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CHRONIC ADMINISTRATION OF 1-METHYL-4-PHENYL-1,2,3,6-TETRAHYDROPYRIDINE TO MONKEYS: BEHAVIOURAL, MORPHOLOGICAL AND BIOCHEMICAL CORRELATES

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Abstract—The behavioural, biochemical and morphological effects of a chronic administration of low doses of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) were studied in the common marmoset. Monkeys received the toxin (1 mg/kg i.p.) twice a week for four months. Group A monkeys were studied one week after the last injection of MPTP; group B monkeys were studied eight months after the last toxic injection. The monkey behaviour was observed throughout the experiment; the biochemical and morphological correlates were studied *post mortem* in the neostriatum and in the substantia nigra, respectively. Data collected from MPTP-treated marmosets were compared to those obtained from sham-injected control monkeys. The results can be summarized as follows. (1) In all MPTP-treated marmosets a progressive Parkinsonism occurred. In group B monkeys, a gradual behavioural recovery was observed after MPTP was discontinued. (2) Biochemical analysis of group A marmosets showed a depletion of dopamine, of 3,4-hydroxyphenylacetic acid and of homovanillic acid, and no variations in dopamine turnover in the neostriatum of MPTP-treated marmosets. In group B, biochemical analysis showed no differences between controls and MPTP-treated animals. (3) Morphological analysis showed that the density of midbrain dopaminergic neurons located in the substantia nigra was unchanged in group A monkeys, but was reduced by 6.8% in MPTP-treated monkeys of group B. The measurement of cross-sectional area showed that midbrain dopaminergic neurons were swollen in MPTP-treated monkeys of group A, with a 11.0% increase of cell size as compared to controls. In group A the nuclei were also swollen, being 304.8% larger in MPTP-treated monkeys, with a nucleus-to-cytoplasm ratio of 65.9% (as compared to 34.0% of controls). In group B monkeys cell size was increased by 18.4% in MPTP-treated marmosets, but the nuclei were of comparable size.

The present data show that a chronic administration of low doses of MPTP brings about biochemical and morphological abnormalities. The first occur acutely in terminals and are reverted early after discontinuance of exposure to the toxin; the latter occur in dopaminergic perikarya, last longer than biochemical abnormalities and, at variance with them, increase in severity after MPTP is discontinued. Morphological abnormalities include early events, such as a transient swelling of nuclei or a long-lasting swelling of neurons, and late events, such as a decrease in the number of tyrosine hydroxylase-positive perikarya. The different time course of events occurring in terminals and in the perikarya of midbrain dopaminergic neurons suggests that the damage occurring in these two compartments is different in nature.

It is well established that the administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) to human beings or to non-human primates leads to a progressive syndrome, which closely resembles idiopathic Parkinson's disease.[24] With only few noticeable exceptions,[17] human cases of accidental exposure to MPTP were caused by the acute or subacute administration of this toxin (i.e. by a single or few repeated injections).[16] Similarly, in order to obtain monkeys which are clinically parkinsonian, MPTP is commonly administered in repeated doses for two to five days. The regimen varies according to the species. For marmosets, four or five daily injections of 1–4 mg/kg per day i.p. are usually effective to make the animals definitely parkinsonian, but capable to maintain themselves.[14]

The resemblance of MPTP-induced Parkinsonism to idiopathic Parkinson's disease is still a matter of dispute. It is commonly accepted, however, that the experimental model of MPTP-induced Parkinsonism may provide clues to the understanding of the events leading to the selective degeneration of nigrostriatal neurons.[1] The time-course of subcellular events occurring in the perikarya and in the terminals of dopaminergic neurons is a crucial issue in the pathophysiology of either the toxic or the idiopathic degeneration of nigrostriatal dopaminergic neurons. The possibility that cell bodies and perikarya may be

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Abbreviations: DA, dopamine; DOPAC, 3,4-hydroxyphenylacetic acid; HPLC, high pressure liquid chromatography; HVA, homovanillic acid, MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PBS, phosphate-buffered saline; TH, tyrosine hydroxylase.

damaged at different times of the degenerative process produced by MPTP has been raised recently.[28] However, this has proven to be difficult to test, particularly because Parkinsonism occurred soon after an acute administration of MPTP.

Considering that Parkinson's disease is a chronic and slowly progressive clinical condition, we performed a dose–response study to evaluate whether low doses of MPTP may actually induce the behavioural and pathological signs of Parkinsonism.[2,3,7] Based on these data, in the present study we treated marmosets with a chronic regimen of MPTP at doses which bring about moderate, but definite, parkinsonian signs approximately after two months of exposure. Aim of this research was to evaluate whether: (i) chronically induced Parkinsonism may be reliably studied by means of a specific disability scale; (ii) the clinical syndrome induced by chronic administration of MPTP differs from that induced by subacute administration; (iii) recovery occurs after such a chronic treatment with MPTP; (iv) damage to nigrostriatal dopaminergic perikarya and terminals occur simultaneously or at different time-intervals after the intoxication.

EXPERIMENTAL PROCEDURES

Fifteen common marmosets (*Callithrix jaccus*) of either sex were used in the study. The animals were housed, in couples, in cages kept at 25°C with a natural daylight cycle.

Treatments and behavioural evaluations

Eight marmosets of either sex, aged between 2.5 and seven years (average six years) were poisoned with MPTP. The toxin was dissolved in saline and injected i.p. twice a week for four months at the dose of 1 mg/kg. Seven monkeys were used as controls; they were injected with 0.5 ml saline solution, using the same schedule of administration. The monkeys were observed before each treatment (either MPTP solution or vehicle only) and for half an hour afterwards. Parkinsonian disability was monitored by means of a rating scale specifically devised for assessing Parkinsonism in this animal species (Table 1). This scale was adopted, as it proved valuable in our hands to monitor parkinsonian disability and behavioural recovery in marmosets treated subacutely with MPTP.

Every monkey behaviour was rated at least twice before starting the experiment, twice a week at the time of MPTP administration, and periodically during survival time after the last injection. Every evaluation was performed blindly by two experienced observers, whose scores were averaged. The following items were considered. Spontaneous motility was globally rated normal (score 0) or impaired (score 1). Reaction to stimuli, following a standard threat brought by the observer into the cage, normally produced a series of defensive movements, after which the monkey moved toward a far away corner of the cage; this reaction was rated 1 when the defensive movements were reduced in their number and complexity, it was rated 2 when, in addition, escaping movements were slow, it was rated 3 when no run-away occurred. Head checking movements, which typically occur in marmosets, could be normal (score 0), reduced (score 1) or absent (score 2). Eye motility was globally rated as normal (score 0) or abnormal (score 1); the latter condition included either the occurrence of blepharospasm or a reduction of blinking. Postural abnormalities were evaluated at rest and during movements; the occurrence of gross

Table 1. Rating scale for marmosets with parkinsonian signs

Item	Evaluation criteria	Score
Spontaneous motility	Normal	0
	Impaired	1
Reaction to stimuli	Normal	0
	Reduced	1
	Severely reduced (monkey is very slow)	2
	Absent	3
Head checking	Normal	0
	Reduced	1
	Absent	2
Eye motility	Normal	0
	Abnormal	1
Posture abnormalities	Normal (at rest and during movements)	0
	Trunk	+1
	Tail	+1
	Limbs	+1
	Grossly abnormal (flexed)	4
Balance	Normal (before and after perch rotation)	0
	Unstable on perch	1
	Spontaneous falls	2
Akinesia	Absent (before and after challenge)	0
	Mild slowing	1
	Significant bradykinesia	2
	Severe akinesia	3
Vocalization	Normal	0
	Reduced	1
	Absent	2
Tremor	Absent	0
	Present	1
Fur condition	Clean	0
	Dirty	1
Total disability		0–20

abnormalities was rated 4, while the observation of abnormal postures of the trunk, tail or limbs added 1 point each to the normal condition (score 0). The balance was evaluated while monkeys were on a perch, either in a normal resting condition or during a regular rotation of the perch in a direction that would make them fall backward; in normal conditions they would easily keep their balance (score 0); monkeys which were unstable during rotation and occasionally fell scored 1; monkeys that fell spontaneously without rotation scored 2. Akinesia was evaluated as the spontaneous reaching behaviour of monkeys toward an inviting piece of food, before receiving their daily meal; it was rated from 0 to 3, according to the occurrence of bradykinesia (mild: score 1, severe: score 2) or of akinesia (score 3). Vocalization was evaluated either in the presence of a threat, or as the response to a global vocalization of the colony; it was rated 1, when reduced, or 2, when absent. Tremor was rated 1 when present. Fur condition was considered as a global index of the animal self-care during the day; the score was 1, when fur was evidently dirty.

Group A consisted of five animals (three MPTP-treated and two controls), which were allowed to survive for one week after treatment was discontinued. Group B consisted of 10 animals (five MPTP-treated and five controls), which were killed eight months after treatment was discontinued. In each experimental group, the analysis of animal behaviour was performed by subtracting the behavioural scores given to controls from the scores given to MPTP-treated monkeys by the same observer during the same observation session.

Biochemical assay

The caudate nucleus and putamen of each hemisphere were dissected, weighed and stored at −20°C pending biochemical analysis. Dopamine, 3,4-hydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA) were extracted with 0.1 M percloric acid and assayed by reverse-phase high-pressure liquid chromatography (HPLC) with an electrochemical detector.[29] The brains of controls and of MPTP-treated monkeys belonging to a single experimental group were assayed at the same time and under identical experimental conditions. Group B marmosets were assayed approximately one year later than group A monkeys, using two different HPLC columns. The data obtained from MPTP-treated animals were compared with those collected from their corresponding controls by means of pooled Student's t-test and of Kolmogorov–Smirnov two-sample analysis (with a significance level of 0.05%). The latter is a non-parametric test, which was used to further verify the data, due to the relatively low number of biochemical samples in group A monkeys.

Histological study

All the animals were killed by decapitation, under deep general anaesthesia with ether. After extraction of the brain, samples for biochemical analysis were taken from the striatum. The brainstem was then fixed by immersion in 4% phosphate-buffered paraformaldehyde (pH 7.4) for approximately two weeks, then transferred to 30% phosphate-buffered sucrose (pH 7.4) for three days, before being processed for morphological analysis. Coronal sections were cut at 40-μm intervals by a freezing microtome.

Adjacent sections were alternatively stained with Cresyl Violet or processed for tyrosine hydroxylase (TH) immunocytochemistry 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% (w/v) bovine serum albumin. Sections were then immersed for 30 min in PBS containing 0.2% (v/v) hydrogen peroxide, and repeatedly washed in PBS. Incubation in TH antisera was overnight at room temperature. Sections were then washed in PBS, incubated with goat anti-rabbit IgG serum (1:20), washed again, and then incubated for 1 h at room temperature with rabbit peroxidase–antiperoxidase antiserum diluted 1:100. After washing, tissue sections were exposed for approximately 10 min to the chromogen solution (1,3-diaminobenzidine, 0.05% w/v) in PBS containing 0.02% (v/v) hydrogen peroxide as substrate.

Sections through the midbrain were studied blindly, by a single observer, by means of a microcomputer-based image analyser. Cell counts were performed in the substantia nigra pars compacta. The relative density of TH-positive cell bodies was computed in medial and lateral segments of this nucleus. In addition, the cross-sectional area of all neurons displaying a well-defined non-stained nucleus was measured in the substantia nigra. The data obtained from MPTP-treated animals were compared with those collected from their corresponding controls by means of pooled t-test two-sample analysis (with a significance level of 0.05%).

RESULTS

Behaviour

In both groups the average total disability score progressively worsened during the first month of treatment, the highest average score being reached by the tenth week after intoxication. In group A monkeys the disability score remained stable afterwards; in group B, instead, a gradual improvement of disability occurred after MPTP was discontinued (Fig. 1). In two monkeys of group B, behavioural abnormalities had almost completely recovered by the time of death.

When individual items of the disability scale were analysed it appeared that some items were affected by the chronic regimen, while others were not. Starting on the fourteenth day after the first administration, all MPTP-treated monkeys were consistently rated abnormal in the following items: reaction to stimuli, head checking movements, akinesia, and vocalization. Posture and eye motility were rated abnormal in all MPTP-treated monkeys starting from the 28th and the 42nd days after the first treatment, respectively. Contrastingly, ratings of spontaneous motility, balance, tremor, and fur self-care did not differ from controls at any time of the evaluation.

In group B monkeys, individual items of the disability scale varied with different time-courses. Reaction to stimuli was impaired quite early; an average disability score of 1.2 was reached on day 10 and gradually increased until a peak value of 1.5 occurred on day 135; by the end of the observation period, the average score was 1.1. Head checking movements were impaired later; a plateau average score of 1 occurred on days 129–144; by the end of the observation period, the average score was 0.75. The average score for eye motility reached a plateau of 0.4 on days 64–96; by the end of the observation period, the average score was almost normal (0.1). Posture abnormalities gradually worsened until the peak value of 1.2 was reached on days 96–135; by the end of the observation period, the average score was back to normal. Akinesia was rated 1.4 on days 58–78; by the end of the observation period, the average score was 0.6. Vocalization was gradually impaired; a plateau average score of 1 occurred on

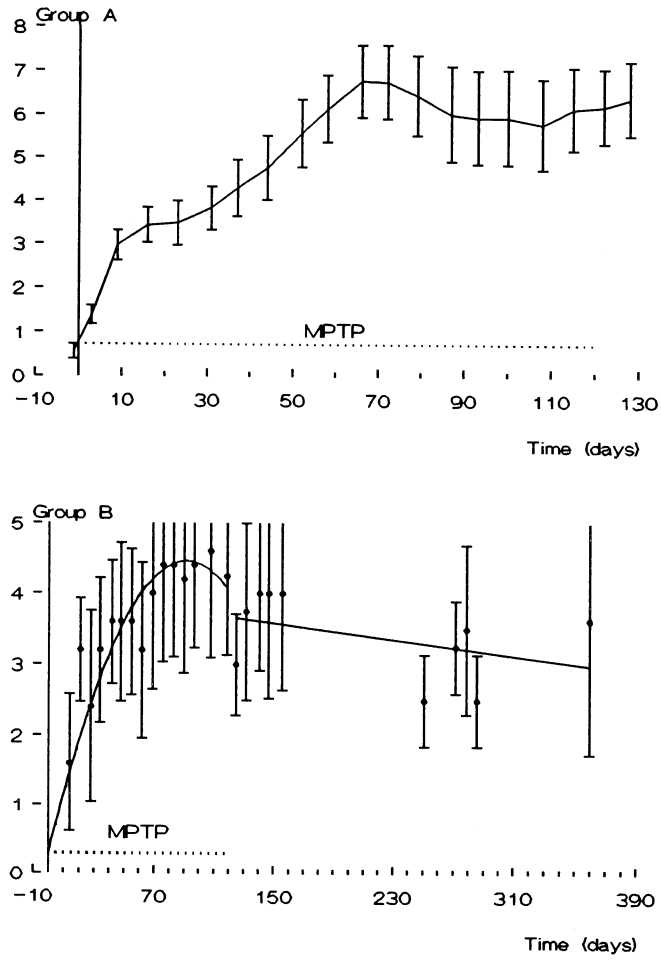

Fig. 1. Mean disability score (±S.E.M.) in group A (top) and in group B (bottom) monkeys. Chronic MPTP treatment is shown by dotted lines. It can be observed that, in both groups, the disability score increased gradually until approximately day 70, when a plateau was reached. In group B monkeys a second order polynomial curve fit and a linear curve fit are also shown for the first four and for the last eight months of observation, respectively.

days 58–85; by the end of the observation period, the average score was 0.9.

Biochemistry

The biochemical analysis of group A monkeys, showed that, as compared to controls, MPTP-treated animals underwent a depletion of dopamine, DOPAC and HVA in the caudate nucleus and in the putamen. The biochemical depletion was slightly more pronounced in the caudate nucleus, in which dopamine, DOPAC and HVA were reduced by 51.4, 63.1, and 48.7%, respectively (Student's t-test: $P < 0.001$; Kolmogorov–Smirnov: $P < 0.05$). In the putamen, dopamine, DOPAC and HVA were reduced by 49.5, 57.3, and 49.8%, respectively (Student's t-test: $P < 0.001$ in each case; Kolmogorov–Smirnov: $P < 0.05$; $P < 0.01$, and $P < 0.05$, respectively). In MPTP-treated monkeys, dopamine turnover (measured as DOPAC + HVA/dopamine ratio) was unchanged either in the caudate nucleus or in the putamen (Fig. 2).

The biochemical study of group B monkeys showed that eight months after discontinuance of MPTP, no significant variations occurred between controls and MPTP-treated animals. Dopamine, HVA, DOPAC and dopamine turnover were all normal, either in the caudate nucleus or in the putamen (Fig. 3).

Morphometry

In all monkeys, histological analysis of the brainstem allowed to observe TH-containing neurons located in the pars compacta of substantia nigra and in the ventral tegmental area (Fig. 4).

In group A, morphometric comparison of MPTP-treated and control monkeys showed no variations in the density of TH-stained neurons (Table 2; $n = 62$ for controls, $n = 112$ for MPTP-treated monkeys). Upon observation of MPTP-treated animals, however, most of the remaining TH-stained cells appeared to be clearly swollen. This was also confirmed by the observation of sections stained with Cresyl Violet and

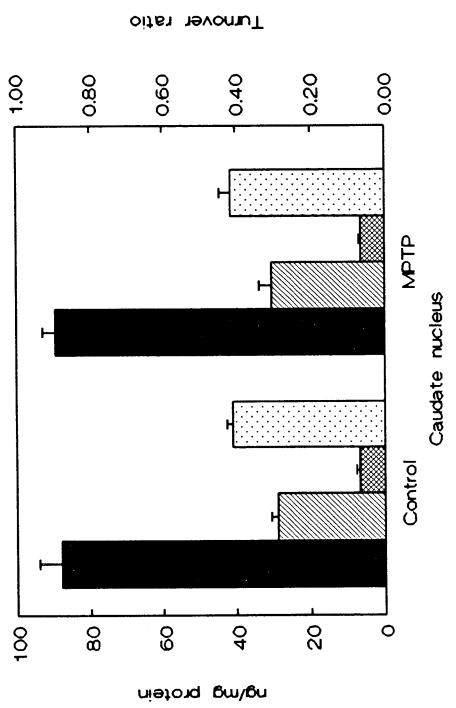

Fig. 3. Biochemical analysis of group B marmosets (mean values ± S.E.M.). No variations were observed in the values of dopamine (solid bars), of HVA (lined bars) and of DOPAC (reticulate bars), and in the turnover ratio (dotted bars), measured in the caudate nucleus and in the putamen of MPTP-treated monkeys.

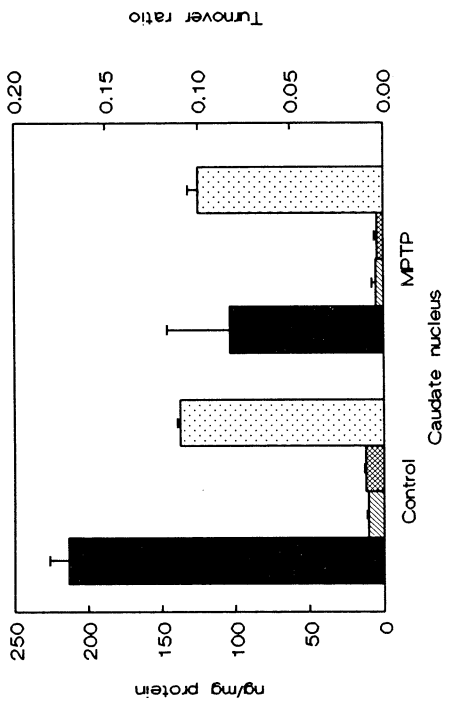

Fig. 2. Biochemical analysis of group A marmosets (mean values ± S.E.M.). As compared to controls, a depletion of dopamine (solid bars), of HVA (lined bars) and of DOPAC (reticulate bars) occurred both in the caudate nucleus and in the putamen of MPTP-treated monkeys. Turnover ratio (measures as DOPAC + HVA/dopamaine ratio) was unchanged (dotted bars).

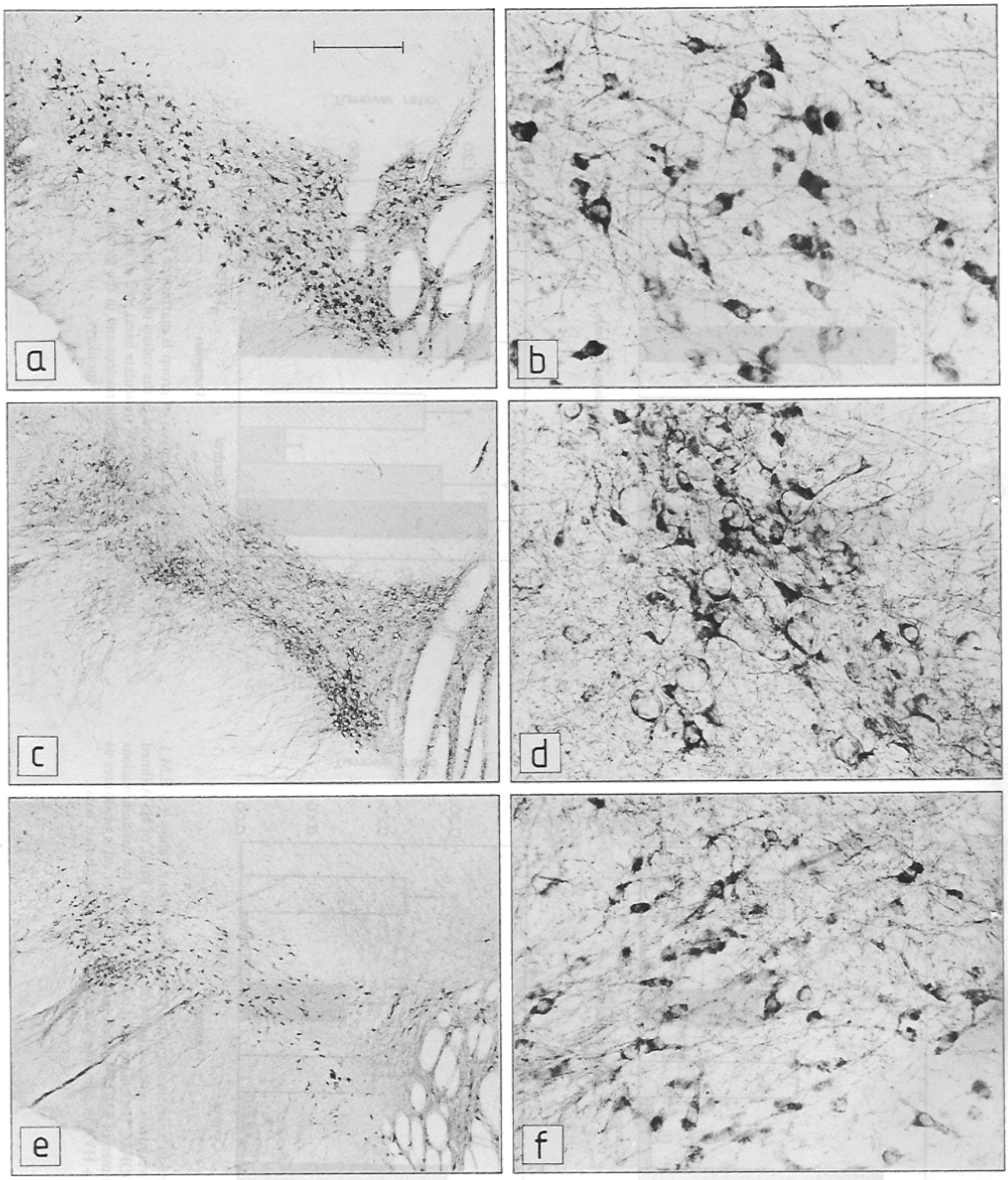

Fig. 4. TH-containing neurons located in the midbrain of controls (a, b), of group A (c, d) and group B monkeys (e, f). Low-power magnifications are represented in the left column, high-power magnifications are represented in the right column. As compared to controls (b) and to group B monkeys (f), neurons of group A are clearly swollen (d). Scale bar = 350 μm for a, c, d; 70 μm for b, d, f.

Table 2. Morphometry of tyrosine-hydroxylase-containing neurons located in the substantia nigra of MPTP-treated marmosets (mean values ± S.E.M.)

Morphometric feature	Controls	MPTP	*P*
Group A			
Cell density (cells/mm^2)	820.6 ± 27.1	816.4 ± 20.3	NS
Cell size (μm^2)	526.2 ± 7.1	584.2 ± 8.5	<0.0001
Nuclear size (μm^2)	150.1 ± 16.2	457.5 ± 42.6	<0.0001
Nucleus to cytoplasm ratio (%)	34.0 ± 2.0	65.9 ± 2.2	<0.0001
Group B			
Cell density (cells/mm^2)	534.2 ± 4.4	498.1 ± 3.9	<0.0001
Cell size (μm^2)	315.5 ± 8.8	373.3 ± 4.2	<0.0001
Nuclear size (μm^2)	150.7 ± 4.8	163.2 ± 4.2	NS
Nucleus to cytoplasm ratio (%)	48.1 ± 1.0	44.4 ± 0.8	<0.0001

by morphometric analysis of TH-stained-cells, that showed a 11.03% increase of the cross-sectional area of neurons located in the substantia nigra ($P < 0.0001$; $n = 622$ for controls, $n = 968$ for MPTP-treated monkeys). In MPTP-treated marmosets, the nuclei were also swollen in the same neurons; the nucleus–cytoplasm ratio was 65.94% (±2.24), as compared to 33.98% (±2.01) in controls ($P < 0.0001$) (Table 2).

In group B, morphometric analysis showed that, as compared to controls, the density of TH-stained neurons was decreased by 6.76% in MPTP-treated marmosets ($P < 0.0001$; $n = 625$ for controls, $n = 599$ for MPTP-treated monkeys) (Table 2). The remaining TH-stained cells were swollen. This was confirmed by morphometric analysis, that showed a 18.35% increase in the cross-sectional area of dopaminergic neurons located in the substantia nigra of MPTP-treated marmosets ($P < 0.0001$; $n = 1518$ for controls, $n = 2951$ for MPTP-treated monkeys) (Table 2). The nuclei were not altered in group B marmosets; as a consequence, in MPTP-treated marmosets the nucleus–cytoplasm ratio was 4% less than in controls.

DISCUSSION

The present study shows that when marmosets are chronically exposed to MPTP, a progressive parkinsonian syndrome occurs. The low doses, which are required for the chronic regimen, do not bring about the acute pharmacologic effects, which commonly appear when repeated maximal doses of MPTP are administered.[13] In addition to behavioural signs, chronic administration of MPTP also produced biochemical and morphological alterations. The first consisted in a depletion of dopamine, DOPAC and HVA in the neostriatum. This depletion was reversible, since eight months after discontinuance of MPTP all the biochemical values were back to normal. Morphological abnormalities lasted longer than biochemical alterations. A swelling of dopaminergic neurons and a reduced nucleus–cytoplasm ratio were the first detectable morphological lesion; these occurred at a time when dopaminergic terminals located in the neostriatum were depleted. Eight months after discontinuance of MPTP, dopaminergic perikarya were still swollen but had a normal nucleus–cytoplasm ratio; in addition, a significant percentage of these neurons did not stain for TH.

As shown by Lams *et al.*,[15] the lack of staining for a metabolic marker does not necessarily imply that non-stained neurons are not viable. Since the derangement from a normal metabolic balance is reversible to a large extent, it may well be the case that a loss of TH staining in the midbrain of marmosets treated chronically with MPTP may be due to some biochemical alterations brought about by the uptake (and, possibly, build-up) of toxic compounds into such neurons.

In monkeys of group A treated chronically with MPTP the depletion of dopamine and of its metabolites occurred both in the caudate nucleus and in the putamen. This observation is in keeping with earlier observations showing that in idiopathic Parkinson's disease the putamen is more severely affected than the caudate nucleus, while in MPTP-treated monkeys either the caudate nucleus is more depleted than the putamen or the two anatomical components of the neostriatum are equally affected.[9,21] In our monkeys, the biochemical depletion was more marked in the caudate nucleus than in the putamen, but the difference was not statistically significant. The present data therefore show that an equal distribution of biochemical depletion is not a consequence of an acute regimen of MPTP administration as it also occurs after a chronic regimen. Thus, it appears that Parkinson's diseases and MPTP-induced Parkinsonism (either following an acute or a chronic exposure) differ with reference to the distribution of dopamine depletion in the neostriatum.

It is commonly accepted that parkinsonian signs develop in man when dopamine depletion reaches the critical threshold of 80% reduction in the neostriatum.[5] In group A monkeys, instead, parkinsonian signs developed while dopamine depletion in the neostriatum was about 50%. This sets another difference between MPTP-induced Parkinsonism and the idiopathic condition, which can be explained by the known inhibitory action of MPTP on tyrosine hydroxylase or monoamine oxidase.[11,19] This possibility is indirectly confirmed by the observation that in group A monkeys

dopamine reduction was not accompanied by a compensatory increase in dopamine turnover. The absence of such an important compensatory mechanism might explain the appearance of parkinsonian symptoms in MPTP-treated primates at more moderate degrees of DA loss, as compared to the human disease.

The present study is in keeping with earlier observations from this laboratory, reporting that a chronic exposure to MPTP is capable of producing a progressive parkinsonian picture.[3] The present data are also in keeping with observations from other laboratories.[20,23,26] Schneider[23] administered MPTP chronically to four macaques, in order to reach the minimal cumulative dose capable to induce an impairment in learning abilities without any detectable parkinsonian sign. The individual dose varied significantly with reference to the concentration of MPTP (from 2.12 to 5.80 mg/month) and to the time of exposure (from five to 13 months). It is remarkable to note that, under these experimental conditions, dopamine was depleted in the caudate nucleus by an average 95.8%, and in the putamen by 89.1%, and that a massive depletion of dopaminergic neurons occurred in the substantia nigra. It is difficult to compare this chronic regimen with ours, since the two were performed in different species of monkeys; furthermore, in our experiments, control cases were sham-treated and were housed in the same environmental setting as MPTP-treated monkeys. Taylor *et al.*[26] treated marmosets for four or six months, using approximately half of the weekly doses that we administered in this study. Their biochemical observations on dopamine, DOPAC and HVA are in full agreement with ours.

In a recent paper, Pérez-Otaño *et al.*[20] also observed that, at variance with subacute regimens, a chronic administration of MPTP brings about a severe depletion of serotonin in the neostriatum, the nucleus accumbens, and the frontal cortex.

The occurrence of a behavioural recovery after discontinuance of MPTP administration is in keeping with earlier observations based on acute or subacute administration of MPTP to monkeys.[8,28] Since recovery of parkinsonian signs is a common phenomenon, the causes of which are still unknown,[28] the occurrence of a certain degree of recovery after discontinuance of a chronic MPTP administration does not necessarily imply that in the latter case parkinsonian signs represent a transient pharmacologic effect. Evidence recently collected has shown that MPTP may produce a progressive retrograde lesion, which is capable to affect the terminals some time before influencing the perikaryon.[10] It has also been shown that, following a series of acute MPTP injections in monkeys, 50% of the nigral cell bodies were still visible after one month, at a time when striatal dopamine levels had fallen to less than 5% of the control value.[6,12] It is possible, however, that the chronic administration of MPTP performed in the present experiment did not produce a significant loss of dopaminergic terminals in the neostriatum. This is suggested by the essentially preserved cellularity in the substantia nigra compacta of group B monkeys and especially by the recovery of behaviour and of dopamine levels eight months after cessation of MPTP treatment. Recovery from MPTP-induced Parkinsonism appears to be species-specific for, in symptomatic rhesus monkeys, neither behaviour nor DA levels recovered for several months after the last MPTP dose.[21] To this respect, it should also be considered that, in our chronically treated marmosets, after few months of a rapid behavioural recovery, parkinsonian signs remained on average quite constant for the following three months (Fig. 1).

When our observations are compared with those of monkeys treated with an acute or subacute regimen, it is concluded that the basic parkinsonian syndrome is quite comparable, but that the severity of each individual sign differs according to the experimental conditions. First, in the chronic regimen motor disabilities, although gradually progressive, impaired motor behaviour without threatening vital functions (e.g. feeding and self-caring). Second, chronic MPTP treatment neither brought about acute toxic reactions nor was it associated with such self-damaging behaviours, which are commonly seen after repeated treatment with high doses of MPTP. As a consequence, higher cumulative doses of MPTP could be given in the chronic regimen. Such high cumulative doses were required in order to produce motor abnormalities comparable to those observed after acute MPTP administration. Third, in this series of experiments, we used a behavioural disability scale that has been previously validated by us on marmosets rendered parkinsonian by a subacute MPTP regimen. Interestingly, marmosets treated subacutely with MPTP, according to a standard protocol,[14] score abnormal on most items of this scale, and reach a global disability score that is customarily higher than 16. Marmosets receiving the chronic intoxication always scored less than 11, instead; in addition, they constantly received normal ratings on items of the scale which are constantly rated abnormal after a subacute regimen (e.g. balance or fur self-care). Taking all these considerations into account, it is considered that (at variance with the syndrome produced by acute or subacute administration of MPTP, and similarly to the human idiopathic condition) chronically treated monkeys suffered from a slowly progressive movement disorder, which was mainly restricted to the motor system.

CONCLUSIONS

The model of chronic Parkinsonism induced by MPTP may be relevant for understanding the supposed pathogenetic role played by environmental toxins in human idiopathic Parkinson's disease.[4,22] Indeed, if environmental pyridines really play a role

in the idiopathic human disease, they must produce pathological and biochemical alterations by the additive effect of low doses over time. This consideration is supported by the observation of Martin *et al.*,[18] who showed by PET scans that subjects exposed to MPTP have a reduced uptake of 6-fluorodopa in the striatum, compared to healthy individuals. The occurrence of cases of mild Parkinsonism, resulting from a discrete exposure to MPTP, has also been described by Tetrud *et al.*[27] The same authors also detected cognitive changes in individuals exposed to MPTP, who were not symptomatic and did not conform with the diagnostic criteria for Parkinson's disease.[25] In summary, it appears that, according to the dose administered, MPTP may induce in different primate species (including man) either the symptoms and signs of Parkinson's disease, or a subclinical Parkinsonism. The relationship between neurobiological changes and the behavioural signs of Parkinsonism require further investigations. The present data indicate that alterations occurring in the cell body and in the terminals have different time-courses.

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