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Methylenedioxymethamphetamine-Induced Serotonin Deficits Are Followed by Partial Recovery Over a 52-Week Period. Part I: Synaptosomal Uptake and Tissue Concentrations[1]

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ABSTRACT

The effects of a high dose methylenedioxymethamphetamine (MDMA) regimen on the serotonin (5-HT) system were evaluated over a 52-wk period. MDMA was administered to rats (20 mg/kg) 8 times at 12-hr intervals. Tissue concentrations of dopamine (DA) and 5-HT, and synaptosomal uptake of ^{3}H-5-HT and ^{3}H-DA were measured at 2, 8, 16, 32 or 52 wk posttreatment. Synaptosomal uptake of ^{3}H-5-HT (hippocampus) was decreased at 2 and 8 wk, but not at 16, 32 or 52 wk after drug. 5-HT tissue concentrations were measured in frontal cortex, frontal-parietal cortex, occipital-temporal cortex, nucleus accumbens/olfactory tubercle, striatum, amygdala, hippocampus, septum, hypothalamus, ventral tegmentum/substantia nigra. Two weeks after MDMA treatment, all regions showed decreased 5-HT tissue concentrations except septum. Recovery over the 52-wk interval was noted for all depleted regions, but the rate and degree of recovery was region dependent. Frontal-parietal cortex, occipital-temporal cortex and hippocampus showed the least recovery, with significant depletions at 52 wk posttreatment. Hypothalamus showed an increase in 5-HT tissue concentrations relative to age-matched controls at 52 wk. These results indicate that a high-dose MDMA regimen results in long-lasting depletions of serotonin. The rate and degree of recovery of serotonin tissue concentrations seen over the 52-wk test period is region specific.

MDMA is an amphetamine analog that, when administered in high doses, results in damage to the serotonergic system of the brain. MDMA causes long-term depletions of serotonin and decreased synaptosomal uptake (Commins *et al.*, 1987; Schmidt, 1987; Schmidt *et al.*, 1986). Terminal degeneration has been identified using the Fink-Heimer technique (Commins *et al.*, 1987) as well as immunocytochemistry (O'Hearn *et al.*, 1988). Decreases in serotonin uptake sites were found using ^{3}H-paroxetine binding (Battaglia *et al.*, 1987) and autoradiographic techniques (Battaglia *et al.*, 1991), further indicating terminal degeneration. In addition to these MDMA-induced deficits in the rat, serotonergic toxicity has been observed in squirrel monkey (Ricaurte *et al.*, 1992) and rhesus monkey (DeSouza *et al.*, 1990; Kleven *et al.*, 1989).

Serotonergic deficits have been noted in rats for up to 52 wk posttreatment. DeSouza *et al.* (1990) reported that MDMA (20 mg/kg, twice daily for 4 days) resulted in significant reductions of serotonin uptake sites up to 6 mo posttreatment; recovery was seen at 12 mo. Serotonin tissue concentrations were significantly decreased for the entire 12 mo, although depletions were greater at early time points. Scanzello *et al.* (1993) found that serotonin tissue concentrations were depleted at 2 wk post-MDMA treatment (10 mg/kg four times at 2-hr intervals) in all regions studied. They reported that different brain regions showed differential patterns of recovery from serotonergic depletions with hypothalamus recovering to control levels at 8 wk, and frontal cortex showing significant depletions at 32 wk post-MDMA. Paroxetine binding to serotonin uptake sites was decreased at 2 but not 32 or 52 wk in cortex and striatum. In contrast, serotonin uptake sites were significantly decreased in hippocampus at 2, 32 and 52 wk posttreatment (Scanzello *et al.*, 1993).

In the experiments described below, we extend the findings discussed above, regarding depletion and recovery of serotonin tissue concentrations in various brain regions after MDMA treatment. In this and the companion paper (Lew *et al.*, 1996) we operationally define recovery as a return to control values for the measure in question. The word recovery in this context does not imply normal regeneration pat-

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ABBREVIATIONS: MDMA, methylenedioxymethamphetamine; METH, methamphetamine; PCA, parachloroamphetamine; 5-HT, serotonin; DA, dopamine; NE, norepinephrine; F. Ctx, frontal cortex; F.P. Cortex, frontal parietal cortex; O.T. Cortex, occipital-temporal cortex; HIPPO, hippocampus; STR, striatum; NA/OT, nucleus accumbens/olfactory tubercle; AMYG, amygdala; SEPT, septum; HYPO, hypothalamus; VTA/SN, ventral tegmental area/substantia nigra.

terns, nor does it imply normal function. We also report that in the hypothalamus and septum, the serotonin tissue concentrations increased to levels greater than age matched controls at 52 wk posttreatment. In addition, we report significant reductions in serotonin but not dopamine synaptosomal uptake at 2 and 8 wk post-MDMA. At 16, 32 and 52 wk, serotonin synaptosomal uptake was not significantly different from age-matched controls.

Methods

Animals. Male Sprague-Dawley rats (Holtzman Co., Madison, WI) weighing 275 to 300 g at the start of the experiment, were housed in pairs under a 12-hr light/dark cycle at room temperature with free access to food (Teklad 4% rat diet) and water.

Drug administration. Rats received s.c. injections with either 0.9% saline or 20 mg/kg MDMA (free base) twice daily (12 hr apart) for 4 days (total MDMA = 160 mg/kg) and allowed to recover for periods of 2, 8, 16, 32 and 52 wk before death. Drug injections were timed so that all rats could be killed within a 7-wk period.

Materials. ^{3}H-Dopamine (specific activity = 24.5 Ci/mmol) and ^{3}H-serotonin (specific activity = 26.4 Ci/mmol) were purchased from NEN-Du Pont (Wilmington, DE). MDMA HCl was obtained from the National Institute on Drug Abuse (Baltimore, MD). Mazindol HCl was purchased from Research Biochemicals International (Natick, MA) and citalopram HCl was purchased from Lundbeck (Denmark). All other chemicals and compounds were purchased from Sigma Chemical Co. (St. Louis, MO) and were of analytical grade.

Synaptosomal uptake procedure. At the end of each recovery period, saline- and MDMA-treated animals were sacrificed and their brains removed and placed on ice. Striatum and hippocampus were dissected from saline-and MDMA-treated animals as previously described (Heffner *et al.*, 1980) and synaptosomes were prepared according to a modified procedure described by Holz and Coyle (1974). Briefly, tissues were suspended in 20 vol of ice-cold 0.32 M sucrose, homogenized with 8 up and down strokes of a Heidolp stirrer set at 800 rpm and centrifuged at 800 × *g* for 20 min. After centrifugation, the supernatants were decanted and centrifuged again at 20,000 × *g* for 20 min. The resulting pellets were resuspended in ice-cold 0.32 M sucrose to a stock concentration of either 20 mg/ml (striatum) or 50 mg/ml original wet weight (hippocampus).

For serotonin uptake, ^{3}H-5-HT (10 nM final concentration) was preincubated with 100 μM pargyline, Kreb's phosphate (containing 126 mM NaCl, 4.8 mM KCl, 1.3 mM $CaCl_2$, 16 mM Na_2HPO_4, 1.4 mM $MgSO_4$, 2 mg/ml dextrose and 1 mg/ml ascorbic acid) pH 7.4, 30°C and increasing concentrations of unlabeled serotonin (0–3 μM) for 10 min at 30°C. Uptake of ^{3}H-5-HT was initiated by the addition of hippocampal synaptosomes (170 μg protein) and allowed to continue for 5 min at 30°C. Nonspecific uptake was defined by 1 μM citalopram. Uptake was terminated by addition of 3 ml of ice-cold 0.32 M sucrose followed by filtration through GF/B filters previously soaked in 0.1% polyethylenimine. Filters were collected and radioactivity measured using a Beckman LS 5000 TD scintillation counter (efficiency = 46%). For dopamine uptake studies with striatal synaptosomes (50 μg protein), a similar procedure was used except ^{3}H-DA (5 nM) was used and uptake was terminated 3 min after addition of synaptosomes. Nonspecific DA uptake was determined by including 1 μM mazindol in the incubation.

Neurochemical analysis procedure. Rats were killed by decapitation. The brains were immediately removed and dissected over ice according to a modification of the method of Heffner *et al.* (1980). The regions dissected in this report came from six sequential rostral-caudal brain slices. Section one: 4.5 to 5.2 mm anterior to Bregma; section two: 3.0 to 4.5 mm anterior to Bregma; section three: 1.5 to 3.0 mm anterior to Bregma; section four: 0 to 1.5 mm anterior to Bregma; section five: 0 to 4 mm posterior to Bregma; section six: 4.0 to 5.5 mm posterior to Bregma (Paxinos and Watson, 1986). Frontal cortex included only the dorsal and medial regions of rostral cortex, and was dissected from sections 1, 2 and 3. Nucleus accumbens/olfactory tubercle was dissected from section 2; striatum and septum from section 4. The frontal-parietal cortex (somatosensory) extended ventrally down to the rhinal fissure and was dissected from section 5; the cortical area below the rhinal fissure was included in the amygdala dissection. Hypothalamus was also dissected from section 5. The hippocampus and occipital-temporal cortex were dissected from section 6; the entire dorsal-ventral extent of the cortex was removed. The ventral tegmentum/substantia nigra dissection included the lower third of the midbrain, also from section 6.

DA, 5-HT and NE levels were determined using high-performance liquid chromatography. Instrumentation and chromatographic conditions included: HPLC pump (Anspec, model 2200); pulse dampener (SSI model LP-21); injection valve (rheodyne model 7010); coulometric detector (ESA model 5200) with applied voltages of −200 and +300 mV, integrator (Shimadzu model C-R5A), and column [Alltech, 250 × 4.6 mm, Econosil (C18) packing, 5-μM particle size]. Mobile phase consisted of 62.5 mM citric acid monohydrate, 7.9 mM sodium phosphate dibasic, 0.27 mM EDTA, 90 mg/L octyl sodium sulfate, 12% acetonitrile; pH was 2.5.

Data analysis. Determination of affinity (K_m) and the number of uptake sites (V_{max}) of ^{3}H-DA and ^{3}H-5-HT in the respective synaptosomal preparations was determined using the EBDA and LIGAND software programs (BIOSOFT, UK). V_{max} was calculated using scatchard analysis (O'Reilly and Reith, 1988; Reith and O'Reilly, 1990).

Statistical analysis of the density of uptake sites and transmitter tissue concentrations was performed using a between groups two-way analysis of variance and Duncan *post hoc* test (CLR analysis of variance). Differences were considered significant when $P < .05$ was observed.

Results

Uptake. Synaptosomal uptake of ^{3}H-5-HT (V_{max}) was significantly changed as a function of time [$F(4,48) = 4.419$, $P < .01$] and drug treatment [$F(1,48) = 71.883$, $P < .001$]. There was also a significant drug-time interaction [$F(4,48) = 13.89$, $P < .001$). *Post hoc* analysis indicates that serotonin uptake in saline-treated rats was significantly decreased as the rats aged, with rats at 8, 16, 32 and 52 wk after drug different from 2 wk posttreatment, but not different from each other. MDMA decreased serotonin uptake at 2 and 8 wk after drug; at 16, 32 and 52 wk posttreatment the MDMA-treated rats were not different from age-matched controls (see fig. 1A). K_m values were not affected.

Synaptosomal uptake of ^{3}H-DA (V_{max}) was significantly affected by time [$F(4,50) = 4.439$, $P < .01$]. There was no effect of drug treatment, nor was there a significant interaction. *Post hoc* analysis indicated that dopamine uptake at 16, 32 and 52 wk posttreatment was significantly decreased compared to 2 and 8 wk posttreatment, regardless of treatment (see fig. 1B). K_m values were not affected by MDMA treatment.

Tissue concentrations. Serotonin tissue concentrations showed significant reductions in all regions studied (except septum), with the rate of recovery dependent on specific region. In septum and hypothalamus there was an MDMA-induced increase in serotonin tissue concentrations at 32 and 52 wk posttreatment relative to age-matched controls. This effect was significant in the septum at 52 wk and in the hypothalamus at 32 and 52 wk.

Table 1 lists the analysis of variance results of serotonin tissue concentration data. Figure 2 shows that MDMA decreased serotonin tissue concentrations in all three cortical

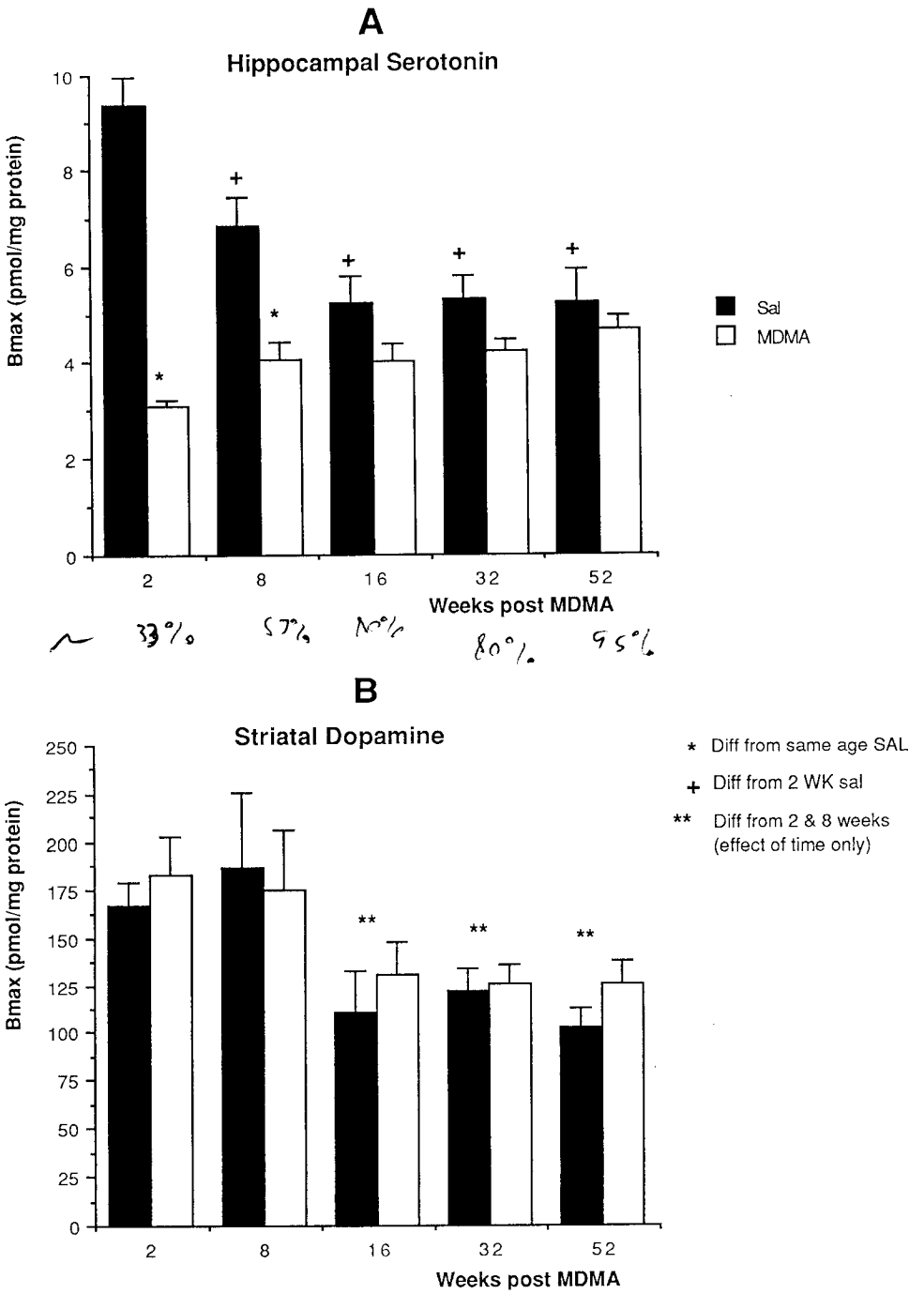

Fig. 1. Synaptosomal uptake of ^{3}H serotonin (A) and ^{3}H dopamine (B) at 2, 8, 16, 32 and 52 wk post-MDMA (20 mg/kg 8 times at 12-hr intervals) or saline treatment.

regions studied, up to 32 wk after treatment. Frontal cortex (fig. 2A) showed partial recovery (partial recovery is defined as significantly increased above 2-wk MDMA, and significantly decreased compared to age-matched controls) at 16 and 32 wk, and complete recovery at 52 wk. Neither frontal-parietal (fig. 2B) nor occipital-temporal cortex (fig. 2C) showed complete recovery, but partial recovery was seen at 16, 32 and 52 wk posttreatment.

From figure 3 it can be seen that serotonin in nucleus accumbens/olfactory tubercle and striatum (fig. 3, A and B) was significantly decreased at 2 and 8 wk, but showed complete recovery by 16 wk posttreatment. Amygdala serotonin (fig. 3C) was significantly decreased compared to age-matched controls at 2 and 32 wk (the decrease at 16 wk was not significant); complete recovery was seen by 32 wk posttreatment. Hippocampal serotonin was significantly decreased for the duration of the study, but showed partial recovery at 16, 32 and 52 wk posttreatment (fig. 3D).

Figure 4A shows that septal serotonin was not reduced significantly at any time point; however, there is a significant increase, compared to age-matched controls, at the 52-wk time point. Hypothalamic serotonin (fig. 4B) was decreased at 2 wk, and increased at 32 and 52 wk compared to age-matched controls. Serotonin in the ventral tegmental area-substantia nigra (fig. 4C) was significantly decreased at 2 wk posttreatment, but showed complete recovery by 16 wk (the 8-wk time point is neither different from the 2-nor nor 16-wk MDMA).

TABLE 1
Serotonin tissue concentrations—ANOVA Results

Region	Treatment			Time			Interaction		
	F	df	P	F	df	P	F	df	P
F. CTX	73.02	1,93	.0000	19.48	4,93	.0000	4.29	4,93	.0031
F.P. CTX	129.18	1,93	.0000	7.26	4,93	.0000	0.65	4,93	.6274
O.T. CTX	78.19	1,92	.0000	9.39	4,92	.0000	0.70	4,92	.5948
HIPPO	104.98	1,93	.0000	8.77	4,93	.0000	2.18	4,93	.0774
STR	28.62	1,93	.0000	4.94	4,93	.0012	2.98	4,93	.0230
NA/OT	12.86	1,93	.0005	6.33	4,93	.0001	2.08	4,93	.0901
AMYG	28.08	1,92	.0000	13.70	4,92	.0000	2.25	4,92	.0701
SEPT	0.010	1,91	.9190	3.41	4,91	.0121	4.36	4,91	.0029
HYPO	2.716	1,92	.1028	4.27	4,92	.0032	6.02	4,92	.0002
VTA/SN	0.442	1,93	.5077	2.84	4,93	.0284	3.103	4,93	.0191

There were no significant treatment or interaction effects for dopamine in any brain region studied. In striatum, amygdala and ventral midbrain there were age-dependent changes in dopamine tissue concentrations. In striatum, the 2-wk time period was significantly decreased compared to all other time points (regardless of treatment). For ventral midbrain, the 2-wk time point was significantly greater than the other four time points. For the amygdala, the 32-wk time point was significantly greater than all other time points. The contrasting time-dependent changes in dopamine in ventral tegmentum *vs.* striatum is interesting and bears further investigation (see table 2).

Norepinephrine tissue concentrations in frontal cortex showed a significant effect of treatment and time (but no significant interaction). However, *post hoc* analysis showed no significant differences between groups at any time point (see table 3).

Discussion

In this report, the term recovery refers to a return to control levels of the measure in question after an initial MDMA-induced decrease. Decreases in uptake sites, functional uptake and tissue concentrations of neurotransmitters are good indicators of terminal degeneration induced by amphetamine-like compounds (Battaglia *et al.*, 1988; Commins *et al.*, 1987; DeSouza *et al.*, 1990; Ricaurte *et al.*, 1992; Scanzello *et al.*, 1993; Seiden and Sabol, 1995). However, a return to control levels with time does not necessarily indicate normal reinnervation or regrowth patterns. For example, Bjorklund *et al.* (1973) observed abnormal axonal sprouting to occur within several days after 5,6-DHT treatment. Thus when we use the word "recovery" to describe our results, we are not suggesting a normal pattern of reinnervation is occurring.

DA and NE. Tissue concentrations of DA and NE were unaffected by MDMA treatment (see tables 2 and 3). Synaptosomal uptake of ^{3}H-DA in striatum was unaffected by MDMA treatment. However, a significant effect of time was observed: rats in the 16- to 52-wk posttreatment periods showed decreased uptake relative to the 2-and 8-wk rats. This suggests an age-related decline in functional uptake of DA.

5-HT. Serotonin tissue concentrations were significantly decreased at 2 wk post-MDMA in all regions studied except septum. Various patterns of recovery of serotonin tissue concentrations were noted, depending on the region. This general pattern of MDMA-induced long-term depletions followed by recovery, is consistent with Scanzello *et al.* (1993). A notable difference between the two studies is the time course of recovery. Our depletions lasted longer, and probably reflect a difference of dose and regimen: Scanzello *et al.* (1993) used 10 mg/kg, 4 times at 2-hr intervals, we used 20 mg/kg, 8 times at 12-hr intervals.

In our study, frontal cortex showed partial recovery of serotonin levels at 16 and 32 wk, but completely recovered at 52 wk posttreatment. In contrast, using the same treatment regimen that was used in our study (20 mg/kg 2 times/day, for 4 days), DeSouza *et al.* (1990) reported frontal cortex serotonin levels at 52 wk posttreatment that were 40 to 50% of control. Inasmuch as the dose regimen was the same, it is more difficult to explain the discrepancy between these two rates of recovery. Other experimental conditions, such as environmental temperature (Bowyer *et al.*, 1992; Schmidt *et al.*, 1990), may contribute to the different rates of recovery for serotonin tissue concentrations observed by Scanzello *et al.* (1993), DeSouza *et al.* (1990) and our laboratory.

Synaptosomal uptake of serotonin (hippocampus) was significantly different from age-matched controls at 2 and 8 wk post-MDMA but showed complete recovery at 16, 32 and 52 wk post-MDMA.

Hippocampal serotonin tissue concentrations remained significantly decreased up to 52 wk posttreatment; partial recovery, however, was apparent at 16, 32 and 52 wk. A similar pattern of partial recovery was seen with autoradiographic analysis of ^{125}I-RTI-55 binding to the 5HT transporter (Lew *et al.*, 1996). Although the recovery pattern for synaptosomal uptake and levels is consistent, the rate of recovery observed in hippocampal serotonin tissue concentrations, as well as ^{125}I-RTI-55 binding, is slower than for functional uptake. This result suggests that one function of the serotonin nerve terminal, removal of serotonin from the synapse, can occur normally when terminal damage is still apparent (as reflected by tissue concentrations of 5HT, and by ^{125}I-RTI-55 binding to the 5HT transporter) (Lew et al., 1996). This faster rate of recovery of synaptosomal uptake may reflect a compensatory increase in function similar to that suggested for the dopaminergic system after partial depletions (Zigmond *et al.*, 1986).

In both the dopamine system and the serotonin system, there was an age-dependent decrease in synaptosomal uptake in the control groups. Although it is possible that the high uptake values during early time points are an artifact of our procedure, this seems unlikely because it was observed in both transmitter systems. We did not see such a pattern in

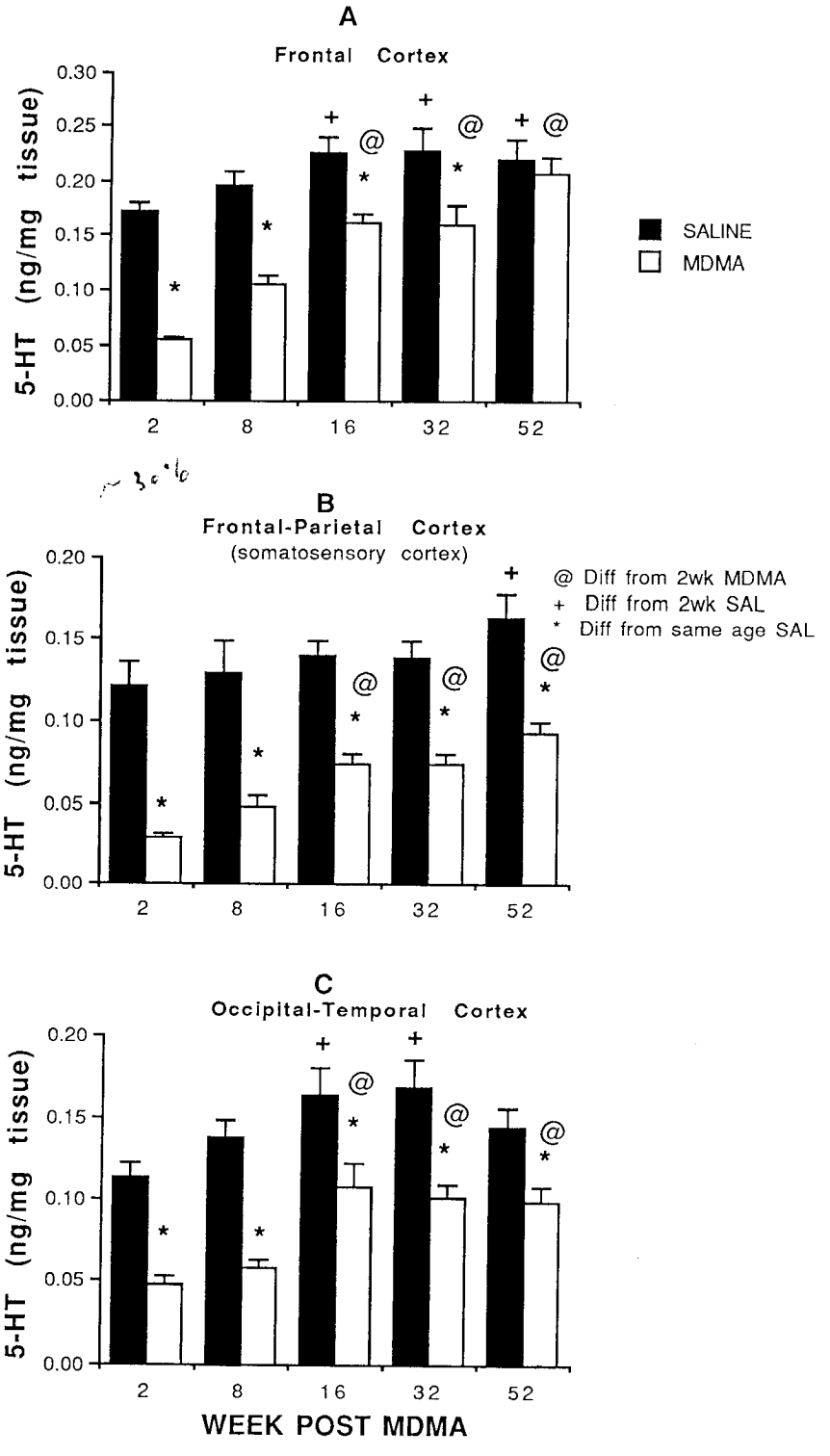

Fig. 2. Serotonin tissue concentrations in frontal cortex (A), frontal parietal cortex (B) and occipital-temporal cortex (C) 2, 8, 16, 32 and 52 wk after MDMA (20 mg/kg 8 times at 12-hr intervals) or saline treatment. Frontal cortex corresponds with plates 4 to 13 of the atlas of Paxinos and Watson (1986); frontal parietal corresponds with plates 18 to 33 and occipital-temporal with plates 33 to 42.

tissues concentrations of transmitter, or in binding to transporter (see accompanying paper by Lew et al., 1996). One possible interpretation of this pattern of results is that the functional uptake of the transporters declines in early adulthood. This is a point that requires further investigation.

Although there may be some questions interpreting the control synaptosomal uptake values, our data suggest that for the MDMA-treated rats, there was initial MDMA-induced damage to 5-HT terminals, which partially recovered. This conclusion is based on the fact that there is a significant difference between controls and MDMA-treated rats at 2 and 8 wk posttreatment.

Using immunohistochemistry, Axt *et al.* (1992) reported a pattern of serotonin reinnervation after para-chloroamphetamine treatment in which frontal cortex was more densely reinnervated than occipital cortex at 4 and 8 mo posttreatment. Axt *et al.* (1992) interpreted this pattern of cortical recovery to reflect the distance between cortical subregions

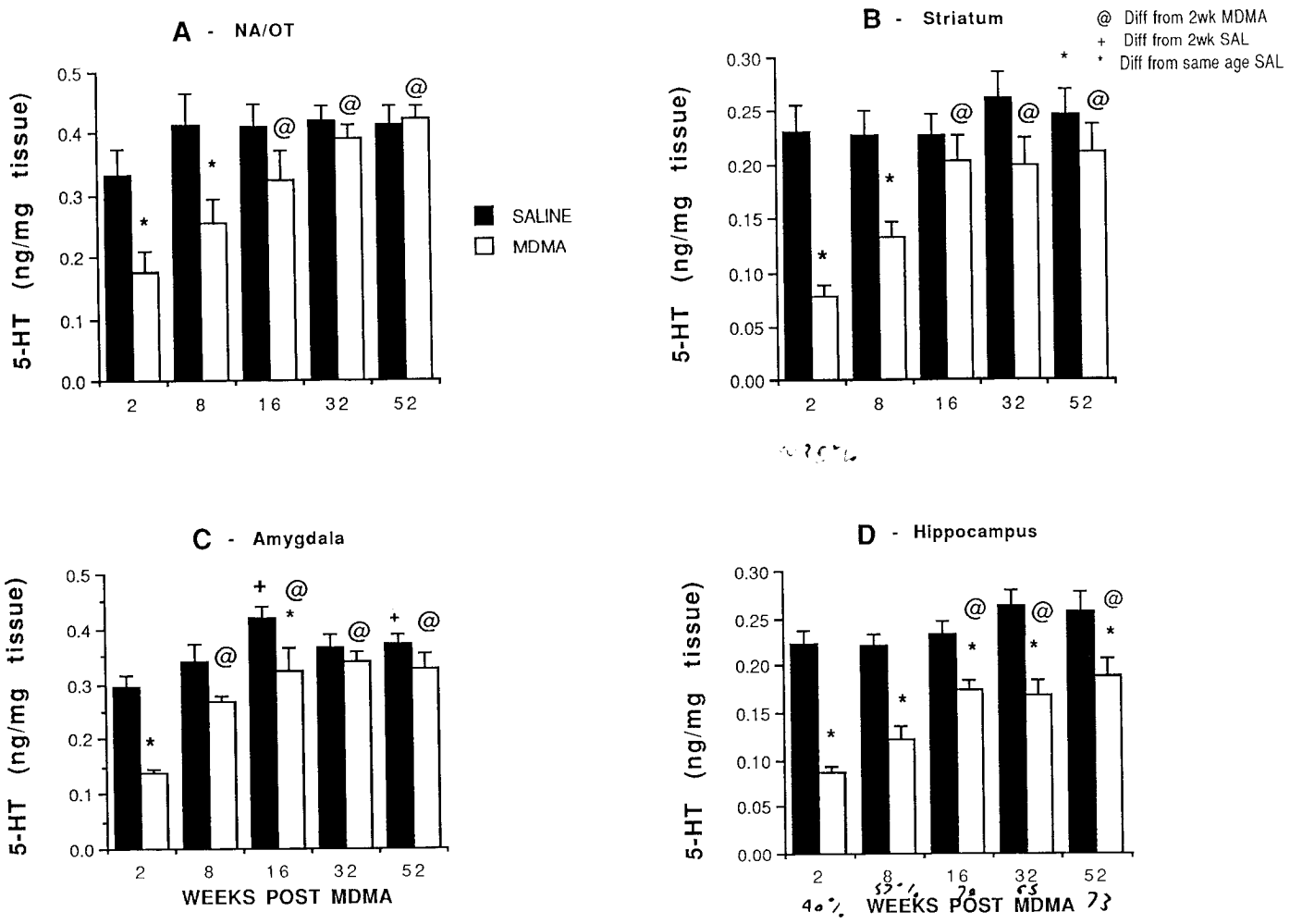

Fig. 3. Serotonin tissue concentrations in nucleus accumbens/olfactory tubercle (A), striatum (B), amygdala (C) and hippocampus (D) 2, 8, 16, 32 and 52 wk after MDMA (20 mg/kg 8 times at 12-hr intervals) or saline treatment. Nucleus accumbens/olfactory tubercle corresponds with plates 10 to 13 of the atlas of Paxinos and Watson (1986); striatum corresponds with plates 13 to 18; amygdala with plates 18 to 33 and hippocampus corresponds with plates 33 to 42.

from the raphe cell bodies. In support of this interpretation, anatomical evidence describes a serotonin-containing fiber bundle (cingulum) that innervates the cortex in a rostral-caudal direction (Azmitia and Segal, 1978; Lidov *et al.*, 1980; Moore *et al.*, 1978; Tohyama *et al.*, 1980); the more caudal cortical areas having a longer fiber length compared to the more rostral cortical areas.

The pattern of recovery of frontal cortex relative to more posterior regions of cortex in the present report is consistent with that found by Axt *et al.* (1992). Frontal cortex showed complete recovery at 52 wk although frontal-parietal and occipital-temporal cortex did not. However, there appears to be a lack of differential reinnervation between frontal-parietal and occipital-temporal cortex. In our study, the frontal-parietal and occipital-temporal dissections included lateral as well as dorsal regions of cortex. The lateral cortical regions are innervated via the serotonergic fibers that pass through the ansa peduncularis; *i.e.*, this pathway does not follow the same rostral-caudal innervation pattern that is demonstrated by the cingulum (Lidov *et al.*, 1980; Moore *et al.*, 1978; Tohyama *et al.*, 1980; Tork, 1985). Recovery patterns of our posterior cortex dissections therefore, would not be expected to follow a strict relationship to the path taken by the serotonin fibers in the cingulum. This may explain why there is little differentiation between the frontal-parietal cortex and the occipital-temporal cortex in the present study.

The pattern of recovery of serotonin tissue concentrations in cortex is consistent with the pattern of recovery of cortical and hippocampal serotonin uptake sites as measured by autoradiography (see adjoining paper, Lew et al., 1996) More rostral regions of cortex and hippocampus recover at earlier time points than more caudal regions.

The focus of the preceding discussion has been to explain our patterns of recovery in terms of distance from neuronal cell bodies. However, alternative explanations are also possible. As is discussed in the accompanying paper (Lew *et al.*, 1996) collateral sprouting from nearby, undamaged serotonin pathways, may also contribute to recovery of serotonin terminal markers.

Hypothalamus and septum showed a pattern of increased serotonin concentrations at the later time points (32 and 52 wk for hypothalamus; 52 wk for septum). The increase in hypothalamic serotonin at 32 (134% of control) and 52 wk (158% of control) is consistent with data obtained with the MDMA analog methamphetamine (METH). Richards *et al.* (1993) found an increase to 123% of control in hypothalamic

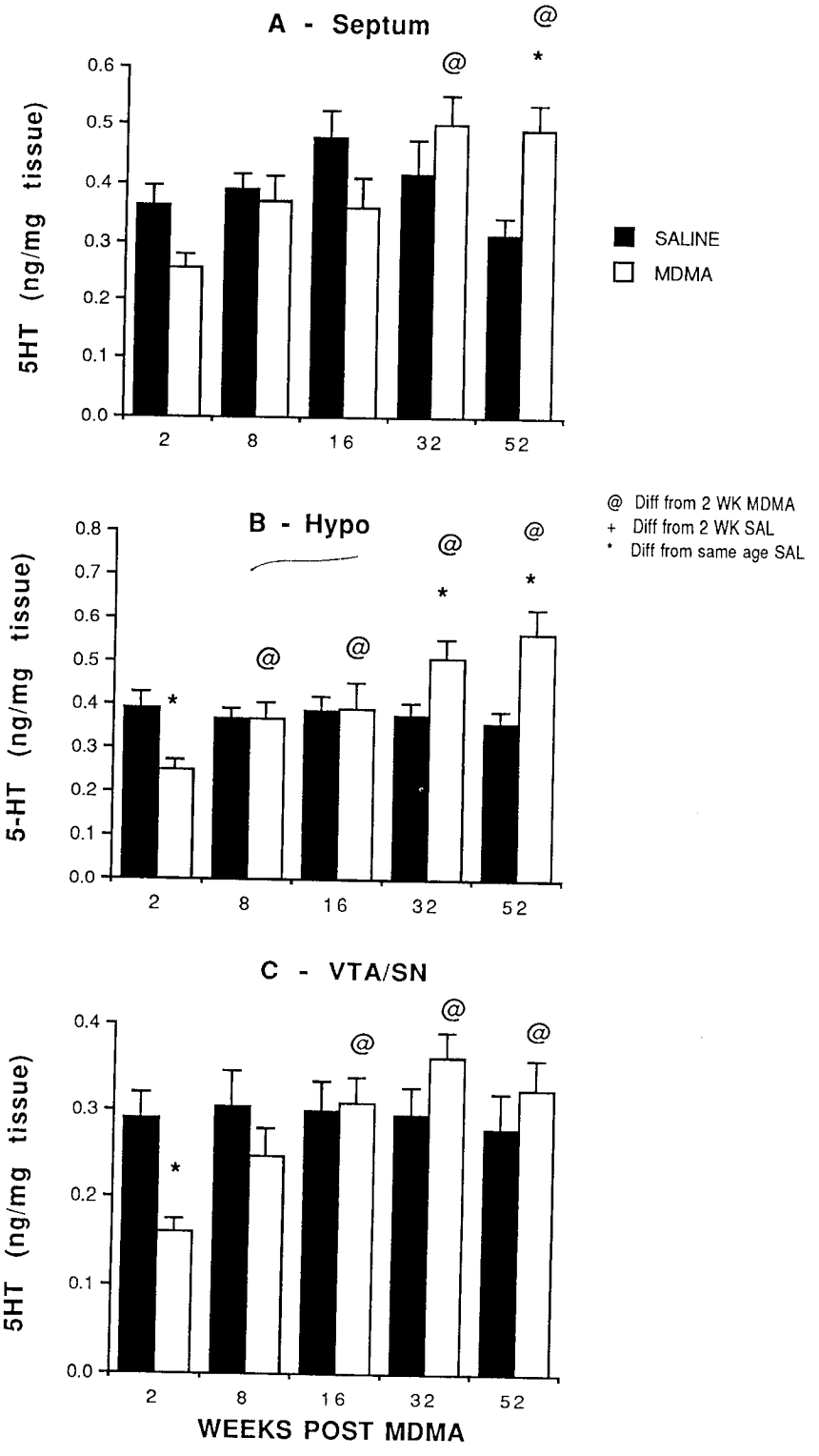

Fig. 4. Serotonin tissue concentrations in septum (A), hypothalamus (B) and ventral tegmentum/substantia nigra (C) 2, 8, 16, 32 and 52 wk after MDMA (20 mg/kg 8 times at 12-hr intervals) or saline treatment. Septum corresponds with plates 13 to 18 of the atlas of Paxinos and Watson (1986); hypothalamus corresponds with plates 18 to 33 and ventral tegmental area-substantia nigra with plates 33 to 42.

serotonin at 12 wk posttreatment. The time course of this effect in the Richards *et al.* (1993) report and in the present MDMA result is different, with the MDMA effect occurring later. This discrepancy may be due to the difference in drugs (METH *vs.* MDMA) and treatment regimens. A similar hypothalamic hyperinnervation after MDMA was reported by Ricaurte *et al.* (1992) for squirrel monkeys, but not by Scanzello *et al.* (1993) for rats.

Similar to the pattern of cortical recovery, hypothalamic innervation may reflect regrowth of serotonin terminals in regions nearest to the raphe cell bodies; in this case however, it may reflect a hyperinnervation. Serotonin levels in another basal region of the brain, ventral tegmentum/substantia nigra, also showed signs of hyperinnervation, but this effect did not reach statistical significance. One might project that given a longer term study (e.g., 18–24 mo), the more anterior regions along the basal forebrain (e.g., nucleus accumbens/olfactory tubercle) would begin to show hyperinnervation.

TABLE 2
Dopamine tissue concentrations (mean ± S.E.M.)

Region	2 Wk		8 Wk		16 Wk		32 Wk		52 Wk	
	MDMA	SAL	MDMA	SAL	MDMA	SAL	MDMA	SAL	MDMA	SAL
STR	9.85	10.44	10.96	11.58	11.45	11.79	11.98	11.91	11.73	12.36
	0.73	0.42	0.66	0.42	0.61	0.42	0.43	0.51	0.4	0.46
NA/OT	5.65	5.63	5.71	5.87	5.51	6.20	5.84	5.44	5.93	5.39
	0.54	0.33	0.22	0.30	0.23	0.38	0.26	0.26	0.27	0.36
AMYG	0.41	0.41	0.40	0.35	0.46	0.43	0.64	0.51	0.40	0.45
	0.05	0.04	0.03	0.03	0.04	0.03	0.11	0.05	0.02	0.05
SEPT	1.11	1.16	0.98	0.94	0.69	1.01	0.83	0.98	0.88	0.93
	0.17	0.15	0.14	0.17	0.07	0.13	0.10	0.20	0.17	0.11
HYPO	0.44	0.30	0.39	0.30	0.25	0.33	0.27	0.23	0.33	0.32
	0.03	0.03	0.06	0.05	0.03	0.07	0.03	0.02	0.05	0.09
VTA/SN	0.28	0.34	0.21	0.20	0.20	0.24	0.20	0.17	0.16	0.15
	0.05	0.03	0.02	0.03	0.02	0.03	0.03	0.01	0.03	0.03

TABLE 3
Norepinephrine tissue concentraions (mean ± S.E.M.)

Region	2 Wk		8 Wk		16 Wk		32 Wk		52 Wk	
	MDMA	SAL	MDMA	SAL	MDMA	SAL	MDMA	SAL	MDMA	SAL
F. CTX	0.18	0.18	0.18	0.19	0.19	0.23	0.21	0.22	0.22	0.23
	0.01	0.01	0.01	0.01	0.01	0.02	0.01	0.01	0.01	0.02
F.P. CTX	0.19	0.19	0.20	0.21	0.23	0.24	0.26	0.28	0.28	0.32
	0.01	0.01	0.01	0.02	0.02	0.01	0.02	0.02	0.01	0.03
O.T. CTX	0.17	0.17	0.19	0.22	0.22	0.23	0.23	0.25	0.21	0.22
	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.02
HIPPO	0.26	0.29	0.29	0.31	0.29	0.32	0.36	0.36	0.34	0.34
	0.01	0.02	0.02	0.02	0.01	0.02	0.01	0.03	0.02	0.02
NA/OT	0.33	0.29	0.24	0.24	0.25	0.31	0.27	0.25	0.31	0.28
	0.03	0.03	0.03	0.04	0.03	0.04	0.02	0.02	0.03	0.03
AMYG	0.29	0.30	0.33	0.34	0.35	0.36	0.38	0.36	0.37	0.40
	0.01	0.01	0.01	0.01	0.03	0.02	0.01	0.01	0.01	0.04
SEPT	1.12	1.12	0.97	1.05	0.85	1.07	1.06	1.00	0.95	0.83
	0.11	0.12	0.09	0.09	0.05	0.06	0.07	0.14	0.09	0.06
HYPO	2.24	2.14	2.10	2.19	2.02	1.94	2.07	2.25	1.89	2.01
	0.09	0.14	0.09	0.11	0.08	0.06	0.05	0.19	0.16	0.13
VTA/SN	0.28	0.26	0.27	0.24	0.27	0.26	0.29	0.28	0.25	0.28
	0.03	0.02	0.03	0.02	0.02	0.03	0.02	0.03	0.03	0.02

Amygdala was completely recovered by 32 wk, nucleus accumbens/olfactory tubercle and striatum were completely recovered by 16 wk, and ventral tegmental area-substantia nigra were completely recovered by 8 wk posttreatment.

Conclusion

High-dose MDMA treatment resulted in long-term decreases in ^{3}H-5-HT synaptosomal uptake (hippocampus), which returned to control values by 16 wk posttreatment. MDMA resulted in decreases in 5-HT tissue concentrations, which showed recovery over the 52-wk test period; the degree of depletion and rate of recovery, however, were region dependent. Cortex and hippocampus were still significantly decreased compared to age-matched controls at 52 wk, although hypothalamus showed a pattern of hyperinnervation at 52 wk in comparison to age-matched controls. The 5-HT innervation of terminal regions after MDMA-induced decreases may represent regrowth from raphe cell bodies or pathways; the time course of innervation may reflect distance from 5-HT containing cells or fiber bundles.

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