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MPTP induced hemiparkinsonism in monkeys: behavioral, mechanographic, electromyographic and immunohistochemical studies

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Summary. A single infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydro-pyridine (MPTP) into the right internal carotid artery of *Macaca mulatta* monkeys resulted in akinesia and rigidity of the contralateral limb. The immunohistochemical study revealed a dramatic reduction in the number of TH-immunoreactive cells in the substantia nigra of the infused side (70–81%). After unilateral MPTP-treatment movement parameters and EMG activity were altered; the agonist muscle developed increased EMG activity associated with a shift of antagonist muscle activity. These results confirm that hemiparkinsonian monkeys are a valuable model of parkinsonism which can be useful in studies of movement disorder physiology and therapy of Parkinson's disease.

Key words: Hemiparkinsonism – MPTP – Dopaminergic neurons – Mechanographic electromyographic studies – Immunohistochemistry – Monkey

Introduction

It has been shown that monkeys systemically treated with MPTP are a valuable model of Parkinson's disease. This model has been used for the measurement of hypokinesia and the testing of the effects of L–DOPA therapies (Doudet et al. 1985; Doudet et al. 1986; Schultz et al. 1985). This quantitative assessment of hypokinesia revealed increased reaction time, delayed onset of muscle activity and prolonged movement time in a forelimb. These parameters of hypokinesia are reversed by L–Dopa treatment. The parkinsonian model has also been used to test transplants (Redmond et al. 1986; Freed et al. 1986) and new surgical treatments including destruction of the subthalamic nuclei (Bergman et al. 1990).

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In order to obtain a sufficient motor deficit, a large cumulative dose of MPTP must be employed (up to 5.4 mg/kg). Often the animals cannot survive these doses without dopamine treatment and constant care throughout the experiment.

To ensure the survival of the MPTP-treated animals, the use of unilateral lesions was suggested by several authors (Joyce et al. 1985; Schmidt et al. 1986; Bankiewicz et al. 1986; Dubach et al. 1988). The only quantitative observations reported to date deal with clinical observations or spontaneous circling activity which are a poor index of parkinsonism. Parkinsonian motor symptoms have never been quantified in this model.

In the present study, we first attempted to determine if unilateral lesions produced a hemiparkinson syndrome. It was important to compare our data with that of the well characterized parkinsonian model (Doudet et al. 1985; Doudet et al. 1986; Schultz et al. 1985) before undertaking further experiments using our hemiparkinsonian model. In addition, we compared the energy developed by the agonist and antagonist muscles in hemiparkinsonian monkeys with data from the same animals prior to injection.

Material and methods

Experiments were performed on two *Macaca mulatta* monkeys weighing 6.5–7 kg. The experimental protocol has already been described (Doudet et al. 1990). Briefly, animals were sat in a primate chair with one arm restrained in a manipulandum consisting of a vertically oriented handle which allowed movement from side to side. The manipulandum was at shoulder height so that only the biceps and triceps were used during the movement. The monkeys were trained to perform fast ballistic elbow movements (flexion, F. or extension, X.) of the left forearm in response to a randomized auditory signal (400 and 1000 Hz respectively). The mechanogram of elbow rotation was obtained from the output of a potentiometer coupled to the hinge of the manipulandum. Monkeys were rewarded with fruit juice when correct movements were performed within 1500 ms after the onset of the auditory signal and with a magnitude of angular displacement of 40 degrees or more. No starting or finishing reference points were imposed.

EMG activity was obtained from the biceps and the triceps brachii with intramuscular electrodes. The two electrodes were implanted 1 cm apart in each muscle, approximately at the same place, before each recording session. EMG activity was amplified, integrated with a time constant of 20 ms (Neurolog system NL 703), filtered and displayed on an oscilloscope.

Kinetic and EMG parameters were studied before and after single unilateral administration of MPTP. The elbow position and the EMG activity data were collected, stored and analyzed with a PDP 11/73 computer from 500 ms before to 1500 ms after the starting signal. Concerning the mechanogram, we determined four values. (1) The behavioral reaction time (RT in ms) i.e. the time elapsed from the beginning of the auditory signal to the onset of movement (OM). OM was determined by the computer from the calculated variance of the position of the forearm (handle position). (2) the movement amplitude (A in d°) and (3) the movement time (MT in ms) were both calculated manually from the mechanogram. (4) The maximum velocity of the movement (V_{max} in d°/s) was calculated by the computer from the velocity which was obtained by differentiation of the mechanogram. Concerning the EMG activity of the agonist and the antagonist muscles, we evaluated: (5) the premotor time (PMT in ms) i.e. the time between the onset of the auditory signal and the beginning of the modification of the EMG activity; (6) the electromechanical delay (EMD in ms) i.e. the time between the onset of the EMG activity and OM, negative values indicate that the EMG activity began after OM; (7) the duration (D in ms); (8) the peak amplitude (PA in mV) and (9) the surface area of the EMG activities (S in mV.ms). The onset of EMG activity in the agonist/antagonist muscles was evaluated manually for each movement. The computer programs provided automatic compilation of the different parameters. Mean values and standard deviations were calculated for each parameter from 100 flexions and 100 extensions recorded in each monkey for several sessions before the MPTP treatment. Then, the same procedure was repeated one week after the MPTP administration when the unilateral syndrome was stabilized. Student's t-test was used to compare the results obtained from the two monkeys both before and after the lesion. No significative difference was observed for any of the parameters recorded. The results obtained from both monkeys were then pooled and the same statistical test was used to compare the results obtained before and after MPTP-treatment.

Drug administration

The monkeys received a single unilateral infusion of MPTP as described by Bankiewicz et al. (1986). The animals were anesthetized with ketamine (Ketalar) (7 mg/kg i.m.) and the right internal carotid artery was exposed. A dose of 0.8 mg/kg was prepared by dissolving 6 mg of crystalline MPTP in 2 ml of ethanol and then diluted in 60 ml of saline. The solution was filtered through a 0.22 μm pore filter and infused into carotid artery at the rate of 1.5 ml/min. To avoid the administration of a lethal dose of MPTP we monitored the ipsilateral pupil and the infusion was stoped if mydriasis appeared.

Histology and immunohistochemistry

The monkeys were sacrified 5–7 months after MPTP administration. The animals were anesthetized with ketamine and perfused through the ascending aorta with 500 ml of heparinized physiological saline followed by 2 liters of 4% paraformaldehyde in a phosphate buffer (pH 7.4) used as fixative. The brain was rapidly removed and sliced into 4 mm frontal sections which were postfixed for 4 h at 4° C in the same fixative. The tissue slices were rinsed for 24 h at 4° C in 20% sucrose in Tris Buffered Saline (TBS) (pH 7.4), frozen in liquid N_2 and cut into 40 μm frontal sections with a cryostat.

Serum containing antibody to Tyrosine Hydroxylase (anti-TH) (Jacques Boy Institut) was diluted 1:2000 in TBS with 0.15% triton X–100 and 1.5% normal goat serum. The sections were incubated overnight in the diluted primary antiserum at 4° C. The sections were rinsed three times in TBS and incubated for 1 h at room temperature in goat antirabbit IgG serum diluted 1:100 in TBS. Sections were rinsed as above and incubated in rabbit peroxidase-anti-peroxidase complex (PAP) for 1 h. After several rinses, the sections were treated with 3,3'diaminobenzidine tetrahydrochloride (DAB) diluted in TBS and H_2O_2. Sections were observed by light microscope.

Pathological alterations in the SN of the lesioned side compared with the control side were analyzed on three anteroposterior parts using an imaging system (CCD camera, Hamamatsu) which was connected to a Macintosh II Cx computer equiped with an image program (image 1.31n, NIMH).

Results

1. Behavioral observations

Persistent motor abnormalities appeared 2 days after the MPTP infusion in both animals. The first sign observed was a turning towards the lesioned side during spontaneous activity. After day 4, a clear asymmetric motor impairment was observed. The contralateral limbs developed progressive akinesia and rigidity and remained in a flexed position. The animals sometimes exhibited postural tremor but parkinsonian tremor was never observed. No sign of hemineglect (during spontaneous activity) was observed but movements to obtain food were limited to the ipsilateral arm. Both monkeys survived without medication and there was no sign of spontaneous amelioration during the experiment period (5–7 months).

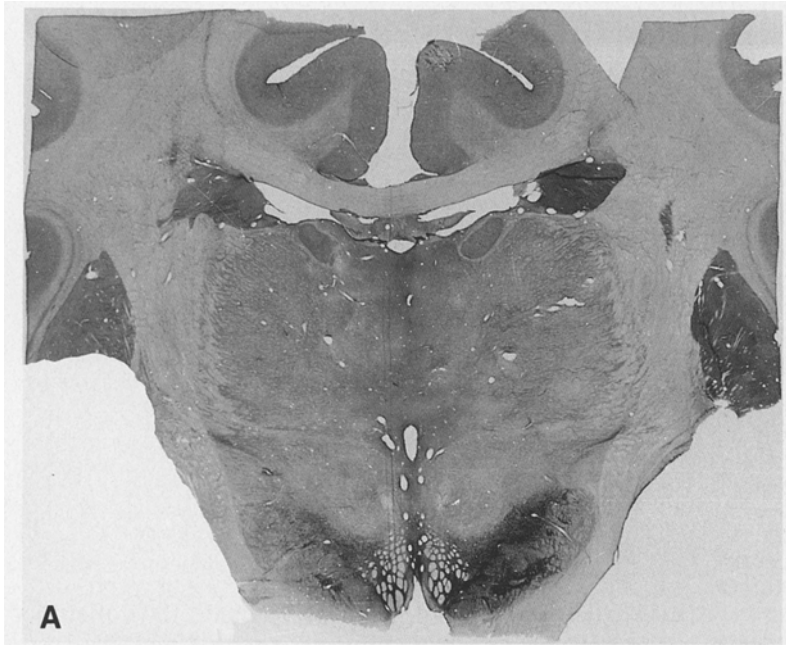
2. Immunohistochemical study

Figure 1 shows an example of the intermediate part of the SN and the body of the caudate nucleus (CD). Microscopic examination revealed that on the MPTP-infused side the density of TH-immunoreactive cell bodies in the SN was dramatically reduced compared with the control side (Fig. 1A, B), and there was a marked reduction in the number of TH-immunoreactive fibers which are normally present throughout the CD. Table 1 shows the results obtained from three anteroposterior parts of SN. Quantitative assessment of TH-immunoreactive cells revealed around 70–71% neuronal loss in the anterior part, 80–81% in the intermediate part and 78–81% in the posterior part of the SN of the lesioned side compared with the control side in both monkeys.

3. Kinetic and electromyographic parameters

In normal animals, inhibition of EMG activity in the antagonist muscle was only seen for extensions (Fig. 2).

The lesioned animals were unable to perform the same movements and executed flexion and extension slowly



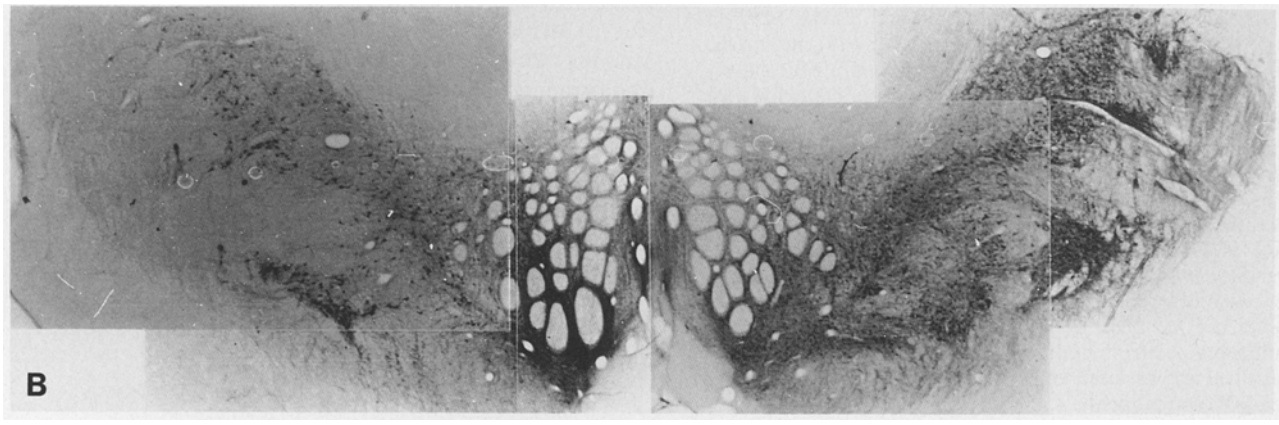


Fig. 1A, B. Tyrosine hydroxylase (*TH*) immunoreactivity showing the nigrostriatal dopaminergic neurons on the control side *(right)* and MPTP-infused side *(left)*. **A** Macroscopic photography showing both cell bodies in the substantia nigra (SN) and TH-immunoreactivity in the caudate nucleus (*CD*). **B** Photomicrograph showing that in the MPTP-infused side the density of TH-immunoreactive cell bodies in the SN was markedly reduced compared to the untreated side

Table 1. Mean values, standard deviation and percentage loss of TH-immunoreactive cells per slice of SN from control and lesioned sides. Data was analyzed on three anteroposterior parts
Mean values were calculated for five frontal sections from each part of the SN

	Monkey 1			Monkey 2		
	Control side	Lesioned side	% of lesion	Control side	Lesioned side	% of lesion
Anterior	657± 73	202±32	70%	721± 66	212±29	71%
Intermediate	1015±114	195±31	81%	1003±101	204±44	80%
Posterior	1225±181	264±42	78%	1304±173	251±41	81%

with small movement amplitude. Examples of mechanographic and electromyographic recordings are shown in Fig. 2 and mean values of kinetic and electromyographic parameters are presented in Table 1.

Movement parameters

The mean value of RT increased significantly for both F and X after MPTP-treatment compared with the normal situation (F: 212±29 ms vs 297±61 ms, $p<0.001$; X: 208±33 ms vs 273±84 ms, $p<0.001$). A significant decrease of V_{max} (F: 356±70 d°/s vs 196±54 d°/s, $p<0.001$; X: 411±85 d°/s vs 188±54 d°/s, $p<0.001$) and A (F: 65±10 d° vs 35±5 d°, $p<0.001$; X: 84±9 d° vs 40±5 d°, $p<0.001$) was found, whereas the mean value of MT did not differ before and after MPTP-treatment for F (299±39 ms vs 308±33 ms) but was significantly decreased for the X (350±24 ms vs 315±47 ms, $p<0.001$).

EMG activity

After MPTP-treatment we observed a disorganization of the EMG activity burst.

In the agonist muscle, PMT increased significantly for both F and X (F: 145±29 ms vs 209±57 ms, $p<0.001$; X: 155±31 ms vs 224±79 ms, $p<0.001$). EMD increased significantly for F (66±17 ms vs 87±24 ms,

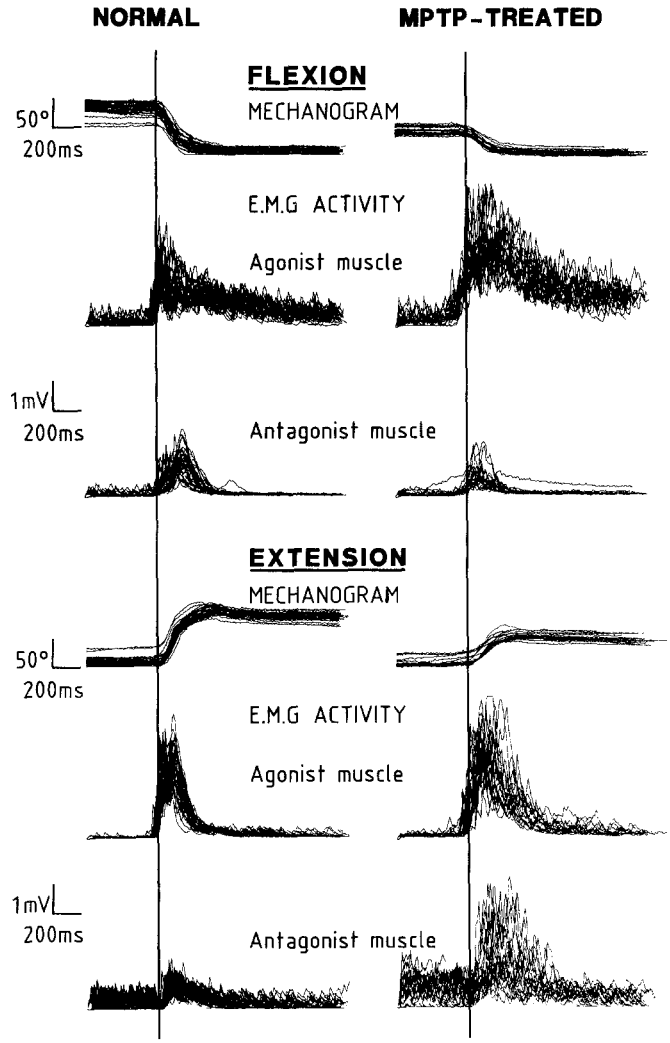


Fig. 2. Representation of the successive mechanogram and the associated EMG activity in the agonist/antagonist muscle for flexion and extension movements in monkeys before (normal) and after MPTP-treatment (MPTP-treated). The vertical bar represents the onset of movement

$p<0.001$) but did not differ for X (53 ± 14 ms vs 50 ± 22 ms). PA of the EMG bursts increased significantly for both F and X (F: 4.2 ± 0.9 mV vs 7.3 ± 1.6 mV, $p<0.001$; X: 6.4 ± 1.7 mV vs 7.1 ± 1.8 mV, $p<0.01$). D increased significantly for both F and X (F: 121 ± 32 ms vs 554 ± 109 ms, $p<0.001$; X: 190 ± 36 ms vs 340 ± 74 ms, $p<0.001$). S increased significantly for F and X (F: 40 ± 13 mV · ms vs 286 ± 91 mV · ms, $p<0.001$; X: 81 ± 25 mV · ms vs 136 ± 55 mV · ms, $p<0.001$).

In the antagonist muscle, PMT did not differ for F (273 ± 40 ms vs 274 ± 65 ms) but increased significantly for X (229 ± 38 ms vs 280 ± 82 ms, $p<0.001$). The EMG activity of the antagonist began after the onset of movement in normal animals for F and X (F: EMD = -61 ± 21 ms; X: EMD = -22 ± 14 ms). After MPTP-treatment, burst activity of the antagonist muscle began significantly earlier than in normal situation (F: EMD = 22 ± 6 ms, $p<0.001$; X: EMD = -6 ± 27 ms, $p<0.001$) and resulted in a coactivation. PA did not differ for F (2.1 ± 0.9 mV vs 2.4 ± 1.1 mV) but increased significantly for X (2.5 ± 0.8 mV vs 6.8 ± 3.1 mV, $p<0.001$). D decreased significantly for F (263 ± 51 ms vs 230 ± 46 ms, $p<0.004$) but increased significantly for X (190 ± 38 ms vs 364 ± 80 ms, $p<0.001$). S did not differ for F (30 ± 15 mV · ms vs 33 ± 15 mV · ms) but increased significantly for X (26 ± 11 mV · ms vs 146 ± 80 mV · ms, $p<0.001$).

Discussion

Behavioral and immunohistochemical observations

Hemiparkinsonian monkeys exhibited the typical akinesia with cogwheel rigidity of the contralateral side. The ipsilateral side was normal and allowed the animals to feed themselves. The monkeys survived without medication.

Our clinical results are in agreement with those previously described for hemiparkinsonian animals produced with low doses of MPTP (Joyce et al. 1985;

Table 2. Mean values and standard deviation of movement parameters: behavioral reaction time (RT), movement time (MT), amplitude (A), maximum velocity (V_{max}) and of EMG parameters of agonist and antagonist muscles: premotor time (PMT), electromechanical delay (EMD), duration (D), peak amplitude (PA), and surface area (S)

		Flexion movement		Extension movement	
		Normal	MPTP-treated	Normal	MPTP-treated
Movement parameters	RT (ms)	212 ±29	297 ± 61*	208 ±33	273 ±84*
	MT (ms)	299 ±39	308 ± 33	350 ±24	315 ±47*
	A (d°)	65 ±10	35 ± 5*	84 ± 9	40 ± 5*
	V_{max}(d°/s)	356 ±70	196 ± 54*	411 ±85	188 ±54*
Agonist muscle activity	PMT (ms)	145 ±29	209 ± 57*	155 ±31	224 ±79*
	EMD (ms)	66 ±17	87 ± 24*	53 ±14	50 ±22
	D (ms)	121 ±32	554 ±109*	190 ±36	340 ±74*
	PA (mV)	4.2± 0.9	7.3± 1.6*	6.4±1.7	7.1± 1.8*
	S (mV · ms)	40 ±13	286 ± 91*	81 ±25	136 ±55*
Antagonist muscle activity	PMT (ms)	273 ±40	274 ± 65	229 ±38	280 ±82*
	EMD (ms)	− 61 ±21	22 ± 6*	− 22 ±14	− 6 ±27*
	D (ms)	263 ±51	230 ± 46*	190 ±38	364 ±80*
	PA (mV)	2.1± 0.9	2.4± 1.1	2.5± 0.8	6.8± 3.1*
	S (mV · ms)	30 ±15	33 ± 15	26 ±11	146 ±80*

(*) indicates a significant difference ($p<0.01$) between values for each parameter before vs after MPTP-treatment for both flexion and extension movements

Bankiewicz et al. 1986; Dubach et al. 1988) with little or no toxic injury of the contralateral side. It seems that both the dose (0.8 mg/kg) and infusion rate (1.5 ml/min) are important parameters in avoiding contralateral poisoning. To ensure the survival of the animal, it is very important to stop the infusion as soon as ipsilateral mydriasis appears.

During spontaneous activity, the animals turned towards the treated side as described previously (Gross et al. 1983; Bankiewicz et al. 1986), but this characteristic is not symptomatic of Parkinson's disease and is not sufficient to validate the model.

Our immunohistochemical results are in agreement with those previously described in hemiparkinsonian animals (Bankiewicz et al. 1986; Joyce et al. 1985). A drastic depletion of TH-immunoreactive cells was apparent and homogeneously distributed throughout the anteroposterior extent of the SN. The high percentage loss of TH positive cells (70–81%) is certainly due to the relatively high dose of MPTP (0.8 mg/kg).

Movement parameters

All the parameters studied were significantly modified in the hemiparkinsonian model except for flexion movement time which was not lengthened. These modifications of movement parameters are in agreement with those obtained from both parkinsonian animals (Gross et al. 1983; Doudet et al. 1990; Schultz et al. 1985) and parkinsonian humans (Evarts et al. 1981; Hallet and Khoshbin 1980) performing large movements.

EMG activity

The two components of the reaction time, the premotor time and the electromechanical delay, which are the central and peripheral components respectively, are significantly increased for both F and X movements. These results are in agreement with those of Sheridan et al. (1987) obtained on parkinsonian subjects and with those of Doudet et al. (1985) which report a consistent increase in these parameters in the parkinsonian monkey.

The EMG peak amplitude of the agonist muscle for both F and X movements is increased while the movement amplitude and maximum velocity decrease in our hemiparkinsonian monkeys. This discrepancy is explained by the coactivation of the antagonist muscle in our model. For the flexion movement, there is a shift in the timing of the antagonist activity, and for the extension movement, there is a large increase in the amplitude of EMG activity of the antagonist. The differences, between biceps and triceps antagonist activity may be related to the results of Marsden et al. (1983) which show, in normal subjects, a decrease of the peak amplitude of the antagonist muscle related to an increase in movement amplitude. This decrease is due to the passive mechanical properties of the limb. It is clear that Parkinson's disease modifies the mechanical characteristics of the muscles which results in rigidity.

This paper confirms that a single intracarotidian injection of MPTP leads to a good hemiparkinsonian model which can be useful in studies of movement disorder physiology and therapy of Parkinson's disease.

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