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Research report

Comparative study of fluoxetine, sibutramine, sertraline and dexfenfluramine on the morphology of serotonergic nerve terminals using serotonin immunohistochemistry

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Abstract

We compared the effects of treatment with high doses of fluoxetine, sibutramine, sertraline, and dexfenfluramine for 4 days on brain serotonergic nerve terminals in rats. Methylenedioxymethamphetamine (MDMA) and 5,7-dihydroxytryptamine (5,7-DHT) were used as positive controls because both compounds deplete brain serotonin. Food intake and body weight changes were also monitored and yoked, pair-fed animals were used to control for possible changes in morphology due to nutritional deficits. Fluoxetine, sibutramine, sertraline and dexfenfluramine all produced a significant reduction in body weight. Fluoxetine, sibutramine and sertraline treatment resulted in no depletion of brain serotonin but produced morphological abnormalities in the serotonergic immunoreactive nerve network. In contrast, dexfenfluramine and MDMA depleted brain serotonin and produced morphological changes in the serotonin nerve network. These results indicate that even though fluoxetine, sibutramine and sertraline do not deplete brain serotonin, they do produce morphological changes in several brain regions (as identified by serotonin immunohistochemistry). Dexfenfluramine and MDMA, on the other hand, markedly deplete brain serotonin and also produce morphological changes. Collectively, these results lend support to the concept that all compounds acting on brain serotonin systems, whether capable of producing serotonin depletion or not, could produce similar effects on the morphology of cerebral serotonin systems.

Keywords: MDMA; SSRI; Anorectic agent; Amphetamine; Neurotoxicity

1. Introduction

It is now well documented that high doses of fenfluramine and methylenedioxymethamphetamine (MDMA) cause marked depletions of brain serotonin [2,12,15,34,35]. These compounds also produce changes in the morphology of serotonergic nerve terminals [1,10,15,25,36,44,45,49]. These morphological changes are characterized by short, thickened, bulbous profiles [25,36]. Recently, in spite of the lack of convincing evidence, these findings of serotonin depletion with or without morphological changes have become accepted as evidence of degeneration [33,36, 44].

Drug-induced morphological changes in the brain usually appear when the serotonergic nerve network is depleted of serotonin [25,36,44]. Since depletion of brain serotonin alone has been considered to be an acceptable indicator of damage [1,2,9,10,21,24,32,36,44,48,52] it has been assumed that compounds that do not deplete brain serotonin could not damage the nerve terminals and therefore no morphological changes are to be expected. In addition, it has been suggested that abnormal morphological profiles demonstrate pathology resulting from the direct action of fenfluramines and MDMA on the brain serotonin system [3,33,34,36,44] through mechanisms that have yet to be elucidated. A logical conclusion from this assumption is that compounds that do not produce any significant measure of depletion of the brain serotonin system (such as fluoxetine, sibutramine, sertraline) [5,8,11] would not produce any abnormality in the morphology of

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this system [39,52]. To our knowledge no one has tested this hypothesis experimentally.

In this study, we have directly compared serotonergic immunoreactivity in the brains of animals after exposure to compounds that deplete brain serotonin (dexfenfluramine and MDMA) [24,26,37] with the brains of animals exposed to compounds that do not deplete brain serotonin (fluoxetine, sibutramine and sertraline [16,53]. The selective serotonin reuptake inhibitors (SSRIs) fluoxetine and sertraline, and the serotonin and norepinephrine reuptake inhibitor (SNRI) sibutramine were selected as the candidate drugs for this study because these agents do not deplete brain serotonin and have not been studied with regard to their effect on the morphology of serotonergic nerve fibers. In addition, the SSRI fluoxetine, which has traditionally been used as an antidepressant, and sibutramine are receiving attention as anorectic agents [31,51] because of their pharmacological similarity to the fenfluramine. Therefore, an evaluation of their effect on brain morphology was considered to be important.

In evaluating the possible morphological changes produced by fluoxetine, sibutramine, sertraline and dexfenfluramine, it should be noted that fluoxetine and sertraline are SSRIs, sibutramine is a SNRI [29,31,51] and dexfenfluramine, like MDMA, is a serotonin releaser and reuptake inhibitor [14,17]. For the neurochemical analysis of 5-HT, we selected 5,7-dihydroxytryptamine (5,7-DHT) as our control compound because it is a known serotonergic neurotoxicant that markedly depletes brain serotonin after intracerebroventricular (i.c.v) injection [4,6,7,19,20,50]. We have used immunocytochemistry to examine the morphology of the serotonergic network in the brain and HPLC analysis of 5-HT levels to demonstrate depletion of brain serotonin. Using an antibody specific for serotonin, we found that high doses of fluoxetine, sibutramine, sertraline produced changes in morphology in several brain regions without depleting the serotonin system [30]. These morphological changes were randomly distributed throughout

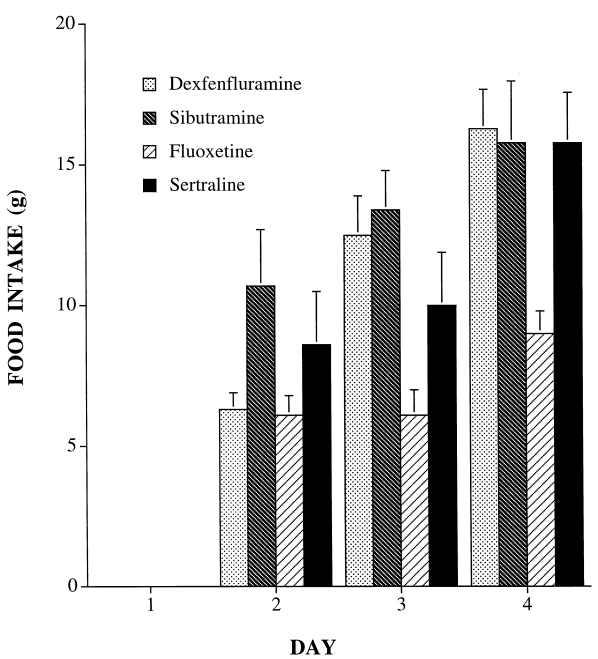

Fig. 1. Effect of 4 days of oral administration of high doses of dexfenfluramine ($n = 10$), sibutramine ($n = 13$), fluoxetine ($n = 10$) and sertraline ($n = 9$) on food intake. Bars represent means ± S.E.M.

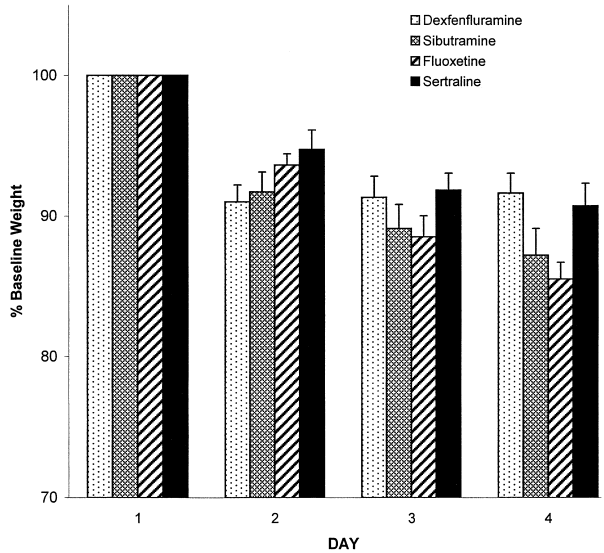

Fig. 2. Effect of 4 days of oral administration of high doses of dexfenfluramine ($n = 10$), sibutramine ($n = 13$), fluoxetine ($n = 10$) and sertraline ($n = 9$) on body weight. Bars represent means ± S.E.M. Baseline weight values were: dexfenfluramine, mean 185.1 g (S.E.M. 11.0); sibutramine, mean 179.5 g (S.E.M. 7.9); fluoxetine, mean 178.8 g (S.E.M. 8.9); sertraline, mean 177.2 g (S.E.M. 10.7).

Table 1
HPLC analysis of 5-HT content in the prefrontal cortex 18 h following a 4-day oral treatment with dexfenfluramine, fluoxetine, sibutramine and sertraline and 4 days after bilateral intraventricular injection of 5,7-DHT Shown are the mean values ± S.E.M. in the frontal cortex. Drug treatment values are compared to vehicle (Veh) in pair-fed controls (PFC).

Group	Dose	5-HT
Dexfenfluramine ($n = 6$)	4.3 mg/kg	224.0 ± 37.1**
PFC (Veh) ($n = 6$)		309.3 ± 36.0
Dexfenfluramine ($n = 6$)	43.0 mg/kg	10.00 ± 0.0***
PFC (Veh) ($n = 6$)		311.4 ± 17.5
Fluoxetine ($n = 6$)	11.4 mg/kg	284.0 ± 27.9
PFC (Veh) ($n = 6$)		323.5 ± 22.8
Fluoxetine ($n = 6$)	114.0 mg/kg	264.8 ± 28.2
PFC (Veh) ($n = 6$)		316.8 ± 15.6
Sibutramine ($n = 6$)	2.8 mg/kg	401.5 ± 29.9
PFC (Veh) ($n = 6$)		372.9 ± 57.5
Sibutramine ($n = 6$)	28.0 mg/kg	366.9 ± 61.3
PFC (Veh) ($n = 6$)		348.2 ± 45.0
Sertraline ($n = 6$)	28.6 mg/kg	306.3 ± 19.2
PFC (Veh) ($n = 6$)		319.8 ± 11.6
Sertraline ($n = 6$)	286.0 mg/kg	213.0 ± 17.6**
PFC (Veh) ($n = 6$)		311.3 ± 15.9
5,7-DHT ($n = 6$)	100 μg intraventricularly	112.57 ± 36.23***
PFC (Veh) ($n = 6$)		335.12 ± 17.69

** Significantly different at $P < 0.05$.
*** Significantly different at $P < 0.001$.

the neuropil and resemble the distribution patterns previously observed with MDMA and dexfenfluramine [33,34,36,52]. These results demonstrate that morphological changes occur without any depletion of brain serotonin and show that high doses of SSRIs, SNRIs, dexfenfluramine and MDMA have similar effects on brain serotonergic neurons. The relationship of these morphological changes to the physiology of the serotonergic system needs to be examined.

2. Materials and methods

2.1. Animals

Experiments were carried out on male Sprague–Dawley rats (150 g initial weight) obtained from Harlan Industries (Walkerville, MD). All experiments were conducted in full compliance with the animal care guidelines of the National Institutes of Health.

2.2. Housing and pair-feeding

Animals were housed individually in hanging wire cages in a pathogen-free environment under standard conditions (room temperature of 18°C and controlled 50% humidity), with a 12 h on/12 h off light/dark cycle. All animals were weighed daily at the same time. Because the doses at which abnormal morphological profiles are only seen with high doses of fenfluramine, we used similar multiples of the therapeutic doses with fluoxetine, sibutramine, sertraline. Since high doses of fluoxetine produce an increase in core body temperature and increased mortality, we lowered the ambient temperature to 18°C, and housed the animals singly in hanging wire cages with increased airflow around the cages.

During the initial period of adjustment to the laboratory conditions, animals were given rat chow pellets ad libitum and the body weights of the animals were recorded. All pair-feedings were done with the same starting body weight. Each experimental animal was matched with a yoked, pair-fed control animal.

After the animals had acclimatized, the experimental group was given free access to pre-weighed rat chow pellets and water. Body weight and food intake were monitored daily for the duration of the study. Pair-fed controls were fed rat chow pellets equal to the weight of the food their yoked, experimental animal had eaten on the previous day to ensure that any observed change in morphological or biochemical endpoints was not due to nutritional deficits.

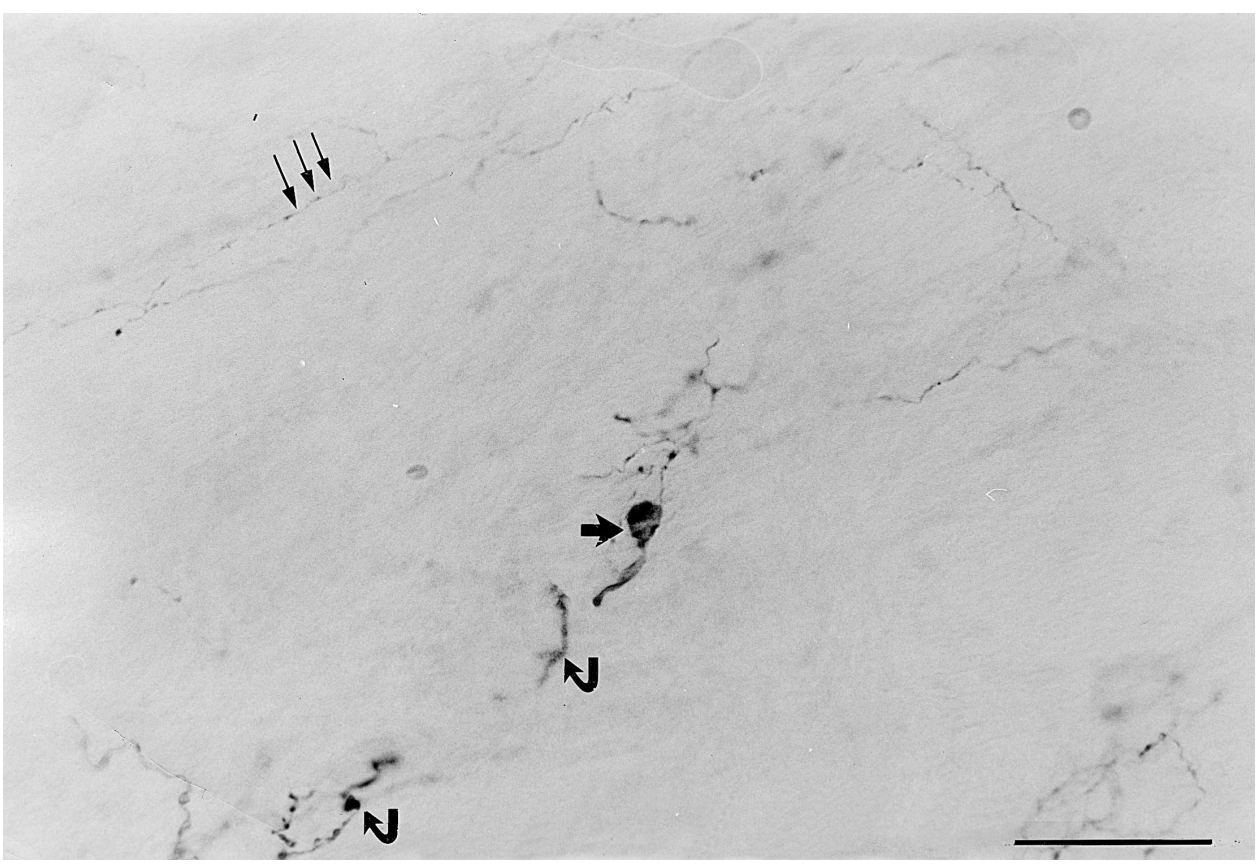

Fig. 3. High-magnification, bright-field photomicrograph of the prefrontal cortex of a rat following treatment with 43 mg/kg of dexfenfluramine for 4 days followed by an 18-h recovery period. This section is 20 μm thick and was stained for 5-HT immunocytochemistry. Bold and curved arrowheads point to abnormal swollen profiles. Three fine straight arrowheads point to one fine ''normal'' serotonergic nerve fiber. Magnification bar = 100 μm.

2.3. Statistical analysis

Brain serotonin levels and body weights of the drug-treated and the pair-fed controls were compared using two-sided Student's paired *t*-test. Food intake was compared using two-sided unpaired *t*-test. A p-value < 0.05 was considered to be significant in both cases.

2.4. Drug treatments

Aqueous solutions of fluoxetine (11.4 and 114 mg/kg), sibutramine HCl (2.8 and 28 mg/kg), sertraline (28.6 and 286 mg/kg) and dexfenfluramine (4.3 and 43 mg/kg) were administered (in two divided doses per day) by gavage. The drug treatment doses were selected based on multiples of the oral therapeutic dose in humans. Since this was a toxicity study, we selected a low-dose level as 10 times the therapeutic dose and a high-dose level as 100 times the therapeutic dose. The selection of these low- and high-dose levels, duration of treatment and post-treatment survival periods are consistent with those used in previous toxicity studies with these agents [1–3,13,16,17,25–27,33,34,36,45]. Yoked, pair-fed controls received the same volume of the vehicle. The animals were treated with the drugs for 4 days and were sacrificed either 18 h or 30 days post-treatment. Twelve animals per drug, per dose, per survival period were used. Four doses of MDMA (20 mg/kg) were administered s.c., every 2 h; 12 animals were used with this compound. A single injection of 50 μg (in 10 μl) of 5,7-DHT solution was made into each lateral ventricle using stereotaxic coordinates [41]. Six animals were treated with this compound. Control animals received intracerebroventricular injections of the vehicle.

2.5. Perfusion and immunocytochemical procedure

The animals were perfused and brain tissue processed for 5-HT immunohistochemistry on serial 30 μm para-sagittal sections [25,42].

Following the appropriate survival period the animal and its pair-fed control was heparinized with 5000 IU given intraperitoneally. The animals were anesthetized with sodium pentobarbital (Nembutal) 35 mg/kg and perfused transcardially with buffered saline (pH 7.4) followed by 4% freshly prepared paraformaldehyde. Following perfusion the brains were rapidly removed and cryprotected by storing them in 0.1 M phosphate buffer containing 10–30% sucrose at 4°C prior to processing. Brains were coded so that all further processing was done in a double blind manner.

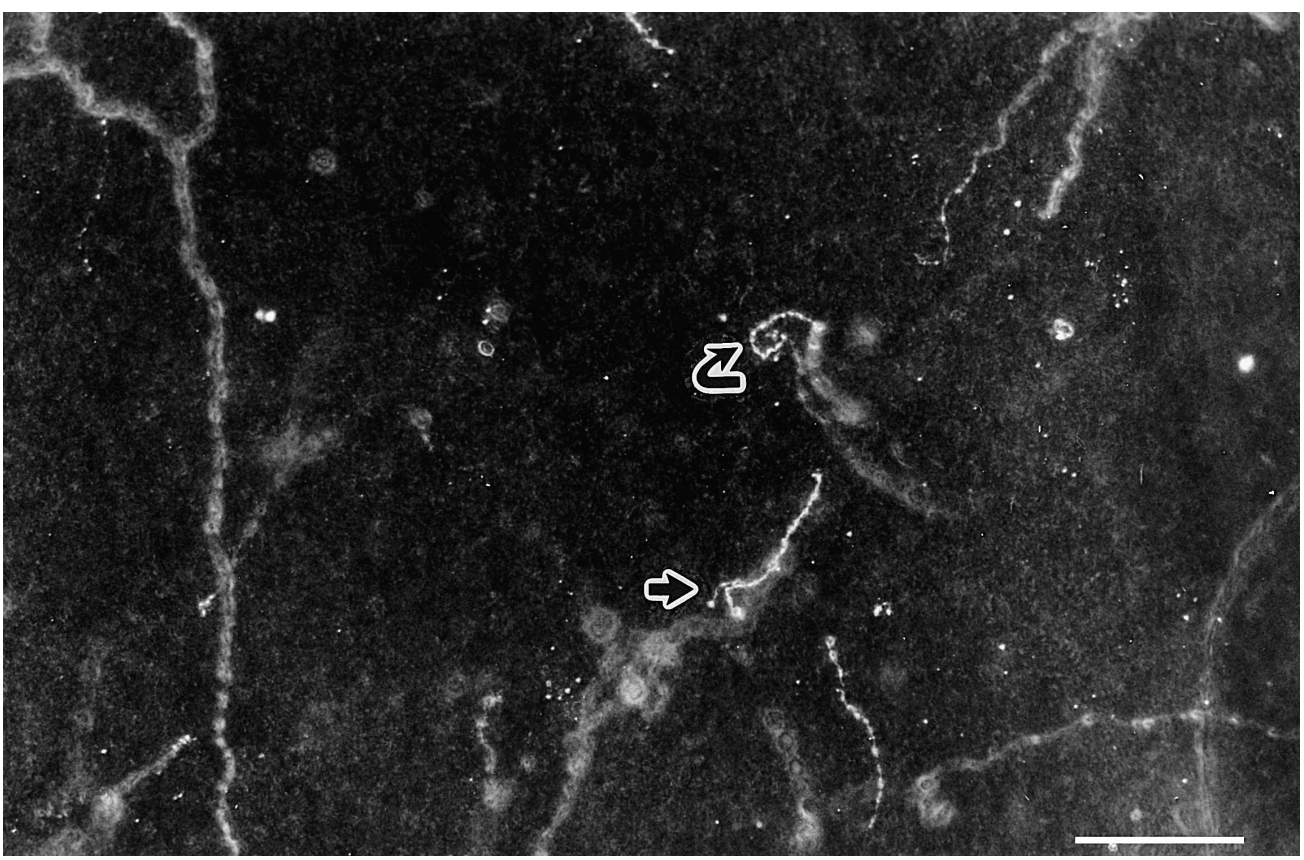

Fig. 4. High-magnification, dark-field photomicrograph of the prefrontal cortex of a rat following treatment with four doses of 20 mg/kg of MDMA followed by an 18-h recovery period. This section is 20 μm thick and was stained for 5-HT immunocytochemistry. Notice the swollen and cork-screw-shaped profiles indicated by arrowheads. Magnification bar = 100 μm.

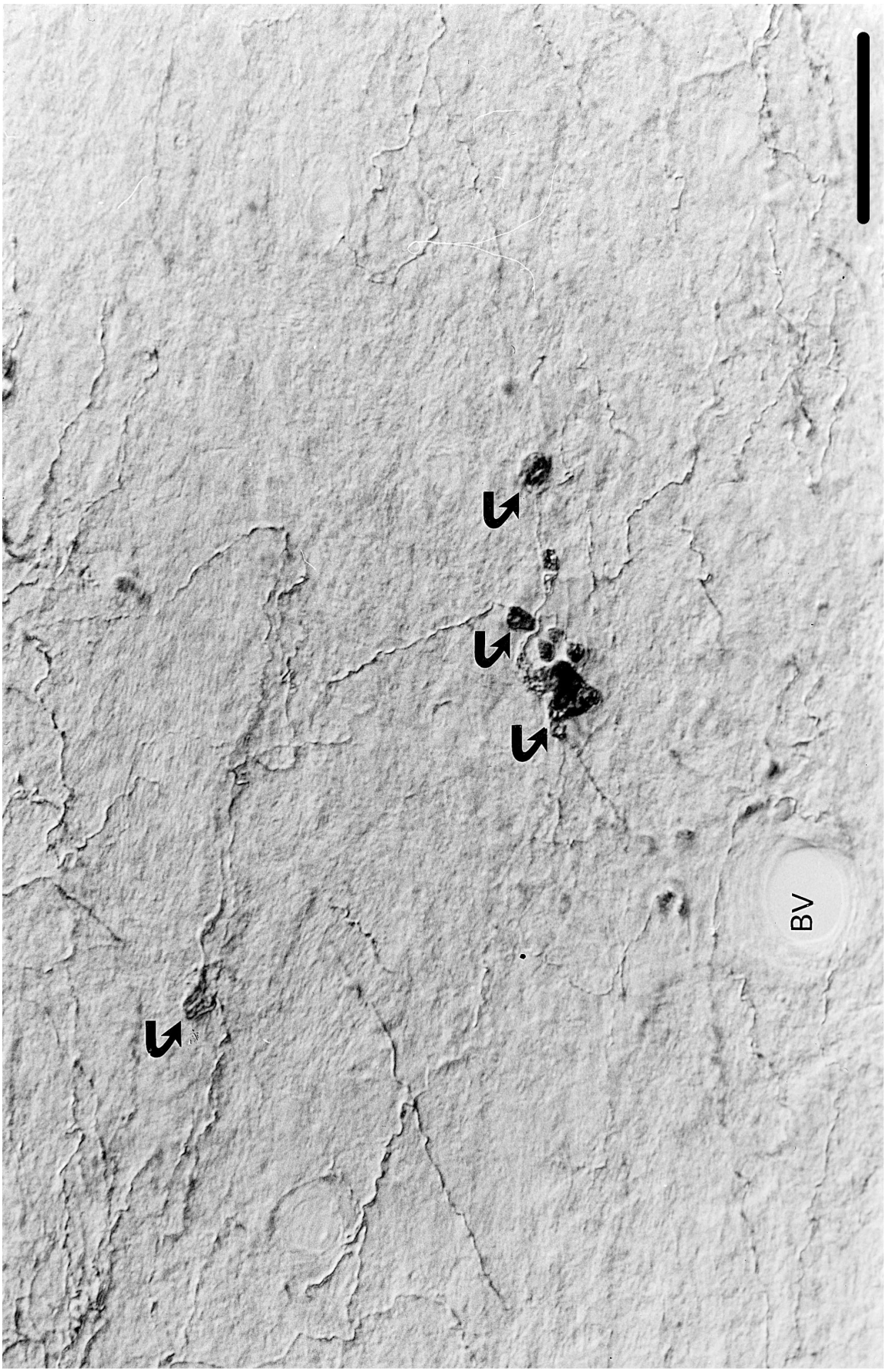

Fig. 5. High-magnification, bright-field photomicrograph of the prefrontal cortex of a rat following treatment with 114 mg/kg of fluoxetine for 4 days followed by an 18-h recovery period. This section is 20 μm thick and was stained for 5-HT immunocytochemistry. Notice the swollen profiles marked by curved arrowheads. A number of fine ''normal'' serotonergic nerve fibers can be seen in the background. Magnification bar = 100 μm.

The two cerebral hemispheres were separated by sectioning the corpus callosum and 30-μm-thick, serial, frozen sections were cut in the para-sagittal plane with a Leitz sledge microtome equipped with a freezing stage. The sections were collected in phosphate buffer at 4°C following which they were immersed in 0.3% Triton solution and phosphate buffer for 15 min and then into 5-HT antiserum 1:3000 dilution (Incstar rabbit 5-HT antiserum). The sections were incubated in the antiserum overnight on an orbital shaker table and the following day the sections

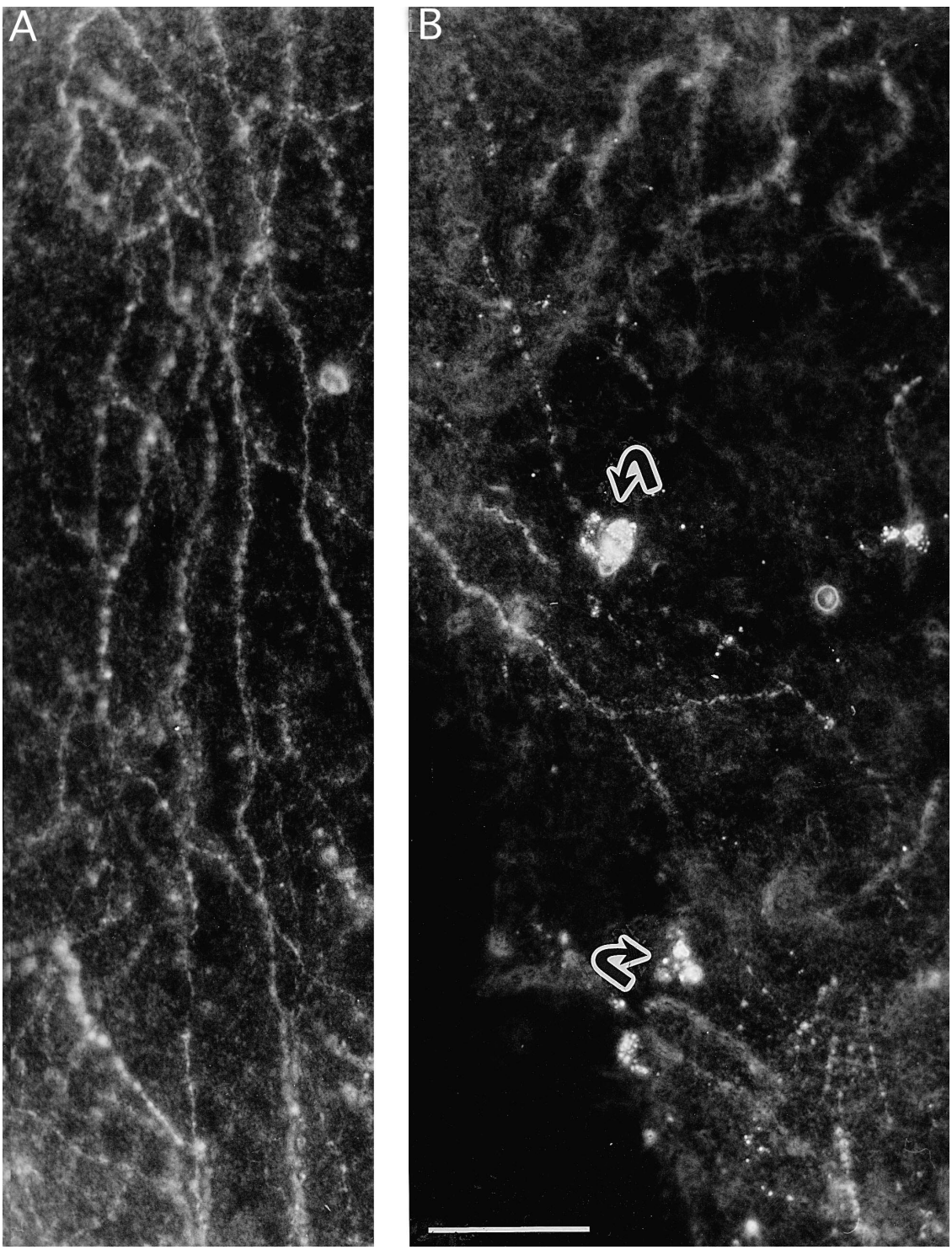

Fig. 6. High-magnification, dark-field photomicrograph of the prefrontal cortex of a rat following treatment with 11.4 mg/kg of fluoxetine for 4 days followed by an 18-h recovery period. This section is 20 μm thick and was stained for 5-HT immunocytochemistry. Notice the swollen profiles marked by curved arrowheads. Numerous fine serotonergic nerve fibers are visible. Magnification bar = 100 μm.

were rinsed three times in phosphate buffer at 4°C. They were then incubated for 2 h in rabbit Igg (Vector Labs) in a 1:100 dilution in 0.3% Triton. Afterwards, the sections were rinsed three times in phosphate buffer at 4°C, and were then placed in ABC elite kit 1:50 dilution for 1 h following which they were rinsed twice in phosphate buffer and twice in Tris buffer 0.05 M and were then reacted with diaminobenzidine along with hydrogen peroxide. The reaction was carefully monitored throughout so that background staining in all sections remained minimal and the staining was optimal. The reaction was stopped by transferring the sections into Tris buffer, and the sections were mounted out of Tris buffer at 4°C onto gelatin-coated slides. The tissue was dehydrated and coverslipped with Permount for light microscopic examination. Identical regions of the brain from experimental and control animals were examined and photographed.

2.6. Brain dissection and tissue preparation

Brains from animals in each treatment group, each control group, each survival period and the 5,7-DHT treatment group and sham-injected animals were used for 5-HT analysis. Animals from each drug-treated group were sacrificed 18 h and 30 days after the treatment or pair-feeding period. Animals from the 5,7-DHT-treated group and the sham intraventicular injection group were sacrificed 4 days after the intracerebroventricular injection.

Immediately after decapitation, the whole brain was removed from the skull with blunt curved forceps and placed on a cold plate at 4°C. The cortex was separated from the diencephalon and the cortex was flattened and then divided in four equal quadrants. Each of these quadrants was weighed, frozen on dry ice and stored at −70°C for subsequent analysis of 5-HT levels.

2.7. 5-HT analysis

5-HT was assayed by high performance liquid chromatography with electrochemical detection [40]. Samples were homogenized in ice-cold 0.2 N perchloric acid containing 30 pM of *N*-methyl-5-HT as the internal standard. After centrifugation at $10{,}000 \times g$ for 10 min, a 20-μl aliquot of the supernatant was injected via a pump (Model 515, Waters, Milford, MA) and a refrigerated (4°C) automatic sample injector (717 Plus Autosampler, Waters) into a C-18 reverse-phase column (Waters Symmetry #WAT046980, Waters) equipped with a precolumn filter.

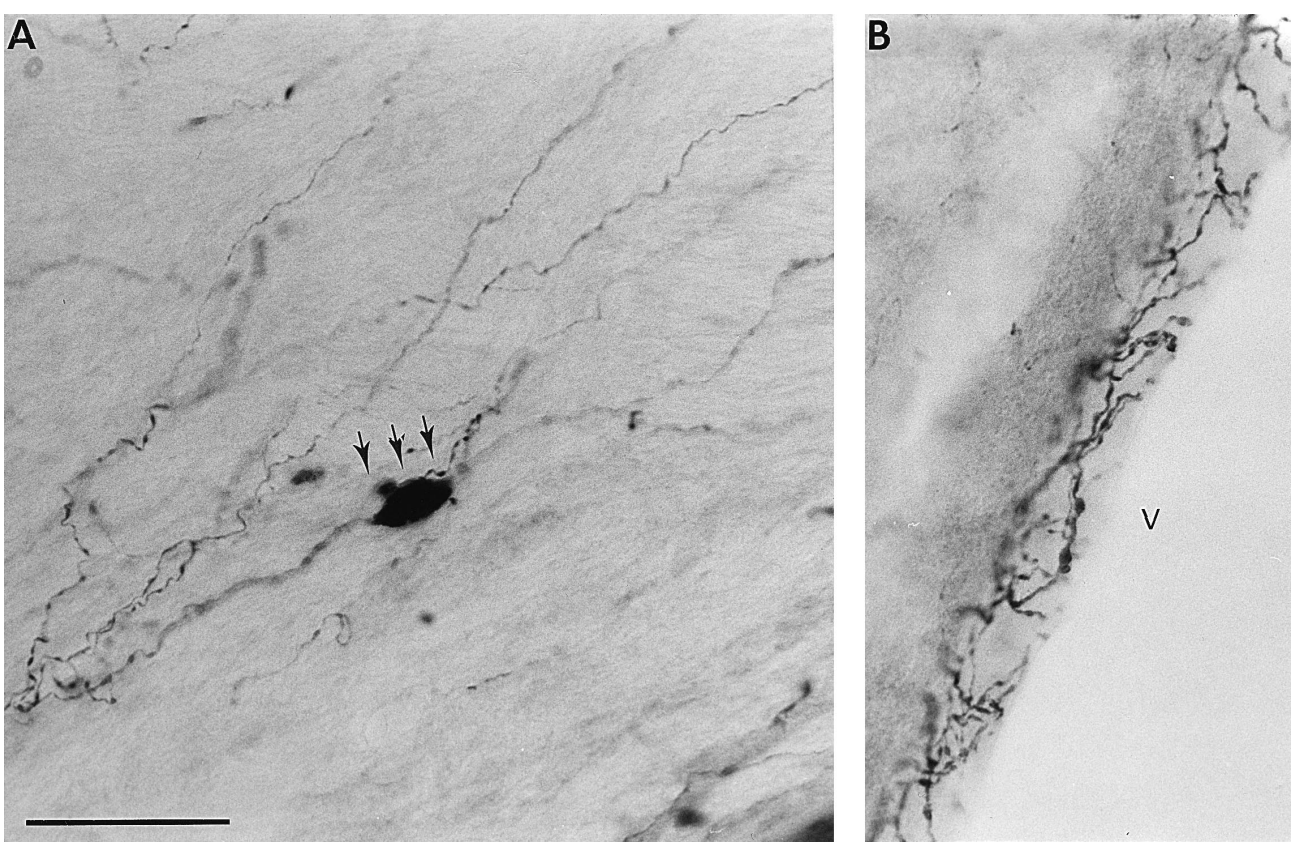

Fig. 7. (A) High-magnification, bright-field photomicrograph of the prefrontal cortex of a rat following treatment with 28 mg/kg of sibutramine for 4 days followed by an 18-h recovery period. This section is 20 μm thick and was stained for 5-HT immunocytochemistry. Three arrowheads point to a very swollen profile on an otherwise normal serotonergic nerve fiber. (B) Serotonergic nerve fibers in the wall of the lateral ventricle (V). Magnification bar = 100 μm.

A pulsed electrochemical detector (model # 464, Waters) was used to detect elution peaks. Acquisition and integration of peaks was accomplished by a Digital Venturis 466 computer running Millenium 2010 Chromatography software (Waters). The mobile phase consisted of 3.96 mM heptane sulfonic acid, 0.1 μM EDTA dihydrate, 15% methanol and 0.074 M H_3PO_4 maintained at a pH of 2.5.

3. Results

3.1. Food intake and weight loss

The effect of high doses of fluoxetine, sibutramine, sertraline and dexfenfluramine, on food intake following oral treatment for 4 days is shown in Fig. 1. Following 1 day of treatment (i.e., on day 2 of observation), animals who had received fluoxetine and dexfenfluramine had consumed a significantly less food as compared to animals who had received either sibutramine or sertraline ($p = 0.025$). After 3 days of treatment, the animals treated with dexfenfluramine and sibutramine had begun to increase their food intake (Fig. 1) while fluoxetine-treated animals were still eating less. Four days after treatment, dexfenfluramine-, sibutramine- and sertraline-treated animals were consuming the same amount of food. Fluoxetine-treated animals were consuming considerably less.

Fig. 2 shows the effect of high-dose dexfenfluramine, sibutramine, fluoxetine and sertraline treatment for 4 days on body weight (as a percentage of baseline). After 1 day of treatment (i.e., on day 2 of observation), all animals showed a reduction in body weight. At the end of the 3-day treatment, the body weight of all the animals was lower than at the baseline. By the fourth day, dexfenfluramine-treated animals had begun to gain weight, whereas the sibutramine-, fluoxetine- and sertraline-treated animals continued to lose weight.

Treated animals lost significantly more weight than their pair-fed controls. After 1 day of treatment the *p*-values were as follows: dexfenfluramine < 0.001, fluoxetine $= 0.004$, sibutramine < 0.001 and sertraline $= 0.015$. After 3 days of treatment, the *p*-values were as follows: dexfenfluramine $= 0.008$, fluoxetine $= 0.009$, sibutramine < 0.001 and sertraline 0.022.

3.2. Neurochemical consequence of these drug treatments on cortical serotonin levels

Table 1 gives the mean values of serotonin content in the prefrontal cortex of rats treated with dexfenfluramine,

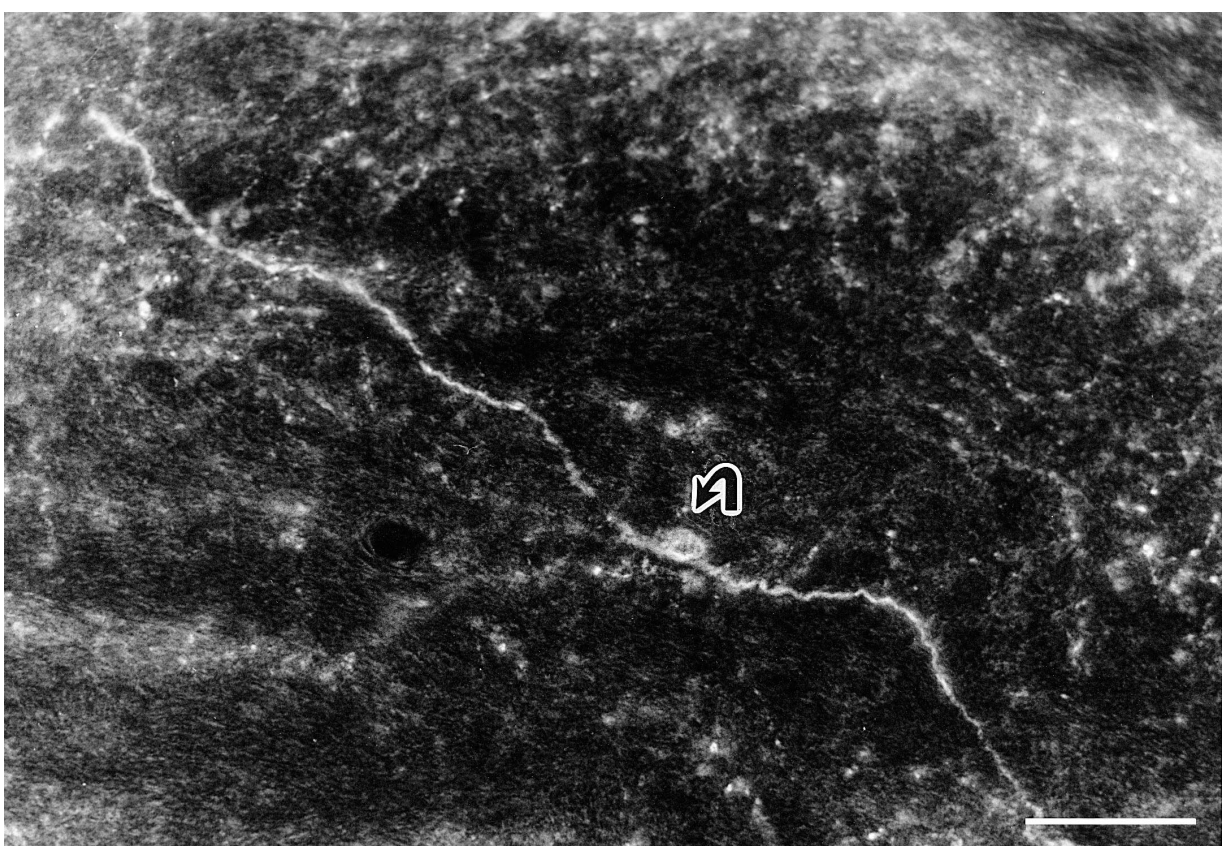

Fig. 8. High-magnification, dark-field photomicrograph of the prefrontal cortex of a rat following treatment with 2.8 mg/kg of fluoxetine for 4 days followed by an 18-h recovery period. This section is 20 μm thick and was stained for 5-HT immunocytochemistry. Notice the swollen profile (arrowhead) on a fine serotonergic nerve fiber. Magnification bar = 100 μm.

fluoxetine, sibutramine, sertraline and one dose 5,7-DHT and their respective pair-fed controls following an 18-h post-treatment survival period. We selected the quadrant containing the prefontal cortex for neurochemical analysis because this region receives dense serotonergic innervation from the median raphe nuclei [28]. Brain serotonin levels decreased significantly following treatment with both high and low doses of dexfenfluramine and with the high dose of sertraline. None of the other treatment groups showed any statistically significant change.

3.3. Morphological changes

3.3.1. Dexfenfluramine

Examination of serotonin immunoreactive nerve terminals in several regions of the brain (frontal and occipital cortex, hippocampus, superior and inferior colliculi) 18 h following treatment with high doses (43 mg/kg) of dexfenfluramine revealed the same morphological features (swollen axon terminals, thick axons ad cork-screw like profiles) that have been described earlier [25,36]. These morphological features were seen against a background devoid of serotonin immunoreactive nerve fibers (Fig. 3). This pattern in the serotonergic nerve network was apparent in all the brain regions examined and was characteristically random in its distribution. Lower doses of dexfenfluramine produced no abnormal morphology in these regions, even when examined 18 h post-treatment. No morphological abnormality was found with either dose at the 30-day post-treatment time point. The pair-fed control animals were all examined and showed no abnormal profiles.

3.3.2. MDMA

MDMA-treated rats were included in this study because the morphological changes produced by this compound have been described as similar to those produced by dexfenfluramine. Therefore, MDMA served as a useful positive control for the morphological changes. 5,7-DHT was used as a positive control for the neurochemical changes. Examination of the brain of rats 18 h after four doses of 20 mg/kg (given at 2-h intervals) were administered revealed showed abnormal serotonergic immunoreactive profiles in the frontal and occipital cortex, hippocampus, superior and inferior colliculi. The morphology of these profiles, their distribution and the background of the tissue closely resembled the profiles found following treatment with high doses of dexfenfluramine. The profiles were heterogeneous in their morphological characteristics. We found curved, corkscrew-like swellings and thickened short axons randomly distributed in several brain regions (Fig. 4). These profiles were easily distinguishable under bright- and dark-field illumination and were similar to those observed by other investigators. Only one time point

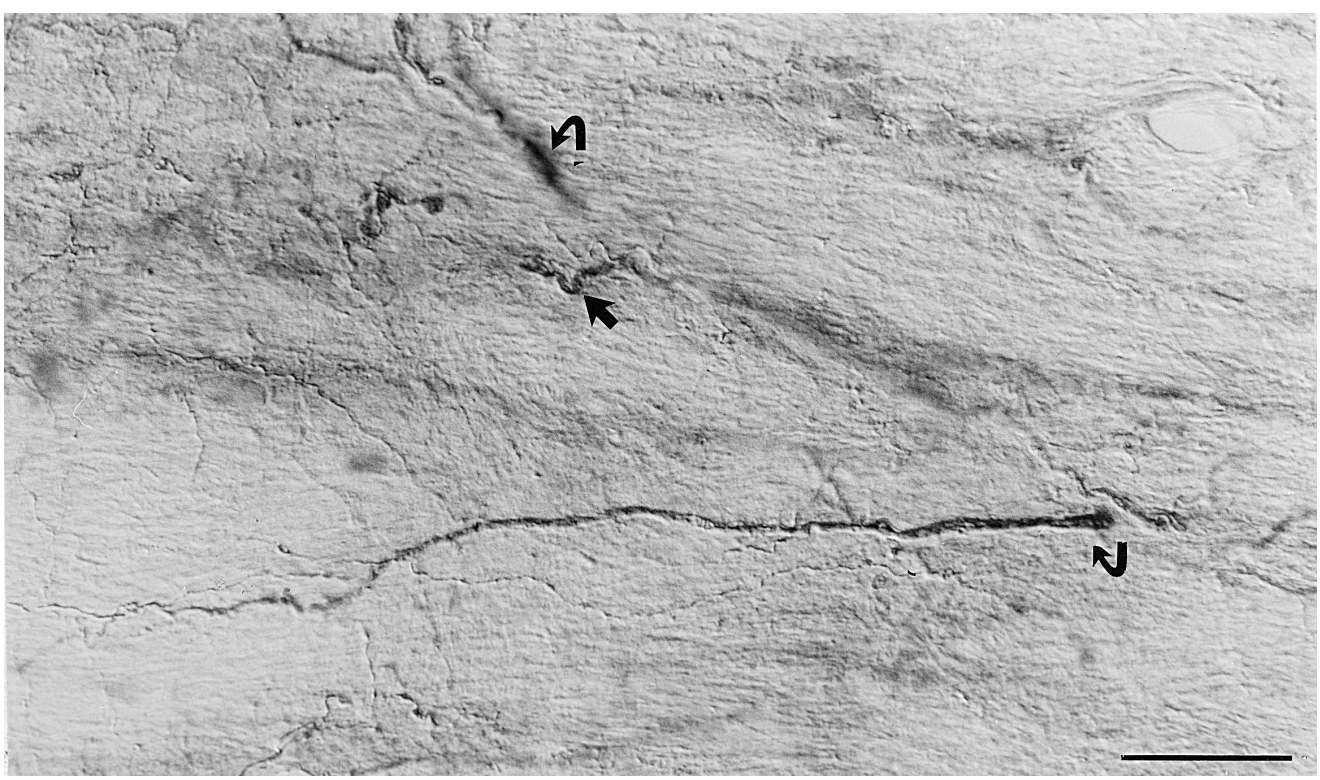

Fig. 9. High-magnification, bright-field photomicrograph of the prefrontal cortex of a rat following treatment with 286 mg/kg of sertraline for 4 days followed by an 18-h recovery period. This section is 20 μm thick and was stained for 5-HT immunocytochemistry. Notice the swollen profiles marked by curved arrowheads. Magnification bar = 100 μm.

(18 h post-treatment) was examined following MDMA treatment. The age and weight-matched control animals showed no morphological abnormality in the serotonergic immunoreactive nerve network.

3.3.3. Fluoxetine

In the high-dose (114 mg/kg)-treated animals, a 4-day exposure to fluoxetine following an 18-h post-treatment survival period showed the presence of abnormal profiles in the frontal and occipital cortex, hippocampus, superior and inferior colliculi. These profiles were seen against a background of normal intact serotonergic nerve network. (Fig. 5). The most commonly observed profile was that of a large swelling which was visible both under bright- and dark-field illumination. The low-dose-treated animals (Fig. 6) showed an occasional swelling. No swellings were found either in the brains of the 30-day survival animals or any of the pair-fed controls.

3.3.4. Sibutramine

In the high-dose (128 mg/kg)-treated animals, a 4-day exposure to sibutramine following an 18-h post-treatment survival period showed the presence of randomly distributed abnormal profiles (Fig. 7). In the frontal and occipital cortex, hippocampus, superior and inferior colliculi. This picture was remarkably similar to the swellings seen 18 h following high-dose fluoxetine treatment. These profiles were also found against a background of normal and intact serotonergic nerve fibers. Animals in the low-dose, 18-h survival group (Fig. 8) showed a few swellings along normal serotonin immunoreactive nerve fibers. The control and 30-day post-treatment survival animals showed no abnormalities in the serotonergic immunoreactive nerve network.

3.3.5. Sertraline

Similar morphological changes were also seen in the high-dose (286 mg/kg) sertraline-treated animals with a 4-day exposure following an 18-h post-treatment survival period. The frontal and occipital cortex, hippocampus, superior and inferior colliculi showed abnormal profiles (Fig. 9). The most commonly observed profile was that of swollen and truncated axons, which were visible both under bright and dark-field illumination. Some corkscrew-like profiles were also observed. Similar to the profiles observed with fluoxetine and sibutramine, these profiles could be seen against a background of normal intact serotonergic nerve network (Fig. 9). The low-dose, 18-h post-treatment animals (Fig. 10) also showed swellings. Pair-fed controls and 30-day post-treatment survival ani-

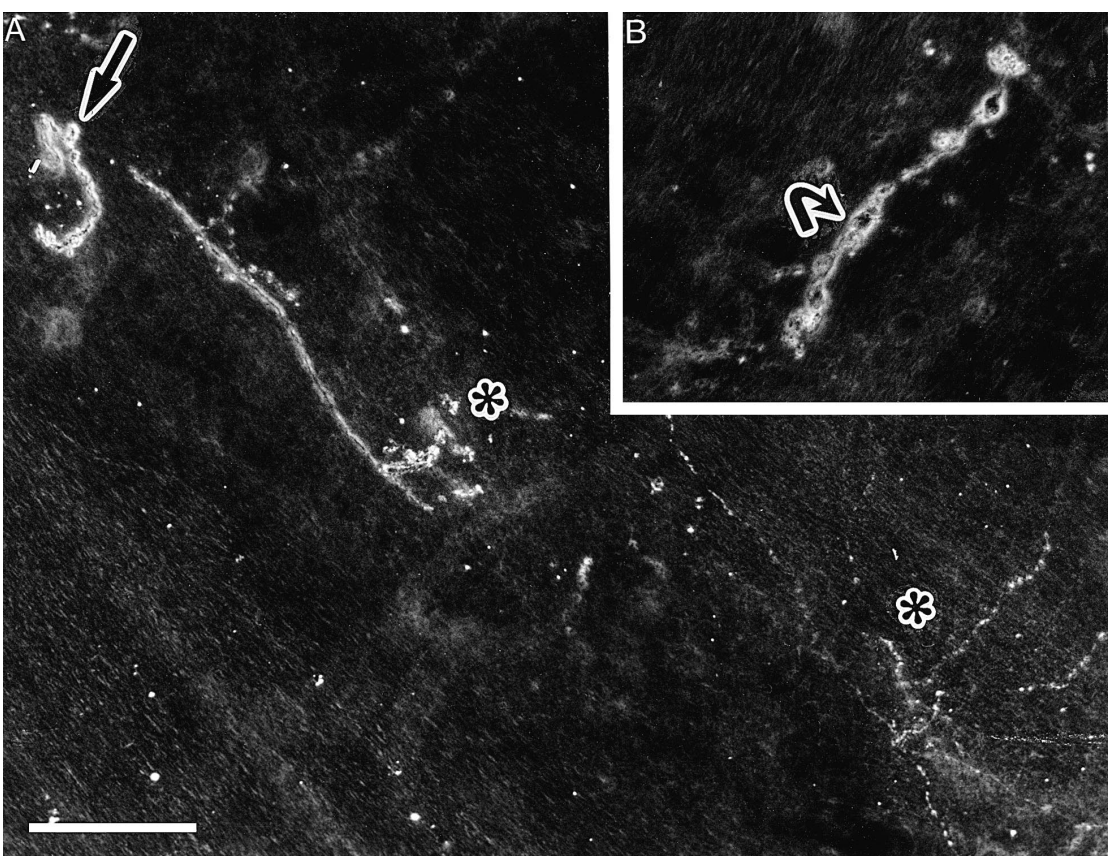

Fig. 10. High-magnification, dark-field photomicrograph of the prefrontal cortex of a rat following treatment with 28.6 mg/kg of sertraline for 4 days followed by an 18-h recovery period. This section is 20 μm thick and was stained for 5-HT immunocytochemistry. Notice the swollen profiles marked by arrowheads and asterisks. Magnification bar = 100 μm.

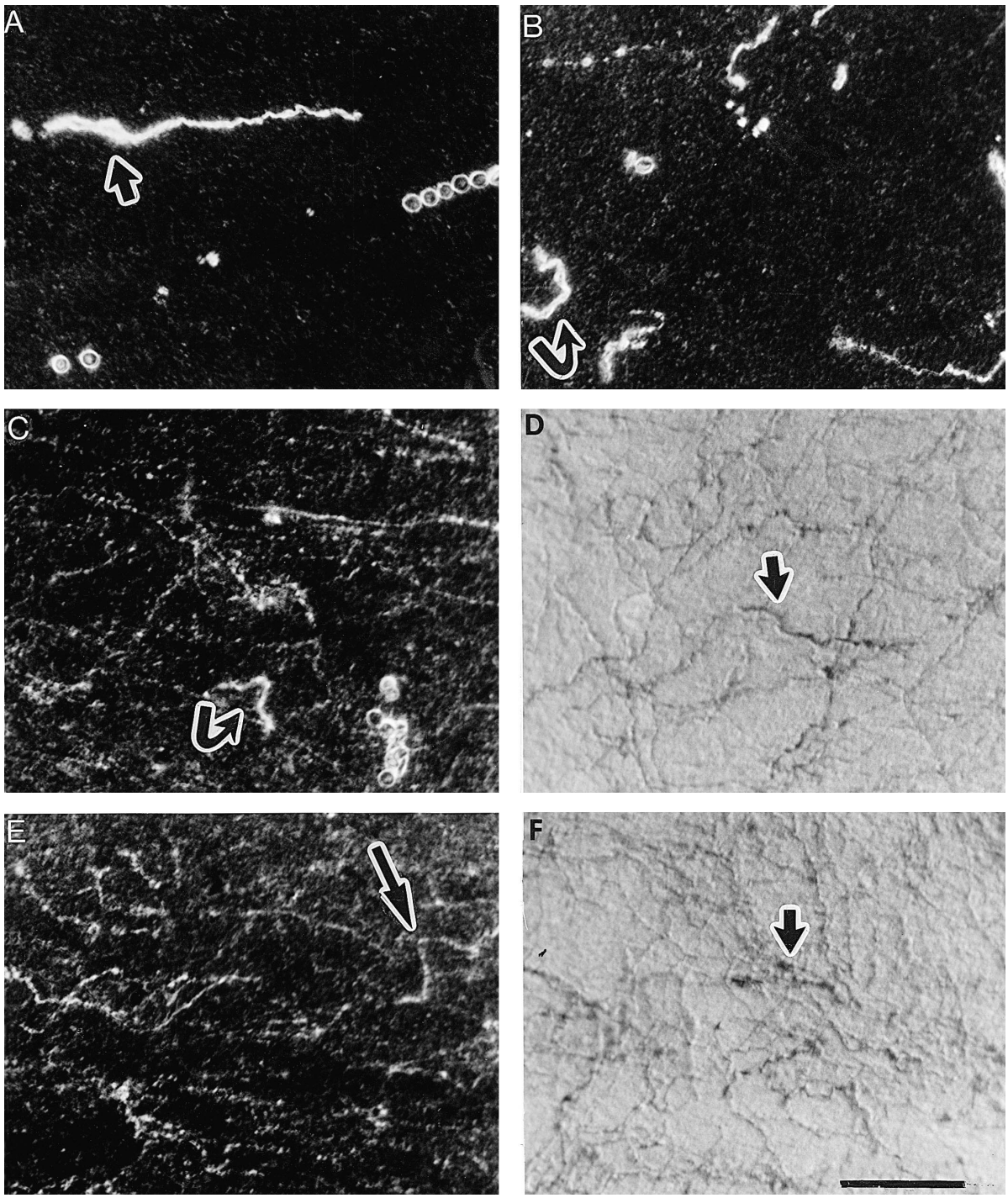

Fig. 11. (A) High-magnification, dark-field photomicrograph of the prefrontal cortex of a rat following treatment with 20 mg/kg of MDMA every 2 h followed by an 18-h recovery period. Notice the swollen profile marked by an arrowhead. (B) High-magnification, dark-field photomicrograph of the prefrontal cortex of a rat following daily treatment with 4.3 mg/kg of dexfenfluramine followed by an 18-h recovery period. Notice the cork-screw-like profile marked by a curved arrowhead. (C) High-magnification, dark-field photomicrograph of the prefrontal cortex of a rat following treatment with 11.4 mg/kg of fluoxetine for 4 days followed by an 18-h recovery period. Notice the cork-screw-like profile marked by a curved arrowhead. (D) High-magnification, bright-field photomicrograph of the prefrontal cortex of a rat following treatment with 11.4 mg/kg of fluoxetine for 4 days followed by an 18-h recovery period. Notice the thick and truncated profile (marked by an arrowhead) in a background of several fine serotonergic nerve fibers. (E) High-magnification, dark-field photomicrograph of the prefrontal cortex of a rat following treatment with 28.6 mg/kg of sertraline for 4 days followed by an 18-h recovery period. Notice the curved, truncated and swollen profile marked by an arrowhead. (F) High-magnification, dark-field photomicrograph of the prefrontal cortex of a rat following treatment with 2.8 mg/kg of sibutramine for 4 days followed by an 18-h recovery period. Notice the swollen profile marked by an arrowhead. All these sections are 20 μm thick and were stained for 5-HT immunocytochemistry. Magnification bar = 50 μm.

mals showed no abnormality in the serotonin immunoreactive nerve network.

Fig. 11 is a comparative illustration of the typical morphological features seen in the frontal cortex with low-dose treatment with all the agents examined in this study. Many of the abnormal profiles described in previous sections with high-dose treatment can be seen.

4. Discussion

4.1. Background

Immunocytochemical changes in the serotonergic system in response to agents such as fenfluramine or MDMA that perturb this system is now well documented [1–3,10,12,13,15,34,36,45]. However, the claim that these immunocytochemically identifiable morphological changes can be linked to serotonin depletion remains unsubstantiated. In addition, the assumption that this depletion reflects damage to serotonergic nerve terminals has not been demonstrated. In the present study, we have shown that morphological changes are produced by several compounds that act on the brain serotonin system. Some of these compounds deplete brain serotonin and others do not [16–18,27,31,51]. Therefore, depletion of brain serotonin can no longer be used to be as a predictor of morphological changes. A better understanding of the effects of all agents exerting any pharmacologic effect on the serotonergic system is clearly necessary to explain the observed short-term morphological effects.

4.2. Food intake and body weight

The anorexic effect of dexfenfluramine [48] and fluoxetine was dramatic and was observed as early as 1 day after exposure. Sibutramine and sertraline had less anorexic effects. The anorexic effect of dexfenfluramine decreased over the next three days of treatment, but fluoxetine-treated animals remained anorexic. This observation supports the theory that SSRIs in general, and fluoxetine in particular, have anorexic properties [9,38,53]. The magnitude of the anorexic effect of fluoxetine in rats was surprising. It will be of considerable interest to examine the anorexic effect of long-term low-dose treatment. The species specificity of this effect needs to be examined.

4.3. 5-HT levels

The reduction in brain serotonin 18 h following exposure to dexfenfluramine and 5,7-DHT closely matched the morphological findings. This pattern has been observed previously by other investigators. Brain serotonin levels following exposure to the high doses of the three SSRIs (fluoxetine, sibutramine and sertraline), however, resulted in no change in brain serotonin levels. Even though the actions of serotonin are mediated by more than 15 different types of receptors a single 5-HT transporter (SERT) is responsible for extracellular clearance of 5-HT [4,43]. SSRIs like fluoxetine preferentially block SERT, enhance serotonergic neurotransmission, decrease the number of 5-HT binding sites of 5-HT transporter (determined with [3H] imipramine or [3H] binding sites in the postmortem brain [41]. MDMA also inhibits imipramine binding to serotonin transporter and trigger SERT-mediated release of 5-HT [46,47]. Even though SERTs are the site for uptake by the compounds examined in this study their role in the observed effects remains unclear. Measurement of SERT by ligand binding following treatment with several of the drugs examined in this study has been used by some investigators to assess the integrity of the serotoninergic nerve plexus [1–3,26,27]. It should also be noted that since SERT itself is subject to pharmacologic regulation [41,43,46,47] changes in SERT or in SERT mRNA cannot be considered to be evidence of neurotoxicity. Nevertheless, several investigators using [3H] imipramine binding have concluded that dexfenfluramine and MDMA, on the one hand, and SSRIs and SNRIs, on the other hand, are on the opposite sides of the neurotoxicity spectrum. Our morphological findings however, reveal that high doses of dexfenfluramine, SSRIs and SNRIs produce very similar abnormalities in the brain serotonergic nerve network in the short term. We did not assay brain serotonin at the 30-day post-treatment time point because the observed morphological changes did not persist for 30 days.

4.4. Morphological changes

Whereas a study of only the short-term effects of high doses of agents perturbing the serotoninergic system such as SSRIs, dexfenfluramine and MDMA is notably incomplete, the observation of morphological changes with these agents can only be made in the time interval immediately following the cessation of the treatment [1,25,36]. Furthermore, these morphological changes cannot been detected by routine histological methods such as H&E staining. For this reason, in this study, we limited our observations to the examination of serotonergic nerve terminals with immunocytochemical techniques. In the case of fenfluramine and MDMA, which are known depleters of brain serotonin, these abnormal serotonergic profiles are easily identified against an unstained background. However, following exposure to the SSRIs fluoxetine [16,17,23] and sertraline [18], and the SNRI sibutramine [31,51] which do not deplete brain serotonin, these abnormalities could be seen in several brain regions against a stained background in which several serotonin immunoreactive nerve fibers were visible. This is important, because it indicates that the morphological changes are not a consequence of serotonin depletion/axonal damage as previously hypothesized [34,36]. The timing of the appearance of these abnormal morphological profiles following high-dose, short-term ex-

posure to the SSRIs fluoxetine and sertraline, and the SNRI 18 h post-treatment is in keeping with observations with the fenfluramine but is in distinct contrast to MDMA which produces morphological changes that last considerably longer [15]. These results indicate that immunocytochemical morphological changes in brain serotonin neurons are produced by a number of drugs such as dexfenfluramine, MDMA, SSRIs and SNRIs that exert their pharmacologic effect by acting on the brain serotonin system [22]. A series of treatment and post-treatment, time-related studies will be needed to fully evaluate the differences and similarities between each of these individual serotonergic agents.

In summary, short-term immunocytochemical changes in brain serotonergic neurons appear to be unrelated to depletion of the serotonergic system by agents that exert their pharmacological effect on this system. In the short term, either changes in the serotonin content of serotonergic nerve terminals or the immediately surrounding perikaryon could be responsible for these serotonin immunoreactive profiles. However, which of these is primarily responsible and to what degree these morphological changes reflect longlasting effects on serotonergic neurons, are issues to be resolved in the future.

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