Reorganization of Ascending 5-HT Axon Projections in Animals Previously Exposed to the Recreational Drug ($\pm$)3,4-Methylenedioxymethamphetamine (MDMA, “Ecstasy”)

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The recreational drug ($\pm$)3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”) is a methamphetamine derivative that selectively destroys central 5-HT axons and axon terminals in animals and, possibly, humans. The fate of 5-HT neurons following MDMA injury is uncertain. In particular, while it is known that central 5-HT axons can undergo regenerative sprouting after MDMA injury, it has not been determined whether they reestablish the original innervation pattern. To address this question, the present studies examined 5-HT innervation patterns in animals lesioned with MDMA 12–18 months previously. Both rodents (rats) and nonhuman primates (squirrel monkeys) were examined, since there is indication that serotonergic recovery after MDMA injury may be species dependent. 5-HT axon projections were studied neurochemically, autoradiographically and immunocytochemically. In both rodents and nonhuman primates previously lesioned with MDMA, substantial serotonergic axonal sprouting was observed. However, in a few rats and in most squirrel monkeys, the reinnervation pattern was highly abnormal: distant targets (e.g., dorsal neocortex) remained denervated, while some proximal targets (e.g., amygdala, hypothalamus) were reinnervated or hyperinnervated. Although the specific determinants of axonal recovery after MDMA injury remain to be identified, it appears that axons which initially sustain more severe damage, are longer, or are more highly arborized have low probability of recovering. The observation that some brain regions remain denervated, while others are reinnervated or hyperinnervated suggests that, under some circumstances, MDMA injury can lead to a lasting reorganization of ascending 5-HT axon projections. Such lasting changes in brain innervation, documented here in MDMA-treated animals, may have implications for humans using MDMA recreationally.

[Key words: 5-HT, neurotoxicity, amphetamines, sprouting, regeneration, reinnervation]

Despite multiple regulatory efforts (Anonymous, 1985; Lawn, 1986, 1988), the synthetic amphetamine derivative ($\pm$)3,4-methylenedioxymethamphetamine (MDMA, “ecstasy”) has gained

status as a popular recreational “designer” drug in various parts of the world (Haislip, 1989; Abbot and Concar, 1992; Solowij and Hall, 1992; Cregg and Tracey, 1993). In the United States, where recreational use of MDMA first became apparent in 1985 (Baum, 1985; Siegel, 1986), recent reports indicate that MDMA use is increasing (Cuomo and Dymont, 1994; McDowell and Kleber, 1994; Newmeyer, 1994). In Great Britain, increasing use of MDMA has also been noted (Henry, 1992; Randall, 1992), and one survey indicates that illicit use of MDMA and related drugs in the United Kingdom ranks second only to the use of marijuana (Anonymous, 1992).

This apparent widespread increase in recreational MDMA use is of concern because there is extensive preclinical evidence that MDMA is a potent 5-HT neurotoxin (for reviews, see McKenna and Peroutka, 1990; Steele et al., 1994). Animals given MDMA show large depletions of brain 5-HT, as well as marked reductions in the activity of tryptophan hydroxylase, the concentration of 5-hydroxyindoleacetic acid (5-HIAA), and the number of 5-HT transporter sites (Stone et al., 1986; Battaglia et al., 1987; Commins et al., 1987; Schmidt, 1987; Ricaurte et al., 1988b; Slikker et al., 1988; Insel et al., 1989; Kleven et al., 1989; De Souza et al., 1990). Correlative anatomic studies indicate that loss of presynaptic 5-HT axonal markers in MDMA-treated animals is related to damage of 5-HT axons and axon terminals (Battaglia et al., 1987; Commins et al., 1987; O’Hearn et al., 1988; Wilson et al., 1989; Molliver et al., 1990; Mamounas and Molliver, 1991). Since doses of MDMA that produce neurotoxic effects in nonhuman primates (Ricaurte et al., 1988) closely approach those typically used by humans (Seymour, 1986; Doblin, 1989), increasing recreational use of MDMA may pose a significant public health problem.

Although the neurotoxic potential of MDMA in animals is now well established, the fate of 5-HT neurons after MDMA injury remains uncertain. In particular, while it is known that central 5-HT neurons can undergo regenerative sprouting after MDMA injury (Battaglia et al., 1988; Molliver et al., 1990; Scanzello et al., 1993), it has not been determined whether they reestablish a normal innervation pattern. Further, there is indication that axonal sprouting after MDMA injury may be species dependent, occurring in rodents (Battaglia et al., 1988; Molliver et al., 1990; Scanzello et al., 1993), but not nonhuman primates (Insel et al., 1989; Ricaurte et al., 1992). In light of these uncertainties, the present studies were undertaken to characterize the organization of ascending 5-HT axon projections in rodents and nonhuman primates lesioned with MDMA 12–18 months previously. The purpose of these studies was to determine if

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Figure 1. 5-HT axon markers in various regions of rat brain 2 weeks after MDMA treatment (10 mg/kg every 2 hr $\times$ 4, for a total dose of 40 mg/kg). Values shown represent the mean $\pm$ SEM. Values of 5-HT and 5-HIAA were expressed in ng/mg of tissue and transformed to percentages of controls. Values for 5-HT in control rats were as follows: prefrontal cortex, 0.34 ± 0.03 ; frontal cortex, 0.32 ± 0.02 ; parietal cortex, 0.31 ± 0.03 ; striatum, 0.72 ± 0.10 ; hippocampus, 0.31 ± 0.04 ; hypothalamus, 0.70 ± 0.07 . Values for 5-HIAA in control rats were as follows: prefrontal cortex, 0.16 ± 0.01 ; frontal cortex, 0.16 ± 0.01 ; parietal cortex, 0.17 ± 0.01 ; striatum, 0.50 ± 0.07 ; hippocampus, 0.32 ± 0.03 ; hypothalamus, 0.28 ± 0.02 . Values of ^3H -citalopram binding were expressed in nCi/mg tissue, and also transformed to percentages of controls. Values for ^3H -citalopram binding in control rats were as follows: prefrontal cortex, 5.1 ± 0.1 ; frontal cortex, 6.3 ± 0.2 ; parietal cortex, 5.7 ± 0.2 ; striatum, 5.2 ± 0.2 ; hippocampus, 4.2 ± 0.6 ; hypothalamus, 17.1 ± 1.9 . * Designates significant difference compared to control (ANOVA followed by Duncan's multiple range test, $P < 0.05$).

after MDMA injury normal 5-HT innervation patterns were reestablished.

Materials and Methods

Subjects. Thirty-two male albino Sprague-Dawley rats (Charles River, Wilmington, MA) 80 d of age, weighing approximately 350 gm, and 15 adult squirrel monkeys (*Saimiri sciureus*) of both sexes, ranging in weight from 499 to 906 gm, were used in this study. Rats were housed individually in suspended wire-mesh cages; squirrel monkeys were housed two per cage in standard stainless steel primate cages, except during drug treatment (when they were housed singly). All animals were used in accordance with institutional guidelines, and procedures were approved by an animal welfare committee. Temperature in the colony rooms was maintained at $22 \pm 2^\circ\text{C}$. Artificial lighting in the colony rooms was maintained on a 12 hr light/12 hr dark cycle. Animals had free access to food and water throughout. The facility for housing and care of the animals is accredited by the American Association for the Accreditation of Laboratory Animal Care.

Drug treatment. MDMA, as the hydrochloride salt, was dissolved in a sterile 0.9% sodium chloride solution and administered on an mg/kg basis. Rats received four intraperitoneal doses of 10 mg/kg MDMA at

Figure 2. 5-HT axon markers in various regions of the rat brain 52 weeks after MDMA treatment (10 mg/kg every 2 hr $\times$ 4, for a total dose of 40 mg/kg). Values shown represent the mean ($\pm$ SEM) expressed in ng/mg (5-HT and 5-HIAA) or nCi/mg tissue (^3H -citalopram binding). Absolute values for controls are given in the Figure 1 caption. * Designates significant difference compared to control (ANOVA followed by Duncan's multiple range test, $P < 0.05$).

2 hr intervals, for a total dose of 40 mg/kg. Monkeys also received a total dose of 40 mg/kg, but they received MDMA subcutaneously, at a dose of 5 mg/kg twice daily (0900 and 1700 hr) for four consecutive days, for a total of eight doses. Control animals received equivalent volumes of saline. Dose was expressed as the salt. The aforementioned drug regimens were employed because they have been previously shown to reliably produce large serotonergic deficits in rodents (Scan-zello et al., 1993) and nonhuman primates (Ricaurte et al., 1988b; Ricaurte et al., 1992). Further, these regimens are well tolerated by the majority of the animals, and their use allowed comparison of the present results with those of previous studies.

Quantitative autoradiography. Autoradiographic studies of the 5-HT transporter were carried out using ^3H -citalopram (D'Amato et al., 1987; Lidow et al., 1989). Serial coronal 20 μm brain sections were cut using a cryostat at -20°C , mounted onto gel/alum coated microscope slides and refrozen at -20°C prior to transfer to a -80°C freezer until needed. A series of kinetic and pharmacologic studies was carried out to define the incubation parameters and to ascertain the properties of ^3H -citalopram binding on slide-mounted rat and monkey sections and tissue homogenates. The sections were subsequently incubated with a saturating concentration of ^3H -citalopram (4.5 nM), rinsed with 50 mM Tris buffer containing 120 mM NaCl and 5 mM KCl (pH 7.4), then quickly dipped in distilled water and dried on a slide warmer at 33°C . Autoradiograms were generated by apposing the sections (along with standards) to tritium-sensitive Ultrosfilm (Leica, Inc.) for appropriate (empirically determined) periods of time. After exposure, the film was developed with Kodak D-19 developer, placed in stop bath for 30 sec, and fixed in Kodak Rapid Fixer for 4 min. Developed films were illuminated with a Northern Light Box and digitized with a solid-state video camera coupled to an imaging system designed for quantitative densitometry (MCID System, Imaging Research Inc., St. Catherine's, Canada). Regional brain densitometric readings were performed on at least four adjacent sections at each level (for levels, see Figs. 3, 6 and captions),

Figure 3. Autoradiograms showing the rostrocaudal distribution of ^3H -citalopram-labeled 5-HT transporter sites in coronal brain sections of a control rat (*top panels*) and a rat treated with MDMA 52 weeks previously and showing an abnormal reinnervation pattern. From left to right, section levels correspond to plates 12, 18, 29, and 32 in the Palkovits and Brownstein (1988) atlas. The scale on right shows the density of binding sites designated by color, expressed in nCi/mg tissue.

and a calibration curve was obtained from standards processed in parallel. Standards containing the same isotope as that used to label the ligand were used, and optical density values outside the linear range were discarded. Specific binding was defined as the difference between total binding and nonspecific binding, with nonspecific binding being defined as the amount of binding taking place in the presence of 1 μ M paroxetine hydrochloride. Data were expressed in nCi/mg tissue. Scatchard transformation and calculation of K_D and B_{max} values were performed using EBDA and LIGAND software (Biosoft).

Indole determinations. Regional brain levels of 5-HT and 5-HIAA were measured by means of high performance liquid chromatography coupled with electrochemical detection (HPLC-EC), as described previously (Ricaurte et al., 1992).

Immunocytochemical studies. Anatomic studies of ascending 5-HT-containing axon projections were performed using a rabbit antiserum directed at 5-HT, employing the method of Wilson et al. (1989), with minor modification (Scanzello et al., 1993). Briefly, 1–2 hr prior to sacrifice, animals received 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 perfused by an intracardiac route. After an initial rinse with ice-cold PBS (850 ml perfused at a rate of approximately 150 ml/min), perfusion was continued using 4% paraformaldehyde and 0.1% glutaraldehyde (pH 7.4) (3600 ml perfused at a rate of approximately 150 ml/min). Tissue blocks were placed in buffered 4% paraformaldehyde for 4–6 hr and then in 10% dimethylsulfoxide in PBS overnight. Frozen sections (30 μ m) were incubated in an anti-5-HT antiserum diluted 1:14,000 in PBS with 0.2% Triton X-100 and 1% normal serum at 4°C for 3 d. 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 analysis of variance (ANOVA) was used to detect significant overall effects. Post hoc Duncan's multiple range tests were performed to assess the significance of differences between group means. Correlations were assessed by Pearson's product moment. Differences were considered significant when a P value less than 0.05 was obtained. All tests were two-tailed.

Drugs and chemicals. 5-Hydroxytryptamine (5-HT, 5-HT) creatinine sulfate complex and 5-hydroxyindole-3-acetic acid (dicyclohexylammonium salt) were purchased from Sigma Chemical Co. (St. Louis, MO). Perchloric acid was purchased from J. T. Baker Inc. (Phillipsburg, NJ). 3 H-Citalopram (specific activity, 38.1 Ci/mmol) was purchased from New England Nuclear (Boston, MA). ($\pm$)-3,4-Methylenedioxymethamphetamine hydrochloride was obtained from the National Institute on Drug Abuse. The 5-HT antibody was purchased from the Incstar Corp., Stillwater, MN.

Results

Rodents. Two weeks after MDMA treatment, serotonergic axonal markers were markedly reduced in multiple regions of the rat brain. Reductions were most severe in the cerebral cortex, striatum, and hippocampus; serotonergic deficits were least pronounced in the hypothalamus (Fig. 1). In keeping with previous results (Battaglia et al. 1988; Molliver et al., 1990; Scanzello et al., 1993), rats examined 52 weeks after MDMA treatment, as a group, showed evidence of serotonergic recovery (Fig. 2). Recovery was complete or nearly complete by 52 weeks for all markers in all brain regions examined (no intermediate time points were examined). The only exception was 3 H-citalopram binding in the hippocampus, which remained partially reduced. In the hypothalamus, all markers were above control levels 52 weeks after MDMA treatment (Fig. 2).

Within the group of rats examined 52 weeks after MDMA treatment, a few (3 of 16) rats were found that had large, persistent reductions in serotonergic axonal markers in multiple regions of the forebrain (e.g., dorsal neocortex, striatum, hippocampus) but an increase in serotonergic markers in the basal forebrain (Fig. 3). For example, as can be seen in the figure, these animals had marked decreases in 3 H-citalopram binding in

Figure 4. Density of 3 H-citalopram-labeled 5-HT uptake sites in the dorsal neocortex and lateral hypothalamus of five rats treated with MDMA 52 weeks previously. Note that the total number of sites is relatively constant across animals. Also note the inverse relation between the number of sites in the dorsal neocortex and hypothalamus.

the dorsal neocortex, but increases in the hypothalamus and other regions of the basal forebrain.

Further analysis of the autoradiographic data revealed a high correlation between the magnitude of decreases in binding sites in the dorsal neocortex and the magnitude of increases in binding sites in the hypothalamus ($r = 0.94$) (Fig. 4). Moreover, while there was a redistribution of 3 H-citalopram binding sites in these animals, the density of transporter sites at three different coronal brain levels (plates 15, 25, and 31 of the rat brain atlas by Palkovits and Brownstein, 1988) was the same as in controls (control, 9.25 ± 1.46 fmol/mg; MDMA, 9.41 ± 1.85 fmol/mg).

Changes in 5-HT, 5-HIAA, and 3 H-citalopram binding were associated with changes in the density of 5-HT-immunoreactive axons (Fig. 5). Of 16 rats examined 52 weeks after MDMA exposure, 3 showed an altered pattern of distribution of 5-HT axon markers such as that depicted in Figures 3 and 5; the others had patterns that approached, to varying degrees, those of control animals.

Nonhuman primates. To explore the species generality of these findings, similar studies were carried out in squirrel monkeys treated with MDMA (5 mg/kg, s.c., twice daily for 4 d) 2 and 72 weeks previously. Two weeks after MDMA treatment, profound decrements in 5-HT axonal markers were evident in all regions of the monkey brain examined (see below). Seventy-two weeks after MDMA treatment, autoradiographic studies revealed persistent and equally profound reductions in 3 H-citalopram binding in many regions of the monkey forebrain, including the neocortex, hippocampus, caudate nucleus, putamen, and many nuclei of the thalamus (Fig. 6). However, as is evident in the figure, there were marked increases in 3 H-citalopram binding in the hypothalamus and other basal forebrain regions.

Immunocytochemical studies revealed a close association between changes in the density of 5-HT-immunoreactive axons and changes in the density of 3 H-citalopram binding sites. For example, the marked decrease in 3 H-citalopram binding in the dorsal neocortex (Fig. 6) was associated with a marked decrease in the density of 5-HT-immunoreactive axons (Fig. 7); similarly, the increase in 3 H-citalopram binding in the hypothalamus (Fig. 6) was associated with an increase in the density of 5-HT-immunoreactive axons (Fig. 7). Of 17 MDMA-treated monkeys

Figure 5. 5-HT-immunoreactive axons in the dorsal neocortex and hypothalamus of a control rat (*A*, *C*) and a rat treated with MDMA 52 weeks previously (*C*, *D*). Note the reduction in axon density in the cerebral cortex (*B*), and the apparent increase in axon density in the hypothalamus (*D*). Dark-field photomicrographs; scale bar, 100 μ m.

Figure 6. Autoradiograms showing the rostrocaudal distribution of ^3H -citalopram-labeled 5-HT transporter sites in coronal brain sections of a control monkey (*top panels*) and a monkey treated with MDMA (*bottom panels*) 18 months previously. Note the redistribution of sites in the MDMA-treated monkey. The scale on right shows the density of binding sites designated by color, expressed in nCi/mg tissue.

Control

MDMA - 2 Wks

MDMA - 18 Mos

Cerebral Cortex

Hypothalamus

Globus Pallidus

thus far evaluated, 14 have shown changes consistent with abnormal reinnervation, although the degree of abnormality has varied among animals.

Discussion

The major finding of the present study is that following MDMA injury there is a lasting reorganization of ascending 5-HT axon projections, with projections to distant forebrain targets (e.g., dorsal neocortex) showing little or no evidence of recovery, while projections to some more proximal targets (e.g., hypothalamus) recover fully or in excess. The present results also indicate that altered reinnervation patterns develop much more frequently in MDMA-treated primates than in MDMA-treated rodents. While the factors responsible for this striking species difference are poorly understood, it may be related to the size or severity of the initial lesion, since 5-HT axon markers are typically more severely reduced by MDMA than those in rodents (Insel et al., 1989; Ricaurte et al., 1992). Other factors that could contribute to the observed species difference include species differences in (1) the length of 5-HT axon projections; (2) the degree of axon myelination (5-HT axons in the monkey are thinly myelinated, whereas those in the rat are unmyelinated) (Azmitia and Ganong, 1983); (3) possible involvement of the nerve cell body in monkeys (Ricaurte et al., 1991a); (4) the composition of the extracellular matrix; (5) the ability to generate growth factors; or (6) the nature and extent of the glial response to MDMA injury (Wilson and Molliver, 1994). Which, if any, of these factors plays a role in the species differences in serotonergic recovery observed after MDMA injury remains to be established.

The redistribution of ³H-citalopram-labeled 5-HT transporter sites observed in MDMA-treated animals is in keeping with previous results indicating that neurons may have an inherent tendency to preserve the quantity of their terminal field. Based on studies involving lesions of retinotectal projections in newborn hamsters, Schneider (1973) was the first to note that axons had a tendency to conserve the quantity of their terminal arborizations, such that loss of synaptic contacts in one brain region was compensated by increased synaptic contacts in another. This "pruning" effect has been observed in numerous neuron systems, including monoamine neuron systems (for review, see Bjorklund et al., 1979). Although most often observed after lesions inflicted during the neonatal period (Jonsson et al., 1974; Sachs and Jonsson, 1975; Jonsson et al., 1978; Levitt and Moore, 1980; Jonsson et al., 1982; Gustafson and Moore, 1987), synaptic rearrangements consistent with a pruning effect have also been documented in animals lesioned as adults (Wiklund et al., 1978; Bjorklund and Wiklund, 1980; Frankfurt and Azmitia, 1984; Frankfurt and Beaudet, 1987; Pare et al., 1987, 1992; Fritschy and Grzanna, 1992). The fact that altered reinnervation patterns develop after MDMA injury is consistent with these reports, and noteworthy in two respects: (1) it mitigates against the possibility that the changes in organization of axonal projections previously observed after intraventricular administration of neurotoxins are related to the mechanical insult associated

with intracerebroventricular injection, and (2) it establishes that such neuronal changes can occur after exposure to a drug abused by humans. The possibility that similar long-term changes in neuronal organization may occur after exposure to other toxic amphetamine derivatives such as methamphetamine (for review on neurotoxicity, see Seiden and Ricaurte, 1986) or dexfenfluramine (Ricaurte et al., 1991b; McCann et al., 1994a) used by humans for recreational (Cho, 1990) or medical (Guy-Grand et al., 1989) purposes, respectively, needs to be considered.

The present results add to growing evidence that, in addition to their acute neurotoxic effects, amphetamine derivatives such as MDMA can produce tardive effects on brain 5-HT neurons. For example, Molliver and colleagues have reported development of premature age-related morphologic changes in 5-HT fibers, as well as delayed 5-HT axon loss, in the neocortex of rats previously treated with *p*-chloroamphetamine (*p*-CA) (Wilson et al., 1993; Axt et al., 1994). Using biochemical markers, Zaczek et al. (1990) have made similar observations after fenfluramine. The extent to which such delayed neuronal changes contribute to development of aberrant reinnervation patterns such as those characterized in this study is unclear. However, it is conceivable that axonal reorganization observed after MDMA injury represents the combined effect of regionally specific regenerative processes and subsequent degenerative changes.

The factors which influence the extent and pattern of serotonergic recovery after MDMA injury have yet to be identified. Initial lesion severity would appear to be important, since axons in brain regions that are initially least severely injured (e.g., hypothalamus) recover to the greatest degree, while axons in more severely damaged brain regions (e.g., dorsal neocortex) show little or no evidence of recovery (Figs. 3, 5). Lesion severity alone, however, is not the sole determining factor, since there are some regions of the hypothalamus that are more severely injured than others (e.g., dorsomedial hypothalamus is more severely injured than the lateral hypothalamus), yet the entire hypothalamus shows evidence of reinnervation. This observation suggests that the regional pattern of reinnervation is influenced not only by initial lesion severity but also perhaps by specific anatomic features of the lesioned neuron system. Specifically, brain regions such as the occipital cortex that are distant from 5-HT nerve cell bodies in the raphe, and/or far removed from a major 5-HT axon bundle (e.g., MFB) may be less likely to be reinnervated than regions such as the lateral hypothalamus, which are close to the raphe as well as the MFB (and therefore contain 5-HT axons that are less arborized). Further studies are needed to better define the factors that govern the extent and pattern of serotonergic axonal regeneration after MDMA injury.

The clinical implications of the present findings are uncertain, although the more frequent occurrence of altered reinnervation patterns in MDMA-treated monkeys than in MDMA-treated rodents raises the question of whether similar neuronal alterations might develop in MDMA-exposed humans. As noted previously, the toxic dose of MDMA in the monkey closely approaches that typically used by humans (5 mg/kg vs 1.7 mg/kg) (Seymour,

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Figure 7. 5-HT-immunoreactive axons in several brain regions of a control monkey (*A, D, G*), a monkey treated with MDMA 2 weeks previously (*B, E, H*), and a monkey treated with MDMA 18 months previously (*C, F, I*). Note the reduction in axon density in the cerebral cortex (*B*), hypothalamus (*E*), and globus pallidus (*H*) 2 weeks after MDMA exposure. Also note that by 18 months, there is little or no evidence of reinnervation in the cerebral cortex (*C*), whereas in the hypothalamus (*F*) and globus pallidus (*I*) of the same animal, there is indication of reinnervation and/or hyperinnervation. Dark-field photomicrographs; scale bar, 100 μ m.

1986; Ricaurte et al., 1988; Doblin, 1989), and many humans use MDMA repeatedly and at high dosage (for reviews, see McKenna and Peroutka, 1990; Steele et al., 1994). Further, results from a recent controlled study indicate that MDMA-exposed humans have lasting reductions in cerebrospinal fluid (CSF) 5-HIAA (McCann et al., 1994b), a finding that in MDMA-treated monkeys is known to reflect 5-HT neurotoxicity (Ricaurte et al., 1988a).

The functional consequences of aberrant serotonergic brain reinnervation are unknown. Hyperinnervation of the hypothalamus may lead to neuroendocrine abnormalities, but this has yet to be documented. Hyperinnervation of other limbic structures (e.g., amygdala) might be anticipated to produce changes in emotion, motivation, learning or memory (Aggleton, 1992) but, again, few such changes have been documented (for review, see Steele et al., 1994). Along with the possible functional consequences of hyperinnervation, behavioral changes related to persistent denervation of certain brain regions (e.g., dorsal neocortex) should also be considered. For instance, as discussed by Hunter (1989), serotonergic denervation of the neocortex could result in cognitive impairments. Under usual circumstances, such impairments might not develop in later life because of sufficient neural reserve; however, if 5-HT function declines with age, MDMA-exposed individuals could be at increased risk for developing age-related cognitive impairment.

In summary, the results of the present study indicate that in some rats and in most monkeys there is a lasting reorganization of ascending 5-HT axon projections following severe MDMA injury. In particular, while some projections (e.g., those to the neocortex) fail to recover for up to 18 months after drug administration, others (e.g., projections to the basal forebrain) recover fully, sometimes in excess. The factors governing 5-HT axon regeneration after MDMA injury await elucidation, as do the functional consequences of aberrant reinnervation. The possibility that aberrant reinnervation such as that seen after MDMA injury may also occur during the course of neurodegenerative diseases (e.g., Parkinson's disease, Alzheimer's disease) should be considered, since aberrant reinnervation could influence not only the manifestations of the disease process but also its treatment.

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