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Ibotenic acid-induced lesions of striatal target and projection neurons: ultrastructural manifestations in dopaminergic and non-dopaminergic neurons and in glia

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Summary. The cytological changes elicited by central microinjections of the excitotoxin, ibotenic acid (IBO) were examined in the adult rat striatonigral system using electron microscopic immunocytochemistry. The chemical markers included tyrosine hydroxylase (TH), a biosynthetic enzyme in dopaminergic neurons, and glial fibrillary acidic protein (GFAP). Both short (1-7 day) and long (30-60 days) term effects were evaluated at the site of IBO-injections in the striatum and more distally in the *substantia nigra,* which both contributes afferents and receives efferents from the striatum. In the *neostriatum* at every survival period examined, TH-labeled axonal processes appeared equally numerous in the control and IBO-injected hemispheres. However, the TH-labeled axons in the striatum ipsilateral to the IBO-injection were slightly enlarged, and generally lacked synaptic densities. In the early period the remaining neuropil showed signs of edema and contained perikarya and dendrites with vacuolar or dense cytoplasm as well as intact, unlabeled terminals. Numerous astrocytes, and apparently demyelinated axons were more commonly seen at the 7 day period. At 30 and 60 days, bundles of myelinated axons, unlabeled axon terminals, and astrocytes containing a variety of cytosomes and other cytoplasmic inclusions were in close apposition to TH-labeled axon terminals. These results suggest that the dopaminergic terminals may serve neuromodulatory functions with respect to glia or other afferent axons remaining after IBO-injections in the striatum. In the *substantia nigra,* homolateral to the injection, a dense type of degeneration was seen in a few perikarya and dendrites at 7 days of survival. At this stage, electron dense anterograde degeneration also was seen in terminals contacting both TH-labeled and unlabeled dendrites. The secondary long term changes in nuclear groups located distal to the primary lesion are characteristic of certain types of progressive human neuropathological disorders.

Key words: Tyrosine hydroxylase - Ibotenic acid - Demyelination - Electron microscopy - Immunocytochemistry

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Introduction

In the striatum microinjections of the excitotoxins, ibotenic (IBO) and kainic acid (KA), reduce the levels of transmitters such as gamma-aminobutyric acid (GABA) which is found in perikarya, but do not change the levels of L-glutamate or dopamine, the putative transmitters in cortical and nigral afferents (Kohler and Schwarcz, 1983; Steiner et al., 1984). Histofluorescence and immunocytochemical localization of the catecholamine synthesizing enzyme, tyrosine hydroxylase (TH) have provided further light microscopic evidence for survival of the dopaminergic innervation to the striatum following IBO-lesions (Campochiaro and Coyle, 1978; Saji and Reis, 1985). The persistence of nigral dopaminergic efferents for periods of up to 60 days following IBO-induced lesions of their striatal target and afferent neurons raises a number of questions regarding possible ultrastructural changes in the dopaminergic neurons of the nigrostriatal system and their relationships to intact and degenerating neuronal profiles and to glia. We used electron microscopic immunocytochemistry of TH and glial fibrillary acidic protein (GFAP) to determine: (1) whether the TH-labeled terminals in the striatum or their perikarya or dendrites in the substantia nigra exhibited ultrastructural alterations following striatal injections of IBO, (2) the anatomical relation between the TH-labeled processes and other components of the striatal neuropil including glial cells, and (3) whether anterograde and/or retrograde changes were seen in non-dopaminergic neurons of the substantia nigra following striatal injections of IBO. The time-courses included short (1 and 7 day) and long (30 and 60 day) term survival periods.

Materials and methods

Animals and Surgical procedures

Male Sprague-Dawley rats weighing 220-250 grams were anesthetized with a mixture of 2% halothane and 98% oxygen and then placed in a stereotaxic apparatus with the same anesthetic. Four-tenths of a microliter of a 1% solution of (IBO) in 0.1 M phosphate buffered saline was infused into each of three rostro-caudal sites in the striatum using the co-ordinates of Paxinos and Watson (1982). The drug was administered through a glass micropipette having a tip-diameter of 30-40 μm and attached to an air pressure delivery system calibrated to infuse 0.1 μl/min (Amaral and Price, 1983). A total of 15 animals, three or more at 1, 7, 30 and 60 days post injection, were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and then perfused through the ascending aorta with 50 ml of 3.75% acrolein and 2% paraformaldehyde in 0.1 M phosphate buffer at pH 7.4. The perfusion was continued with 200 ml of 2% paraformaldehyde in the same buffer.

Antiserum

A polyclonal antiserum to TH was produced in rabbits by previously described methods (Joh et al., 1973). The antiserum was shown to yield an immunoprecipiate only against purified TH and not with other catecholamine synthesizing enzymes such as dopamine beta-hydroxylase on phenylethanolamine N-methyltransferase. Substitution of normal serum or antiserum absorbed with the purified antigen has previously been shown to remove all detectable immunoreactivity in rat brain (Pickel, 1981). A polyclonal rabbit antiserum produced against GFAP was generously donated by Drs. D. Dahl and A. Bignami. (See Schachner et al., (1977) for characterization of antiserum.

Immunocytochemical Labeling

Following the perfusion fixation, the brains were removed, postfixed for an additional 30 min in 2% paraformaldehyde, and sectioned coronally at 30 μ m on a vibrating microtome. Representative sections through the striatum were collected just anterior to the decussation of the anterior commissure; whereas those from the substantia nigra were collected at the level of the medial geniculate. For improved immunocytochemicall labeling, the aldehyde groups were reduced by placing the sections for 5 min in 1% sodium borohydride in 0.1 M phosphate buffer, pH 7.4 (Schachner, 1977). These were rinsed through several changes of the same buffer followed by several washes in 0.1 M Tris-saline, pH 7.6. These sections were then immunocytochemically labeled using a modification (Pickel, 1981) of the peroxidase-antiperoxidase (PAP) method of Sternberger (1979). In brief, they were incubated for 18 hr with 1:2,000 dilution of the rabbit antiserum to TH or to GFAP, for 1 hr with a 1:50 dilution of goat anti-rabbit immunoglobulin (IgG), and for 1hr with a 1:100 dilution of rabbit PAP complex. Each incubation was separated by several washes in 0.1 M Tris-saline. The bound peroxidase was demonstrated by reaction with 3,3'-diaminobenzidine and hydrogen peroxide. These sections were then either mounted on glass slides and examined with interference contrast or dark field optics on a Nikon microscope. One micron sections from the outer surface of plastic-embedded (see next section) tissues also were collected on glass slides, counterstained with toluidine blue, and examined by light microscopy using interference contrast-optics.

Electron microscopy

For electron microscopy, the labeled sections were post fixed in 2% osmium tetroxide for 1 hr, dehydrated and flat-embedded in Epon 812. Ultrathin sections were collected ipsilateral and contralateral to the injection from the striatum and from the border between the pars compacta and pars reticulata of the substantia nigra. The striatal sections included the zone near one of the injection sites and the proximally located tissue reached by diffussion of the drug, but showing no apparent damage from the tip of the micropipette. The ultrathin sections were counterstained with 5% aqueous uranyl acetate and lead citrate (Reynolds, 1963) prior to examination with a Phillips 201 electron microscope.

Results

1. Short-Term Survival Periods

(a) Neostriatum

Light microscopic examination of the striatum in the short (1 and 7 day) periods following local injections of IBO revealed an edematous (vacuole-filled) neuropil containing scattered bundles of myelinated axons, TH-labeled varicosities and irregularly shaped basophilic cells (Fig. 1A). Electron microscopy revealed numerous vesicle-filled axonal profiles containing immunoreactivity for TH both ipsilateral and contralateral to the striatal injection at 1 and 7 day survival periods. The labeled processes on the side of the lesion ranged from 0.5-2.0 μm in cross-sectional diameter (Fig. 2 A&B). In contrast, much smaller (0.1-0.5 μm) labeled terminals were found in the striatum contralateral to the injection at every post-lesion stage examined (Fig. 2C). At one day survival, TH-labeled terminals in the lesioned striatum were proximally located with respect to neuronal perikarya, dendrites and dendritic spines showing electron density, dissolution of cytoplasmic organelles and other signs of degeneration (Fig. 2 A&B). Many of the electron-dense dendritic spines received asymmetric synapses from unlabeled and apparently undamaged axon terminals (Fig. 2B). A similar arrangement was seen in the control striatum, however the symmetric synapses between TH-labeled terminals and dendritic spines were far more numerous at every stage examined (Fig. 2C). Infrequently the TH-labeled axons showed direct appositions with other axon terminals which contained the full complement of vesicles and none of the electron density usually

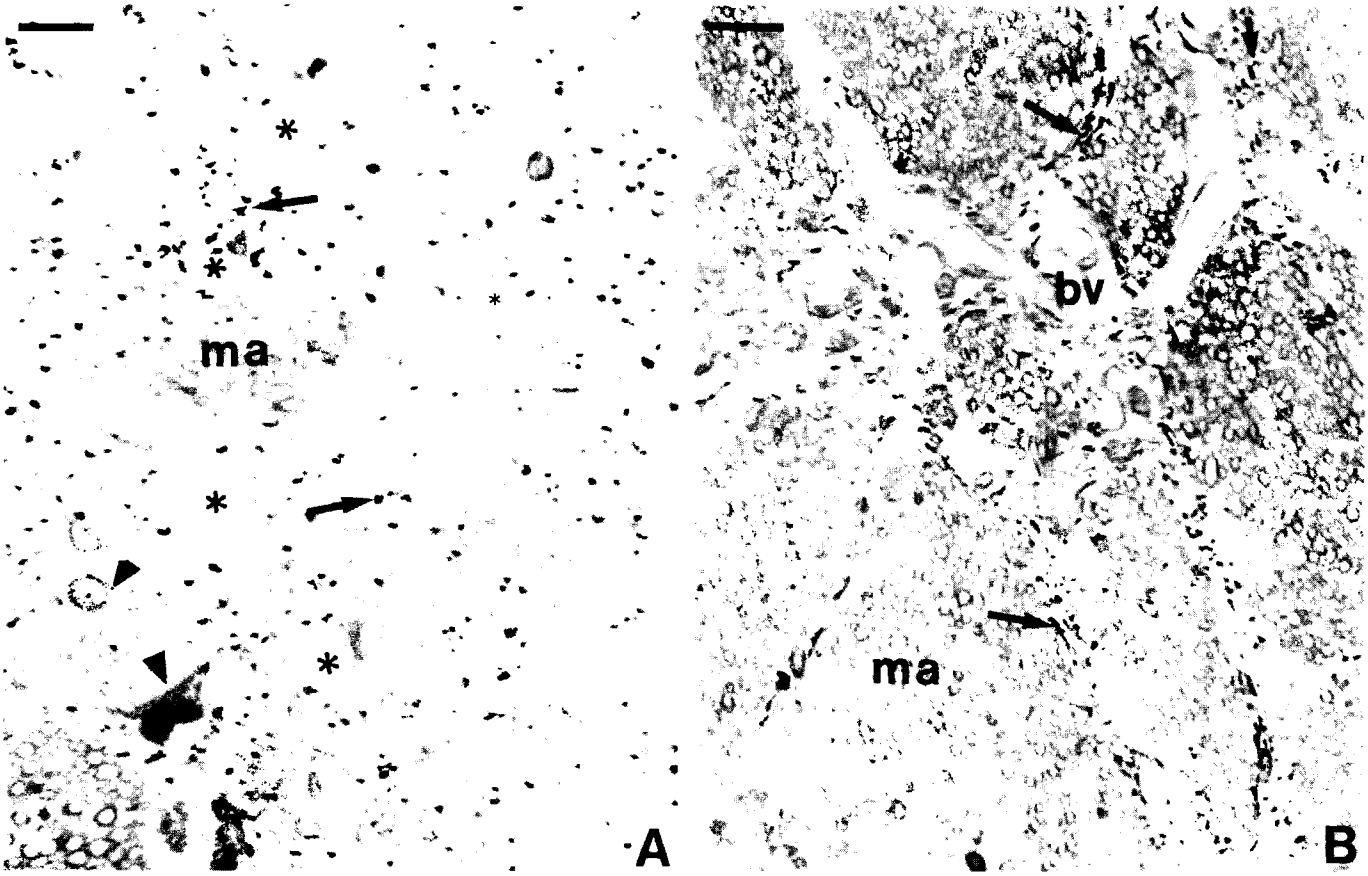

Fig. 1 Light microscopic localization of tyrosine hydroxylase (TH) in the striatum ipsilateral to ibotenic acid injections. **A.** Photomicrograph at 7 days illustrates the punctate varicosities (arrows) with TH-labeling. Numerous vacuoles (asterix), basophilic cells (arrowheads) and a few bundles of myelinated axons (ma) are also seen. **B.** Photomicrograph at 60 days shows TH-labeling in bundles of processes (arrows) coursing between numerous bundles of myelinated axons (ma) and blood vessels (bv). Both micrographs were taken from 1 μm plastic sections collected from the outer surface of vibratome sections immunocytochemically labeled for TH using a Toluidine blue counterstain. Bar = 50 μm.

associated with terminal-degeneration (Fig. 3A). A few extremely large profiles exhibiting TH-immunoreactivity were seen at 1 (Fig. 3B) and 7 (Fig. 4) days near the injection site. The accumulation of organelles in these profiles suggested that they might be transected or damaged axons.

Exclusive of TH-labeled processes, the striatal neuropil ipsilateral to the IBO-injection contained predominantly unlabeled axons and axon terminals which were not electron dense, and had sizeable populations of synaptic vesicles when examined at 1 or 7 days following the lesion. The edematous appearance, including a large number of membrane-bound vacuoles seen 1 day after the IBO injections (Fig. 3B) was replaced by a greater abundance of astrocytic profiles and demyelinated or partially myelinated axons at 7 days (Fig. 4). However, astrocytes identified by GFAP labeling were also readily detected 24 hr after the IBO-injection (Fig. 5A). Many astrocytes were filled with assorted metabolic byproducts representative of ingested neuronal debris, i.e., myelin bodies, multivesicular bodies and lipofuscin-like droplets. Numerous macrophages also invaded the striatal tissue even at 1 day post-injection (Fig. 6). The macrophages typically showed clumped chromatin, distended Golgi apparatus and an electron-dense cytoplasm.

b) Substantia Nigra

The TH-labeled perikarya in the pars compacta and dendrites in the pars reticulata of the substantia nigra showed no observable morphological alterations ipsilateral versus contralateral to the striatal IBO-injections at 1 or 7 days post-lesion. However at 7 days, several unlabeled perikarya and dendrites within the pars reticulata showed morphological changes usually associated with cytotoxicity. These dendrites exhibited electron density and vacuolated mitochondria, both of which may have been obscured by peroxidase reaction product in the TH-labeled dendrites. Unlabeled axon terminals showing a dissolution of synaptic vesicles, electron density and other signs of degeneration were particularly noticeable in the medial portions of the substantia nigra at 7 days after the lesion (Figs. 7 A&B). The degenerating terminals were in synaptic contact with both TH-labeled (Fig. 7A) and unlabeled (Fig. 7B) dendrites. At 1 and 7 days, glial processes identified by GFAP-labeling also were numerous in the substantia nigra ipsilateral to the striatal injection (Fig. 5B).

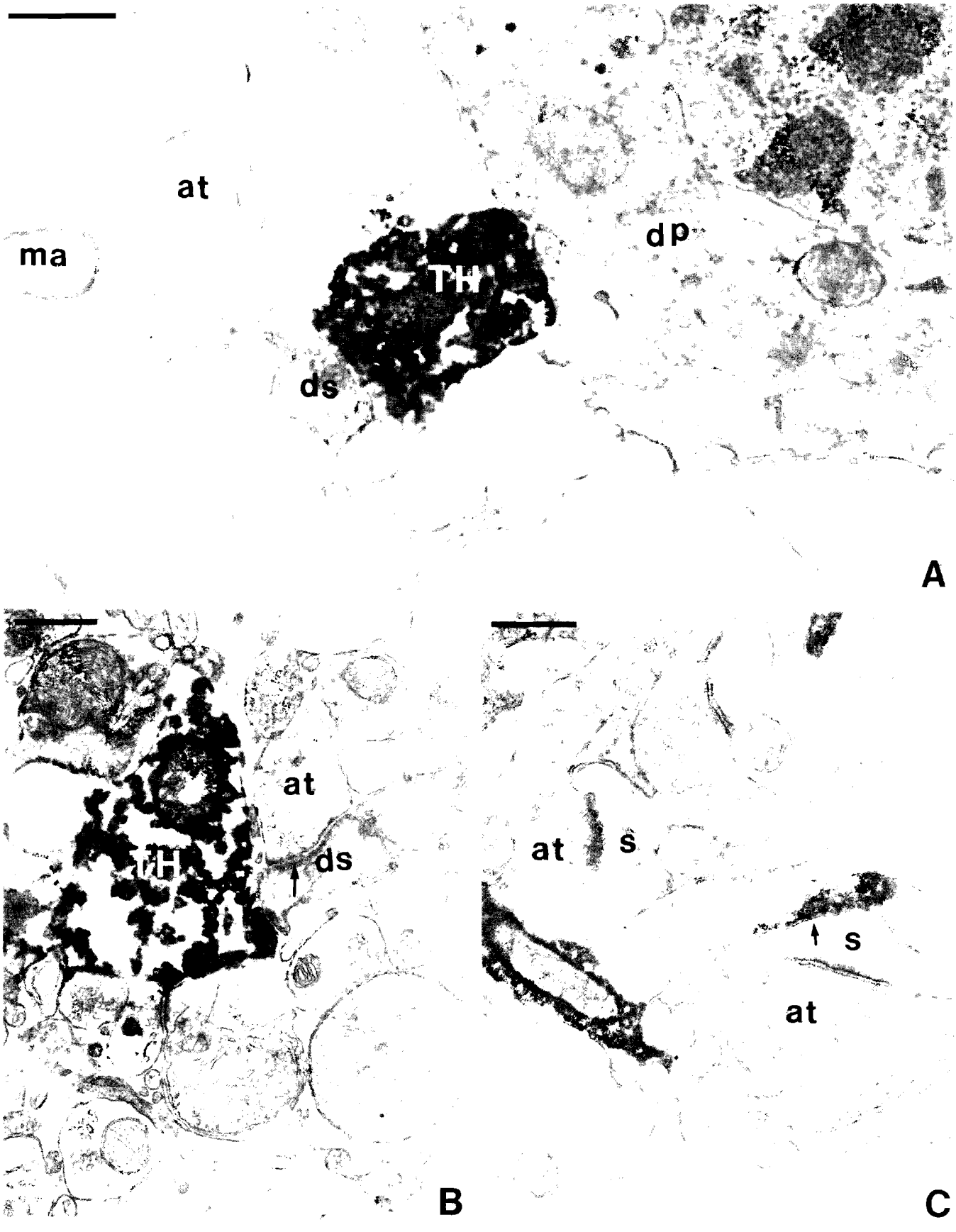

Fig. 2. Localization of tyrosine hydroxylase (TH) in the neostriatal neuropil 24 hr after injection of IBO. **A.** Electron micrograph taken from a section homolateral to the injection shows a TH-labeled process located near the outer soma of an electron dense perikarya (dp) and dense spine (ds). Other intact myelinated axons (ma) and axon terminals (at) without apparent morphological damage are also seen. **B.** Electron micrograph of the striatum homolateral to the injection shows a TH-labeled terminal in direct apposition to a dense degenerating spine (ds) and another unlabeled axon terminal (at) which forms an asymmetric junction (arrow) with the spine. **C.** Electron micrograph from the striatum contralateral to the injection shows a similiar arrangement in which the TH-labeled terminal exhibits a small symmetric specialization (arrow) with the head of a dendritic spine also receiving an asymmetric junction with an unlabeled axon terminal (at). The spines (s) in this micrograph show no obvious signs of degeneration. Bar = 0.5 μm.

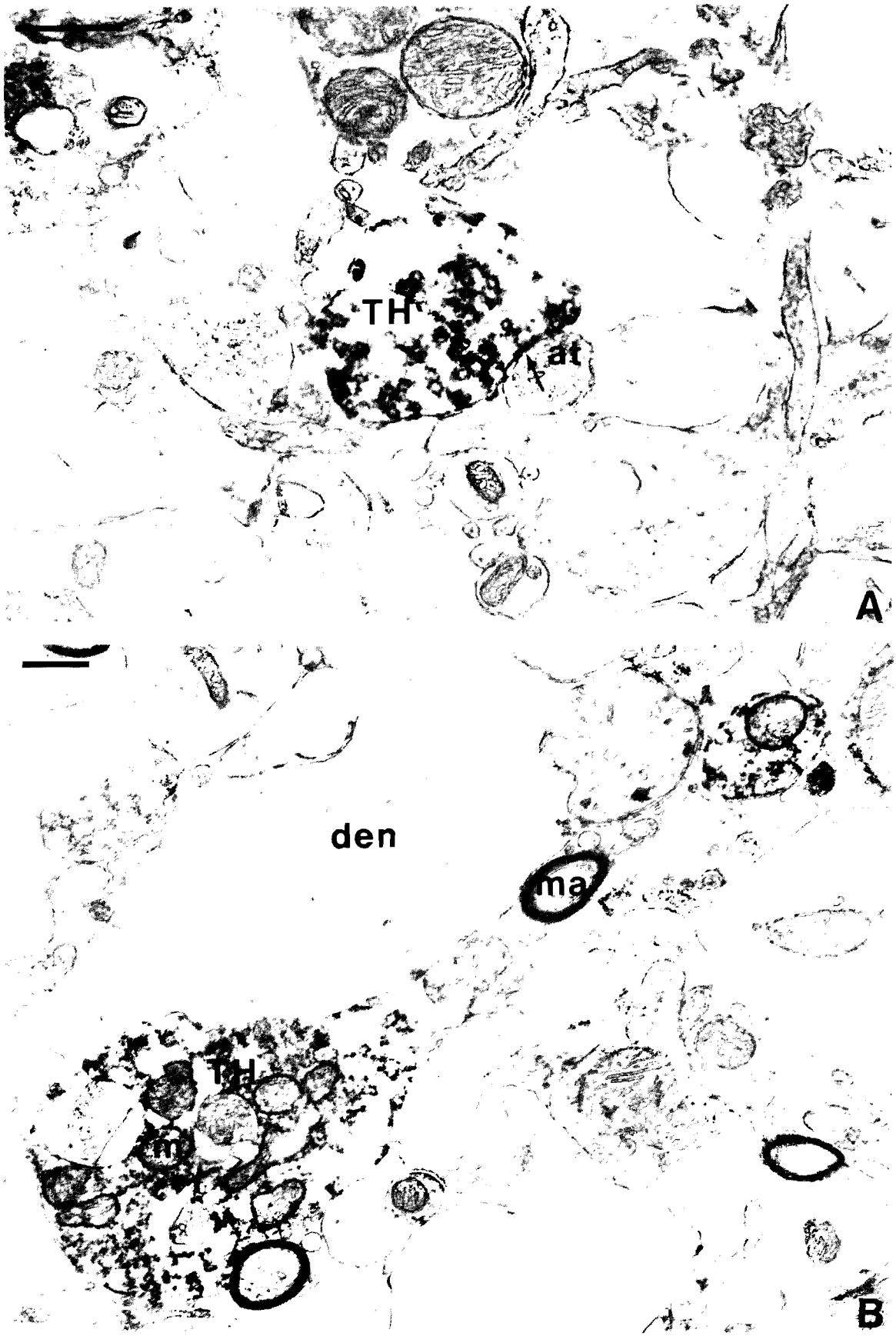

Fig. 3. Striatal neuropil ipsilateral to the IBO-injection at a 24 hr survival period. **A.** Electron micrograph shows an apposition (arrow) between a TH-labeled axon terminal and another unlabeled terminal (at). **B.** Electron micrograph shows a distended, presumably transected, TH-labeled axon near the injection site. The process contains numerous mitochondria (m), multivesicular bodies, vesicles and peroxidase immunoreactivity. The TH-labeled process is adjacent to a large membrane bound presumably dendritic structure which is filled with amorphous material. Intact myelinated axons (ma) are also seen in the neuropil. Bar = 0.5 μm.

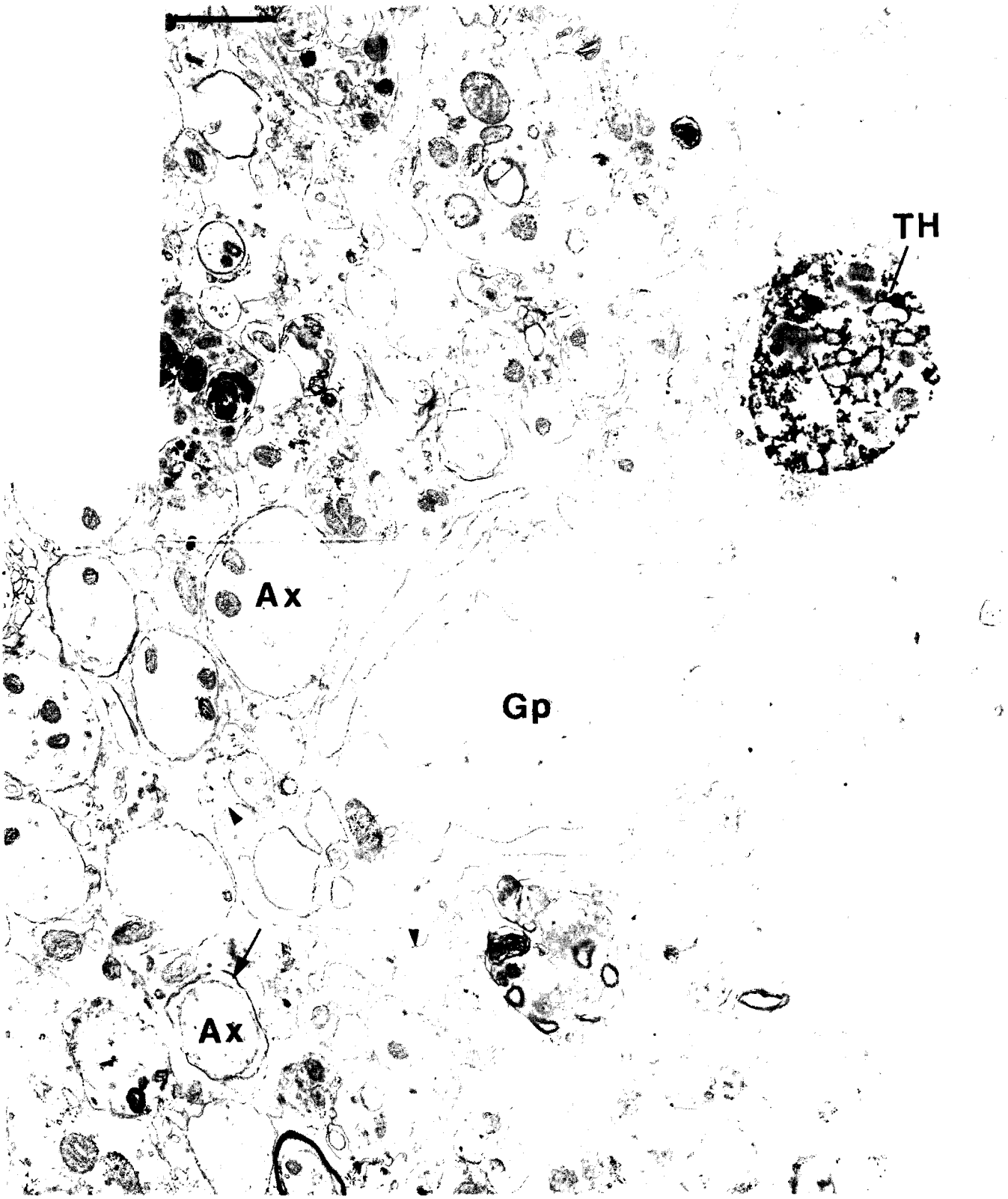

Fig. 4. Localization of TH and ultrastructure of the striatal neuropil at 7 days following IBO-injections. Montage of electron micrographs shows an enlarged TH-labeled axon near the injection site. The surrounding neuropil is edematous, contains large vacuolated profiles, glial processes (Gp), and large axons (Ax) which appear to have lost their myelin. A small rim of myelin (arrow) is seen around some of the axons. Bar = 1.0 μm.

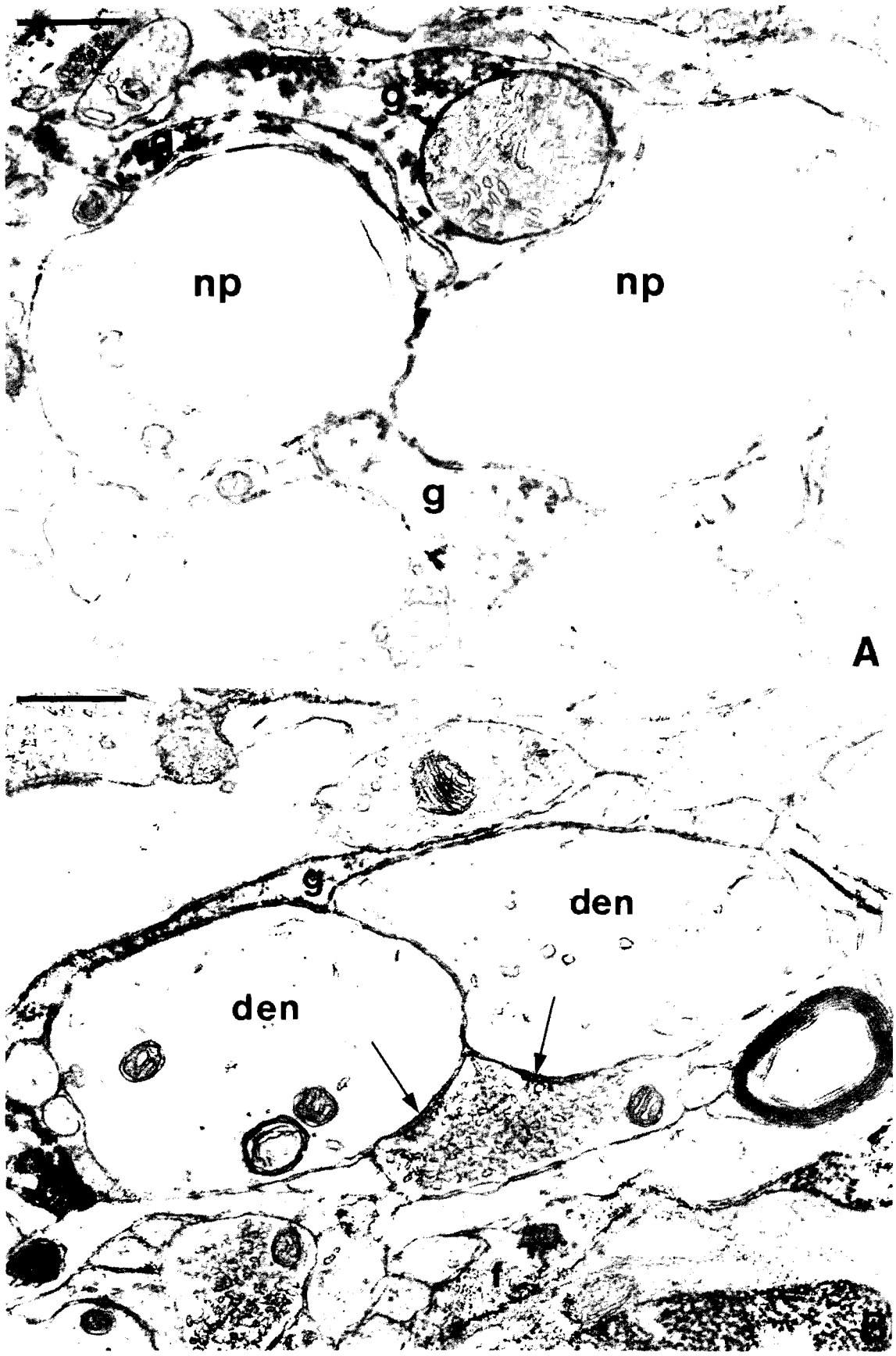

Fig. 5. Localization of GFAP in the striatum **(A)** and substantia nigra **(B)** ipsilateral to the IBO injection. The reaction product is exclusively localized in astrocytic glial processes (g) surrounding dendrites (den) and other neuronal processes (np). **A** is shown at 24 hr after the injection and **B** is 30 days after the injection, but a similar pattern was seen at all other stages examined. The dendrites in **B** are postsynaptic (arrows) to a common unlabeled axon terminal. Bar = 0.5 μm.

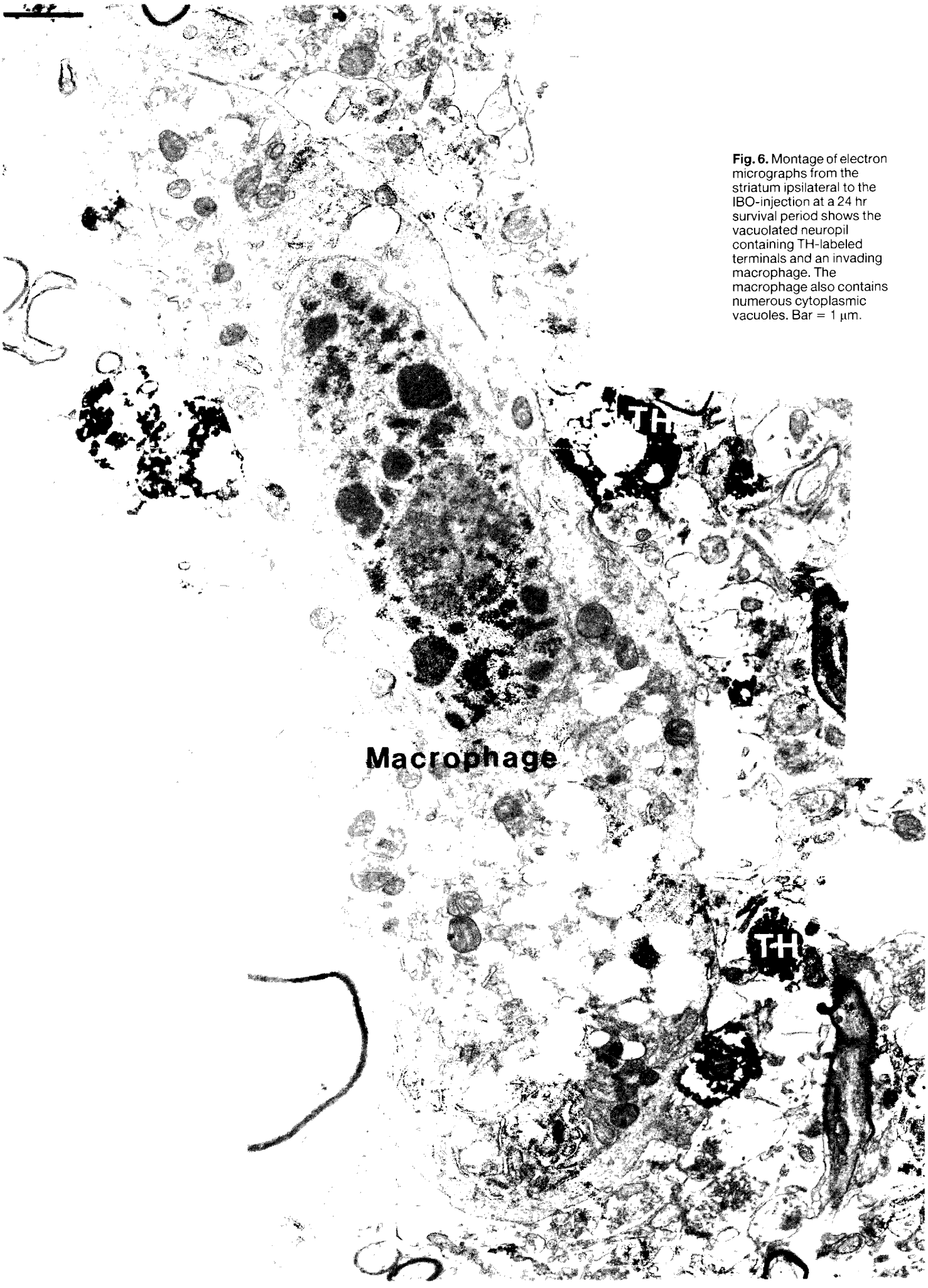

Fig. 6. Montage of electron micrographs from the striatum ipsilateral to the IBO-injection at a 24 hr survival period shows the vacuolated neuropil containing TH-labeled terminals and an invading macrophage. The macrophage also contains numerous cytoplasmic vacuoles. Bar = 1 μm.

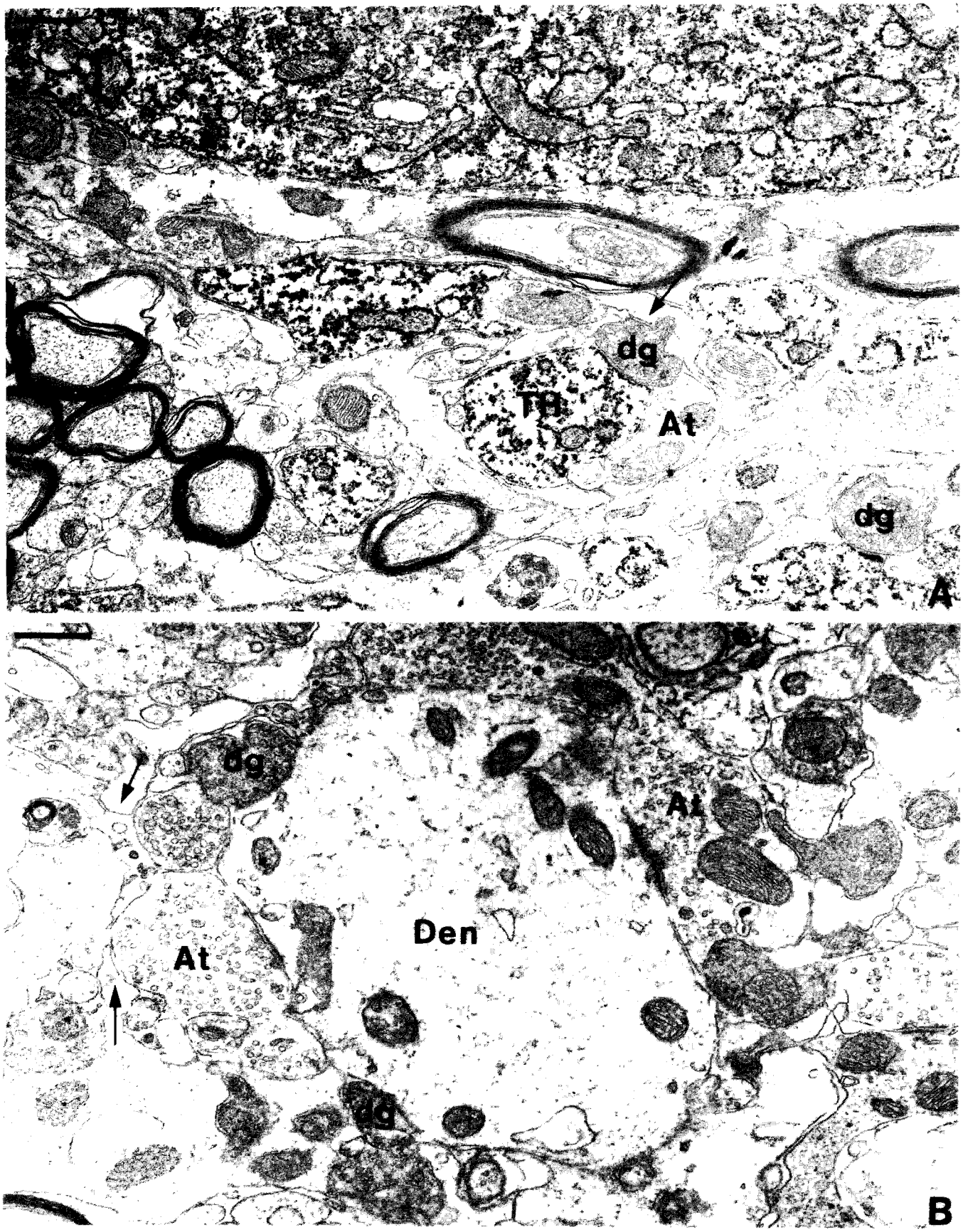

Fig. 7. Micrographs showing the synaptic junctions between degenerating (dg) axon terminals and TH-labeled **(A)** and unlabeled **(B)** dendrite (Den) in the substantia nigra at 7 days following IBO-injections in the homolateral striatum. Both the TH-labeled and unlabeled dendrites also receive numerous synaptic contacts from other axon terminals (At) which contain numerous synaptic vesicles and show no apparent electron density. Glial processes (arrows) surround the degenerating terminals. Bar = 0.5 μm.

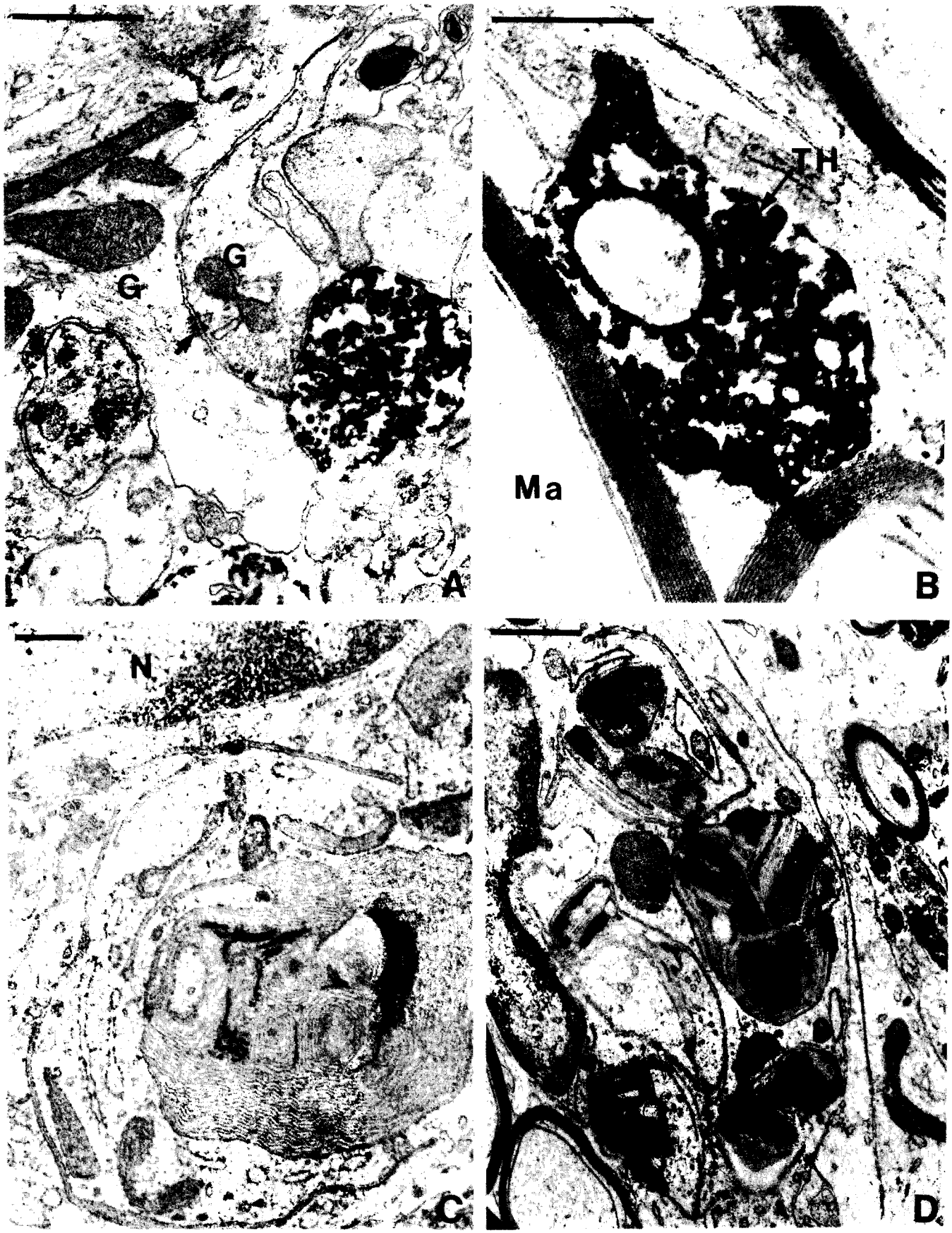

Fig. 8. Ultrastructure of the striatum at long survival periods after injection of IBO. **A.** Electron micrograph shows the proximity between a large TH-labeled axon and other glial (G) processes joined by a punctum adherens (arrow) attachment at 30 days. **B.** Electron micrograph showing the close apposition between the TH-labeled terminal and myelinated axons (ma) at 60 days. **C.** Micrograph showing a finger-print cytosome in an astroglial cell at 30 days. **D.** Electron micrograph showing crystaloid type cytosome in an astrocyte in the striatum at 60 days. Bar = 0.5 μm in **A-C** and 1 μm in D.

2. Long Term (30 and 60 day) Survival periods

a) Neostriatum

At 30 and 60 days, light microscopic examination of 1 μm plastic sections from the striatum ipsilateral to the IBO-lesions revealed a compact neuropil consisting chiefly of highly organized bundles of TH-labeled processes, densely packed myelinated axons and blood vessels (Fig. 1B). On ultrastructural examination, the TH-labeled terminals appeared to be more prevalent than other unlabeled axon terminals. Small curvilinear cytosomes were sometimes seen within terminals containing immunoreactivity for TH. No recognizable junctions were detected between the TH-labeled terminals and remaining unlabeled axon terminals, myelinated or unmyelinated axons or glia. However, close appositions between the TH-labeled axons and myelinated axons and glia were frequently observed at 30 (Fig. 8A) and 60 (Fig. 8B) days following the lesion. The neuropil was otherwise distinguished from that seen at earlier stages by a more compact, less vacuolated appearance and juxtapositions betwen astrocytic processes which were bridged by desmosomes and punctum adhaerens (Fig. 8A). Finger-print (Fig. 8C), curvilinear and crystalloid (Fig. 8D) cytosomes also were seen within the astrocytic cytoplasm in the striatal tissue ipsilateral to the IBO-injection.

b) Substantia Nigra

As seen by electron microscopy, TH-labeled perikarya and dendrites in the substantia nigra remained morphologically unaltered at 30 and 60 days after the striatal injections of IBO. The degenerative changes in unlabeled perikarya and dendrites seen at 7 day survival were not evident at the later stages. In addition, axon terminals with dense agrophilia were rarely detected.

Discussion

The results from this study demonstrate: (1) that TH-labeled terminals in the rat striatum show morphological alterations, but are readily detectable for up to 60 days following lesions of their target neurons by local infusions of IBO, (2) that in the period from 1-60 days following IBO-injections, the striatal tissue shows a complex series of changes including loss of intrinsic neuronal perikarya, a reduction in non-TH-labeled axon terminals and demyelination followed by apparent remyelination and gliosis, (3) that degeneration of presumed striatal efferents and degeneration in a few perikarya and myelinated axons in the substantia nigra are seen at 7-days after striatal injections of IBO, but are virtually undetectable at longer 30-60 day intervals, and (4) that these efferents terminate on both dopaminergic (TH-labeled) and non-dopaminergic neurons in the substantia nigra.

(1) Maintenance of TH-labeled Axons in Striatum

The present detection of TH-labeled axon terminals for up to 60-days following striatal infusions of IBO supports the concept that most afferents are not permanently damaged by the excitotoxin (Olney, 1985). However morphological changes such as the distension of many axonal profiles which display TH-immunoreactivity and the absence of recognizable synaptic specializations suggest that functional alterations may occur in dopaminergic afferents in response to IBO-lesions of their neuronal targets. The appositions between the TH-labeled profiles and the remaining bundles of myelinated axons and glia which are virtually the only remaining profiles at 60 days after the IBO-lesions suggests that the dopaminergic axons may assume a new modulatory role particularly with respect to astrocytes in the reorganized neuropil (Kimelberg, 1983).

(2) Progressive Alterations in Non-dopaminergic Components of Striatum

Our observation that neuronal perikarya are either undetectable or show signs of degeneration within 24 hr following infusion of IBO has already been well documented by light microscopy (Schwarcz et al., 1979; Kohler and Schwarcz, 1983; Steiner et al.; 1984, Saji and Reis, 1985, 1986). Furthermore similar ultrastructural evidence for loss of perikarya within the area of the infusion also has been shown for other cytotoxins such as kainic acid (Coyle et al., 1978; Sperk, et al., 1983; Olney, 1985). However, the present demonstration that unlabeled axon terminals containing full complements of vesicles and other organelles were progressively more difficult to identify at 30 and 60 days following striatal infusions of IBO differs from the reports with kainic acid where axon terminals were reportedly unaltered (Coyle et al., 1978). This difference is likely attributable to the fact that the survival periods with kainic acid were only 10 days after the injection (Coyle et al., 1978; Olney, 1985; Sperk, et al., 1983). Since numerous unlabeled axon terminals were seen at 7 days, but not at 30 or 60 days following the IBO-injections; the depletion is probably accounted for by anterograde degeneration of collaterals of intrinsic neurons. It is also possible that at long survival periods, there is a reduction of cortical or other afferents due to the absence of their neuronal targets.

A second striking change noted in the present study was the sparsity of myelin around large axons at 7 days and the abundance of myelinated axons at 30 and 60 days near the IBO-infusion sites in the striatum. Earlier light microscopic studies following IBO-injections in the striatum failed to note any alterations in the myelinated axons. However, kainic acid has been reported to cause demyelination and loss of oligodendrocytes within 24 hr of subcutaneous injections in the rat (Sperk, et al., 1983).

In the present study, alterations in oligodendrocytes and neurons in the striatal tissues were accompanied by the detection of a number of residual bodies, including lamellar and finger-print cytosomes, in astrocytes of the striatum at 30 and 60 days after the IBO-injections. These lamellar cytosomes may be of clinical interest with

respect to infant-juvenile encephalopathies resulting from inborn errors of lipid metabolism (Figols et al., 1986; Martin et al., 1977). In astrocytes and to a lesser extent in macrophages and neurons from patients with this disorder, Towfighi et al. (1975) reported the presence of cytosomes with lamellar profiles consisting of straight or slightly curved electron dense lines (40-70 Å) separated from each other by an electron lucent zone measuring 30-50 Å. The more complex finger-print cytosomes, seen in the striatum at 30 days after injection of IBO in the present study, have also been reported in patients with neuronal ceroid lipofuscinosis (Carpenter et al., 1977; Greenfield, 1976; Gilroy and Meyer, 1979) and in one case of congenital amaurotic idiocy (Humphreys et al., 1985). However in these disorder the finger-print cytosomes were principally in neurons and only occasionally seen in astrocytes.

Our ability to detect lamellar bodies in astrocytes only 30-60 days after IBO-injections in the striatum seems inconsistent with direct astrocytic cytotoxicity. The induction of lamellar bodies occurs within 2-24 hr after administration of amphiphilic cationic drugs *in vivo* and *in vitro* (Lullmann et al., 1978; Drenckhahn et al., 1976). Furthermore these lamellar structures appear to dissociate following withdrawal of the drug. In the clinical cases cited above, Figols et al. (1986) speculated that the appearance of cytoplasmic lamellar cytosomes may have been induced by the administration of anti-epileptic druges (diphenylhydantoin, clonazepan and netrazepan) soon after birth. The onset of seizures following systematic administration of excitotoxins, which presumably disrupt neuronal pathways in the frontal cortex and hippocampus, has led to the suggestion that the drugs may be used in producing animal models for epilepsy (Sperk et al., 1983; Olney, 1985). The continued release of L-glutamate from cortical afferents, possibly mediated by the remaining dopaminergic terminals, might account for the more long lasting induction of cytosomal profiles within glia of the striatum following IBO-injections. In pathological human tissues several possibilities have been suggested for the formation and composition of cytosomes. These include: peroxidase insufficiency as a result of an inborn error in metabolism such as that found in the various lipoidoses (Gilroy and Meyer, 1979; Zeman, 1974), formation of retinol complexes (Wolfe et al., 1976), and undigested metabolites of amino acid transmitters (Greenfield, 1984). The latter suggestion is particularly appealing in the IBO-lesioned animals of the present study.

3) Degeneration of Afferent Axons and a few Intrinsic Neurons in the Substantia Nigra

The detection of degenerating terminals in the substantia nigra at 7 days following IBO-injections in the ipsilateral striatum is consistent with the earlier demonstration of a significant reduction in the GABA-synthesizing enzyme, glutamate decarboxilase (GAD) immunoreactive nerve terminals in the substantia nigra 7 days after striatal injections of KA (Oertel, et al., 1981). Furthermore, Saji and Reis (1986) have shown that intraventricular infusions of GABA can at least partially prevent the loss of neuronal perikarya in the substantia nigra after striatal infusions of IBO. Thus it seems highly probable that a majority of the degenerating terminals in the substantia nigra represent anterograde degeneration of GABAergic afferents from the striatum.

The present detection of morphological signs of degeneration in perikarya within the pars reticulata of the substantia nigra homolateral to the IBO-injection has not been reported in earlier studies (Guldin and Markowitsch, 1981; Kohler and Schwarcz, 1983; Schwarcz et al., 1979; Steiner et al., 1984). However more widespread cytotoxic effects have been suggested on the basis of a histochemical analysis of GABA-transaminase by Gale et al. (1984). Furthermore Saji and Reis (1985) reported a reduction in the number of neurons in the substantia nigra which was attributed to transneuronal degeneration following IBO-injections in the homolateral striatum. We did not detect signs of degeneration in both pre- and postsynaptic components within the substantia nigra which may indicate a different time course for detectable anterograde degeneration in comparison to later changes which may occur in the deafferented perikarya. The degenerative changes might also be attributable to retrograde transport of toxic substances or local damage to non-dopaminergic terminals in the striatum. Degenerative changes also were not detected in perikarya in the substantia nigra which exhibited labeling for TH. However, the reaction product could easily have obscured the electron density of degenerating cells.

4) Termination of Degenerating Axons on TH-labeled and Unlabeled Dendrites in the Substantiva Nigra

The relative abundance of synapses between degenerating axon terminals and unlabeled versus TH-labeled dendrites confirms earlier reports that the majority of afferents from the striatum terminate on non-dopaminergic neurons (Wassef et al., 1981). However, the relative proportions of synapses on unlabeled dendrites versus those with detectable TH-immunoreactivity may be biased by incomplete penetration of the antisera.

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