Advances in Neurology, Vol. 53: Parkinson's Disease: Anatomy, Pathology, and Therapy, edited by M.B. Streifler, A.D. Korczyn, E. Melamed, and M.B.H. Youdim. Raven Press, New York © 1990.

Chronic Administration of MPTP to Marmosets

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Parkinsonism induced in primates by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has provided an experimental model of parkinsonism (7). In order to obtain parkinsonian symptoms, primates are usually injected with repeated doses of MPTP over 2 to 5 days, according to the species: In marmosets, 1 to 4 mg/kg ip is effective (4).

Although the relevance of MPTP-induced parkinsonism to the idiopathic human disease is still debated (5), environmental toxins may play a role in human pathology (1,6). Such toxic pyridines may induce Parkinson's disease by the additive effect of low doses over time. Therefore, the present study was undertaken in order to evaluate the effect of chronic administration of different regimens of MPTP in the common marmoset.

MATERIALS AND METHOD

Twenty common marmosets (*Callithrix jaccus*) of either sex were used. They were divided into five experimental groups consisting of four sex- and age-matched individuals. Sixteen monkeys were dosed twice a week intraperitoneally, with MPTP: group A received 0.25 mg/kg of the toxin in each injection; group B, 0.50 mg/kg; group C, 0.75 mg/kg; and group D, 1.25 mg/kg. Four monkeys, group E, were injected twice a week with vehicle only (0.5 saline solution, ip). Assessment of parkinsonian disability was performed before starting the experiment and at weekly intervals thereafter by means of a disability scale (Table 1). In addition, animal behavior was observed for at least 1 hr every day and after each injection.

Three months after treatment, the animals were sacrificed and the brainstem was fixed by immersion in 4% phosphate buffered paraformaldehyde for approximately 2 weeks, then transferred to 30% phosphate buffered sucrose for 3 days, before being processed for morphological analysis. Coronal sections were cut at 30 μm intervals, using a freezing microtome. Alternate adjacent sections were stained by cresyl violet or processed for tyrosine hydroxylase (TH) immunohistochemical staining, according to the following procedure. All antisera were diluted in phosphate buffered saline (PBS) containing 0.1% triton X-100, 3% goat serum, and 1% (wt/vol) bovine serum albumin. Sections were treated for 30 min. in PBS containing 0.2% (vol/vol) hydro-

TABLE 1. *Disability scale for marmosets*

Feature	Score
Normal	0
Slowing of movements, with normal balance and posture	1
Abnormal posture of the limbs, trunk, or tail; abnormal balance when pushed or pulled	2
Very slow, unable to keep balance on a perch	3
Immobile, bound to the floor	4

gen peroxide (30 vol) followed by repeated wash in PBS. Incubation in TH antisera (Eugene Technologies) was overnight at room temperature. Sections were then washed, incubated with goat antirabbit IgG serum (1:20), washed again, and then incubated for 1 hr at room temperature with rabbit peroxidase-antiperoxidase antiserum (Miles) diluted 1:100. After washing, tissue sections were exposed to the chromogen solution (1,3-diaminobenzidine, 0.05% wt/vol) in PBS containing 0.2% (vol/vol) hydrogen peroxide (30 vol) as substrate for approximately 10 min. Midbrain sections were studied by a Leitz ASBA image analyzer, blindly, by a single observer. Cell counts were performed in the pars compacta: the relative density of TH-positive cell bodies was computed in medial, intermediate, and lateral segments. In addition, the cross-sectional area of all neurons displaying a well-defined, nonstained nucleus was measured in the substantia nigra and in the ventral tegmental area. Data obtained from the five experimental groups were compared individually, by means of pooled *t* two-sample analysis.

RESULTS

Behavior

During chronic administration of MPTP, the behavior of marmosets differed from what is customarily observed following daily maximal doses. Acute motor abnormalities, which usually appear within a few minutes following the second maximal dose of MPTP (3), were never observed in these experiments. The earliest motor changes consisted in progressive bradykinesia, which affected monkeys of groups C and D, starting 1 month after beginning treatment. Later, limb rigidity and abnormalities of posture and balance appeared in the same groups. Group A and group B monkeys became bradykinetic only during the third month of treatment. Motor symptoms were never as severe as those following short-term maximal treatments: although some monkeys of group D were inactive for long periods, all animals could feed themselves. As bradykinesia progressed during the third month, dystonic postures were observed with increasing frequency in groups C and D. During the third month, tremor was observed in one animal of group B and in two of group D; starting from the seventh week of treatment, blepharospasm occurred in two animals of group B and in two of group D. Tremor and blepharospasm were intermittent, and their frequency gradually increased with time. After 3 months of dosing, group C and D animals were definitely parkinsonian, showing bradykinesia, tremor, and dystonia; group B animals showed only mild bradykinesia and some additional abnormalities, whereas group A animals were almost normal (Fig. 1).

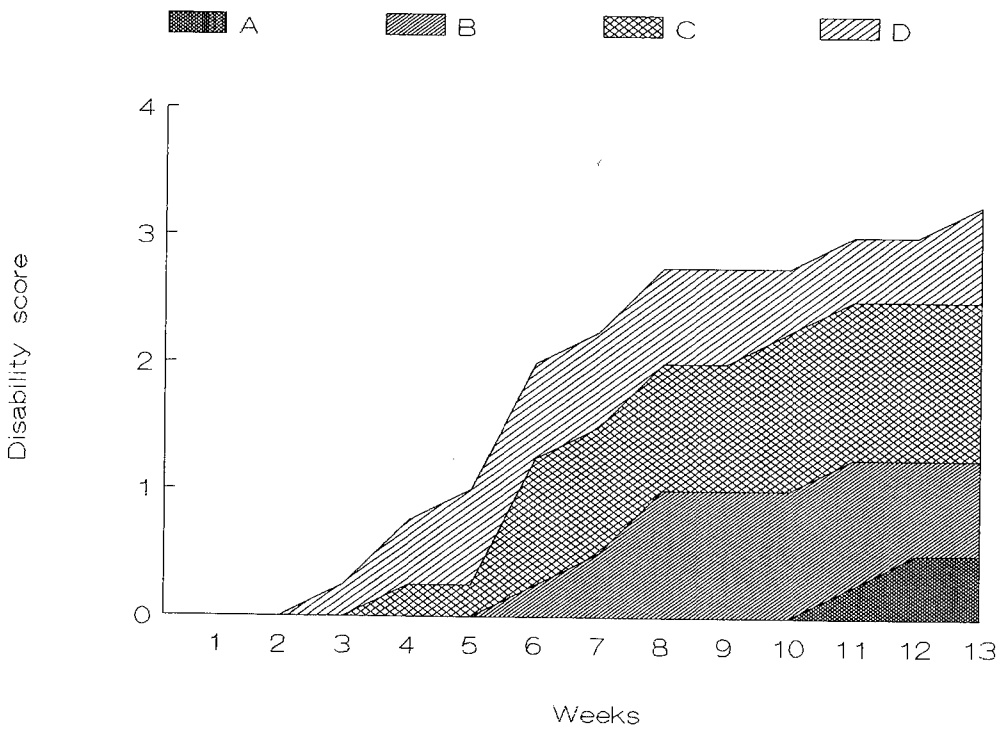

FIG. 1. Weekly evaluations of disability scores in marmosets treated chronically with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). It can be observed that behavioral disability, as observed by the scale listed in Table 1, progresses with time.

Morphology

In all monkeys, both the substantia nigra and ventral tegmental area were only partially depleted of TH immunoreactive perikarya. As compared to controls, the number of TH-positive neurons located in the substantia nigra was decreased in all MPTP-treated groups. No differences were observed among medial, intermediate, or lateral segments of the pars compacta. Average cell density in controls was 483.81 cells/μm^2; in group A it was decreased by 36.88% to 305.40 cells/μm^2, in group B it was 302.33 cells/μm^2 (-37.51%), in group C it was 252.16 cells/μm^2 (-47.88%), and in group D it reached 234.86 cells/μm^2 (-51.46%) (Fig. 2).

Morphometric analysis also showed that, as compared to controls, the size of individual TH-stained neurons is reduced in the substantia nigra and in the ventral tegmental area of MPTP-treated marmosets (Table 2). Cell shrinkage in the pars compacta was clearly graded from groups A to D: neural size in group A was 2.75% smaller than controls, in group B it was reduced by 4.13%, in group C by 10.45% ($t < 0.001$), and by 14.77% in group D ($t < 0.001$). In addition, between-group comparison showed that all values were significantly different from each other ($t < 0.001$), with the exception of groups A and B, and of groups C and D. In the ventral tegmental area, sizing of dopaminergic neurons by groups showed a similar trend and a flatter slope (Fig. 3): neural size in group A was 7.25% smaller than

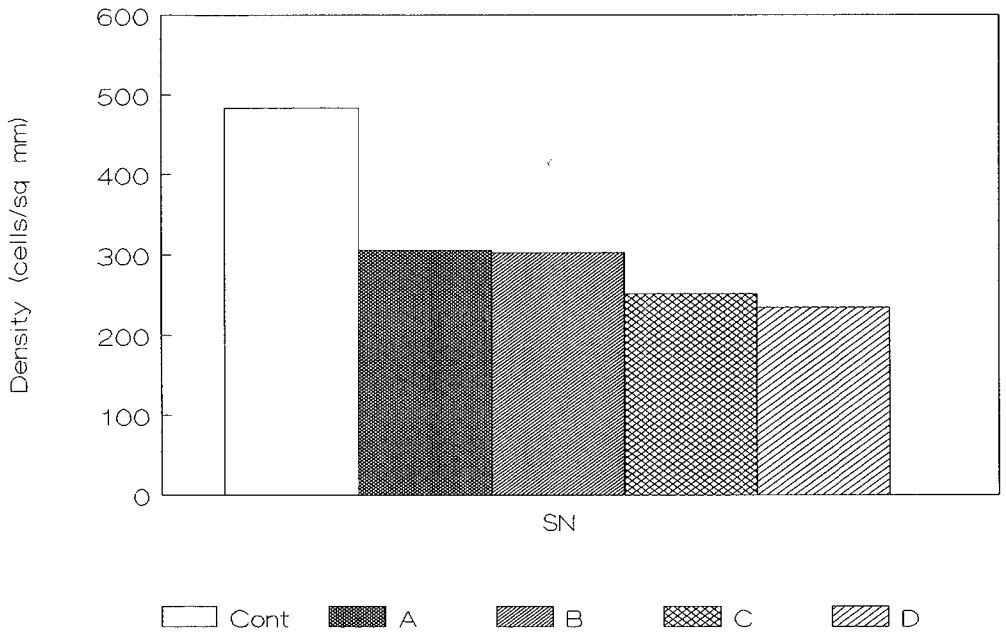

FIG. 2. Density of dopaminergic neurons located in the substantia nigra (SN) of MPTP-treated and control monkeys. Cell density is graded according with the group of treatment.

TABLE 2. *Comparative size (μm^2) of neurons located in the midbrain of MPTP-treated marmosets and controls (mean cross-sectional area ± SEM)*

Group	Substantia nigra	Different from	Ventral tegmental area	Different from
Control	398.54 ± 8.41	C,D[a]	195.47 ± 4.60	A,B,C,D[c]
A	387.59 ± 6.95	C,D[a]	181.29 ± 4.46	Control[d]
B	382.07 ± 6.21	C,D[b]	174.18 ± 4.27	Control[a]
C	359.96 ± 6.23	Control,A,B[b]	173.45 ± 3.14	Control[a]
D	339.67 ± 7.35	Control,A,B[a]	172.57 ± 4.94	Control[a]

[a] $t < 0.001$ in each case.
[b] $t < 0.005$ when comparing B to C; $t < 0.001$ in all other cases.
[c] $t < 0.05$ when comparing controls to A; $t < 0.001$ in all other cases.
[d] $t < 0.05$.
MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

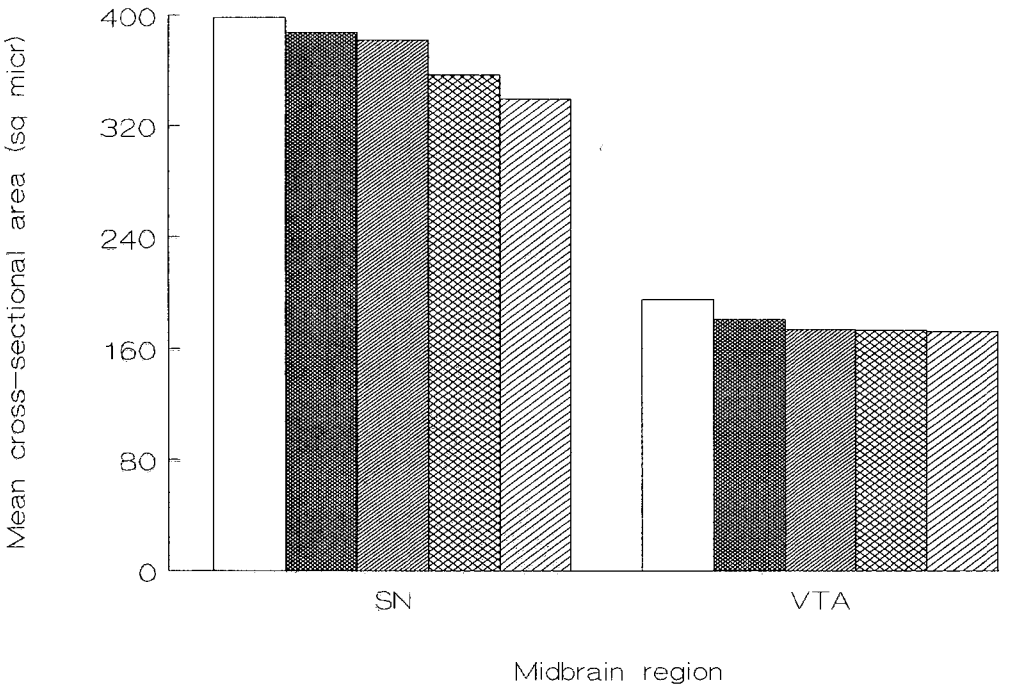

FIG. 3. Mean cross-sectional area (± SEM) of dopaminergic neurons located in the substantia nigra (SN) and in the ventral tegmental area (VTA) of MPTP-treated and control monkeys. As compared to controls, the mean size of perikarya located in groups A through D is reduced in both regions. However, although in the pars compacta a clear progression is observed in the four treated groups, this is not the case with the VTA.

controls ($t < 0.05$), in group B it was reduced by 10.89% ($t < 0.001$), in group C by 11.27 ($t < 0.001$), and in group D by 11.72 ($t < 0.001$). Between-group comparison did not show significant differences among groups A, B, C, and D.

DISCUSSION

This study demonstrates that chronic exposure of common marmosets to different doses of MPTP induces a progressive parkinsonian syndrome characterized by impairment of motor capabilities, tremor, and dystonia. Behavioral and morphological features depend on the total amount of MPTP given over time, and possibly on individual susceptibility. Bradykinesia is most clearly related to the dose regimen of MPTP, for it gradually progresses as toxin administration is pursued. Severity of dystonia and tremor is not directly related to the amount of injected toxin.

Parkinsonian symptoms produced by acute or chronic administration of MPTP have a similar phenomenology, but differ in some important features. First, motor disability produced by a chronic regimen gradually progresses as it impairs motility, without affecting vital functions such as feeding and self-caring. Second, chronic administration does not produce some dangerous effects which characterize acute administration of MPTP, such as self-damaging behaviors (e.g., head bumping). Third, in order to produce motor abnormalities comparable to the ones observed

after acute MPTP administration, higher cumulative doses of the toxin must be given chronically. Therefore, the chronic model of MPTP-induced parkinsonism shares with the human idiopathic condition some features which are not observed in traditional acute or subacute treatments: a gradual onset and a slow progression of symptoms.

The present data also show that morphometric analysis is a useful tool for quantifying the amount of neurotoxic damage to midbrain dopaminergic neurons. Not only cell depletion, but also the volume of surviving neurons located in the substantia nigra and ventral tegmental area, directly reflect the amount of toxin administered. In this study, differences among the four experimental groups were dependent only on different amounts of MPTP dosed in each injection; this affected the total amount of MPTP which any monkey received by the end of the study. Therefore, worsening behavioral scores and a decrease in the density and size of residual dopaminergic neurons correlate directly with the regimen of MPTP administration. Behavioral recovery has been observed in monkeys treated acutely with MPTP (2) and in monkeys subject to repeated acute challenges with the toxin (8). It would be important to know whether any behavioral or morphological recovery occurs in the case of parkinsonism induced by a chronic treatment.

ACKNOWLEDGMENTS

The authors wish to thank Dr. R. Filippetti for his valuable cooperation, and Mr. I. Marchionne for assisting during the experimental procedures. This work was supported in part by CNR (Consiglio Nazionale delle Ricerche) grant no. 87.00028.04.

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