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FURTHER TREATMENT WITH MPTP DOES NOT PRODUCE PARKINSONISM IN MARMOSETS SHOWING BEHAVIOURAL RECOVERY FROM MOTOR DEFICITS INDUCED BY AN EARLIER EXPOSURE TO THE TOXIN

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Summary—1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), or 0.9% saline, was administered to a group of common marmosets initially treated with the toxin 12–18 months previously. Initial treatment had consisted of a cumulative dose of 6–22 mg/kg (i.p.) which caused marked parkinsonism. Subsequently, the animals gradually recovered normal motor function. Further treatment consisted of a cumulative dose of MPTP of 78–83 mg/kg (i.p.) but this produced only modest akinesia.

At 12–18 months after the initial treatment with MPTP, the content of dopamine, HVA and DOPAC in the caudate and putamen was markedly reduced. However, levels of dopamine, HVA and DOPAC in the nucleus accumbens were normal. Three months after the second treatment with MPTP there was no further decrease in the content of dopamine in the caudate-putamen. However, in the nucleus accumbens the content of dopamine, HVA and DOPAC was now reduced.

The initial treatment with MPTP substantially decreased the binding of [^{3}H]mazindol in the caudate-putamen but less so in the nucleus accumbens. Only a small additional decrease occurred upon further treatment with MPTP. The density of tyrosine hydroxylase (TH) immunoreactive cells in substantia nigra was reduced after the initial treatment with MPTP. However, the cell loss was far less marked than the decrease in terminal density, assessed by the binding of [^{3}H]mazindol. Subsequent treatment with MPTP caused a small further loss of tyrosine hydroxylase-positive cells.

Initial treatment with MPTP may kill the majority of MPTP-sensitive dopamine cells in the nigra. Compensation by the remaining nigrostriatal neurones may account for the behavioural recovery observed. Alternatively, initial treatment with MPTP may strip surviving neurones of their terminals so that uptake of the active metabolite MPP^+ is reduced and its toxicity restricted.

Key words—MPTP, parkinsonism, common marmoset, recovery, tolerance.

Administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) causes parkinsonism in humans (Davis, Williams, Markey, Ebert, Caine, Reichert and Kopin, 1979; Langston, Ballard, Tetrud and Irwin, 1984a) and a variety of other primate species (Burns, Chiueh, Markey, Ebert, Jacobwitz and Kopin, 1983; Langston, Forno, Robert and Irwin, 1984b).

In experiments in young common marmosets (Jenner, Rupniak, Rose, Kelly, Kilpatrick, Lees and Marsden, 1984; Jenner, Marsden, Costall and Naylor, 1986), peripheral administration of MPTP (8–10 mg/kg i.p.) over 3–4 days, induced parkinsonian motor deficits, associated with a 60% destruction of the cells in the substantia nigra pars compacta. Cells in the ventral tegmental area were also damaged but to a lesser extent than in the substantia nigra. The content of dopamine (DA) in the caudate-putamen decreased to less than 10% of the normal value and there was a reduction in the uptake of [^{3}H] dopamine. In contrast, there was only a transient and reversible decrease in levels of dopamine in the nucleus accumbens. Over the months after treatment with MPTP there was a gradual reversal of the motor deficits exhibited by the animals. They no longer showed gross akinesia or rigidity and eventually their behaviour was similar to that of normal animals, with the exception of slowness of motion and a lack of coordination in executing complex movements. Recovery was associated with an increased turnover of dopamine in the caudate-putamen, as judged by the dopamine metabolite/dopamine ratio and a recovery of the content of dopamine in the nucleus accumbens. These two effects may contribute to the functional recovery observed (Jenner *et al.*, 1984; 1986).

In view of this recovery of motor function, it was decided to expose such animals to further treatment with MPTP. The effects of MPTP on the dopamine

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neurones in the nigrostriatal tract may be cumulative (Burns, Chiueh, Parisi, Markey and Kopin, 1986), hence, it was expected that the recovered marmosets would show a marked sensitivity to subsequent treatment with MPTP due to further damage to the remaining dopamine cells in the substantia nigra and ventral tegmental area. So, MPTP was administered to a group of common marmosets, which had been treated with MPTP, 12–18 months previously and which had almost completely recovered motor function. Contrary to expectations, sensitivity to MPTP toxicity in the second treatment was decreased.

A behavioural, biochemical and histological analysis of the effects of a second treatment with MPTP, compared to animals treated identically with MPTP on the first occasion only is now reported. A preliminary report of these findings was published previously (Ueki, Chong, Albanese, Nomoto, Rose, Gibb, Jenner and Marsden, 1988).

METHODS

Treatment with MPTP

Eleven common marmosets of both sexes (350–385 g, mean ± SD 338 ± 23 g; 2–3 years old) were used. Animals were divided into three groups. *Group A* comprised 3 animals, which were treated with MPTP and which recovered over the following 12 months, during which time no further treatment with MPTP was given. *Group B* comprised 4 animals treated with MPTP 12–18 months previously, and which were then further subjected to administration of MPTP. *Group C* was a control group of 4 animals not receiving treatment with MPTP.

In the initial treatment of groups A and B, MPTP base (Aldrich Chemicals U.K.) was used. The MPTP base, dissolved in a minimum volume of 70% ethanol and then diluted with sterile 0.9% saline, was injected intraperitoneally once a day in doses of 2.0–4.9 mg/kg. A cumulative dose of 6–22 mg/kg of MPTP was administered over a period of 3–7 days; this induced gross parkinsonism, to the extent that the animals sometimes required hand feeding and rehydration. At the time of the second treatment with MPTP, 12–18 months after the initial treatment, animals in group B had recovered fully and were almost indistinguishable from the controls. Subsequently, they were treated with individual doses of 2.0–5.3 mg/kg of MPTP to a cumulative dose of 78–83 mg/kg, over a period of 5 weeks. Initially MPTP base was employed but, due to its apparent lack of effect, MPTP hydrochloride (Research Biochemicals Inc., U.S.A.) dissolved in 0.9% saline, was substituted part-way through the second treatment to assess the effect of an alternative form of the drug. Nutrition and hydration of the animals were maintained as previously described.

Behavioural assessment

The behaviour of the animals was assessed immediately prior to the start of the second period of administration of MPTP, every day during administration of MPTP and then at intervals following cessation of treatment with MPTP. Motor impairment was rated using the following 0–5 scale: 0 = normal; 1 = minimal slowness and clumsiness; 2 = clear bradykinesia; 3 = postural instability on a perch; 4 = mobile only on the floor of the cage; 5 = total akinesia.

Preparation of the brain

Three months after stopping the administration of MPTP to animals of group B, the animals of all three groups were killed by decapitation under sodium pentobarbitone (50 mg/kg) anaesthesia. The brain was immediately removed and divided into the two hemispheres and the brainstem, for use in biochemical, autoradiographic and morphological examination, as described below.

Biochemical assay

The caudate nucleus, putamen and nucleus accumbens of one hemisphere were dissected, weighed and stored at −20°C pending biochemical analysis. Dopamine, 3,4-hydroxyphenyl acetic acid (DOPAC) and homovanillic acid (HVA) were assayed by reverse-phase high pressure liquid chromatography (HPLC) with an electrochemical detector, according to the technique of Rose, Nomoto, Kelly, Kilpatrick, Jenner and Marsden (1989).

Histological study

The brain stem was fixed by immersion in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4) for approximately 2 weeks, then transferred to 30% sucrose in 0.1 M phosphate buffer (pH 7.4) for 3 days, before being processed for morphological analysis. Coronal sections were cut at 30 μm intervals, by means of a freezing microtome. Alternate adjacent sections were stained by means of 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% (w/v) bovine serum albumin. Sections were treated for 30 min in phosphate buffered saline containing 0.2% (v/v) hydrogen peroxide (30 vol.) followed by repeated wash in phosphate buffered saline. Incubation in tyrosine hydroxylase antisera (Eugene Tech, New York) was overnight at room temperature. Sections were then washed in phosphate buffered saline, incubated with goat, anti-rabbit 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, the sections of tissue were then exposed to the chromogen solution (1,3-diaminobenzidine, 0.5%

w/v) in phosphate buffered saline, containing 0.02% (v/v) hydrogen peroxide (30 vol) as substrate for approximately 10 min. Sections through the midbrain were studied by means of an image analyser, blindly, by a single observer. In every tyrosine hydroxylase-stained section, cell counts were performed in the pars compacta. The relative density of tyrosine-hydroxylase-positive cell bodies was computed in sample areas, located in the medial, intermediate and lateral segments of this nucleus. Data obtained from the three experimental groups were compared individually, by means of pooled two-sample analysis.

Autoradiography

The visualization of sites for uptake of DA in the terminal area was determined by autoradiography, using [^{3}H]mazindol, as described by Javitch, Strittmatter and Snyder (1985b). One hemisphere was rapidly frozen in isopentane at -30°C and stored at -70°C. Coronal sections (20 μ) were cut at -20°C, thaw-mounted onto gelatin-coated slides, dried and stored at -20°C. For autoradiography, the slides were incubated with 4 nM [^{3}H]mazindol (15 Ci/mmol, Amersham International, U.K.) in 50 mM Tris buffer, containing 300 mM NaCl and 5 mM KCl (pH 7.9) for 40 min at 4°C. Desmethylimipramine 0.3 μM) was added to prevent binding to sites uptake for the noradrenaline. Non-specific binding was determined in adjacent sections by the addition of 10 μM of unlabelled mazindol. The sections were dried at room temperature and the slide-mounted tissue was apposed on tritium sensitive film (LKB, Sweden) for 21 days, along with a set of tritium containing standards (Amersham International, U.K.) and then developed with D-19 (Kodak, U.S.A.). The optical density of the autoradiography was quantified with a RAS-DG 1000 autoradiography image analysis system (Amersham, U.S.A.). A calibration curve was prepared for each film, from the tritium microscale autoradiograph (Amersham, U.K.) and non-specific binding was subtracted. Quantitative measurement of optical densities was performed in the lateral and medial region of caudate-putamen and the nucleus accumbens, from the autographs of coronal sections corresponding to levels of A 10.0 and 12.5 (atlas of Stephan, Baron and Schwerdtferger, 1980). Optical readings were made by positioning a joystick-controlled square cursor (0.24 mm^2) over each subregion. Four readings were made in each subregion and they were converted into fentomol of [^{3}H] mazindol bound per milligram of protein by referring to the calibration curves. Readings in the nucleus accumbens were obtained from a level of A 12.5. Readings in the caudate-putamen were combined from levels of both A 10.0 and 12.5 and then averaged in each subregion of the medial and lateral parts. Data obtained in three experimental groups were compared individually by means of pooled two-sample analysis.

RESULTS

Behavioural effects of treatment with MPTP

After the initial treatment of animals of groups A and B with a cumulative dose of 6–22 mg/kg of MPTP (mean $\pm$ SEM, 6.2 $\pm$ 2.4 mg/kg), all animals showed gross motor deficits within a 3–7 day period. The amount of MPTP administered daily was 2.4 $\pm$ 0.1 mg/kg (mean $\pm$ SEM) with a range of 2.0–4.9 mg/kg. In the initial treatment, each administration of MPTP caused an acute reaction, characterised by drowsiness, dystonia and seizure-related events, lasting for about 1 hr. Stable motor deficits (score 4–5) developed rapidly and lasted for 4–6 months, with the animals recovering mobility gradually over the following months. By 12–18 months and immediately prior to the second administration of MPTP to animals of group B, the motor activity of the animals (disability score 0–1) was almost indistinguishable from that of the control group.

The amount of MPTP administered daily in the second treatment was 3.8 $\pm$ 0.1 mg/kg (mean $\pm$ SEM) with a range of 2.0–5.3 mg/kg. The second administration of MPTP did not induce gross parkinsonism and the treatment induced only a minimal slowness of movement. Overall, these animals received a cumulative dose of 78–83 mg/kg MPTP (80.8 $\pm$ 1.1 mg/kg, mean $\pm$ SEM), given in 19–23 injections over a period of 5 weeks. The second administration of MPTP to animals in group B did not induce an obvious acute reaction at a dose of 2.0 mg/kg. When individual doses were increased to 4.0–5.3 mg/kg, a mild acute reaction occurred, lasting for approximately 15 min. Replacement of the MPTP base, with MPTP hydrochloride did not cause an acute reaction to treatment with MPTP. After the 5 week period of administration of MPTP to group B, the animals showed a disability score between 1–2.

Biochemical analysis

At 12–18 months after the initial treatment with MPTP (group A) the content of HVA and DOPAC of the caudate and putamen was markedly reduced, compared to control values (Table 1). The content of HVA and DOPAC in the nucleus accumbens of group A animals was almost normal (87%). There was a significant increase in the turnover of dopamine, as judged by the (DOPAC + HVA)/dopamine ratio in the caudate, with a similar trend in the putamen. Turnover of dopamine in the nucleus accumbens was not altered.

In animals receiving the second treatment with MPTP and studied 3 months later (group B) the content of dopamine, HVA and DOPAC in the caudate or putamen was not further reduced. However, the content of dopamine, HVA and DOPAC in the nucleus accumbens was now reduced, compared to controls. Again, there was a trend for increased

Table 1. The content of dopamine (DA), DOPAC and HVA of the caudate, putamen and nucleus accumbens after an initial administration of MPTP, 12–18 months previously (group A) and after a second treatment with MPTP (group B)

	DA	DOPAC ng/mg tissue	HVA	Ratio (DOPAC + HVA)/DA
Caudate				
Control	13.2 ± 0.7	4.5 ± 0.1	7.1 ± 0.6	0.89 ± 0.05
Group A	0.7 ± 0.2**	0.6 ± 0.1**	1.1 ± 0.4*	2.45 ± 0.40*
Group B	0.8 ± 0.3**	0.5 ± 0.3**	0.7 ± 0.4**	1.78 ± 0.66
Putamen				
Control	13.4 ± 2.2	5.9 ± 0.2	12.3 ± 1.5	1.52 ± 0.22
Group A	1.0 ± 0.6**	1.6 ± 0.6**	2.0 ± 1.0**	6.86 ± 3.78
Group B	0.6 ± 0.2**	1.8 ± 1.0*	1.2 ± 0.4**	5.31 ± 2.45
Nucleus accumbens				
Control	5.2 ± 0.2	4.6 ± 0.5	5.8 ± 0.5	2.02 ± 0.50
Group A	4.5 ± 1.5	3.8 ± 1.0	4.4 ± 1.2	1.89 ± 0.20
Group B	2.7 ± 0.5**	2.2 ± 0.6*	2.8 ± 1.0*	1.79 ± 0.49

The results are shown as the mean ± 1 SEM for groups of 3 or 4 animals. Marmosets were killed 3 months after the cessation of treatment with MPTP to group B animals.
**$P < 0.001$; *$P < 0.05$ compared to control; one way analysis of variance (ANOVA), followed by Dunnett's test.

turnover of dopamine in the caudate and putamen, but not in nucleus accumbens.

Morphological analysis

After the initial treatment with MPTP (group A animals), the number of tyrosine hydroxylase-staining cells in the substantia nigra was markedly decreased (Fig. 1). Depletion of cells, was more pronounced in the lateral part and the ventral portion of the intermediate part of the substantia nigra. Cells in the most medial part of the substantia nigra were constantly spared. The remaining cells had pathological changes, such as pale staining, shrinkage of the perikaria and peripheral displacement of the nuclei. Quantification of the number of cells revealed that the total tyrosine hydroxylase-staining cells in the substantia nigra decreased to 54% of the value for the control group and that the loss of cells was greater, moving from the medial to the lateral region of the substantia nigra (Table 2).

The effect of a second treatment with MPTP (group B) was apparent in the medial and intermediate areas of the substantia nigra, where a small further loss of tyrosine hydroxylase-positive cells was noted. In the lateral part of the substantia nigra no further drop in the number of tyrosine hydroxylase-positive cells was apparent. The total number of cells in the ventral tegmental area was almost normal, although slight pathological changes were noted in individual cells. The total number of tyrosine hydroxylase-positive cells in substantia nigra in group B was 43% of controls, which was significantly less than was found in group A.

Autoradiographic analysis

Treatment with MPTP caused a qualitative loss of the binding of [^{3}H] mazindol in the caudate-putamen, which was prominent in the medial and lateral part of the caudate-putamen, where a low density of specific binding remained in patchy clusters (Fig. 2). There was greater binding of [^{3}H]mazindol in the most medial part of the caudate-putamen and in the nucleus accumbens.

Quantification of the density of terminals showed that the specific binding of [^{3}H]mazindol was decreased markedly in MPTP-treated animals (Table 3). At 12–18 months after the initial treatment with MPTP (group A animals), there was a dramatic decrease in specific binding of [^{3}H]mazindol in the lateral caudate and putamen. There was also a substantial but less marked reduction in the binding of [^{3}H]mazindol in the medial caudate and putamen. There was a small decrease in the specific binding of [^{3}H]mazindol in the nucleus accumbens.

Table 2. Mean density of dopaminergic neurones in the substantia nigra pars compacta, of MPTP-treated marmosets (mean number of cells/mm^2 ± SEM)

	Medial	Intermediate	Lateral	Average
Controls	307.9 ± 15.2	233.3 ± 10.9	224.2 ± 18.4	271.7 ± 13.4
Group A	181.0 ± 6.2* (58.8%)	123.6 ± 4.1* (52.9%)	81.7 ± 5.1* (36.4%)	145.7 ± 5.3* (53.6%)
Group B	140.3 ± 5.4* (45.6%)	94.4 ± 3.7* (40.5%)	72.7 ± 4.1* (32.4%)	116.2 ± 3.0* (42.8%)

The density of dopaminergic neurones, in different portions of the pars compacta when divided in eight mediolateral levels, was determined. Ventral and dorsal cell groupings were considered together, since there was very little distance between the dorsal and ventral borders of the pars compacta. Statistical analysis was performed by considering three regions: a medial segment (including samples 1–3), an intermediate (samples 3–4) and a lateral one (samples 6–8). Statistical evaluations were performed by means of pooled two-sample Student's t-test. *$P < 0.001$ compared to controls; †$P < 0.001$ compared to Group A animals.

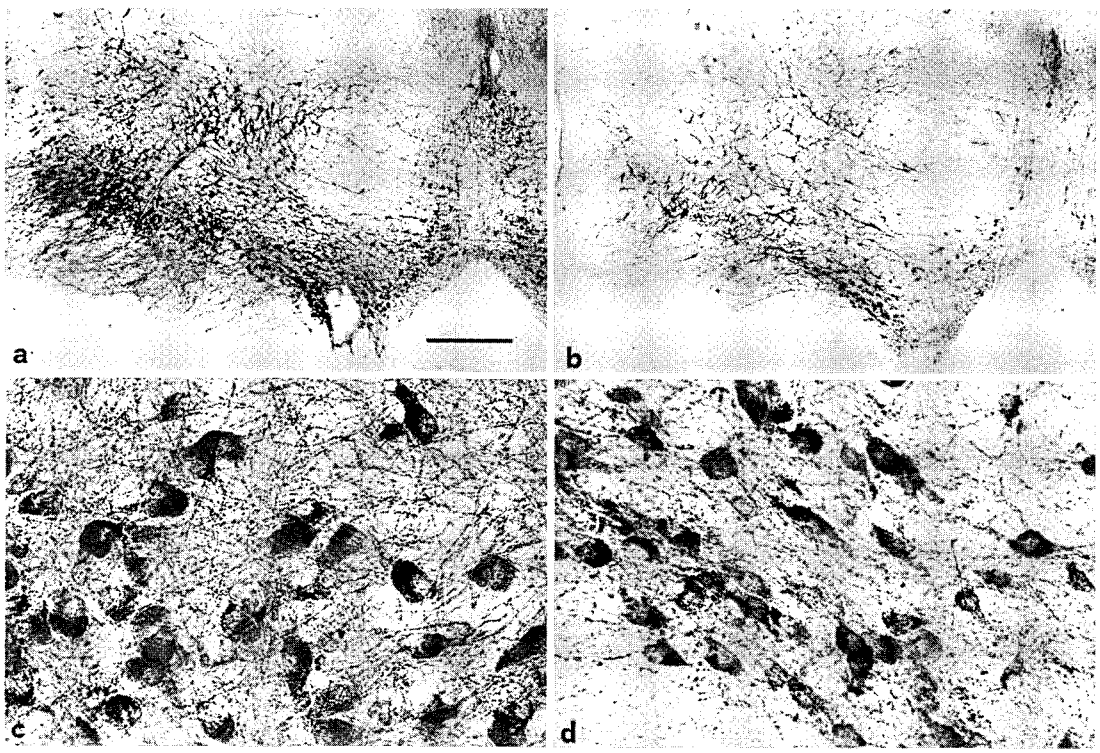


Fig. 1. Morphological appearance of dopaminergic neurones in coronal sections of the one control (a,c) and one MPTP-treated marmoset, belonging to group A (b,d). Low power magnifications (a,b) show the substantia nigra and the ventral tegmental area, at the level of interpeduncular nucleus. The intermediate part of the pars compacta is shown at higher magnification in c and d. It can be observed that, as compared to c, dopaminergic perikarya are shrunken and the neuropil is less represented in the MPTP-treated marmoset (d). Sections were stained, using tyrosine hydroxylase immunocytochemistry. Scale bar: 5 mm for a,b; 500 μm for c,d.

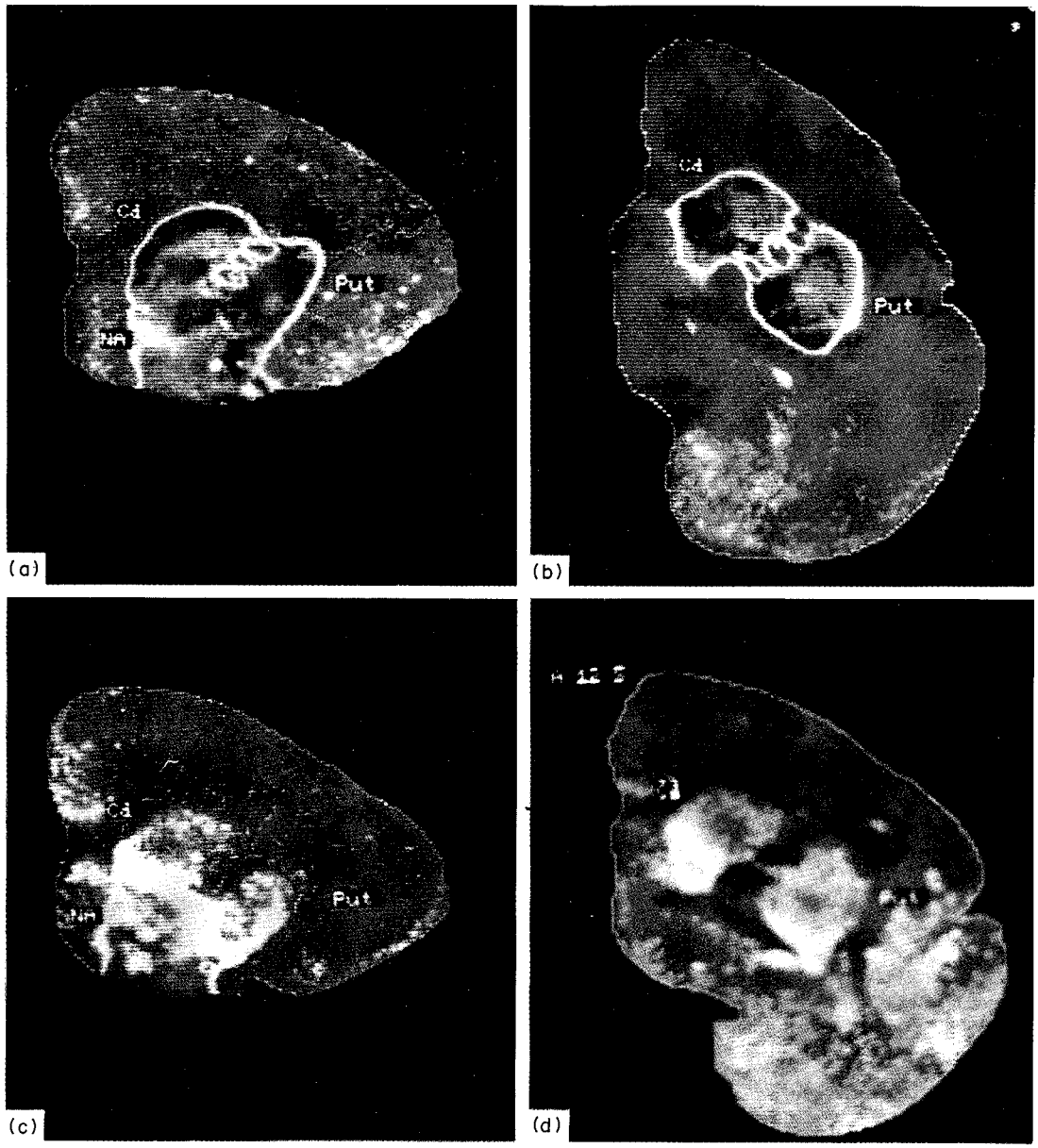
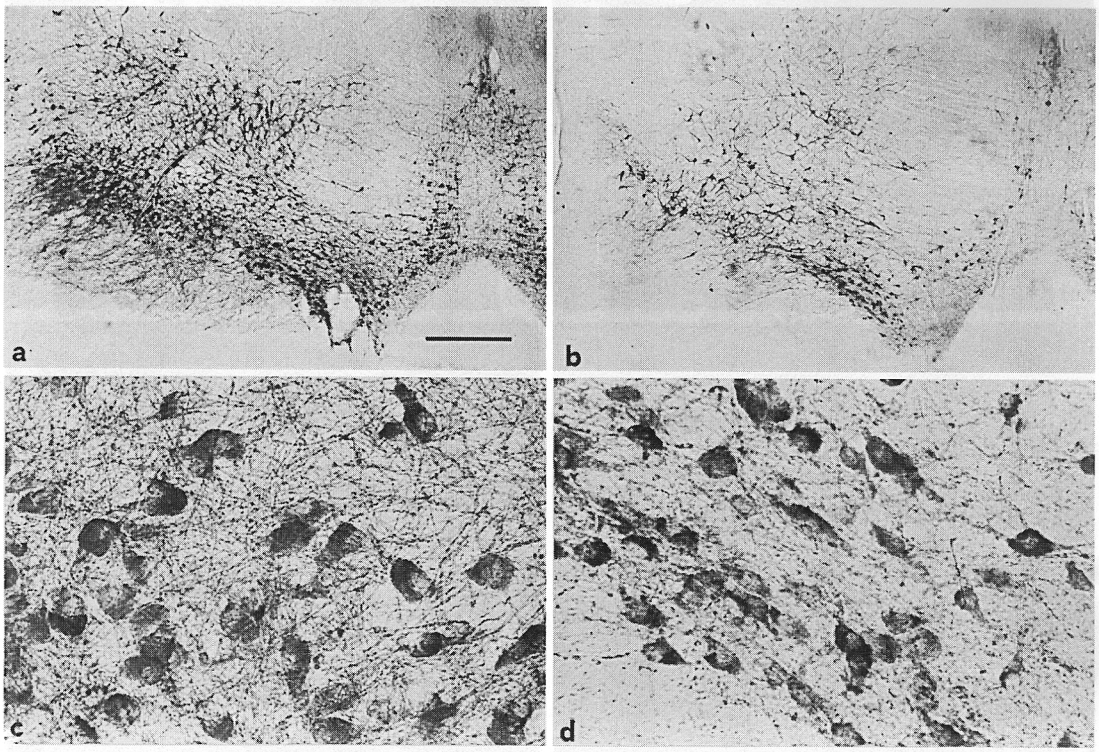


Fig. 2. Linearized autoradiographic images of specific binding of [^{3}H]mazindol (4 nM; defined using 10 μM unlabelled mazindol in the presence of 0.3 μM desmethylimipramine) to coronal slices of forebrain. The scale on the right indicates the amount of binding of [^{3}H]mazindol in colour. Green corresponds to binding of [^{3}H]mazindol less than 80 fmol/mg protein and red to more than 2400 fmol/mg protein. The left-(a & b) and right-(c & d) hand panels represent control and Group A MPTP-treated marmosets, respectively. The upper (a & c) and lower (b & d) panels were taken at the stereotaxic planes of A 12.5 and A 10.0 respectively. Note the decrease in the binding of [^{3}H]mazindol in the lateral caudate-putamen and greater preservation in the most medial part of the caudate-putamen and nucleus accumbens. Co-ordinates were taken from the atlas of Stephan *et al.* (1980). Cd = caudate; Put = putamen; NA = nucleus accumbens.



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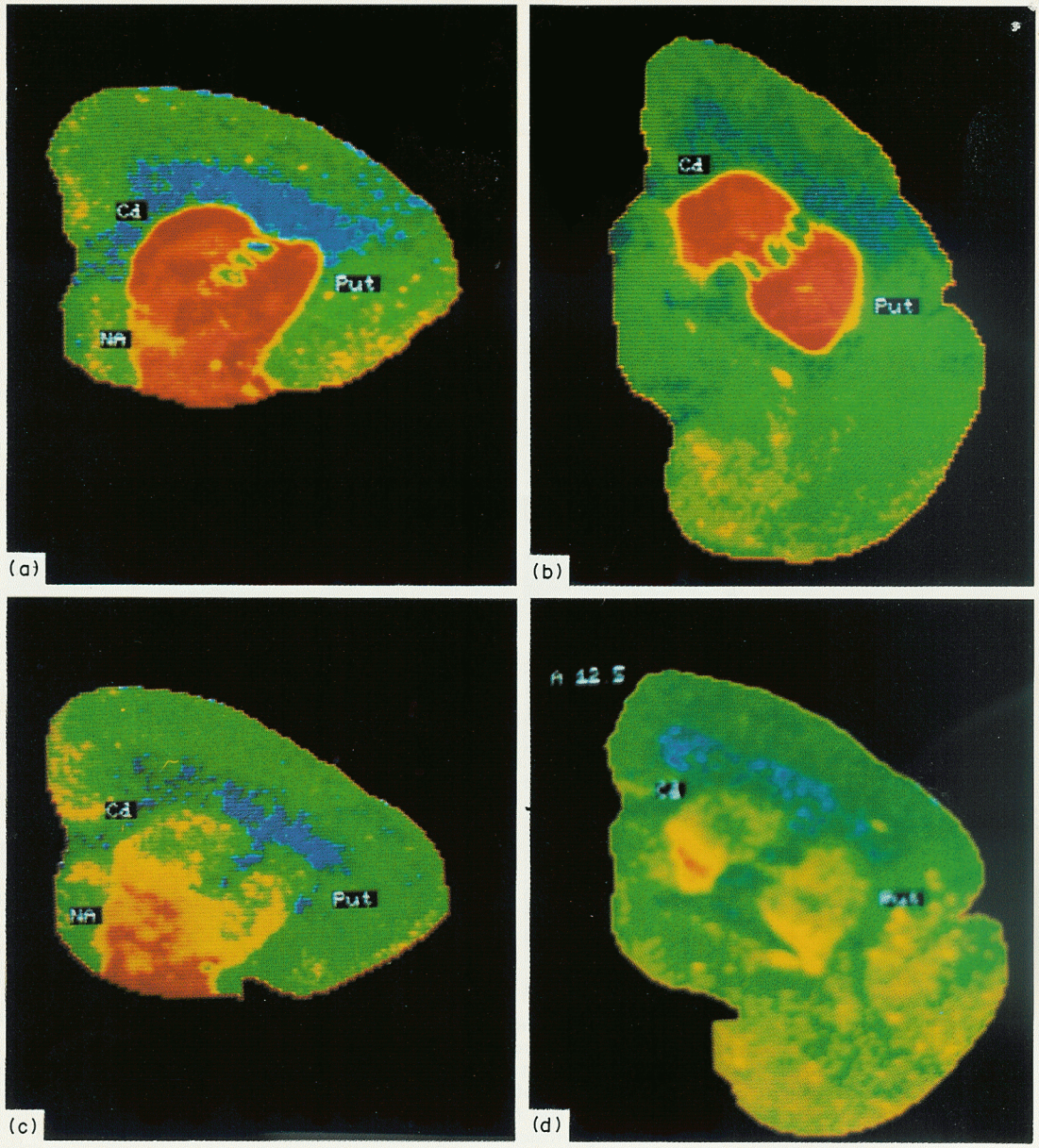

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Table 3. Specific binding of [^{3}H]mazindol to the caudate, putamen and nucleus accumbens after an initial treatment with MPTP, 12–18 months previously (group A) and after a second treatment with MPTP (group B)

	Control	Group A	Group B
	Binding of [^{3}H] mazindol fmol/mg protein		
Caudate			
Lateral	2708 ± 45	244 ± 23 (9.0%)[a]	190 ± 23 (7.0%)[a]
Medial	2830 ± 73	647 ± 66 (22.4%)[a,c]	674 ± 89 (23.8%)[a,c]
Putamen			
Lateral	2881 ± 50	265 ± 34 (9.2%)[a]	205 ± 25 (7.1%)[a,b]
Medial	2766 ± 91	554 ± 73 (20.0%)[a,c]	396 ± 79 (14.3%)[a,b,c]
Nucleus accumbens	2202 ± 74	1440 ± 57 (65.4%)[a]	1225 ± 131 (57.0%)[a]

The results (mean ± 1 SEM) (percentage of control values shown in parenthesis) were obtained from autoradiograms of [^{3}H] mazindol (4 nM; defined using 10 μM unlabelled mazindol, in the presence of 0.3 μM desmethylimipramine) using slices of brain from 3 or 4 animals in each group. The values for the nucleus accumbens were taken at the stereotaxic level of A 12.5 (atlas of Stephan *et al.*, 1980). Readings in the caudate-putamen were combined from levels of both A 10.0 and 12.5 and they were averaged in each medial and lateral subregion. [a] $P < 0.05$ compared to control group; [b] $P < 0.05$ compared to Group A; [c] $P < 0.05$ compared to lateral region; pooled two-sample analysis.

After a second treatment with MPTP (group B animals), the decreases in specific binding of [^{3}H]mazindol in the medial and lateral putamen were very similar to those found after the initial treatment (Table 3). There was only a small further decrease in the binding of [^{3}H]mazindol in the lateral and medial part of the putamen. There was no further decrease in the binding of [^{3}H]mazindol in the nucleus accumbens.

DISCUSSION

The administration of MPTP to common marmosets produces cell death in the nigra, resulting in parkinsonian motor deficits (Jenner *et al.*, 1984). However, the animals slowly recover almost normal motor function, without evidence for the regeneration of nigro-striatal dopamine neurones. A compensatory increase in the turnover of dopamine in the caudate-putamen by the remaining dopamine neurones, and a reversal of the transient decrease in the content of dopamine in the mesolimbic area, probably are responsible for the behavioural recovery (Jenner *et al.*, 1984; 1986). A reversal of MPTP-induced motor deficits has also been reported in the cynomolgous monkey (Eidelberg, Brooks, Morgan, Walden and Kokemoor, 1986).

In the present study, animals treated once with MPTP, between 12–18 months previously, showed only a modest motor impairment, despite having been grossly parkinsonian initially. This residual impairment consisted of a lack of spontaneous movement and poor coordination of activities, such as climbing and jumping. Despite the behavioural recovery, the content of dopamine in the caudate and putamen was approximately 5% of control values. The persistence of the neurotoxicity induced by MPTP was emphasised by the marked decrease in tyrosine hydroxylase-positive cells in the substantia nigra and the loss of specific binding of [^{3}H]mazindol in the caudate and putamen. As reported previously (Gibb, Lees, Jenner and Marsden, 1986; Waters, Hunt, Bond, Jenner and Marsden, 1986), there was a relative sparing of the dopamine neurones in the mesolimbic area in these animals, with only a modest reduction in the content of dopamine and extent of specific binding of [^{3}H]mazindol in the nucleus accumbens and little loss of tyrosine hydroxylase-positive cells in the ventral tegmental area.

The major finding in the present study was that common marmosets, treated with MPTP 12–18 months previously, did not revert to a parkinsonian state when treated over a period of weeks with MPTP on a subsequent occasion. Indeed, while in the initial treatment a cumulative dose of 6–22 mg/kg of MPTP caused gross parkinsonism, on the subsequent administration doses 3–12 times greater (78–83 mg/kg) were not able to precipitate obvious motor deficits (whether MPTP base or the hydrochloride was employed). These findings are at variance with the concept that the toxicity of MPTP is cumulative. Burns, Phillips, Chiueh and Paris (1986) showed that rhesus monkeys, treated with MPTP initially to cause a mild parkinsonian state, subsequently developed a persistent increase in motor deficits when receiving additional treatment with MPTP, some months later. So, the further administration of MPTP may worsen modest parkinsonism by destroying a larger proportion of susceptible neurones. However, in the present experiments on marmosets, the initial treatment with MPTP caused a prominent neurotoxic effect, associated with gross motor disability and, under these circumstances, subsequent treatment with MPTP appeared unable to produce sufficient further impairment of dopamine function to destabilize the compensated parkinsonian state.

Retreatment with MPTP had a limited effect on the remaining nigro-striatal dopamine neurones. There was a small further reduction in tyrosine hydroxylase-positive cells in the substantia nigra and the specific binding of [^{3}H]mazindol in the caudate-putamen. These changes were insufficient to produce

any measurable differences in the already small content of dopamine in the caudate-putamen. More marked effects were observed on the content of dopamine in the mesolimbic area and may be this was responsible for the onset of mild akinesia after the second treatment with MPTP.

The data provide some clues to an explanation for the lack of effect of a second administration of MPTP on the remaining nigro-striatal neurones. Two broad hypotheses can be considered. Either the neurones remaining after the initial exposure to MPTP were insensitive to this neurotoxin; or the initial exposure to MPTP did something which destroyed its ability to act as a neurotoxin subsequently.

Initial treatment with MPTP preferentially affected tyrosine hydroxylase-positive cells in the ventral and lateral portion of the substantia nigra, while the cells in the medial area were relatively spared (see also Forno, Langston, DeLanney, Irwin and Ricaurte, 1986). Similarly, the binding of [^{3}H]mazindol to the terminals of dopamine neurones was markedly reduced in the medial and dorso-lateral portion of the caudate-putamen, whereas the most medial area was less altered, as previously reported in the rhesus monkey (Chiueh, Burns, Markey, Jacobowitz and Kopin, 1985). Some nigro-striatal dopamine neurones appear sensitive to, and others resistant to the toxicity of MPTP, although why this is so is not understood. It is possible that the majority of dopamine neurones, sensitive to the toxicity of MPTP may have been damaged by the initial exposure, leaving few sensitive neurones for subsequent attack by MPTP. It may be these neurones, insensitive to MPTP, which compensate for the motor deficits induced by the initial treatment with MPTP treatment.

Alternatively, some change may occur in the ability of MPTP to produce neurotoxicity on a second administration. One possibility is that treatment with MPTP irreversibly inhibits the activity of monoamine oxidase B (MAO_B) so preventing the formation of the toxic metabolite 1-methyl-4-phenylpyridinium species (MPP^+). Indeed, the activity of MAO_B is decreased in mice treated with MPTP (Melamed, Youdim, Rosenthal, Spanier, Uzzan and Globus, 1985) and also irreversibly inhibited *in vitro* by MPP^+ itself (Salach, Singer, Castagnoli and Trevor, 1984; Singer, Salach and Crabtree, 1985; Kinemuchi, Arai and Toyoshima, 1985). Prior exposure to MPTP might change the activity of MAO_B so as to affect the concentration of MPP^+ achieved in the brain on subsequent treatment. However, the long interval between the initial treatment with MPTP and the subsequent administration makes this unlikely.

Another explanation for an alteration in the ability of MPTP to produce neurotoxicity has attraction. This is based on the disparity apparent between the extent of loss of tyrosine hydroxylase-positive cells, compared to the far greater decrease in the density of their terminals, measured by the binding of [^{3}H]mazindol. Terminal areas appear far more sensitive to the effects of MPTP than the cell bodies. This suggestion is supported by previous data by Bradbury, Costall, Jenner, Kelly, Marsden and Naylor (1986), who showed that focal injection of MPP^+ into the striatum of rodents, caused a greater disruption of nigro-striatal function than occurred on local injection of MPP^+ into substantia nigra. The substance MPP^+ is a substrate for mechanisms for the reuptake of dopamine (Javitch, D'Amato, Strittmatter and Snyder, 1985a; Chiba, Trevor and Castagnoli, 1985), which is partially responsible for its accumulation within dopamine neurones. This suggests that MPTP acts preferentially through dopamine neuronal terminals to cause an extensive axonopathy of terminals but less loss of cell bodies in the nigro-striatal system. Consequently, on a second administration of MPTP, the initial loss of nerve terminals might prevent a sufficient concentration of MPP^+, in the remaining dopamine neurones, to induce neurotoxicity.

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