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Evolution of Nerve Fiber Degeneration in the Striatum in the MPTP-Treated Squirrel Monkey

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Abstract

We have examined the ultrastructure of the striatum in squirrel monkeys 1–5 d after a single sc injection of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) 2.5 mg/kg. One untreated monkey served as control. We expected to find a dense degeneration of the dopamine terminals, but found instead that the main abnormality consisted of a focal vacuolation of the tissue, perhaps related to the striosome/matrix mosaic of the neostriatum. The vacuolation involved not only terminals, but also other parts of the neuropil. The severity of the destructive process increased from d 1–5. We conclude that MPP^+, the toxic metabolite of MPTP, may gain access to the neuropil, either before or after its active uptake into and subsequent destruction of the dopamine terminals.

In the present study, abnormalities were observed simultaneously in the striatum and substantia nigra as early as 24 h after MPTP administration. It is, however, possible that the time-course might differ between the two locations with even shorter time intervals or changes in dosage of MPTP.

Index Entries: Electron microscopy; experimental parkinsonism; nerve cell degeneration; neurotoxin; nonhuman primates; striatum; tyrosine hydroxylase.

Introduction

The present electron microscopic (EM) study of the striatum in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated squirrel monkeys was conceived as a simple way of obtaining important information about the gradual development of the degeneration of dopaminergic fibers in the basal ganglia in MPTP-induced parkinsonism. It is part of a more comprehensive study of the effect of MPTP on brain structure in squirrel monkeys *(saimiri sciureus)* when examined on consecutive days after MPTP administration.

Although the MPTP animal model for Parkinson's disease (PD) has been in existence for 10 yr, much still remains to be learned about the structural changes resulting from administration of this drug and the evolution of the nerve cell degeneration. The MPTP-induced nerve cell degeneration in the substantia nigra (SN) has been the subject of a few EM reports *(1–5)*. Two of these papers *(4,5)* have also touched on EM findings in the striatum in the MPTP-treated mouse, but we are aware of only one detailed description of the effect of MPTP on the ultrastructure of the neostriatum *(6)*.

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In MPTP-treated mice, acute degeneration of nigrostriatal fibers in the neostriatum has been demonstrated with the Fink-Heimer silver impregnation method *(7)*. In the EM study of the mouse by Linder and colleagues *(6)*, the earliest changes in the striatum were found in astrocytic processes. They were followed by dense degeneration of terminals, but after a couple of days, these abnormalities were no longer visible. In unpublished studies in young and middle-aged mice, described briefly in a review *(8)*, we were unable to distinguish clearly the striatal ultrastructure of treated from untreated mice 4 d after ip MPTP, although many nerve cells in the SN from the older animals appeared to be dying. It is, however, possible that the degenerating terminals may already have been eliminated.

Because the primate animal model is more relevant to human PD and important species differences may exist, we undertook the present study in the squirrel monkey. It has generally been considered that the dopaminergic nerve terminals in the striatum are more vulnerable to MPTP and its toxic metabolite MPP^+ than their parent nerve cells in the SN, at least in the mouse *(9)*, but perhaps also in primates *(10)*. In the squirrel monkey, we found changes in the SN as early as 1 d after MPTP and severe nerve cell degeneration after 5 d. Here we report the results in the striatum in the same animals.

Materials and Methods

Electron Microscopy

Squirrel monkeys were examined by EM on consecutive days after a single sc injection of MPTP. Middle-aged monkeys, 8–12 yr old, were selected. Five experimental animals and one untreated control were used. The dose administered, 2.5 mg MPTP/kg, was one that in our experience will cause nerve cell loss in the SN in squirrel monkeys that survive for more than a week. The monkeys were anesthetized and perfused intracardially with a paraformaldehyde-glutaraldehyde fixative (4 and 1%) 1, 2, 3, 4, and 5 d after the MPTP injection. The brains were removed and stored overnight in the fixative. On the following day, they were examined and sectioned, and samples were taken from 16–20 locations. Cores of tissue were taken with a small punch from anterior and posterior putamen, body and head of the caudate, and from the globus pallidus. Examination of other brain areas, including the SN and the locus cerulus, will be the focus of another study. A preliminary report of the findings 2, 3, and 4 d after MPTP has been published *(11)*.

The tissues were postfixed in osmium tetroxide and embedded in epon-araldite according to routine methods used in the EM laboratory. Thick sections (1 μm) were examined, and selected blocks (three or four from each core) were thin sectioned, stained with uranyl acetate and lead citrate, and viewed in a Philips 201 electron microscope.

Light Microscopy and Tyrosine Hydroxylase Immunocytochemistry

The location of the samples taken was verified in paraffin-embedded sections of the corresponding tissues. This light microscopic material was examined with routine neuropathological methods and also reacted with a rabbit polyclonal antibody (from Pel-Freez, Rogers, AK) to tyrosine hydroxylase (TH), using the avidin biotin method (Vectastain ABC Kit, Vector Labs, Burlingame, CA). The antibody was used in dilution 1:400. For control sections, normal rabbit serum was substituted for the primary antibody. Several levels of the basal ganglia were examined in each animal.

Results

Electron Microscopy

Abnormalities were noted as early as 1 d after MPTP and consisted of a focal vacuolation of the tissue, involving axon terminals as well as other portions of the neuropile (Fig. 1A–C). These lesions became generally more numerous and more severe with increasing interval from MPTP injection to death (Figs. 2 and 3).

It could not always be determined what components of the neuropile were involved. In addition to axon terminals, dendrites were clearly affected also, and some of the dilated spaces were most likely within astrocytes. Synaptic vesicles were, however, commonly found in distended terminals when the devastation was mild. Convincing dense degeneration in terminals was not found. The evaluation was made difficult by the fact that occasionally a slight vacuolation could also be found in the untreated control monkey. Rare dystrophic axons could be seen in both control and MPTP-treated striatum, and were only questionably more pronounced in the MPTP-treated monkeys.

A characteristic feature was the patchy distribution of the vacuolation. We have not established how this uneven involvement relates to the neo-

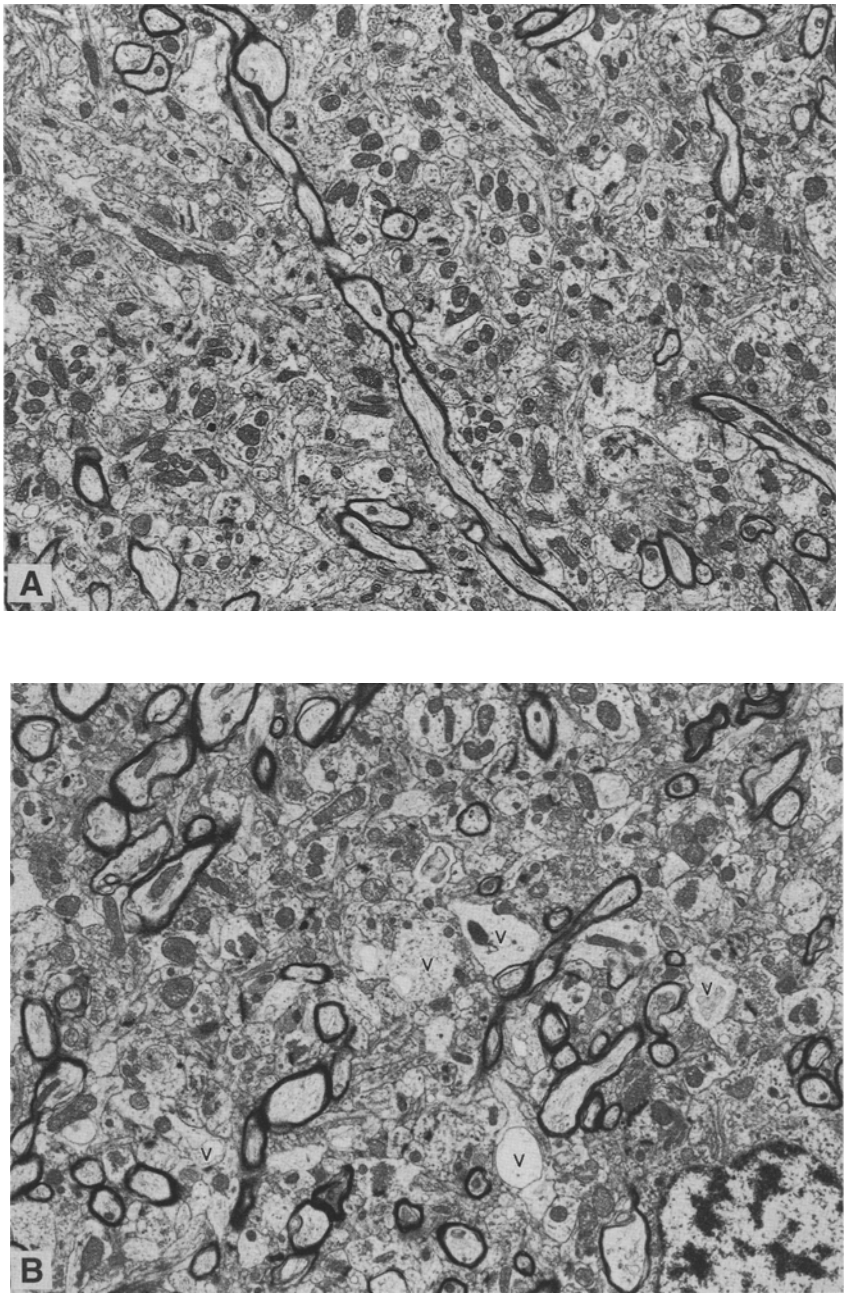

Fig. 1. **(A)** Normal neostriatum in untreated control monkey, ×7750. **(B)** Neostriatum 1 d after MPTP. There is a slight vacuolation (V) in the neuropile, ×7750.

striatal mosaic of striosome (also called patch) and matrix *(12,13)*, but dopaminergic terminals have been found mainly in the matrix compartment in humans *(14)* and perhaps also in the squirrel monkey *(13)*. From d 3, the focal nature of this vacuolation could be observed by light microscopy in the 1 μm toluidine blue-stained plastic section (Fig. 4). At d 5 (Figs. 1C and 3), the vacuolation was quite severe in several locations, but even here essentially normal areas could still be found. There was no predilection for certain parts of the striatum, although variation in degree of involvement was found between individual animals. The 5-d monkey, for example, showed more severe changes in the head of the caudate than in the posterior putamen, whereas in the 3-d monkey, the posterior putamen

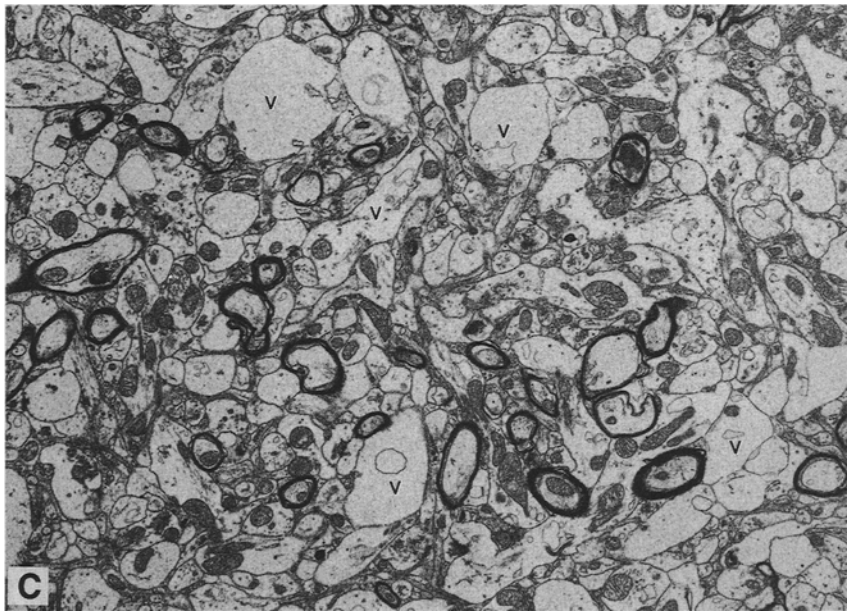

Fig. 1. **(C)** Neostriatum 5 d after MPTP. Note the marked vacuolation (V) of the tissue, ×7750.

was more affected. We had the impression that these differences occurred by chance, depending on the location of the probe and perhaps its relation to the striosome/matrix compartments.

The effect on astrocytes has been briefly mentioned in a previous publication *(15)*. In general, astrocytes appeared to be fewer than normal, and glial filaments were sparse in those present, especially compared to the situation in the SN and the globus pallidus. This was not an entirely consistent finding, however, and may require more detailed study. A direct effect on astrocytes may be present very early after MPTP. In the 1-d monkey, we found in a few sections (not shown) some dense profiles with wisps of dense tissue surrounding cell processes. These profiles, which did not contain vesicles and did not resemble nerve terminals, were indeed very similar to the early astrocytic changes demonstrated by Linder and colleagues *(6)* in the MPTP-treated mouse striatum.

Nerve cells within the striatum did not show any significant abnormalities. Dark neurons were rarely seen. The globus pallidus in all five animals displayed a few examples of vacuolation of tissue, but not sufficient to convince us that it resulted from the MPTP treatment, rather than being an artifact of tissue preparation or of other pathology in these middle-aged monkeys.

Light Microscopy and Tyrosine Hydroxylase Immunocytochemistry

Routine stains with hematoxylin-eosin, Luxol fast blue-cresyl violet, Bielschowsky, and others did not reveal any abnormality in the striatum. The vacuolation observed by EM was not visible in 8-μm thick paraffin sections. There was no iron deposition in the basal ganglia. The immunoreaction for the antibody to TH remained unaffected and fairly uniform in the 1-, 2-, and 3-d monkey, when compared to the untreated control (Fig. 5A,B). No definite matrix pattern could be demonstrated in paraffin sections. By d 4 (not shown), the reaction was much weaker and very uneven, and the 5-d monkey exhibited only a reaction in a few thin fibers.

Discussion

We had embarked on this study anticipating that we would be able to demonstrate a discrete dense degeneration in presumed dopamine terminals in the striatum in the earliest time-points after MPTP injection. This expectation was not fulfilled. The abnormalities presented instead as focal pale areas of destruction of the neuropil with disruption of membranes and vacuolation. Synaptic terminals, usually with preservation of synaptic vesicles, did,

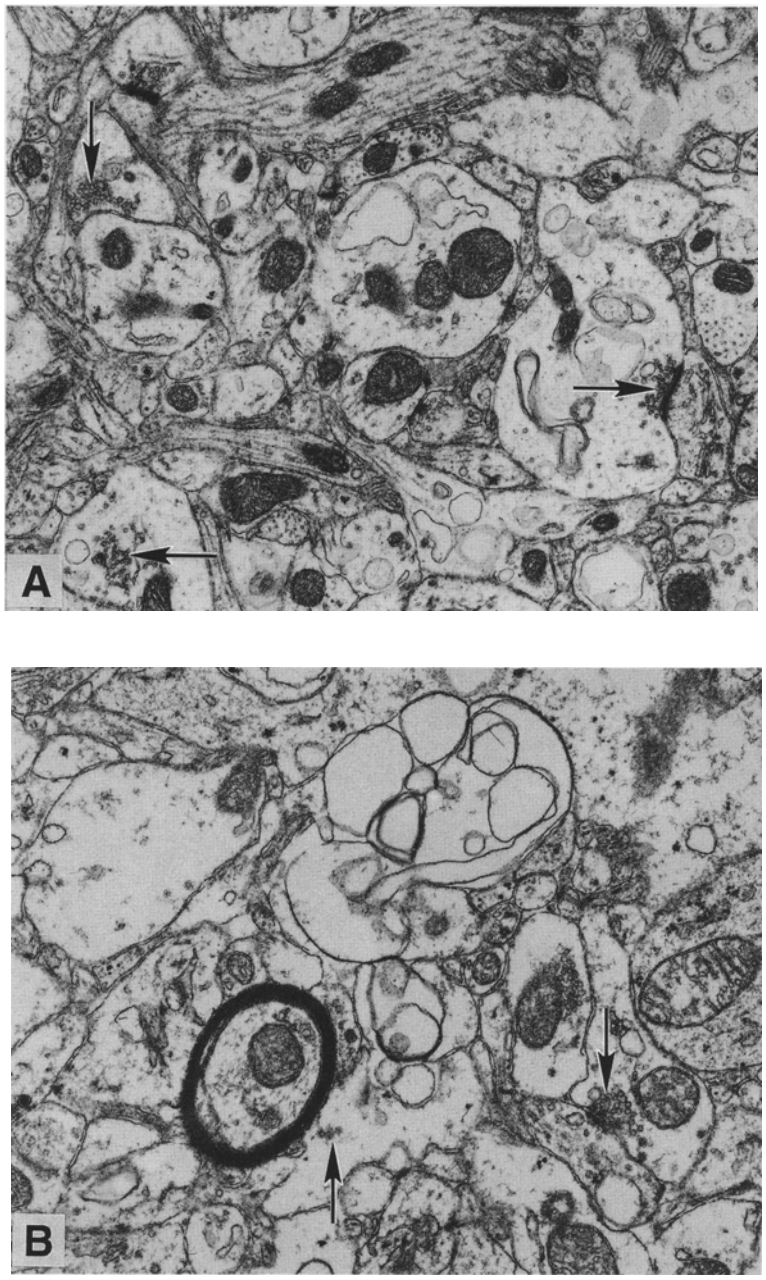

Fig. 2. **(A)** Neostriatum 3 d after MPTP at higher magnification. Synaptic vesicles (arrows) are seen in some of the distended processes. ×18,000. The mitochondria are fairly well preserved. **(B)** Neostriatum 4 d after MPTP. Marked vacuolation of the neuropile. Synaptic vesicles (arrows) can be seen in a few of the pale and dilated processes.

however, appear to be main targets for the process. In the 1-d monkey, this was particularly evident.

The character of the dopaminergic synaptic terminal in the striatum has for many years been a matter of debate. For a while, it was thought that the dopamine innervation exerted a rather nonspecific modulation of the activity of striatal neurons. There is now convincing evidence (*see* ref. *16* for review) that medium spiny output neurons in the striatum receive symmetrical inputs on their distal dendritic spines and shafts from dopaminergic terminals. Since the same dendritic spines also receive

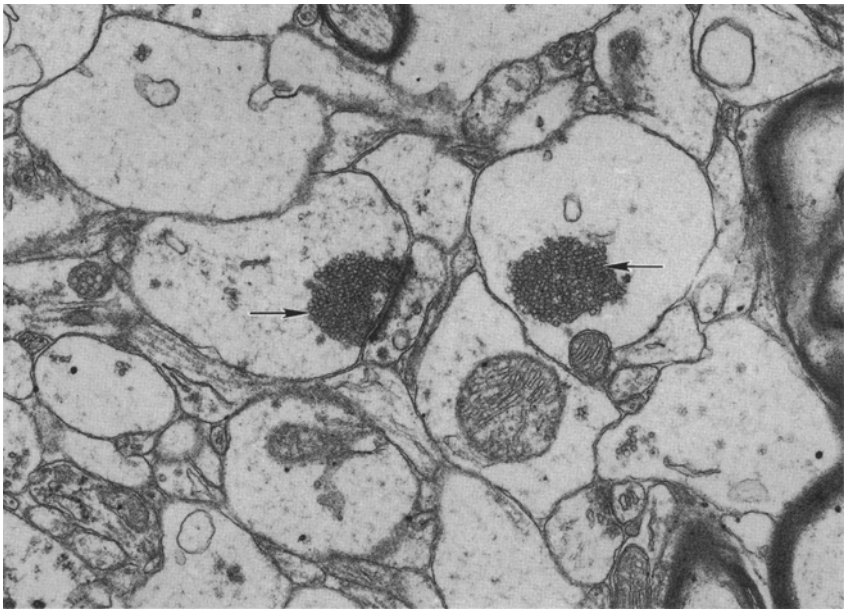

Fig. 3. Five days after MPTP. Although the neuropil is quite abnormal, synaptic vesicles are still present (arrows) in several of the vacuoles.

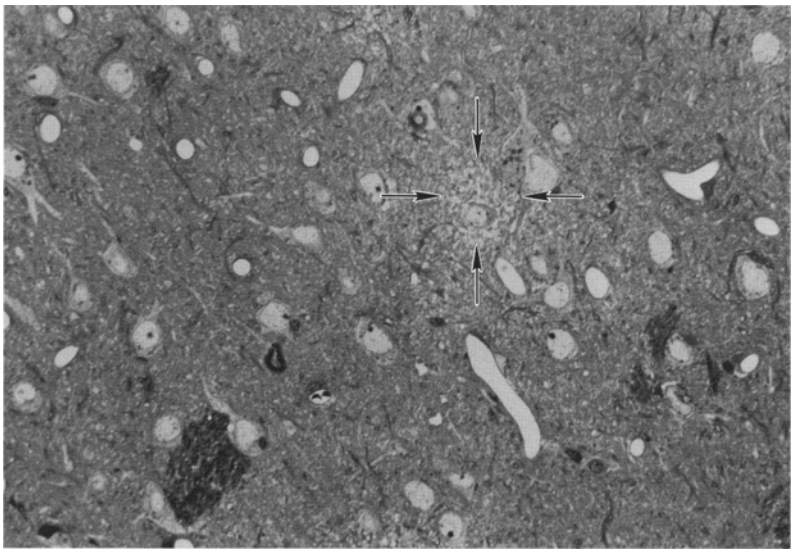

Fig. 4. One-micrometer thick plastic section from the neostriatum, stained with toluidine blue. Note the focal vacuolation (arrows). Three days after MPTP, ×700.

an asymmetrical (excitatory) input from the cortex, this arrangement may permit important interactions between these two afferent systems. We looked for abnormal distended terminals with this particular relationship to dendritic spines, but did not find them, perhaps because the tissue destruction was too extensive. Serial thin sections might also have been helpful. The reaction to the antibody to TH pointed to a considerable loss of dopamine by d 4 and 5, but the intense reactivity for the first 3 d was surprising. It does, however, agree with the results obtained in squirrel monkeys by Irwin and

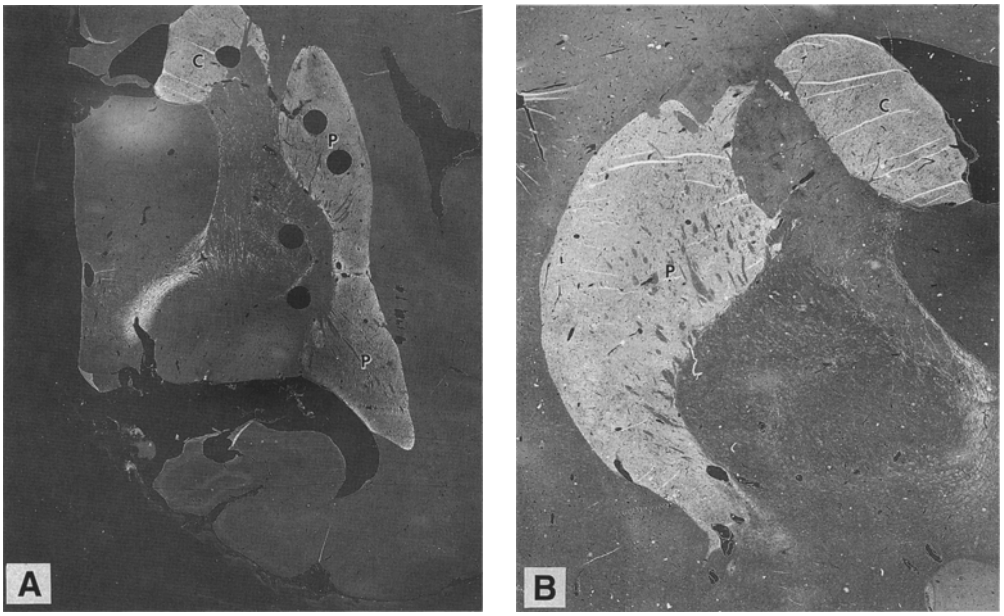

Fig. 5. **(A)** Paraffin section from untreated control monkey, demonstrating reaction to antibody to tyrosine hydroxylase in the basal ganglia. C = caudate. P = putamen. Tissue for EM was removed as shown by the punch holes. Negative print, ×7. **(B)** Paraffin section of a slightly different (more anterior) level of the basal ganglia in squirrel monkey, 3 d after MPTP, showing similar vigorous reaction to antibody to tyrosine hydroxylase. C = caudate; P = putamen. Neg. print, ×12.

colleagues *(17)*. In their squirrel monkeys, examined 1, 3, 5, and 10 d after a similar dose of MPTP as in the present study, they did not find a decrease in striatal dopamine until d 5.

If the interpretation of our findings is correct, the process observed after MPTP appears to be more complex than a simple degeneration of the terminals. It is well established that astrocytes, known to contain monoamine oxidase (MAO) B, play an important part in the MAO B-mediated conversion of MPTP to its toxic metabolite MPP^+ *(18)*. If MPP^+ gets access to the extracellular space and adjacent neuropil, before its uptake into the dopaminergic terminals, it might damage not only terminals, but also dendrites and astrocytic processes. Another explanation for the vacuolation might be that MPP^+ is released into the adjacent neuropile after having destroyed the terminals. Since MPP^+ is not formed within the nerve terminals, it might well exert its effect both before and after uptake into the terminal and with or without killing the astrocytes.

Our earliest observations in this study were made 24 h after administration of MPTP. This leaves open the possibility that some changes may have taken place at an even earlier time interval. One of the aims of this ongoing study is to compare the time course of the MPTP-induced degenerative process in the neostriatum with that in the SN, a subject about which there is some controversy *(10)*. In our study, abnormalities were observed simultaneously in the striatum and the SN as early as 24 h after MPTP administration. It is possible that differences in time course between the two locations might be revealed at even shorter time intervals or with changes in dosage of MPTP. More work will be needed to clarify these issues.

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