

# Long-Term Impairment of Anterograde Axonal Transport Along Fiber Projections Originating in the Rostral Raphe Nuclei After Treatment With Fenfluramine or Methylenedioxymethamphetamine

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**KEY WORDS** anterograde transport; fenfluramine; methylenedioxymethamphetamine; neurotoxicity; serotonin

**ABSTRACT** To further evaluate the serotonin (5-HT) neurotoxic potential of substituted amphetamines, we used tritiated proline to examine anterograde transport along ascending axonal projections originating in the rostral raphe nuclei of animals treated 3 weeks previously with ( $\pm$ )fenfluramine (FEN, 10 mg/kg, every 2 h  $\times$  4 injections; i.p.) or ( $\pm$ )3,4-methylenedioxymethamphetamine (MDMA, 20 mg/kg, twice daily for 4 days; s.c.). The documented 5-HT neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT, 75  $\mu$ g; ICV; 30 min after pretreatment with pargyline, 50 mg/kg; i.p., and desipramine 25 mg/kg; i.p.), served as a positive control. Along with anterograde axonal transport, we measured two 5-HT axonal markers, 5-HT and 5-hydroxyindoleacetic acid (5-HIAA). Prior treatment with FEN or MDMA led to marked reductions in anterograde transport of labeled material to various forebrain regions known to receive 5-HT innervation. These reductions were associated with lasting decrements in 5-HT axonal markers. In general, decreases in axonal transport were less pronounced than those in 5-HT and 5-HIAA. However, identical changes were observed after 5,7-DHT. These results further indicate that FEN and MDMA, like 5,7-DHT, are 5-HT neurotoxins. **Synapse 40:113–121, 2001.**

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## INTRODUCTION

The long-term neurotoxicity of certain ring-substituted amphetamine derivatives is an area of much interest and concern (see Adelekan et al., 1997; McCann et al., 1997a, for reviews). One of these substituted amphetamines, ( $\pm$ )3,4-methylenedioxymethamphetamine (MDMA), is a recreational drug of abuse whose use is on the rise (Johnston et al., 2000). Another, ( $\pm$ )fenfluramine (FEN), was used as an appetite suppressant by over 40 million people (Derome-Trembley and Nathan, 1989) until reports of valvular heart damage (Connolly et al., 1997; Wee et al., 1998; Kancherla et al., 1999), possibly mediated by serotonin (5-HT) (Fitzgerald et al., 2000), prompted its removal from the market.

Over the last two decades, a large body of data has accrued strongly suggesting that FEN and MDMA are 5-HT neurotoxins. Animals treated with FEN or MDMA develop long-term reductions in a number of markers unique to central 5-HT axons and axon terminals including the level of 5-HT and its major metabo-

lite 5-hydroxyindoleacetic acid (5-HIAA) (Commins et al., 1987; Schmidt, 1987; Kleven and Seiden, 1989; Zaczek et al., 1990), the number of 5-HT transporters (5-HTT) (Schuster et al., 1986; Commins et al., 1987; Battaglia et al., 1987; Lew et al., 1996), the activity of tryptophan hydroxylase (Steranka and Sanders-Bush, 1979; Stone et al., 1989; Schmidt and Taylor, 1987), and the density of 5-HT and 5-HTT-immunoreactive axons (Appel et al., 1989; O'Hearn et al., 1988; Molliver et al., 1990; Molliver and Molliver, 1990; Fischer et al., 1995; Hatzidimitriou et al., 1999). In addition, there is evidence of 5-HT axon fragmentation shortly after drug exposure (O'Hearn et al., 1988; Appel et al., 1989; Molliver et al., 1990; Molliver and Molliver, 1990). This constellation of findings, coupled with anatomic obser-

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vations at the level of the raphe nuclei, has led most, although not all (Kalia, 1991; Sotelo, 1991; O'Callaghan and Miller, 1994), investigators in the field to conclude that ring-substituted amphetamines such as FEN and MDMA destroy 5-HT axons and axon terminals in the forebrain while sparing 5-HT cell bodies in the brain stem (see McCann et al., 1997a, for review).

The notion that the lasting loss of 5-HT presynaptic axonal markers observed after treatment with substituted amphetamines is perhaps related to downregulation of certain proteins unique to serotonin axons, rather than to frank axonal destruction, merits consideration. According to this view, loss of axonal markers could occur even though the axons and axon terminals remain functionally viable (Kalia, 1991; O'Callaghan and Miller, 1997). To address this issue, the present study was undertaken to measure anterograde axonal transport along ascending 5-HT axonal projections originating in the rostral raphe nuclei of the brain stem of animals previously treated with FEN or MDMA (Dahlstrom and Fuxe, 1965; Anden et al., 1965). This tract-tracing technique, coupled with a neuron-specific lesion induced by 5,6-dihydroxytryptamine (5,6-DHT), was previously used by Halaris et al. (1976) to trace ascending 5-HT neuronal pathways in the rat brain. We hypothesized that if substituted amphetamines produced a distal axotomy of central 5-HT neurons, anterograde axonal transport along 5-HT axons would be reduced after treatment with FEN and MDMA.

## MATERIALS AND METHODS

### Drugs and chemicals

The following test compounds were purchased or obtained from the sources indicated: ( $\pm$ )3,4-methylenedioxymethamphetamine hydrochloride and ( $\pm$ )fenfluramine hydrochloride from the National Institute on Drug Abuse (Rockville, MD); 5,7-dihydroxytryptamine creatinine sulfate, pargyline hydrochloride, desipramine hydrochloride, serotonin creatinine sulfate and 5-hydroxyindoleacetic acid from Sigma Chemical Co. (St. Louis, MO); and [ $^3$ H]proline (L-proline [2,3- $^3$ H (N), 40 Ci/mmol) from New England Nuclear (Boston, MA).

### Animals and housing

Male albino rats (200–225 g) of the Sprague-Dawley strain (Harlan, Indianapolis, IN) were used for these studies. Animals were housed individually in hanging wire mesh cages in a temperature-controlled room ( $22 \pm 1^\circ\text{C}$ ), maintained on a 12-h light/dark cycle (0600–1800). Food (PMI Feeds, St. Louis, MO) and water were provided ad libitum throughout. All procedures were approved by the Animal Care and Use Committee of the Johns Hopkins Medical Institutions.

### Drug treatments

Rats ( $n = 15$ – $18$  rats/group) were treated with FEN (10 mg/kg, every 2 h  $\times$  4 injections; i.p.) or MDMA

(20 mg/kg, at approximately 0900 and 1700 h for 4 days; s.c.). FEN and MDMA were dissolved in 0.9% saline and were administered on a ml/kg basis. Control animals received equal volumes of vehicle. The dosage regimens of FEN and MDMA were selected because they have previously been shown to produce long-lasting 5-HT neurochemical deficits in rats (Commins et al., 1987; Battaglia et al., 1987; Zaczek et al., 1990). 5,7-DHT was given by an intracerebroventricular (ICV) route to rats ( $n = 13$ – $15$  rats/group) pretreated with desipramine (25 mg/kg, i.p.) and pargyline (50 mg/kg, i.p.). Thirty minutes after desipramine/pargyline pretreatment, a 75  $\mu\text{g}$  dose of 5,7-DHT (dose calculated as the free base) was given into the right lateral ventricle using a 5  $\mu\text{l}$  Hamilton syringe (Reno, NV) in 3  $\mu\text{l}$  of sterile 0.9% saline with 0.2% ascorbic acid over 5 min. A 75  $\mu\text{g}$  dose of 5,7-DHT was used in order to produce a lesion of 5-HT neurons that is limited to 5-HT axons (Neale et al., 1972; Bjorklund et al., 1973, 1974), and thus serve as an appropriate positive control group. Three weeks after treatment, five animals from each group (FEN, MDMA, 5,7-DHT), along with an equal number of controls, were sacrificed by decapitation and analyzed for regional brain 5-HT and 5-HIAA content, as below. The remaining animals in each group were used for axon tracing experiments with [ $^3$ H]proline.

### Anterograde axonal transport

Three weeks after drug treatment, animals were anesthetized with chloral hydrate (50 mg/kg, i.p.) and placed in a Kopf stereotaxic device (Tujunga, CA). Just prior to use, [ $^3$ H]proline was evaporated under a stream of nitrogen gas, then dissolved in sterile physiological saline (pH 7.4) to a final concentration of 100  $\mu\text{Ci}$  in 1.0  $\mu\text{l}$ . Using a 1  $\mu\text{l}$  Hamilton syringe, rats received injections of [ $^3$ H]proline (100  $\mu\text{Ci}$ ) into the rostral raphe nuclei (nucleus raphe dorsalis, nucleus centralis superior) over a period of 20 min (rate of injection .05  $\mu\text{l}/\text{min}$ ). Syringe needles were left in place for 5 min following the completion of injections to minimize diffusion of [ $^3$ H]proline from the injection site. Coordinates for the rostral raphe injections were as follows: perpendicular plane at 2.0 mm anterior from the ear bars, vertical 2.0 mm above horizontal zero, with the tooth bar at 2.5 mm below the ear bar (Paxinos and Watson, 1986). Animals were sacrificed 48 h after injection of the labeled amino acid by decapitation. Immediately after sacrifice, whole brains were removed from the skull and dissected over ice as previously described (Halaris et al., 1976). For determination of regional brain radioactivity, evaluated as activity of isotope (DPMs/mg tissue), tissue sections were dissolved overnight in 450  $\mu\text{l}$  of Soluene tissue solubilizer (Packard Instrument Co., Downers Grove, IL) at a temperature of  $37^\circ\text{C}$ . The following day, samples were combined with 10 ml of Hionoflour scintillation fluid (Packard Instrument Co.) and radioactivity

was measured by a Packard scintillation counter. In order to visualize the injection sites, tissue sections containing the raphe nucleus were frozen quickly with powdered dry ice and serial 20- $\mu$ m frozen sections were obtained by using a cryostat at  $-20^{\circ}\text{C}$ . The sections were then thaw-mounted onto glass slides and autoradiograms were generated by apposing the dry, slide-mounted sections, to tritium-sensitive Amersham film. After an exposure period of 2 days the film was developed and the mounted sections were counterstained with cresyl violet. Injection sites were evaluated by superimposing the autoradiograms over the stained tissue sections.

**Determination of 5-HT and 5-HIAA levels**

Three weeks after drug treatment rats were killed and the brain was removed and regionally dissected as previously described (Halaris et al., 1976). Tissue samples were placed in aluminum foil and frozen in liquid nitrogen until analysis of monoamines and major monoamine metabolites by reverse-phase HPLC coupled to electrochemical detection (Ricaurte et al., 1992). Briefly, frozen tissue samples were weighed, placed in 10–15 volumes (wt/vol) of 0.4 N perchloric acid, and homogenized using a Brinkman Polytron Homogenizer (setting of 5 for 12 sec). The homogenates were then centrifuged for 20 min at approximately 25,000g in a refrigerated Sorvall RC2B centrifuge at 0–4°C. The supernatant was removed and 0.3 ml aliquots were placed in polypropylene tubes, which were then stored in liquid nitrogen until assay. Monoamines and their metabolites were separated using a reverse phase C-18 column from Brownlee Applied Systems (Santa Clara, CA) using a mobile phase that was 100% aqueous and contained citric acid (125 mM), sodium phosphate (125 mM), EDTA (0.27 mM), sodium octyl sulfate (0.12 mM), pH 3.0–3.5. The flow rate was 1 ml/min. The column was housed at 40°C in a Shimadzu (Columbia, MD) CTO-6A column oven. A Shimadzu amperometric L-ECD-6A detector containing a glassy carbon working electrode and a silver/silver chloride reference electrode with a potential difference of 0.70 V was used. Shimadzu Chromatopacs C-R 4A and 7A were used to quantify the electrochemical response by measuring the area under a given curve and comparing it to that of a standard processed identically.

**Data analysis**

Regional brain 5-HT and 5-HIAA and axonal tracing data were analyzed by one-way ANOVA followed by Duncan's Multiple Range post hoc comparisons. The relationship between regional amount of labeled material and regional brain 5-HT levels was investigated through determination of Pearson's correlation coefficients. For these purposes, all control data was pooled. Results were considered significant at  $P < 0.05$ , us-

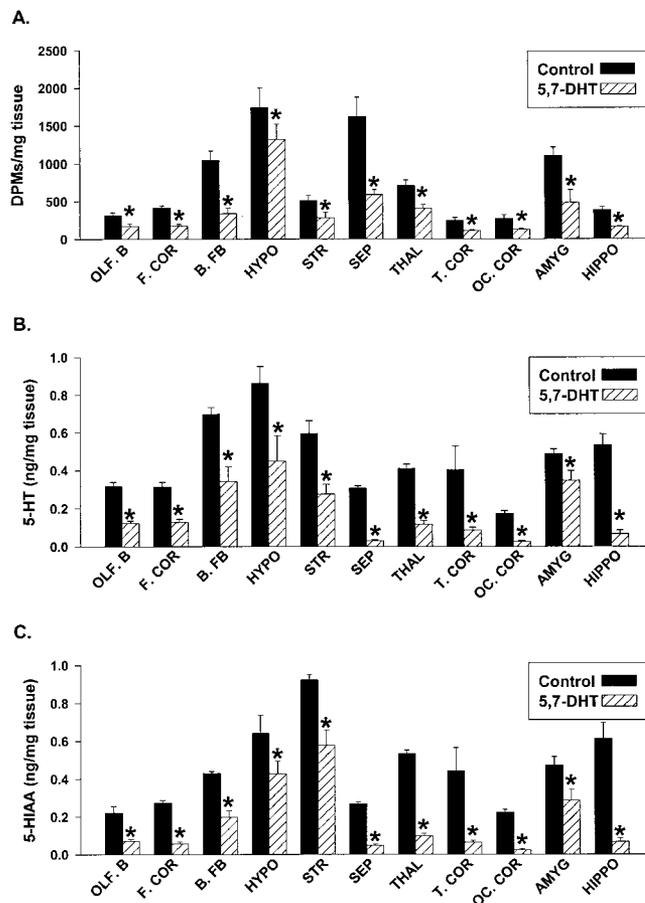


Fig. 1. Effect of treatment with 5,7-DHT (75  $\mu$ g; ICV; 30 min after pretreatment with pargyline, 50 mg/kg; i.p., and desipramine 25 mg/kg; i.p.; 3-week survival) on regional distribution of radioactivity following injection of 100  $\mu$ Ci [ $^3$ H]proline into the rostral raphe nuclei (A), and on regional brain 5-HT (B) and 5-HIAA (C). Values shown are the means  $\pm$  SEM ( $n = 5-8$  rats/group). Significance level was set at  $*P < 0.05$ . OLF.B, olfactory bulb; F.COR, frontal cortex; B.FB, basal forebrain; HYPO, hypothalamus; STR, striatum; SEP, septum; THAL, thalamus; T.COR, temporal cortex; OC.COR, occipital cortex; AMYG, amygdala; HIPPO, hippocampus.

ing a two-tailed test. SPSS for Windows, v.7.5 (Chicago, IL) was employed for all data analysis. Results were considered significant at  $P < 0.05$ .

**RESULTS**

**Effect of treatment with 5,7-DHT/DMI/pargyline (positive control)**

As shown in Figure 1A, animals treated with 5,7-DHT/DMI/pargyline 3 weeks previously had significantly lower regional brain concentrations of labeled material when examined 2 days after stereotactically guided microinjections of [ $^3$ H]proline (100  $\mu$ Ci) into the rostral raphe nuclei. Reductions in regional brain concentrations of labeled material were associated with long-term reductions in the levels of regional brain 5-HT (Fig. 1B) and 5-HIAA (Fig. 1C). These findings confirm and extend those of Halaris et al. (1976) and attest to the validity of the anterograde axonal trans-

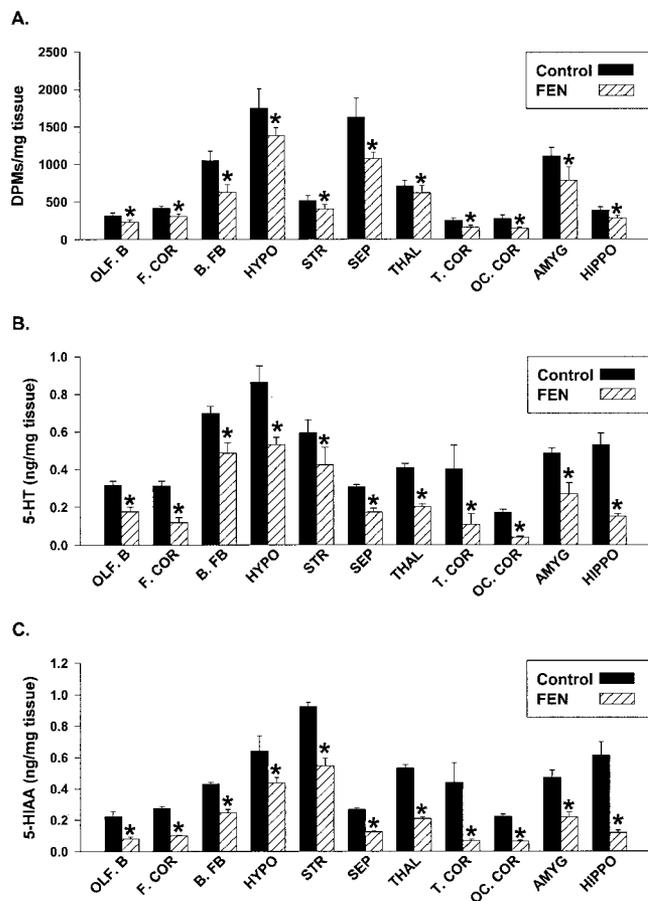


Fig. 2. Effects of treatment with FEN (10 mg/kg, Q2×4; i.p.; 3-week survival period) on regional distribution of radioactivity following injection of 100  $\mu$ Ci [ $^3$ H]proline into the rostral raphe nuclei (A), and on regional brain 5-HT (B) and 5-HIAA (C). Values shown are the means  $\pm$  SEM ( $n = 5-8$  rats/group). Significance level was set at  $*P < 0.05$ . OLF.B, olfactory bulb; F.COR, frontal cortex; B.FB, basal forebrain; HYPO, hypothalamus; STR, striatum; SEP, septum; THAL, thalamus; T.COR, temporal cortex; OC.COR, occipital cortex; AMYG, amygdala; HIPPO, hippocampus.

port method for detecting drug-induced neurotoxicity of ascending 5-HT axonal projections.

#### Effect of treatment with FEN

Rats previously treated with FEN (10 mg/kg, every 2 h  $\times$  4 injections; i.p.) showed similar effects as those previously treated with 5,7-DHT/DMI/pargyline. In particular, FEN-treated animals showed significant decreases in regional brain concentrations of labeled material when examined 2 days after [ $^3$ H]proline injections into the rostral raphe nuclei (Fig. 2A), as well as long-term reductions in regional brain levels of 5-HT (Fig. 2B) and 5-HIAA (Fig. 2C). Reductions in regional brain 5-HT paralleled reductions in regional brain concentrations of labeled material in all brain regions examined except the hypothalamus, striatum, and thalamus, where transport reductions did not achieve statistical significance.

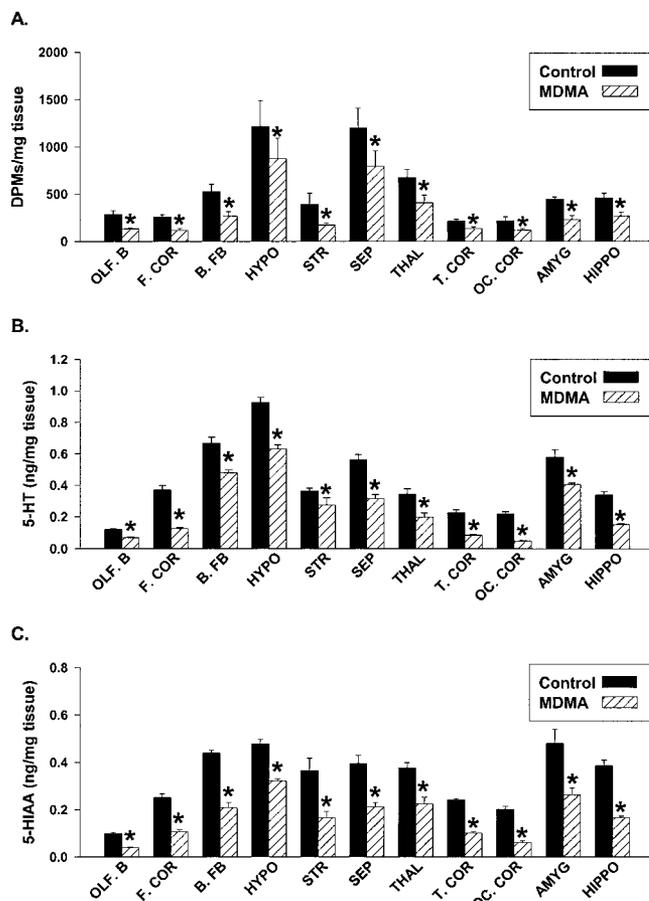


Fig. 3. Effect of treatment with MDMA (20 mg/kg, BID×4; s.c.; 3-week survival period) on regional distribution of radioactivity following injection of 100  $\mu$ Ci [ $^3$ H]proline into the rostral raphe nuclei (A), and on regional brain 5-HT (B) and 5-HIAA (C). Values shown are the means  $\pm$  SEM ( $n = 5-8$  rats/group). Significance level was set at  $*P < 0.05$ . OLF.B, olfactory bulb; F.COR, frontal cortex; B.FB, basal forebrain; HYPO, hypothalamus; STR, striatum; SEP, septum; THAL, thalamus; T.COR, temporal cortex; OC.COR, occipital cortex; AMYG, amygdala; HIPPO, hippocampus.

#### Effect of treatment with MDMA

Prior treatment with MDMA (20 mg/kg, twice daily for 4 days; s.c.) also led to significant decreases in anterograde axonal transport of labeled material (Fig. 3A), and lasting decreases in regional brain levels of 5-HT (Fig. 3B) and 5-HIAA (Fig. 3C). Again, decreases in labeled material tended to parallel decreases in 5-HT and 5-HIAA, but decreases in regional concentrations of labeled material were generally less severe, as was the case after 5,7-DHT/DMI/pargyline.

#### Verification of injection sites

Autoradiographic analysis of injection sites revealed labeling of [ $^3$ H]proline along the entire rostral raphe complex (Fig. 4), with labeling primarily centered over the dorsal and medial raphe nuclei. There was also a small amount of labeling along the injection tract. Notably, there was a positive correlation, ( $r = 0.78$ )

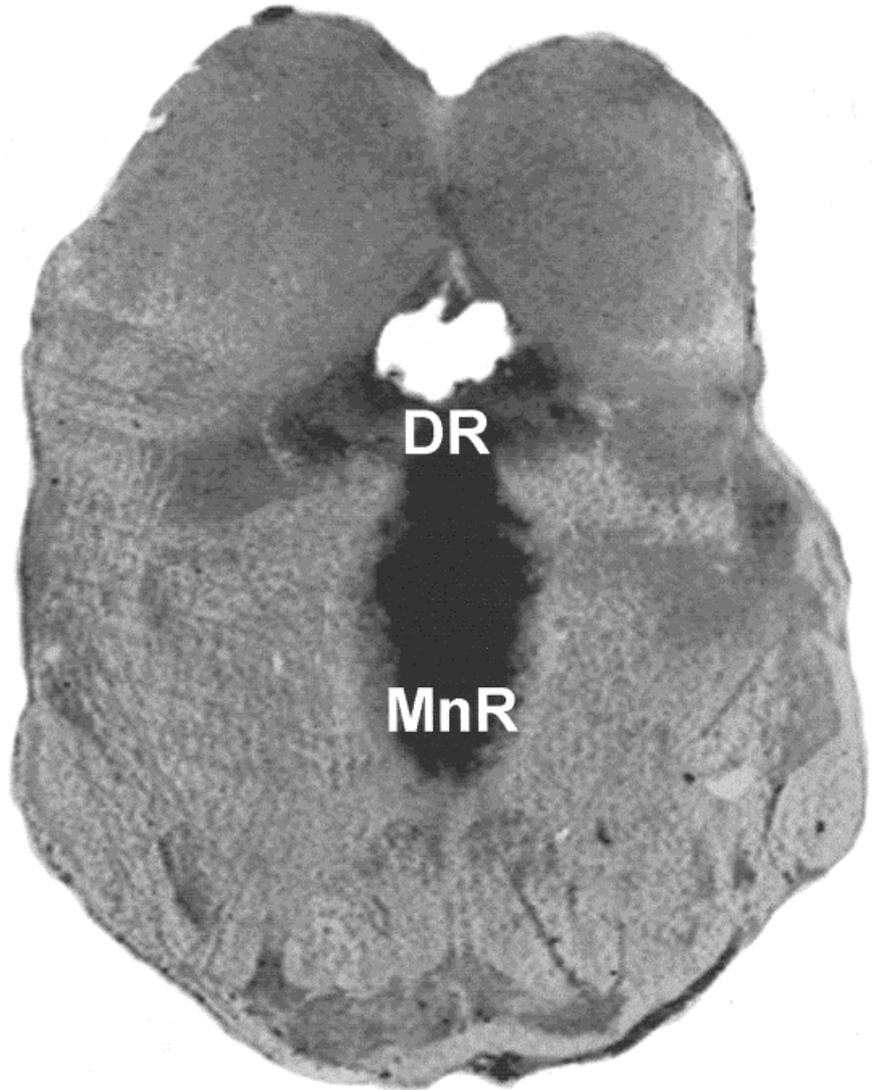


Fig. 4. Frontal section of a [ $^3\text{H}$ ]proline injection site showing the distribution of radiolabel over the rostral raphe complex. Note that radiolabel is centered primarily over the dorsal (DR) and medial (MnR) raphe nuclei.

between concentrations of labeled material and regional brain 5-HT levels (Fig. 5), suggesting that greater amounts of labeled material were transported to areas high in 5-HT axon density.

#### DISCUSSION

Ascending 5-HT axonal projections in normal rats and in rats treated 3 weeks previously with FEN or MDMA have been studied by tracing the transport of radioactive material after [ $^3\text{H}$ ]proline injections into the rostral raphe nuclei (nucleus raphe dorsalis and nucleus centralis superior) (Fig. 4). The results indicate that prior treatment with either FEN or MDMA leads to a marked reduction of anterograde transport of radioactive label to forebrain regions that receive 5-HT innervation. Along with reductions in radioactive label, there are lasting reductions in two 5-HT axonal markers, 5-HT and 5-HIAA (see Results). In addition, similarly treated animals show comparable decrements in

the number of 5-HT transporters (Schuster et al., 1986; Commins et al., 1987; McCann et al., 1994; Lew et al., 1996). Given that animals treated with 5,7-DHT, a well-documented 5-HT neurotoxin (for review, see Baumgarten et al., 1982), develop an identical constellation of neuronal deficits (Fig. 1), it seems reasonable to conclude that FEN and MDMA, like 5,7-DHT, produce these long-lasting effects by producing a distal axotomy of ascending 5-HT neuron projections.

The fiber-tracing technique used in the present study has been used in numerous previous studies to examine the trajectory of various neuronal pathways, including projections to the thalamus and brain stem (Beckstead et al., 1980), spinal cord (Hung et al., 1994), substantia nigra (Sakai, 1988), and striatum (Kelley et al., 1982). In all of these studies, axonal projections have been mapped by tracing the transport of radioactive material after injection of [ $^3\text{H}$ ]proline (or [ $^3\text{H}$ ]leucine) into brain region that contain the cell bod-

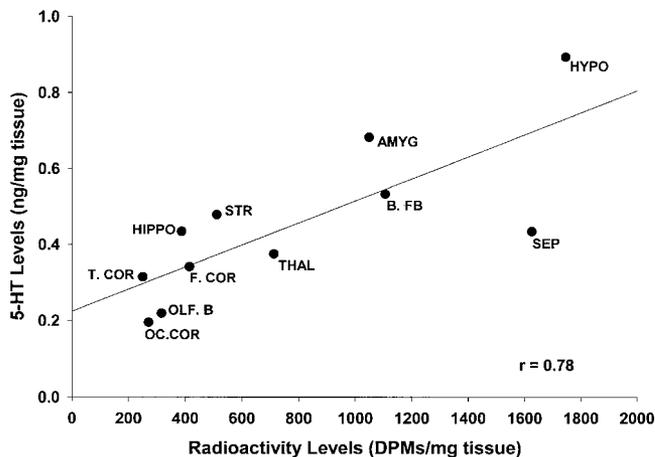


Fig. 5. Graph representing the correlation ( $r = 0.78$ ) between regional brain 5-HT levels and amounts of labeled material in various brain regions 2 days after microinjection of [ $^3\text{H}$ ]-proline into the rostral raphe nuclei. Note that regions high in 5-HT levels also exhibit elevated levels of labeled material, indicating that radioactive material is transported to regions high in 5-HT density.

ies of origin. A refinement of this method was introduced by Halaris et al. (1976), who coupled the [ $^3\text{H}$ ]-proline method with selective lesions induced by 5,6-dihydroxytryptamine (5,6-DHT) to successfully trace ascending 5-HT neuronal pathways in the rat brain. The present study used a similar approach but introduced two further refinements. First, we used 5,7-DHT instead of 5,6-DHT because of reports that it is a more selective 5-HT neurotoxin (Breese et al., 1974). Second, we used 5,7-DHT in combination with DMI and pargyline to prevent toxic effects of 5,7-DHT on brain noradrenergic neurons (Breese and Mueller, 1978). As might be expected, we found a significant correlation between regional brain 5-HT levels and [ $^3\text{H}$ ]-proline concentrations in normal animals (Fig. 5). Moreover, the pattern of regional concentrations of [ $^3\text{H}$ ]-proline we observed coincides closely with that observed by Halaris et al. (1976). Collectively our findings support the hypothesis that substituted amphetamines produce a distal axotomy of central 5-HT neurons.

Decreases in axonal transport measured with [ $^3\text{H}$ ]-proline tended to parallel lasting depletions of 5-HT and 5-HIAA after 5,7-DHT (Fig. 1), FEN (Fig. 2), and MDMA (Fig. 3) but were generally smaller. In addition, there were some brain regions (e.g., hypothalamus) where significant long-term depletions of 5-HT and 5-HIAA were associated with more modest reductions in radioactive material that did not achieve statistical significance. Although the basis for these differences is not fully understood, it may be that nonserotonergic neurons located in (or passing through) the rostral raphe nuclei incorporated labeled material and transported it to terminal fields in the diencephalon and telencephalon. It is also possible that at least some of the damaged 5-HT axons are still capable of transporting labeled material, at least as far as the site of most

severe axonal injury (Tsuiki et al., 1994; Curtis et al., 1993). Either of these possibilities could lead to reductions in axonal transport that underestimate the true extent of axonal injury. Therefore, while use of the axonal tracing method to assess neurotoxic potential of substituted amphetamines offers an important complement to other existing methods, it may have a tendency to underestimate the actual extent of axonal damage induced by substituted amphetamines.

It could be argued that despite the decrease in axonal transport measured with [ $^3\text{H}$ ]-proline and the loss of 5-HT axonal markers documented in this study, 5-HT axons in FEN- and MDMA-treated animals, although metabolically and functionally impaired, remain structurally intact. Although perhaps theoretically possible, we regard this possibility as highly improbable for several reasons. First, immunocytochemical studies performed a few days after substituted amphetamine exposure reveal the presence of markedly swollen, seemingly fragmented 5-HT-containing axons (O'Hearn et al., 1988; Appel et al., 1989; Molliver, 1990; Molliver and Molliver, 1990) which are identical in appearance to those seen after 5,7-DHT (Frankfurt and Azmitia, 1984; Rowland et al., 1993), an accepted 5-HT neurotoxin. Second, the apparent fragmentation of preterminal 5-HT axons is followed by an extremely long-lasting loss of all 5-HT axonal markers measured to date (Zaczek et al., 1990; Scanzello et al., 1993; McCann et al., 1994; Hatzidimitriou et al., 1999). Given that the reduction of 5-HT axonal markers after substituted amphetamines has been documented as long as 7 years after MDMA (Hatzidimitriou et al., 1999) and 14 months after FEN (McCann et al., 1994), it seems unlikely that the observed axonal changes are due to a drug-related down-regulation of 5-HT neuronal metabolism, especially since the biologic half-lives of FEN and MDMA in animals are relatively short (2 to 8 h, depending on species). Moreover, in those species where recovery of 5-HT axonal markers is observed, the time-course of recovery coincides more closely with the anticipated time-course of axonal regeneration, the pattern of which is abnormal and in keeping with evidence of prior "pruning" (see Fischer et al., 1995). Third, recent studies of yet another axonal marker said to be less amenable to pharmacologic regulation, the vesicular monoamine transporter (VMAT), show that it too is reduced on a long-term basis after substituted amphetamine exposure (Ricaurte et al., 2000).

The neurotoxic potential of substituted amphetamines toward brain 5-HT neurons has been questioned because no increases in glial fibrillary acidic protein (GFAP) have been observed after drug treatment in rodents (O'Callaghan and Miller, 1994). However, it is not known if the GFAP method has the sensitivity for detecting small lesions of fine unmyelinated 5-HT axons (Rowland et al., 1993; Bendotti et al., 1994), particularly after treatments of drug that pro-

duce only 15–20% reductions in 5-HT axonal markers (O'Callaghan and Miller, 1994). In another study, young rats treated with escalating doses of the dextro-rotatory isomer of fenfluramine, dexfenfluramine, developed only transient decreases in 5-HT, and these were not associated with lasting decreases in the density of serotonin immunoreactive fibers, increases in GFAP, or increases in a growth-associated protein (GAP 43) (Rose et al., 1996). However, given that escalating dose-regimens may influence fenfluramine's long-term effects (Caccia et al., 1992; Rose et al., 1997), and given that young rats are known to be less sensitive to the neurotoxic effects of amphetamines than more mature animals (Seiden, 1991; Teuchert-Noodt and Dawirs, 1991; Broening et al., 1994, 1995; D'Almeida et al., 1996), this report also has to be interpreted with caution. Moreover, as noted above, the reliability of the GFAP method for detecting 5-HT axonal injury has not been fully documented (Rowland et al., 1993; Bendotti et al., 1994; Stewart et al., 1999; but see O'Callaghan and Miller, 1993), nor has it been established that axotomy of central 5-HT neurons is necessarily followed by increases in GFAP or GAP 43. Thus, methodologic issues may help explain the different conclusions reached in these investigations.

There are a number of important differences between 5,7-DHT and substituted amphetamines that deserve emphasis. First, 5,7-DHT can destroy 5-HT nerve cell bodies (Baumgarten et al., 1982), whereas the toxicity of FEN and MDMA is largely, if not exclusively, restricted to 5-HT axons and axon terminals (McCann et al., 1994; Hatzidimitriou et al., 1999). No doubt, sparing of the nerve cell bodies accounts for the regrowth of some 5-HT axons after substituted amphetamines (Scanzello et al., 1993; Fracasso et al., 1995; Lew et al., 1996). Second, 5,7-DHT has the potential to damage NE neurons, whereas FEN and MDMA neurotoxicity tends to be highly selective for 5-HT neurons. In this regard, it is noteworthy that in the present studies we used a dose of 5,7-DHT (75  $\mu$ g) which, when given after pretreatment with desipramine and pargyline, is known to be selectively toxic to 5-HT neurons (Breese and Mueller, 1978; Gerson and Baldessarini, 1975). Third, there is some indication that there may be an increase in GFAP after 5,7-DHT (O'Callaghan and Miller, 1993) but not after FEN or MDMA; however, this is likely to be related to the fact that intracerebroventricular administration of 5,7-DHT directly damages a variety of nerve fibers, inducing a glial response. In contrast, both FEN and MDMA are administered systemically.

Detecting or documenting degeneration of fine-caliber axons in the central nervous system (CNS) poses a significant challenge, particularly when the axons in question do not achieve a high density in any particular brain region (such as dopamine axons in the striatum). As noted elsewhere (McCann et al., 1997b), the GFAP

method is useful for detecting many forms of neural injury but its sensitivity for detecting 5-HT axonal injury remains to be fully established. Moreover, it may be that lesions of 5-HT axons are not necessarily followed by increases in GFAP or other proteins associated with neuronal degeneration or regeneration (e.g., GAP 43 and other growth factors). Finally, there is some indication that the glial response to 5-HT axonal injury may involve microglia rather than astroglia (Wilson and Molliver, 1994). It may well be that studies of 5-HT axonal injury associated with substituted amphetamines will lead to new insights regarding the response of CNS elements to axonal injury.

The relevance of the present studies to humans previously exposed to repeated doses of MDMA or FEN deserves comment. In particular, the dosage regimens utilized in the present study were not intended to mimic those that have been used by humans. Rather, the dose regimens were selected because they had previously been shown to produce a large and selective lesion of 5-HT neurons, as measured by reductions in 5-HT axonal markers. However, other studies have demonstrated loss of 5-HT axonal markers following dosages of FEN and MDMA that overlap those used by humans (see McCann et al., 1994, 1998).

In summary, the present results extend previous findings regarding the neurotoxic potential of FEN and MDMA. More specifically, they show that following substituted amphetamine exposure there is a lasting reduction in anterograde axonal transport along ascending neuronal projections originating in the rostral raphe nuclei. This finding, coupled with the fact that every 5-HT axonal marker measured to date is reduced on a long-term basis after FEN and MDMA exposure, supports the view that substituted amphetamines can damage central 5-HT axon projections. This view is further supported by the fact that the effects of FEN and MDMA on axonal transport closely parallel those of 5,7-DHT, a well-documented 5-HT neurotoxin.

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