, it is unclear from the present data whether there is actual regeneration of neurons that have previously undergone axon or terminal degeneration or whether the increased density of uptake sites is a consequence of increased collateral sprouting of neurons unaffected by the drug treatment, It has previously been reported that following 5,6-dihydroxytryptamine-induced axotomy, axonal sprouting occurs within 4 to 5 days, and the appearance of new axonal sprouts correlates with the recovery of [^{3}H]serotonin uptake (Bjorklund et al. 1973). Evidence from both immunocytochemical data (O'Hearn et al. 1988) and autoradiographic studies quantifying changes in the density and distribution of [^{3}H]paroxetine-labeled serotonin uptake sites (see figure 10) indicates that serotonin cell bodies appear to be insensitive to the neurotoxic effects of repeated systemic administration of MDMA in rats. The fact that serotonin cell bodies are unaffected by MDMA treatment provides a mechanism by which terminal regeneration of MDMA-affected neurons may occur in rats.

NEUROANATOMICAL AND MORPHOLOGICAL SPECIFICITY OF THE EFFECTS OF MDMA AND MDA

The data described above clearly demonstrate the specific and marked neurodegenerative effects of MDMA on serotonin axons and terminals in the cerebral cortex. Two approaches have been taken to investigate whether the effects of MDMA on brain serotonin neurons are ubiquitous or whether the effects of MDMA show neuroanatomical specificity. The first approach involved the measurements of various monoamines, their metabolites, and monoamine uptake sites in homogenates of discrete areas of rat brain following treatment with MDMA or MDA. Second, autoradiographic techniques were used to visualize the effects of MDMA treatment on the localization and density of [^{3}H]paroxetine-labeled serotonin uptake sites and [^{3}H]mazindol-labeled NE and DA uptake sites in slide-mounted sections of rat brain. In addition, to address further the neurochemical specificity of the effects of MDMA on brain serotonin neurons, effects of MDMA and MDA treatment on the content of DA, NE, and their respective mctabolites and DA and NE uptake sites in homogenates of various brain regions will be described. In some studies, the changes induced by the N-ethyl derivative of MDA (MDE) have been investigated.

Effects of MDMA on Serotonin and 5-HIAA Content and [^{3}H]Paroxetine-Labeled Serotonin Uptake Sites in Discrete Regions of Rat Brain

The effects of repeated systemic administration of MDMA and MDA on various serotonergic parameters were investigated at 2 weeks following the last injection of the treatment regimen previously described (i.e., 20 mg/kg, sc, twice daily for 4 days). As shown in figure 6, MDMA and MDA produced marked decreases in the content of serotonin and 5-HIAA in various brain regions. Both MDMA and MDA caused dramatic decreases in 5-HIAA levels in cerebral cortex, hippocampus, striatum, and hypothalamus (figure 6B). In hypothalamus, the reduction in 5-HIAA levels elicited by MDA was significantly greater ($p<0.05$) than that observed with MDMA (figure 6B). When plotted as a percent of control values in the respective brain regions, it was apparent that, while decreases in 5-HIAA content were observed in all the brain regions examined. the reductions in cerebral cortex and in hippocampus (40 to 60 percent) were greater than those observed in striatum and in hypothalamus (30 to 40 percent). With respect to serotonin levels, marked decreases were observed in cerebral cortex and hypothalamus in both MDMA- and MDA-treated rats (figure 6A). While small decreases were observed in hippocampal and striatal serotonin content following either MDA or MDMA treatment, these reductions were found to be statistically significant only in striatum ($p<0.01$) of MDMA-treated rats. Data calculated as a percent of control serotonin levels in their respective brain regions (figure 6A) indicate a more marked reduction in serotonin in cerebral cortex (40 to 60 percent) than in hypothalamus (18 to 33 percent).

To determine whether changes in serotonin and/or 5-HIAA were a consequence of long-term suppression of serotonergic function in structurally intact neurons or whether MDMA and MDA may be affecting a neurodegenerative process in each of the brain regions, we measured the density of serotonin uptake sites in these brain regions. Both MDMA and MDA caused substantial reductions in the densities of [^{3}H]paroxetine-labeled serotonin uptake sites in all the brain regions examined. The densities of serotonin uptake sites were calculated as a percent of the respective control levels in cerebral cortex, hippocampus, striatum, hypothalamus, and midbrain and are shown in figure 7. Significant reductions (all $p<0.001$) were observed in cerebral cortex (60 to 70 percent), hippocampus (70 to 75 percent), striatum (50 percent), hypothalamus (40 to 50 percent) and midbrain (50 to 60 percent). Interestingly, MDA produced a significantly greater reduction in the density of serotonin uptake sites in cerebral cortex than did MDMA. It has also been observed that MDE causes 40 percent reductions in serotonin uptake sites in cerebral cortex with a comparable treatment regimen, suggesting that this compound may be less toxic than either MDA or MDMA. Scatchard analysis of [^{3}H]paroxetine saturation

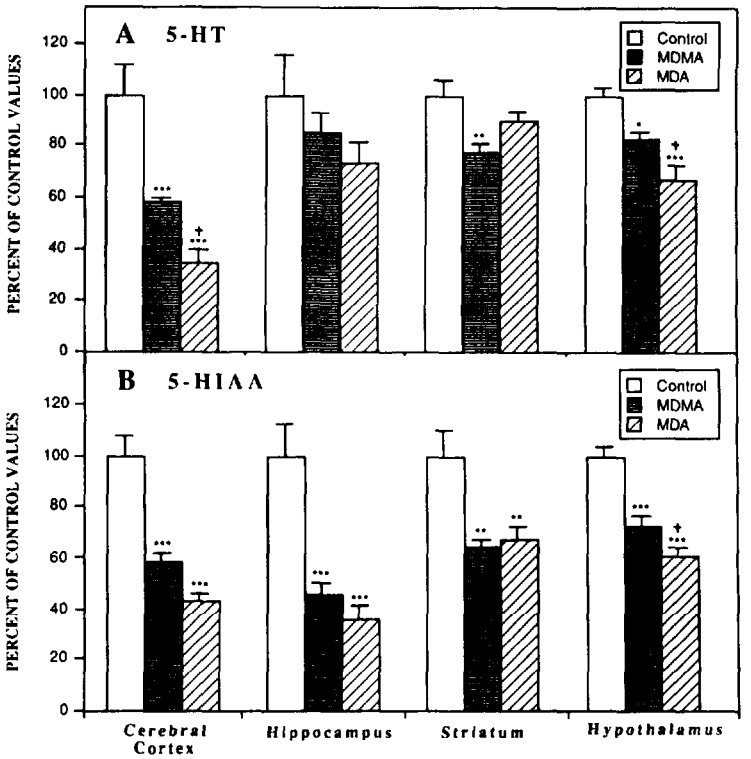


FIGURE 6. *Effect of repeated systemic administration of MDMA and MDA on the concentration of (A) serotonin (5-HT) and (B) 5-HIAA in various brain regions*

*Significantly different from control, $p<0.05$.

**Significantly different from control, $p<0.01$.

***Significantly different from control, $p<0.001$.

†Significantly different from MDMA-treated rats, $p<0.05$.

NOTE: Rats were injected subcutaneously twice daily for 4 days with drug (20 mg/kg) or saline vehicle (1 mL/kg) and sacrificed 2 weeks after the last injection. 5-HT and 5-IIIAA levels were measured using reversed phase HPLC. Data, plotted as percent of control values in each brain region, represent the mean and SEM from four to six control and drug-treated rats. Control values for 5-HT and 5-HIAA in each region were: cerebal cortex, 504±58 and 422±32; hippocampus, 4l0±67 and 684±89; striatum, 363±22 and 492±50; hypothalamus, 1605±55 and 997±42 pg/mg tissue, respectively. Data were analyzed by one-way ANOVA and Duncan's multiple range test.

SOURCE: Battaglia et al. 1987.

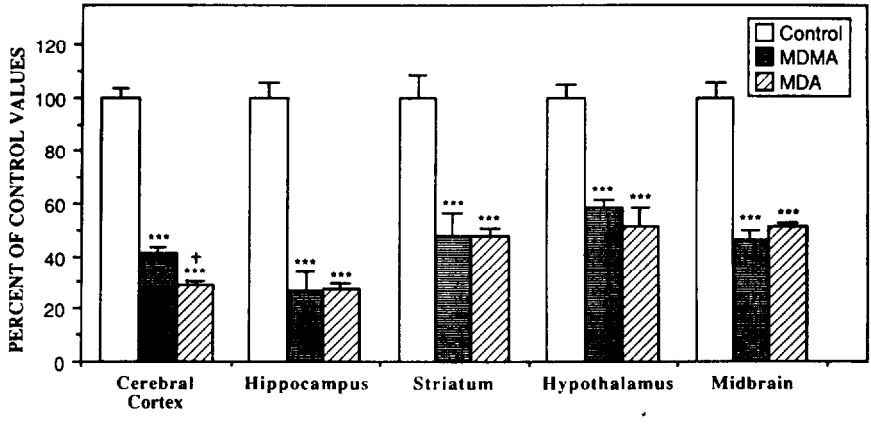


FIGURE 7. *Effect of repeated systemic administration of MDMA and MDA on the density of serotonin (5-HT) uptake sites in various brain regions*

***Significantly different from control. $p<0.001$.

†Significant difference between MDA and MDMA treatments, $p<0.001$.

NOTE: Rats were injected subcutaneously twice daily for 4 days with MDMA or MDA (20 mL/kg) or saline vehicle (1 mL/kg) and sacrificed at 2 weeks a fter the last injection. Values were determincd from saturation studies in each region except striatum and hypothalamus, where the density of 5-HT uptake sites was assessed using a saturating concentration (0.25 nM) of [^{3}H]paroxetine. No significant different from control K_D values (10-20 pM) were observed in either MDMA- or MDA-treated rats. Data, plotted as percent of the 5-HT uptake site density observed in controls in each brain region, represent the mean and SEM from three to six rats per group. Control values were: cerebral cortex, 338±10; hippocampus, 360±17; striatum, 344±30; hypothalamus, 775±36; and midbrain, 570±16 fmol/mg protein. Data were analyzed by one-way ANOVA and Duncan's multiple range test

SOURCE: Battaglia et al. 1987.

data in control and drug-treated rats indicated that, in cerebral cortex, [^{3}H]paroxetine binding was through a single population of binding sites as indicated by the Hill coefficient values (1.02, 1.01, and 1.03 in control, MDMA-. and MDA-treated rats, respectively). Furthermore, there were no significant differences in the affimity of [^{3}H]paroxetine for the serotonin uptake site (i.e., K_D) between control and drug-treated rats (18.8, 20.8, and 17.9 pM in control, MDMA-, and MDA-treated rats, respectively). Differences in the sensitivity in various brain regions to the effects of MDMA and MDA are not unique, as previous data have demonstrated differential sensitivity to the effects of methamphetamine (Ricaurte et al. 1980) and parachloroamphetamine (Kohler et al. 1978; Fuller 1978) on serotonergic systems in various brain regions. Biochemical and

histochemical data suggest that parachloroamphetamine primarily affects the ascending serotonin systems, whereas the descending pathways are left intact (Kohler et al. 1978; Fuller 1978).

Effects of MDA and MDMA on Catecholamine Neurons

In contrast with the marked and consistent effects of MDMA and MDA on serotonergic systems, neither drug produced any widespread or consistent changes in the levels of NE, DA, or their metabolites 3,4-dihydroxyphenylacetic acid (DOPAC) or homovanillic acid (HVA) in the various brain regions examined (table 1). Small changes, however, were observed in some brain regions. Both MDMA and MDA produced statistically significant increases in striatal DOPAC and cerebral cortical HVA content, whereas only MDMA treatment resulted in an increase in hippocampal DOPAC levels (table 1). Furthermore. neither MDMA nor MDA treatment caused any significant reduction in the levels of [^{3}H]mazindol-labeled NE uptake sites in cerebral cortex, hippocampus, or midbrain when compared with the respective saline-treated controls (figure 8). Although a small reduction was noted in NE uptake sites in hippocampus, this change was not statistically significant. Similarly, no significant decreases were observed in the number of [^{3}H]mazindol-labeled DA uptake sites in cerebral cortex, hippocampus, striatum, and midbrain following treatment with MDA. MDMA caused a statistically significant reduction (37 percent) in the density of DA uptake sites only in midbrain.

The neurotoxic effects of MDMA and MDA appeared to be exerted preferentially on serotonergic neurons, as no widespread changes in a variety of catecholamine markers were seen after chronic administration of these drugs. The small increases in DOPAC and/or HVA that were seen in the cerebral cortex, hippocampus, and striatum after chronic administration of these MDA derivatives are comparable to similar increases in DA metabolite levels that have been previously reported following both acute (Schmidt et al. 1986) and chronic (Stone et al. 1986) administration of MDMA and MDA. These alterations may reflect increases in DA turnover. Because serotonin-containing terminals are present in high concentrations in midbrain areas (substantia nigra and ventral tegmental area) and DA cell bodies (Steinbusch 1983). the degeneration of serotonin terminals in these regions after MDMA or MDA treatment may be responsible for the observed changes in DA metabolites. Despite the small effects on DA turnover, MDMA and MDA do not appear to produce any widespread destruction of catecholaminergic terminals, as the only significant change observed was a reduction in DA uptake sites in midbrain after administration of MDMA. The reasons for the decrease in DA uptake sites are not clear at present. Preliminary immunocytochemical data indicate that there are no changes in the density or morphology of catecholamine axons after chronic administration of MDMA or MDA (O'Hearn et al. 1988).

TABLE 1. *Effect of repeated systemic administration of MDMA and MDA on NE, DA, and DA metabolite levels in various regions of rat brain*

Brain Region	Concentration (pg/mg tissue) NE	DA	DOPAC	HVA
Cerebral Cortex				
Control	447 ± 53	59 ± 12	96 ± 14	19 ± 4
MDMA	424 ± 13	72 ± 3	73 ± 5	32 ± 4*
MDA	404 ± 26	63 ± 4	94 ± 19	36 ± 5*
Hippocampus				
Control	528 ± 62	31 ± 9	39 ± 6	6 ± 2
MDMA	573 ± 34	13 ± 5	65 ± 12*	15 ± 5
MDA	608 ± 46	16 ± 4	32 ± 13	8 ± 52
Striatum				
Control	ND	6,091 ± 596	3,212 ± 159	788 ± 58
MDMA	ND	6,974 ± 228	3,954 ± 320*	767 ± 46
MDA	ND	6,168 ± 569	3,669 ± 189*	890 ± 48
Hypothalamus				
Control	3,320 ± 209	569 ± 40	228 ± 43	54 ± 4
MDMA	3,052 ± 159	475 ± 38	200 ± 30	54 ± 5
MDA	3,577 ± 148	585 ± 67	229 ± 26	58 ± 3

*Significant difference from saline-treated control rats at $p<0.05$.

KEY: ND=levels below the sensitivity of the assay. Data were analyzed by one-way ANOVA and Duncan's multiple range test.

NOTE: NE, DA, DOPAC, and HVA measured 2 weeks after administration of 20 mg/kg MDMA or MDA, subcutaneously. every 12 hours for 4 consecutive days. Values represent the mean ± SEM. n=4 to 6 rats.

SOURCE: Battaglia et al. 1981.

Autoradiographic Studies on [^{3}H]Paroxetine-Labeled Serotonin Uptake Sites

In vitro autoradiographic studies of [^{3}H]paroxetine-labeled serotonin uptake sites in control and MDMA-treated brains were carried out as previously described (De Souza and Kuyatt 1987) to assess the neuroanatomic localization of lesions induced by MDMA. Substantial reductions in serotonin uptake sites (50 to 100 percent decreases) were observed in all areas of cerebral cortex as early as 18 hours after a 4-day treatment

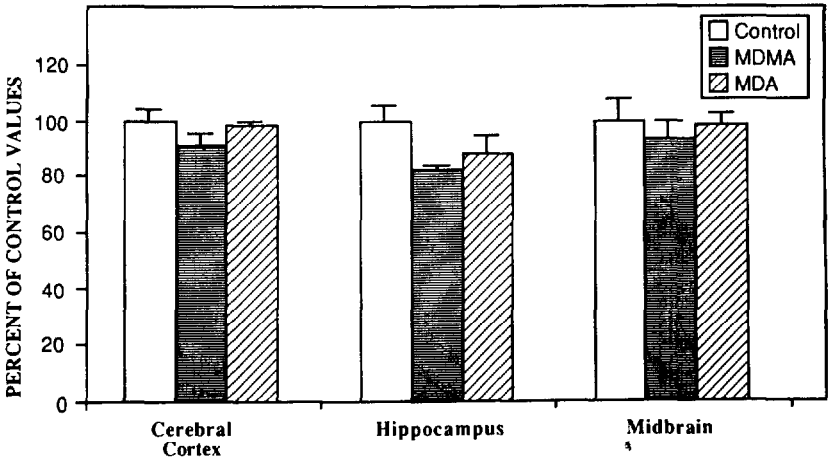


FIGURE 8. *Effect of repealed systemic administration of MDMA and MDA on the density of NE uptake sites in various brain regions*

NOTE: Rats were injected subcutaneously twice daily for 4 days with MDMA or MDA (20 mg/kg) or saline vehicle (1 mL/kg) and sacrificed 2 weeks after the last injection. NE uptake sites were measured using 6 nM [^{3}H]mazindol in the presence of selective blockers as previously described (Javitch et al. 1984). Data, plotted as percent of control values in each brian region, represent the mean and SEM from six control. MDMA-, and MDA-treated animals. Control values of NE uptake sites were: cerebral cortex. 164±6; hippocampus, 176±9; midbrain, 157±13 fmol/mg protein.

SOURCE: Battaglia et al. 1987.

regimen (20 mg/kg MDMA twice daily); the reductions were maintained for at least 2 weeks. As shown in table 2, cerebral cortical regions that showed the most extensive destruction of serotonin uptake sites (i.e., more than 90 percent) were the prefrontal (area 32), anterior cingulate (area 24). entorhinal, and parietal cortex. Comparable decreases in serotonin uptake sites were observed between day 0 (18 hours after last injection) and day 14 (14 days after last injection) in several regions of cerebral cortex such as prefrontal, pyriform, frontal areas 8 and 10, entorhinal, and primary auditory regions. In other areas of cerebral cortex, such as the sensory motor regions, significant reductions in serotonin uptake sites were observed only 2 weeks after the treatment.

As shown in table 2 and figure 9, marked decreases in serotonin uptake sites were observed following MDMA administration in all regions of caudate putamen, olfactory tubercle, endopiriform nucleus, islands of Calleja, and nucleus accumbens. Within the caudate putamen, some time-dependent

TABLE 2. *Effects of repeated systemic administration of MDMA on the regional decreases in [^{3}H]paroxetine-labeled serotonin uptake sites*

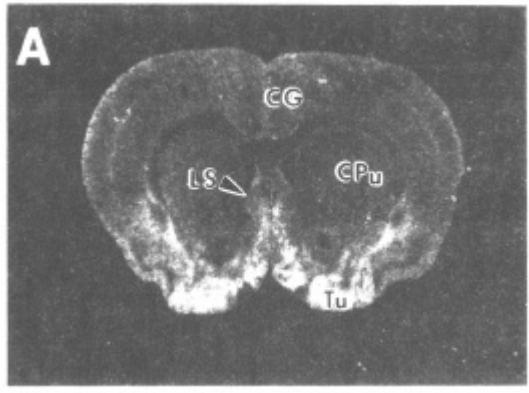
Brain Region	Control	MDMA
Cerebral Cortex		
Prefrontal area 32	2-	1
Cingulate area 24	2	1
Indusium griseum	1	2
Piriform	2	1
Frontal area 8	2	1+
Frontal area 10	1+	1-
Sensorymotor	2	1-
Parietal	1+	0
Entorhinal	4	1+
Primary auditory	1	1-
Primary visual	2+	2
Olfactory tubercle	4	2
Endopiriform nucleus	3	2
Islands of Calleja	3+	1+
Basal Ganglia		
Caudate putamen		
dorsolateral	2	1-
dorsomedial	1+	1-
ventrolateral	3	2
ventromedial	2+	1+
Nucleus accumbens	2	1-
Septal Area		
Medial septal nucleus	4-	3+
Lateral septal nucleus	3	2
Amygdala basolateral nucleus	4-	3+
Thalamus and Epithalamus		
Anteroventral nucleus	3-	0
Anteromedial nucleus	3	0
Anteroventral dorsomedial nucleus	3-	0
Reuniens	4+	1
Lateroposterior nucleus	3	1
Posterior nucleus	1	1
Posterioventromedial nucleus	1	1
Parafascicular nucleus	2	1+
Lateral geniculate body	5-	1+
Medial geniculate body	2	1
Lateral habenula	3	1-

TABLE 2. (Continued)

Brain Region	Control	MDMA
Hypothalamus		
Lateral nucleus	4	4-
Hippocampus		
CA3 region	2+	1
Dentate gyrus	2	1
Molecular layer	2+	1
Parasubiculum	3+	2
Presubiculum	3	2
Midbrain		
Inferior colliculus	3	1-
Interpeduncular nucleus	3+	3
Central gray	5+	5+
Superior colliculus		
superficial layers	3	1
profundum	2+	1
Substantia nigra		
pars compacta	3+	2
pars reticulata	3	2+
paranigral nucleus	2	3
Ventral tegmental area	2+	2
Dorsal raphe nuclei	5+	5+
Median raphe nuclei	5+	5+
Pons-Medulla		
Locus coeruleus	5+	5
Pontine reticular formation	2	2
Cerebellum (all lobules)	2	2

KEY: Relative density of [^{3}H]paroxetine binding sites: 1=0-50 fmol/mg tissue; 2=50-150 fmol/mg tissue; 3=150-250 fmol/mg tissue; 4=250-400 fmol/mg tissue and 5=>400 fmol/mg tissue; + and - values indicate the upper and lower limits, respectively, of each range.

NOTE: Data are based on observations from three animals per group. Rats were injected subcutaneously twice daily for 4 days with MDMA (20 mg/kg) or saline (1 mL/kg) (control) and sacrificed 14 days after the last injection. Anatomical terminology is derived from Paxinos and Watson (1982). [^{3}H]Paroxetine binding sites were visualized by using a saturating concentration (0.25 nM) of [^{3}H]paroxetine. Autoradiograms of rat brain were generated using [^{3}H]Ultrofilm. Analysis of [^{3}H]paroxetine-labeled serotonin uptake site densities in the various brain regions was performed by computerized image analysis densitometry. No correction for "grey-white" quenching of tritium was used.



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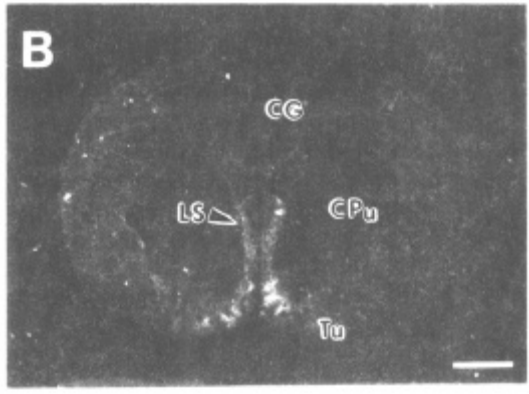

MDMA

FIGURE 9. *Autoradiographic distribution of [^{3}H]paroxetine-labeled serotonin uptake sites in coronal sections at the level of the caudate putamen from (A) saline-treated and (B) MDMA-treated rats*

NOTE: In these darkfield photomicrographs (tritium-sensitive Ultrofilm), autoradiographic grains (i.e., binding sites) appear as white spots; the tissue is not visible. The degree of nonspecific binding defined in the presence of 2 μM citalopram was comparable for both treatments. In (A), note the high density of serotonin uptake sites in cingulate cortex (CG), caudate putamen (CPu), olfactory tubercle (Tu), islands of Calleja, and lateral septal nuclei (LS) in control brains. In MDMA-treated animals (B), marked reductions were observed in most regions except for the septal nuclei, which were relatively unaffected.

reductions in serotonin uptake sites were observed following MDMA treatment. For example, equivalent decreases in serotonin uptake sites were observed between day 0 and day 14 groups in the ventrolateral region, while in dorsolateral and dorsomedial areas a significantly greater reduction in serotonin uptake sites was observed only at the later timepoint. Other brain regions that were sensitive to the neurodegenerative effects of MDMA included various thalamic nuclei and regions of hippocampus. In contrast, the dorsal and medial septal nuclei appeared to be less sensitive to the neurotoxic effects of MDMA as the reductions (approximately 25 percent) in serotonin uptake sites in these brain regions were not statistically significant. Likewise, no significant reductions were observed in the indusium griseum, which contains primarily serotonin axons of passage.

Within midbrain structures, regions containing serotonin projections appeared to be more dramatically affected by MDMA than those containing scrotonin cell bodies (figure 10). For instance, in both the superficial layers of superior colliculus and profundum, serotonin uptake sites were reduced 85 to 90 percent, while in dorsal median raphe, central grey, and the ventral tegmental region, there was little or no change after MDMA treatment. Likewise, serotonin projections to substantia nigra pars compacta and reticulata were markedly affected, while no changes in serotonin uptake sites were observed in the interpeduncular nucleus and pontine reticular formation up to 14 days following MDMA administration.

To assess the serotonergic selectivity of the neurodegenerative effects of MDMA in brain, additional autoradiographic studies of NE and DA uptake sites in brain regions containing catecholamine terminals and cell bodies were carried out. NE and DA uptake sites were labeled using [^{3}H]mazindol in the presence of specific blockers as previously published (Javitch et al. 1985). With respect to NE uptake sites, no change was observed from control levels of [^{3}H]mazindol binding sites in midbrain regions such as locus coeruleus, interpeduncular nucleus, substantia nigra pars compacta, or reticulata up to 14 days following MDMA treatment. In a number of cerebral cortical regions that receive NE projections, [^{3}H]mazindol-labeled NE uptake sites were not decreased 18 hours after treatment (i.e., day 0) but rather appeared to be slightly increased at day 14. Consistent with the minimal effects of MDMA on metabolic parameters associated with catecholamine neurons, there were no changes in the density of DA uptake sites, when compared to levels in saline-treated rats, in either cell body regions such as substantia nigra pars compacta and reticulata or terminal regions such as caudate putamen, nucleus accumbens, and olfactory tubercle. These results are therefore consistent with what has been observed in neurochemical studies in brain homogenates and indicate that the neurodegenerative effects of MDMA appear to be confined primarily to serotonergic pathways, since this treatment regimen did not reduce the density of uptake sites associated with catecholamine-containing neurons. Additional studies are necessary to assess further the neuroanatomic localization of any long-term

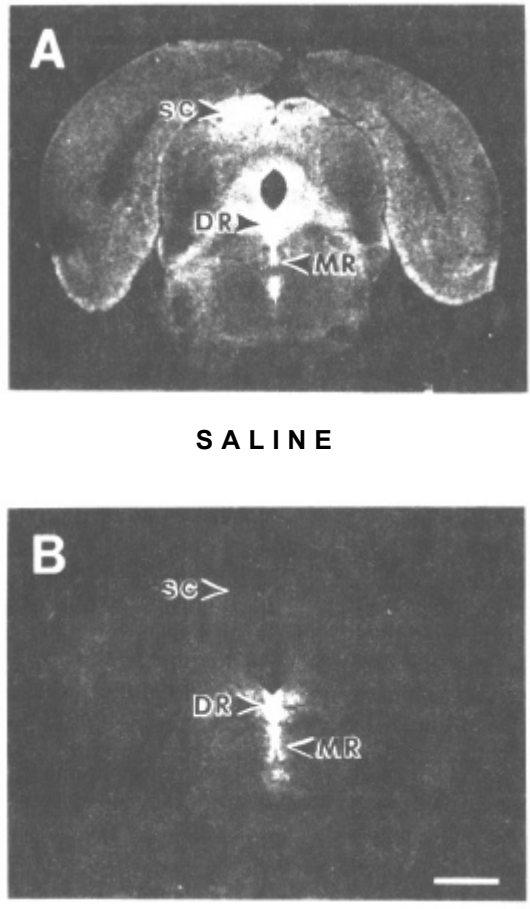

FIGURE 10. *Autoradiographic distribution of [^{3}H]paroxetine-labeled serotonin uptake sites in coronal sections at the level of midbrain in (A) saline-treated and (B) MDMA-treated rats*

NOTE: In (A), note the high density of serotonin uptake sites in control brain in regions containing serotonin projections such as entorhinal cortex, superior colliculus (sc), presubiculum and parasubiculum, as well as cell body regions such as dorsal (DR) and median (MR) raphe and central grey. MDMA-treated animals exhibited marked reductions in [^{3}H]paroxetine binding rites in presubiculum and parasubiculum, entorhinal cortex, and superior colliculus (sc), while no changes in densities were observed in areas containing primarily serotonin perikarya such as the raphe nuclei (DR and MR).

compensatory changes in NE projections or other neurotransmitter recognition sites that may occur as a consequence of MDMA lesion of scrotonin pathways.

SUMMARY AND CONCLUSIONS

The data presented in this chapter provide strong evidence, from both neurochemical and neuroanatomical studies, demonstrating that, following *in vivo* administration of a number of methylenedioxy-substituted amphetamine derivatives, there is widespread and long-lasting degeneration of scrotonin neurons in brain, without any major or consistent effects on catecholamine neurons. A detailed examination of the parameters involved in the neurotoxic and neurodegencrative effects of MDMA on brain scrotonin neurons indicates that:

(1) the severity of the lesion by MDMA is dependent on both the dose and frequency of drug administration;

(2) the neurodegenerative effects of MDMA can be elicited in a number of animal species including primates;

(3) the neurodegenerative effects on brain serotonin neurons can be prevented by the serotonin uptake blocker, suggesting a role for the active uptake of MDMA, a neurotoxic metabolite of MDMA, or an unidentified endogenous neurotoxin; and

(4) the neurodegenerative effects of the drug are long-lasting (up to 1 year) with respect to neuronal recovery, while functional recovery may be permanently impaired.

In addition, the neurochemical and autoradiographic data suggest that there is some neuroanatomical and morphological specificity to the neurodegenerative effects of MDMA and MDA, as evidenced by predominant reductions in serotonin uptake sites in brain regions containing primarily serotonin terminals, while regions containing serotonin axons of passage and cell bodies are relatively unaffected.

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