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Modifications of precentral cortex discharge and EMG activity in monkeys with MPTP-induced lesions of DA nigral neurons

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Summary. 1. Individual neurons were recorded extracellularly in the precentral forelimb area of two monkeys trained to perform rapid, large amplitude flexion and extension movements of the contralateral forearm in response to auditory signals. Electromyographic (EMG) activity in the biceps/triceps muscles was recorded separately under the same conditions. The dopaminergic (DA) neurons of the substantia nigra (SN) were destroyed selectively by repeated series of intravenous injections of MPTP. The lesion was verified on serial slices using both tyrosine hydroxylase immunocytochemistry and classical staining methods. 2. In normal monkeys, the frequency of firing of precentral neurons shows rapid changes shortly before the onset of displacement. In our sample ($n=102$), most of the neurons (49%) tested during movement in both directions (flexion, extension) showed a reciprocal pattern of activity for the two directions of movement, a small percentage (19%) exhibited a change for only one direction (unidirectional neurons), and the remaining 32% displayed a similar change for both directions of movement (bidirectional neurons). 3. In MPTP-treated monkeys, movement-related modification of neuronal activity was more gradual, beginning earlier and lasting longer relative to the onset of movement. The cellular reaction time (the time between the auditory cue and a significant change in neuronal activity) was not significantly altered. Spontaneous firing of precentral neurons ($n=124$) did not increase significantly, and the dynamic discharge rate was unchanged after the nigral lesion. However, only 18% of cortical neurons still presented a reciprocal pattern of discharge for the two directions of movement, while the percentage of unidirectional neurons increased (50%), and the percentage of bidirectional neurons remained the same (32%). 4. After MPTP treatment, alterations in movement parameters and EMG activity were observed. Mean reaction time and movement duration increased by 20–25% and 25–30% respectively. The movements were slower and were associated with a generalized depression in the shape and the amplitude of EMG activity in the agonist muscle. 5. The neuronal basis for the observed central and peripheral disturbance in the MPTP-treated monkeys is discussed. We conclude that SN lesion leads to two main disturbances of cortical activity: i) the loss of the reciprocal pattern of response of movement-related cortical cells, and ii) an inability of the motor cortex to modify its activity in response to peripheral input.

Key words: Motor cortex activity – EMG – MPTP – Parkinson's disease – Immunocytochemical histology – Monkey

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Introduction

There is considerable pathological and experimental evidence for a role of the substantia nigra (SN) in motor function (Delong and Georgopoulos 1981; Féger 1981; Marsden 1982). At least one major motor disorder, Parkinson's disease, is associated with a loss of pigmented dopamine (DA)-containing neurons in the pars compacta of the SN (Flowers 1978; Selby 1975). The extent of the DA nigral lesions and the corresponding striatal DA deficiency have been correlated with the intensity of such Parkinsonian motor abnormalities as akinesia and rigidity (Hornykiewicz 1982). Although a role for the SN in both oculomotor and skeletomotor activity has long been recognized, its exact function remains poorly understood. Investigations in the awake primate using either neurochemical and electrolytic lesions (Gross et al. 1983; Viallet et al. 1983) or electrophysiological measurements of neuronal discharge (Delong et al. 1983; Hikosaka and Wurtz 1983; Schultz et al. 1983) are hard to carry out and interpret due to the

particular position of this nucleus in the brainstem circuitry and its involvement in both motor and limbic systems (Carpenter 1981). For instance, lesions of the pars compacta whose DA neurons are scattered throughout the nucleus, are usually not specific, and invariably include other portions of the brainstem, such as the ventral tegmental area of Tsai (VTA) and the SN pars reticulata. Lesions may also damage fibers in the neighboring medial forebrain bundle. Moreover, the interpretation of unit activity during a simple motor behavior is hindered by the different motivational and motor parameters involved (type of movement, amplitude required, proposed reward) in the different studies (Delong et al. 1983; Schultz et al. 1983).

In order to investigate further the role of the DA nigrostriatal pathway in aimed movement in the rhesus monkey, we analyzed both central and peripheral sites of motor activity before and after nigral lesions. We studied the movement-related unit activity of motor cortex neurons, and the kinematic and electromyographic components of a trained forearm displacement.

Systemic injection of the neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), is known to induce in humans and subhuman primates a behavioral extrapyramidal syndrome with Parkinsonian-like features including akinesia or bradykinesia and rigidity (Davis et al. 1979; Burns et al. 1983; Langston and Ballard 1984; Doudet et al. 1985). MPTP destroys DA nigral neurons in both species (Davis et al. 1978; Burns et al. 1983; Jacobowitz et al. 1984; Langston et al. 1984), although some doubt has been cast on the selectivity of this compound.

It seems clear that a number of factors (breed, age, administration route and schedule, dosage) affect selectivity (for review see Langston and Irwin 1986; Kopin and Markey 1988). The MPTP-induced motor deficiencies are also time-dependent. After an initial acute injury, although the nigro-striatal system remains depleted, there is some recovery in the other affected catecholaminergic systems (for instance, DA or noradrenergic systems in the VTA and Locus coeruleus (LC)). In the long-term recovered rhesus monkey, cell body damage appears to be confined to the DA nigral neurons (Jacobowitz et al. 1984). Motor cortical neurons, from which originate portions of the pyramidal tract, are a site of convergence for sensori-motor inputs from various structures: i) the basal ganglia and the neocerebellum via relays in the ventrolateral thalamus and premotor and supplementary motor areas, ii) other cortical areas involved in both initiation and execution processes (parietal association and prefrontal cortex, supplementary and premotor areas), and iii) sensory inputs via somatosensory cortex and thalamus (for reference see Asanuma 1981). Elimination of DA modulation from the SN may disrupt, via several pathways, the build-up of the pattern of movement-related responses in the motor cortex. It is known that the build-up of EMG activity and movement parameters is disrupted in both Parkinsonian patients and SN-lesioned monkeys performing motor tasks (Hallet and Khosbin 1980; Gross et al. 1983).

Methods

Subjects and behavioral task

Four young adult female rhesus monkeys (Macaca mulatta, 5–6 years old) were used in this study. Two non-treated animals were used for control histology, and the other two were treated with MPTP. Recording were performed through one hemisphere in one animal and through both hemispheres in the other. The animals were seated in a primate chair with the active arm abducted approximately 90 degrees at the shoulder. The forearm was restrained supinated in a manipulandum consisting of a vertically oriented handle which the monkeys moved from side to side.

Supination was imposed so that biceps and triceps brachii became prime flexors and extensors of the elbow. At the level of the elbow, the manipulandum was coupled to a potentiometer whose output indicated the position of the handle. Each animal was trained over a period of 4 to 6 weeks to perform fast ballistic extension (X) or flexion (F) movements of the forearm in response to the relevant auditory cue (1000 and 400 Hz respectively). This was achieved by delivering a liquid reward when the animal performed the correct movement within 1500 msec after the onset of the auditory signal and with a magnitude of angular displacement of at least 40 degrees. No starting or finishing reference points were imposed. Sessions were recorded when there was stability in both task reaction time and movement duration. Recordings were performed every week-day, at the same time each day, and with experimental sessions of the same duration. The animals received 100–300 ml reward during each recording session.

Chamber placement and drug administration

The surgery was performed after completion of training. The animal was then pretreated with Atropine (0.04 mg/kg i.m.) and anesthetized with Ketamine hydrochloride (10 mg/kg i.m.) followed by the minimal dose of i.v. barbiturate (Nembutal) required to induce a deep state of anesthesia. Barbiturate was given throughout surgery when required. A cylindrical plastic chamber was implanted over the arm area of the motor cortex contralateral to the trained limb, using the technique of Lamarre et al. (1978b). The position of the craniotomy was such that both central and arcuate sulci were visible through the exposed dura. The cortical zones projecting to the forelimb muscles and elbow joint were identified in the anesthetized monkey by intracortical microstimulation using the technique developed by Asanuma and Rosen (1972). Movement or muscle twitches must be evoked by stimulation of the precentral motor cortex (MI) (area 4) by intensities below 60 μA (Weinrich and Wise 1982). Following surgery, Gentamicin sulfate (5 mg/kg i.m.) was given once a day for 5 days. Throughout the experiment, the dura was kept intact, and antibiotics were applied daily to the exposed surface.

Recordings from MI neurons were performed as described elsewhere (Gross et al. 1983). Unit activity was recorded using conventional techniques with tungsten microelectrodes insulated with epoxylite, having an impedance of about 1 MΩ at 1000 Hz.

After collection of control data from MI neurons in the normal monkey, the animal was treated with MPTP (crystalline MPTP: Aldrich 97%). The MPTP solution was prepared according to Burns et al. (1983), and injected intravenously as previously described (Doudet et al. 1985). Briefly, the two animals received MPTP in 3 or 4 series of 4 or 5 daily injections (0.35 mg/kg) according to their behavioral response to the drug. The cumulative doses were: 5.25 mg/kg (1.75+1.75+1.75) for one animal and 5.4 mg/kg (1.4+1.4+1.4+1.2) for the other. Recordings were resumed 4–5 weeks after the last administration of MPTP, i.e. when the monkeys had recovered from the acute and sub-acute effects of the drug and were in a relatively steady state of motor impairment. Recordings from cortical neurons in the treated monkeys were performed

over a period of 2 to 3 months in the same cortical subareas as in the intact animal.

Data acquisition and analysis

During the recording sessions, the animal's head was kept immobile by fixing the upper-part of the chamber to the platform of the chair where the microdrive (La Précision Cinématographique) was mounted. A program developed on a PDP 11/34 computer (Digital Equipment Corporation) controlled the task, and collected and analyzed the data. The position of the handle was obtained by A/D conversion of the output voltage of a linear potentiometer.

In addition, electromyographic (EMG) activity from the agonist/antagonist muscle pair was recorded in similar experimental sessions. Intramuscular electrodes made of Teflon-insulated silver wires (Clark Electromedical Instrument AG5T) were implanted in the biceps and the triceps brachii. EMG activity was recorded differentially, filtered (100 Hz–3 KHz), monitored on an oscilloscope, and integrated with a time constant of 20 ms (Neurolog System NL 703) before data acquisition.

The position of handle (forearm), the muscular (EMG) and neuronal activity were collected, stored and analyzed from 500 ms before to 1500 ms after the beginning of the auditory signal (OS). Statistical analysis and graphic representation of the data were carried out off-line. The parameters studied were those schematized in Fig. 1: the duration of the movement (MD, in msec) and the maximal velocity (V, in d/s) were determined by differentiation of the mechanogram; the maximal amplitude of the movement was also calculated from the mechanogram; the behavioral reaction time (BRT, in ms) was the time elapsed from OS to onset of movement (OM); OM was determined by the computer from the calculated variance of the position of the forearm (handle position); the premotor time (PMT in ms) was the time elapsed from OS to the beginning of the EMG activity in the agonist muscle; the electromechanical delay (EMD, in ms) represented the duration of the EMG activity in the agonist muscle before OM; the cellular reaction time (CRT, in ms) was the time from the auditory cue to the change in neuronal activity (Evarts 1974); the duration of changes in the neuron discharge in area 4 (MI) preceeding (TA, in ms) and following (TB, in ms) OM were also analyzed. The spontaneous discharge rate of motor cortex neurons (spikes/s) was obtained during 500 ms of data acquisition before OS and "the dynamic" frequency (spikes/s) was calculated over the whole duration of the increased discharge of cortical neurons. The onset of EMG activity in the agonist muscle was evaluated manually, trial by trial, in both normal and lesioned situations, and the estimated onset time was input to the computer. The computer programs provided automatic computation of the different parameters for each trial as well as the mean and standard deviation for the group of trials recorded for each cell, and the averages for EMG, neuronal and logic signals. Mean values were calculated for each parameter for both F and X in normal and MPTP-treated monkeys. Student's *t*-test was used to compare the results. In addition, three classes of movement-related neurons were distinguished according to their activity during the movement: i) neurons showing a reciprocal discharge pattern for F and X (RO neurons); ii) neurons whose activity changed for only one direction: unidirectional neurons (UD neurons) and iii) neurons whose activity decreased or increased for both directions of movement: bidirectional neurons (BD neurons).

Histology

After completion of the experiments, the monkeys were deeply anesthetized with pentobarbital and perfused through the heart

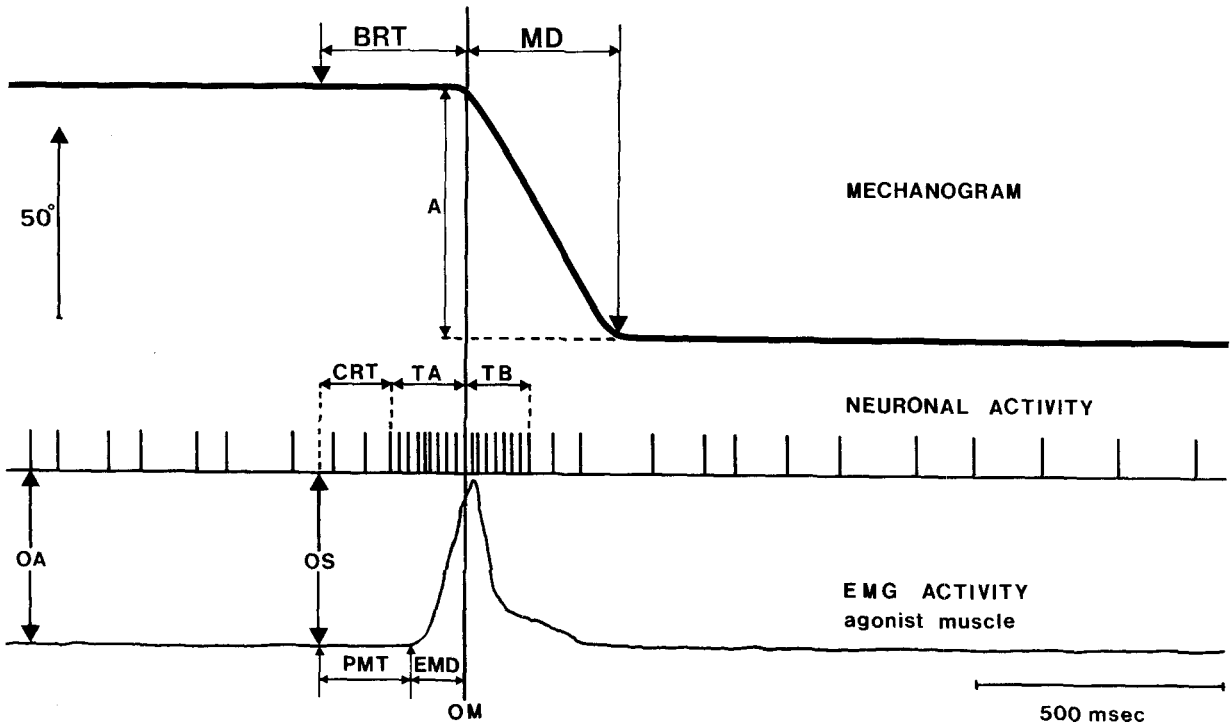

Fig. 1. Schematic representation of the parameters studied. Data acquisition (OA) began 500 ms before the onset of the auditory signal (OS) and continued for 1500 ms. *Movement parameters* (*upper diagram*): behavioral reaction time (BRT in ms); movement duration (MD in ms); movement amplitude (A in d°); *Neuronal activity parameters* (*middle diagram*): cellular reaction time (CRT in ms); duration (ms) of burst discharge preceding (T_A) and following (T_B) the onset of movement (OM) represented by the vertical line. *EMG activity parameters* (*lower diagram*): premotor time (PMT in ms) and electromechanical delay (EMD in ms)

with artificial cerebrospinal fluid. The center of the recording chamber was marked on the cortical surface, and the brain was then dissected out.

Large blocks of tissue corresponding to the brainstem (SN and adjacent areas) and cortical sensori-motor regions were taken for histological analysis. The remaining portions of the neostriata were assayed for homovanillic acid (HVA) by high performance liquid chromatography. Since the number of subjects was too small (2 normals and 2 MPTP-treated) for statistical analysis, the data are given only as an indication of the extent of DA depletion. The right SN and sensori-motor cortical areas were sliced at 10 μm and processed histologically using Kluver and Barrera's technique (1953).

Immunocytochemistry

The left SN of the two monkeys were examined using Thyrosine hydroxylase (TH) immunocytochemistry. For controls, two drug-free rhesus monkeys of similar age were sacrificed under the same conditions, and the neostriata and SN areas processed using identical techniques.

Pathological alterations in the SN of the lesioned animals compared to controls were evaluated in frontal sections. The results were combined in composite diagrams based on the atlas of François et al. (1985).

Results

Immunocytochemical and histological studies

The results are summarized in Fig. 2. In control monkeys, throughout the anterior-posterior extent of the SN and adjacent areas, 5 groups of neurons were distinguished and designated A, B, C, D and E according to their location and the shape of their somata. Schematic sections of anterior, intermediate and posterior portions of the SN with the respective location of the 5 groups are shown in Fig. 2. Group A corresponded to the DA A10 group medial to the SN. Its multipolar neurons, oval or round in shape, were found among the fibers of the oculomotor nerve (III) in the VTA of the mesencephalon. Groups B, C and D represented the large complex of intensely immunoreactive neurons found in the dorsal pars compacta and intermingled with pars reticulata neurons: the DA group A9. In normal animals, all these neurons gave rise to numerous immunoreactive processes, and the entire SN background was carpeted with these dendritic processes (Fig. 3). Group B consisted of bipolar neurons mainly oriented obliquely in a dorso-lateral to ventro-medial direction. The cell bodies were located dorsally in the anterior two-thirds of the SN. Group C was composed of multipolar neurons invading ventrally the pars reticulata as deep oblique indentations, and found throughout the entire nucleus. Group D neurons presented the same appearance as those of group C, but were located caudally in the extreme ventro-lateral portion of the SN pars reticulata.

The posterior group E corresponded to the DA retrorubral A8 group. Most A8 cells were found caudally, dorsal and lateral to the SN, above and within fibers of the medial lemniscus. They were similar in appearance

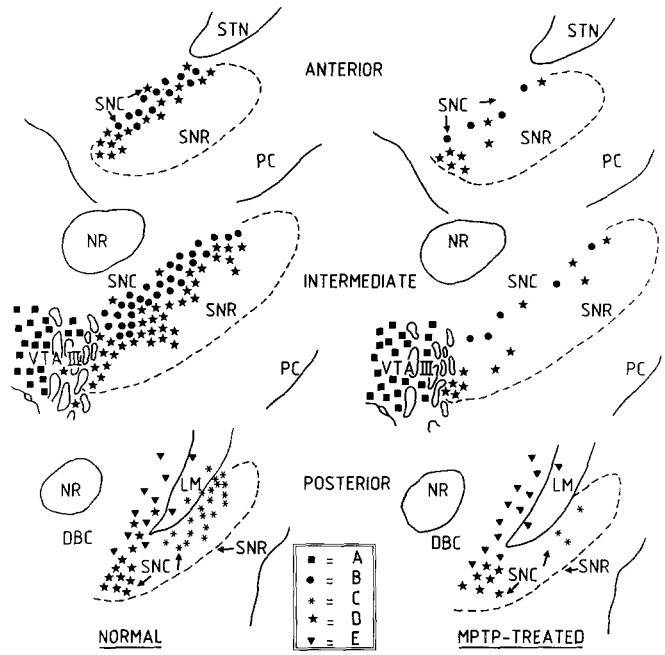

Fig. 2. Schematic sections at the anterior, intermediate and posterior level of the SN and respective locations and density of the 5 groups of tyrosine hydroxylase-immunoreactive neurons in both normal and MPTP-treated animals (average of both animals in each group). Group A (■) neurons were located in the ventral tegmental area (VTA) among and medial to the fibers of the oculomotor nerve (III); Group B (●) neurons in the dorsal bank of the SN pars compacta (SNC); Group C (*) neurons invaded ventrally the pars reticulata (SNR); Group D (★) neurons were located in the most posterior and ventral portion of the SN: Group E (▼) neurons represented the A8 group, dorsal and medial to the lemniscus medialis (LM). DBC: decussatio brachio conjunctivum; NR: nucleus ruber; PC: pedunculus cerebri: STN: subthalamic nucleus

to group C and D neurons, albeit slightly smaller. The cells were less abundant, and were more scattered than those in the SN. No staining was observed in any of these areas when the TH antiserum was omitted from the incubation medium.

Similar data were obtained from the two MPTP-treated monkeys. A dramatic decrease in the density of TH immunoreactivity was clearly apparent, but it was not homogeneously distributed throughout the dorsal/ventral and medial/lateral extent of the SN and neighboring areas. The density of immuno-reactive neurons within the VTA remained unaffected (Fig. 3), and the size and staining of the neurons was unchanged. In contrast, at all levels of the SN, the great majority of B and C neurons had disappeared. However, some neurons were spared in the more medial portion of the SN. The ventral neurons intermingled with the pars reticulata neurons appeared more affected than the dorsal neurons. In addition, the ventro-lateral TH-containing D neurons had almost completely degenerated. This decreasing gradient of immunoreactivity from medial to lateral was due to death of TH-containing cell bodies rather than a loss of TH-immunoreactivity since a similar gradient of depopulation was observed with classical

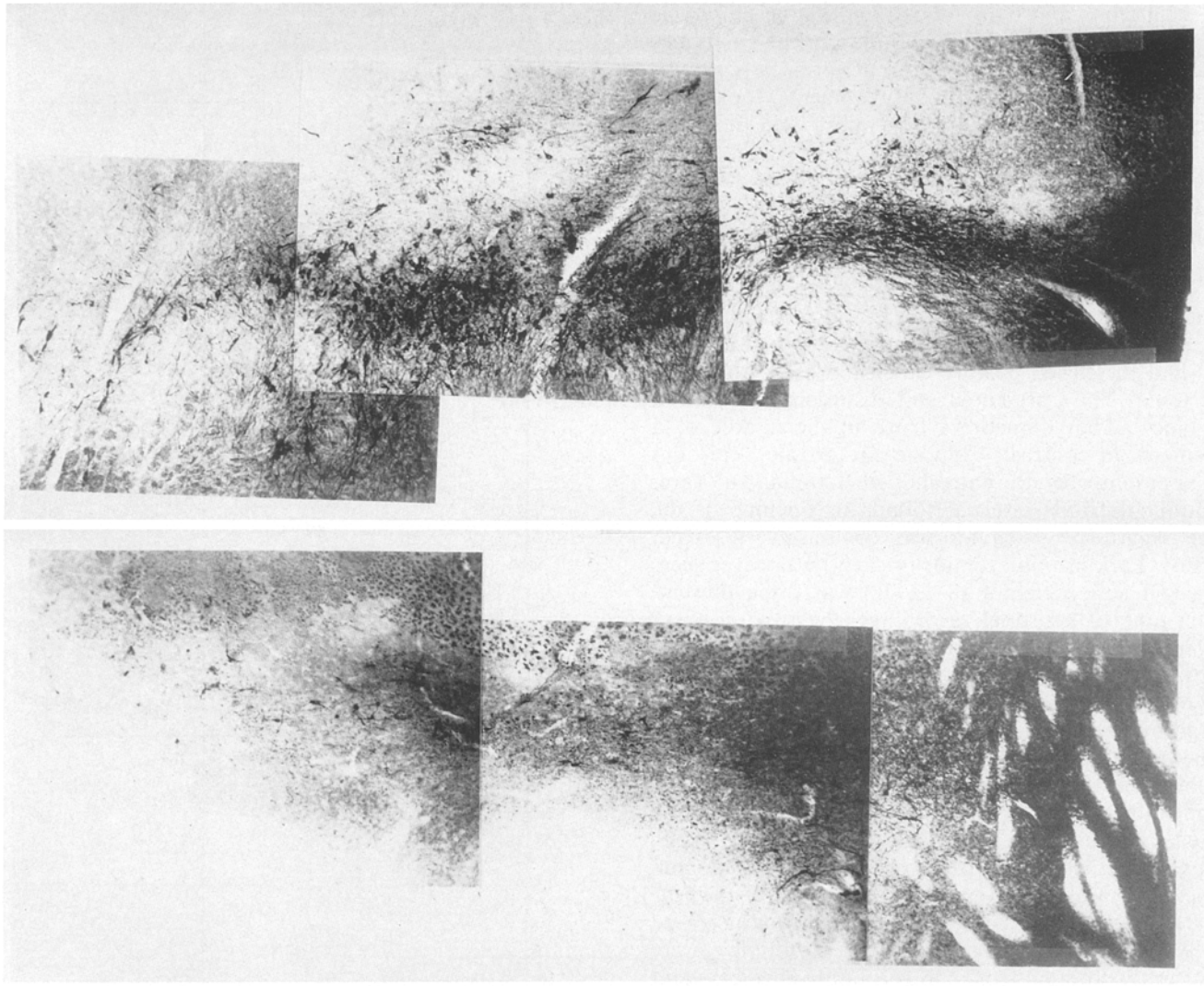

Fig. 3. Comparison of substantia nigra pars compacta dopaminergic neurons as shown by tyrosine hydroxylase immunocytochemistry (×80) in control and MPTP treated animal. *Top*: SN of intact animal; *bottom*: SN of MPTP-treated monkey. Note the almost inexistent staining in the lesioned animal

staining techniques. Compared to the morphology of A9 neurons in normal monkeys, the surviving cells in the MPTP-treated animals appeared smaller. They also seemed to have fewer dendrites and a smaller dendritic field. Furthermore, despite the variability of staining from one section to another depending on the exact incubation conditions, it seemed that the TH-immunoreactivity was stronger in the lesioned than in the normal monkeys. Counts from classical stains revealed around 70–85% neuronal loss in the lateral half (85–95% in the so-called D group) and 45–55% loss in the medial half of the SN of MPTP-treated animals. Despite the small number of neurons in the retrorubral E group, it was possible to detect a slight decrease in the number of stained cells in the lesioned animals. The cell loss was evaluated at about 5%, mainly restricted to the most lateral part of A8.

The HVA content was found to be decreased in the striatum of the two MPTP-treated animals (52% and 60% reduction). This fall represents an average for the whole striatum including the dorso-lateral as well as the more ventro-medial (nucleus accumbens) structures. Under our experimental conditions, the gradient of depletion throughout the neostriatum, reflecting the pattern of DA nigral cell loss, reported by Jacobowitz et al. (1984), was not observed.

Behavioral observations

The behavior of the animals during MPTP administration (acute phase: 10 to 20 min) and the following few days (sub-acute phase) has been described in a previous publication (Doudet et al. 1985). During the sub-acute phase (ranging from day 2 to day 10 following MPTP treatment), the animals needed constant care. They were given fluids intravenously, force-fed via a bucco-gastric tubing or hand-fed at least twice a day (crushed monkey

food with fruits and baby foods), and temperature was maintained around 35–37° C. No antiparkinsonian medication such as L-DOPA or bromocriptine was administered. Rapid recovery (daily improvements) took place during the two to three following weeks, and the monkeys fed spontaneously.

Recordings were resumed one or two weeks later, when no gross changes were observed in behavior. The chronic phase of motor deficits was reached 4 to 5 weeks after the last administration of MPTP. At that time, the animals exhibited an extrapyramidal syndrome. Spontaneous movements were rare and of small amplitude, sometimes showing the "cogwheel" phenomenon. They had the flexed posture classically associated with hypertonia. They presented gait disorders and moved "en bloc". They sometimes froze in the middle of a movement. In contrast, "kinesie paradoxale" episodes were apparent when the animals were "stimulated" (presentation of fruits, unusual sounds or opening of the door). Aggressive behavior was commonly observed. The slow Parkinsonian tremor (4–5 Hz) was never seen, but a fast action tremor (8–12 Hz) was often present. At the time of neuronal recordings, the two monkeys were in a mildly deficient stage. Throughout the whole duration of the experiment, the hypertonia and rigidity were more prominent than the akinesia/bradykinesia.

Although the motor disabilities appeared clearly when the animals were free in their cage, the slowing of movement was not so clear-cut during the execution of the trained task. During the recording sessions, the trained movement seemed to be initiated more easily, and executed faster and with larger amplitude compared to the self-initiated movements. The values of the MT and BRT, which we expected to be markedly increased in view of the spontaneous behavior of the animals, were significantly different from controls but to a lesser extent than expected. Cogwheel rigidity and freezing episodes were seldom observed during the recordings. These discrepancies will be considered in the discussion.

Neuronal activity

Control animals. 102 precentral neurons were recorded. Their neuronal discharge pattern during both F and X movements was closely related to the elbow-displacement. In agreement with other studies, an abrupt change in the firing rate occurred before the onset of displacement and stopped rapidly afterwards (Fig. 4) (Tanji and Evarts 1976; Fetz et al. 1980; Evarts 1981; Lamarre et al. 1981). The distribution of TA and TB is shown in Fig. 5. The mean value of the spontaneous discharge was low: 10 ± 6 spikes/s. The dynamic firing frequency was 32 ± 11 spikes/s. The percentages of RO, BD and UD movement-related neurons are shown in Fig. 6. Approximately half (49%) of the precentral cells recorded were directionally reciprocal, 32% were bidirectional and only 19% exhibited a change in their activity for only one direction of movement; for both UD and BD neurons, a majority of cells (75%) presented an excitation. The three populations were intermingled throughout the recording area, and could be found during the same penetration within a few hundred microns of each other.

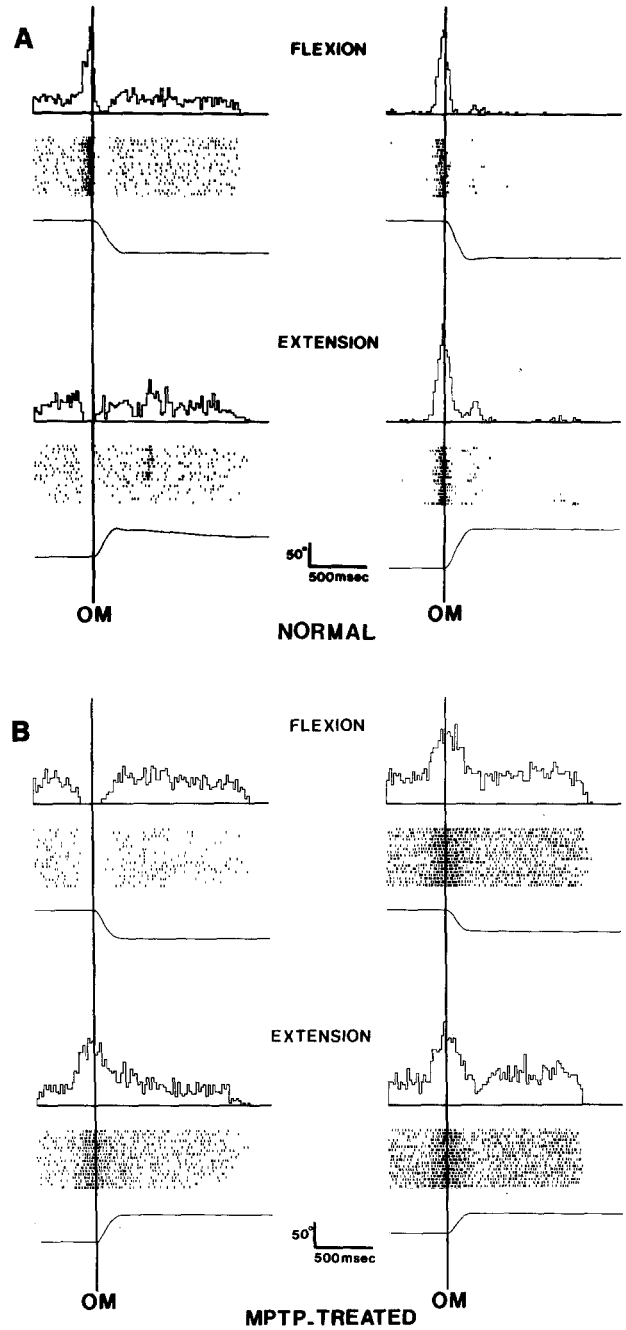

Fig. 4A, B. Changes in the activity of a reciprocally organized neuron (*left*) and a bidirectional neuron (*right*) for flexion and extension movements in a normal monkey (**A** $n=30$) and in a MPTP-treated animal (**B** $n=30$). For each part of the figure: *top*: histogram (bin width: 20 ms); *middle*: raster display; *bottom*: mean mechanogram. The vertical bars indicate the onset of movement (OM)

MPTP-treated animals. 124 movement-related neurons were recorded during both F and X movements. The

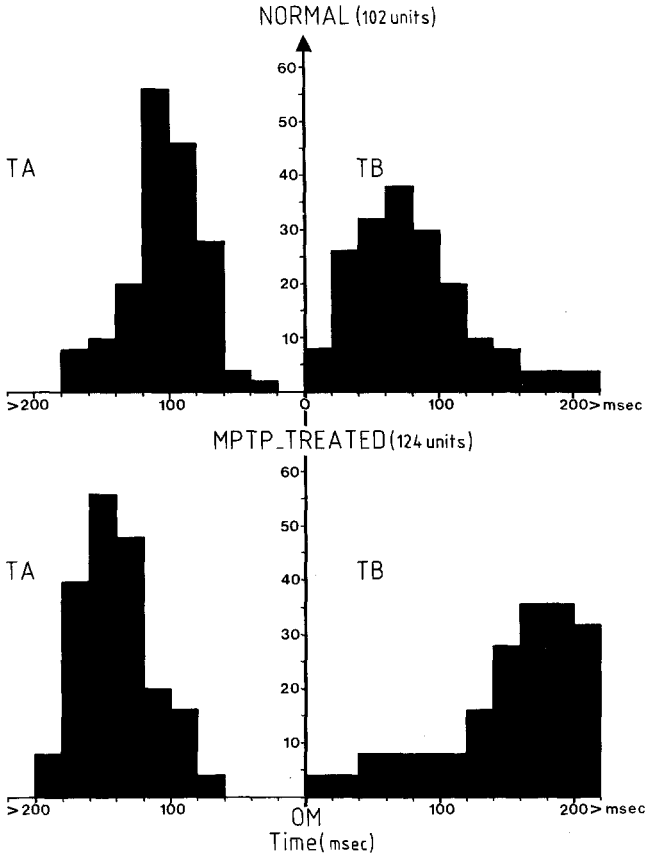

Fig. 5. Distribution of TA (*left*) and TB (*right*) of precentral units recorded in two monkeys, before (*upper part*) and after MPTP-treatment (*lower part*). The abcissa shows the time of neuronal changes (intervals 20 ms) before and after (OM). The ordinate represents the number of neuronal changes for F and X combined (see text). Both TA and TB were significantly different in MPTP-treated animals versus normal ones (X^2, $p<0.01$)

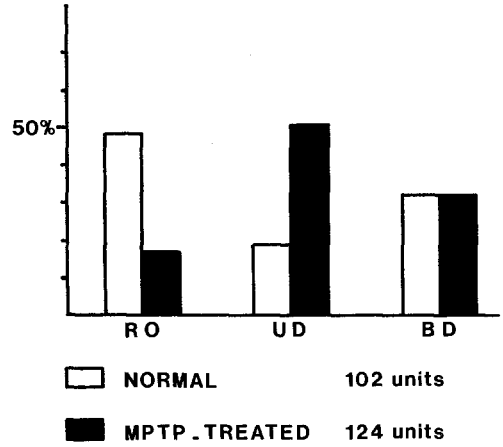

Fig. 6. Percentage of movement-related neurons with a reciprocal organization (RO), undirectional (UD), and bidirectional (BD) changes in activity during movement in normal and MPTP-treated monkeys. (Significantly different for RO and UD neurons, $p<0.05$, see text)

activity of these cells is represented in Fig. 4. The firing rate changed earlier before the onset of displacement, increased more gradually, but lasted much longer than in normal animals, resulting in an increased total duration of the altered precentral activity (TA+TB) (Fig. 5). Statistical analysis of the data (X^2) showed that TA was only slightly affected in the MPTP-treated animals, whereas there was a dramatic change in TB. There were no significant differences in the CRT in the lesioned versus the normal monkeys. The cortical data are summarized in Table 1.

There was no significant increase in the spontaneous frequency of the neurons (13 ± 7 in the lesioned versus 10 ± 6 spikes/s in the normals). The burst discharge rate was unchanged (32 ± 10 spikes/s). It is to be noted that in MPTP-treated animals the percentage of RO neurons decreased (18%), the percentage of UD increased (50%) and the percentage of BD neurons was unchanged (32%). Most of the UD and BD neurons (70%) still presented an excitation. This means that one of the actions of MPTP was to reduce inhibition from reciprocal cells; this can explain muscular co-contraction.

Table 1. Student's *t*-test (*) indicates a significant difference ($p<0.01$) between the respective values of behavioral reaction time (BRT), movement time (MT), amplitude (A), velocity (V), electromechanical delay (EMD), TA and TB in normal animal vs MPTP-treated animals for both flexion and extension. There is no significant difference ($p>0.1$) between the values of premotor time (PMT) and cellular reaction time (CRT) in normal and lesioned animal for flexion and extension. ↗ ↘: percentage of increase or decrease of mean values on MPTP-treated monkeys vs normal animals

	Flexion movement			Extension movement		
	Normal	MPTP-treated	%	Normal	MPTP-treated	%
BRT (ms)	215±46	270±37*	↗26	219±39	283±38*	↗29
MT (ms)	170±35	211±40*	↗24	209±41	255±43	↗22
A (d°)	65±10	55±7*	↘15	63±11	52±6	↘17
V (d°/s)	558±72	420±80*	↘25	458±98	351±66	↘23
EMD (ms)	64±6	83±15*	↗30	69±9	85±9*	↗23
PMT (ms)	151±73	187±84	NS	150±72	198±88	NS
(ms)	117±48	140±40	NS	123±48	144±46	NS
TA (ms)	98±25	130±23*	↗33	96±27	139±30*	↗42
TB (ms)	69±41	145±62*	↗110	76±49	149±60*	↗96

Movement parameters and EMG activity

In normal animals, recording sessions began when the animals reached a stable daily percentage of successfully rewarded trials (89–95%; mean: 93%). The percentage of daily successful trials remained high in the MPTP-treated animals (86–93%; mean: 90%).

The pattern of muscular activity in normal animals is illustrated in Fig. 7. The resting EMG activity level was low, and agonist/antagonist muscles were reciprocally active and silent during extension and flexion movements. They usually exhibited the characteristic triphasic pattern of activity (agonist-antagonist-agonist) reported in human subjects in similar situations (for references see Hallet and Khosbin (1980)). Characteristically, during fast movements, the agonist muscle generated a short, large amplitude burst of activity of relatively fixed length, beginning before OM and usually followed by a subsequent firing of the antagonist and agonist muscles (see superimposed traces in Fig. 7). The EMG activity returned to the baseline level promptly after execution of the movement.

As summarized in Fig. 7 and Table 1, the primary effect of the MPTP-induced nigral lesions on motor behavior was a slowing of the contralateral arm movement, (both behavioral reaction time and movement duration were increased) associated with the expected fall in the velocity and amplitude of the movement. As illustrated

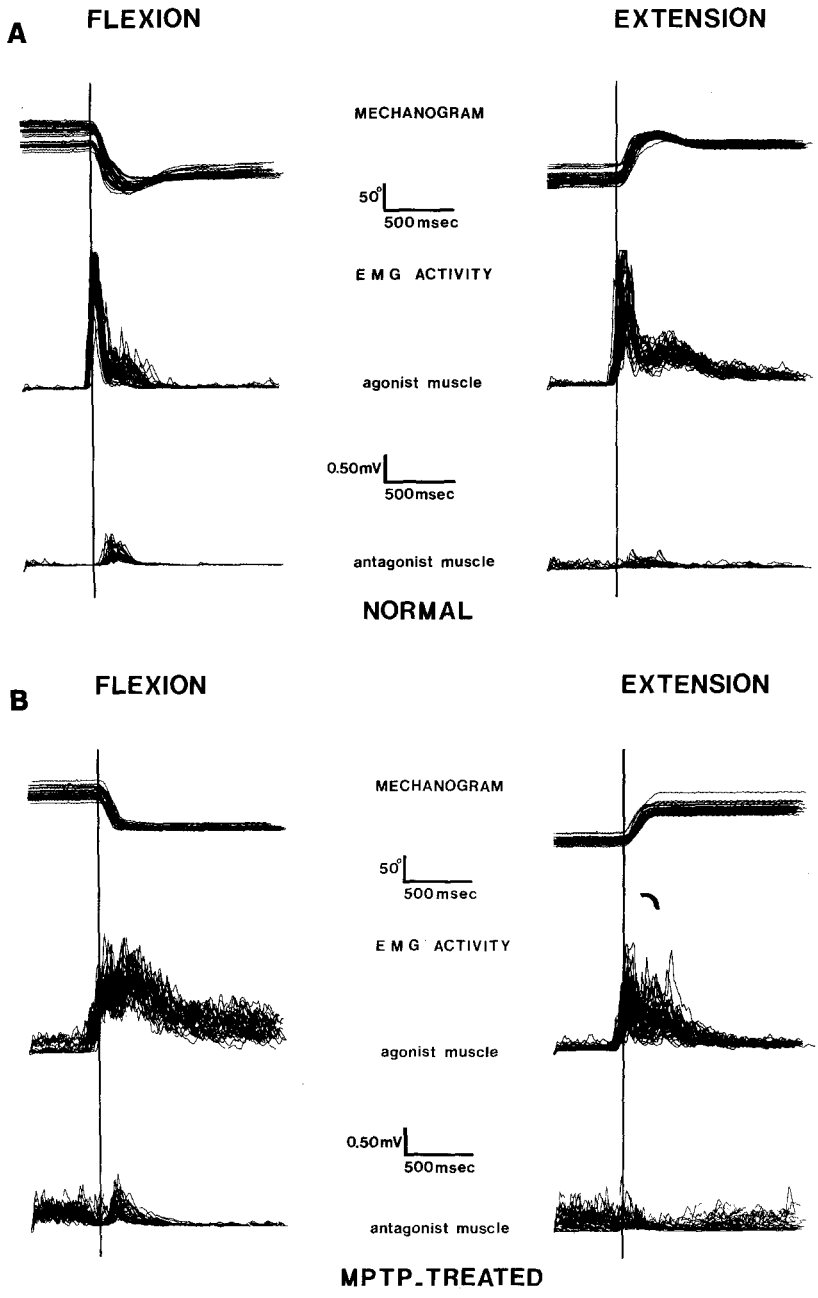

Fig. 7A, B. Successive mechanograms ($n=30$) and associated EMG activity in the agonist/antagonist muscles (biceps/triceps) in normal (**A**) and MPTP-treated monkey (**B**)

in Fig. 7, the background level of EMG activity was slightly increased, and the rate of development and peak amplitude of the first agonist burst of EMG activity were depressed. It appeared as if the EMG activity in the first burst discharge reached a ceiling. The inadequate initial burst was then followed by additonal successive bursts of lower amplitude and duration to achieve the full amplitude of the required movement.

Discussion

Behavioral and histological alterations

From the behavioral point of view, the MPTP-treated monkeys presented the classical Parkinsonian symptoms of rigidity and hypokinesia. However, this behavior, with lack of resting tremor, was more reminiscent of striato-nigral degeneration (Adams et al. 1961) or juvenile Parkinson's disease (Narabayashi 1987). In addition, the kinematic (increase in MD and BRT, decrease in movement velocity and amplitude) and electromyographic data (depression and disorganization of the EMG bursts of activity) are in good agreement with the findings reported for Parkinsonian patients (Evarts et al. 1981; Flowers 1978; Hallet and Khosbin 1980) as well as for monkeys with unilateral stereotaxic lesions of the SN (Gross et al. 1983; Viallet et al. 1983). These results give further support to the attribution of a primary role of nigral degeneration with corresponding striatal DA deficiency in the rigidity and hypo/bradykinesia observed in this disease. The data on MPTP-treated subhuman primates collected over the last few years seem to indicate that a restricted lesion of the DA nigrostriatal system cannot by itself generate Parkinsonian tremor, at least in the rhesus monkey. This is consistent with studies demonstrating that Parkinsonian tremor could be induced in non-human primates by lesions in the SN, which also involved adjacent structures (medial forebrain bundle or nucleus ruber for example) (Lamarre 1975; Ohye 1981).

Histological studies in one human subject accidentally injected with a meperidine compound (MPTP analogue) (Davis et al. 1979), and MPTP-treated rhesus monkeys have demonstrated a selective destruction of DA nigral neurons (Davis et al. 1979; Jacobowitz et al. 1984). However, doubt has been cast on the selectivity of MPTP, and recent investigations have shown that it causes damage in the VTA, LC and hypothalamus (Schultz 1988). The lack of cell loss in these other areas in our animals may be accounted for by a number of factors such as species, sex, age, or injection route. Moreover, schedule and time of histological analysis after the administration may also be critical. Much of the anatomical data on the selectivity of MPTP was obtained shortly after drug administration (Deutch et al. 1986; Mitchell et al. 1985). In long-term treated and recovered animals, the lesions appear to be restricted to the SN pars compacta (Jacobowitz et al. 1984; Schneider and Markham 1986). One must also bear in mind that the age of the animal is rarely specified, although it seems to be one of the most critical factors. Older animals display a wider range of behavioral deficits and lesions than younger ones, and show more similarities to Parkinson's disease (Degryse and Colpaert 1986; Gupta et al. 1986). Furthermore, it has been shown that there are species differences in the primary lesion sites, the lesion usually described in the rhesus monkey selectively involves the SN pars compacta (Deutch et al. 1986; Jacobowitz et al. 1984; Langston et al. 1984; Mitchell et al. 1985). Thus, a selective nigral lesion in our recovered young rhesus monkeys does not appear to be in contradiction with other reports.

We observed that the TH immunoreactivity of the nigral neurons was increased. This suggests an increased synthesis and turn-over of DA in the nigro-striatal system. Such a phenomenon may account in part for the behavioral recovery usually observed in MPTP-treated monkeys.

However, discrepancies remain between spontaneous versus trained movements. Recent experiments have shown that after training, there is an increased excitability of cortical neurons related to the particular muscles involved in the trained task (Brons and Woody 1980). This is accounted for by an increased efficiency in the particular polysynaptic corticoperipheral loop involved in the movement (Asanuma and Arissian 1984). Whatever the underlying mechanisms, such a facilitatory phenomenon might be independent of contributions from the basal ganglia. Thus, the existence of a facilitated corticoperipheral loop for the expression of a trained movement may account for two observations: 1) there were no significant differences in the number of successfully rewarded trials in the recovered animals versus controls; 2) there was only a weak (but significant) disruption of movement parameters during the trained task, whereas there was a marked impairment in the spontaneous movements of the MPTP-intoxicated animals.

Alterations in the firing of precentral neurons

The response pattern of cortical cells recorded in the lesioned monkeys during flexion and extension of the contralateral elbow was similar to that described in monkeys with SN electrolytic lesions (Gross et al. 1983). In the MPTP-intoxicated animals, the time between the start of the alterations in the neuronal discharge and the onset of movement was increased by 20%. The change in discharge rate was prolonged long after the onset of the movement although the frequency was not changed. The total duration of the neuronal response was increased by about 130% (Table 1). Studies on central coding of cortical and non-cortical motor outputs have demonstrated a functional relationship between the duration of the change in central neuronal activity and the duration of the associated movement (Cheney and Fetz 1980). Various explanations can be advanced for this long-lasting alteration in the cortical modification of discharge. One explanation focuses on a predominant

role of the SN. To date, anatomical data suggest that there are no direct connections between the SN and the primary motor cortex (Asanuma 1981; Evarts 1981; Schell and Strick 1984). However, a DA innervation of motor cortical areas (areas 4, 6 and SMA) in the cynomolgus monkey has been reported (Berger et al. 1986). These authors did not indicate the origin of the DA inputs but proposed a mesencephalic origin (VTA and/or SN) in view of the known projections of these structures to other prefrontal cortical areas. Such pathways in the rhesus monkey might account for the cortical disturbances observed. Nevertheless, it appears reasonable to assume that the action of the DA nigral neurons is mainly mediated via indirect pathways.

Anatomical and physiological observations have shown that the DA nigrostriatal pathway exerts mostly a tonic modulatory influence on the activity of the basal ganglia efferent nuclei by controlling the striatal DA concentration and, hence, the degree of DA receptor stimulation (Besson et al. 1971; Filion 1979; Lindvall and Björklund 1979; Ruffieux and Schultz 1980). The DA nigral neurons may thereby influence the activity of thalamic followed by cortical relays, and hence control the duration of the changes in the activity of precentral neurons.

On the other hand, in view of Asanuma and Arissian's (1984) suggestions that the "sensory inputs function by selectively changing the excitability of cortical efferent zones before and during the execution of voluntary movement", it is possible that the "motor initiation signals" coming, directly and indirectly, from other cortical areas (such as premotor and association cortex) and deep nuclei (such as cerebellum and basal ganglia via thalamic relays) impinge upon precentral neurons whose excitability is already modified by an altered sensory input from muscles. These motor initiation signals would not, therefore, be able to alter the dynamic discharge of precentral cells sufficiently to compensate for the peripheral load.

This would be seen as a rigidity opposing the movement. The motor messages conveyed by the pyramidal tract would have less effect on the hypertonic muscles. In terms of coding, it could be assumed that the same quantity of central information is produced (the frequency of the burst discharge is unchanged) to initiate the movement against a supplementary load as to initiate the movement with no peripheral load. As the movement proceeds, the structures involved in the regulation of the movement receive the information that the movement has not been completed and send corresponding signals to the motor cortex which perseveres in its output of movement execution messages.

The marked decrease in the percentage of neurons showing a reciprocal organization in MPTP-treated animals (18% in treated animals versus 49% in controls) may account for the bradykinesia observed. Indeed, in control animals the neurons involved in the contraction of the antagonist muscle would be expected to be inhibited during the movement. As these neurons remained active in the MPTP-treated animals they represent an additional load, which would account for the bradykinesia.

Central dysfunction and disturbed movement parameters

It seems unlikely that the alterations in area 4 activity could be solely responsible for all the peripheral deficits (decrease in movement amplitude and velocity, disturbances in patterns of muscular activity). It has been shown that pallidal dysfunction produces alterations in the speed of movement as well as changes in the absolute amplitude and rate of rise of EMG activity (Horak and Anderson 1984a; Horak and Anderson 1984b). Our mechanographic and EMG data are in agreement with, at least, a pallidal disturbance. Recent reports have shown that, after MPTP treatment, discharge pattern of pallidal neurons are profoundly disorganized (Filion 1985; Miller et al. 1986). Some reports have shown that motoneuronal excitability may be influenced by SN stimulation (York 1978), and alterations in motoneuronal excitability have been found in Parkinsonian patients i.e. with SN lesion (Bathien 1978). A disregulation in basal ganglia, may be expressed at the periphery through a number of possible pathways, participating in/or enhancing peripheral disturbances. A disinhibition of the cortico-thalamocortical feedback loop may then account in part for the reduced buildup of EMG activity.

Parkinson's disease: defect in movement initiation or execution?

BRT significantly increased for both F and X, while CRT was not affected. This suggests that the nigral lesion did not significantly affect central processes involved in the initiation of movement i.e. occurring between the perception of the auditory signal and the onset of the response of the precentral cortex neurons. Similarly, no significant difference was observed in PMT, suggesting a lack of disruption in the conveyance of the motor message to the muscles. Under our experimental conditions at least, it seems more likely that the SN and the basal ganglia intervene in the execution phase of the motor process.

The last point we want to discuss is the difference between the results with MPTP-treated monkeys and those with unilateral electrolytic lesion of the substantia nigra (Gross et al. 1983). In this previous study, there was a difference between movements of extension and flexion which was certainly due to an incomplete lesion; destruction may have been principally localized in the region of the SN implicated in extension movement. Furthermore, a decrease in discharge frequency was observed in the electrolytically lesioned monkeys, while the MPTP-treated animals showed no alteration in frequency.

The present observations are similar to those of Hallet and Khosbin (1980) in Parkinsonian subjects performing large elbow movements. A tendency to co-con-

traction was seldom noticed in the antagonist muscle. This observation differs from our previous published study in MPTP-treated animals where co-contraction was normal. The pattern of agonist/antagonist muscular activity was usually normal and, in fact, was quite similar to that of normal monkeys performing ramp movements (weaker amplitude of burst, increased EMD) (Schieber and Thach 1985a), except for the enhanced background activity. It has been demonstrated that, during slow ramp movements, the amplitude and increased rate of agonist EMG activity in normal monkeys are strongly dependent on the amplitude and the velocity of the trained movement (Schieber and Thach 1985a). Since in our MPTP-treated monkeys, both amplitude and velocity decreased, these modifications were to be expected. In that experiment, BRT could have been influenced by both the time of initiation of muscle activity and the time required to reach the necessary muscular activation to induce movement. EMG data suggest that the increase of BRT in the MPTP-treated animals may be due to the depression in the rate of development and the peak amplitude rather than to delayed initiation of the agonist EMG activity. Indeed, the premotor time, i.e. time from OS to the onset of EMG activity in the agonist muscle, was not significantly altered in the MPTP-treated monkeys.

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