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Neuroanatomic Specificity and Time Course of Alterations in Rat Brain Serotonergic Pathways Induced by MDMA (3,4-Methylenedioxymethamphetamine): Assessment Using Quantitative Autoradiography

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ABSTRACT The widely abused "designer" drug MDMA (3,4-methylenedioxymethamphetamine) has been shown to cause marked and long-lasting changes in brain serotonergic systems. The present study uses quantitative in vitro autoradiography of ^{3}H-paroxetine labeled 5-HT uptake sites to assess the time-dependent effects of MDMA on 5-HT neurons in specific neuroanatomic loci. Following treatment with MDMA (20 mg/kg, b.i.d. for 4 days), marked decreases in 5-HT uptake sites were observed in a number of brain regions known to receive projections of 5-HT neurons. These regions included cerebral cortex, caudate nucleus, hippocampus, nucleus accumbens, olfactory tubercle, superior and inferior colliculi, geniculate nuclei, and most thalamic nuclei. In contrast, other areas such as the septal nuclei and some thalamic nuclei which also receive 5-HT projections were not substantially affected by this drug. In most regions, decreases in 5-HT uptake sites occurred within 24 hours of the last dose of MDMA and persisted at the 2 week time point. Some regions such as dorsal striatum exhibited a time-dependent reduction with greater reductions occurring at 2 weeks rather than immediately following the MDMA treatment regimen. The density of 5-HT uptake sites in other regions such as endopiriform nucleus and substantia nigra at the 2 week versus 18 hour time point indicated some degree of region-specific recovery. Regions which demonstrated no significant reduction in 5-HT uptake sites included the dorsal and median raphe nuclei, ventral tegmental area, central grey, interpeduncular nucleus, locus coerulus, pontine reticular formation and cerebellum. Likewise, regions containing 5-HT axons of passage (e.g., indusium griseum and lateral hypothalamus) appeared to be insensitive to the neurotoxic effects of MDMA on 5-HT neurons. Furthermore, the neurotoxic effects of MDMA showed specificity in that the catecholamine neurons labeled by ^{3}H-mazindol were unaffected by the treatment regimen. These data indicate that the preferential degeneration of serotonergic neurons by MDMA is mediated primarily at 5-HT terminal regions, whereas regions containg 5-HT perikarya and axons of passage remain relatively unaffected. In addition, the observed time-dependent reductions and recovery of 5-HT uptake sites which were detected within 2 weeks of the treatment regimen in certain brain regions suggest region-specific differences in recovery of 5-HT systems from MDMA-induced lesion.

INTRODUCTION

A great deal of concern has been raised regarding the potential hazards associated with the increasing abuse of designer drugs such as 3,4-methylenedioxymethamphetamine (MDMA) among certain segments of the population (Adler et al., 1985; Barnes, 1988; Gertz,

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1986). Drugs such as MDMA (3,4-methylenedioxymethamphetamine) and MDA (3,4-methylenedioxyamphetamine) have been found to be toxic to brain neurons (Commins et al., 1987) and cause widespread and long-lasting suppression of brain serotonin (5-HT) neurotransmission in animals (Battaglia et al., 1987; Insel et al., 1989; Ricaurte et al., 1985). Short-term administration of MDMA results in decreased levels of serotonin (5-HT), its major metabolite 5-hydroxyindoleacetic acid (5-HIAA), and marked reductions in the density of 5-HT uptake sites (Battaglia et al., 1987) and in the density of 5-HT immunoreactive neuronal fibers (O'Hearn et al., 1988). These neurotoxic effects of MDMA on serotonin neurons appear to be dependent on both dose and frequency of administration of the drug (Battaglia et al., 1988). While the acute decreases in 5-HT and 5-HIAA content may be attributed to the effects of MDMA on suppression of tryptophan hydroxylase activity (Schmidt and Taylor, 1987; Stone et al., 1987), the long-term decreases in serotonergic markers, particularly the reductions in 5-HT uptake sites, are indicative of the destruction of serotonin axons and terminals (Battaglia, 1990; Battaglia et al., 1987, 1988). Reductions in both 5-HT immunoreactivity and 5-HT uptake sites have also been observed following administration of methamphetamine (Fukui et al., 1989; Kovachich et al., 1989) and fenfluramine (Appel et al., 1989, 1990; Zaczek et al., 1989), two additional amphetamine derivatives which also markedly affect brain serotonergic systems. The neurotoxic effects of MDMA on serotonergic systems have been observed in a number of animal species (Battaglia et al., 1988) including nonhuman primates (Insel et al., 1989; Ricaurte et al., 1988). The marked neurochemical deficits and consequent behavioral changes observed in primates following MDMA administration may be of particular relevance to the postulated effects in humans following chronic abuse of this drug.

We have previously demonstrated that following an initial 90% reduction in rat cerebral cortical 5-HT uptake sites in rat brain, a recovery of these sites occurs over a very protracted period of time with control levels not being attained for at least 6 months (Battaglia et al., 1988). Since the recovery of 5-HT uptake sites was monitored in only one brain region, the cerebral cortex, it is unclear if recovery of 5-HT uptake sites may occur more rapidly in other more discrete brain regions which are affected by MDMA. A recent report indicates region-specific differences in the recovery of 5-HT uptake sites following lesion of 5-HT neurons by high-dose methamphetamine administration (Kovachich et al., 1989). Furthermore, while comparable reductions in serotonergic parameters have been observed in a number of gross brain regions following MDMA administration, it is unclear if all 5-HT pathways and/or areas receiving 5-HT projections are equally affected or if there is some neuroanatomic specificity to the effects of the drug. There is accumulating evidence which suggests that fine versus coarse serotonergic fibers, originating from dorsal versus median raphe, respectively, may exhibit differential sensitivity to the neurotoxic effects of substituted amphetamine derivatives (Mamounas and Molliver, 1988; Molliver, 1987; O'Hearn et al., 1988). In order to address these questions, we have carried out detailed quantitative in vitro autoradiographic studies of ^{3}H-paroxetine labeled 5-HT uptake sites in control and MDMA-treated rats. The feasibility of using ^{3}H-paroxetine for the in vitro autoradiographic visualization of 5-HT uptake sites (Appel et al., 1990; De Souza and Kuyatt, 1987) and the utility of measuring reductions in 5-HT uptake sites as a valid index of the absence of 5-HT neurons has been previously substantiated (Battaglia, 1990; D'Amato et al., 1987). In addition, we have measured changes in 5-HT uptake sites at two time points following MDMA administration in order to assess region-specific differences in the time course of 5-HT lesion and recovery. For comparative purposes, the integrity of catecholamine neurons following MDMA and MDA (3,4-methylenedioxyamphetamine) administration was assessed by measuring the regional densities of ^{3}H-mazindol-labeled catecholamine (dopamine and norepinephrine) uptake sites.

MATERIALS AND METHODS

All studies were performed in accordance with the National Institutes of Health animal care guidelines.

Male Sprague-Dawley rats weighing 175–200 g were housed under standard laboratory conditions (22°C, 12 hour light/dark cycle) and had access to food and water ad libitum. Rats were injected subcutaneously with either saline (1 ml/kg) or MDMA (20 mg/kg as the free base) twice a day (approximately 12 hours apart) for 4 consecutive days and then sacrificed at 18 hours and 2 weeks following the final injection. The brains were rapidly removed and frozen in powdered dry ice. Coronal sections of brains were obtained at −16°C using a cryostat (Hacker Instruments, Inc.) set to a thickness of 10 μm. Sections were thaw-mounted onto chrome alum/gelatin-coated microscope slides, and stored at −20°C until used.

Prior to all autoradiographic studies, slide-mounted brain sections were brought to room temperature. Autoradiographic localization of 5-HT uptake sites was accomplished using the protocol of De Souza and Kuyatt (1987) which utilized a saturating concentration of ^{3}H-paroxetine. Slide-mounted brain sections were incubated for 2 hours at room temperature with 0.25 nM ^{3}H-paroxetine (Du Pont NEN, Boston, MA; specific activity 19 Ci/mmol) in 50 mM Tris HCl containing 120 mM NaCl and 5 mM KCl (pH 7.7 at 23°C). Nonspecific binding was defined as that in the presence of 4 μM citalopram (Pfizer; Groton, CT). Following incubation, tissue sections were washed in the same buffer at room temperature, dipped in deionized water, and dried rap-

idly under a stream of cold, dry air. The dry, labeled slide-mounted sections and tritium autoradiographic standards (Amersham; Arlington Heights, IL) were apposed to ^{3}H-Ultrofilm (LKB, Gaithersburg, MD) for 8 weeks. Following exposure, autoradiograms were developed for 5 minutes in D-19 developer (Kodak, Rochester, NY), washed for 1 minute in water, fixed for 5 minutes in GBX fixer (Kodak, Rochester, NY), washed in circulating water for 25 minutes and then air dried.

Autoradiographic localization of ^{3}H-mazindol-labeled catecholamine uptake sites was accomplished using the protocol of Javitch et al. (1985). Slide-mounted brain sections were thawed and pre-incubated for 5 min in ice-cold 50 mM Tris buffer (pH 7.9) containing 120 mM NaCl and 5 mM KCl. Sections were then incubated for 40 minutes with 4 nM ^{3}H-mazindol (Du Pont NEN; Boston, MA; specific activity 15.8 Ci/mmol) in the absence and presence of selective uptake inhibitors in ice-cold buffer. Specific binding to norepinephrine(NE) uptake sites was defined as the ^{3}H-mazindol binding inhibited by 0.3 μM desipramine (DMI). Specific binding to dopamine (DA) uptake sites was defined as the difference between ^{3}H-mazindol in the presence of 0.3μM DMI and that in the presence of 1 μM unlabeled mazindol (RBI, Natick, MA). Subsequently, slides were washed for two consecutive 1-minute periods in ice-cold deionized water and dried rapidly under a stream of cold, dry air. Autoradiograms were generated as described above.

Quantification of autoradiograms was accomplished using a personal computer based digital image analysis system (Loats; Westminster, MD). Best-fit standard curves of film optical density generated by tritium standards co-exposed with labeled slides resulted when a power function was used to describe the relationship between radioactivity and optical density. Results were not corrected for differential quenching of ^{3}H by gray and white matter.

RESULTS

Distribution of 5-HT uptake sites in control brain

The distribution of densities of ^{3}H-paroxetine labeled 5-HT uptake sites at various levels of brain from saline treated rats are shown in Table I and the left panels of Figure 1. All levels of cerebral cortex exhibited a low to moderate density of 5-HT uptake sites (55–167 fmol/mg tissue equivalent) with the exception of entorhinal cortex (Fig. 1M) which exhibited a substantially higher density of sites (413 fmol/mg tissue equivalent) than any other cortical region examined. Olfactory tubercle, endopiriform nucleus and islands of Calleja also contained approximately twice the density of 5-HT uptake sites observed in most cortical regions. In striatum, the greatest density of sites was observed in the ventromedial and ventrolateral regions (171–256 fmol/mg tissue equivalent), while lower densities were detected in dorsal regions and in the nucleus accumbens. Septal nuclei contained densities of 5-HT uptake sites comparable to those observed in ventral striatal regions. In hippocampus, the highest density of 5-HT uptake sites was observed in presubiculum and parasubiculum (202 and 255 fmol/mg tissue equivalent, respectively).

While 5-HT uptake sites were also observed in a number of thalamic nuclei, some regions such as posterior and posterior ventromedial nuclei were found to contain only a minimal number of 5-HT uptake sites (20 fmol/mg tissue equivalent). Marked densities of 5-HT uptake sites, however, were localized in reuniens nucleus (392 fmol/mg tissue equivalent) and lateral geniculate nucleus (414 fmol/mg tissue). There was also a substantial number of 5-HT uptake sites (360 fmol/mg tissue equivalent) in lateral hypothalamus, an area containing 5-HT axons of passage as part of the medial forebrain bundle. Among the midbrain regions investigated (Fig. 1K), 5-HT uptake sites were most dense in the central gray (687 fmol/mg tissue equivalent) and regions containing serotonin perikarya such as the dorsal (1292 fmol/mg tissue equivalent) and median (836 fmol/mg tissue equivalent) raphe nuclei. The densities of serotonin uptake sites in dopamine cell body regions such as the substantia nigra, pars reticulata, and pars compacta (see Fig. 1I) were comparable (213 and 234 fmol/mg tissue equivalent, respectively) but substantially lower than those in raphe regions. The locus coeruleus (Fig. 1O), a region containing norepinephrine cell bodies and serotonergic projections to these perikarya, also contained a very high density of 5-HT uptake sites (591 fmol/mg tissue equivalent). In contrast, only a minimal number of sites (64 fmol/mg tissue equivalent) were observed in cerebellum.

Effects of MDMA-induced lesion

As mentioned previously, short-term administration of the designer drug MDMA has been shown to cause marked destruction of 5-HT neuronal fibers in various gross brain regions (Battaglia et al., 1987). Consistent with previous results and as shown in Figure 1 and Table I, in vitro autoradiographic studies demonstrate that a 4-day treatment with MDMA causes decreases in the density of 5-HT uptake sites in numerous discrete regions of rat forebrain, midbrain and hindbrain. These changes were observed as early as 18 hours after the last injection and persisted for at least 2 weeks in most regions. Across all areas of cerebral cortex, there were substantial reductions (50–100% decreases). Changes in some areas of cortex such as the sensorimotor region were time-dependent as significant reductions in 5-HT uptake sites were only observed at the 2-week time point. Decreases in 5-HT uptake sites in a number of cerebral cortical areas such as prefrontal, cingulate, piriform, frontal, entorhinal, and primary auditory regions exhibited comparable decreases in 5-HT uptake sites at day 0 (18 hours) and day 14. Cortical regions which showed the most extensive depletion of 5-HT

TABLE I. Effects of repeated systemic administration of MDMA on the time-dependent regional alterations in 5-HT uptake sites as a percent of control values[1,*]

Brain region	Control (fmol/mg tissue equivalent)	Day 0	Day 14	Change in % from day 0
Cerebral cortex				
Prefrontal (Fr)	100 ± 5 (64 ± 3)	3 ± 3	14 ± 5	+11
Cingulate (Cg)	100 ± 16 (143 ± 23)	36 ± 10	9 ± 3	−27
Indusium griseum	100 ± 4 (55 ± 2)	69 ± 33^{NS}	163 ± 33^{NS}	—
Piriform	100 ± 23 (128 ± 30)	12 ± 3	19 ± 4	+7
Frontal (Area 8)	100 ± 3 (106 ± 3)	42 ± 4	47 ± 6	+5
(Area 10)	100 ± 15 (66 ± 10)	15 ± 8	15 ± 9	0
Sensorimotor	100 ± 11 (104 ± 11)	64 ± 24^{NS}	20 ± 10	−44**
Parietal	100 ± 20 (61 ± 12)	N.D.	N.D.	0
Entorhinal (Ent)	100 ± 4 (413 ± 16)	8 ± 1	13 ± 3	+5
Primary auditory	100 ± 10 (57 ± 10)	54 ± 7	35 ± 2	−19
Primary visual	100 ± 19 (167 ± 31)	53 ± 2	50 ± 8	−3
Olfactory tubercle (Tu)	100 ± 6 (368 ± 24)	47 ± 6*	22 ± 4*	−25**
Endopiriform nucleus	100 ± 3 (234 ± 7)	25 ± 5*	47 ± 7*	+22**
Islands of Calleja	100 ± 9 (278 ± 26)	32 ± 4	21 ± 7	−11
Caudate putamen (CPu)				
Dorsolateral	100 ± 5 (80 ± 4)	45 ± 1*	15 ± 10*	−30**
Dorsomedial	100 ± 5 (62 ± 3)	47 ± 2*	13 ± 8*	−34**
Ventrolateral	100 ± 7 (256 ± 18)	44 ± 14	29 ± 7	−15
Ventromedial	100 ± 11 (171 ± 19)	69 ± 19^{NS}	31 ± 2	−38**
Nucleus accumbens	100 ± 17 (92 ± 16)	10 ± 3	22 ± 4	+12
Septal area				
Medial septal nucleus	100 ± 6 (280 ± 16)	60 ± 10	75 ± 4^{NS}	+15
Lateral septal nucleus	100 ± 8 (223 ± 18)	71 ± 4*	52 ± 2*	−19**
Amygdala				
Basolateral nucleus (BL)	100 ± 13 (268 ± 36)	108 ± 20^{NS}	99 ± 7^{NS}	—
Thalamus and epithalamus				
Anterioventral nucleus	100 ± 2 (175 ± 3)	N.D.	N.D.	0
Anteriomedial nucleus	100 ± 2 (255 ± 5)	N.D.	N.D.	0
Anterioventral dorsomedial nucleus	100 ± 3 (252 ± 8)	N.D.	N.D.	0
Reuniens (Re)	100 ± 2 (392 ± 7)	N.D.	4 ± 1	+4
Lateroposterior nucleus	100 ± 7 (189 ± 14)	13 ± 1	18 ± 2	+5
Posterior nucleus	100 ± 45 (20 ± 9)	100 ± 20^{NS}	120 ± 20^{NS}	—
Posterioventromedial nucleus	100 ± 14 (22 ± 3)	91 ± 9^{NS}	82 ± 9^{NS}	—
Parafascicular nucleus	100 ± 6 (106 ± 6)	62 ± 8	46 ± 2	−16
Lateral geniculate body (LG)	100 ± 8 (414 ± 34)	19 ± 2	12 ± 38	−7
Medial geniculate body	100 ± 5 (88 ± 4)	33 ± 11	19 ± 1	−14
Habenula	100 ± 3 (180 ± 5)	14 ± 7	5 ± 3	−9
Hypothalamus				
Lateral nucleus (LH)	100 ± 1 (360 ± 4)	82 ± 6	86 ± 4	+4

(*continued*)

TABLE I. Effects of repeated systemic administration of MDMA on the time-dependent regional alterations in 5-HT uptake sites as a percent of control values[1,] (continued)*

Brain region	Control (fmol/mg tissue equivalent)	Day 0	Day 14	Change in % from day 0
Hippocampus				
CA3	100 ± 7 (138 ± 10)	34 ± 5	24 ± 4	−10
Dentate gyrus	100 ± 6 (90 ± 5)	47 ± 9*	20 ± 2*	−27**
Molecular layer	100 ± 7 (134 ± 9)	30 ± 3	20 ± 2	−10
Parasubiculum	100 ± 4 (255 ± 11)	39 ± 4	36 ± 2	−3
Presubiculum	100 ± 12 (202 ± 24)	32 ± 1	40 ± 2	+8
Midbrain				
Inferior colliculus (IC)	100 ± 4 (244 ± 10)	N.D.	N.D.	0
Interpeduncular nucleus	100 ± 17 (275 ± 47)	87 ± 19^{NS}	73 ± 13^{NS}	—
Central gray (CG)	100 ± 1 (687 ± 8)	98 ± 9^{NS}	85 ± 7^{NS}	—
Superior colliculus (SC)				
Superficial layers	100 ± 6 (198 ± 11)	12 ± 3	12 ± 3	0
Profundum	100 ± 5 (175 ± 9)	16 ± 4	15 ± 1	−1
Substantia nigra				
Pars compacta (SNC)	100 ± 7 (234 ± 16)	30 ± 1*	50 ± 1*	+20**
Pars reticulata (SNR)	100 ± 10 (213 ± 21)	27 ± 2*	66 ± 6*	+39**
Paranigral nucleus	100 ± 10 (87 ± 9)	71 ± 10	317 ± 50*	+246**
Ventral tegmental area	100 ± 10 (133 ± 14)	157 ± 31^{NS}	90 ± 2^{NS}	—
Dorsal raphe (DR) nuclei	100 ± 2 (1292 ± 25)	113 ± 5^{NS}	116 ± 5^{NS}	—
Median raphe (MR) nuclei	100 ± 4 (836 ± 35)	88 ± 2^{NS}	86 ± 5^{NS}	—
Pons-medulla				
Locus coeruleus (LC)	100 ± 6 (591 ± 34)	65 ± 8	74 ± 7	+9
Pontine reticular formation	100 ± 2 (83 ± 2)	105 ± 11^{NS}	104 ± 12^{NS}	—
Cerebellum (Ce) (all lobules)	100 ± 29 (64 ± 19)	143 ± 28^{NS}	117 ± 23^{NS}	—

[1]These data represent the means ± S.E.M. of observations in from at two to four adjacent sections in individual rats in three treatment groups (n = 3 rats/group). Data in parentheses represent the number of 5-HT uptake sites expressed as fmol/mg tissue equivilent in saline-treated control animals. Rats were injected subcutaneously with either saline vehicle, 1 ml/kg or 20 mg/kg MDMA (as a free base), twice a day for 4 consecutive days and sacrificed at 12–18 hours and 14 days after the last injection. Quantification of autoradiograms was performed using computerized digital image analysis as described in Materials and Methods. Results are not corrected for differential quenching of ^{3}H by gray and white matter. Anatomical terminology is from Paxinos and Watson (1982). Data were analyzed using a one-way ANOVA and Newman-Keules test. Differences between MDMA-treated and saline-injected control rats were determined to be significant ($P < 0.05$) in all cases except where denoted by NS. N.D. indicates the number of sites was below the level of detection.

*Indicates a significant difference ($P < 0.05$) from both the control and the other treatment group.

**Indicates a significant difference ($P < 0.05$) in the change in percent from Day 0.

uptake sites (i.e., >90%) were prefrontal, cingulate, entorhinal, and parietal cortex. The density of uptake sites in the olfactory tubercle and islands of Calleja were reduced by approximately 80% at 2 weeks after drug treatment. Within the caudate-putamen, there were clear-cut differences in the effects of MDMA on dorsal versus ventral striatal 5-HT uptake sites (see Table I). For example, in dorsolateral and dorsomedial regions of

Fig. 1. *(overleaf)* Effects of MDMA treatment on the densities of ^{3}H-paroxetine-labeled 5-HT uptake sites in rat brain. Animals received either saline vehicle, 1 ml/kg (A,C,E,G,I,K,M,O) or *d,l*-MDMA HCl, 20 mg/kg, as the free base (B,D,F,H,J,L,N,P), subcutaneously twice a day for 4 consecutive days. The sections in this figure are from rats which were sacrificed 14 days after the final injection. The coronal sections depict the rostro-caudal autoradiographic distribution of ^{3}H-paroxetine-labeled serotonin uptake sites in rat brain. These are darkfield photomicrographs in which the autoradiographic grains (i.e., ^{3}H-paroxetine binding sites) appear as lighter areas and the tissue is not visible. The degree of non-specific binding as defined by 4 μM citalopram was comparable in all treatment groups. Note that while MDMA decreases ^{3}H-paroxetine binding in most areas measured, there are some brain regions which are less sensitive or insensitive the effects of MDMA. For example, cerebral cortex (A,B), caudate-putamen (C,D), lateral geniculate nuclei (G,H), and colliculi (K,L and N,M) exhibited greater reductions in 5-HT uptake sites than did the lateral hypothalamus (E,F), basolateral amygdaloid nucleus (G,H), substantia nigra (I,J), dorsal and median raphe nuclei (K,L), or locus coeruleus (O,P). Abbreviations are defined in Table I. Bar = 2 mm.

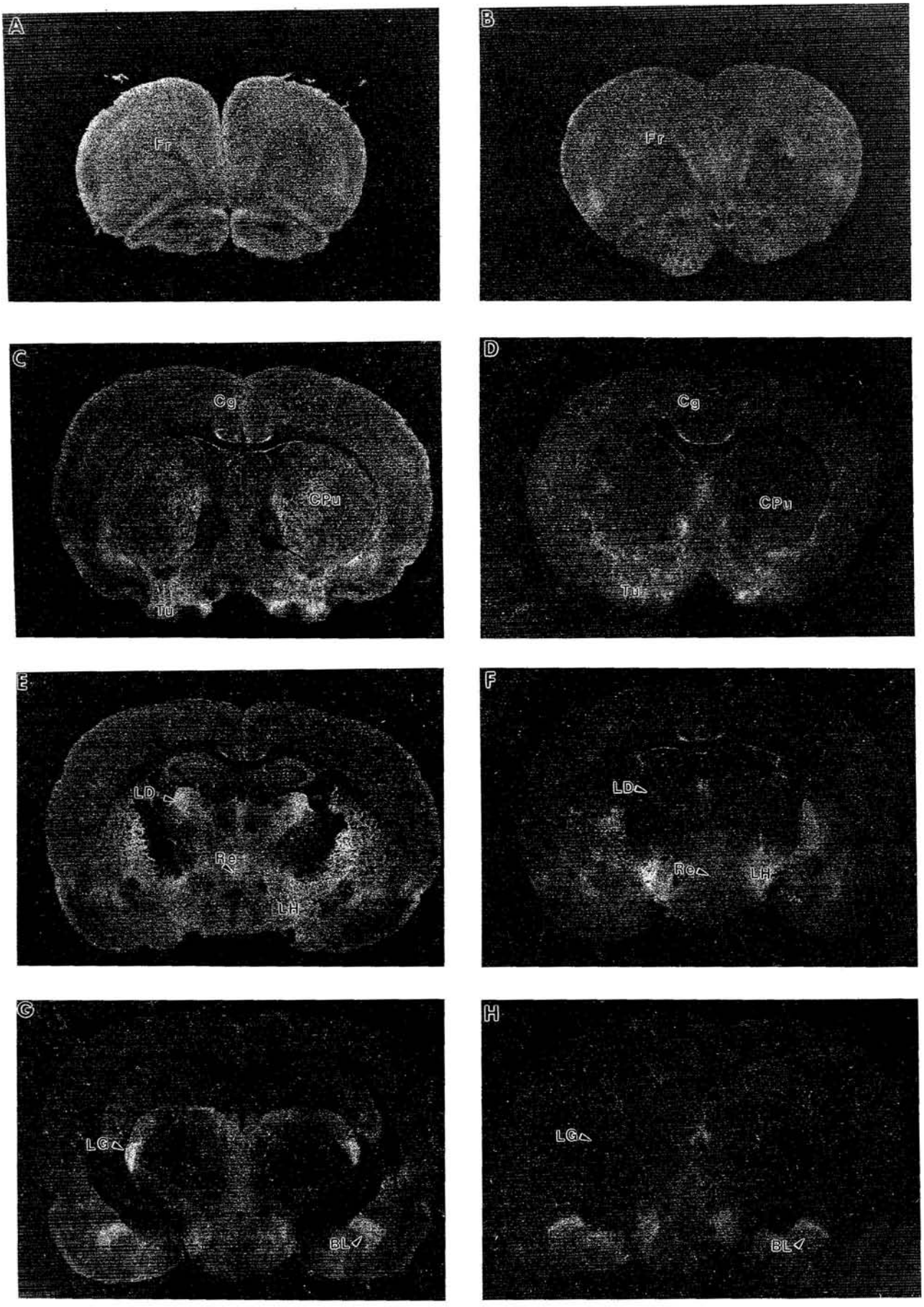

Figure 1A–H.

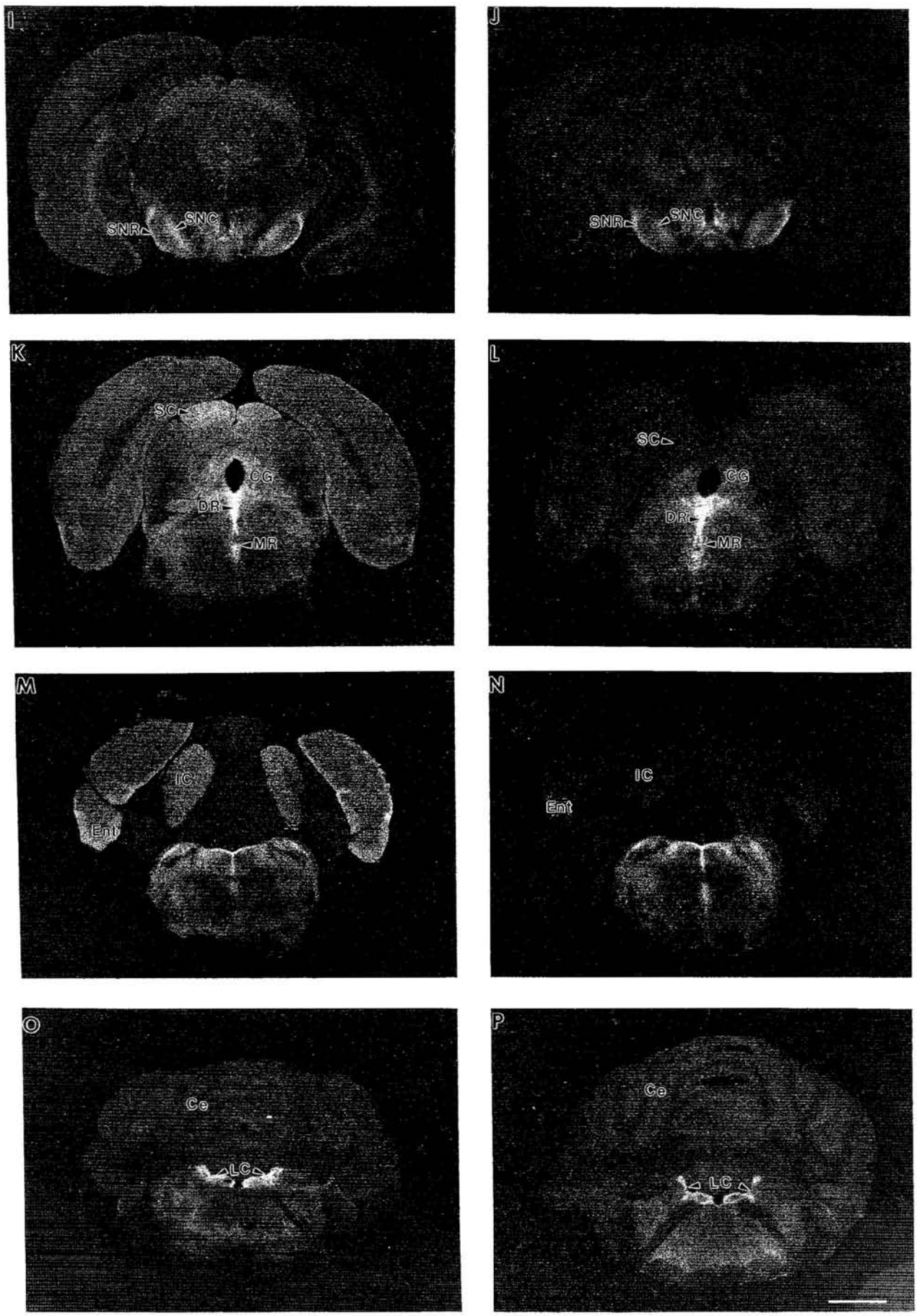
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Figure 11–P.

caudate-putamen, where significant time-dependent reductions in 5-HT uptake sites were noted, only 30–60% decreases occurred at day 0, while by day 14 significantly greater reductions of 70–85% were obtained. In contrast, ventrolateral and ventromedial regions displayed dissimilar patterns of reductions in 5-HT uptake sites. In ventrolateral regions, comparable reductions in 5-HT uptake sites were observed at both time points, while in ventromedial striatum a significant reduction in 5-HT uptake sites was observed only at the 14-day time point following MDMA treatment. In nucleus accumbens, comparable 80–90% decreases in 5-HT uptake sites were observed at both time-points after treatment.

Brain regions which appeared to be less sensitive or insensitive to the neurotoxic effects of MDMA were the dorsal and medial septal nuclei (25–50% decrease) and the basolateral nucleus of amygdala, which exhibited no significant change in 5-HT uptake sites up to 14 days after drug administration (see Table I; Fig. 2). Similarly, the lateral nucleus of the hypothalamus, which contains numerous 5-HT axons of passage, was also relatively unaffected (15% decreases which were not significant) by MDMA up to 14 days of the treatment regimen (Fig. 1F).

As shown in Table I, MDMA caused very marked decreases in the density of 5-HT uptake sites in some thalamic nuclei but was without effect on others. Thalamic nuclei in which the density of 5-HT uptake sites were below the level of detection (N.D.) following administration of MDMA include anterioventral, anteriomedial, anterioventral dorsomedial and reuniens. Marked reductions (95%) following MDMA administration were also observed in 5-HT uptake sites in habenula. In contrast, other thalamic nuclei which were far less sensitive to MDMA were the posterior nucleus and posterioventromedial regions where no significant reductions in 5-HT uptake sites were observed at any of the time points examined. However, lateral and medial geniculate bodies exhibited marked and comparable decreases (≈80%) at both time points following drug treatment (Fig. 1H). Within hippocampal regions, 60–80% reductions were observed in CA3, parasubiculum, presubiculum, and the molecular layer with the dentate gyrus revealing a significantly greater loss of 5-HT uptake sites at 2 weeks compared with 18 hours after MDMA treatment.

Within midbrain structures, regions containing 5-HT projections appeared to be more dramatically affected than those containing 5-HT cell bodies (see Fig. 1L). For instance, in both the superficial layers of superior colliculus and profundum, 5-HT uptake sites were reduced 85–90%. Serotonergic projections to substantia nigra pars compacta and reticulata also appeared to be affected by MDMA as moderate reductions in 5-HT uptake sites were noted in both regions. Interestingly, 5-HT uptake sites in substantia nigra revealed a time-dependent recovery, as significantly more sites were present 14 days following MDMA treatment than at the 18 hour time point. In contrast, in midbrain regions containing 5-HT cell bodies such as the dorsal and median raphe, there were no significant reductions in 5-HT uptake sites noted at either time point (Table I). Likewise, in the central grey, the ventral tegmental region and the pontine reticular activating system, no significant reductions in 5-HT uptake sites were observed immediately after or up to 14 days following the MDMA treatment regimen.

MDMA-induced lesion specificity

In order to assess the serotonergic selectivity of the MDMA-induced neurodegenerative effects in brain, we have carried out additional autoradiographic studies of norepinephrine (NE) and dopamine (DA) 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 blockers as previously published by Javitch et al. (1985). With respect to NE uptake sites, we have observed no significant change from control levels of binding sites in locus coeruleus (1,211 ± 66 fmol/mg tissue equivalent), interpeduncular nucleus (177 ± 57 fmol/mg tissue equivalent), or substantia nigra pars compacta (74 ± 9 fmol/mg tissue equivalent) or reticulata (109 ± 26 fmol/mg tissue equivalent) at either time point following MDMA treatment. While no decreases were observed in the density of NE uptake sites, in some cerebral cortical regions such as cingulate (area 8 and area 10), NE uptake sites in these cerebrocortical areas appeared to be slightly increased by day 14, although the variability in the data precluded obtaining statistical significance for this effect. Consistent with the minimal effect of MDMA on NE uptake sites, there were no statistically significant changes in DA uptake sites from control levels in either dopamine cell body regions such as substantia nigra pars compacta (119 ± 32 fmol/mg tissue equivalent) and reticulata (47 ± 6 fmol/mg tissue equivalent) or dopamine terminal regions such as caudate putamen (439 ± 61 fmol/mg tissue equivalent), nucleus accumbens (158 ± 32 fmol/mg tissue equivalent), or olfactory tubercle (215 ± 46 fmol/mg tissue equivalent). Therefore, the lack of statistically significant effects of MDMA on catecholamine containing neurons in either cell body or terminal regions is consistent with the data from homogenate studies indicating that the neurodegenerative effects of MDMA appear to be selectively mediated on serotonergic systems (see Fig. 2A,B,D,E).

We have previously reported that the changes in 5-HT uptake sites following administration of another designer drug, MDA (3,4 methylenedioxyamphetamine), were comparable in various brain regions to those observed for MDMA (Battaglia et al., 1987). As shown in Figure 2, short-term administration of MDA resulted in decreases in cerebral cortical, caudate-putamen, and

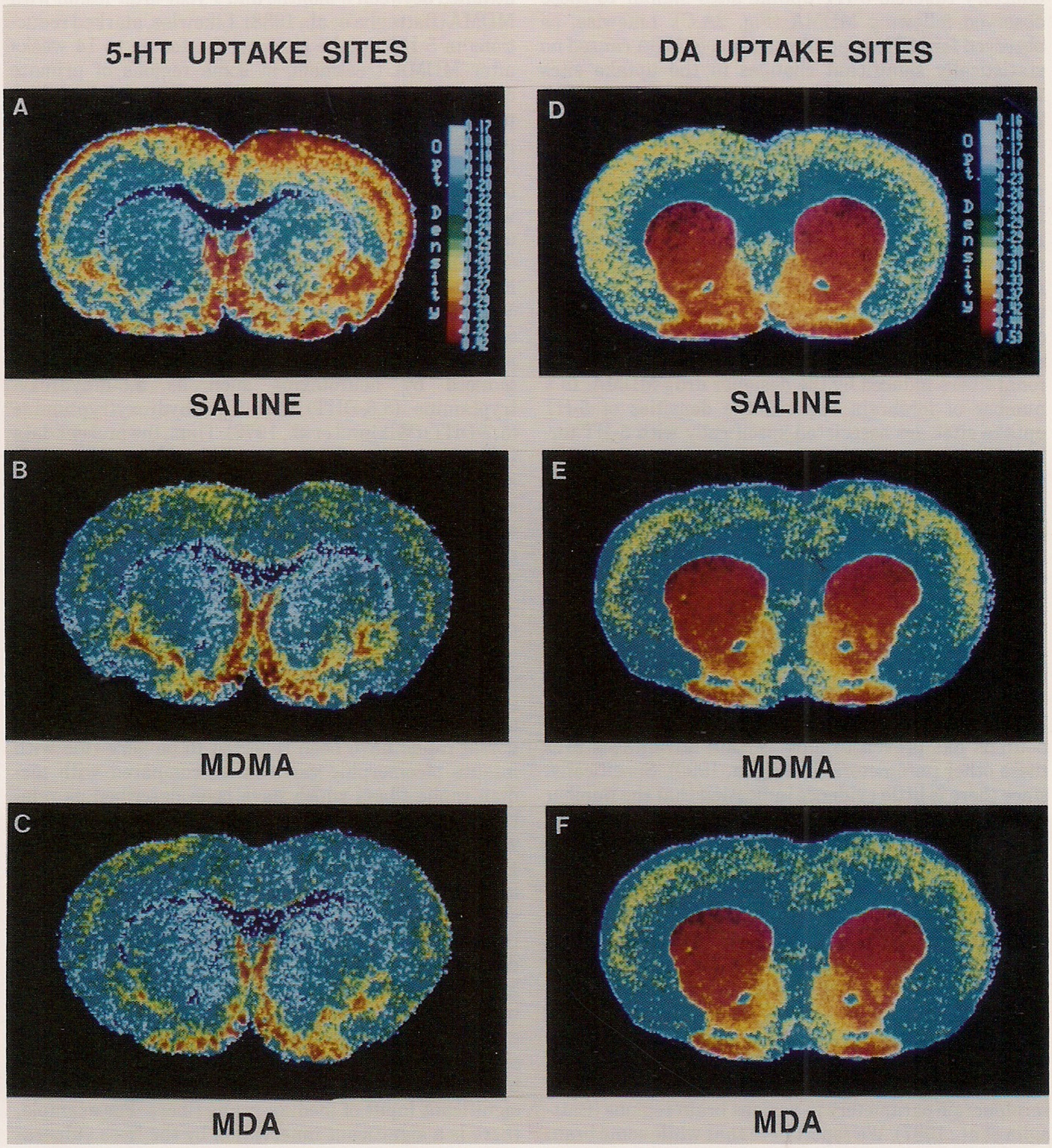

Fig. 2. Comparative effects of MDMA and MDA treatment on the densities of ^{3}H-paroxetine-labeled 5-HT uptake sites (A–C) and ^{3}H-mazindol-labeled dopamine uptake sites (D–F) in a coronal section of rat brain taken at the level of the caudate-putamen. Animals received either saline vehicle, 1 ml/kg (A,D), or 20 mg/kg (as the free base) *d,l*-MDMA HCl (B,E), or *d,l*-3,4-methylenedioxyamphetamine (MDA), subcutaneously twice a day for four consecutive days. The sections in this figure are from rats which were sacrificed 14 days after the final injection. These pictures represent color-coded autoradiograms of the densities of 5-HT and DA uptake sites in which the "warmer" colors (red and yellow) correspond to higher densities while the "cooler" colors (blue and violet) depict areas having lower numbers of sites. The optical density scale is located to the right for each of the uptake sites. Note the marked reduction in cortical 5-HT uptake sites but relative insensitivity of the septum to changes following MDA and MDMA (B,C). Note also the lack of any statistically significant reduction by MDA or MDMA on the density of DA uptake sites (E,F).

olfactory tubercle 5-HT uptake sites similar to those observed following MDMA (Fig. 2A,C). Likewise, as observed for MDMA, administration of MDA caused no statistically significant changes in the uptake sites associated with catecholamine containing neurons (Fig. 2D,F).

DISCUSSION

We report here that short-term administration of MDMA produces region-specific damage to 5-HT neuronal fibers and that the time course of lesion and recovery also demonstrates region specificity. In vitro autoradiography of ^{3}H-paroxetine-labeled 5-HT uptake sites has been used to quantitate the regional densities of 5-HT uptake sites and elucidate the neuroanatomic specificity and time-dependence of lesions of 5-HT neuronal fibers induced by the designer drug MDMA. In a number of forebrain regions, the densities of 5-HT uptake sites are associated specifically with 5-HT terminals and axons, while in midbrain areas, we have observed that radiolabeled 5-HT uptake sites are also associated with 5-HT perikarya (Fig. 1K). We have previously provided evidence that reductions in ^{3}H-paroxetine-labeled 5-HT uptake sites correspond to decreases in other 5-HT parameters and thus provide a valid index to assess neurodegeneration (Battaglia et al., 1987). While decreases in 5-HT and 5-HIAA content and active uptake of ^{3}H-5-HT have also been used to assess the extent of lesions of serotonergic systems, direct labeling and measurement of the density of 5-HT uptake sites as a marker for the integrity of 5-HT neurons has proven to have certain advantages over these other parameters (Battaglia, 1990). Specifically, since there is little evidence indicating that the number of 5-HT uptake sites changes in response to alterations in 5-HT metabolism (Kovachich et al., 1990), reductions in 5-HT uptake sites would indicate a loss of the 5-HT neuronal fibers within which they are contained. This contention is consistent with all existing data from immunocytochemical, radioligand binding and autoradiographic studies (Appel et al., 1990; Battaglia et al., 1987; D'Amato et al., 1987; De Souza and Kuyatt, 1987; O'Hearn et al., 1988).

The present autoradiographic results support previous data from homogenate studies which demonstrate that MDMA causes extensive reductions in a number of serotonergic parameters in various brain regions (Battaglia et al., 1987). We report here that while some brain regions may require 14 days for the neurodegenerative process to be fully expressed (e.g., dorsal caudate), in other brain regions, at the same time point, there is a maximal and persistent deficit in 5-HT innervation (e.g., cerebral cortex) or a regenerative process which has already begun to occur (e.g., substantia nigra). We have previously demonstrated, using rat brain homogenates, that there is a very protracted recovery of frontal cortex 5-HT uptake sites, with control values only being attained between 6 months and 1 year after lesion with MDMA (Battaglia et al., 1988). Likewise, marked reductions in 5-HT uptake sites persist for up to 14 weeks after MDMA treatment in some regions of primate cerebral cortex (i.e., somatosensory and occipital), hippocampus and caudate (Insel et al., 1989). A similar prolonged recovery of cerebral cortical and hippocampal 5-HT uptake sites has been reported following administration of high-dose fenfluramine, with levels of 5-HT uptake sites remaining significantly below control values for up to 32 weeks following drug administration (Zaczek et al., 1989). The present autoradiographic data indicate neuroanatomic differences in the time course of 5-HT axonal recovery which are consistent with the temporal differences in region-specific regeneration reported following extensive axotomy of 5-HT systems induced by neurotoxins such as 5,6-dihydroxytryptamine (5,6-DHT) and 5,7-dihydroxytryptamine (5,7-DHT) (Wiklund et al., 1978). Thus, the present data indicate that not all brain regions may be equally sensitive to the neurotoxic effects of MDMA. Our data also support the hypothesis that following short term administration of MDMA, there is region-specificity in the degenerative as well as the regenerative processes which occur for 5-HT neurons.

It has been postulated that 5-HT neurons may exhibit differential neurotoxic sensitivities to the amphetamines based on the morphologic differences between 5-HT fibers originating from dorsal versus median raphe nuclei (Kosofsky and Molliver, 1987; Mamounas and Molliver, 1988; Molliver, 1987). Axons rising from dorsal raphe have been reported to be very fine with minute pleomorphic varicosities, compared with median raphe fibers which have been described as relatively course, are beaded, and have large spherical varicosities (Kosofsky and Molliver, 1987; Molliver, 1987). The fine dorsal raphe fibers have been proposed to be more vulnerable to chemical destruction by drugs such as parachloroamphetamine (Mamounas and Molliver, 1988) and MDMA (O'Hearn et al., 1988). While the data from the present study are consistent with this hypothesis in some regions of cerebral cortex which receive extensive innervation by dorsal raphe fibers and exhibit broad and marked reductions in 5-HT uptake sites following MDMA, this hypothesis is not supported by the data in other regions of cortex which receive a significant input of median raphe fibers. For example, there is a significant innervation by the "less vulnerable" median type raphe axons in some limbic structures such as the dentate gyrus of the hippocampus, entorhinal, cingulate and parietal cortices, yet these same brain regions exhibit rapid and marked deficits in 5-HT uptake sites following MDMA. Interestingly, the septum, which receives serotonergic input from both dorsal and median raphe (Swanson and Cowen, 1979), is one of the limbic structures which is more resistent to the neurodegenerative effects of MDMA. In both lateral and

medial septal nuclei, less marked decreases (approximately 40%) in 5-HT uptake sites were observed up to 2 weeks following MDMA administration. These effects contrast with those reported for the dihydroxytryptamine serotonin neurotoxins. Both 5,6-dihydroxytryptamine (5,6-DHT) and 5,7 dihydroxytryptamine (5,7-DHT), produce marked changes in septum, hypothalamus, and pons-medulla, areas which are less affected by halogenated amphetamines (Fuller, 1978) or MDMA (Table I).

Data from immunocytochemical studies support both the time-dependence and regional neurotoxicity of MDMA in that decreases in 5-HT immunoreactivity were observed across all layers of neocortex as early as 1 day following MDMA administration. Evidence from 5-HT immunocytochemical studies supports the hypothesis that 5-HT terminals are primarily affected by MDMA (O'Hearn et al., 1988) and other substituted amphetamines (Appel et al., 1989, 1990; Fukui et al., 1989). While it had been reported that following MDMA-induced lesion the number of cortical 5-HT axons remaining was roughly proportional to the neuronal innervation density (O'Hearn et al., 1988), the present autoradiographic studies using 5-HT uptake site densities as an index of cortical 5-HT innervation do not support such a hypothesis. For example, more marked and earlier MDMA-induced reductions in 5-HT uptake sites were detected in entorhinal cortex compared with sensorimotor regions, although entorhinal cortex contains approximately 4 times the number of 5-HT uptake sites as the sensorimotor region (see Table I). A similar lack of correspondence between 5-HT uptake site densities and the magnitude of reductions has been observed following high-dose fenfluramine treatment (Appel et al., 1990).

The present study supports the contention that MDMA does not produce any predominant effects in brain regions containing either 5-HT perikarya or 5-HT axons of passage. For example, MDMA produced no significant reductions in 5-HT uptake sites in indusium gresium, basolateral nucleus of the amygdala, the lateral nucleus of the hypothalamus, the interpeduncular nucleus, the central gray, the ventral tegmental area, pontine reticular formation and the dorsal and median raphe nuclei. The relative insensitivity of these brain regions to MDMA-induced deficits is consistent with similar findings following high-dose fenfluramine (Appel et al., 1990). In addition, the lack of effect of MDMA in brain regions containing 5-HT perikarya is consistent with 5-HT immunocytochemical data (O'Hearn et al., 1988) indicating no changes in 5-HT cell bodies. In addition to the regional selectivity of MDMA on 5-HT neurons, the present autoradiographic data of radiolabeled catecholamine uptake sites (see Results section, Fig. 2) support previous studies using homogenates (Battaglia et al., 1987) which indicate that MDMA exhibits marked neuroselectivity, in preferentially affecting serotonergic versus catecholaminergic neurons. No significant effects of MDMA on dopamine or norepinephrine uptake sites were observed in brain regions containing either catecholamine perikarya or terminals. We have also observed that 3,4-methylenedioxyamphetamine (MDA), the desmethyl analog of MDMA, exhibits a similar pattern of selective neurotoxicity as MDMA, with respect to both the brain regions affected and the preferential effects on serotonergic versus catecholaminergic neurons (Fig. 2). Although other amphetamine derivatives such as parachloroamphetamine (Sanders-Bush and Steranka, 1978) and fenfluramine (Appel et al., 1989, 1990; Zaczek et al., 1989) exhibit a similar selective neurotoxicity of 5-HT neurons, these findings differ from those reported for the dihydroxytryptamine neurotoxins. While both 5,6-DHT and 5,7-DHT are potent serotonin neurotoxins, they exhibit less selectivity toward serotonergic versus catecholaminergic systems (Nobin and Bjorklund, 1978).

In summary, the present study supports the contention that the predominant effects of MDMA on serotonergic systems throughout the brain are mediated on 5-HT axons and terminals. The data from the present report, however, indicate that not all regions containing 5-HT projections may be equally vulnerable to the neurodegenerative effects of MDMA. We report here region-specific differences in the effects of MDMA with respect to 1) the magnitude of the reduction in 5-HT uptake sites, 2) the time required to obtain a maximal effect, and 3) the ability to recover from these effects of MDMA.

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