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**MOUSE NIGROSTRIATUM IN INDUCED
PARKINSONISM: AN ULTRASTRUCTURAL
STUDY**

**A Thesis
Presented to
The Faculty of the Department of Biology
San Jose State University
In Partial Fulfillment of the Requirements for the Degree
Master of Science**

**By
Jeffrey A. Cochiolo
December 2001**

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ABSTRACT

MOUSE NIGROSTRIATUM IN INDUCED PARKINSONISM: AN ULTRASTRUCTURAL STUDY

by Jeffrey A. Cochiolo

Acute doses of MPTP elicited degenerative and apparently compensatory changes in the nigrostriatum of injected animals within one day of treatment. In the SNc, low-dose (20 mg/kg) administration elicited early degeneration of mitochondria in the absence of other effects. In the striatum, a low MPTP dose resulted in myelin unwinding, demyelination, cytoplasmic shrinkage, and disturbance of synaptic communication, as evinced by a profound reduction in synaptic vesicle production. High-dose (40 mg/kg) administration generated more drastic axonal degeneration leading to cell elimination in the striatum. At neither dose was mitochondrial disturbance evident. Evidence is presented that darkened synaptic boutons, visible at this level of MPTP administration, were part of healthy enlarged axons with an elevated number of synaptic contacts. These spared neuronal processes, therefore, were hypothesized to compensate for the MPTP-induced death of dopaminergic neurons by adaptive structural modifications that would serve to enhance their functional capability.

INTRODUCTION

The neurotoxin, MPTP, selectively targets the dopaminergic (DA) neurons of the substantia nigra pars compacta (SNc), resulting in a parkinsonian condition clinically nearly indistinguishable from idiopathic Parkinson's disease in humans (Ballard et al., 1985; Davis et al., 1979; Langston et al., 1983; Tetud and Langston, 1989). Non-human primate models (Burns et al., 1983; Forno et al., 1988; Langston et al., 1984; Tanaka et al., 1988) and several non-primate animal models (Aria et al., 1990; Kaakkola and Teravainen, 1990) have been characterized that accurately portray most of the behavioral and neuropathological aspects of the human disease. The C57BL/6 mouse reproduces much of the neuropathological degeneration found in other animal models (Aria et al., 1990; Heikkila et al., 1984a; Sundström et al., 1990). Studies of the MPTP-treated C57BL/6 mouse have shown substantial depletion in striatal dopamine (DA) (Saitoh et al., 1987; Sundström et al., 1990) and severe lesions of the SNc, with cell deaths comparable in number to those in primates (Heikkila et al., 1984a,b; Sundström et al., 1987, 1988).

DA depletion due to lesions, however, is not permanent in all species. In fact, in several animals, including the mouse, some level of neuronal function is restored in DA neurons spared from destruction (Hornykiewicz, 1993; Reinhard et al., 1985; Snyder et al., 1990). C57BL/6 mice can recover both behaviorally and biochemically from the effects of MPTP (Hallman et al., 1984, 1985; Ricaurte et al., 1986). For instance, treated mice could not be distinguished from controls within a day after cessation of MPTP treatment (Ricaurte et al., 1986). Neurochemically, four months were required in young (6-8 week-old) mice for DA levels to return to normal (Ricaurte et al., 1986). In older (10 month-old) mice, neurochemical recovery was slower, with only a slight increase in DA levels evident within three months (Saitoh et al., 1987). Other studies have indicated that some level of DA

recovery occurs at earlier times (Arai et al., 1990; Irwin et al., 1987; Reinhard et al., 1988; Sundström et al., 1987, 1990).

Previous ultrastructural studies of MPTP-induced nigrostriatal lesions focused primarily on high-dose, chronic (multiple injections over days or weeks) exposure to MPTP in mice (Arai et al., 1990; Mizukawa et al., 1990), dogs (Rapisardi et al., 1990), and monkeys (Forno et al., 1988; Langston et al., 1984; Tanaka et al., 1988). One report of acute ultrastructural effects of MPTP in mice by Linder et al. (1987), described damage only in striatal DA axon terminals. While high-dose, chronic studies examining the SNc in mice (Arai et al., 1990; Mizukawa et al., 1990) and dogs (Rapisardi et al., 1990) have shown relatively consistent results between studies, less consistency exists among studies of the striatum.

Our goal with respect to the SNc was to identify the first effect of MPTP on the DA cell bodies to distinguish it from secondary effects arising from generalized cellular death. Therefore, we used a low-dose, single injection regime for MPTP exposure. We felt that this objective would be most appropriate for testing the currently most accepted model for MPTP action, that of accumulation of and destruction by MPTP metabolites in mitochondria of DA cells. In addition, we wished to better characterize early effects in the DA cell projections into the striatum.

MATERIALS AND METHODS

Forty-one middle aged (6-8 month-old) male C57BL/6 mice ("retired breeders"), obtained from Simonsen Labs (Gilroy, CA), were housed one per Plexiglas cage with free access to food and water in a colony room maintained at 23 ± 1 °C. Thirteen of the mice were given a single subcutaneous injection of 20 mg/kg and another thirteen mice 40 mg/kg of the hydrochloride salt of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Fifteen (control) mice were injected with 0.85% saline (vehicle). All animals were anesthetized

subcutaneously with Phenobarbital and perfused at 24 h post-injection via the left atrium first with heparin (10 units/ml 0.85% saline) and then with 2.5% (w/v) paraformaldehyde and 3% (w/v) gluteraldehyde in 0.05 M sodium cacodylate buffer, pH 7.4. The brains were immediately removed from their skulls, and coronal sections were made at two different levels, one of which included the striatum and the other the substantia nigra. The striatal and nigral tissues were dissected out and cut in random orientations and positions into approximately 0.5 mm³ cubes. These tissue fragments were bathed in the primary fixative for 5 days and then post-fixed in 2% (w/v) osmium tetroxide in sodium cacodylate buffer, pH 7.4, for 2 h. They were pre-stained *en bloc* with 2% aqueous (w/v) uranyl acetate, dehydrated in a graded series of ethyl alcohol, and infiltrated with and embedded in Epon 812 resin. The blocks were thin sectioned (60-90 nm) on a Porter-Blum MT-2 ultramicrotome. Sections were stained with 2% uranyl acetate and Reynold's lead citrate or Sato's lead stain and viewed on a Zeiss EM109 or JEOL 100S transmission electron microscope. Observations were made on at least five distant sections of each of five different brains at each dose of MPTP and in controls. One of the authors (Bruck) was blind to the treatments until his observations were completed.

It was vital in this study to distinguish between effects of MPTP and preparation artifacts. Because we found that MPTP affected both myelin and mitochondria, both of which are particularly sensitive to preparation conditions, fixation quality was assessed in each region by observation of mitochondrial intactness, myelin integrity, and maintenance of periplasmic space volume. When any two of these features were absent, the brain tissues from which the section was taken were discarded and the section was excluded from the study.

RESULTS

Control SNc

Nigral neurons within the control group were relatively large with one visible dendrite and a single axon. They contained large, rounded nuclei with smooth (Fig. 1) or slightly indented (Fig. 2) envelopes, sparse amounts of rough ER only rarely arranged in parallel as in most primate Nissl substance but more often randomly distributed in the cell body (Fig. 1-3), many mitochondria with lamellar, tubular, or both kinds of cristae (Fig. 2), an extensive Golgi apparatus (Fig. 2), and many lipofuscin-containing lysosomes (Fig. 2, 3). Glial cells observed were almost exclusively oligodendrocytes with little cytoplasm, few mitochondria, and large nuclei containing plentiful peripheral heterochromatin.

Low-Dose (20 mg/kg) MPTP-Treated SNc

Mitochondria of MPTP-treated nigral neurons showed marked swelling and deformation (Fig. 4-6). Severe disruption of cristae occurred, including disintegration and displacement (Fig. 4-6). Cleared regions formed in the matrix (Fig. 4-6). Damaged mitochondria were more numerous with greater proximity to the nucleus. Those immediately beyond the axon hillock appeared undamaged (Fig. 5). Residual bodies did not appear to increase in number nor was lipofuscin density greater than in control neurons (Fig. 6). No increase in cellular vacuolation was evident. Other cellular components, including the rough ER and Golgi apparatus, remained unchanged (Fig. 4-6). Oligodendrocytes were also unaffected.

Control Striatum

Within the striatum of the control animals, the neuropil was heterogeneous with some areas containing large fields of parallel-aligned myelinated axons (Fig. 7) and other areas containing mostly unmyelinated axons and axon terminals (Fig. 8), dendrites, cell bodies of

striatal interneurons, and oligodendrocytes. Extracellular spaces were limited. In the fields of myelinated axons, the cells abutted one another sufficiently to produce a kind of jigsaw-puzzle pattern (Fig. 7). Myelinated axons throughout the striatum showed tight winding of the myelin (Fig. 7, 9), although short stretches were sometimes disrupted, presumably as a result of incomplete fixation (Fig. 10). All axons were somewhat irregular in contour in that they bended abruptly and deviated from a cylindrical shape. Within them were evenly spaced and parallel-arrayed microtubules and neurofilaments (Fig. 9, 10), long mitochondria with dense (Fig. 9) or fine (Fig. 8, 10) cristae, and an occasional ER cisternum (Fig. 9). However, many axons appeared nearly devoid of organized contents (Fig. 10). Synaptic boutons contained evenly distributed vesicles with lightly stained contents (Fig. 8, 10, 11). Dendritic contents were usually sparse and consisted largely of ribosomes and amorphous substances (Fig. 8, 10).

Low-Dose (20 mg/kg) MPTP-Treated Striatum

Cytoplasmic shrinkage occurred in localized regions of axons, manifested in the PM receding from the myelin sheath (Fig. 12). Shrinkage was accompanied by displacement and clumping of cellular contents, including neurofilaments and microtubules. Vacuolation was occasionally seen, but more often vacuole-like structures were actually beyond the PM of myelinated axons (Fig. 13). Myelin sheath layers separated (Fig. 13), and dense particles accumulated in the separation sites (Fig. 12-14), as if the particles were myelin breakdown products. In many cases, demyelination was evident by short interruptions in the sheath (Fig. 14). Microglial processes were often in close proximity to these axons (Fig. 15). Little or no change was detectable in unmyelinated axons. Axon terminals were often swollen and contained few or no synaptic vesicles (Fig. 16). Vesicles that were present were aggregated into clumps with the remaining bouton appearing empty (Fig. 16).

Mitochondria were normal in morphology (Fig. 16). We also encountered a few cells undergoing severe degeneration, as evident by the nearly complete vacuolation of all cytoplasmic contents (Fig. 17). These cells always contained a large nucleus and thus were either nondopaminergic interneurons or oligodendrocytes. Other than in these unusual cells, striatal interneurons, dendrites, and glial cells remained unchanged from controls.

High-Dose (40 mg/kg) MPTP-Treated Striatum

Effects were pronounced, including further deterioration of the myelin sheaths of axons (Fig. 18). Mitochondria were again unaffected (Fig. 19). Large spaces were visible in the neuropil, often bordering or in the location of axons in terminal stages of decline and presumably digestion (Fig. 18, 19). Clearly, these spaces were axonal remnants, as the majority still contained mitochondria (Fig. 18, 19) and myelin fragments could often be seen on their periphery (Fig. 18). Swelling had occurred, as their diameters were greater than those of normal axons (Fig. 18, 19). Microglial processes increased throughout damaged areas (Fig. 20). Although one cannot determine it conclusively from thin sections, the number of lateral *en passant* boutons appeared to increase dramatically in remaining fibers in comparison to low-dose specimens and controls. These synaptic boutons stood out as an outcome of their numerous, tightly packed synaptic vesicles, which produced a starkly dark appearance (Fig. 21). Because we had demonstrated that morphological degradation was appreciable in afferents of putative DA neurons, we made a concerted effort to search our sections for PM continuities between the dense boutons and their axons to definitively establish whether these boutons were associated with healthy or damaged axons. Because of the extreme curvature of the PM in the vicinity of these terminals, such continuities are rarely seen. Nonetheless, several of these connections were located and revealed that the dark boutons were associated with intact axons lacking any of the morphological features (shrinkage, myelin disruption, microtubule and neurofilament

disarrangement or tangling, or autolysis) that characterized injured axons (Fig. 22, 23). Moreover, the axons were larger than control axons. The exact dimensions of these axons could rarely be determined, as they appeared to assume irregular patterns by perhaps expanding into areas vacated by degenerative cells. Before conducting this analysis, we had earlier observed evenly distributed, parallel arrays of cytoskeletal elements traversing the neuropil (Fig. 24). These microtubules and neurofilaments looked as though they were external to axons in the extracellular environment, as the confining cell membranes were indistinct. We now believe that they are, in fact, components of the large diameter axons that represent enlarged fibers of spared DA neurons. Because these cytoskeletal elements appear to be in an unusual location, they are easily overlooked and may account for the absence of their description in the literature. Multiple (mostly paired) and perforated asymmetric synaptic densities were also commonly encountered in the dense terminals (Fig. 21, 23) and were absent in control and low-dose tissues.

DISCUSSION

SNc Degeneration

MPTP destroys DA neurons in the SNc of a variety of species (Arai et al., 1990; Burns et al., 1983; Forno et al., 1988; Heikkila et al., 1984; Langston et al., 1984; Mizukawa et al., 1990; Rapisardi et al., 1990; Tanaka et al., 1988). Ultrastructurally, deterioration of SNc neurons has been manifested in cell shrinkage (Langston et al., 1984), pyknosis (Aria et al., 1990), chromatin clumping (Tanaka et al., 1988), loss and swelling of RER (Arai et al., 1990; Langston et al., 1984; Tanaka et al., 1988; Mizukawa et al., 1990), vacuolar changes (Mizukawa et al., 1990; Tanaka et al., 1988), dilated Golgi cisternae (Mizukawa et al., 1990; Tanaka et al., 1988), cytoplasmic inclusion bodies (Forno et al., 1986), mitochondrial swelling (Rapisardi et al., 1990), disarranged cristae (Mizukawa et al., 1990), dense mitochondrial inclusions (Tanaka et al., 1988), and dark neuronal degeneration (Tanaka et

al., 1988; Aria et al., 1990). Previous studies, however, have been conducted using high-dose injections over long periods of time.

In the present study, acute, low-dose administration of MPTP elicited mitochondrial aberrations in the nigral cells, including disruption and disintegration of cristae, clearing of the matrix, and overall distortion of the organelle. The other effects reported in higher dose studies were absent. Given the absence of disruption of myelin sheaths in the surrounding neuropil and of other morphological anomalies commonly associated with fixation or other artifact, the mitochondrial abnormalities were considered a specific result of MPTP treatment. No other degenerative changes were observed in the SNc. These results concur with neurochemical evidence indicating that MPTP enters the glial cells (astrocytes) of the brain where it is cleaved by monoamine oxidase B to form MPP⁺ (1-methyl-4-phenylpyridinium), the neurotoxic metabolite (Heikkila et al., 1984; Ranson et al., 1987). MPP⁺ is subsequently concentrated in dopaminergic cells following its uptake by the dopamine endocytotic, re-uptake system at the striatal synaptic bouton or by the PM-borne dopamine uptake system in the nigral cell body (Markham and Diamond, 1993). Once concentrated within the dopaminergic cells, MPP⁺ is believed to accumulate in the mitochondrial matrix, where it combines with NADH dehydrogenase (Ramsay and Singer, 1986; Ramsay et al., 1991). Electron transport is blocked, preventing ATP synthesis and leading to ATP depletion in the cell and cell death (DiMonte et al., 1986; Nicklas et al., 1985; Singer and Ramsay, 1990).

Our evidence implicates the mitochondrion as the first and primary site of action of MPTP or its metabolites as well as the site of the most significant damage during parkinsonism. Other effects observed in other studies are likely to be secondary effects, that is, those arising as the cell death process proceeds in the face of ATP depletion. Due to the short (24 h) period of this study, it could not be determined if mitochondrial degradation is the first step in irreversible damage to the DA neuron leading subsequently to its demise

or if the damage is reversible at this dose of MPTP. Further long-term evaluation is warranted and in progress.

Striatal Degeneration

At the low MPTP dose, we saw considerable evidence of axon degeneration in the form of myelin separation, demyelination, localized cytoplasmic shrinkage, microtubule and neurofilament disruption, and axon terminal swelling, compatible with some other striatal investigations of MPTP. At the high MPTP dose, demyelination was accentuated, resulting in axonal segments that resembled extracellular spaces. Before degradation of these segments, cytoplasmic contraction was accompanied by myelin sheath expansion, so that the axonal remnants were greater in diameter than normal axons. At low and high doses, mitochondria were unaltered morphologically in the striatum, providing convincing evidence that the changes observed were not fixation artifacts.

In previous ultrastructural studies following MPTP administration in various animal species, the striatal damage documented has been more variable than that in the SNc. Similar to our findings, Rapisardi et al. (1990) described axonal swelling and myelin sheath disruption in DA neuronal fibers of the rostral midbrain of dogs. Mizukawa et al. (1990) reported dilated neuronal processes in the striatum of mice exposed to repeated injections of 30 mg/kg MPTP. Their specimens were similar to ours under the single low dose (20 mg/kg), as terminals appeared pale from a lack of synaptic vesicles. The only difference from normal striatal tissue noted by Arai et al. (1990) was the presence in axons of membranous bodies containing electron-dense inclusions. These structures were morphologically similar to late-stage lysosomes (residual bodies). In our study, the lack of axonal structures of this type in the striatum could be entirely due to acute MPTP exposure. The intensified injury and accumulation of effects accruing from chronic neurotoxin exposure would more likely result in the prevalence of lysosomal deposits.

Other differences between studies may be due to different dosing schedules, time frames, or species and strains examined (Gupta et al., 1886; Irwin et al., 1992; Ricaurte et al., 1986; Saitoh et al., 1987; Seniuk et al., 1990; Sonsalla and Heikkila, 1986). Our mice were middle aged and may have had the combined features of young and aged mice reported by others, namely, the recuperative capacity of young mice and the greater sensitivity to MPTP of aged mice.

The only acute study of mouse striatum known to us, that by Linder et al. (1987), revealed darkened glial processes, disrupted mitochondria in axon terminals, occasionally swollen axons, postsynaptic degeneration, and electron-dense synaptic boutons interpreted as “dark degenerating” terminals. Our specimens bore neither mitochondrial nor postsynaptic cell aberrations. Glial processes also showed no darkening. Low-dose MPTP administration was insufficient to generate the darkened terminals described by Linder et al. (1987), prompting us to administer a high dose. At high dose, degenerative processes were accentuated. The axon terminals under low dose were swollen and contained few vesicles beyond occasional clumps, suggesting reduced signal activity of the cells and imminent death. This kind of terminal was absent at high doses, perhaps indicating that the cells bearing these terminals were eliminated and therefore no longer visible. The incidence of microglial processes in the vicinity of these axons supports this proposal. The dense boutons described by Linder et al. (1987) did occur at the high dose.

Striatal Compensation

Some DA neurons survive and restore normal DA metabolism and signaling interactions. Upregulation of DA turnover in surviving DA neurons is well documented (Hornykiewicz, 1993). For example, dopamine efflux from striatal slices was enhanced after 6-OHDA lesioning (Snyder et al., 1990), providing evidence for compensatory hyperactivity of remaining neurons after deafferentation. In the high-dose striatum in our

investigation, densely vesiculated boutons corresponded to those of Linder et al. (1987), who described them as undergoing a process of “dark degeneration.” The question is whether they were undergoing degradation or, exactly the opposite, were instruments of the recovery process. There were no low-dose terminals with transitional character between control and high-dose terminals, our first indication that the dark boutons were not reflections of cellular damage. We have provided further evidence that these heavily vesiculated boutons increased in number along the flanks of axons (*en passant* boutons) and were associated with large healthy axons, confirming that degeneration was not involved. The darkened appearance resulted solely from the abundance of the vesicles, their tight packing, and perhaps an increase in density of their contents. These properties attest to an increase in synaptic efficacy rather than its decline. Our main misgiving about this conclusion involves the occasional contiguousness of dark boutons to the degraded axonal segments that appear as spaces. However, the axons of which these boutons were a part might have been positioned perpendicular to the plane of the sections and thus been unseen. In addition, in all fairness to Linder et al. (1987, 1995), we should point out that they reported a further bouton darkening in the absence of vesicles in post 24 h specimens. Neither the timing of this event nor illustrations of it were included in their papers. Preliminary results in our lab of a time course following MPTP administration failed to reveal this kind of darkening. We, therefore, continue to maintain that the darkening processes are agents of recovery rather than degeneration.

An increase in the number of boutons (Kerns et al., 1992) and in synaptic vesicles in boutons (Lnenicka et al., 1991; Pierce and Lewin, 1994; Sasaki and Iwata, 1996) has been associated with increased functional capacity of the synapse in a number of studies of nervous tissue. Darkened boutons have been observed in other systems and have been interpreted to be indications of healthy, signaling neurons (Sasaki and Iwata, 1996). Increased spine density, as characterize high-dose fibers, has also been associated with

associated with functional improvement (Ingham et al., 1989). Enlargement of axons has been correlated with increased DA release and turnover in residual neurons under 6-OHDA lesioning of the nigrostriatal pathway (Pickel et al., 1992; Pickel and Chan, 1995). In these studies, TH-labeled (dopaminergic) boutons of unsusceptible neurons were significantly larger in mean cross-sectional diameter than in TH-labeled control neurons.

Dense terminals were frequently associated with multiple or perforated synaptic densities in our study. Lateral enlargement of synaptic densities and synaptic perforations, as occur in reinnervation of dendrites after lesioning, have been implicated in many studies as plastic, potentiating changes leading to improved synaptic function and axon regeneration (Carlin and Siekevitz, 1983; Ingham et al., 1998; Kerns et al., 1992; Lnenicka et al., 1991; Pierce and Lewin, 1994). Perforated synaptic densities are associated with division and multiplication of synapses, thereby increasing post-synaptic target innervation and the surface area for exocytosis of neurotransmitter (Ingham et al., 1998; Kerns et al., 1992). Thus we believe that the densely vesiculated boutons and their perforated synaptic densities were indications of adaptive plasticity.

This morphological evidence coupled with that of behavioral and biochemical recovery from MPTP-induced damage suggests compensatory mechanisms were responsible for the initial recovery in the MPTP-lesioned striatum. We believe that the neuronal fibers described here with dense boutons, increased bouton density, perforated synapses, and enlarged axonal processes were parts of spared DA neurons responsible for the recovery of the mouse from the effects of MPTP. The relative uniqueness of the C57BL/6 mouse with regard to recovery most likely accounts for the uniqueness of the characteristics in this report. By the methods we employed, we could not discern whether the fibers observed in the striatum were ones present before lesioning or instead were newly sprouting fibers branching off of existing neurons. Our results do not preclude the possibility of sprouting from surviving DA neurons to contribute to the recovery process, a phenomenon for which

an increasingly large body of evidence has recently been amassed (Ho and Blum, 1998; Kerns et al., 1992; Leonard et al., 1993; Mitsumoto et al., 1998; Wong et al., 1997). However, sprouting to this extent within 24 h seems unlikely, driving us to lean instead toward an adaptive response of existing axons and their terminals. These results thus appear to indicate that, rather than repairing existing fibers to engender mouse recovery from parkinsonism, surviving DA neurons compensate for the loss of susceptible DA neurons by enhanced synaptic efficacy through increased DA-containing vesicle production, increased bouton formation, increased synaptic surface area and number of synaptic connections with postsynaptic targets, and increased axon size.

FIGURE LEGENDS

Fig. 1-6. Transmission electron micrographs of neuronal cell bodies of the substantia nigra pars compacta. All bars = 1.0 μ m. Fig. 1-3. Controls. **1:** Cell body illustrating the relatively smooth envelope of the nucleus (N) and the dense cytoplasm containing intact mitochondria (M), scattered rough endoplasmic reticulum (ER), conspicuous Golgi apparatus (G), and abundant ribosomes.

2: Indented nuclear envelope (N). The endoplasmic reticulum (ER) is more organized, and dense lysosomes (L) are visible. Mitochondria (M) are intact, and the Golgi apparatus (G) is actively vesiculating. **3:** Late-stage lysosomes (L) containing lipofuscin. Fig. 4-6. Low-dose MPTP-treated neurons. **4:** Cleared and swollen mitochondria (M). The Golgi apparatus (G), ER, and other contents appear normal. **5:** Mitochondria (M) in the cell body show cleared matrix regions where cristae have disintegrated or been displaced. Mitochondria (m) at and beyond the axon hillock are normal in morphology. **6:** Lysosomes appear no denser nor more numerous than in control neurons. Note the distorted mitochondrion at M.

Fig. 7-11. Transmission electron micrographs of the striatum of untreated mice. All bars = 1.0 μ m. **7:** Field of parallel, tightly packed myelinated axons. **8:** Unmyelinated axons (A) and axon terminals (B). Synaptic contacts (S) can be seen to small dendrites (D) with sparse contents.

9: Myelinated axons in transverse and longitudinal section bearing parallel microtubules and neurofilaments, mitochondria with dense (M) or fine (m) cristae, and an occasional ER cisternum. **10:** Axon with slight disruption of its myelin (at unlabeled arrowhead). Most axons and synaptic boutons contain normal components, but some (A) appear nearly empty of cytoskeletal elements and synaptic vesicles. **11:** Synaptic boutons, containing abundant synaptic vesicles and occasional mitochondria, forming an asymmetric synapse (S) with dendrites (D) bearing few contents but sometimes with membranous components.

Fig. 12-17. Striatum of low-dose (20 mg/kg) MPTP-treated mice. All bars = 1.0 μ m.

12: Cytoplasmic shrinkage as evident by retraction of cell membrane from the myelin sheath of myelinated axons (at unlabeled arrowheads). **13:** Myelin disruption, including incidence

of dense particles at the sites of unraveling (unlabeled arrowheads) and severe cytoplasmic distortion.

14: Interruption of myelin sheath as well as disorganization and dissolution of axonal contents. **15:** Increase in occurrence of apparent microglial processes (mg) in damaged areas. **16:** Axon terminal with visible postsynaptic density but nearly devoid of synaptic vesicles. The mitochondrion (M) and dendrite (D) appear normal. **17:** Neuron with prominent nucleus (N) in which many cytoplasmic contents have been converted to vacuoles (V).

Fig. 18-24. Striatum of high-dose (40 mg/kg) MPTP-treated mice. All bars = 1.0 μ m.

18: Dismantling of myelin and axonal contents. The enlarged space is continuous with the myelinated axon at A, suggesting the space arose from digestion of the intervening section of the axon. Mitochondria can be seen in the space. **19:** Mitochondria-bearing axons (A) in the process of degeneration. **20:** Abundance of microglial processes (mg). **21:** Numerous dark terminals (B) with densely packed vesicles, intact mitochondria (M), and apparently normal synaptic contacts (S). **22:** Dark *en passant* boutons (B) with undamaged mitochondria (M) synapsing (S) off of axon showing normal distribution of microtubules (mt) and other contents. **23:** Another dark *en passant* bouton (B) with a perforated synapse in continuity with an undamaged, enlarged axon. **24:** Cytoskeletal elements (mt) traversing the neuropil but without any clear axonal boundaries.

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