Brain Research, 619 (1993) 1–14

BRES 19073

Research Reports

Methylenedioxyamphetamine: neurotoxic effects on serotonergic projections to brainstem nuclei in the rat

John A. Harvey, Scott E. McMaster and Anthony G. Romano

Division of Behavioral Neurobiology, Department of Pharmacology, The Medical College of Pennsylvania, Philadelphia, PA 19129 (USA)

(Accepted 2 March 1993)

Key words: Serotonergic axon; Neurotoxicity; Brainstem nuclei; Methylenedioxyamphetamine; Immunocytochemistry; Rat

This study employed immunocytochemistry to visualize the neurotoxic effects of methylenedioxyamphetamine hydrochloride (MDA) on serotonergic projections to brainstem structures. Separate groups of animals were injected twice a day, for 4 consecutive days, with: saline; MDA (40 mg/kg/day); or fluoxetine hydrochloride (10 mg/kg) prior to each injection of MDA. In agreement with previous reports, MDA produced a pronounced loss of 5-HT immunoreactivity in the forebrain, most notably in neocortex and hippocampus. However, our results revealed that MDA also produced a loss of 5-HT fibers in brainstem that was as severe as that seen in any region of forebrain. Regions most severely affected included: superior colliculus; superior olivary complex; trigeminal sensory complex and vestibular nuclei. The brains of animals treated with MDA demonstrated a relative absence of fine 5-HT axon terminals within these forebrain and brainstem regions, while thicker axonal elements were still present. The neurotoxic effects of MDA on serotonergic projections to forebrain and brainstem were completely blocked by the prior administration of the 5-HT reuptake inhibitor, fluoxetine. It was suggested that the denervation of the superior colliculus, superior olive and vestibular nuclei could alter visually guided eye movements and the vestibulo-ocular reflex while the loss of serotonergic inputs to the trigeminal sensory complex might be expected to alter tactual reflexes.

INTRODUCTION

The neurotoxic actions of some halogenated amphetamines (e.g., *p*-chloroamphetamine) on serotonergic neurons were first suggested on the basis of long-term changes in neurochemical measures[15,16] and morphological evidence derived from studies using the Fink-Heimer silver-impregnation methods[3,4]. Subsequently, the substituted amphetamines, methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) were also suggested to produce a selective neurotoxic action on 5-HT neurons based on a long-term depletion of 5-HT and other neurochemical markers[11,19,21] as well as morphological evidence of degenerative changes based on Fink-Heimer methods[11]. These latter findings have gained considerable attention because of the psychedelic action of MDA and MDMA[20] and the current abuse of the latter under the street name of ecstasy[10].

More recently, a series of elegant immunocytochemical studies have described in great detail the neurotoxic actions of *p*-chloroamphetamine, MDA and MDMA in the rat and non-human primate[7,8,12,23]. These studies have demonstrated that the neurotoxic actions of the substituted amphetamines were due to the degeneration and structural loss of serotonergic axon terminals. The major emphasis of the immunocytochemical studies cited above has been to describe the selective pattern of neurotoxicity in the forebrain. In the rat, MDA and MDMA were reported to produce the greatest damage to 5-HT axon terminals innervating neocortex, striatum and thalamus with smaller reductions observed in hippocampal formation, septum, olfactory bulb and amygdala[8]. There was also a selective action within brain regions. For example, in hippocampus, 5-HT projections to field CA_1 were the most affected. In neocortex, spared axon terminals were most visible in frontal cortex with greater neuro-

Correspondence: J.A. Harvey, Division of Behavioral Neurobiology, Department of Pharmacology, The Medical College of Pennsylvania, 3200 Henry Avenue, Philadelphia, PA 19129, USA. Fax: (1)(215)843-1515.

toxicity seen as one moved to the occipital cortex. The medial hypothalamus including the ventromedial hypothalamic nucleus was more denervated than the lateral hypothalamus. The high degree of selectivity in the neurotoxic actions of *p*-chloroamphetamine, MDA and MDMA in forebrain structures was shown to be due to a greater susceptibility of fine axons projecting from the dorsal raphe nucleus[7,8].

The brainstem was not analyzed systematically as part of the studies cited above except to note that the raphe cell bodies were spared[8,17]. Evidence for a possible brainstem effect of 5-HT neurotoxins was provided by Fritschy et al.[2] who reported that *p*-chloroamphetamine selectively destroyed the fine 5-HT axons projecting from the dorsal raphe nucleus to the trigeminal motor nucleus while sparing 5-HT projections from the raphe obscurus and raphe pallidus. This latter finding suggests that both ascending and descending fine axons from dorsal raphe may be equally susceptible to the 5-HT neurotoxins. Consequently, one might expect that these drugs would produce a selective denervation of nuclei in the brainstem in a manner similar to that observed for forebrain. The present study employed immunocytochemistry to further examine the neurotoxic effects of MDA on 5-HT axons in brain with special emphasis on its possible selective effects on brainstem nuclei. In addition, the 5-HT uptake inhibitor, fluoxetine, was used to determine whether neurotoxicity required the neuronal uptake of MDA into 5-HT neurons.

MATERIALS AND METHODS

Subjects

Male Sprague–Dawley rats, weighing 150 g on arrival, were obtained from Ace Animals, Inc. (Boyertown, PA, U.S.A.). Rats were housed 3 per cage and had free access to food and water. The colony room was illuminated on a 12:12 h light:dark cycle.

Drugs

MDA (D,L-methylenedioxyamphetamine hydrochloride), obtained from the National Institute on Drug Abuse, was dissolved in sterile physiological saline and fluoxetine hydrochloride, a gift from Eli Lilly and Co. (Indianapolis, IN, U.S.A.), was dissolved in sterile distilled water. Fluoxetine hydrochloride (10 mg/kg) or MDA (20 mg/kg) were prepared just prior to use and their doses were expressed as the salt form. All injections of drug or control injections of sterile

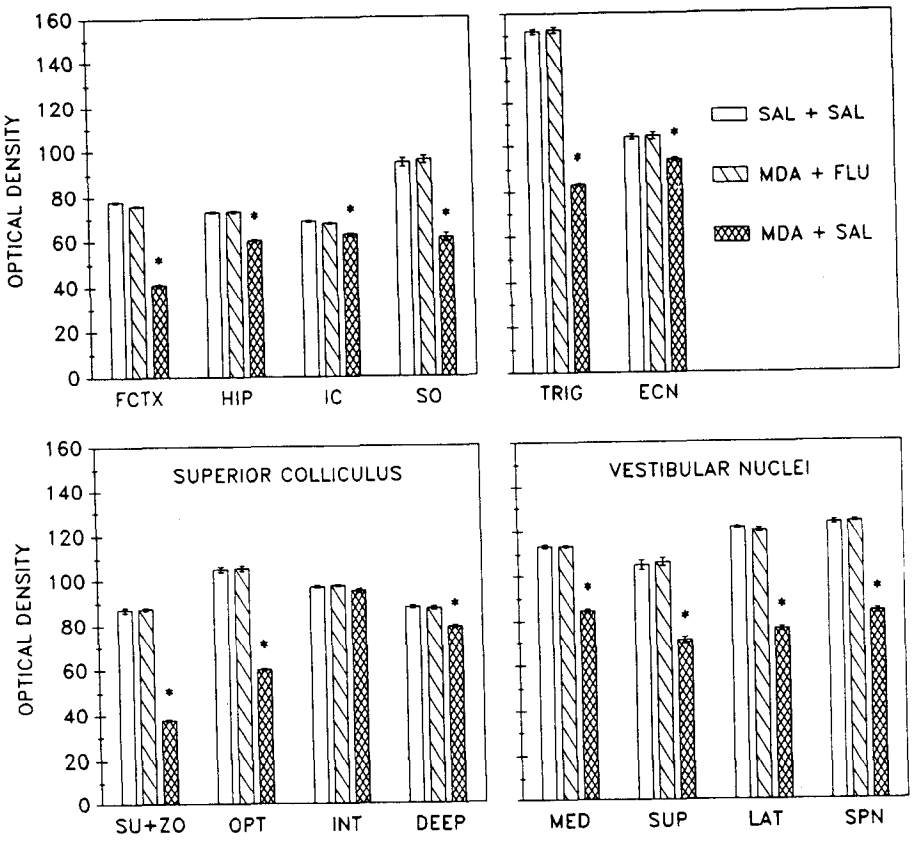


Fig. 1. Average optical density of 5-HT immunoreactivity obtained in designated brain structures. The optical density was measured in successive coronal sections taken at intervals of 0.5 mm through each structure. Each vertical bar represents the mean of 5 animals. The brackets above and below each vertical bar indicate one S.E.M. Asterisks indicate a mean value significantly ($P < 0.001$) different from that of saline controls. Abbreviations are: DEEP, deep gray layer of the superior colliculus; ECN, external cuneate nucleus; FCTX, frontal cortex; HIP, hippocampus; IC, inferior colliculus; INT, intermediate gray layer of superior colliculus; LAT, lateral vestibular nucleus; MED, medial vestibular nucleus; OPT, optic nerve layer of superior colliculus; SPN, spinal vestibular nucleus; SO, superior olivary complex; SU+ZO, superficial and zonal layer of superior colliculus; SUP, superior vestibular nucleus; TRIG, trigeminal sensory complex.

physiological saline were given in a volume of 1 ml/kg. Sodium pentobarbital (Sigma Chemical Co., St. Louis, MO, U.S.A.) was injected i.p. at a lethal dose of 75 mg/kg.

Procedures

Three groups of animals were employed. The control group ($n = 5$) received an i.p. injection of saline followed 20 min later by a s.c. injection of saline; a second group ($n = 5$) received an i.p. injection of saline followed 20 min later by a s.c. injection of MDA (20 mg/kg); and a third group ($n = 5$) received an i.p. injection of fluoxetine (10 mg/kg) followed 20 min later by a s.c. injection of MDA (20 mg/kg). Each group was injected twice a day (every 12 h) for 4 days. Thus, the total daily dose of MDA was 40 mg/kg.

Histological procedures

Standard procedures were employed. Fourteen days after the last injection, rats were perfused transcardially, under deep pentobarbital anesthesia (75 mg/kg, i.p.), with ice-cold phosphate buffered saline (PBS; pH 7.4) followed by ice-cold 4% paraformaldehyde dissolved in a 0.15 M phosphate buffer (PB; pH 7.4). The brains were removed and placed sequentially in ice-cold solutions for the times indicated: 48 h in the 4% paraformaldehyde dissolved in PB; 12 h in 10% (w/v) sucrose dissolved in PB; and 24 h in 20% (w/v) sucrose dissolved in PB. Brains were then frozen and 30-μm thick coronal sections were cut in a cryostat and placed in ice-cold PBS. The free floating sections were then incubated for 24 h on a rotating platform at room temperature with an anti 5-HT antisera obtained from Incstar Corporation (Stillwater, MN). The antibody was diluted 1:10000 in PBS containing 1% normal goat serum and 0.3% Triton X-100. 5-HT-like immunoreactivity was visualized with the avidin-biotin-peroxidase complex (ABC) kit supplied by Vector Laboratories, Inc. (Burlin-

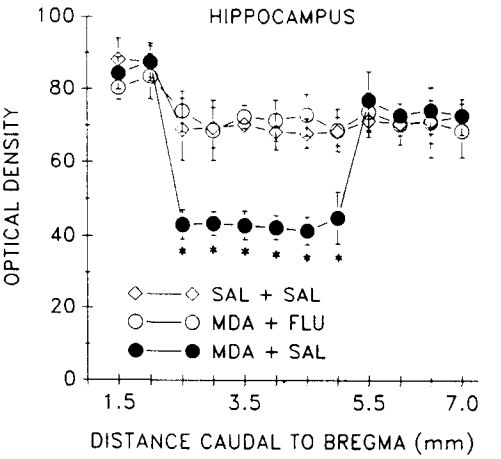


Fig. 3. Average optical density of immunoreactivity in hippocampus. Optical density was measured in successive coronal sections spaced 0.5 mm apart through the entire extent of the hippocampus. These coronal sections correspond to Plates 18 to 28 of Paxinos and Watson[9]. Each point is the average of 5 animals. Vertical bars indicate one S.E.M. Asterisks indicate a significant difference from saline controls at $P < 0.001$.

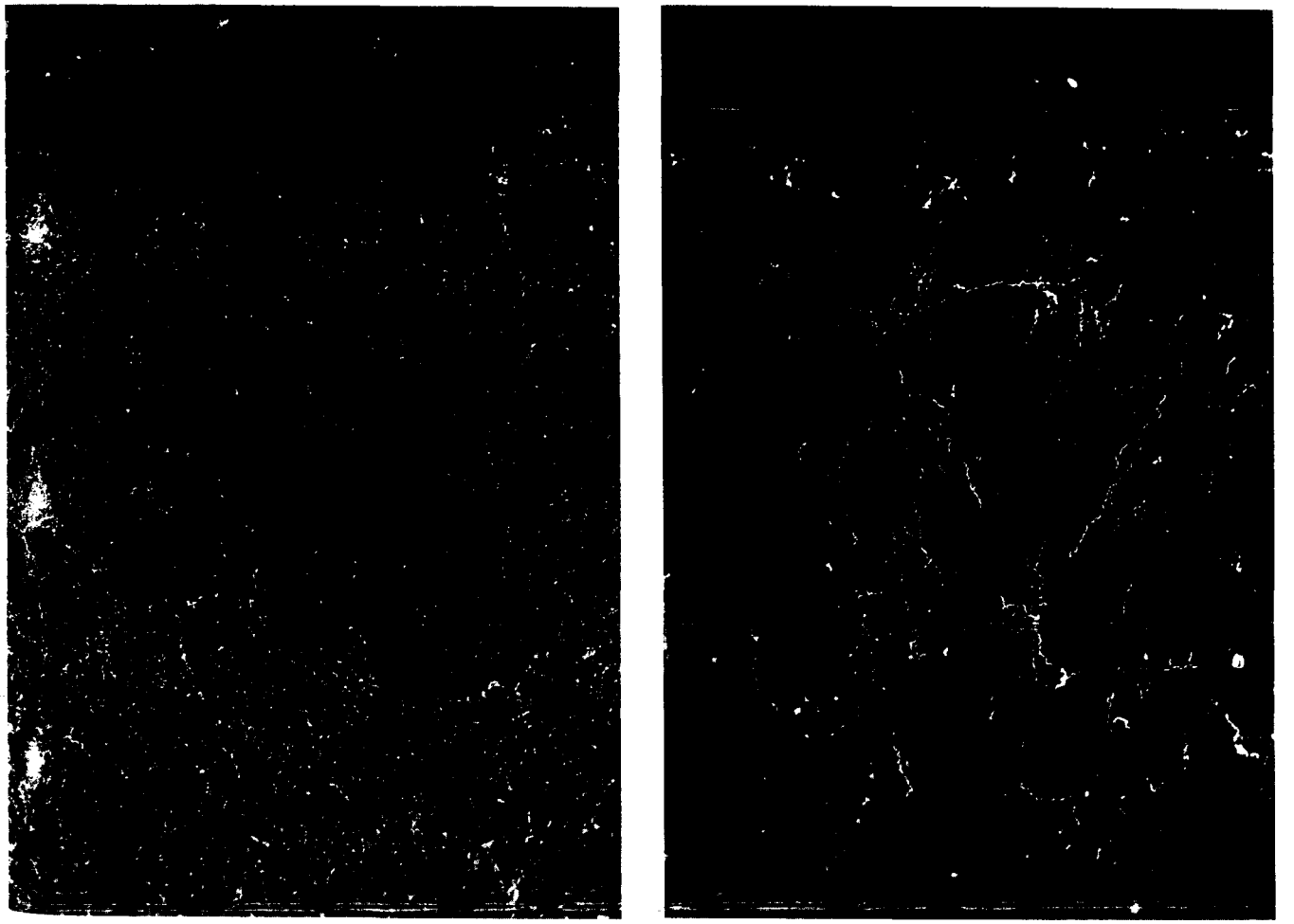


Fig. 2. 5-HT axons in frontal cortex. The coronal sections were taken approximately 3 mm rostral to bregma and correspond to Plate 8 of Paxinos and Watson[9]. A high density of fine axon terminals is seen in frontal cortex of a control rat (left panel). After MDA (right panel) nearly all fine axon terminals are ablated, but thicker preterminal fibers are still present. Dark-field photomicrograph, ×30.

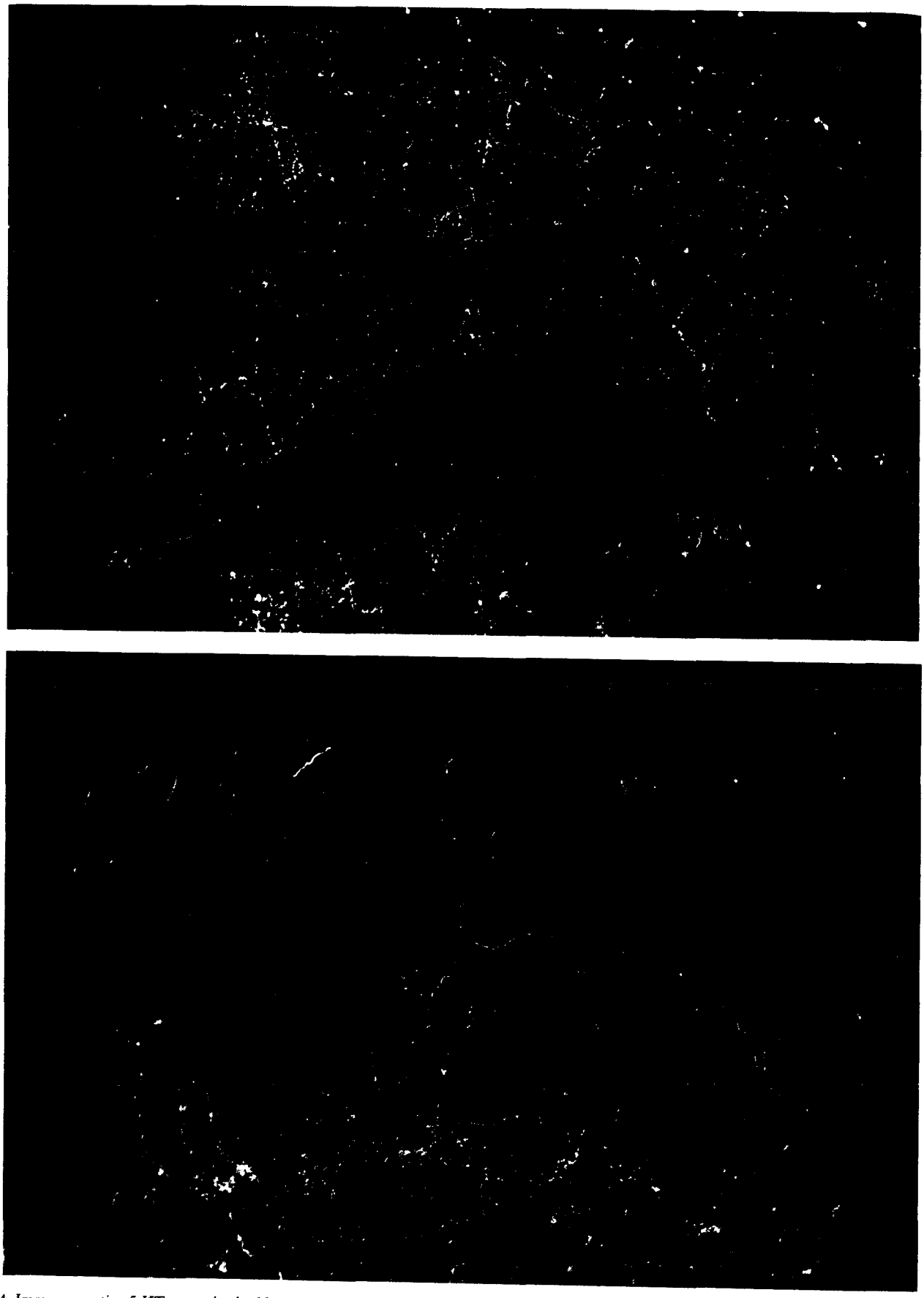


Fig. 4. Immunoreactive 5-HT axons in the hippocampus. The coronal sections were taken approximately 3 mm caudal to bregma and correspond to Plate 21 of Paxinos and Watson[9]. Top panel: a photomicrograph taken from a control animals showing a high density of fine axon terminals in field CA_1. Bottom panel: after MDA nearly all fine axon terminals are ablated in field CA_1 but thicker preterminal fibers are still present. Dark-field photomicrograph, ×40.

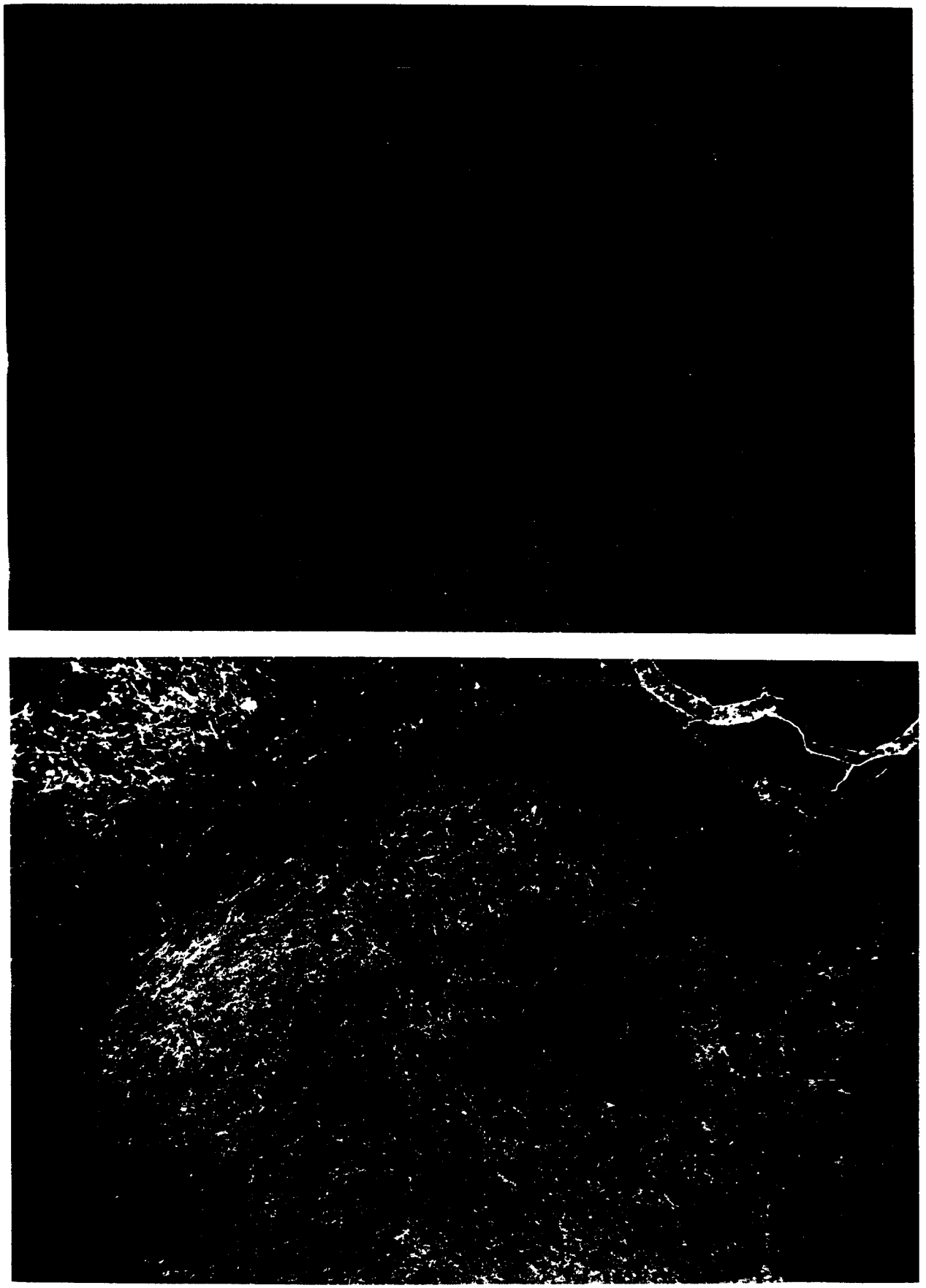


Fig. 5. 5-HT axons in the superior colliculus. The coronal sections were taken approximately 7 mm caudal to bregma and correspond to Plate 28 of Paxinos and Watson[9]. A high density of 5-HT axons can be seen in all layers of the superior colliculus of a control rat (top panel). After MDA (bottom panel) nearly all fine axon terminals are ablated, but thicker preterminal fibers are still present especially in the intermediate and deep gray layers. Dark-field photomicrograph, ×20.

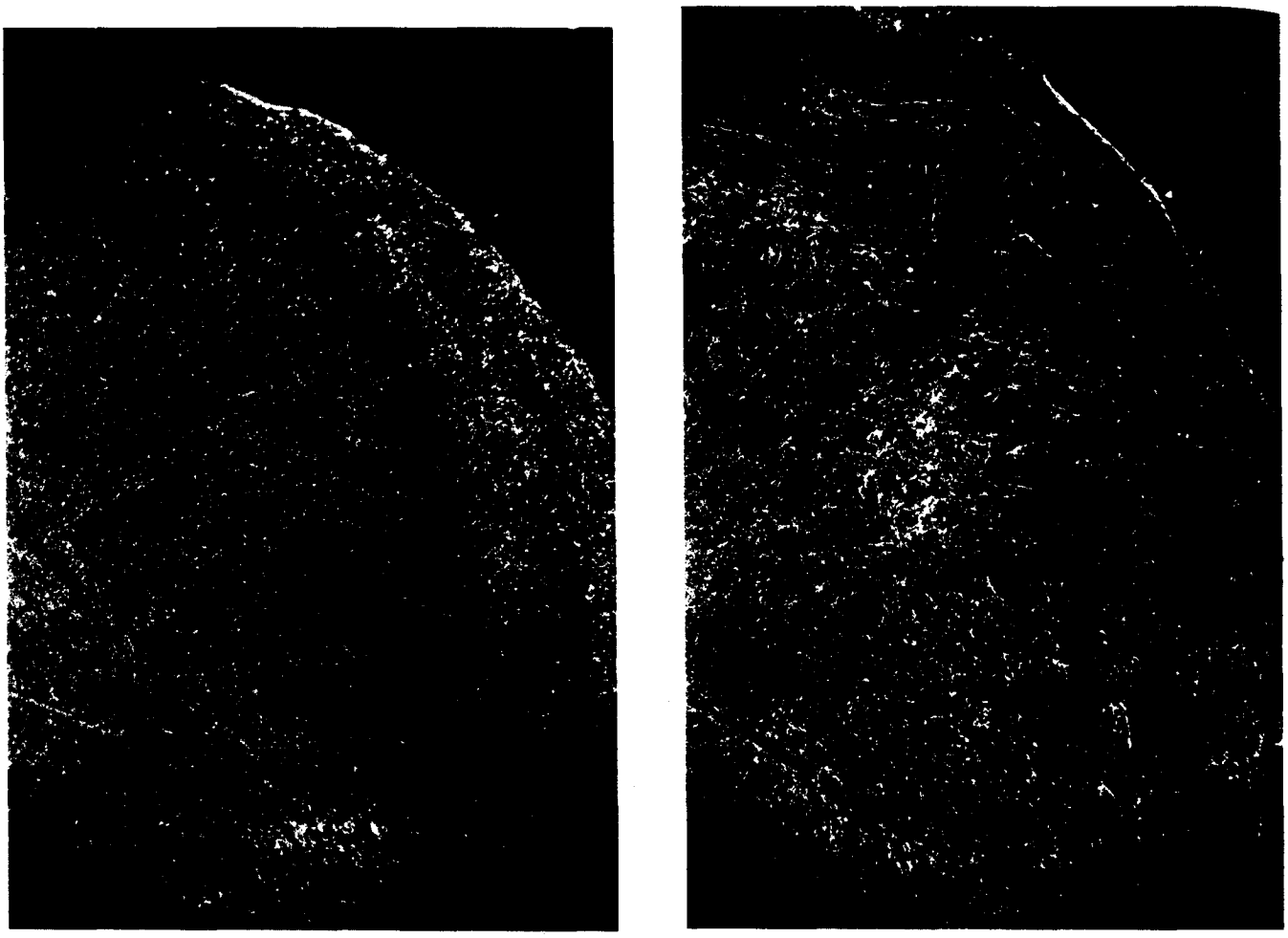


Fig. 6. 5-HT axons in the inferior colliculus. The coronal sections were taken approximately 8 mm caudal to bregma and correspond to Plate 31 of Paxinos and Watson[9]. The high density of 5-HT axons in a control rat (left panel) was reduced after treatment with MDA (right panel). Dark-field photomicrograph, ×15.

game, CA, U.S.A.) which is based on the method of Hsu et al.[5]. Sections were then mounted on chrome alum-gelatin coated slides. The average optical density of the immunoreactivity was measured in various brain regions by means of a Bioquant Image Analysis System (R&M Biometrics, Inc., Nashville, TN, U.S.A.) and expressed as average optical density as previously described by Wang et al.[22]. Statistical measurement of mean differences were calculated by Student's *t*-test with corrections for multiple comparisons with controls.

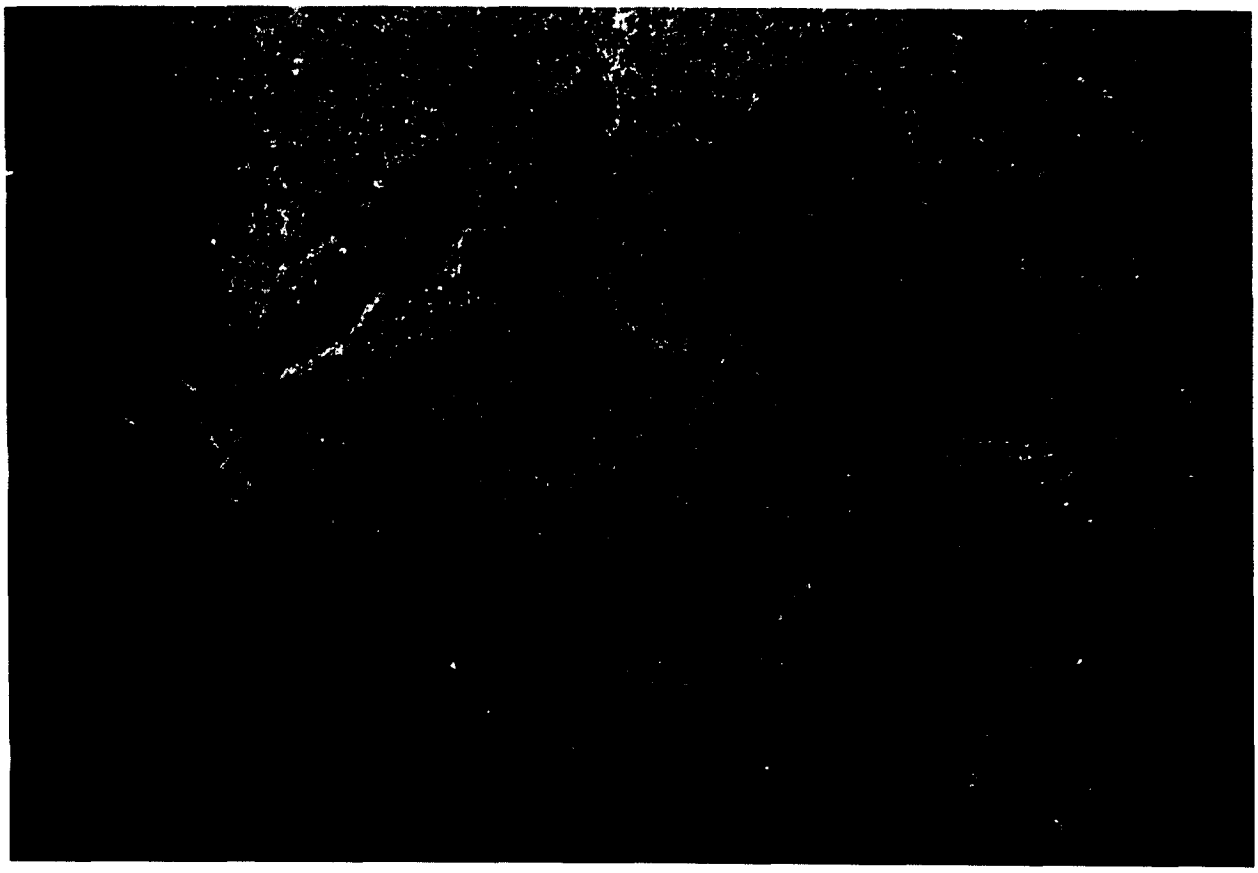
RESULTS

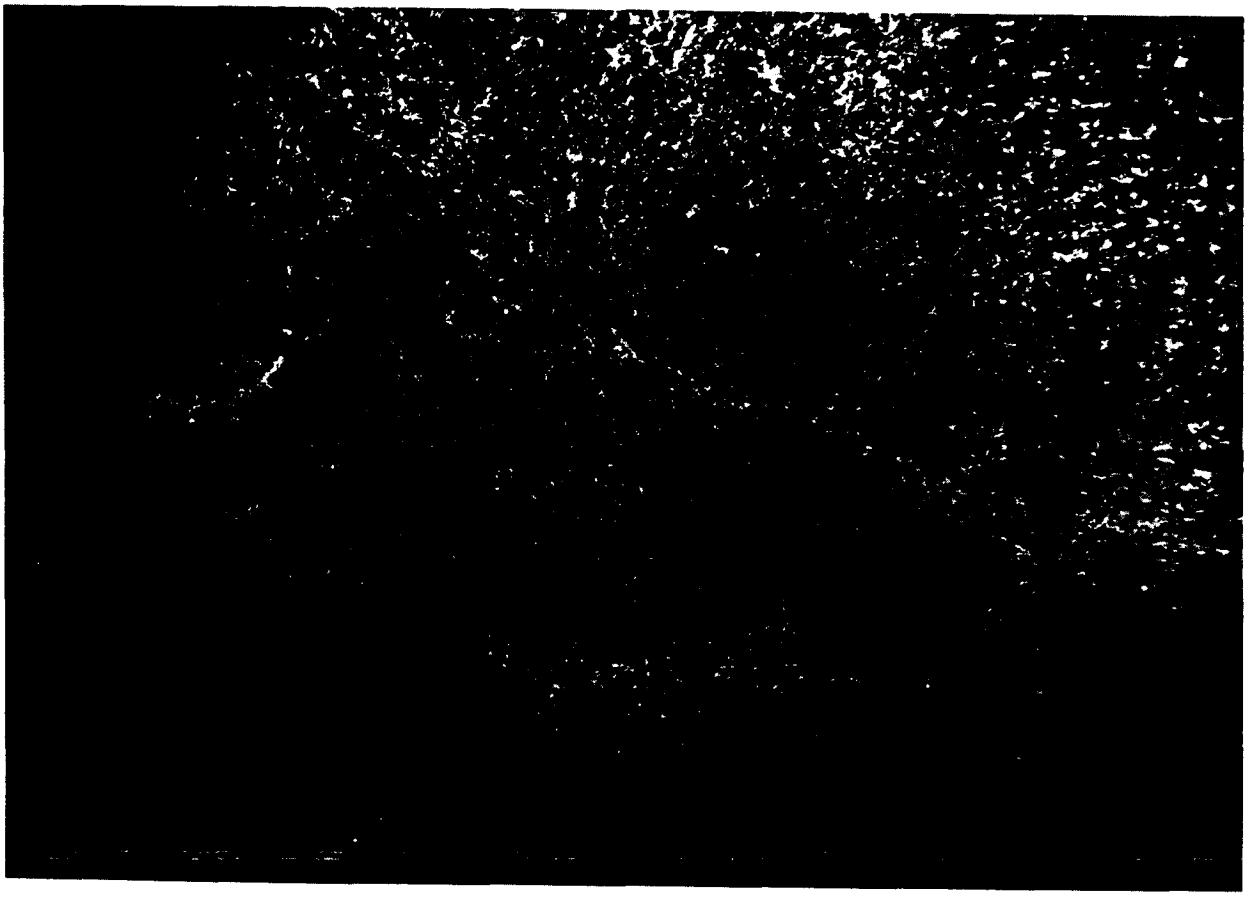
Rats treated with MDA (40 mg/kg/day for 4 days) demonstrated a reduction in 5-HT reactive neurons throughout the brain (Fig. 1). These reductions occurred in both forebrain and brainstem structures and were completely blocked by pretreatment with fluoxetine (Fig. 1). The reductions in 5-HT axon terminals in forebrain and in brainstem were highly variable between and within various structures.

Effects in forebrain

Rats treated with MDA demonstrated a loss of immunoreactive neurons in forebrain structures that was essentially identical with the neurotoxic effects previously described by O'Hearn et al.[8]. In agreement with those findings, the dense innervation of frontal cortex by 5-HT axons seen in control animals (Fig. 2, left panel) was greatly reduced by MDA (Fig. 2, right panel). There was an almost complete loss of fine axons as described by O'Hearn et al.[8] with a selective

Fig. 7. 5-HT axons at the level of the superior olivary nucleus. Coronal sections were taken approximately 10 mm caudal to bregma and correspond to Plate 34 of Paxinos and Watson[9]. The top panel shows 5-HT immunoreactive fibers in a control animal. 5-HT axons can be seen in the principal sensory trigeminal nucleus located just dorsal and lateral to the root of the facial nerve and in the lateral and medial superior olivary nuclei located medial to the facial nerve. Further medially some 5-HT axons can be seen in both the medial and lateral nuclei of the trapezoid body. MDA produced a decrease in the number of 5-HT axons in each of these areas (bottom panel) but had no effect on the dense 5-HT innervation of the pontine reticular formation just dorsal to the superior olivary nuclei. Dark-field photomicrograph, ×20.





sparing of thicker axons (Fig. 2, right panel). The overall reduction in optical density in frontal cortex produced by MDA (48%) was much greater than the overall reduction in hippocampus (18%; Fig. 1). However, the neurotoxic effect of MDA on hippocampus was highly selective as shown in Fig. 3. There was no loss of 5-HT axons at the most rostral or most caudal levels of the hippocampus, however the average optical density of 5-HT axons was reduced by 34–39% at coronal levels lying approximately 2.5–5 mm caudal to bregma. MDA had its largest effect on 5-HT axons in field CA_1. Fig. 4 (top panel) illustrates the innervation of field CA_1 by 5-HT axons in a control animal. After treatment with MDA there is a near total loss of these fine axons in field CA_1 while thicker 5-HT axons are still present (Fig. 4, bottom panel), an effect identical with previous findings[8].

Effects in brainstem

There was also a selective effect of MDA in the brainstem. The greatest decrease in the density of 5-HT axons occurred in the superior colliculus, superior olivary complex, trigeminal sensory complex and vestibular nuclei with smaller reductions in the inferior colliculus and external cuneate nucleus (Fig. 1). There were no observable changes in the raphe nuclei or in the trigeminal motor nucleus and only subtle changes in remaining brainstem structures, which were not further analyzed. The neurotoxic effect of MDA in the brainstem appeared similar to that observed in forebrain in that the affected areas demonstrated a selective loss of fine 5-HT axons. Consequently, thicker 5-HT axons were usually still present within an otherwise denervated area (see Figs. 5–11).

As in the case of forebrain structures, the neurotoxic effects of MDA varied within some nuclear regions and this was the most dramatic for the superior colliculus. 5-HT axons in control animals were densely distributed in all layers of the superior colliculus (Fig. 5, top panel). MDA produced large decreases in 5-HT axons within the superficial and zonal layers (57%) and the optic nerve layer (43%) of the superior colliculus as

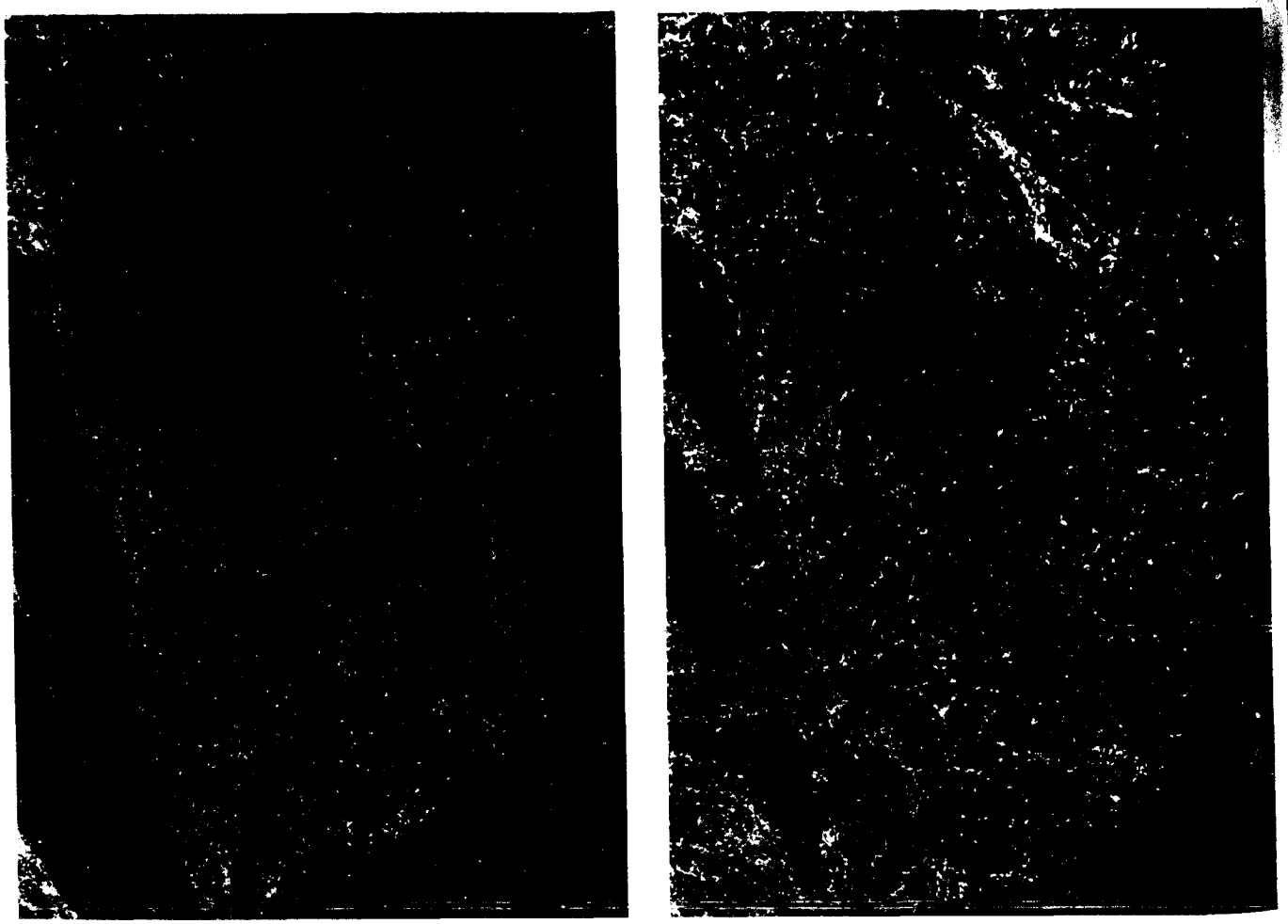


Fig. 8. 5-HT axons in the trigeminal sensory complex at a level approximately 10.5 mm caudal to bregma corresponding to Plate 35 of Paxinos and Watson[9]. The high density of 5-HT axons which can be seen in pars oralis of a control rat (left panel) was greatly reduced by MDA (right panel). Dark-field photomicrograph, ×30.

well as a small (10%) decrease in the deep gray layer but had no significant effect on 5-HT axons in the intermediate gray layer (Fig. 1). These effects of MDA are illustrated in Fig. 5 (bottom panel), where it can be seen that the smaller effects of MDA in the deep and intermediate gray layers of the superior colliculus were due to the presence of a dense plexus of thick 5-HT fibers that were spared by MDA. In contrast with the large effects of MDA on 5-HT fibers in the superior colliculus, the dense 5-HT innervation of the inferior colliculus (Fig. 6, left panel) demonstrated only a small (9%) but significant decrease in 5-HT axons (Fig. 1). This small decrease was due primarily to the elimination of fine 5-HT axons in the rostral portions of the inferior colliculus and within its dorsolateral margins (Fig. 6, right panel).

The superior olive demonstrated an overall 35% decrease in 5-HT immunoreactivity (Fig. 1). There was a greater innervation of the lateral as compared with the medial superior olive as well as a sparse innervation of the medial and lateral nuclei of the trapezoid body (Fig. 7, top panel). MDA produced a decrease in the number of 5-HT axons in each of these areas (Fig. 7, bottom panel). Fig. 7 also illustrates that the dense 5-HT innervation of the pontine reticular formation dorsal to the superior olive (top panel) was not affected by MDA (bottom panel).

MDA produced a substantial (45%) decrease in density of 5-HT neurons throughout the trigeminal sensory complex (Fig. 1). For example, the dense 5-HT innervation of the principal sensory trigeminal nucleus (see area just dorsal and lateral to the root of the facial nerve in Fig. 7, top panel), pars oralis (Fig. 8, left panel) and at the juncture of pars interpolaris and pars caudalis (Fig. 9, left panel) was greatly reduced by MDA in each of these structures (Fig. 7, bottom panel; Fig. 8 and 9, right panels). Fig. 8 and 9 clearly demonstrate that MDA tended to eliminate fine 5-HT axons throughout the trigeminal sensory complex while sparing the thicker 5-HT axons. Note that the 5-HT axons

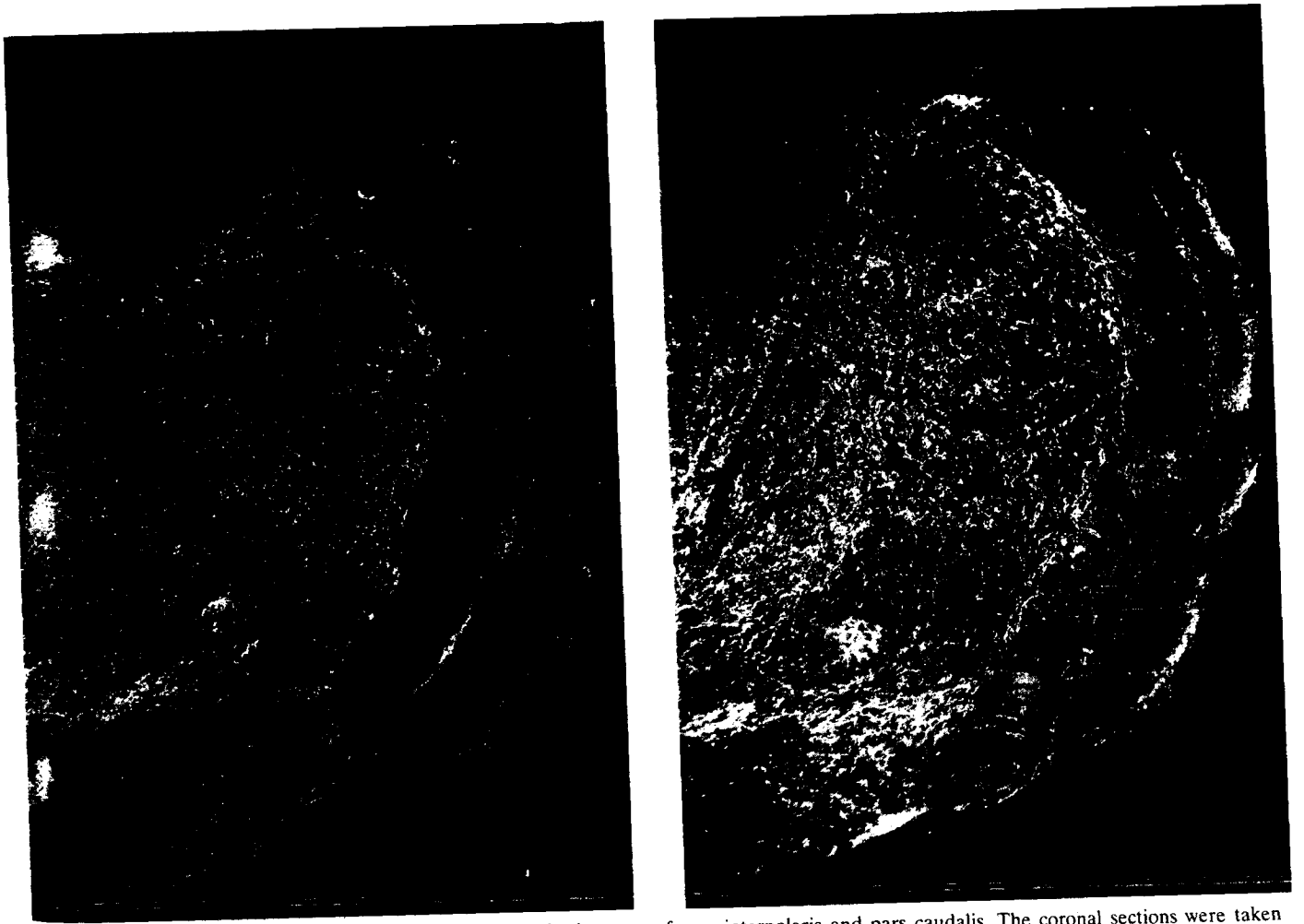


Fig. 9. 5-HT axons in the trigeminal sensory complex at the juncture of pars interpolaris and pars caudalis. The coronal sections were taken approximately 13.5 mm caudal to bregma corresponding to Plate 41 of Paxinos and Watson[9]. The high density of 5-HT axons which can be seen in pars interpolaris of a control rat (left panel) was greatly reduced by MDA (right panel). Dark-field photomicrograph, ×7.5.

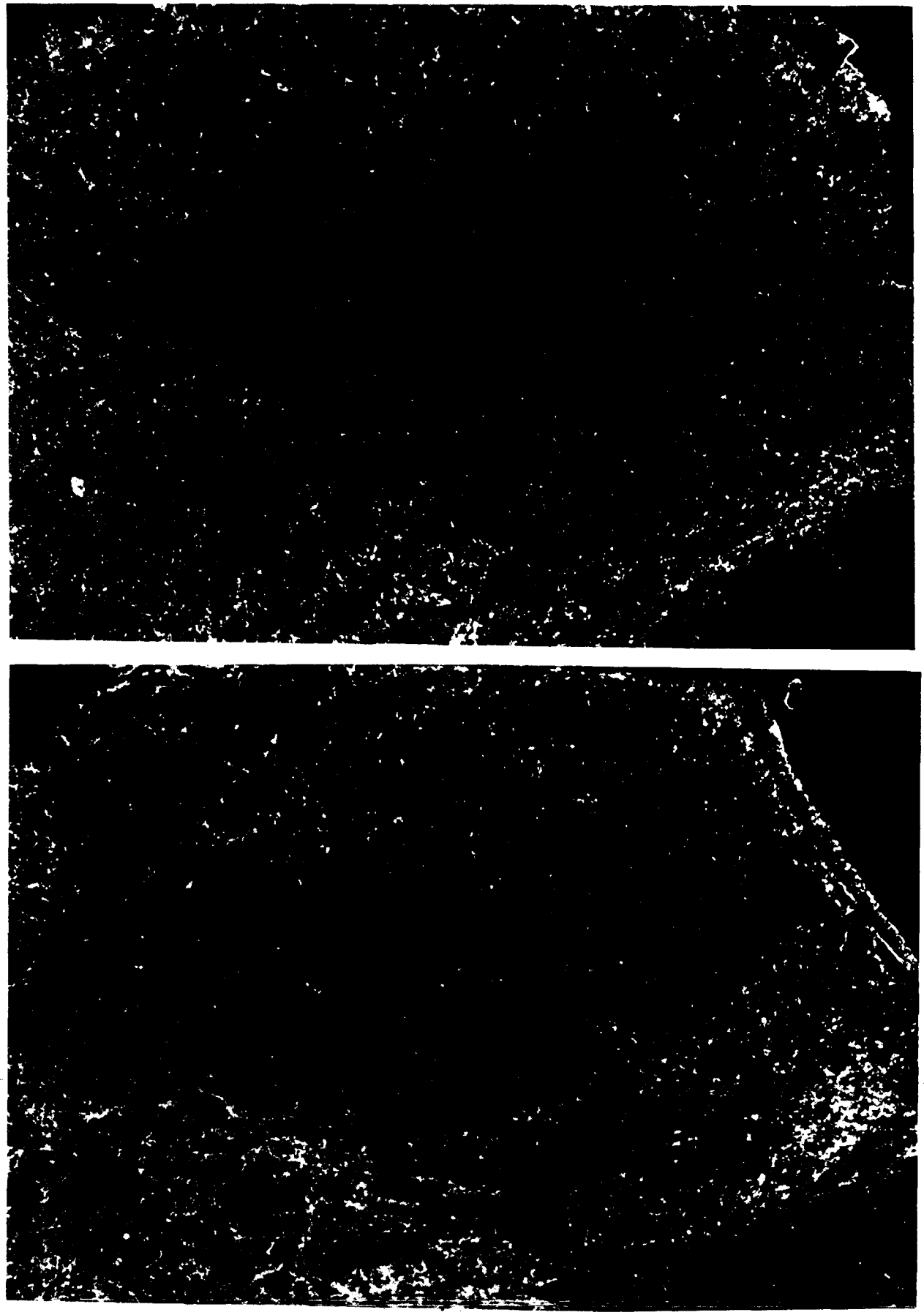


Fig. 10. 5-HT axons in the medial, superior and lateral vestibular nuclei at a level approximately 11 mm caudal to bregma corresponding to Plate 36 of Paxinos and Watson[9]. The dense 5-HT innervation of all 3 nuclei in a control animal (top panel) was greatly reduced by MDA (bottom panel). Dark-field photomicrograph, ×40.

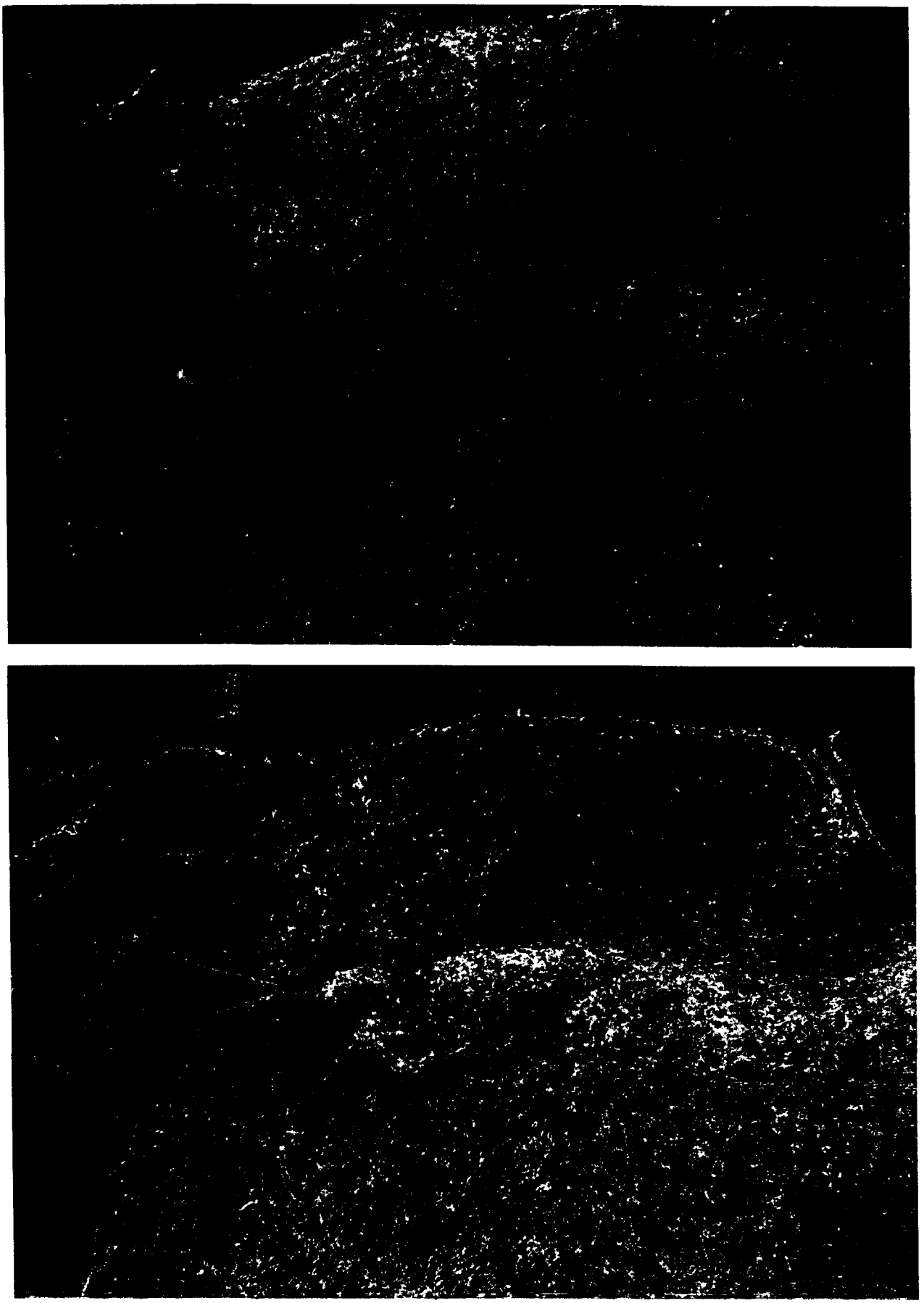


Fig. 11. 5-HT axons in the medial and spinal vestibular nuclei at a level approximately 12 mm caudal to bregma corresponding to Plate 38 of Paxinos and Watson[9]. The external cuneate nucleus can be seen just medial to the inferior cerebellar peduncle and trigeminal spinal nerve. The 5-HT innervation of all 3 nuclei in a control animal (top panel) was reduced by MDA (bottom panel). Dark-field photomicrograph, ×20.

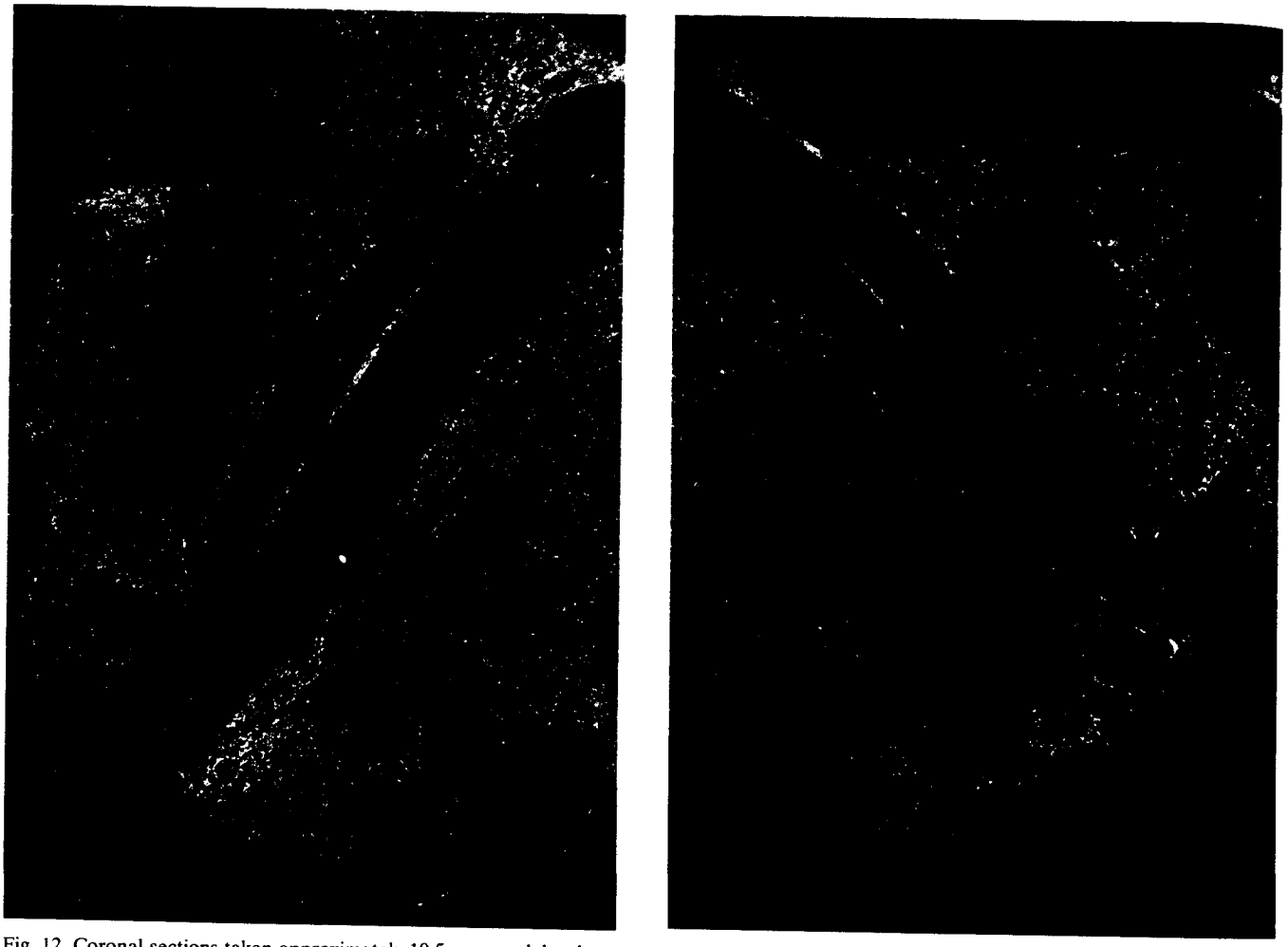


Fig. 12. Coronal sections taken approximately 10.5 mm caudal to bregma corresponding to Plate 35 of Paxinos and Watson[9]. The section was cut at an angle that allows one to view the rostral portions of the trigeminal sensory nucleus (just lateral to the facial nerve), the rostral portions of the vestibular nuclei located just lateral to the facial nerve and dorsal to the trigeminal sensory nucleus, and the superior olivary complex located at the base of the brain just medial to the facial nerve. The 5-HT innervation of these nuclei in a control animal injected with saline (right panel) does not differ from that of an animal injected with fluoxetine just prior to each injection of MDA (left panel). Dark-field photomicrograph, ×15.

in the parvocellular reticular formation just medial to the trigeminal sensory nuclei (Fig. 9) appear to have been largely spared by MDA.

The vestibular nuclei exhibited approximately equivalent neurotoxicity demonstrating 26 to 37% decreases (Fig. 1). Fig. 10 (top panel) shows the distribution of 5-HT axons in the medial, superior and lateral vestibular nuclei of a control animal and Fig. 11 (top panel) the distribution, at a more caudal level, in the medial and spinal vestibular nuclei. MDA produced a loss of fine 5-HT axons in each of these nuclei (see bottom panels of Fig. 10 and 11), while thicker axons are still present. In addition, there was a small (9%) but significant decrease in the 5-HT innervation of the external cuneate nucleus which can be seen just lateral to the spinal vestibular nucleus in Fig. 11 (compare the top and bottom panels).

As indicated in Fig. 1, fluoxetine was able to completely block the neurotoxic effects of MDA on 5-HT axons in all brain regions. This can be seen by the similar density of 5-HT immunoreactive axons in the trigeminal sensory nucleus, vestibular nucleus and superior olivary nucleus of a control animal (Fig. 12, right panel) and an animal given fluoxetine prior to each injection of MDA (Fig. 12, left panel).

DISCUSSION

This study has demonstrated that MDA destroys a much greater number of 5-HT axons in brain than had been previously reported. The pattern and degree to which MDA denervated forebrain structures in our study was identical with that previously described by O'Hearn et al.[8] who used the same dose of MDA and the same survival time. However, in addition to the previously reported damage to 5-HT axons in forebrain, we observed equivalent selective damage to 5-HT axons within highly circumscribed regions of the brain-

stem. For example, the zonal, superior and optic nerve layers of the superior colliculus were highly denervated while intermediate and deep gray levels were relatively unaffected. In addition, there was a selective neurotoxic effect in the brainstem on the trigeminal sensory complex, vestibular nuclei and the superior olivary complex. All of the neurotoxic effects of MDA in forebrain and brainstem were blocked by fluoxetine suggesting that neurotoxicity results from the uptake of MDA into 5-HT neurons. Schmidt[18] had previously demonstrated that fluoxetine also blocks the long term depletion of brain 5-HT produced by MDMA.

In agreement with O'Hearn et al.[8], MDA appeared to selectively eliminate fine axon terminals in the forebrain and this was most prominent in neocortex and field CA_1 of the hippocampus. Although these areas of the telencephalon receive 5-HT inputs from both the dorsal and median raphe nuclei[1], it has been suggested that the fine axon terminals that are particularly sensitive to the toxic effects of MDA project predominantly from the dorsal raphe nucleus[7,23]. The denervation of brainstem nuclei also appeared to be due to a selective loss of fine axon terminals. Fritschy et al.[2] demonstrated that *p*-chloroamphetamine, selectively destroyed the fine 5-HT axons projecting from dorsal raphe nucleus to the trigeminal motor nucleus without affecting inputs from other 5-HT raphe nuclei. It is possible, therefore, that at least part of the denervation of brain stem nuclei in our study is due to a loss of fine 5-HT projections from the dorsal raphe nucleus.

The neurotoxic effects of MDA in the brainstem suggest that one might expect long term alterations in visually guided eye movements and the vestibulo-ocular reflex due to denervation of the superior colliculus, superior olivary complex and vestibular nuclei and in tactile and nociceptive reflexes due to the denervation of the trigeminal sensory complex. It had been previously suggested that the selective neurotoxicity of MDA, MDMA and/or *p*-chloroamphetamine on 5-HT axons innervating the ventromedial hypothalamic nucleus and the trigeminal motor nucleus may reflect the greater sensitivity of these brain regions to smaller, acute and possibly non-toxic doses of these drugs[2,8]. On this basis, it was suggested that the anorexic effects of MDA, MDMA and related drugs could well be due to an increased release of 5-HT onto the ventromedial hypothalamic nucleus which is known to be important for processing satiety signals[8]. Similarly, the intense jaw-clenching reported after ingestion of MDMA was suggested to result from the increased release of 5-HT in the trigeminal motor nucleus[2]. An increased release of 5-HT in the trigeminal sensory complex would also be consistent with the reported increase in the tactile startle reflex produced by MDMA in the rat[6] and the reported increase in the tactually elicited nictitating membrane reflex produced by MDA in the rabbit[13,14]. The results of this and previous studies suggest that further knowledge of the regional neurotoxic effects of substituted amphetamines may provide important insights into the behavioral functions regulated by 5-HT innervation of various anatomical structures.

Acknowledgments. This work was supported by United States Public Health Service Grant DA04944 from the National Institute on Drug Abuse. We thank P. Iannuzzelli for his excellent assistance.

REFERENCES

1 Azmitia, E.C. and Segal, M., An autoradiographic analysis of the differential ascending projections of the dorsal and median raphe nuclei in the rat, *J. Comp. Neurol.*, 179 (1978) 641–668.
2 Fritschy, J.-M., Lyons, W.E., Molliver, M.E. and Grzanna, R., Neurotoxic effects of *p*-chloroamphetamine on the serotoninergic innervation of the trigeminal motor nucleus: a retrograde transport study, *Brain Res.*, 473 (1988) 261–270.
3 Harvey, J.A., Neurotoxic action of halogenated amphetamines, *Ann. N.Y. Acad. Sci.*, 12 (1978) 289–304.
4 Harvey, J.A., McMaster, S.E. and Yunger, L.M., *p*-Chloroamphetamine: selective neurotoxic action in brain, *Science*, 187 (1975) 841–843.
5 Hsu, S.M., Raine, L. and Fanger, H., The use of avidin-biotin peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures, *J. Histochem. Cytochem.*, 29 (1981) 577–580.
6 Kehne, J.H., McCloskey, T.C., Taylor, V.L., Black, C.K., Fadayel, G.M. and Schmidt, C.J., Effects of the serotonin releasers 3,4-methylenedioxymethamphetamine (MDMA), 4-chloroamphetamine (PCA) and fenfluramine on acoustic and tactile startle reflexes in rats, *J. Pharmacol. Exp. Ther.*, 260 (1992) 78–89.
7 Mamounas, L.A. and Molliver, M.E., Evidence for dual serotonergic projections to neocortex: Axons from the dorsal and median raphe nuclei are differentially vulnerable to the neurotoxin *p*-chloroamphetamine (PCA), *Exp. Neurol.*, 102 (1988) 23–36.
8 O'Hearn, E., Battaglia, G., De Souza, E.B., Kuhar, M.J. and Molliver, M.E., Methylenedioxyamphetamine (MDA) and methylenedioxymethamphetamine (MDMA) cause selective ablation of serotonergic axon terminals in forebrain: immunocytochemical evidence for neurotoxicity, *J. Neurosci.*, 8 (1988) 2788–2803.
9 Paxinos, G. and Watson, C., *The Rat Brain in Stereotaxic Coordinates*, Academic Press, New York, 1982.
10 Peroutka, S.J., Newman, H. and Harris H., Subjective effects of 3,4-methylenedioxymethamphetamine in recreational users, *Neuropsychopharmacology*, 1 (1988) 273–277.
11 Ricaurte, G.A., Bryan, G., Strauss, L., Seiden, L. and Schuster, C., Hallucinogenic amphetamine selectively destroys brain serotonin nerve terminals, *Science*, 229 (1985) 986–988.
12 Ricaurte, G.A., Forno, L.S., Wilson, M.A., Delanney, L.E., Irwin, I., Molliver, M.E. and Langston, J.W., (±)3,4-Methylenedioxymethamphetamine (MDMA) selectively damages central serotonergic neurons in non-human primates, *J. Am. Med. Assoc.*, 260 (1988) 51–55.
13 Romano, A.G., Bormann, N.M. and Harvey, J.A., A unique enhancement of associative learning produced by methylenedioxyamphetamine, *Behav. Pharmacol.*, 2 (1991) 225–231.
14 Romano, A.G. and Harvey, J.A., Enhanced learning following a single, acute dose of MDA, *Pharmacol. Biochem. Behav.*, in press.
15 Sanders-Bush, E., Bushing, J.A. and Sulser, F., Long-term effects of *p*-chloroamphetamine on tryptophan hydroxylase activity and on levels of 5-hydroxytryptamine and 5-hydroxyindole acetic acid in brain, *Eur. J. Pharmacol.*, 20 (1972) 385–388.

16 Sanders-Bush, E., Bushing, J.A. and Sulser, F., Long-term effects of *p*-chloroamphetamine and related drugs on central serotonergic mechanisms, *J. Pharmacol. Exp. Ther.*, 192 (1975) 33–41.
17 Scallet, A.C., Lipe, G.W., Ali, S.F., Holson, R.R., Frith, C.H. and Slikker, W., Jr., Neuropathological evaluation by combined immunohistochemistry and degeneration-specific methods: application to methylenedioxymethamphetamine, *Neurotoxicology*, 9 (1988) 529–537.
18 Schmidt, C.J., Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine, *J. Pharmacol. Exp. Ther.*, 240 (1987) 1–7.
19 Schmidt, C.J., Wu, L. and Lovenberg, W., Methylenedioxymethamphetamine: a potentially neurotoxic amphetamine analogue, *Eur. J. Pharmacol.*, 124 (1986) 175–178.
20 Shulgin, A.T. and Nichols, D.E., Characterization of three new psychotomimetics. In R. Stillman and R. Willette (Eds.), *The Psychopharmacology of Hallucinogens*, Pergamon, New York, 1978, pp. 74–83.
21 Stone, D.M., Stahl, D.C., Hanson, G.R. and Gibb, J.W., The effects of 3,4-methylenedioxymethamphetamine (MDMA) and 3,4-methylenedioxyamphetamine (MDA) on monoaminergic systems in the rat brain, *Eur. J. Pharmacol.*, 128 (1986) 41–48.
22 Wang, Shwun-De, Goldberger, M.E. and Murray, M., Plasticity of spinal systems after unilateral lumbosacral dorsal rhizotomy in the adult rat, *J. Comp. Neurol.*, 304 (1991) 555–568.
23 Wilson, M.A., Ricaurte, G.A. and Molliver, M.E., Distinct morphologic classes of serotonergic axons in primates exhibit differential vulnerability to the psychotropic drug 3,4-methylenedioxymethamphetamine, *Neuroscience*, (1989) 121–137.