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Frontal Cognitive Impairments and Saccadic Deficits in Low-Dose MPTP-Treated Monkeys

HAMUTAL SLOVIN,1 MOSHE ABELES,1,2 EILON VAADIA,1,2 IRIS HAALMAN,1 YIFAT PRUT,1 AND HAGAI BERGMAN1,2
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Slovin, Hamutal, Moshe Abeles, Eilon Vaadia, Iris Haalman, Yifat Prut, and Hagai Bergman. Frontal cognitive impairments and saccadic deficits in low-dose MPTP-treated monkeys. J. Neurophysiol. 81: 858–874, 1999. There is considerable overlap between the cognitive deficits observed in humans with frontal lobe damage and those described in patients with Parkinson’s disease. Similar frontal impairments have been found in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) primate model of Parkinsonism. Here we provide quantitative documentation of the cognitive, oculomotor, and skeletonmotor dysfunctions of monkeys trained on a frontal task and treated with low-doses (LD) of MPTP. Two rhesus monkeys were trained to perform a spatial delayed-response task with frequent alternations between two behavioral modes (GO and NO-GO). After control recordings, the monkeys were treated with one placebo and successive LD MPTP courses. Monkey C developed motor Parkinsonian signs after a fourth course of medium-dose (MD) MPTP and later was treated with combined dopaminergic therapy (CDoT). There were no gross motor changes after the LD MPTP courses, and the average movement time (MT) did not increase. However, reaction time (RT) increased significantly. Both RT and MT were further increased in the symptomatic state, under CDoT. Self-initiated saccades became hypometric after LD MPTP treatments and their frequency decreased. Visually triggered saccades were affected to a lesser extent by the LD MPTP treatments. All saccadic parameters declined further in the symptomatic state and improved partially during CDoT. The number of GO mode (no-response, location, and early release) errors increased after MPTP treatment. The monkeys made more perseverative errors while switching from the GO to the NO-GO mode. Saccadic eye movement patterns suggest that frontal deficits were involved in most observed errors. CDoT had a differential effect on the behavioral errors. It decreased omission errors but did not improve location errors or perseverative errors. Tyrosine hydroxylase immunohistochemistry showed moderate (~70–80%) reduction in the number of dopaminergic neurons in the substantia nigra pars compacta after MPTP treatment. These results show that cognitive and motor disorders can be dissociated in the LD MPTP model and that cognitive and oculomotor impairments develop before the onset of skeletal motor symptoms. The behavioral and saccadic deficits probably result from the marked reduction of dopaminergic neurons in the midbrain. We suggest that these behavioral changes result from modified neuronal activity in the frontal cortex.

INTRODUCTION

Parkinson’s disease (PD) is characterized by motor symptoms; however, many studies have now established that patients with PD also develop deficits across a range of cognitive functions (Brown and Marsden 1990). Recent studies of PD patients indicate that functions dependent on the visual, parietal, and temporal association cortices are mostly intact, whereas functions dependent on the association frontal cortex are disrupted (Taylor et al. 1986). For example, PD patients are impaired on attentional set-shifting (Raskin et al. 1992) and delayed-response tasks (De Lancy Horne 1971; Labutta et al. 1994) as is the case for humans with frontal lobe deficits (Owen et al. 1991; Pascual Leone and Hallett 1994).

Additional evidence for frontal lobe malfunction in PD arises from studies of the effects of the dopaminergic neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). Humans exposed to this neurotoxin, or monkeys treated with high doses of MPTP, develop clinical symptoms as well as biochemical and anatomic signs that closely resemble those of PD (Burns et al. 1983). The pattern of cognitive dysfunction of humans with MPTP-induced symptomatic or asymptomatic Parkinsonism is similar to the pattern exhibited by PD patients (Stern and Langston 1985; Stern et al. 1990). Monkeys, when chronically treated with low doses of MPTP, are motor asymptomatic but show frontal signs (Schneider and Kovelowski 1990; Schneider and Roelting 1993).

The main pathological sign of PD is the destruction of dopaminergic cells in the brain stem. Electrophysiological studies of neurons in the basal ganglia (BG) of MPTP-treated monkeys (Bergman et al. 1994; Filion and Tremblay 1991) have suggested that the inhibitory output of the BG is increased, leading to excessive inhibition of the frontal cortex. Reduced activity in the frontal cortex may underlie the cognitive impairments of PD. However, the frontal cortex of Parkinsonian patients also can be affected by regional depletion of dopamine (Brozoski et al. 1979; Sawaguchi and Goldman-Rakic 1991) caused by the degeneration of mesofrontal dopaminergic innervation. This pathway also is damaged in PD (Playford et al. 1992) and in the MPTP primate model of Parkinsonism (Pifl et al. 1991).

The aim of this study is to gain more insight into the frontal deficits associated with low-dose (LD) MPTP treatment. To do so, we studied performance on a frontal behavioral task (spatial delayed-response with frequent alternation between 2 behavioral modes), before and after MPTP treatments.

Parts of this study have been reported in abstract form (Slovin et al. 1993).
FIG. 1. Spatial delayed-response task with behavioral mode alternations. Trials were initiated when the monkey touched a central key and a central red light-emitting diode (LED) was turned on ("ready signal"). After a variable delay (3–6 s), 1 of the 2 target keys (±15° on the horizontal plane) was illuminated for 200 ms ("spatial cue"). Then after a further variable delay (1, 2, 4, 8, 16, or 32 s), the central red light was dimmed ("trigger signal"), instructing the monkey to exhibit the appropriate behavioral response. In the 1st mode (GO), the monkey was rewarded for releasing the central key within 0.6 s (MRT, maximal reaction time) after the trigger signal and touching the correct target key within the next 0.6 s (MMT, maximal movement time). In the 2nd mode (NO-GO), the monkey was rewarded for continuing to hold the central key for ±1.2 s after the trigger. A nonspatial cue (5 LEDs turned on for 3–4 s, "mode switch signal") instructed the monkey to switch behavioral modes after every 4th correct trial. The mode switch signal was identical for GO to NO-GO and NO-GO to GO transitions, and there was no external cue for the requested behavioral mode (GO or NO-GO) during trial performance. Thus the monkey had to remember or figure out the current requested behavioral mode by trial and error. Only short delay (1, 2, and 4 s) trials were given after the mode switch signal until the monkey performed the first correct trial in the new mode. Otherwise, assignment of delay and location was semirandom.

METHODS

Animals and behavioral conditioning

Two monkeys (B and C, Macaca mulatta, females, 3–4 kg) were trained on a spatial delayed-response task with alternations between GO and NO-GO behavioral modes (Fig. 1). All experiments were carried out in compliance with the regulations of the Hebrew University for care and use of laboratory animals.

Time schedule of the experiment, MPTP treatment, and therapy

The monkeys were trained until they performed the behavioral task at >85% correct trials and then were operated upon. The surgery was performed under aseptic conditions and deep anesthesia. A recording chamber was positioned above the frontal cortex, and a head holder was attached to the skull. Two Ag-AgCl cup electrodes were implanted in the sphenoid bone for electrooculographic (EOG) recording of horizontal eye movements. After 3–5 days the monkeys were retrained with head fixation, and the control (behavioral and neuronal) recording sessions began.

At the end of the control recording sessions, a course of placebo saline injections was given, followed by 1 wk of recording sessions. The monkeys then were treated with courses of LD MPTP hydrochloride, (Aldrich, Milwaukee, WI) injections. Each course was composed of daily intramuscular injections of 0.1 mg/kg of MPTP for 4 days. Monkey B received four such courses and monkey C received three such courses. The fourth MPTP course of monkey C consisted of 4 × 0.2 mg · kg⁻¹ · day⁻¹ im injections [medium dose (MD) MPTP]. The monkey developed severe motor Parkinsonian symptoms and 3 wk later was treated with 16 days with combined dopaminergic therapy [CDoT; P. O. 1/4-1 carbidopa and levodopa (Sinemet) 250/25 twice per day and Bromocriptine 2.5–5 mg 3 times per day]. The drugs were crushed mechanically and were given to the monkey hidden in a dried fruit under close supervision to make sure drugs were swallowed.

The MPTP courses were separated by 2–4 wk. After each course, behavioral and neuronal activities were studied (MPTP recording sessions).

Behavioral data collection and analysis

The spontaneous behavior of the monkeys in their home cages was estimated by daily 30-min observations. A human observer depressed a single key each time the monkey moved, and the number of times the key was depressed was compared before and after the MPTP treatments.

During the controlled task, all behavioral events were recorded with 1-ms resolution on an Intel 310 system. Several behavioral intervals were defined: precue period, the period between the ready signal onset and the spatial cue onset; delay period, from the spatial cue onset to the trigger signal onset; reaction time (RT), the time from the trigger signal until the release of the central key; and movement time (MT), the time from the release of central key to the touch of the target switch.

Trials in which the monkey responded with the required response within the time constraints were defined as correct trials. Incorrect trials were divided into three major groups: GO mode errors, NO-GO mode errors, and perseverative errors.

GO MODE ERRORS. These errors included early-release errors when the central key was released before the trigger signal. We discriminated between precue and delay early release, depending on when the key was released. Reaction time miss, which was when the monkey released the central key after the maximal RT had elapsed (600 ms) and within the next 600 ms (the maximal MT allowed). Omission error—after the trigger signal, the monkey did not release the central key within the next 1,200 ms and no target was touched until the next trial started. If the monkey made three or more consecutive omission errors, they were defined as low-responsive (LR) trials. An LR state was defined as all successive LR trials. Location error—the time...
constraints of the trial were adhered to but the monkey touched a wrong target key.

NO-GO MODE ERRORS. These errors included early-release errors, as in the go mode, no-go miss in which the central key was released during the hold interval (1,200 ms).

PERSEVERATIVE ERRORS. To quantify perseverative errors, we defined the trials that followed the mode switch signal up to and including the first correct trial as go or no-go mode switching trials (GmST, NmST). Go perseverative errors were defined as no-go miss errors (go response) in the NmST. No-go perseverative errors were defined as omission errors (no-go response) in the GmST. The number of perseverative errors during a recording session was divided by the total number of trials in the GmST or NmST.

Trials following a correct behavioral mode switch were defined as go or no-go mode nonswitching trials (GmNST, NmNST), and they reflect the performance on the acquired behavioral mode. All four groups of trials were analyzed for all types of errors.

Eye movements: data collection and analysis

The EOG signal was amplified, low-pass filtered at 40 Hz, sampled at 100 Hz, and compressed according to the transients of the signal. Eye movement amplitude was measured in A/D converter (AD) units (range of ±5 V, 12 bits) and converted to degrees, assuming that the average amplitude of visually triggered saccades in the control state was equal to the target amplitude (15°).

An eye movement was off-line defined as a saccade based on its velocity (≥40°/s), duration (≥20 ms), and amplitude (≥3.0 and 3.4° for monkeys B and C, respectively, based on the signal-to-noise ratio of the EOG signal). Two types of saccades were defined: visually triggered saccades (VTS), and self-initiated saccades (SIS). The VTS analysis included only the first saccade the monkey made toward the location of the left spatial cue within 50–400 ms after cue onset. SIS analysis included saccades initiated from 8 to 32 s after the onset of the left spatial cue of correct no-go trials with a 32-s delay to maximize the temporal difference between VTS and SIS. Saccadic velocity, duration, and latency depend on amplitude (Becker 1989). Saccades therefore were grouped into bins of amplitude of 2 and 2.3° (monkeys B and C, respectively). For each such bin, we calculated the mean velocity, duration, and latency before and after MPTP.

The saccadic strategy was evaluated by the saccadic frequency pattern (the frequency of saccades over sequential time windows in the delay period, e.g., Fig. 3, B and C) and by the saccadic eye position pattern (the position of the eyes at the end or beginning of a saccades as function of time during the delay period; e.g., Fig. 5).

Tremor: data collection and analysis

A triaxial accelerometer (model 354B17, PCB, Depew, NY) was attached to the limb of monkey C after the MD MPTP treatment. The output of the accelerometer was filtered, sampled, and compressed with the same algorithm as for eye movements.

Histology

Monkey W (M. mulatta, 5 kg) had been trained on other behavioral tasks, and after recording in the visual cortex it was used as the histological normal control.

At the completion of the experiments, the control and the MPTP-treated monkeys were killed with an overdose of pentobarbital sodium, followed by transcardial perfusion of saline and then by paraformaldehyde. The fixed brains were cut into two hemispheres, sectioned in the coronal plane and stained for tyrosine hydroxylase (TH) immunohistochemistry (Bolam 1992). Neurons were defined as TH positive if their cytoplasm stained for TH and had a clearly visible unstained nucleus or a large portion of a cell without a visible nucleus. Neurons were counted in parallel anatomic slices for the different monkeys, and estimates of the neuronal count data were corrected by the Abercrombie method (Konigsmark 1970), assuming that the mean diameter of dopaminergic neurons equals 33.4 μm (Poirier et al. 1983).

Statistical analysis

A two-tailed t-test, assuming unequal variances, was used to calculate significance levels between the means of parameter distributions, before and after MPTP treatment. A χ² test was used to evaluate the fit of the MPTP data to control state frequencies.

RESULTS

Table 1 shows the number of recording sessions used for behavioral assessment in the control, saline (placebo) periods and for each MPTP course. The average number of executed (correct and error) trials and of LR trials per recording session is shown in Table 2.

Gross behavioral effects of MPTP treatment

Acute effects of LD MPTP injections lasted a few hours and included increased arousal, hyperventilation, mydriasis, and tail erection. After recovery from the acute effects, no skeletal-motor dysfunction was observed in the monkeys. The number of spontaneous movements in their home cage, posture, and appetites did not change as compared with their control state and compared with other monkeys. There were no signs of postural abnormalities, bradykinesia, rigidity, or tremor.

To intensify Parkinsonian symptoms and to test the efficacy of CDoT, monkey C was treated with a MD MPTP fourth course. It developed severe akinesia, rigidity, and a high-frequency (~10 Hz) postural/action tremor. Functional recovery began several days later, and from the 9th day after injections the monkey moved freely, no tremor was observed, and

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Placebo</th>
<th>MPTP-1</th>
<th>MPTP-2</th>
<th>MPTP-3</th>
<th>MPTP-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monkey B</td>
<td>38</td>
<td>6</td>
<td>19</td>
<td>14</td>
<td>22</td>
<td>37</td>
</tr>
<tr>
<td>Monkey C</td>
<td>22</td>
<td>5</td>
<td>7</td>
<td>13</td>
<td>7</td>
<td>23 + 16*</td>
</tr>
</tbody>
</table>

*aDopamine replacement therapy started 23 days after the last injection in the fourth (MD MPTP, medium-dose 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) course and lasted for 16 days. The data base was divided into two groups: recording sessions in the symptomatic Parkinsonian untreated state (the first 23 days) and recording sessions under combined dopaminergic therapy (CDoT; the next 16 days).

TABLE 2. Averaged number of executed trials (error + correct) and low-responsive trials per recording session

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>MPTP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Executed Trials</td>
<td>LR Trials</td>
<td>Executed Trials</td>
</tr>
<tr>
<td>Monkey B</td>
<td>548.3 ± 133.8</td>
<td>19.6 ± 27.0</td>
</tr>
<tr>
<td>Monkey C</td>
<td>774.2 ± 156.0</td>
<td>1.7 ± 3.5</td>
</tr>
</tbody>
</table>

Values are means ± SD. Executed trials were defined as the sum of incorrect and correct trials for both behavioral modes [low-responsive (LR) trials were not included]. The number of executed and LR trials was calculated separately for each recording session and then averaged for all the controls or low-dose (LD) MPTP periods. *P < 0.05, t-test.
and the monkey performed the GO mode but failed to switch to the NO-GO behavioral mode. CDoT was given for 16 days. Shortly after the first dose, saccadic eye movements reappeared and did not improve the monkey’s performance on the task. (e.g., she easily reached fruits placed on target keys.) Training on the rigidity had almost disappeared. However, the monkey declined to perform the behavioral task, even though it had no motor disability that could prevent it from performing the task (e.g., she easily reached fruits placed on target keys.) Training did not improve the monkey’s performance on the task.

CDoT was started on the 23rd day after the last injection. Shortly after the first dose, saccadic eye movements reappeared and the monkey performed the go mode but failed to switch to the no-go behavioral mode. CDoT was given for 16 days. Peak-dose dyskinesia was observed in the final days of the treatment. The beginning and the end of the dyskinesia episodes during recording sessions were marked and were not included the behavioral database for CDoT. The monkey did not perform the behavioral task when we tested her 1 day after the termination of CDoT (40 days after the last MPTP injection.)

**RT and MT**

RT was dependent on the duration of the delay period (Fig. 2A) both in the control and in the MPTP states. The average RT values increased with sequential MPTP courses (Fig. 2A) but were stable within the 2- to 4-wk intervals between the MPTP courses. When averaged over all LD MPTP courses and delays, RT increased significantly by 31 and 56 ms from control values for monkeys B and C, respectively (P < 0.05 for the different delays.)

The average MT for correct trials after all MPTP injections did not increase but rather decreased (Fig. 2B). The average decrease (over all the delays and MPTP courses) was 10 and 25 ms for monkeys B and C, respectively (P values ranged from nonsignificant values to P < 0.05 for the different delays). These changes were smaller than the changes in RT. Finally, unlike RT, the changes in MT were not consistent across successive MPTP courses (Fig. 2B) and MT did not exhibit delay dependence before or after LD MPTP.

After the MD MPTP course, monkey C’s RT and MT only could be retested when it was treated with CDoT. During this period, we observed significant increases in RT and MT compared with both control and LD MPTP values (P < 0.05).

**Eye movements**

The database for eye movement analysis was derived from 21 to 27 recording sessions in the control and MPTP state for each monkey and state. Figure 3A shows the EOG signal during GO mode correct trials with a 32-s delay period in the control and the LD-MPTP state.

**VISUALLY TRIGGERED SACCADES.** The average saccadic amplitude of VTS decreased moderately for both monkeys (Fig. 4A, Table 3A). After MPTP treatments, the changes in VTS velocity (Fig. 4B) and latency were not consistent for the two monkeys.

To assess the monkeys’ ability to orient gaze toward the spatial cue, we defined ‘‘cue responsiveness’’ as the number of VTS the monkey executed toward the left target divided by the number of left spatial cues. After MPTP treatment, cue responsiveness fell by moderate values (Table 3A).

**SELF-INITIATED SACCADES.** SIS also became hypometric after MPTP treatment. The average amplitude decreased by moderate values (Fig. 4C and Table 3B). SIS velocity decreased in the MPTP state, and the velocity difference between the control and MPTP state was greater for saccades with larger amplitudes (Fig. 4D). After MPTP treatment, SIS frequency decreased severely (Table 3B, Fig. 3A). The changes in SIS after MPTP treatment were more consistent and more significant than the changes in VTS.

In monkey C’s motor symptomatic Parkinsonian state, all saccadic parameters declined significantly more than in the LD MPTP state and CDoT significantly improved all saccadic parameters.

**Saccadic strategy and saccadic velocity in go and no-go behavioral modes**

The saccadic frequency pattern in the control state for the GO mode indicates that there was a high frequency of saccades after onset of the spatial cue (Fig. 3B). In the next time window, the saccadic frequency decreased to very low values. The saccadic position patterns (Fig. 5B) show the spatial details of these saccades. The monkey started the delay period looking toward the location of the ready cue. Within 0.2 s after...
A

Control

MPTP

B

GO mode

C

NO-GO mode

Saccadic frequency [1/s]

Time after cue onset [s]
The spatial cue onset, the monkey made saccades to the target, fixated on it for ~0.6–0.8 s, and then returned its gaze to the ready cue. The monkey fixated on the ready cue for another 2 s, waiting for a randomly timed trigger signal (Figs. 3, A and B, and 5B). In the subsequent time windows during the delay, saccadic frequency increased as the monkey made saccades near the location of the ready cue (Figs. 3, A and B, and 5A).

The monkey used a different eye movement strategy in the NO-GO state. After the spatial cue, the monkey made a saccade toward the spatial cue but maintained high saccadic frequency over the entire delay duration (Figs. 3C and 5C). One possible explanation for this behavior is that the spatial cue and the trigger signal (dimming of the ready cue) in the NO-GO mode lose their spatial and attentional trigger relevance, thus prompting the monkey to look around.

The difference in saccadic strategy between the GO and NO-GO modes may be related to changes in the monkey’s attention level. Another sensitive estimation of attention level is saccadic velocity (Becker 1989). Our data show that when the monkey is alert and attentive, saccades are significantly faster than saccades with the same amplitude in the LR state. Saccadic eye movements in the NO-GO mode (which requires less attention) were significantly slower than saccades in the GO mode.

After MPTP treatment, there was a general decrease in saccadic frequency for the GO and the NO-GO behavioral modes (Figs. 3 and 5; Table 3) but the global strategy of saccadic eye movements was preserved (Figs. 3 and 5). The monkeys’ field of view decreased after MPTP treatment (Fig. 5A) consistent with the decrease in saccadic amplitude after MPTP.

**Behavioral performance**

**LR STATE IN THE GO MODE.** In most of LR trials, the monkeys did not respond to the trial stimuli. In many cases, they closed their eyes and slow waves on the EOG were frequently observed (Fig. 6A). The saccadic frequency pattern was flat and was not modulated by the visual stimuli (Fig. 6B). The LR state could last from tens of seconds to several minutes.

During recording in the control state, the monkeys usually reached the LR state at the end of the recording session. After MPTP, the LR state appeared earlier and the number of GO mode correct trials to the first LR state dropped from control (Fig. 7A and Table 4). The average number of LR episodes during a single recording session increased significantly (Fig. 7B and Table 4).

Figure 7, C and D, shows that the LR state was more pronounced in the first 9 or 6 days after MPTP injections in monkeys B and C, respectively. Thereafter, LR state parameters recovered partially and became more similar to the control state.

The LR state was the main characteristic of the symptomatic Parkinsonian state of monkey C. CDoT had a dramatic effect on the monkey’s condition. The LR state practically disappeared after the morning dosage of CDoT (and the monkey performed the behavioral task) but reappeared 2–4 h later.

LR state expression increased with the level of Parkinsonian symptoms [from control to LD MPTP (asymptomatic state)] to MD MPTP (symptomatic state) and decreased under CDoT.

**PERFORMANCE ON THE BEHAVIORAL TASK.** The fraction of total errors was defined as the percentage of incorrect trials out of the total number of executed trials. After MPTP treatment, there was a severe increase in the number of errors, mainly in the GO mode (Fig. 8A and Table 4). After MD MPTP, monkey C did not perform the task at all (100% errors). Under CDoT, the percentage of total errors on the GO mode was more than tripled (45.4% errors) in comparison with the control state.

The LD MPTP effect on performance of the NO-GO mode was weaker than in the GO mode (Fig. 8B and Table 4). On developing Parkinsonian symptoms and under CDoT, monkey C was unable to perform the NO-GO mode correctly at all.

To assess the dependency of task performance on the delay, we analyzed the GmNST and NmNST database (GO mode and NO-GO mode nonswitching trials). Task performance in the GO mode depended on the delay, and the monkeys exhibited a higher percentage of incorrect trials with longer delays, for the control, LD MPTP state (Fig. 8C) and after MD MPTP under CDoT. Performance in the NO-GO mode was much less dependent on the delay.

**OMISSION ERRORS.** Omission errors were committed frequently in both the control and the MPTP state. The fraction of omission errors increased after LD MPTP treatment (Table 4), but under CDoT monkey C made only a few omission errors (2.9%). Omission error values did not change significantly within the 2- to 4-wk intervals between the MPTP courses. The number of omission errors increased with the delay (Fig. 8C).

To assess behavioral strategy in omission errors, saccadic strategies between correct trials and omission errors were compared. Although omission errors are GO mode errors, their saccadic strategy resembled the NO-GO (the less attentive mode) correct trials (Fig. 9). The correlation coefficient between NO-GO correct trials and omission errors (for eye position and saccadic frequency patterns) was higher than the correlation between GO correct trials and omission errors. The saccadic velocity of both VTS and SIS in omission errors decreased as compared with correct trials by 7–15%. Together, these findings suggest that omission errors result from a low level of attention.

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**FIG. 3.** Saccadic frequency pattern of correct trials before and after MPTP treatment. A: electroocculographic (EOG) signal in GO mode correct trials, before and after MPTP treatments. EOG signal during a 32-s delay of 13 correct GO mode trials, in the control state (top) and the LD MPTP state (bottom). t = 0 is the left spatial cue onset (duration 200 ms). Plotted EOG signal starts 1.5 s before the spatial cue onset and continues ±34 s after the cue onset, 1.8 s after the trigger signal. Fast upward and downward deviation of the line represents saccadic eye movements to the left or right, respectively. Visually triggered saccades are shaded. Data are taken from monkey B, B and C: saccadic frequency pattern shows a scaling down in the frequency of saccades after MPTP treatments. Saccadic frequency pattern during the delay period, in the GO (B) and the NO-GO (C) mode, before (■) and after (●) MPTP treatment. x axis shows the time windows after spatial cue onset, and saccadic frequency (saccades to the left and right are included) is plotted on the y axis. Data are from monkey B, correct trials, 32-s delay. B: frequency pattern was calculated from 13,910 saccades in 630 GO mode trials in the control state. In the MPTP state, the frequency pattern was calculated from 5,090 saccades in 509 trials. C: frequency pattern was calculated from 23,646 saccades in 638 NO-GO mode trials in the control state. In the MPTP state, the frequency pattern was calculated from 8,512 saccades in 524 trials.
LOCATION ERRORS. The number of location errors (LEs) increased significantly after MPTP treatment (Table 4). Figure 10A shows the percentage of LE as a function of time. The probability of making a LE increased in the first few days after each MPTP course and subsequently decreased slowly toward control values. The percentage of LE increased with delay duration (Fig. 8C).

Location errors may result from different causes, such as a deficit in attending to the spatial stimulus, malperception of the stimulus, perseveration or memory dysfunction. The “cue responsiveness” of LE was calculated to assess the monkey’s ability to orient gaze toward the spatial stimulus. This decreased after MPTP treatments by 13 and 26% in monkeys B and C, respectively. Thus in most LEs, the monkeys oriented their gaze toward the spatial cue, but the way they treated the stimulus was very different from GO mode correct trials. The average eye positions around the ready cue (before spatial cue onset) and around the spatial cue were more variable, and the timing of saccades toward the spatial target was less well organized. Fixation time over the spatial cue for LE was 120 and 80 ms shorter than control values for monkeys B and C, respectively (P < 0.005), a value much closer to fixation time in the NO-GO mode. The saccadic frequency pattern showed that the monkey used roughly the same saccadic strategy as in GO mode correct trials, suggesting this was not a general attention failure, as was observed for omission errors.

LEs were the most frequent errors (16.9%) made by monkey C after MD MPTP under CDoT. In contrast to the LD MPTP state, the monkey now touched several different target keys during the LE trials. The monkey did not make a saccade toward the target stimulus in many LE trials, and saccadic behavior was disorganized in space and time compared with GO mode correct trials.

EARLY-RELEASE ERRORS. The number of precue early-release errors showed no significant change after LD MPTP. The number of delay early-release errors was dependent on the delay duration (Fig. 8C) and increased significantly after LD MPTP in monkey B (Table 4). Monkey C showed the same tendency, but this was not significant.

Precue early-release errors increased significantly in monkey C during the MD MPTP and CDoT (from 0.1% in the control state to 16.3%). The percentage of delay early-release errors increased from 0.74% in the control state to 6.3%. Many of these errors included complete movement to the instructed target, and about half were trials in which the monkey touched the target repetitively.

PERSEVERATIVE ERRORS. After MPTP treatment the monkeys had severe problems in shifting between the two behavioral modes and made many perseverative errors (Table 4). Figure 10B shows the time course of perseverative GO errors (i.e., the monkey responded with a GO response when it was instructed to switch to the NO-GO mode). The probability of making GO perseverative errors increased after each course of MPTP and then later decreased toward control values. The saccadic strategy in GO perseverative errors revealed that long before the trigger signal, the monkey continued to behave as in the “GO” mode (Fig. 9).

To assess whether the increase in perseverative errors was merely part of a general decrease in the monkey’s performance, we compared the number of GO perseverative errors with the number of NO-GO misses in matched delays. These errors are similar to GO perseverative errors but they appear after the monkey correctly changed its behavioral mode. The number of NO-GO misses in short-delay NmNST increased after MPTP treatments by a factor of 1.4 and 0.9 for monkeys B and C, respectively. However, the number of perseverative errors (NO-GO misses in NmST) increased by a factor of 3.8 and 1.7, respectively. This
was not the case for no-go perseverative errors (i.e., trials immediately after the no-go to go switching signal in which the monkey did not release the central key). For monkeys B and C, the expected value, based on the increase in the number of omission errors after MPTP in the GmNST, was 2.7 and 2.0, respectively, whereas the actual ratio was 2.3 and 2.1, respectively. CDoT was ineffective in restoring monkey C’s ability to shift behavioral mode. It did not perform the no-go mode, and most of the errors were go perseverative errors or early-release errors.

**Histology**

The monkeys that were treated with LD and MD MPTP showed moderate to extensive loss of dopaminergic neurons in the substantia nigra-pars compacta (SNpc). Semi quantitative analysis of TH immunohistochemistry of dopaminergic neurons in the SNpc showed a 68% loss in monkey B (164.0 ± 53.7 dopaminergic neurons/50 μm coronal section; P < 0.001) and a 78% reduction in monkey C (111.8 ± 17.5; P < 0.001) as compared with monkey W (511.2 ± 85.4). Cell loss was higher in monkey C, which may explain the Parkinsonian symptomatic state that developed after MD MPTP. The depletion of dopaminergic neurons was more evident in the posterior lateral side of SNpc, whereas the medial anterior SNpc was less damaged, indicating relative sparing of the ventral tegmental area.

**DISCUSSION**

The present study shows that LD MPTP treatment can produce frontal cognitive and eye movement impairments with minimal motor disorders. After LD MPTP treatment, the monkeys appeared asymptomatic for Parkinsonian gross motor...
symptoms. Quantitative analysis of their task performance revealed that the average MT did not increase. RT increased, saccades became hypometric, and their frequency decreased. The frequency of errors on the behavioral paradigm increased after LD MPTP. Most of these error types commonly are seen with frontal lobe damage. However, the frontal lobes are only part of many circuits. Thus the frontal cognitive impairments of PD may arise from abnormal discharges in the cortico-basal ganglia-thalamocortical circuit or from dopamine depletion in the frontal cortex. We therefore will use the more general term of frontal deficits without implying that the primary defect is in the frontal lobes. Our preliminary physiological studies (Slovin et al. 1993) indicate that these impairments are correlated with changes in neuronal activity in the frontal cortex.

**LD MPTP primate model**

Primates treated with moderate to high doses of MPTP develop most of the anatomic, biochemical, and clinical symptoms found in humans with PD (DeLong 1990). However, because of the rapid evolution of the symptoms (usually a few days) these models cannot shed light on the initial stage of the disease. Moreover, there are some differences in the distribution of the main dopaminergic loss between MPTP-treated monkeys and human Parkinsonian patients (Pifl et al. 1991).

Recent studies have shown that the LD (chronic) MPTP models may overcome some of these shortcomings of the full-dose MPTP models. In these studies, MPTP is given at low doses, 0.01–0.175 mg/kg, one to three times per week, for several weeks (Schneider and Kovelowski 1990; Schneider et al. 1994) until the development of stable and chronic symptoms. The cumulative MPTP doses achieved in these studies range between 15 and 175 mg per monkey. The behavioral effects of these treatments are limited to cognitive effects with minimal to moderate motor symptoms.

The MPTP effect is highly variable from monkey to monkey and is not a simple function of the cumulative dose of MPTP. Rather the time course of the treatment might be a more critical factor. In the current study, we chose to give sequential courses of LD MPTP (4 × 0.1 mg · kg⁻¹ · day⁻¹) and to record the behavioral effects of these treatments in the 2–4 wk after each course. The cumulative MPTP dose in our monkeys (6.4–8.0 mg per monkey) is far below the range used in chronic MPTP studies. We repeated the treatment every 2–4 wk to partially overcome the compensatory mechanism and to follow the progression of symptoms in the “initial” stage of Parkinson’s disease. Our definition of LD MPTP treatment is therefore...
mainly a functional one, emphasizing the development of cognitive symptoms in the absence of severe motor symptoms. We started the behavioral testing 72 h after the last MPTP injection, and we continued the recording session for 2–4 wk. The acute pharmacological effects of MPTP treatment (which are probably complex responses involving many neurotransmitter systems) subside within 24–48 h (Jenner et al. 1986). We therefore assume that most of the effects described below are due to the moderate dopaminergic damage induced by the low doses of MPTP.

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**RT but not MT is prolonged after LD MPTP treatment**

RT increased after MPTP treatment. With successive MPTP courses, RT showed successive increases. The average MT

**TABLE 4. Summary of the most significant behavioral changes between control and MPTP**

<table>
<thead>
<tr>
<th>Error type</th>
<th>Monkey B</th>
<th>Monkey C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>LD MPTP</td>
</tr>
<tr>
<td>Number of correct trials up to first LR state</td>
<td>159.8 ± 75.6</td>
<td>96.6 ± 78.3*</td>
</tr>
<tr>
<td>Frequency of LR episodes per session</td>
<td>2.2 ± 2.2</td>
<td>5.2 ± 3.0*</td>
</tr>
<tr>
<td>Total errors, go mode, %</td>
<td>13.6 ± 5.1</td>
<td>23.2 ± 8.6*</td>
</tr>
<tr>
<td>Omission errors, %</td>
<td>3.8 ± 2.3</td>
<td>7.6 ± 5.2*</td>
</tr>
<tr>
<td>Location errors, %</td>
<td>6.7 ± 2.6</td>
<td>9.4 ± 4.0†</td>
</tr>
<tr>
<td>Delay early release errors, %</td>
<td>2.4 ± 2.7</td>
<td>6.6 ± 4.6*</td>
</tr>
<tr>
<td>Go preservative errors, %</td>
<td>0.74 ± 0.78</td>
<td>1.8 ± 1.3*</td>
</tr>
<tr>
<td></td>
<td>3.5 ± 3.0</td>
<td>13.0 ± 10.8*</td>
</tr>
</tbody>
</table>

Values are means ± SD over all recording sessions, in each state. The percentage of errors were calculated as follows: the number of errors (e.g., location errors) divided by the total number of trials (correct and incorrect). Significance level (using t-test): *P < 0.001; †P < 0.01.
decreased rather than increased. This may be related to faster movements the monkey learned to execute with excess training on the behavioral task. After MD MPTP and CDoT, monkey C performed the behavioral task again, but its RT and MT were still much longer than in the LD MPTP state. These findings suggest a differential effect of the LD MPTP treatments on RT and MT and different dependence of RT and MT on the degree of Parkinsonian symptoms.

Our results concur with studies that report a prolongation of RT and MT in Parkinsonian patients (Pullman et al. 1988) human patients and monkeys with MPTP-induced Parkinsonism, either with or without motor symptoms (Mandir and Watts 1990; Schultz et al. 1989b; Stern et al. 1990), and after frontal lobe lesions (Stuss and Benson 1986). Accumulative increase in RT over successive MPTP courses is in line with finding that RT increases with the severity of human Parkinsonian symp-
Hypometria and other deficits of saccadic eye movements have been observed in PD patients (Lueck et al. 1992a,b; Rottach et al. 1996) and in humans with MPTP-induced Parkinsonism (Hotson et al. 1986). Dopaminergic drugs make a remarkable improvement in oculomotor abnormalities (Nakamura et al. 1991; Rascol et al. 1989). Saccadic eye movement deficits were reported in MPTP-treated monkeys (Brooks et al. 1986; Schultz et al. 1989a) and after caudate local dopamine depletion (Kato et al. 1995; Kori et al. 1995). Our results are congruent with those studies showing that saccades related to external stimulus in PD patients are less affected than internally triggered saccades. This phenomenon is in line with the general decline in self-initiated behavior in Parkinsonism (Jahanshahi et al. 1995) and argues for a differentiation between the effects of internal and external cues in PD.

Analysis of the saccadic strategy reveals that the monkeys used different spatio-temporal strategies in the go and in the no-go behavioral modes. The difference in saccadic velocity and strategy probably is related to the differential attention level in the go and no-go modes (Aschoff et al. 1975). The saccadic strategy in each behavioral mode did not change after MPTP treatments. However, the spatio-temporal organization of saccades was much more variable. Saccadic parameters exhibited high correlations with both RT and MT as well as with cognitive errors. A particularly, SIS velocity correlated with several types of behavioral errors (data not shown). This may indicate that many errors are related to attention or alertness deficits.

The frontal and the supplementary eye field are associated directly with control and generation of saccadic eye-movements (Bruce and Goldberg 1985). Lesions of these areas, as well as manipulation of dopamine content in the frontal cortex, causes saccadic deficits (Passingham 1993; Sawaguchi and Goldman Rakic 1991; Williams and Goldman Rakic 1995). The abnormal eye movements found here after LD MPTP treatments may be due to abnormal activation of the frontal cortex or from abnormal activity in the oculomotor related nuclei in the basal ganglia.

Disinhibition and switching behavioral modes after MPTP treatment

Monkeys with lesions in the dorso-lateral frontal cortex suffer from the inability to suppress behavioral reactions evoked by external stimuli (Fuster 1997). NO-go potentials have been recorded in the frontal cortex of humans and monkeys (Gemba and Sasaki 1990; Sasaki et al. 1993), suggesting an active neuronal process that controls the suppression of movement. The frequency of postcue (but not the precue) early-release errors increased after LD MPTP treatment. Both error types increased in monkey C under MD MPTP and CDoT. Together these finding suggest disinhibition behavior of the monkeys in the MPTP state.

Saccadic eye movements are impaired after MPTP treatments

Saccadic eye movements were impaired after MPTP treatments. SIS became hypometropic and their frequency decreased. VTS were less affected. All saccadic parameters declined further in the motor symptomatic Parkinsonian state of monkey C. CDoT dramatically improved all saccadic parameters.

**FIG. 9.** Saccadic strategy of omission errors is similar to the 1 in the no-go mode and saccadic strategy in no-go perseverative errors is similar to the previous, now incorrect mode (the go mode). A: saccadic eye-position pattern during the delay period in go mode correct trials (n = 125), no-go mode correct trials (n = 125), omission errors (n = 125 trials), and go perseverative errors (n = 36 trials). B: saccadic frequency pattern in the delay period, as a function of time windows after onset of the left spatial cue. GO perseverative errors (■), GO (EE) and NO-GO (□) mode correct trials are plotted on the left. Saccadic frequency pattern of omission errors (■) GO and NO-GO mode correct trials are plotted on the right. Data are taken from monkey B. NGcor, no-go correct trials; GOcor, go mode correct trials; Pers Err, go perseverative errors. Omiss Err, omission errors. r between GOcor to Pers Err frequency pattern was 0.995, P < 0.01; r between NGcor to Pers Err was 0.8, P < 0.05, and r between NGcor to GOcor frequency pattern was 0.75, P > 0.05.
After MPTP treatment, the monkeys made more frequent go perseverative errors. The saccadic strategy indicated that the monkey was persevering in the go mode during these errors. The tendency of the MPTP-treated monkeys to make go but not no-go perseverative errors could be due to frontal-lobe disinhibition. However, the characteristic go eye movements, starting early as the onset of the spatial cue, indicate that these errors were not merely motor disinhibition provoked by the trigger signal.

Impaired ability to shift between sets is considered to be one of the characteristic signs of cognitive impairments related to frontal lobe lesions in humans (Fuster 1997; Levin et al. 1991) and monkeys (Iversen and Mishkin 1970). Problems with shifting between mental sets also have been described in human patients with early PD (Brown and Marsden 1988; Flowers and Robertson 1985; Richards et al. 1993). Our findings suggest that the observed set-shifting deficits are due to problems in changing rules that requires “internal” suppression of a behavioral mode (Owen et al. 1993). At a different level, problems with set-shifting may be due to perseveration of the previously correct “set” or result from slower refocusing of attention to the previously irrelevant set. Although such a distinction recently has been used to differentiate between Parkinsonian patients and frontal-lobe patients (Owen et al. 1993), the present study was not designed to discriminate between these alternatives.

The ability of monkeys to perform extradimensional set shifts is improved significantly after 6-hydroxydopamine lesions of the frontal cortex (Roberts et al. 1994). In this study, neurochemical measures showed adaptive elevation of dopamine levels in the striatum. The mild destruction of dopaminergic neurons in the ventral tegmental area of our monkeys suggests that the observed behavioral deficits are less due to direct dopamine depletion of the frontal cortex. Further direct studies of the dopamine contents in the striatum and the frontal cortex of LD MPTP-treated monkeys are needed to pinpoint the most critical changes leading to set-switching deficits.

**LE and attentional deficits after MPTP treatment**

The frequency of LE increased after MPTP treatments. These errors may emerge from several different dysfunctions, e.g., visual neglect of visuo-spatial deficits. The existence of visual neglect in PD patients and in MPTP-treated hemi-Parkinsonian monkeys is debatable (Apicella et al. 1991; Bankiewicz et al. 1991). In most LE trials, our monkeys oriented their gaze toward the spatial cue, suggesting no neglect.

The saccadic eye movements associated with the onset of the spatial cue in LE trials differed from those in correct trials: they had longer latencies, and fixation times on the target were typically shorter, as in correct NO-GO trials. This may indicate that the monkeys had problems in assessing the relevant information from the cue, although they still could orient attention toward the cue. LE also may result from memory dysfunction; there was a slight reduction in frequency of saccades that were oriented toward the target position during the delay period of LE trials. Other possibilities for LE, such as perseverative response to a previously rewarded spatial cue may exist; however, these effects were rather small.

Omission errors were very frequent in our monkeys. Our results suggest that omission errors may reflect a low attention level and that the LR states are transition states ranging from a low attention level to a short “napping” period. Attentional deficit and task impersistence have been described in LD MPTP-treated monkeys (Roelert and Schneider 1994) and patients with early PD (Cooper et al. 1991; Levin et al. 1989; Mohr et al. 1990).

Our results show that CDoT decreased omission errors but did not improve commission errors such as location errors and go perseverative behavior. These results are in line with a previous study (Schneider et al. 1994), which showed that D2 agonists decreased the number of no-response errors but not other errors. Our monkey performed more trials under CDoT, but it did not make more correct trials, as found for medicated children with attention deficit hyperactivity disorder (Charles et al. 1979; Pelham et al. 1985). L-Dopa has been shown to affect cognitive performance in PD patients in tests sensitive to frontal lobe dysfunction (Gotham et al. 1988; Lange et al. 1992). However, CDoT induced dyskinesia in our monkeys. Although we omitted all the sections that may have pointed to dyskinesia, it is still possible that some of the deficits observed under CDoT may be attributed to side effects of medications.

Delay dependence of behavioral performance has been reported in chronic LD MPTP-treated monkeys (Schneider and Kovelowski 1990) and in MPTP monkeys with no motor impairments (Fernandez Ruiz et al. 1995). Omission errors and other types of errors (location errors, postcue early release errors) were dependent on the duration of the delay. The delay period in our behavioral paradigm requires activation of frontal functions such as memory, attention, and inhibition; failures in each of these processes may cause different types of errors. Finally, PD patients show an increase in the severity and broadening of cognitive impairments with increasing clinical disability (Owen et al. 1992). This is similar to our results, which show that the maximal level of most errors increased twice, both from control to LD MPTP state and from LD MPTP to MD MPTP under CDoT.

In conclusion, LD MPTP treatments can produce frontal cognitive errors in primates similar to those observed in humans and other animals with frontal lobe damage. PD patients, and humans exposed to MPTP. The intellectual changes in Parkinson’s disease are not a simple function of motor impairments (Tomer et al. 1993). Indeed, some evidence suggests that cognitive changes precede motor impairments (Cooper et al. 1991; Levin et al. 1989; TsaI et al. 1994). The cognitive impairments observed here in monkeys treated with LD MPTP were not associated with Parkinsonian motor dysfunction. However, self-initiated saccades showed clear deficits. We therefore suggest that frontal-type cognitive impairments and saccadic deficits are the first signs of MPTP-induced Parkinsonism and that careful study of eye movements and frontal cognitive functions may be used for early diagnosis of Parkinsonism.

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