

Serotonergic Recovery after ($\pm$)3,4-(Methylenedioxy) Methamphetamine Injury: Observations in Rats

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ABSTRACT

($\pm$)-3,4-Methylenedioxymethamphetamine (MDMA) is a recreational drug of abuse which damages serotonin (5-HT) neurons in animals. In monkeys, the damage appears to be permanent. By contrast, in rats there is indication that neuronal recovery takes place, although there is question as to whether the recovery is sustained. The purpose of the present study was to examine the fate of 5-HT neurons in MDMA-treated rats, and to compare findings in the rat with those in the monkey. Rats were treated with MDMA (10 mg/kg i.p.) every 2 hr for a total dose of 40 mg/kg. Two, 8, 16, 32 and 52 weeks later, groups ($n = 8$) of MDMA-treated rats, along with age-matched controls ($n = 8$), were analyzed for regional brain 5-HT, 5-hydroxyindoleacetic acid and [3]paroxetine-labeled 5-HT uptake sites. Two weeks after MDMA, 5-HT neuronal markers were reduced markedly. Reductions ranged from 42 to 82% depending on brain region. By 16

weeks, there was evidence of recovery in some brain regions (e.g., hypothalamus and striatum) and by 32 weeks, recovery was nearly complete in most brain regions examined. One year after MDMA, recovery was still evident in all brain regions evaluated, although closer inspection of the group data revealed that whereas most MDMA-treated rats recovered, some did not. These few animals had severe and enduring serotonergic deficits in multiple brain regions. Morphologic immunocytochemical studies yielded results which corroborated the neurochemical findings. Together, these observations suggest that 5-HT neurons in most (but not all) rats recover from MDMA injury, and that in those rats which recover, recovery is maintained for at least 1 year after MDMA treatment. Further studies are needed to determine if recovery is sustained for longer than 1 year, and to define the factors which govern 5-HT neuronal recovery after MDMA injury.

MDMA ("Ecstasy"), a ring-substituted methamphetamine derivative, is used for recreational purposes in North America (Peroutka, 1987; Bost, 1988) and Western Europe (Creighton *et al.*, 1991; McGuire and Fahy, 1991; Anonymous, 1992). Since appearing on the illicit drug market, MDMA has been found to exert selective toxic effects on central serotonergic neurons in various species. For example, MDMA neurotoxicity has been documented in rats (Stone *et al.*, 1986; Schmidt, 1987; Commins *et al.*, 1987; Battaglia *et al.*, 1987; O'Hearn *et al.*, 1988; Slikker *et al.*, 1988), guinea pigs (Commins *et al.*, 1987), squirrel monkeys (Ricaurte *et al.*, 1988a,b), cynomolgus monkeys (Wilson *et al.*, 1989), rhesus monkeys (Insel *et al.*, 1989; Kleven *et al.*, 1989; Slikker *et al.*, 1989) and, possibly, humans (Price *et al.*, 1989; Ricaurte *et al.*, 1990).

Although the neurotoxic potential of MDMA is well established, relatively little is known regarding the fate of damaged 5-HT neurons. In particular, it is not known if 5-HT axonal regeneration takes place, such that the original synaptic con-

tacts are re-established. In primates, it appears that MDMA-induced 5-HT neural damage is persistent, and possibly permanent (Insel *et al.*, 1989; Ricaurte *et al.*, 1992). By contrast, in rodents, there is indication that the neurotoxicity of MDMA and related drugs is reversible. For example, rats treated with MDMA show partial recovery of 5-HT, and complete recovery of [3 H]paroxetine-labeled 5-HT uptake sites 1 year after MDMA treatment (Battaglia *et al.*, 1988; De Souza and Battaglia, 1989, 1990). Similarly, rats exposed to 3,4-methylenedioxymphetamine and other toxic amphetamine derivatives show extensive regenerative sprouting of 5-HT axons during the first 6 to 8 months after drug exposure (Molliver *et al.*, 1989, 1990). These findings are in keeping with previous reports of 5-HT neuronal recovery after PCA (Sanders-Bush *et al.*, 1972) and fenfluramine (Harvey and McMaster, 1975; Clineschmidt *et al.*, 1978; Kleven *et al.*, 1988).

Two recent reports suggest that 5-HT recovery in rats exposed to toxic amphetamine derivatives may not be sustained. Zaczek and colleagues (1990) indicate that whereas there is initial recovery of 5-HT neuronal markers in rats treated with

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ABBREVIATIONS: MDMA, 3,4-methylenedioxymethamphetamine; 5-HT, 5-hydroxytryptamine (serotonin); PCA, *p*-chloroamphetamine; 5-HIAA, 5-hydroxyindoleacetic acid; PFC, prefrontal cortex; FC, frontal cortex; PBS, phosphate-buffered saline; ANOVA, analysis of variance.

fenfluramine, recovery is not maintained because severe 5-HT deficits are once again apparent 6 to 8 months after fenfluramine treatment. Similarly, Mamounas and Molliver (1991) report that although there is initial regenerative sprouting of 5-HT axons after PCA injury, 6 to 12 months after PCA 5-HT axons appear reduced in number and abnormal. Together, these reports raise the possibility that 5-HT neuronal damage in the MDMA-treated rat, like that in the MDMA-treated monkey (Insel *et al.*, 1989; Ricaurte *et al.*, 1992), is persistent rather than reversible. As inability of 5-HT neurons to recover from MDMA injury could have implications for recreational MDMA users, the present study was undertaken to: 1) characterize the fate of central 5-HT neurons in MDMA-treated rodents and 2) compare findings in the rat with those obtained recently in the squirrel monkey (Ricaurte *et al.*, 1992).

Materials and Methods

Animals. Male albino Sprague-Dawley rats (Charles River, Wilmington, MA), 77 days of age and weighing approximately 350 g, were used. Rats were housed three per cage in standard polypropylene cages (17 inches $\times$ 10 inches $\times$ 8 inches) in a colony room maintained at $22 \pm 2^\circ\text{C}$, and on a 12-hr light/12-hr dark cycle. Rats had free access to food and water throughout.

Drug treatment. Rats were treated with 10 mg/kg of MDMA (as the hydrochloride salt) 4 times at 2-hr intervals for a total dose of 40 mg/kg. MDMA was given by i.p. Pilot studies showed that this regimen of MDMA was well tolerated, and that it produced a 60 to 80% depletion of regional brain 5-HT and 5-HIAA 2 weeks later.

Brain dissection. Two, 8, 16, 32 and 52 weeks after treatment, eight control rats and eight MDMA-treated rats were sacrificed by decapitation. The brain was removed and dissected over ice. Samples of the striatum and hippocampus were obtained as described previously (Ricaurte *et al.*, 1983). Samples of prefrontal cortex (PFC), frontal cortex (FC), parieto-temporal and occipital cortex were obtained using the König and Klippel (1963) atlas as a reference. Brain samples were wrapped in aluminum foil and stored frozen in liquid nitrogen until assay.

5-HT and 5-HIAA determinations. Regional brain samples were analyzed for their content of 5-HT and 5-HIAA as described recently (Ricaurte *et al.*, 1992). Briefly, samples were homogenized in 0.4 N PCA for 10 to 15 sec and centrifuged at 15,000 rpm in a Dupont Sorvall RC2-B refrigerated centrifuge ($0-4^\circ\text{C}$) for 20 min. The supernatant was collected and stored frozen in liquid nitrogen. 5-HT and 5-HIAA in the supernatant were quantitated by reverse-phase high-performance liquid chromatography coupled to electrochemical detection (Ricaurte *et al.*, 1992).

[^3H]Paroxetine binding measurements. [^3H]Paroxetine-labeled 5-HT uptake sites were measured using the method of Habert *et al.* (1985), with minor modifications described elsewhere (Ricaurte *et al.*, 1992).

Immunocytochemistry. Morphologic immunocytochemical studies of 5-HT-containing nerve fibers were performed in MDMA-treated animals sacrificed 2 ($n = 3$) and 52 weeks ($n = 3$) after drug treatment. Three age-matched control rats were studied in parallel at each time point. Studies were performed with a rabbit antiserum directed at 5-HT using the method of Wilson *et al.* (1989), with minor modification. Briefly, 1 to 2 hr before sacrifice, animals were injected with 10 mg/kg of the monoamine oxidase inhibitor trans-2-phenylcyclopropylamine (i.p.). Under deep chloral hydrate anesthesia (400 mg/kg i.p.) the animals were then perfused by an intracardiac route. After an initial rinse with ice-cold PBS, perfusion was continued using 4% paraformaldehyde and 0.1% glutaraldehyde (pH 7.4). Tissue blocks were placed in buffered 4% paraformaldehyde for 4 to 6 hr and then in 10% dimethylsulfoxide in PBS overnight. Frozen sections (30 μm) were incubated in an anti-5-HT antisera diluted 1:14,000 in PBS with 0.2% Triton X-100 and 1% normal serum at 4°C for 3 days. The antibody

was visualized with the Vectastain ABC-peroxidase method (Vector Laboratories, Inc., Burlingame, CA), and staining was enhanced with the osmiophilic reaction sequence of Gerfen (1985).

Statistical analysis. One-way ANOVA was used to detect significant overall effects. Post-hoc Newman-Keuls multiple range tests were performed to assess the significance of differences among group means. Differences were considered significant when a P value less than .05 was obtained.

Drugs and chemicals. 5-HT creatinine sulfate complex and 5-HIAA dicyclohexylammonium salt were purchased from Sigma Chemical Co. (St. Louis, MO). Perchloric acid was purchased from J. T. Baker, Inc. (Phillipsburg, NJ). [^3H]Paroxetine (specific activity, 21.0 Ci/mmol) was purchased from New England Nuclear (Boston, MA), citalopram hydrobromide was obtained compliments of H. Lundbeck (Copenhagen, Denmark). MDMA was obtained from the National Institute on Drug Abuse (Baltimore, MD). The 5-HT antibody was purchased from the Instar Corp. (Stillwater, MI).

Results

Chemistry

5-HT. Two weeks after MDMA treatment, regional brain 5-HT levels were reduced markedly (fig. 1). The most severely affected region was the occipital cortex, where 5-HT was reduced by 82%. The least affected brain region was the hypothalamus, where 5-HT was reduced by 42%.

Time course studies showed that the earliest recovery of 5-HT occurred in the hypothalamus where 8 weeks after MDMA treatment, 5-HT concentrations were back to control levels (fig. 1). Eight weeks later (16 weeks after MDMA treatment), 5-HT levels had also recovered in the striatum, hippocampus, FC and PFC. By 32 weeks, there was no statistically significant difference between 5-HT levels in control and MDMA animals in any brain region examined, except the FC where a slight depletion was present (fig. 1). Notably, 5-HT levels in this same brain region had been within normal limits in rats examined 16 weeks after MDMA treatment, and there was no significant difference between 5-HT levels in 16- and 32-week MDMA rats (the significant difference was between the 32-week MDMA group and its age-matched control group). Moreover, 20 weeks later (1 year after MDMA treatment), 5-HT levels in the FC were again within normal limits, casting doubt on the significance of the 5-HT depletion found at 32 weeks.

One year after MDMA treatment (5 months after recovery appeared to be complete), 5-HT levels remained within normal limits in all brain regions examined, indicating that the recovery of 5-HT noted at 32 weeks was maintained.

5-HIAA. Recovery of 5-HIAA followed a similar, but not identical, pattern (fig. 2). For example, hippocampal and striatal 5-HIAA levels were recovered completely by 16 weeks, but hypothalamic 5-HIAA levels did not recover totally until 32 weeks after MDMA treatment. Recovery of 5-HIAA in frontal cortical regions also lagged slightly behind that of 5-HT. By 32 weeks, 5-HIAA levels were within normal limits in all brain regions analyzed.

Similar to the recovery of 5-HT, the recovery of 5-HIAA appeared to be maintained for at least 52 weeks after MDMA treatment in most brain regions examined (fig. 2). The only exceptions were the PFC and FC, where 5-HIAA levels in MDMA rats were slightly lower than in age-matched controls. However, as before, there was no significant difference between the 32- and 52-week MDMA groups, again raising doubt regarding the significance of 5-HIAA depletions in the PFC and FC at 52 weeks.

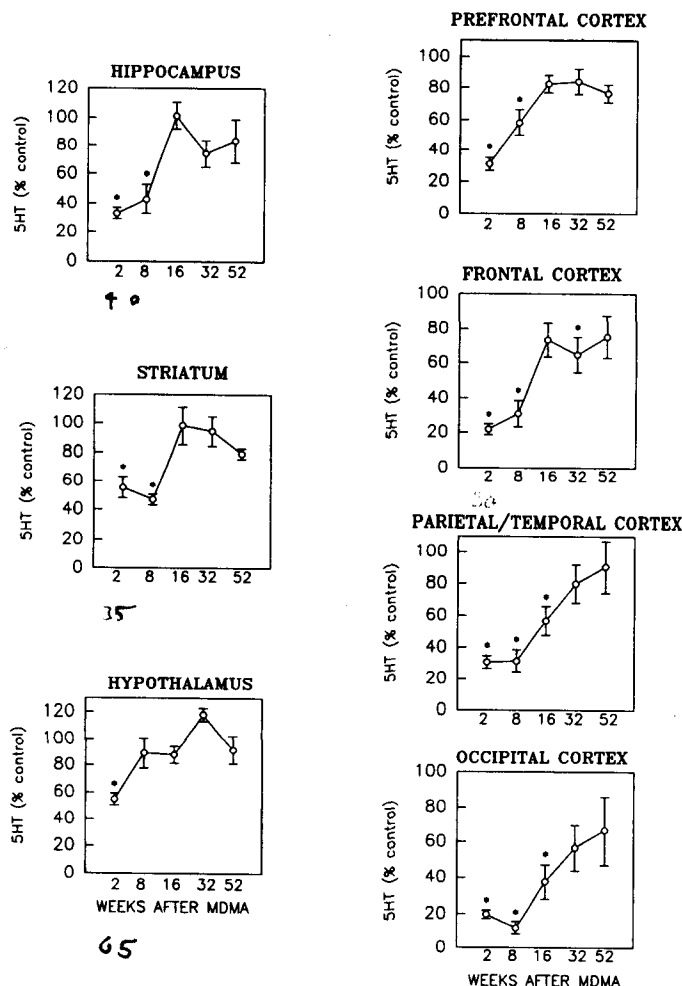


Fig. 1. Regional brain 5-HT levels over time in MDMA-treated rats. MDMA (10 mg/kg) was given i.p. 4 times at 2-hr intervals for a total dose of 40 mg/kg. Rats were sacrificed 2, 8, 16, 32 and 52 weeks after MDMA treatment. Values of 5-HT were expressed in nanograms per milligram of tissue and transformed to percentages of age-matched controls. Values for the 2-week control rats were: hippocampus, 0.170 ± 0.009 ; striatum, 0.246 ± 0.019 ; hypothalamus, 0.480 ± 0.027 ; PFC, 0.257 ± 0.013 ; FC, 0.163 ± 0.010 ; parieto-temporal cortex, 0.201 ± 0.014 ; and occipital cortex, 0.087 ± 0.011 . There were no significant differences between regional brain 5-HT levels in 2- and 52-week controls. Each point represents the mean $\pm$ S.E.M. *A significant difference from age-matched controls, $P < .05$, ANOVA followed by Newman-Keuls' multiple range test.

[³H]Paroxetine binding. [³H]Paroxetine binding (fig. 3), like 5-HT (fig. 1) and 5-HIAA (fig. 2), recovered over time in MDMA-treated rats. In the cerebral cortex and striatum, [³H]paroxetine binding returned to control levels by 32 weeks and remained within normal limits at 52 weeks. In the hippocampus, [³H]paroxetine binding also recovered, but not completely because it was still reduced by 29% at 52 weeks (fig. 3).

Individual data points. Closer inspection of the group data revealed that whereas most rats recovered, some did not. In particular, there appeared to be a few rats which had severe and persistent serotonergic deficits in multiple brain regions. To assess the significance of these deficits, it was necessary to develop means by which to determine whether a low value reflected lack of recovery or possible laboratory error. Therefore, criteria were established to define rats as recovered or unrecovered. Recovered rats were defined as those that had 5-

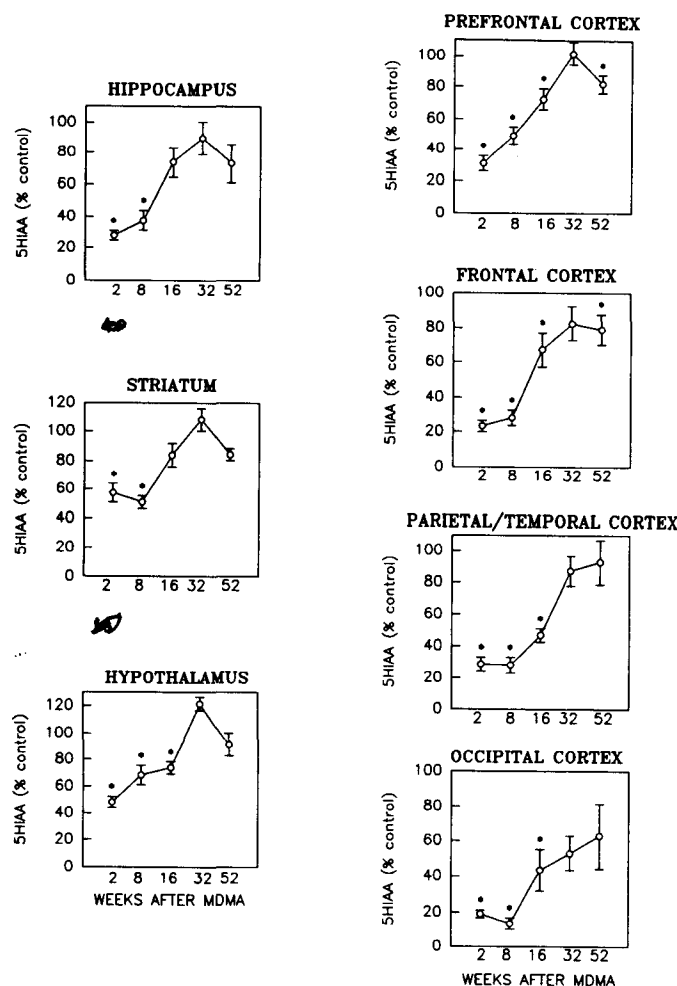


Fig. 2. Regional brain 5-HIAA levels over time in MDMA-treated rats. Rats were treated with 10 mg/kg of MDMA 4 times at 2-hr intervals for a final dose of 40 mg/kg. Rats were sacrificed 2, 8, 16, 32 and 52 weeks after treatment. Values of 5-HIAA expressed in nanograms per milligram of tissue were transformed to percentages of age-matched controls. Values for the 2-week control rats were: hippocampus, 0.242 ± 0.015 ; striatum, 0.394 ± 0.033 ; hypothalamus, 0.382 ± 0.012 ; PFC, 0.168 ± 0.009 ; FC, 0.141 ± 0.007 ; parieto-temporal cortex, 0.208 ± 0.007 , and occipital cortex, 0.090 ± 0.007 . Each point represents the mean $\pm$ S.E.M. *A significant difference from age-matched controls, $P < .05$, ANOVA followed by Newman-Keuls' multiple range test.

HT, 5-HIAA and [³H]paroxetine binding values that were at least two S.D.s higher than the mean 2-week value (defined as the time of maximal deficit measured in this study). By contrast, unrecovered rats were defined as those that had values which were within 2 S.D.s of the mean 2-week value. Furthermore, to be considered unrecovered, a rat had to have low values in four or more brain regions.

By using these arbitrary but stringent criteria, at least one animal in each of the 16-, 32- and 52-week MDMA groups met requirements to be considered unrecovered. When age-matched control rats at each time point were tested in an identical manner (relative to 2-week controls), none met the same criteria (i.e., no animals in the 8-, 16-, 32- and 52-week control groups had such low values).

Anatomy

Immunocytochemical studies. Two weeks after drug treatment, three of three MDMA-treated rats showed a reduc-

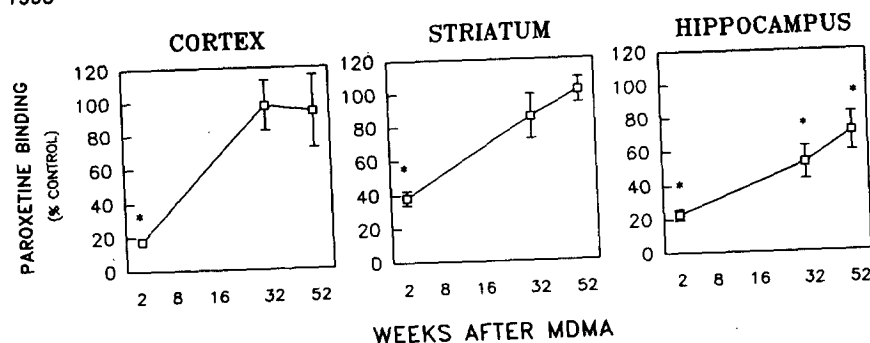


Fig. 3. [^3H]Paroxetine-labeled 5-HT uptake sites over time in MDMA-treated rats. Rats were treated with MDMA (10 mg/kg) 4 times at 2-hr intervals for a total dose of 40 mg/kg. Rats were sacrificed 2, 32 and 52 weeks after MDMA treatment. Values were transformed to percentages of age-matched controls. [^3H]Paroxetine binding site densities in control rats were as follows: cortex, 22.44 ± 2.38 pmol/mg; striatum, 23.25 ± 1.90 pmol/mg; and hippocampus, 16.84 ± 1.64 pmol/mg. Each point represents the mean $\pm$ S.E.M. *A significant difference from control, $P < .05$, ANOVA followed by Newman-Keuls' multiple range test.

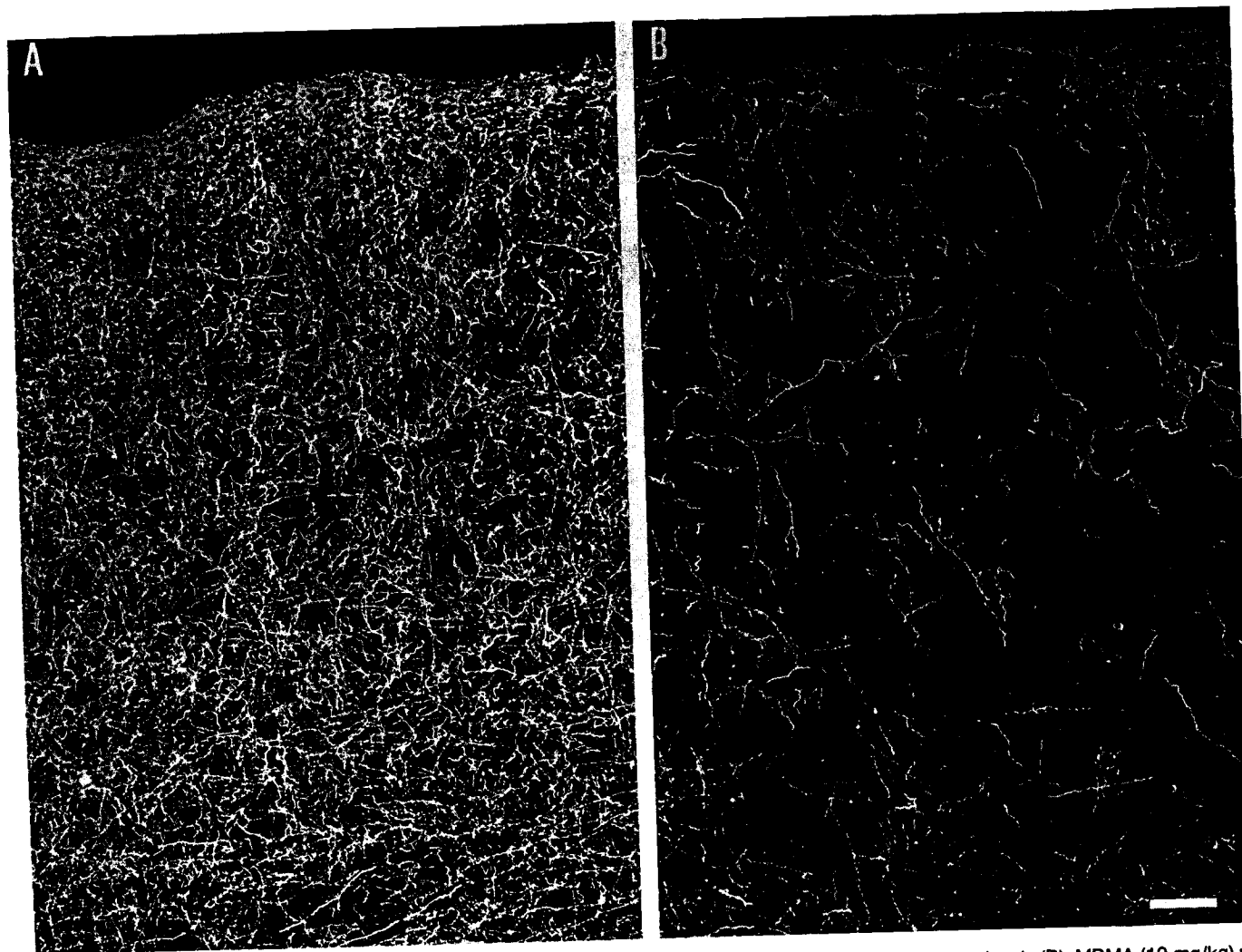


Fig. 4. 5-HT-immunoreactive axons in the parietal cortex of a control rat (A) and a rat given MDMA 2 weeks previously (B). MDMA (10 mg/kg) was given i.p. 4 times at 2-hr intervals for a total dose of 40 mg/kg. Note reduced 5-HT axon density in MDMA-treated animal. Bar indicates 100 μm .

tion in 5-HT axon density (fig. 4). Fifty-two weeks later, one of the three MDMA-treated rats had recovered (fig. 5). By contrast, the other two animals appeared to belong to the small group of animals that does not recover, because they continued to show reduced 5-HT axon density (fig. 6).

Notably, the density of 5-HT axons in the more caudal portions of the dorsal neocortex of older control rats appeared to be reduced relative to younger control animals (compare fig. 5A with fig. 4A). Whether this is due to some aspect of the tissue preparation (e.g., perfusion) or whether it represents a true age-related loss of 5-HT axons is unclear, but older rats (both control and MDMA-treated) also showed 5-HT axonal

tangles (fig. 5A, arrow) of the type described recently by various investigators (van Luijckelaar *et al.*, 1988, 1989; Mamounas and Molliver, 1991; Marielle *et al.*, 1992). Although quantitative studies of these axonal tangles were not carried out, they appeared to occur with equal frequency in the cerebral cortex of older control rats and older MDMA-treated rats.

Discussion

The results of this combined chemical and anatomical study indicate that 5-HT neurons in most, but not all, rats recover from MDMA injury. The neurochemical data show that in rats

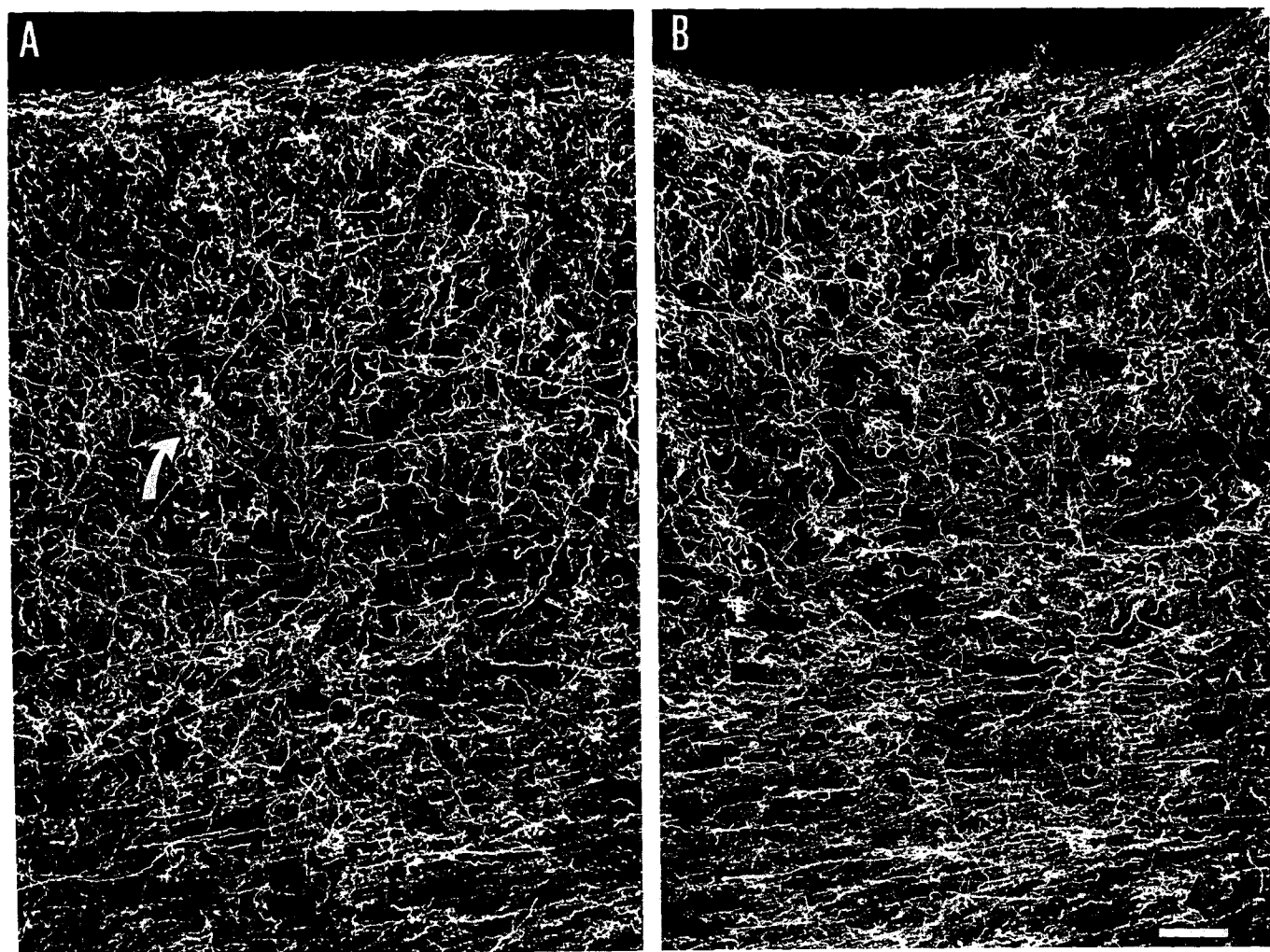


Fig. 5. 5-HT-immunoreactive axons in the parietal cortex of an older control rat (A) and a rat given MDMA 1 year previously (B). MDMA (10 mg/kg) was given i.p. 4 times at 2-hr intervals for a total dose of 40 mg/kg. Note axonal changes in the older control (A, arrow). Also note that 5-HT axon density in the control and the MDMA-treated rat (recovered animal) does not appear to be appreciably different. Bar indicates 100 μ m.

which recover, 5-HT, 5-HIAA, and [3 H]paroxetine-labeled 5-HT uptake sites return to control levels 16 to 32 weeks after MDMA treatment. The neuroanatomical data, although more limited, corroborate the biochemical data, and show that recovery of presynaptic 5-HT neuronal markers is most likely related to regeneration of 5-HT-containing axons, as suggested by Molliver and colleagues (1989, 1990). Taken together, these results attest to the plasticity of central 5-HT neurons (Azmitia *et al.*, 1978; Bjorklund *et al.*, 1981; Jacobs and Azmitia, 1992) and indicate that under certain conditions, 5-HT neurons in most MDMA-treated rats are able to recover from MDMA injury.

Although ascending 5-HT axonal projections in most MDMA-treated rats recover, those in some do not. In particular, some MDMA-treated rats continue to show severe 5-HT deficits as long as 52 weeks after MDMA exposure. Serotonergic deficits in these few animals are present in multiple brain regions, and there is reasonably good (although not perfect) correspondence among the various markers (*i.e.*, 5-HT, 5-HIAA and paroxetine binding are comparably reduced). These parallel reductions, coupled with the fact that "unrecovered" animals could only be identified in the MDMA group (*i.e.*, no animals in the control group had such low values) argue against the

possibility that persistent 5-HT deficits observed are related to laboratory error or variability among animals. The factors responsible for lack of recovery in some MDMA-treated rats are unknown. However, it may be that 5-HT neurons in rats showing persistent 5-HT deficits are initially damaged more severely than those in rats which recover. Alternatively, it may be that recovery is influenced by factors other than the extent of initial neuronal damage [*e.g.*, availability of a target-derived neurotrophic substance (Zhou *et al.*, 1987)]. More information is needed regarding the factors which govern 5-HT axonal regeneration after MDMA injury.

An important feature of the serotonergic recovery described here is that it is sustained for at least 52 weeks after MDMA injury. This contrasts with reports that serotonergic deficits reappear in rats treated with fenfluramine (Zaczek *et al.*, 1990) and PCA (Mamounas and Molliver, 1991). Although the basis for the differing observations is unclear, sustained recovery in this study may be related to the fact that serotonergic damage induced by MDMA was less pronounced than that induced by PCA and fenfluramine in the aforementioned studies. If correct, the proposal that the likelihood of recovery after neurotoxic amphetamines is related inversely to the severity and extent of initial neuronal damage could help explain not only why most

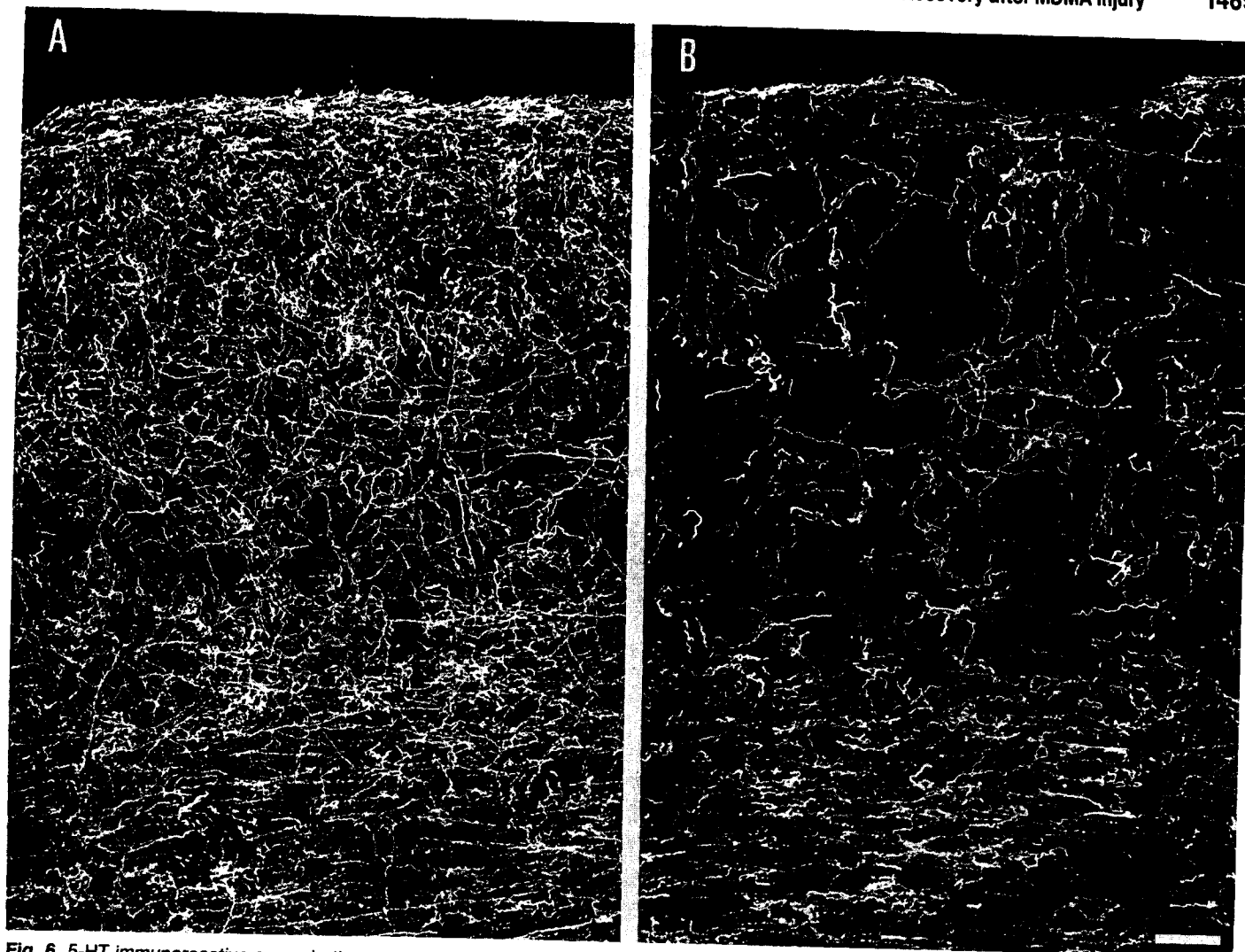


Fig. 6. 5-HT-immunoreactive axons in the parietal cortex of a control rat (A) and a rat given MDMA 1 year previously (B). MDMA (10 mg/kg) was given i.p. 4 times at 2-hr intervals for a total dose of 40 mg/kg. Note that 5-HT axon density appears reduced relative to that in an age-matched control processed in parallel. Bar indicates 100 μ m.

MDMA-treated rats recover, but also why MDMA-treated monkeys [which tend to be more severely affected than rats (Ricaurte *et al.*, 1988b; De Souza and Battaglia, 1989; Slikker *et al.*, 1989)] fail to recover from MDMA injury (Insel *et al.*, 1989; Martello *et al.*, 1990; Ricaurte *et al.*, 1992).

Comparison of recovery in different regions of the rat brain reveals that serotonergic recovery is slowest (and possibly also least complete) in the occipital cortex (fig. 1). This is in keeping with the suggestion that 5-HT axonal recovery after neurotoxic amphetamine derivatives follows a fronto-occipital gradient (Molliver *et al.*, 1989, 1990). However, slow recovery in the occipital cortex could also be related to the fact that, 2 weeks after MDMA treatment, the occipital cortex is the most severely affected brain region. The possibility that extent of 5-HT axonal recovery is influenced by both 1) the distance that a severed axon has to travel before re-establishing synaptic contact and 2) the extent of the initial neuronal insult needs to be considered.

The present results are not in full agreement with some of the findings of De Souza and Battaglia (1989). In particular, these investigators found complete recovery of [3 H]paroxetine-labeled 5-HT uptake sites, but only partial recovery of 5-HT

levels 1 year after MDMA treatment. By contrast, both 5-HT levels and uptake site density recovered completely in this study. Moreover, rats in this study also showed near total recovery of a third serotonergic presynaptic marker, 5-HIAA (fig. 1). It is conceivable that the results of the two studies differ because different MDMA treatments were used (10 mg/kg/2 hr $\times$ 4 *vs.* 20 mg/kg twice daily for 4 days). This possibility seems unlikely, however, because both MDMA treatments produced comparable initial 5-HT deficits, and the two studies are similar in most other respects. Another possibility is that the different results are related to sampling factors. Specifically, it may be that one or two of the animals in the De Souza and Battaglia (1989) study had persistent (rather than reversible) 5-HT deficits, and that averaging of low 5-HT levels in these animals with normal 5-HT levels in the recovered animals could give rise to "partial" recovery for the group. Although speculative, this explanation is in keeping with the findings of this study and, if correct, would reconcile the findings of the two investigations.

One brain region that did not show complete recovery of all presynaptic 5-HT neuronal markers 1 year after MDMA treatment was the hippocampus. This brain region showed complete

recovery of 5-HT and 5-HIAA (fig. 1), but only partial recovery of [3 H]paroxetine labeled 5-HT uptake sites (fig. 2). Further studies are needed to ascertain whether the incomplete recovery of 5-HT uptake is indicative of an abnormality in the 5-HT axons reinnervating the hippocampus or whether, after survival times longer than 1 year, the density of 5-HT uptake sites returns to control levels.

The results of this study do not support the hypothesis that exposure to MDMA and related drugs in young adulthood leads to what has been termed "accelerated aging" of brain 5-HT neurons in later life (Mamounas and Molliver, 1991). In chemical studies, there was little indication that 5-HT parameters declined with age, either in control rats or in rats treated with MDMA 1 year previously. In anatomical studies, MDMA-treated rats did show evidence of reduced 5-HT axon density and altered 5-HT axon morphology (tangles). However, these changes were also evident in age-matched control animals, and appeared to develop as a function of age, as maintained by van Lijstelaar *et al.* (1989) and Marielle *et al.* (1992), rather than as a consequence of MDMA exposure. Further studies are needed to assess the effect of aging on 5-HT neurons damaged previously by MDMA.

Because this study relied upon post-mortem analysis of presynaptic 5-HT neuronal markers, it was impossible to monitor the status of 5-HT projections in MDMA-treated rats longitudinally. This makes it difficult to exclude the possibility that the few rats which showed persistent 5-HT deficits did not in fact, at some early time point, undergo transient recovery of presynaptic 5-HT neuronal markers which then dissipated. Although this possibility merits consideration, it seems unlikely for two reasons. First, MDMA-treated rats were examined with some frequency (2, 8, 16, 32 and 52 weeks after MDMA), and there was never a time at which all animals showed complete recovery. Second, neurochemical recovery related to axonal regeneration is not a rapid event but a slow gradual process which takes months to complete (Bjorklund *et al.*, 1981; Jacobs and Azmitia, 1992).

Comparison of the present results with those in the primate reveals two major differences. The first is that the initial neurotoxic effects of MDMA in the rat tend to be less severe than those in the monkey, where greater than 90% depletions of cortical 5-HT are obtained (Ricaurte *et al.*, 1988b). The second is that although the monkey shows little tendency to recover (Insel *et al.*, 1989; Ricaurte *et al.*, 1992), the rat shows considerable recovery over time. Whether these differences are related to pharmacokinetic or pharmacodynamic factors remains to be determined.

In summary, the results of the present study indicate that central 5-HT neurons in most (but not all) rats recover from MDMA injury. Furthermore, they indicate that, in those rats which recover, recovery is maintained for at least 1 year after MDMA treatment. The sustained nature of the recovery in the MDMA-treated rat is in keeping with the view that central 5-HT neurons in the rodent have marked regenerative potential (Azmitia *et al.*, 1978; Bjorklund *et al.*, 1981; Jacobs and Azmitia, 1992), and suggests that, at least under some conditions, sustained recovery from MDMA injury is possible. Finally, the finding that there are individual differences which render some animals more susceptible than others to MDMA neurotoxicity is noteworthy. Such individual differences could account for some of the untoward effects of MDMA in humans (Creighton *et al.*, 1991; McGuire and Fahy, 1991; McCann and Ricaurte,

1991), and may suggest that some recreational MDMA users are at higher risk than others for sustaining permanent 5-HT neural injury.

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ERRATUM

D. R. Brown, A. M. Parsons and S. M. O'Grady: Substance P produces sodium and bicarbonate secretion in porcine jejunal mucosa through an action on enteric neurons. *J. Pharmacol. Exp. Ther.* **261**(3): 1206-1212, 1992.

The polarities indicated for the net residual flux (J^R) values reported in this paper should display the opposite sign (*i.e.*, "-" values should have appeared as "+" and *vice versa*). Moreover, the formula used in calculating the J^R (appearing on page 1207) should read: $J^R = (J^{Na}_{net} - J^{Cl}_{net}) + I_{sc}$.

The authors regret these typographical errors.