10. MDMA EFFECTS IN BRAIN: PHARMACOLOGIC PROFILE AND EVIDENCE OF NEUROTOXICITY FROM NEUROCHEMICAL AND AUTORADIOGRAPHIC STUDIES

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1. INTRODUCTION

3,4-Methylenedioxymethamphetamine (MDMA), a ring-substituted derivative of methamphetamine, has been reported to exhibit both stimulant and psychotomimetic properties [1–3]. MDMA has recently attracted a great deal of attention due to its increasing abuse among certain segments of the population [4, 5] and has been the focus of a number of review articles [6, 7] and symposia [8, 9]. Recent data demonstrating that MDMA is self-administered by both rhesus monkeys [10] and baboons [11] suggest that MDMA may have high abuse potential in man. These reports are particularly disturbing, as we and others have recently demonstrated that MDMA is a potent neurotoxin that appears to cause selective degeneration of brain serotonin neurons [12–16], comparable to that reported for its structural analogue, 3,4-methylenedioxyamphetamine (MDA) [12, 17–18].

This chapter will address both the pharmacologic profile of MDMA at various brain recognition sites and the neurotoxic effects of MDMA on brain monoamine systems. We will first describe the in vitro pharmacologic profile of MDMA at a number of established brain recognition sites and receptors and the characteristics of [^{3}H]-MDA and [^{3}H]-MDMA association with brain membranes. With respect to the neurochemical consequences of in vivo administration of MDMA on brain monoamine systems, we will discuss: (1) the selective neurodegenerative effects on serotonin (5-HT) systems, (2) the effects of dose and frequency of drug administration, (3) the relative sensitivity of

Peroutka S.J. (ed), Ecstasy.

various animal species to MDMA, (4) the potential neuronal mechanisms involved in the neurotoxic effects of the drug, (5) the time course of recovery following neurodegenerative changes, and (6) the neuroanatomic and morphological specificity of MDMA-induced neurotoxicity. For comparative purposes, we will also describe the effects of some other designer drugs, such as MDA and N-ethyl-3,4-methylexdioxyamphetamine (MDE).

2. IN VITRO EFFECTS OF MDMA

In order to elucidate the putative sites of action of MDMA in brain, we have taken two approaches: (1) we have carried out an extensive in vitro pharmacologic profile of MDMA at known brain receptors and recognition sites to assess which neuronal systems may play a role in the central nervous system (CNS) actions of this drug, and (2) we have carried out preliminary studies investigating the characteristics and pharmacology of [^{3}H]MDMA binding in rat brain synaptosomes to assess whether these sites represent a novel binding site distinct from other established neurotransmitter receptors or recognition sites.

2.1. Pharmacologic profile of MDMA at various brain recognition sites

We have used radioligand binding techniques to determine the relative potencies of MDMA at various brain neurotransmitter receptors and recognition sites, in order to elucidate the sites through which MDMA might elicit its behavioral, psychotomimetic, and neurotoxic effects.

The in vitro pharmacologic profile of MDMA demonstrates a broad range of affinities of the drug for various brain recognition sites [19]. The relative potencies of MDMA at the various brain recognition sites were assessed from the inhibitory affinity constants (K_i values) derived from MDMA competition data and calculated using the non-linear curve fitting program, LIGAND [20]. These data are summarized in Table 1.

MDMA is most potent at a number of serotonin recognition sites and at α_2-adrenergic receptors with affinity constants in the high nanomolar to low micromolar range. MDMA had highest affinity for 5-HT uptake sites ($<1\mu M$), with lower but comparable affinities at 5-HT_2 serotonin, α_2-adrenergic, M-1 muscarinic cholinergic, and H-1 histamine receptors (K_i values $\leqq 5\mu M$). The rank order of affinities of MDMA at various brain receptors and uptake sites was as follows: 5-HT uptake $>$ α_2-adrenergic = 5-HT_2 serotonin = M-1 muscarinic = H-1 histamine $>$ norepinephrine (NE) uptake = M-2 muscarinic = α_1-adrenergic = β-adrenergic $\geqq$ dopamine uptake = 5-HT_1 serotonin $>>$ D-2 dopamine (DA) $>$ D-1 dopamine (Table 1). MDMA exhibited negligible affinities ($>500\ \mu M$) at μ, δ, and κ opioid, central-type benzodiazepine, and corticotropin-releasing factor receptors, at choline uptake sites, and at calcium channels. MDA had a similar pharmacologic profile, with affinities comparable ($<$ twofold difference) to those of MDMA at each of the respective brain recognition sities investigated.

These data suggest that a number of the behavioral, psychotomimetic, and neurochemical effects of MDMA may be explained, in part, by interactions of this drug at multiple serotonin recognition sites in brain. MDMA may alter serotonergic transmission in brain through direct actions at post- as well as pre-synpatic 5-HT recognition sites. With respect to actions mediated at postsynaptic receptors, a number of hallucinogenic phenylisopropylamine derivatives have been shown to exhibit potent agonist-like activity at brain 5-HT_2 serotonin receptors [21, 22]. The in vitro affinities of these hallucinogens at 5-HT_2 serotonin receptors are significantly correlated with both their behavioral potencies in animals, in generalization to other hallucinogens, and with their human hallucinogenic potencies [23, 24]. As observed for other ring-substituted amphetamine derivatives, we have found that MDMA and other methylenedioxy derivatives of amphetamine (MDA and MMDA) exhibit high affinity agonist-like binding characteristics at 5-HT_2 serotonin receptors and a stereospecificity consistent with that observed for other hallucinogenic compounds at this receptor [25, 26]. Data in support of agonist-like characteristics of MDMA at 5-HT_2 serotonin receptors include interactions of MDMA with the high affinity state of 5-HT_2 serotonin receptors, which are sensitive to the effects of guanine nucleotides; similar interactions have been previously reported for 5-HT and other classical tryptamine agonists at this site [27, 28]. Furthermore, while the overall apparent affinity of MDMA for [^{3}H]-kentanserin-labeled 5-HT_2 serotonin receptors is in the low micromolar range, MDMA interactions with the high affinity state of 5-HT_2 serotonin receptors labeled directly by [3-H]-DOB [29] are markedly more potent, with an affinity in the nanomolar range ($K_i \approx 300$ nM). Since MDMA exhibits agonist-like properties at this site similar to those of other hallucinogens, it is feasible that a significant component of the "mood-altering" effects of MDMA may be mediated via direct agonist actions at 5-HT_2 serotonin receptors. A comparison of the relative affinities of MDMA and MDA at postsynaptic 5-HT_2 serotonin receptors with those of other ring-substituted amphetamine hallucinogens suggests that MDMA and MDA would be weaker hallucinogens than would be compounds such as DOM (STP) or DOI (4-iodo, 2,5 dimethoxy amphetamine). A recent study demonstrating that the 5-HT receptor antagonist methysergide can potentiate the MDMA-induced increases in locomotor activity [30] further supports the contention that some of the actions of MDMA are mediated by postsynaptic 5-HT_2 serotonin receptors. In addition to interactions with 5-HT_2 serotonin receptors, some of the effects of MDMA on serotonergic systems could be mediated via 5-HT_{1A} receptors; MDMA has a relatively high affinity for 5-HT_{1A} receptors. Direct agonist effects at this site might contribute to the "centering" and calming subjective reports, since similar effects are reported for novel, non-benzodiazepine anxiolytics, such as ipsapirone and buspirone, which interact with 5-HT_{1A} sites [31].

In addition to its relatively high affinity at postsynaptic serotonin receptors,

Table 1. Pharmacologic profile of MDMA at various brain recognition sites.

Brain recognition site	Affinity K^i (μM)	Radioligand/displacer	Brain region	Assay time, temperature	Buffer
Uptake sites					
Serotonin	0.61 ± .05	0.25nM ^{3}H-Paroxetine/1μM citalopram	1	120min, Rm T	A
Norepinephrine	15.8 ± 1.7	4.0nM ^{3}H-Mazindol/0.3μM desipramine	1	90min, 4°C	A
Dopamine	24.4 ± 1.9	1.0nM ^{3}H-GBR 12935/1μM mazindol	2	60min, Rm T	A
Choline	> 500	10nM ^{3}H-Hemicholinium-3/10μM Hemicholinium-3	2	30min, 25°C	B
Adrenoceptors					
α_1	18.4 ± 1.2	0.5nM ^{3}H-Prazosin/10μM phentolamine	1	30min, 37°C	C
α_2	3.6 ± 0.8	0.5nM ^{3}H-Para-aminoclonidine-10μM phentolamine	1	30min, 37°C	C
β	19.2 ± 2.1	0.5nM ^{3}H-Dihydroalprenalol/1μM propranolol	1	30min, 37°C	C
Dopamine receptors					
D-1	148 ± 14	0.2nM ^{3}H-SCH 23390/0.1μM flupenthixol	2	30min, 37°C	C
D-2	95 ± 15	0.2nM ^{3}H-Spiperone/1μM (+)butaclamol	2	30min, 37°C	C
Serotonin Receptors					
5-HT_1	23 ± 1.5	2.5nM ^{3}H-Serotonin/10μM serotonin	1	30min, 37°C	C
5-HT_2	5.1 ± 0.3	0.4nM ^{3}H-Ketanserin/0.5μM cinanserin	1	30min, 37°C	C
Cholinergic receptors					

M-1 Muscarinic	5.8 ± 0.3	0.1nM ^{3}H(−)QNB/1μM atropine	1	90min, Rm T	D
M-2 Muscarinic	15.1 ± 0.1	0.1nM ^{3}H(−)QNB/1μM atropine	3	90min, Rm T	D
Opioid receptors					
μ	> 500	2nM ^{3}H-Dihydromorphine/1μM levallorphan	4	45min, 25°C	E
δ	> 500	4nM ^{3}H-D-Ala^2-D=leu^5-enkephalin (30nM morphine)/1μM levallorphan	4	45min, 25°C	E
κ	> 500	1.6nM ^{3}H-Ethylketazocine (30 nM morphine + #00nM D-ala^2-D-leu^5-enkephalin)/1μM levallorphan	4	45min 25°C	E
Other sites					
H-1 Histamine receptors	5.7 ± 2.4	2nM ^{3}H-Mepyramine/1μM doxepin	1	60min, Rm T	F
Benzodiazepine receptors	> 500	0.2nM ^{3}H-Flunitrazepam/1μM clonazepam	1	60min, Rm T	G
Corticotropin-releasing factors (CRF) receptors	> 500	0.1nM ^{125}I-Tyr°-rat CRF/1μM, ovine CRF	5	120min, Rm T	H
Calcium channels	> 500	0.2nM ^{3}H-Nitredipine/0.1μM nifedipine	1	60min, Rm T	G

Affinities of MDMA at various brain recognition sites. Data represent the mean and SEM from three to five competition curves at each of the sites. K_i values were determined using the nonlinear least-squares curve fitting program LIGAND. Assay buffers were as follows: A, 50 mM TRIS-HCl, 120 mM NaCl, 5 mM KCl (pH 7.4 at Rm T); B, 50 mM glycylglycine, 200 mM NaCl (pH 7.8 at 25°C); C, 50 mM TRIS-HCl, 10 mM $MgSO_4$, 0.5 mM K_2EDTA (pH 7.4 at 37°C); D, 50 mM TRIS-HCl, 10mM $MgSO_4$ (pH 7.7 at Rm T); E, 0.17 M TRIS-HCl (pH 7.6 at 25°C); G, 50mM TRIS-HCl (pH 7.7 at Rm T); F, 50 mM Na^+K^+ phosphate (pH 7.4 at Rm T); H, 50 mM TRIS-HCl, 10 mM $MgCl_2$, 2 mM EGTA 0.1% Bovine Serum Albumin, 0.1 mM bacitracin, aprotinin (100 KIU/ml) (pH 7.2 at 22°C). Brain regions were as follows: 1. frontal cortex; 2. striatum; 3. brain stem; 4. whole brain; and 5. olfactory bulb.

MDMA also exhibits high affinity for 5-HT uptake sites. These data suggest that some of the actions of MDMA may be mediated at presynaptic 5-HT binding sites. MDMA has been reported to competitively inhibit [^{3}H]-5-HT uptake in vitro [3] and to increase the release of [^{3}H]-5-HT from brain synaptosomes [32] and hippocampal slices [33]. Furthermore, the neurotoxic effects of in vivo administration of MDMA on 5-HT terminals can be blocked by concomitant administration of the 5-HT uptake blockers citalopram [13, 34]. Additional evidence in support of the hypothesis that MDMA produces some of its effects through presynaptic serotonergic mechanisms is provided by data demonstrating that MDMA generalizes to a fenfluramine cue in discrimination studies [35]. As mentioned previously and as shown in Table 1, MDMA exhibits relatively high affinity for α_2-adrenergic receptors. Classic α-adrenergic receptor antagonists such as phentolamine have been reported to increase the release of ^{3}H-5-HT via effects on α_2-adrenergic receptors [36]. Thus one might speculate that the serotonin releasing effects of MDMA may be mediated, in part, by high affinity antagonist-like effects at α_2-adrenergic receptors localized to presynaptic serotonin terminals. Thus the relatively high affinity of MDMA at the 5-HT uptake site and α_2-adrenergic receptor may contribute, in part, to the neurochemical, neurotoxic, and behavioral effects mediated at presynaptic 5-HT terminals.

Interestingly, the "anxiolytic-like" effects of MDMA do not appear to be mediated through agonist actions at benzodiazepine receptors or antagonist effects at corticotropin-releasing factor receptors, as evidenced by the low affinity of MDMA (>500 μM) at each of these receptors. In addition, neither the reinforcing, analgesic or mood-altering properties of the drug appear to be mediated through interactions with any of the opioid receptor subtypes since MDMA has relatively low affinities for these binding sites. While brain serotonin systems may play a key role in mediating some of the effects of MDMA on analgesia and body temperature, as well as in the reported anxiolytic-like, mood altering, and subjective effects of the drug, additional neurotransmitter systems may contribute to some of the unique subjective experiences reported for MDMA and other drugs in this class.

2.2. ^{3}H-MDMA binding studies

In an attempt to further elucidate the molecular interactions between MDMA and neuronal tissue that may underlie MDMA's actions in brain, we examined the incorporation of both [^{3}H]-MDMA and the related compound [^{3}H]-MDA into rat brain synaptosomes. [^{3}H]-MDMA was previously reported to bind to a "high affinity" site ($K_i = 99.2$ nM) in whole rat brain membranes [37]. A subsequent study [38] reported that the apparent "high affinity" MDMA binding was due to a "nonspecific" interaction of [^{3}H]-MDMA with glass fiber filters. In addition, the latter study did not detect any uptake of [^{3}H]-MDMA into rat brain synaptosomes.

While the previous studies of [^{3}H]-MDMA incorporation into brain mem-

branes were performed using standard filter binding techniques, we have used centrifugation assays and have employed intact synaptosomes prepared from various regions of rat brain to investigate the incorporation of [^{3}H]-MDMA and [^{3}H]-MDA [39]. We observed that [^{3}H]-MDA was incorporated into three saturable pools. First, the radioligand was sequestered into a saturable-nonspecific site that was resistent to boiling of the membranes. Analysis of saturation data indicated that, in addition to this nonspecific site, there was also a high affinity site (K_D = 0.89 μM, B_{max} = 23 pmoles/mg synaptosomal protein) and a low affinity site (k_D = 45 μM, B_{max} = 3 nmoles/mg synaptosomal protein). The low affinity site was dependent on the presence of 0.27 M sucrose. This sucrose-dependence was not due to the maintenance of iso-osmotic conditions, since [^{3}H]-MDA incorporation was reduced by 74% when synaptosomes were incubated in iso-osmotic saline. [^{3}H]-MDMA interacts with sites similar to those characterized for [^{3}H]-MDA. In addition to saturable-nonspecific sites (i.e., resistant to boiling), two specific [^{3}H]-MDMA sites were observed on analysis of saturation data (K_D high: 2.9 μM, B_{max}: 79 pmole/mg protein; K_D low: 128 μM, B_{max}: 7.4 nmole/mg protein). The pharmacological profiles of [^{3}H]-MDA and [^{3}H]-MDMA binding were also similar. The order of potency of inhibition of [^{3}H]-MDA or [^{3}H]-MDMA incorporation was paroxetine = desipramine > mazindol > serotonin.

The high binding capacity of the MDA/MDMA site suggests that the binding does not represent a bimolecular ligand-protein interaction. In addition, [^{3}H]-MDA and [^{3}H]-MDMA do not appear to be internalized or sequestered in a intrasynaptic pool, since the binding is not dependent on temperature and is relatively insensitive to the effects of detergents. However, the heterogenous distribution of [^{3}H]-MDA binding in different brain regions indicates that this site is not a nonspecific interaction of MDA or MDMA with brain lipid, as has been suggested for an apparent low affinity component of [^{3}H]-imipramine binding [40]. Further studies are required to determine the exact nature of the interactions of [^{3}H]-MDA and [^{3}H]-MDMA with novel brain recognition sites and the possible relevance of these interactions to the clinical, biochemical, and toxic actions of these compounds.

In order to address the question of pharmacologic relevance of micromolar affinities of MDMA and MDA at various brain recognition sites and the micromolar affinites of [^{3}H]-MDMA and [^{3}H]-MDA binding sites, we have carried out preliminary studies to assess brain concentrations of drug following systemic administration of MDA and MDMA. Concentrations of drug were measured at 45 minutes after systemic administration of the compounds, as peak locomotor activity as well as peak levels of [^{3}H]-MDA and [^{3}H]-MDMA in brain were present during this period. As shown in Table 2, following a single subcutaneous injection of 20mg/kg [^{3}H]-MDMA or [^{3}H]-MDA, fairly comparable concentrations of each drug were observed in all brain regions, with slightly higher levels of drug measured in liver. Assuming a conversion factor of 1 gm of tissue being equivalent of 1 ml, then the values

Table 2. Regional distribution of [^{3}H]-MDA and [^{3}H]-MDMA in rat brain and peripheral tissue.

Region	*μmol/g tissue* [^{3}H]-MDMA	[^{3}H]-MDA
Frontal cortex	0.22	0.42
Rest of cortex	0.19	0.32
Striatum	0.22	0.42
Hippocampus	0.22	0.44
Thalamus	0.21	0.44
Hypothalamus	0.18	0.37
Midbrain	0.17	0.36
Cerebellum	0.15	0.39
Brainstem	0.15	0.23
Pituitary	0.31	—
Liver	0.48	1.26
Spleen	0.25	0.56

Rats were injected with 20 mg/kg of [^{3}H]-MDMA or [^{3}H]-MDA, sacrificed at 45 minutes, and brain regions and peripheral tissues were dissected. A portion of the respective tissues was weighed, solubilized overnight in protosol, and counted by liquid scintillation spectrometry.

of [^{3}H]-MDMA and [^{3}H]-MDA found in all brain regions would convert to concentrations of MDMA and MDA in the high micromolar range for a 20 mg/kg dose of drug. This dose of MDA and MDMA has previously been shown to produce a variety of behavioral and neurochemical effects [12, 14, 17, 31]. These data suggest that the micromolar affinity interactions of MDMA and MDA with binding sites and receptors in brain appear to be pharmacologically relevant with respect to the behavioral and/or neurotoxic effects of the drugs.

3. IN VIVO EFFECTS OF MDMA: NEUROCHEMICAL STUDIES

Typically, neurotoxic effects of drugs on 5-HT neurons have been assessed from changes in a number of serotonergic parameters including (1) reductions in brain levels of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA), (2) decreases in the maximal activity of tryptophan hydroxylase (TPH), and (3) decreases in the activity of the serotonin active uptake carrier. Since MDMA can inhibit the activity tryptophan hydroxylase, the rate-limiting enzyme in 5-HT synthesis [18, 41], it is unclear whether MDMA-induced reductions in 5-HT and 5-HIAA may be due to suppressed neurotransmission in otherwise structurally intact 5-HT neurons or whether these changes may represent the consequence of the destruction of 5-HT axons and terminals.

The following studies were designed to assess and quantify both the neurochemical and neurodegenerative effects of short-term administration of MDMA on monoamine neurons in rat brain. In some studies we have also investigated the changes induced by MDA and MDE. Since monoamine uptake sites are highly concentrated on their respective nerve terminals in brain [42], we can directly quantify the degree of neurodegeneration of particular monoamine terminals by measuring reductions in the density of their respective uptake

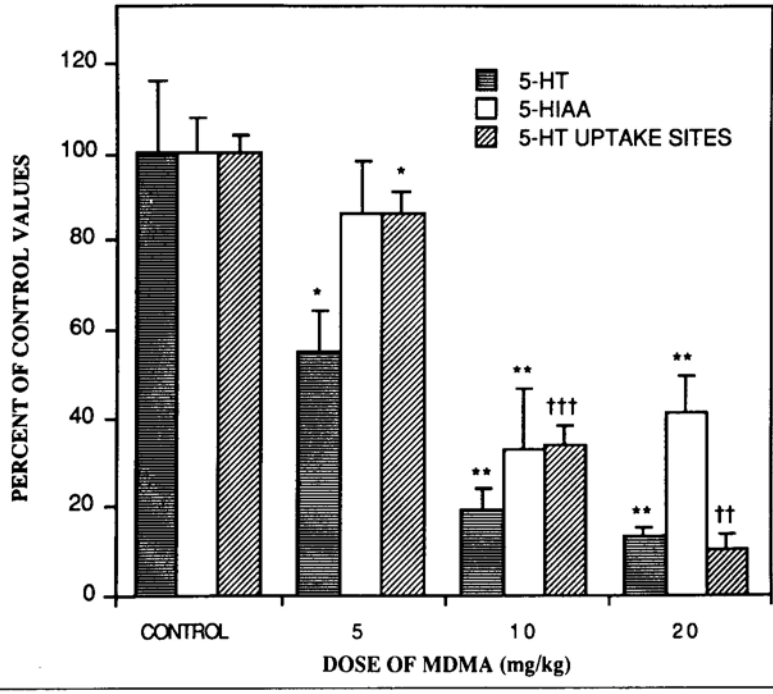


Figure 1. The effect of repeated systemic administration of various doses of MDMA on the content of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) and on the density of 5-HT uptake sites in rat frontal cerebral cortex. Rats were administered either saline or MDMA twice a day for 4 consecutive days and sacrificed 18 hours after the last injection. Data represent the mean ± SEM from 3–5 rats and are 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. * and ** indicate significant differences at $p < 0.05$ and $p < 0.01$, respectively, from control saline-treated rats. †† and ††† indicate significant differences at $p < 0.01$ and $p < 0.001$, respectively, from all other MDMA-treated groups. (From Battaglia et al., 1988.)

sites. We have recently reported the feasibility of using radioligand binding techniques and high affinity ligands for 5-HT, dopamine, and norepinephrine uptake sites to monitor the loss of monoamine axons and terminals [12, 13]. We report here that MDMA, MDA, and MDE cause marked reductions in various serotonergic markers (i.e., 5-HT, 5-HIAA, and 5-HT uptake sites). Marked long-term reductions in the density of 5-HT uptake sites in brain, which reflect the loss 5-HT axons and/or terminals, indicate that both MDMA and MDA are potent, long-lasting 5-HT neurotoxins.

3.1. Dose dependence

As shown in Figure 1, a repetitive dosing regimen (i.e., subcutaneous administration twice a day for four consecutive days) of MDMA at various doses up

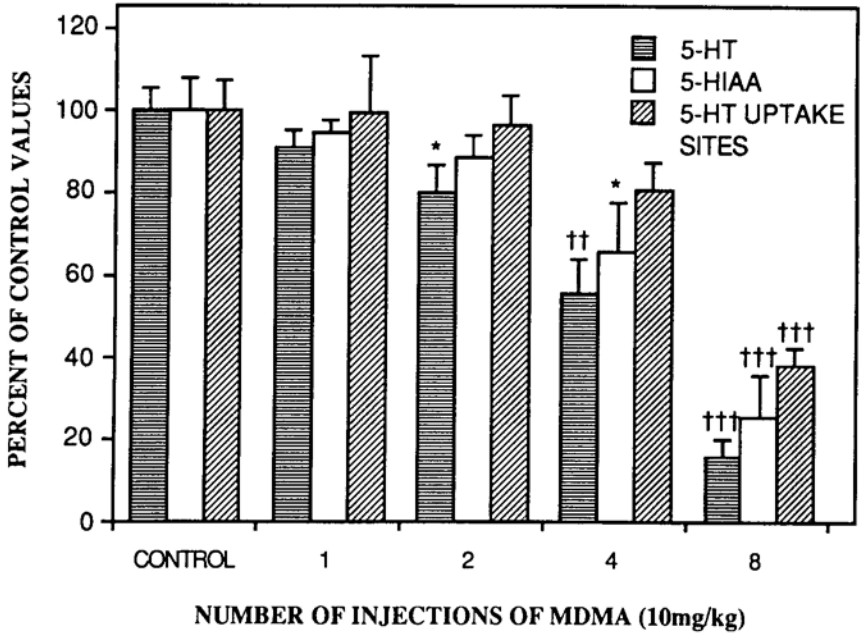


Figure 2. The effects of single and multiple injections of MDMA on the content of serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) and on the density of 5-HT uptake sites in rat frontal cerebral cortex. Rats were injected subcutaneously the specificed number of times with either saline or 10 mg/kg MDMA and sacrified 18 hours after the last injection. Data that represent the mean and SEM from three to five animals are plotted as a percent of respective values for each of the markers in control, saline-injected rats. Control levels of 5-HT and 5-HIAA were 475 ± 24 and 332 ± 24 pmol/mg tissue, respectively. The density of 5-HT uptake sites was 349 ± 24 fmol/mg protein in controls. Data were analyzed by one-way ANOVA and Duncan's multiple range test. * indicates a significant difference at $p < 0.05$ from corresponding control saline-injected rats; †† and ††† indicate significant differences at $p < 0.01$ and $P < 0.001$, respectively, from all other groups. (From Battaglia et al., 1988.)

to 20 mg/kg resulted in dose-dependent decreases in the content of 5-HT and 5-HIAA and in the density of 5-HT uptake sites in rat frontal cerebral cortex at 18 hours following the last injection. At the lowest dose of MDMA tested (5 mg/kg), 5-HT content was markedly reduced (45%), while only a small (14%) but statistically significant decrease in the density of 5-HT 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 (60–70%), while the decrease in 5-HT content was significantly greater at 20 mg/kg (90%) than at 10 mg/kg (80%). The density of 5-HT uptake sites decreased progressively as the dose of MDMA was increased, with a maximal reduction of 90% observed at 18 hours following repetitive administration of 20 mg/kg MDMA. In contrast, following the identical treatment regimen

of 20 mg/kg MDMA, there were no significant differences in the density of [^{3}H]-mazindol-labeled norepinephrine uptake sites (fmol/mg protein) in the frontal cerebral cortex between saline-treated (159 ± 17) and drug-treated (152 ± 5) animals.

3.2. Effect of single versus multiple injections

Since repeated systemic administration of 10 mg/kg MDMA caused marked caused marked neurodegeneration of cerebral cortical 5-HT neurons, we chose to investigate the neurodegenerative effects of single versus multiple subcutaneous injections of MDMA at this dose. Although neurotoxicity studies for MDMA typically employ a subcutaneous route of drug administration, whereas humans self-administer this drug orally, recent reports indicate comparable neurotoxic effects with either type of drug administration [43]. As shown in Figure 2, increasing the number of injections of MDMA (10 mg/kg s.c.) resulted in significant and progressively greater reductions in 5-HT and 5-HIAA content. While one injection of MDMA was without effect on any of the serotonergic markers examined, two doses were sufficient to elicit a significant reduction (~20%) in 5-HT content. A significant reduction (~34%) in 5-HIAA content was observed only after four injections of MDMA. Marked reductions of 84% and 75% in 5-HT and 5-HIAA, respectively, were observed following an eight injection treatment regimen of 10 mg/kg MDMA. The density of [^{3}H]-paroxetine-labeled 5-HT uptake sites was only significantly decreased (~64%) following eight injections of MDMA at this dose. While there was no change in dopamine content in any of the groups examined, a small and consistent decrease in norepinephrine content (~20%) was observed in all MDMA-treated rats. This small change in norepinephrine following MDMA treatment was not accompanied by a reduction in the density of [^{3}H]-mazindol-labeled norepinephrine uptake sites (data not shown).

3.3. Potential mechanisms

As the neurotoxic effects of drugs, such as para-chloroamphetamine, on 5-HT neurons can be prevented by 5-HT uptake blockers [44, 45], we investigated if the 5-HT uptake carrier protein was likewise involved in the neurotoxic effects of MDMA. As shown in Figure 3, pretreatment of rats with the selective 5-HT uptake blocker citalopram (10 mg/kg) prior to each injection of 10 mg/kg MDMA resulted in nearly complete protection against the neurotoxic and neurodegenerative effects of MDMA. Citalopram-pretreated rats showed only a small (15%) decrease in 5-HT uptake sites following MDMA treatment, in comparison with a 60% reduction in 5-HT uptake sites observed in rats treated with an identical dose of MDMA alone. In addition, citalopram pretreatment completely prevented the MDMA-induced decreases in the concentration of both 5-HT and 5-HIAA. Similar protective effects of serotonin uptake blockers, such as citalopram and fluoxetine, on MDMA-induced neurotoxicity have previously been demonstrated [15, 34]. These data would suggest that the

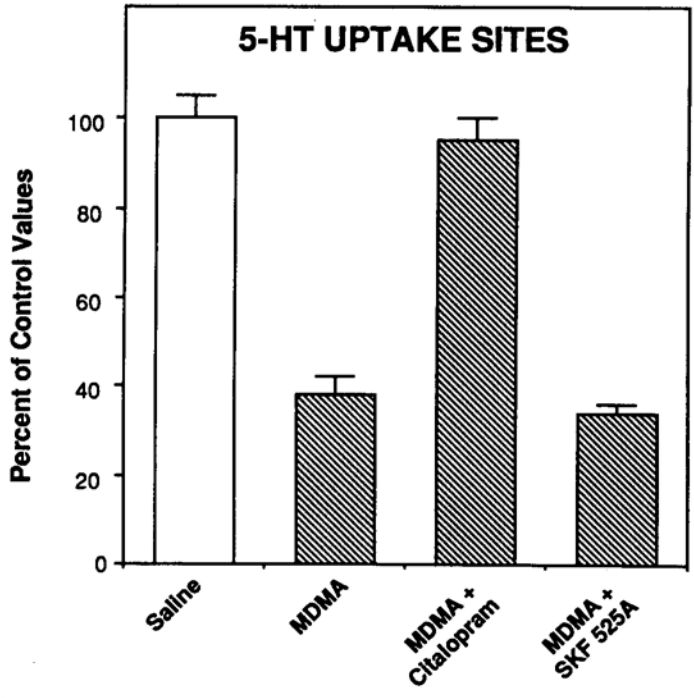


Figure 3. The effect of repeated systemic administration of 10 mg/kg MDMA, MDMA plus 10 mg/kg citalopram, and MDMA plus 25 mg/kg SKF 525A on the density of serotonin (5-HT) uptake sites in homogenates of rat frontal cerebral cortex. Data are expressed as a percent of values in control saline-treated rats and represent the mean and SEM from 4–6 animals. Control levels of 5-HT uptake sites were 356 ± 15 fmol/mg protein.

active uptake of MDMA, some related metabolite, or endogenous neurotoxin is required for MDMA-induced degeneration of serotonin neurons.

We have previously reported that in contrast to the marked neurodegenerative effects following systemic administration of MDMA or MDA on brain 5-HT neurons, single, direct intracerebral injections of MDMA or MDA had no effect on cerebral cortical 5-HT neurons, as visualized directly using serotonin immunocytochemistry [46]. Our observation of marked differences in 5-HT neurotoxicity to central versus systemically-administered MDMA would be consistent with the hypothesis of a peripherally produced neurotoxic metabolite of MDMA. Since MDMA has been reported to interact with the hepatic microsomal enzyme cytochrome P-450 [47], we investigated whether inhibition of this enzyme could alter the neurotoxic effects of MDMA. In preliminary studies, we found that in rats pretreated with the cytochrome P-450 enzyme inhibitor, SKF 525A, 45 minutes prior to each administration of MDMA, there was neither potentiation nor attenuation of the neurodegenera-

tion found following repeated administration of 10 mg/kg of MDMA. As shown in Figure 3, no changes in the density of 5-HT uptake sites were observed between MDMA and MDMA plus SKF 525A-treated rats. While these data suggest that it is unlikely that the putative neurotoxic species is a cytochrome P-450-dependent metabolite of MDMA, the involvement of some other peripheral and/or central metabolite of MDMA cannot be ruled out.

3.4. Regeneration of serotonin neurons

In order to assess whether 5-HT neurons regenerate in MDMA-treated rats, we recently investigated the time course of changes in 5-HT content and 5-HT uptake sites in frontal cerebral cortex for up to one year following a repeated systemic administration (i.e., twice daily s.c. injections for four days) of 20 mg/kg MDMA [13]. As shown in Figure 4, at all time points up to six months during the recovery time course, the density of 5-HT uptake sites was significantly below the corresponding values in age-matched, saline-treated controls. At the six-month time point, the density of 5-HT uptake sites was only 75% of the values of saline-treated controls, whereas by 12 months after MDMA treatment, the density of 5-HT uptake sites returned to control levels. The shape of the recovery curve suggests that there may be a faster initial rate of recovery of 5-HT uptake sites that occurs between 18 hours and four weeks, which is followed by a slower rate of recovery that occurs between four weeks and 12 months. These data indicate that more than six months are required for a complete recovery of 5-HT uptake sites to control levels.

It was of interest that despite the recovery of 5-HT uptakes sites to control levels, the content of 5-HT in the same brain region remained markedly (40–50%) below age-matched controls for as long as one year after MDMA administration. It is unclear from these data whether there is a regeneration of axons that have previously degenerated or whether the increased density of 5-HT uptake sites is a consequence of increased collateral sprouting of neurons unaffected by the drug treatment. It is also possible that axonal regeneration or collateral sprouting is associated with considerably greater densities of uptake sites per neuron, thereby making it more difficult to assess neuronal recovery from this index. The observation that 5-HT levels remain 40–50% below age-matched controls for up to one year despite control-level densities of 5-HT uptake sites indicates that following lesion by MDMA, the 5-HT neurons that "recover" may not be functionally identical to those present in age-matched control brains.

3.5. Species differences

Since amphetamines have been shown to be metabolized by different pathways in rat, mouse, and guinea pig [48], studies were carried out to investigate whether MDMA neurotoxicity can be demonstrated in other species, such as mouse and guinea pig. Animals in these studies were treated repeatedly (i.e.,

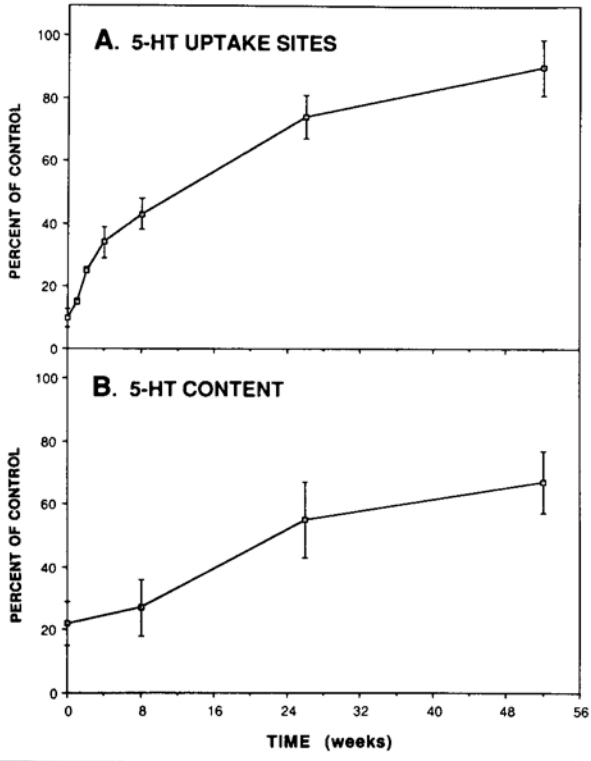


Figure 4. Time course of recovery of (A) serotonin (5-HT) uptake sites and (B) 5-HT content in rat cerebral cortex following repeated systemic administration of MDMA. Rats were injected subcutaneously with either saline or 20 mg/kg MDMA twice a day for 4 consecutive days and then sacrificed at various times up to 12 months later following the last injection of the drug. Saline-injected control rats were killed at each of the time points, and the data that represent the mean ± SEM of five rats per group are plotted as a percent of the value of age-matched saline-injected control rats. (Adapted from Battaglia et al., 1988.)

twice a day for four consecutive days) with 20 mg/kg MDMA, and levels of 5-HT, 5-HIAA, and 5-HT uptake sites were measured seven days later to assess the long-term effects of the treatment [13]. As shown in Figure 5, MDMA caused comparable and marked decreases in 5-HT and 5-HIAA content and in the density of 5-HT 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 [49] have also suggested that mice are less susceptible to the neurotoxic effects of MDMA. In more recent studies, we have also demonstrated that administration of 2.5 or 10 mg/kg MDMA for four consecutive days results in neurotoxic effects in primates, with decreases in the density of 5-HT uptake sites observed following the higher dose [50]. The

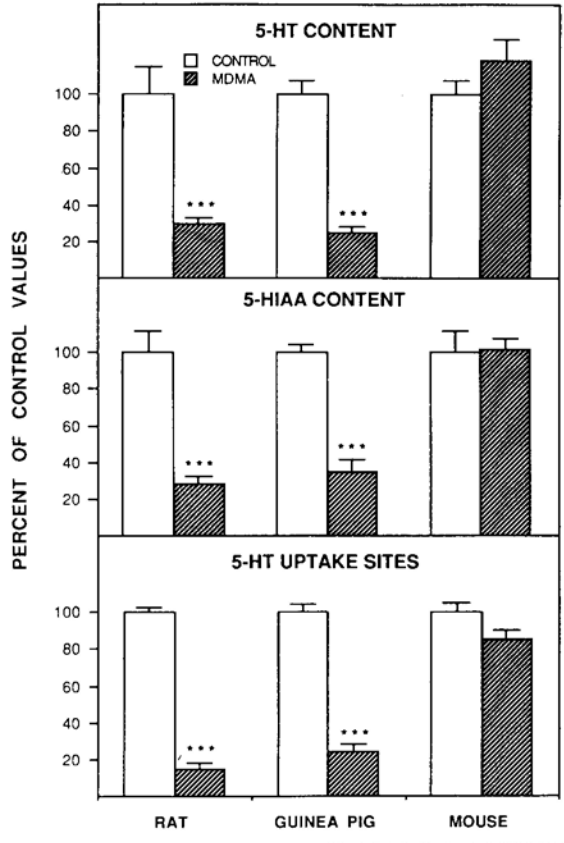


Figure 5. The effects of repeated systemic administration of MDMA on (A) the content of serotonin (5-HT), (B) the content of 5-hydroxyindoleacetic acid (5-HIAA), and (C) the density of 5-HT uptake sites in rat, guinea pig, and mouse frontal cerebral cortex. Animals were treated with saline or 20 mg/kg MDMA twice a day for 4 consecutive days and sacrificed 7 days after the last injection. Data represent mean ± SEM of five animals per group and are expressed as a percent of saline-injected control values in the 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 sites were 397 ± 10, 210 ± 6, and 233 ± 12 fmol/mg protein, respectively. Data were analyzed by Students t-test. *** indicates a significant difference at $p < 0.001$ from respective control values. (From Battaglia et al., 1988.)

neurotoxic effects of MDMA observed in all species included reductions in the content of 5-HT and 5-HIAA and decreases in the density of 5-HT uptake sites. In primates, marked reductions in CSF 5-HIAA levels were also observed after drug administration [50]. These findings and other reports of neurotoxic effects of MDMA in primates [51, 52] raise serious concern for its hazards in humans.

4. NEUROTOXIC EFFECTS OF MDMA IN VARIOUS BRAIN REGIONS

The results of the studies described previously demonstrate the specific and marked neurodegenerative effects of MDMA on 5-HT axons and terminals in cerebral cortex. The following studies will address the neuroanatomical and morphological specificity of the neurotoxic actions of MDMA. Specifically we will describe (1) the neurotoxic and neurodegenerative effects of MDMA and MDA in homogenates of gross brain regions and (2) the neuroanatomic and morphological specificity of MDMA-induced degeneration of serotonergic pathways in rat brain. We have used radioligand binding studies and in vitro autoradiography of [^{3}H]-paroxetine-labeled 5-HT uptake sites to address the latter point.

4.1. Effects of MDMA on monoamine content

The effects of repeated systemic administration of MDMA and MDA on brain monoamine and monoamine metabolite levels were investigated at two weeks following the last injection. As shown in Figure 6, MDMA and MDA produced marked decreases in the content of 5-HT 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, 5-HIAA content was observed to decrease in all the brain regions examined, but the reductions in cerebral cortex and hippocampus (40–60%) were greater than those observed in striatum and hypothalamus (30–40%). With respect to 5-HT levels, marked decreases were observed in cerebral cortex and hypothalamus in both MDMA-treated and MDA-treated rats (Figure 6A). While small decreases were observed in hippocampal and striatal 5-HT 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. When calculated as a percent of control 5-HT levels in the respective brain regions (Figure 6A), the data indicate a more marked reduction in 5-HT in cerebral cortex (40–60%) than in hypothalamus (18–33%).

In contrast with the marked and consistent effects of these compounds on serotonergic systems, neither MDMA nor MDA administration produced any widespread or consistent changes in levels of norepinephrine, dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), or homovanillic acid (HVA) in the various brain regions examined; however, small changes 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 3). Since the changes in catecholamine levels were not accompanied by corresponding changes in the density of dopamine or norepinephrine uptake

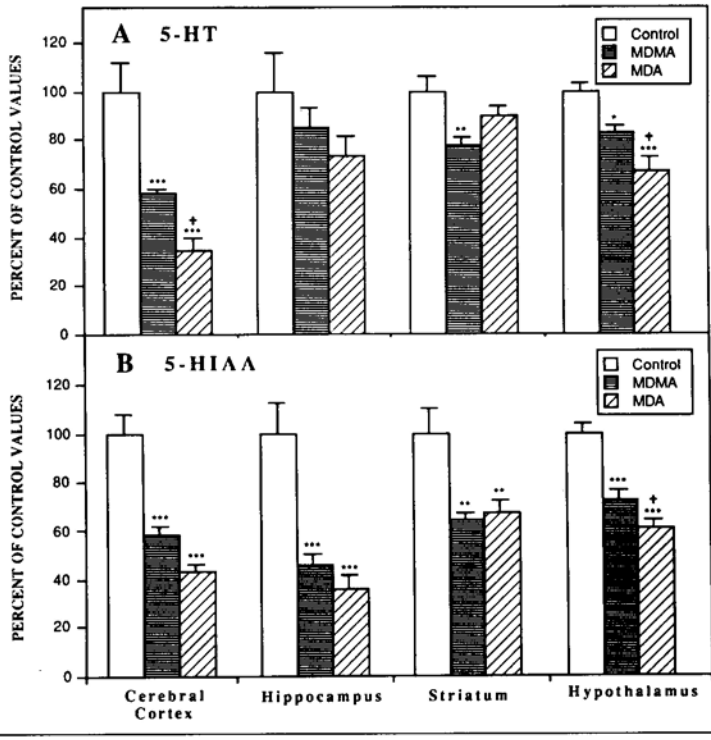


Figure 6. Effect of repeated systemic admininstration of MDMA and MDA on the concentration of (A) serotonin (5-HT) and (B) its metabolite 5-hydroxyindoleacetic acid (5-HIAA) in various brain regions. Rats were injected subcutaneously twice daily for 4 days with drug (20 mg/kg) or saline vehicle (1 ml/kg) and sacrificed at 2 weeks after the last injection. 5-HT and 5-HIAA levels were measured using reversed phase HPLC. Data are plotted as a percent of control values in each brain region and represent the mean and SEM from four to six control and drug-treated rats. Control values for 5-HT and 5-HIAA in each of the regions were as follows: cerebral cortex, 504 ± 58 and 422 ± 32; hippocampus, 410 ± 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. *, **, and *** indicate significant differences at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, from control saline-treated rats. † indicates a significant difference at $p < 0.05$ from MDMA-treated rats. (From Battaglia et al., 1987.)

sites, these effects may be mediated indirectly via MDMA- or MDA-induced alterations in serotonergic pathways.

4.2. Effects of MDMA on monoamine uptake sites

To determine whether changes in 5-HT and/or 5-HIAA were the consequence of long-term suppression of serotonergic function in structurally intact neurons or whether MDMA or MDA may be affecting a neurodegenerative process in each of the brain regions, we measured the density of monoamine uptake sites in these brain regions. Densities of uptake sites for 5-HT [12],

Table 3. Effect of repeated systemic administration of MDMA and MDA on norepinephrine, dopamine, and dopamine metabolite level 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	572 ± 34	13 ± 5	65 ± 12*	15 ± 5
MDA	608 ± 46	16 ± 4	32 ± 13	8 ± 5
Striatum				
Control	N.D.	6091 ± 596	3212 ± 159	788 ± 58
MDMA	N.D.	6974 ± 228	3954 ± 320*	767 ± 46
MDA	N.D.	6168 ± 569	3669 ± 189*	890 ± 48
Hypothalamus	3320 ± 209	569 ± 40	228 ± 43	54 ± 4
Control	3052 ± 159	475 ± 38	200 ± 30	54 ± 5
MDMA	3577 ± 148	585 ± 67	229 ± 26	58 ± 3
MDA				

Regional brain levels of norepinephrine (NE), dopamine (DA), 3,4-dihydroxy phenylacetic acid (DOPAC), and homovanillic acid (HVA) in rats 2 weeks after administration of 20 mg/kg 3,4-methylenedioxymethamphetamine (MDMA) or 3,4-methylenedioxyamphetamine (MDA). Drugs were administered subcutaneously every 12 hours for 4 consecutive days. Values (in picograms per milligram of tissue) represent the mean and standard error of the mean (SEM.) of determinations in four to six individual rats. N.D. indicates that levels were below the sensitivity of the assay. Data were analyzed by one-way ANOVA and Duncan's multiple range test. * indicates a significant difference from saline-treated control rats at $p < 0.05$.

dopamine and norepinephrine [53] were carried out as previously described. Both MDMA and MDA caused substantial reductions in the densities of [^{3}H]-paroxetine-labeled 5-HT uptake sites in all brain regions examined. As shown in Figure 7, the densities of 5-HT uptake sites are represented as a percent of the respective control values in cerebral cortex, hippocampus, striatum, hypothalamus, and midbrain. Significant reductions (all $p < 0.001$) were observed in cerebral cortex (60–70%). hippocampus (70–75%), striatum (50%), hypothalamus (40–50%), and midbrain (50–60%). Interestingly, MDA produced a significantly greater reduction in the density of 5-HT uptake sites in cerebral cortex than that observed with MDMA. We have also observed that MDE, the N-ethyl derivative of MDA, with a comparable treatment regimen, causes a 40% reduction in 5-HT uptake sites in cerebral cortex, suggesting that this compound may be less toxic than MDA or MDMA. Scatchard analyses of [^{3}H]-paroxetine saturation data in control and drug-treated rats indicated that, in cerebral cortex, [^{3}H]-paroxetine binding was to a single population of sites (Hill coefficient values = 1.02, 1.01, and 1.03 in control, MDMA-treated and MDA-treated animals, respectively) and that there were no significant differences in the K_D values between control and drug-treated rats (18.8, 20.8, and 17.9 pM in control, MDMA-treated, and MDA-treated rats, respectively).

In contrast with the effects of these drugs on the density of 5-HT uptake

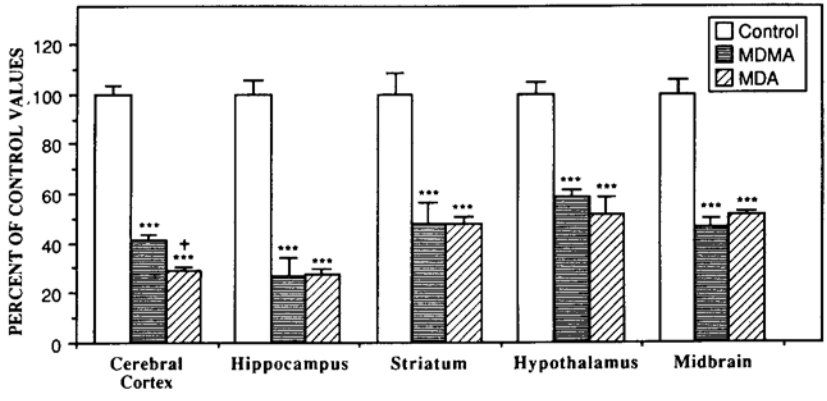


Figure 7. Effect of repeated systemic administration of MDMA and MDA on the density of serotonin (5-HT) uptake sites in various brain regions. Rats were injected subcutaneously twice daily for 4 days with MDMA and MDA (20 mg/kg) or saline vehicle (1 ml/kg) and sacrificed at 2 weeks after the last injection. Values were determined from saturation studies in each of the regions 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 differences from control K_D values (10–20 pM) were observed in either MDMA- or MDA-treated rats. Data are plotted as a percent of the 5-HT uptake site density observed in controls in each brain region and represent the mean and SEM from 3–6 rats per group. Control values were as follows: 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. Significant differences at $p < 0.001$ from control values are denoted by ***, while differences at $p < 0.001$ between MDA and MDMA treatments are denoted by †. (From Battaglia et al., 1987.)

sites, neither MDMA nor MDA treatment caused any significant reduction in the levels of [^{3}H]-mazindol-labeled norepinephrine 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 norepinephrine uptake sites in hippocampus, this change was not statistically significant. Similarly, no significant decreases were observed in the density of [^{3}H]-mazindol-labeled dopamine uptake sites in cerebral cortex, hippocampus, striatum, and midbrain, following treatment with MDA. MDMA caused a statistically significant reduction (37%) in the density of dopamine uptake sites only in midbrain. These findings are consistent with the results from our measurements of the content of catecholamines and catecholamine metabolites in various brain regions and support the contention that neither MDMA nor MDA cause any marked widespread alterations in the integrity of catecholaminergic neurons.

4.3. Autoradiographic studies of 5-HT uptake sites

In vitro autoradiographic studies of [^{3}H]-paroxetine-labeled 5-HT uptake sites in brains of saline-treated (control) and MDMA-treated rats were carried out as previously described [54] in order to assess the neuroanatomic localization

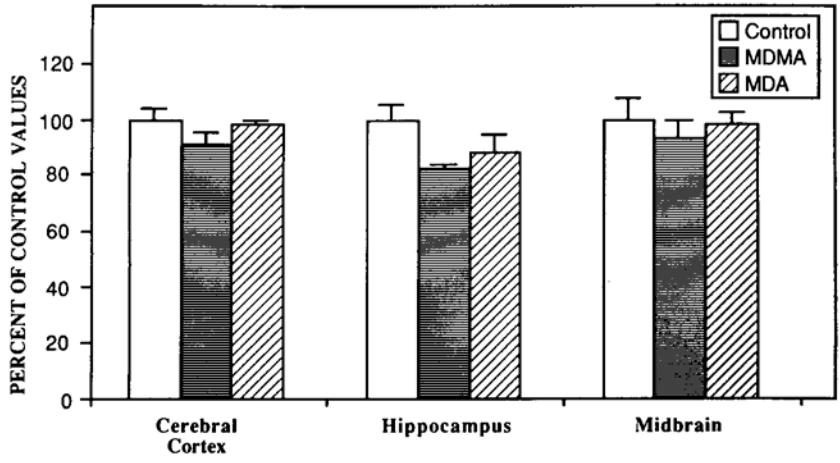


Figure 8. Effect of repeated systemic administration of MDMA and MDA on the density of norepinephrine (NE) uptake sites in various brain regions. Rats were injected subcutaneously twice daily for 4 days with MDMA or MDA (20 mg/kg) or saline vehicle (1 ml/kg) and sacrificed at 2 weeks after the last injection. NE uptake sites were measured using 6 nM [^{3}H]-mazindol in the presence of selective blockers as previously described [53]. Data are plotted as a percent of control values in each brain region and represent the mean and SEM from six control, MDMA-treated, and MDA-treated animals. Control values of NE uptake sites were as follows: cerebral cortex, 164 ± 6; hippocampus, 176 ± 9; midbrain, 157 ± 13 fmol/mg protein. (From Battaglia et al., 1987.)

of lesions induced by MDMA. In a detailed quantitative study, substantial reductions in 5-HT uptake sites (50–100% decreases) were observed in all areas of cerebral cortex as early as 18 hours after a four-day treatment regimen (20 mg/kg MDMA twice daily) and lasted for at least two weeks. As shown in Table 4, cerebral cortical regions that showed the most extensive destruction of 5-HT uptake sites (i.e., $>90\%$) were the prefrontal (area 32), anterior cingulate (area 24), entorhinal, and parietal cortex. Comparable decreases in 5-HT 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 area 8 and 10, entorhinal, and primary auditory regions. In other areas of cerebral cortex, such as the sensory motor regions, significant reductions in 5-HT uptake sites were observed only two weeks after the treatment.

As shown in Table 4 and Figure 9, marked decreases in 5-HT uptake sites were observed in all regions of caudate-putamen, olfactory tubercle, endopiriform nucleus, islands of calleja, and nucleus accumbens, following MDMA administration. Within the caudate-putamen, some time-dependent reductions were observed. For example, equivalent decreases in 5-HT uptake sites were observed between 18 hours and two weeks in the ventrolateral region, while in dorsolateral and dorsomedial areas, a significantly greater reduction in 5-HT

Table 4. 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
Pyriform	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
Island 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−
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+

Table 4. Cont.

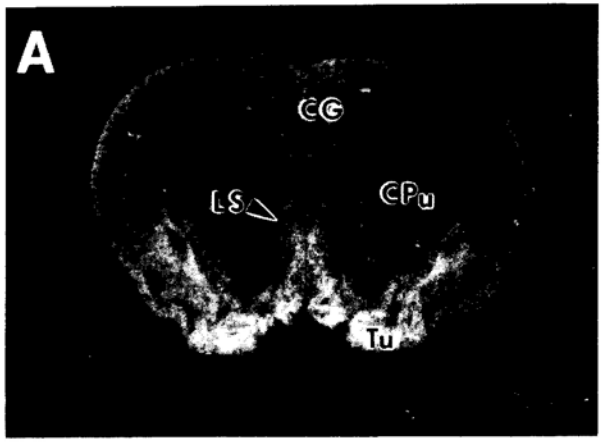
Brain region	Control	MDMA
Superior colliculus:		
Superficial layers	3	1
Profundum	2+	1
Substantia nigra:		
Pars compacta	3+	2
Pars reticulata	3	2+
Paranigral nucleus	2	3
Ventral tegmental area	2+	2
Dorsal raphe nuclei	5+	5+
Median raphe nuclei	5+	5+
Pons-medulla		
Locus coeruleus	5+	5
Pontine reticular formation	2	2
Cerebellum (all lobules)	2	2

The data are based on observations from three animals per group. Rats were injected twice daily subcutaneously for four days with MDMA (20 mg/kg) or saline (1 ml/kg) (control) and sacrificed 14 days after the last injection. The anatomical terminology is derived from Paxinos and Watson [56]. [^{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. The relative density of [^{3}H]-paroxetine binding sites corresponds to the following range: 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. + amd − values indicate the upper and lower limits, respectively, of each range. No correction for "grey-white" quenching of tritium was used.

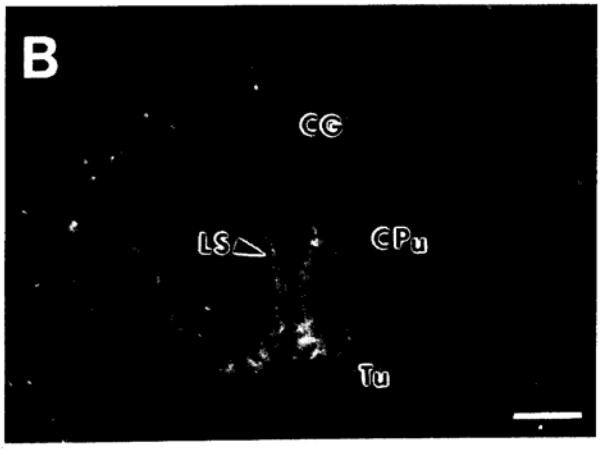
uptake sites was observed only at the later time point. Other brain regions that were sensitive to the neurodegenerative effects of MDMA included various thalamic nuclei and regions of hippocampus. In contrast, the dorsal and medial septal nuclei appeared to be less sensitive to the neurotoxic effects of MDMA, as the reductions (~25%) in 5-HT uptake sites in these regions were not statistically significant. Likewise, no significant reductions were observed in the indusium griseum, which contains primarily 5-HT axons of passage.

Within midbrain structures, regions containing 5-HT projections appeared to be more dramatically affected by MDMA than those containing 5-HT cell bodies (see Figure 10). For example, in both the superficial layers of superior colliculus and profundum, 5-HT uptake sites were reduced 85–90%, while in dorsal and median raphe, central grey, and the ventral tegmental region, there was little or no change after MDMA. Likewise, 5-HT projections to substantia nigra pars compacta and reticulata were markedly affected, whereas no changes in 5-HT uptake sites were observed in the interpreduncular nucleus and pontine reticular formation up to 14 days following MDMA administration.

In order to assess the serotonergic selectivity of the neurodegenerative effects of MDMA in brain, we have carried out additional autoradiographic studies of norepinephrine and dopamine uptake sites in brain regions containing catecholamine terminals and cell bodies. Norepinephrine and dopamine uptake sites were labeled using [^{3}H]-mazindol in the presence of specific



SALINE



MDMA

Figure 9. Autoradiographic distribution of [^{3}H]-paroxetine-labeled serotonin uptake sites in coronal sections at the level of the caudateputamen from (A) saline-treated and (B) MDMA-treated rats. These are darkfield photomicrographs (Tritium-sensitive Ultrofilm) in which the autoradiographic grains (i.e., binding sites) appear as white spots and 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.

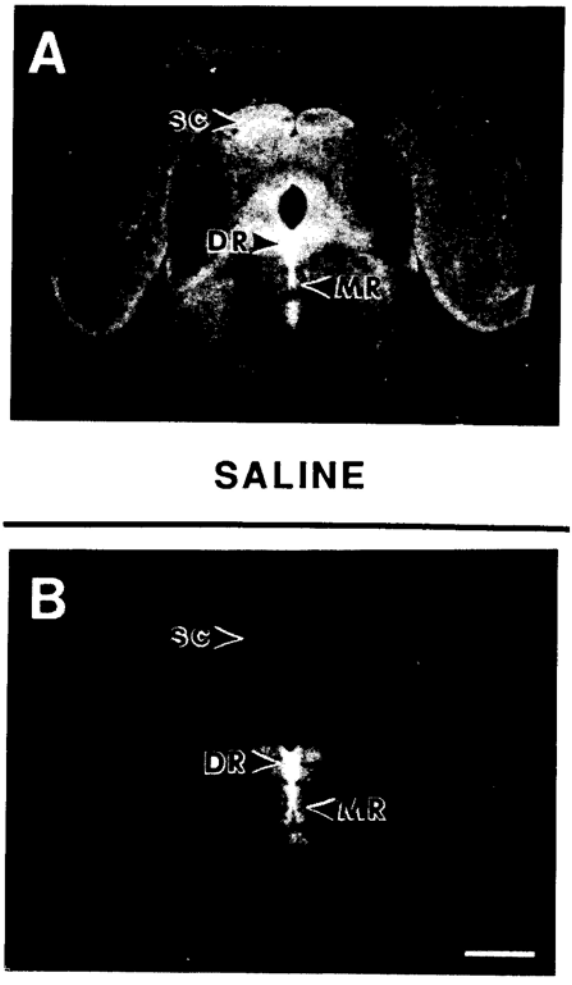


Figure 10. Autoradiographic distribution of [^{3}H]-paroxetine-labeled serotonin uptake sites in coronal sections at the level of midbrain in (A) saline-treated and (B) MDMA-treated rats. In A, note the high density of serotonin uptake sites in control brain in regions containing serotonin projections, 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 sites in pre- and para-subiculum, entorhinal cortex, and superior colliculus (SC), while no change in densities were observed in areas containing primarily serotonin perikarya, such as the raphe nuclei (DR and MR).

blockers, as previously published [55]. With respect to norepinephrine uptake sites, we observed no change from control levels of binding sites in midbrain regions, such as locus coeruleus, interpeduncular nucleus, or substantia nigra pars compacta or reticulata, up to 14 days following MDMA treatement. In a number of cerebral cortical regions that receive norepinephrine projections, norepinephrine uptake sites were not decreased at 18 hours after treatment but appeared to be slightly increased at Day 14. Consistent with the minimal effect of MDMA on parameters associated with catecholamine neurons, there were no changes in the density of dopamine 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 homogenate studies and indicate that the neurodegenerative effects of MDMA appear to be confined primarily to serotonergic neurons since this treatment regimen did not reduce the density of uptake sites associated with catecholamine-containing neurons. Additional studies are necessary to further access the neuroanatomic localization of any long-term compensatory changes in norepinephine projections or other neurotransmitter recognition sites that may occur as a consequence of MDMA lesion of 5-HT pathways.

5. SUMMARY AND CONCLUSIONS

The present chapter attempts to elucidate some of the in vitro and in vivo properties of the drug 3,4-methylenedioxymethamphetamine (MDMA). We have utilized in vitro radioligand binding studies to obtain a detailed pharmacologic profile of MDMA at various established brain receptors and recognition sites, as well as to assess the putative role of novel [^{3}H]-MDMA binding sites in brain. With respect to established neurotransmitter receptors and recognition sites, the relatively high affinities of MDMA at pre-synaptic and post-synaptic serotonin recognition sites (i.e., 5-HT uptake sites, 5-HT_2 and 5-HT_{1A} serotonin receptors) and α_2-adrenergic receptors suggest that these sites may play a significant role in both the psychotomimetic and neurodegenerative properties of this drug. In addition, some preliminary data from [^{3}H]-MDMA binding studies suggest that MDMA may interact with a novel recognition site in brain, which may play some role in the actions of this compound. It is unlikely that the anxiolytic-like or reinforcing properties of MDMA are mediated via benzodiazepine or opioid receptors since the drug exhibits much lower affinities for these binding sites.

We have also addressed the neurochemical effects of MDMA following in vivo administration of the drug and have provided substantial evidence that this drug causes widespread and long-lasting degeneration of serotonin neurons in brain. With respect to the parameters involved in the neurotoxic and neurodegenerative actions of MDMA, the data indicate that: (1) the severity of lesion by MDMA is dependent on both the dose and the frequency of drug

administration; (2) MDMA-induced neurodegenerative effects are preferentially localized to serotonergic neurons while sparing catecholamine neurons; (3) the neurodegenerative effects of MDMA can be elicited in a number of animal species, including primates; (4) the neurodegenerative effects of MDMA on 5-HT neurons can be prevented by 5-HT uptake blockers, suggesting a role for the active uptake of the drug or a neurotoxic metabolite of MDMA; and (5) the neurodegenerative effects are long-lasting (up to one year) with respect to structural recovery, while functional recovery may be permanently impaired.

In addition, we have reported that the neurotoxic and neurodegenerative effects of MDMA are widespread, with marked reductions in all 5-HT parameters observed in a number of brain regions. In vitro autoradiographic studies have revealed that there is neuroanatomic and morphologic specificity to the neurodegenerative effects of MDMA, as evidenced from the predominant reductions in 5-HT uptake sites in brain regions containing primarily 5-HT terminals whereas regions containing 5-HT axons of passage and perikarya are relatively unaffected. These findings have been confirmed by additional data from 5-HT immunocytochemical studies [16] that demonstrate the specificity of MDMA for neurodegeneration of 5-HT axons and terminals, while 5-HT perikaya are spared.

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