Nigrostriatal Dopamine System in Learning to Perform Sequential Motor Tasks in a Predictive Manner

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Matsumoto, Naoyuki, Toru Hanakawa, Shinichiro Maki, Ann M. Graybiel, and Minoru Kimura. Role of nigrostriatal dopamine system in learning to perform sequential motor tasks in a predictive manner. J. Neurophysiol. 82: 978–998, 1999. Neurons in the primate striatum and the substantia nigra pars compacta change their firing patterns during sensory-motor learning. To study the consequences of nigrostriatal dopamine depletion for learning and memory of motor sequences, we used a neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), to deplete dopamine unilaterally in the striatum of macaque monkeys either before or after training them on sequential push-button motor tasks. We compared the monkeys’ performance with the arms ipsilateral and contralateral to dopamine depletions. During training and retraining on the tasks, we measured initial and serial movement times and reaction times for the push button movements, electromyographic patterns of arm and orofacial muscle activity during button pushing and reward licking, and saccadic eye movements during the button push sequences. With the arm ipsilateral to the side of dopamine depletions, each monkey showed progressive shortening of movement times and initial and serial reaction times, and each developed consistent strategies of hand-orofacial and hand-eye coordination in which single button push movements were linked efficiently to succeeding movements so that performance of the whole sequence became predictive. These patterns did not develop for contralateral arm performance in this monkey treated with MPTP before training. With the arm contralateral to dopamine depletions, the monkey showed significant quantitative deficits in all parameters measured except initial reaction times. Movement times and serial reaction times were longer than those for the ipsilateral arm; anticipatory saccadic eye movements were not well time-locked to individual button pushes made with the contralateral hand; and push and licking movements were not smoothly coordinated. This monkey further showed striking differences in performance when using the ipsilateral and contralateral arms in switch trial tests in which reward was delivered unexpectedly one button early. He continued to make movements to the previously rewarded button with the ipsilateral arm but showed no such automatic movements when he used his contralateral arm. For the monkey treated with MPTP after training, performance on the push-button task was skilled for both arms before dopamine depletions, but the unilateral dopamine depletion produced deficits in contralateral arm performance for all parameters measured, again excepting initial reaction times. With retraining, however, his performance with the contralateral arm improved. We conclude that the striatum and its nigrostriatal afferents function in the initial learning underling performance of sequences of movements as single motor programs. The nigrostriatal system also operates during the retrieval of these programs once learning is accomplished, but lesions of the nigrostriatal system spare the ability to relearn the previously acquired programs.

I N T R O D U C T I O N

A long-standing question in motor control is how motor sequences are built up from individual movement components (Lashley 1951; Rosenbaum 1991). New approaches to this problem now are being generated by virtue of evidence that regions of the frontal cortex may be specialized for coding motor sequences and that these cortical areas together with the basal ganglia may form part of the central control mechanism for motor sequencing (Brotchie et al. 1991; Cromwell and Berridge 1996; Graybiel 1995, 1998; Kermadi and Joseph 1995; Kimura et al. 1992; Marsden 1987; Miyachi et al. 1997; Mushiake and Strick 1996; Roland et al. 1980; Tanji and Shima 1994; Tanji et al. 1987). Neuronal activity in the primate premotor cortex (Mitz et al. 1991) and supplementary eye field (Chen and Wise 1995a,b, 1996) is modified during the acquisition of sensory-motor associations. Tanji and coworkers have shown that many neurons in the supplementary motor area (SMA) fire preferentially in relation to particular learned movement sequences. These authors have suggested further that neurons in the cortical area anteriorly adjoining the SMA (the pre-SMA) are differentially active when a motor sequence is to be switched (Matsuzaka and Tanji 1996; Shima et al. 1996). In accord with these findings, inactivation of the SMA and particularly of the pre-SMA in monkeys produces deficits in learning and performing sequential motor tasks (Miyashita et al. 1996).

For the basal ganglia, it has been reported that neurons in the striatum and globus pallidus of monkeys are activated specifically at the first movement of a motor sequence or at other specific phases of the sequence (Brotchie et al. 1991; Kermadi and Joseph 1995; Kimura 1990; Kimura et al. 1992; Mushiake and Strick 1996). Similar task-specific activation patterns have been reported in the rat (e.g., Aldridge and Berridge 1998; Gardiner and Kitai 1992; Jog et al. 1997; Kubota et al. 1998; West et al. 1990; Woodward et al. 1995). Lesions of the striatum in the rat have been shown to impair sequential grooming behavior (Cromwell and Berridge 1996), but with the exception of a recent report by Miyachi et al. (1997), little has been reported about the consequences of lesions of the primate striatum for the ability of monkeys to learn and to retrieve motor sequences.

In the study reported here, we used the strategy of inacti-
vating the dopamine-containing innervation of the striatum to impair selectively striatal function in monkeys before or after training on sequential push-button tasks. In previous work, we have found that local inactivation of dopaminergic function in the striatum by infusion of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) greatly decreases conditioned responses of striatal neurons previously acquired in a sensory-motor learning task (Aosaki et al. 1994a). These observations suggest that the nigrostriatal dopamine system is necessary for retrieving acquired neuronal activity from long-term storage of activity patterns in the striatum (Aosaki et al. 1994a; Graybiel et al. 1994). We reasoned that if the nigrostriatal system were critical for learning movement sequences or for retrieving them, we should detect deficits in performance of sequential motor tasks in monkeys with intrastral MPTP-induced dopamine depletion. The experiments reported here were designed to test this notion.

Our goals were to assess abilities of unilaterally dopamine-depleted animals to acquire programs for new motor sequences and to retrieve or to relearn previously learned motor programs. Our findings, briefly reported elsewhere (Matsumoto et al. 1994), strongly implicate the striatum and its dopamine-containing innervation as being involved in the learning of sequential motor tasks and in the retrieval of previously learned motor sequences.

METHODS

Behavioral paradigms

Three male monkeys (Macaca fuscata, 5.5, 7.0, and 7.2 kg) were studied. The acquisition and care of the animals were based on National Institutes of Health guidelines (publication No. 80–23). The monkeys were trained to sit in a primate chair facing a small panel placed 50 cm in front of the animal. For experiments on monkeys KE and GN, three push buttons were situated on the panel so as to form the apices of a triangle ca. 9° of visual angle (10 cm) apart (Fig. 1). These buttons could be illuminated under computer control. The monkeys learned to push the three buttons sequentially in an instructed order for a total of three pushes (monkey KE) or four pushes (monkey GN). For monkey TZ, a touch panel (NEC 9873L) was attached to the surface of the computer display. A 1.6 × 1.6 cm blue rectangular light could be illuminated at any one of nine possible locations on the display (see Fig. 5). The monkey was trained to touch three sequentially illuminated rectangles on the touch panel with his index and middle fingers, in an instructed order, for a total of three pushes. All three monkeys were trained to make the button pushes with each arm. In a given trial, the unused arm was loosely restrained by a yoke.

The first step of training was the one-push task. As shown for the button task in Fig. 1, button 1 was illuminated, and the monkey received a drop of water as reward when he pushed the lighted button. During this phase of training, button 1 was illuminated at variable intertrial intervals of 5–7 s. In this and all subsequent behavioral sessions, an audible click was given 280 ms before the water reward. The second step of training was the two-push task (Fig. 1). After the monkey depressed button 1 for 1 s, the button 1 light was turned off, and simultaneously the second button was lighted. This served as a go signal for the monkey to release button 1 and push button 2 to obtain a reward. The third training step was the three-push task, in which the monkey pushed the three buttons in either clockwise or counterclockwise sequences (Fig. 1). Button 2 was lighted after the monkey pushed the illuminated hold button (button 1) for 1 s; the monkey then had to release the hold button and push the illuminated second button, which in turn illuminated the third button, which the monkey had to push to receive reward. Monkey TZ was similarly trained on the touch panel to perform two blocks of trials. The first block was 30 trials of a fixed, cued sequence. The second block consisted of a total of 60 trials in which three different cued sequences of screen touches were triggered in random order but with equal probabilities (Fig. 5). Monkey GN, but not the other monkeys, was trained on a four-push task after he had learned the three-push task. The fourth button was illuminated after the third push, and then the monkey pushed the fourth button to receive reward (Fig. 1).

The monkeys performed a total of 800–1,500 correct trials in a day. In an effort to have identical training conditions for right and left hands, we adjusted the number of trials so that the total trials performed with the right and left arms did not differ by >25–100 trials in a day. Thus when the monkeys moved from one step to the next step of learning with the right hand, they also moved to the same next task with the left hand on the same day. Late in the experiment, task switch trials were introduced for monkey KE at irregular 4- to 7-day intervals. In these, the monkey unexpectedly had to perform a previously performed push task, rather than the current push task (for example, a 2-push instead of a 3-push task). This meant that reward and the associated click were delivered unexpectedly, one push early. When the task was switched, the monkey performed a total of 80–200 switch trials and 700–1,300 regular trials in a day. We carried out six switch experiments.

![Sequential button-push task training protocol. Monkeys were trained to push buttons in response to illumination of the buttons in a clockwise or counterclockwise sequence.](http://jn.physiology.org/DownloadedFrom/120/32.2.on.August.27.2017)
Before behavioral training, each monkey underwent surgery for implantation of a head restraint bolt and a recording chamber to permit recording of single unit activity in the caudate nucleus and putamen. The activity of neurons in the striatum of these animals will be described in a separate report. Surgery was performed under anesthesia induced with ketamine hydrochloride (6–12 mg/kg im) and maintained with pentobarbital sodium (Nembutal, 28 mg/kg ip). Supplemental Nembutal (10 mg ⋅ kg\(^{-1}\) ⋅ h\(^{-1}\) im) was given as needed. Neuronal activity was recorded extracellularly with glass-insulated elygo microelectrodes inserted through the recording chamber at the start of each recording session.

Electromyographic (EMG) activity was recorded from the triceps and biceps brachii muscles (prime mover muscles of the arm) as well as from other supporting muscles and from the digastic muscle of the tongue (activated for licking the reward water) through chronically implanted, multistranded teflon-coated stainless steel wire electrodes (Cooner Wire AS631) with leads that led subcutaneously to the head implant. The EMG signals were amplified, rectified, integrated, and monitored on-line on a computer display simultaneously with the recorded neuronal activity. Licking movements also were recorded by means of a strain gauge fixed to the spoon in front of the animal’s mouth.

The behavior of the animals was monitored routinely by a video camera. Eye movements were monitored by measuring the corneal reflection of an infrared light beam through the video camera at a sampling rate of 250 Hz. The computer system (RMS R-21C-A) determined the two-dimensional (x and y) signal of the center of gravity of the reflected infrared light beam. The spatial resolution of this system was ca. ±0.15°. The muscle activity and eye position signal from the video system were recorded at a sampling rate of 100 Hz by a laboratory computer (NEC 9801RA). The behavioral tasks also were controlled by the computer system. For the button tasks, the times of onset and offset of the button light stimuli, the time of depression and release of the push buttons and the onset of reward delivery and associated click stimuli were recorded during task performance. For the touch screen, the horizontal and vertical locations at which the monkey touched and released were detected as analogue data by means of a hardware device at a sampling rate of 500/s, and the computer system recorded the data at a rate of 100 Hz.

Unilateral dopamine depletion

To destroy the nigrostriatal dopamine system within the striatum, we locally infused MPTP (Sigma), a dopaminergic neurotoxin, into the caudate-putamen complex of one hemisphere in *monkey KE* and *monkey GN* (Aosaki et al. 1994a; Imai et al. 1988). MPTP (4 mg) was dissolved in 200 μl of 0.15 M NaCl and was infused over a 14-day period into the right (*monkey KE*) or the left (*monkey GN*) hemisphere by means of an Alzet osmotic minipump (average rate, 0.5 μl/h). The infusion needle (0.6 mm OD, 0.3 mm ID) was connected to a minipump by a polyethylene tube, and it was implanted through the recording chamber with the monkey under Nembutal anesthesia (28 mg/kg im) at a depth of 11–12 mm and atlas coordinates A15.5-A16, a site between the putamen and the caudate nucleus. The infusion needle was fixed to the inner wall of the recording chamber by means of dental cement, and the minipump was implanted subcutaneously at the nape of the neck (Aosaki et al. 1994a; Imai et al. 1988). The infusion needle and minipump were implanted for 3 wk and then were removed. The monkeys were observed carefully throughout this period and were given injections of apomorphine (0.1 mg/kg im) to test for contralateral turning indicative of unilateral dopamine depletion (Imai et al. 1988). The apomorphine test was done once in *monkey KE* and three times in *monkey GN*.

We used two different protocols for the unilateral nigrostriatal dopamine depletion. In *monkey KE*, MPTP was given before behav-
The depletion of dopamine in the striatum and in the substantia nigra pars compacta, which gives rise to the nigrostriatal dopamine innervation, was confirmed histologically by staining for tyrosine hydroxylase (TH), the synthetic enzyme expressed in the cell bodies and axons of the nigrostriatal tract. The extent of dopamine depletion in the striatum, as judged by TH immunoreactivity, was different in the two animals (Fig. 3). In monkey KE, in which MPTP was infused into the right hemisphere before training (Fig. 3A, 4), depletion of TH immunoreactivity occurred over ~7 mm in the caudal and midanteroposterior levels of the striatum, and the rostral striatum was left intact. The putamen was affected more severely than the caudate nucleus (Fig. 3A). In monkey GN, in which MPTP was infused into the left hemisphere after training (Fig. 3B), the depletion of TH immunoreactivity was much more extensive. It covered a large part of both the caudate nucleus and the putamen. Only the most ventral part of the rostral striatum and the rostrodorsal part of the caudate nucleus were left with dense TH immunoreactivity (Fig. 3B). The loss of TH immunoreactivity in the midbrain of the two monkeys differed in parallel to the levels of striatal depletion. There was no obvious loss of TH staining in the ventral tegmental area. Photographs of histological sections through the striatum and substantia nigra are shown for monkey KE in Fig. 4.

Sequential push movements are preprogrammed after learning

The push movements of the monkeys became faster as they were trained to perform particular fixed sequences of pushes over the course of several weeks. To test for the effects of predictability of the triggers for the movements, monkey TZ was trained first to perform >300 trials of three fixed sequences of pushes per day for 3 mo (Fig. 5A, left). The monkey then was asked to perform three new sequences of screen touches (Fig. 5B, left), in which the first and second targets were the same as that in the original sequence, but in which the third target was different. These three sequences occurred at random but with equal probabilities. The screen touches in the
fixed and in the randomized sequences were performed in different 30 trial blocks. In a given day, we usually asked the monkey to perform 10–15 blocks of fixed sequences and 2–5 blocks of randomized sequences.

Figure 5A, right, shows the serial reaction times for the pushes performed after 3 mo of training. These were defined as the time between the onset of the target 3 light and the release of the target 2 light. The average serial reaction time was $81.7 \pm 52.0$ ms. The serial reaction times for the third push in the randomized sequences (time between onset of the 3rd target and release of the 2nd) are shown in Fig. 5B, right. They were significantly longer than those in the fixed sequence ($131.5 \pm 51.1$ ms; $P < 0.005$, Wilcoxon single rank test). During the first and sometimes the second trial after the task was switched from the fixed sequence to the random sequence, the monkey tried to touch the target at the top right corner, which had been the third button of the fixed sequence, even though one of the other targets was illuminated. Only after that did he then press the illuminated button.

These observations suggest that the push movements from the second to the third target in the well-learned sequences no longer actually were guided by the visual stimuli, but were performed in a predictive, preprogrammed manner. By contrast, the longer serial reaction times for the movements in the randomized sequences suggest that these movements were guided by the visual cue for the third push, which was not predictable. In the random sequence task, the serial reaction times for movements from the second to the third button at the top right corner ($124.8 \pm 50.9$ ms) were not significantly different from those to the third button at the top left ($118.8 \pm 50.8$ ms) and bottom left ($137.5 \pm 35.5$ ms), but they were significantly longer than those in the fixed sequence task ($81.7 \pm 52.0$ ms, $P < 0.005$, Wilcoxon single rank test). These results indicate that the longer serial reaction times in the random sequence task did not occur because the random sequence task was less well learned than the fixed sequence task, but because the position of the third button was not predictable in the randomized sequence task. In the experiments with monkeys KE and GN, we tested the effects of dopamine depletion on the acquisition and performance of fixed sequences of push movements.

**Unilateral dopamine depletion and movement times during sequence learning**

In monkey KE, training on the sequential push button task started after dopamine depletion. The MPTP infusion needle and minipump were removed 3 wk before training onset. After 5 wk of training, the monkey had mastered both the one- and two-push tasks with his right and left hands. He then learned the three-push task. Figures 6 and 7 illustrate the activity of prime mover muscles (triceps brachii) for the arms ipsilateral and contralateral to the side of MPTP infusion as the monkey learned this new three-push task. The EMG traces illustrated were selected as representative of the activities occurring during the first 10 days of learning. The traces shown are aligned to the third button push, which elicited reward. Figure 6 illustrates performance in the clockwise sequence, and Fig. 7 illustrates performance in the counterclockwise sequence.

Over the course of the 10 days of training, there were marked changes in the kinetics of the button presses when the monkey used the arm ipsilateral to the side of the MPTP lesion (Figs. 6 and 7). On the first day of training, when the monkey...
pushed the second button, which previously had elicited reward but now did not, he depressed the second button for long times, suggesting that he detected that something was wrong. The second button light was turned off, and the third button was illuminated simultaneously when the monkey pushed the second button. The monkey soon learned to release the second button and to push the illuminated third button to receive...
reward. He also depressed the third button for a long time. The time interval between the second and third button pushes became shorter and shorter when the monkey used his ipsilateral arm (from 836 ± 184 ms on day 1 to 319 ± 122 ms on day 10 for the clockwise sequence, and from 639 ± 98 ms on day 1 to 424 ± 92 ms on day 10 for the counterclockwise sequence).

The pattern of activation of the triceps also changed through the early period of monkey KE’s training on the three-push task when he used his ipsilateral arm (Figs. 6 and 7). On the first 2–3 days, the duration of activation of the triceps at the second push was long (508 ± 134 ms for clockwise sequence, 375 ± 78 ms for counterclockwise sequence), as though the monkey did not understand that he had to move to the third button. But by the fourth day of training, the monkey moved his hand smoothly from the first to the second and then to the third button. The duration of the second and third button pushes became short, as shown in the brief periods of triceps activation (~ 334 ± 48.8 and 348 ± 71.4 ms for clockwise, 292 ± 48.0 and 275 ± 46.5 ms for counterclockwise).

In contrast to the large changes in movement kinetics and muscle activation shown when the monkey used the arm ipsilateral to MPTP infusion, relatively little change occurred when the monkey, over the same trials, used the arm contralateral to the MPTP lesion. As shown in Figs. 6 and 7, the interval between the second and third button presses remained long (592 ± 150 ms on day 1, 445 ± 131 ms on day 10 for the clockwise sequence; 804 ± 100 ms on day 1, 652 ± 172 ms on day 10 for the counterclockwise sequence).

After 3–4 days of training, the duration of activation of the triceps brachii muscle became short when the monkey pushed the third button. This shortening occurred for both the ipsilateral and the contralateral arm (and for both clockwise and counterclockwise sequences), as if the monkey realized that the third button was the final target to push in order to get reward.

Quantitative changes in task performance during learning of the three-push task are summarized in Fig. 8 and Table 1. The plots show the movement times of monkey KE for the first 18 days of training on the three-push task. An initial movement time (Fig. 8, A and C) was defined as the time interval from the release of the first button to the push of the second one. A serial movement time (Fig. 8, B and D) was defined as the time from release of the second button to the push of the third button. The initial movement times for movements with the arm contralateral to the MPTP infusion were significantly longer than those of the ipsilateral arm except for the first 3 days of learning in the clockwise series (Fig. 8A, for the 18 day averages, \( P < 0.0001 \), 2-tailed \( t \)-test). The serial movement times for the contralateral arm were also significantly longer than those of the ipsilateral arm, except for the first 4 days of learning (Fig. 8B, for the 18 day averages, \( P < 0.0001 \), 2-tailed \( t \)-test). Particularly striking was the variability in serial movement times for the contralateral arm, indicated by the large standard deviations (Fig. 8, B and D). Significant slowness of push button movements made with the limb contralateral to the dopamine depletion and large standard deviations also were observed in monkey GN (Table 1). These results confirmed the “hemi-Parkinsonian” status of the monkeys.

Unilateral dopamine depletion and changes in reaction times during learning of sequential motor tasks

To determine how prior dopamine depletion would affect the learning of motor sequences, we measured initial and serial reaction times through the learning process in monkey KE. This allowed us to compare performance in a situation that in the normal case would be reactive (initial reaction time) or predictive (serial reaction time). The initial reaction time was defined as the time between the onset of the second button light and the release of the first button. The serial reaction time was defined as the interval between the push of button 2 and the release of the same button in response to illumination of button 3.
When monkey KE underwent initial training on the two-push task having mastered the one-push task, his reaction times decreased for both arms. After the long hold time for button 1 (1 s), the reaction times for release of button 1 after illumination of button 2 decreased for the ipsilateral arm by ca. 91 ms from days 1 and 2 to days 5 and 6 (457–65 ms to 366–65 ms, \( P < 0.0001, \) 2-tailed \( t \)-test). For the arm contralateral to the MPTP infusion, the decrease was ca. 41 ms (332–119 ms to 286–65 ms). Thus the percent declines for the ipsilateral and contralateral arms were quite similar (20 and 14%, respectively).

The initial and serial reaction times of monkey KE during training on the three-push sequential task are shown in Fig. 9 for the first 18 days after he had mastered the one- and two-push tasks. The initial reaction times were quite similar for the arms ipsilateral and contralateral to the MPTP infusion. The average initial reaction times for the full 18 days of learning were not significantly different for the ipsilateral and contralateral arms in the clockwise direction (Fig. 9A). Those for the ipsilateral arm were actually longer than those for the contralateral arm for the counterclockwise direction on some days of testing (\( P < 0.0001, \) 2-tailed \( t \)-test, Fig. 9C). The initial reaction times did not undergo significant shortening for either arm throughout the 18 day learning period.

In contrast to the initial reaction times, the serial reaction times were significantly different for the arms ipsilateral and contralateral to the side of MPTP infusion. As illustrated in

![FIG. 9. Initial and serial reaction times for monkey KE during learning of the 3-push task. The period of training shown is the same as that for Fig. 8. Plots show mean values of 20–300 trials in a day. Error bars indicate SD. A and C: initial reaction times. B and D: serial reaction times for movements from the 2nd to the 3rd button. Best fitting logarithmic curves are superimposed on the plots (\( B, y = −378 \log x + 555 \) for \( \circ; y = −114 \log x + 319 \) for \( ●; y = −150 \log x + 384 \) for \( ○\)). *, significant difference between the values for the ipsilateral and contralateral arms at \( P < 0.0001 \) (2-tailed \( t \)-test).]
Fig. 9B, the serial reaction times for the ipsilateral arm showed a decrease of >200 ms during the first 4 days of training (from ~520 ms on the first 2 days to ~310 ms on the 3rd and 4th days of learning the clockwise sequence; \( P < 0.0001, \) 2-tailed t-test). The high values for the ipsilateral arm reflect the long time during which the monkey continued to press the second button, which had provided reward (cf. Figs. 6 and 7). During this time, he also kept licking the spoon without reward (cf. Figs. 13 and 14). By the third and fourth days, the serial reaction times had shortened significantly in parallel with the rapid decline in duration of the triceps muscle activity in the ipsilateral arm (Figs. 6 and 7). Average serial reaction times for the ipsilateral arm decreased further to 120 ms by day 12. Values for the counterclockwise sequence fell less precipitously but otherwise showed similar patterns.

The serial reaction times for the arm contralateral to the side of the MPTP infusion did not undergo a rapid early decline and showed a much smaller decline during the rest of training. For the clockwise sequences, they fell only ~120 ms from an average of 320 ms (day 1–2) to an average of 200 ms (day 10–18), and values for the contralateral arm were significantly longer than those for the ipsilateral arm after 12 days of training (\( P < 0.0001, \) 2-tailed t-test). For counterclockwise sequences, the serial reaction times for the contralateral arm were also longer than for the ipsilateral arm throughout the learning period (Fig. 9D). Thus the effects of unilateral MPTP infusion on the acquisition of the sequential-button-push task appeared selectively for the arm contralateral to the side of MPTP infusion in both clockwise and counterclockwise sequences.

In performing the three-push task with the arm contralateral to the side of MPTP infusion, monkey KE continued for >12 days of training to lick for reward after the second push, which had been correct behavior in the previously learned two-push task. In the ipsilateral arm task, monkey KE almost completely abandoned the previously correct behavior in 6 days of training. This suggests that the monkey realized that the third rather than the second button was the reward-associated button after a few days of training when he performed with his ipsilateral arm, but that it took over 12 days for the monkey to realize the change of reward-associated button when he performed with his contralateral arm. This perseveration appeared to account for the lack of early decline in the serial reaction time for the contralateral arm.

Unilateral dopamine depletion abolishes the ability to learn predictive, preprogrammed movements in sequential motor tasks performed with the limb contralateral to the side of depletion

To visualize the temporal characteristics of the sequential button pushes by monkey KE, we constructed time trajectories for the three button pushes (Fig. 10). These time trajectories illustrate the averaged envelope of the movements by showing the initiation of the presses (rise times), the hold times (flat phases), and the releases (falling phases). Figure 10A depicts schematically how the time trajectory was calculated. The average time trajectories for clockwise sequences are plotted in Fig. 10, B and D, and those for counterclockwise sequences in Fig. 10, C and E. These plots were based on the performance of the three-push task during the fourth week of training on the three-push task. The average value for the hold times for each button are shown in Table 2.

The time trajectory plots show that the temporal pattern of sequential push movements was different for the arms ipsilateral and contralateral to the side of dopamine depletion. With the ipsilateral arm (Fig. 10, D and E), after the required long hold time for the first button, the second button was depressed for a brief time (230 ms for clockwise and 350 ms for counterclockwise sequences, determined by the time trajectory plot), and this was followed by a relatively long hold time for the third button (610 ms for clockwise and 580 ms for counterclockwise sequences), after which the monkey received reward water. The respective peak times for the second and third pushes in the average time trajectories (i.e., most frequent times of occurrence of the pushes) were at 490 and 800 ms after the go signal for clockwise sequences and 560 and 920 ms afterward for counterclockwise sequences. There was considerable overlap of the average first-, second-, and third-push time trajectories.

By contrast, for the pushes that monkey KE made with his contralateral arm (Fig. 10, B and C), the average time trajectories for the three button pushes did not overlap. The different push movements clearly were separated. The average durations of the button pushes determined by the average time trajectories for the second and third buttons were, respectively, 340 and 910 ms for clockwise sequences and 460 and 600 ms for counterclockwise sequence. The average durations of pushes for the second and third buttons were longer for the contralateral arm than for the ipsilateral arm (by, respectively, 110 and 300 ms for clockwise, and 110 and 20 ms for counterclockwise directions). The total times from the go signal to the release of the third button were also longer, by 710 ms in the clockwise direction and by 370 ms in the counterclockwise direction.

Unexpected task switch exposes automatic preprogrammed movement sequence

Despite the quantitative differences between movements made with the ipsilateral and contralateral arms during learning, these differences were not obvious on qualitative inspection. However, when we tested the monkey KE in an experimental paradigm involving an unexpected task switch, we found a clear qualitative difference in his performance when using the arms ipsilateral and contralateral to dopamine depletion.

The switch-task trials were introduced after the monkey had mastered the three-push task and his serial reaction times had shortened to asymptotic levels after 4–6 wk of training on the task. Then, during infrequent, randomly spaced trials, the task was switched from the three-push task currently being performed to the previously learned two-push task. The task switch experiments were performed once every 4–7 day during otherwise normal training. In the switched two-push task, when the monkey pushed the illuminated second button, the button 2 light was turned off and the liquid reward and associated click were given. The third button was left unilluminated. Thus, the monkey was given both visual and auditory stimuli indicating that the reward now would come after the button 2 press.

Figure 11 shows schematically the timing of button presses before (a) and after (b and c) the task switch for pushes made with the contralateral (Fig. 11B) and ipsilateral (Fig. 11D)
FIG. 10. Temporal patterns of 3 sequential button pushes ("time trajectories") made by monkey KE. A: diagram showing how the time trajectory curves were obtained. In each trial, 3 button pushes are represented as 3 rectangles in which the time axes of both the abscissa and ordinate are durations of button pushes (a in A), and, in the abscissa, the 3 rectangles are arranged based on the times of push and release relative to the onset of go signal (time 0). Time from the push of button 1 to the onset of the go signal was fixed and was 1 s. In A, 3 rectangles obtained in 3 trials calculated in this way are superimposed. A time trajectory curve was then obtained by averaging ordinate values of the rectangles. B and D: average time trajectories in clockwise sequence. Values of means and SD are plotted. C and E: counterclockwise sequence. Vertical tick marks below the traces indicate the times at which the monkey pushed the 3rd button. Ipsilateral hand performance is shown on the right (D and E), contralateral hand performance on the left (B and C). In the average time trajectories, the onset of a push was determined as a time when the average hold time became significantly greater than the baseline (0), 2-tailed t-test, P < 0.01. Button-release time was determined as the time when the average hold time became so short that it was not significantly greater than the baseline. Hold times determined by the average time trajectories for the 3-push movements relative to the go signal are longer than the average of hold times of each button push (Table 2), because they include movement times and reaction times.
after the task switch had a temporal pattern similar to that of before the task switch (700 vs. 710 ms). The average onset of the automatic third button pushes was 700 ms after the GO signal. The average durations of pushes for the second and third buttons after the switch were 1,440, 410, and 550 ms, respectively, as compared to values of 1,370, 350, and 580 ms before the switch. The peak trajectory times of pushes for the second and third buttons after the GO signal were 610 and 930 ms, respectively, after the switch, and 560 and 920 ms before the switch. The average time trajectories for button 2 and 3 pushes overlapped each other considerably even more than they did before the switch. In the switched task, the reward-associated clicks (vertical ticks in Fig. 12E) appeared immediately after the second-button push (520 ± 72.7 ms after the GO signal). The average onset of the automatic third button pushes was 700 ms after the GO signal, indicating that the automatic pushes occurred as late as 180 ms after the onset of the reward-associated clicks. Thus the monkey should have had enough time to detect the clicks before the third button push. Nevertheless, the average onset time of the third button pushes after the task switch was similar to that before the task switch (700 vs. 710 ms).

For the arm contralateral to the side of the MPTP infusion, the three-push task was missing, but the two pushes that were made after the task switch had a temporal pattern similar to that of the first and second pushes in the three-push task before the task switch. Both contralateral arm pushes showed a greater separation between the first and second holds and longer second hold durations than the ipsilateral arm pushes (Fig. 12, D and E). The average durations of pushes for the first, second, and third buttons after the switch were 1,440, 410, and 550 ms, respectively, as compared to values of 1,370, 350, and 580 ms before the switch. The peak trajectory times of pushes for the second and third buttons after the GO signal were 610 and 930 ms, respectively, after the switch, and 560 and 920 ms before the switch. The average time trajectories for button 2 and 3 pushes overlapped each other considerably even more than they did before the switch. In the switched task, the reward-associated clicks (vertical ticks in Fig. 12E) appeared immediately after the second-button push (520 ± 72.7 ms after the GO signal). The average onset of the automatic third button pushes was 700 ms after the GO signal, indicating that the automatic pushes occurred as late as 180 ms after the onset of the reward-associated clicks. Thus the monkey should have had enough time to detect the clicks before the third button push. Nevertheless, the average onset time of the third button pushes after the task switch was similar to that before the task switch (700 vs. 710 ms).

Unilateral dopamine depletion not only induced slow movement times and prolonged serial reaction times for movements made with the contralateral arm but also affected other aspects of the monkeys’ behavior. As they performed the sequential

### TABLE 2. Hold times of button push in monkey KE

<table>
<thead>
<tr>
<th></th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
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<tbody>
<tr>
<td></td>
<td>Hold time</td>
<td>Hold time</td>
<td>Hold time</td>
</tr>
<tr>
<td>n</td>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
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<tr>
<td></td>
<td>n (trials)</td>
<td>1st (ms)</td>
<td>2nd (ms)</td>
</tr>
<tr>
<td>Ipsilateral hand</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Before task switch</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CW</td>
<td>87</td>
<td>1332.8 ± 59.6</td>
<td>107.4 ± 22.5</td>
</tr>
<tr>
<td>CCW</td>
<td>93</td>
<td>1324.5 ± 41.9</td>
<td>207.0 ± 52.1</td>
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<tr>
<td>After task switch</td>
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<td></td>
<td></td>
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<tr>
<td>CW</td>
<td>36</td>
<td>1341.6 ± 68.4</td>
<td>105.3 ± 40.5</td>
</tr>
<tr>
<td>CCW</td>
<td>84</td>
<td>1368.2 ± 54.5*</td>
<td>161.6 ± 50.2*</td>
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<tr>
<td>Contralateral hand</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before task switch</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CW</td>
<td>99</td>
<td>1356.1 ± 43.1</td>
<td>188.6 ± 31.8</td>
</tr>
<tr>
<td>CCW</td>
<td>114</td>
<td>1314.5 ± 31.5</td>
<td>287.0 ± 46.0</td>
</tr>
<tr>
<td>After task switch</td>
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<tr>
<td>CW</td>
<td>51</td>
<td>1349.0 ± 45.1</td>
<td>195.9 ± 52.4</td>
</tr>
<tr>
<td>CCW</td>
<td>62</td>
<td>1337.2 ± 39.2*</td>
<td>279.1 ± 62.7</td>
</tr>
</tbody>
</table>

Values are means ± SD in milliseconds. The task was switched from three push to two push in monkey KE. —, no data because of no trial. * Significant difference between before and after the task switch for the same direction at $P < 0.0001$ (2-tailed t-test). n, number of trials. CW and CCW, clockwise and counterclockwise sequence, respectively.
motor tasks, the monkeys made orofacial movements to consume liquid reward in addition to movements of the arms and eyes. To perform the tasks efficiently, they needed to learn a coordinated action strategy, which required activation of different body parts in the correct temporal order. For instance, arm muscles such as the triceps and biceps brachii and wrist extensor and flexors were activated during the sequential push-button movements, and then orofacial muscles were activated as the monkey consumed the reward delivered after the last push button movement. With training, the temporal pattern of activation of different body parts during the task performance became stereotyped when the monkeys used the arm ipsilateral to the side of dopamine depletion. By contrast, there was a striking retardation of the acquisition of such coordinated sequential movement patterns when the monkey used the arm contralateral to the side of dopamine depletion.

Figure 13 illustrates for monkey KE, representative activities of arm (triceps brachii) and orofacial (digastric) muscles during the course of the initial 12 days of training on the three-push task after the monkey had learned the one- and two-push tasks. During the first 2–3 days of training with the right arm (ipsilateral to the MPTP infusion), the monkey licked the spoon in front of his mouth when he pushed the second button, which had been the appropriate temporal order in the previous two-push task in which he expected reward delivery at button 2. The licks at the second push occurred in both clockwise (Fig. 13, right) and counterclockwise sequences (Fig. 14, right). The reward and the reward-associated click were now delivered after the third push, and the monkey licked the spoon again, after the third push, and he consumed the delivered liquid reward. The licks after the second push suggest that the monkey did not recognize that the task had been switched from the well-learned two-push task to the new three-push task. After 5 days of training, the monkey appeared to realize that reward would not be delivered after the second push because he stopped licking after the second push in most of the trials. In parallel with this change, activation of prime mover muscles for the second push became shorter in duration and shifted to the next activation for the third push (cf. Figs. 6 and 7). During the following several days of training, the duration of prime mover muscle activation and the interval between the second and the third push became shorter and shorter.

Remarkably, when monkey KE used the arm contralateral to
MPTP infusion during the same training period, he kept trying to lick at the second push for 8–12 days, even though by day 5 he no longer licked at button 2 when he used his ipsilateral arm (compare Figs. 13 and 14, left and right). Figure 15 plots the probability of occurrence of licks at the second push as a function of days of training. When the monkey used the arm ipsilateral to the MPTP infusion, licks at the second push occurred in <30% of trials after 6 days of training and in <5% of trials after 12 days of training. The declines occurred for both clockwise (Fig. 15A) and counterclockwise (Fig. 15B) sequences. When the monkey used his contralateral arm, there was no steady decline in licks after the second push. They declined only briefly, mainly for the clockwise series, for the first 3 days after the switch. The duration of activation of prime mover muscles for the second push also was still long even on the 8–10th day of training (Figs. 13 and 14, left). These observations suggest that the monkey was still expecting reward at the second push when he used his contralateral arm even after 8–12 days of training, when he was no longer showing signs of expecting reward at the second button when he used the ipsilateral arm.

Eye movements before and after unilateral dopamine depletion

In agreement with the findings of Miyashita et al. (1995), we observed that both monkeys showed a tendency to pay less attention to visual objects appearing in the visual field contralateral to the MPTP infusion and tended to make fewer saccades to that side. We therefore examined eye movements as well as hand movements during the sequential button push tasks. Saccadic eye movements to each of the buttons occurred in addition to the arm movements. After learning, the saccades led movements of the ipsilateral arm during performance of the push button tasks (Fig. 16), suggesting that a coordinated, predictive eye-hand strategy had been acquired. There was greatly increased variability in the relative timing of the saccades and hand releases when the monkeys used the arm contralateral to the side of dopamine depletion.

Figure 16 illustrates the timing of the onset of saccades from the first button to the second one relative to the times of hand release from button 1 made by monkey KE. In this monkey, not only the putamen but also the caudate nucleus was severely depleted of TH-like immunoreactivity, including the region.
representing oculomotor function (Hikosaka et al. 1989; Parthasarathy et al. 1992). Figure 16, A–D, left, illustrates superimposed traces of vertical saccades from the first (hold) button to the second button. The eye movement traces are centered at the saccade onset, and the times at which the monkey released the first button after illumination of the second button are marked by the vertical ticks above the traces. Two representative blocks of eye movement traces are illustrated. Figure 16, A–D, right, plots times of release of the first button relative to saccade onset. The plots show that saccade

FIG. 13. Activities of arm muscles (triceps brachii) and orofacial muscles (digastrics) during the course of the initial 12 days of training of monkey KE on the 3-push task after the monkey had learned the 1- and 2-push tasks. Numbers on the right of traces indicate the occurrence of licks between the 2nd and 3rd button pushes (cf. Fig. 15). Muscle activity during clockwise sequence is shown. Symbols are the same as in Figs. 6 and 7.
onset led hand release by \(-120\) ms on average when the monkey used the arm ipsilateral to the MPTP infusion (Fig. 16, C and D). With the contralateral arm, however, the lead times were smaller (average, 87.9 ms in the clockwise sequence; 99.2 ms in the counterclockwise sequence), and hand release occurred first and thus without guidance of saccades in a considerable number of trials, especially for the clockwise sequence (Fig. 16A). The trial-by-trial variations in relative saccade onset-hand release timing were greater when the monkey used the arm contralateral to the MPTP infusion than when he used his ipsilateral arm (6,128 vs. 3,592 for the clockwise sequence, and 3,896 vs. 2,935 for the counterclockwise sequence).

In addition to these ipsilateral and contralateral arm asymmetries, variations in relative saccade-hand release timing were...
larger in the clockwise sequences in which the initial saccade and hand movement went toward the hemifield contralateral to the MPTP infusion (ipsilateral hand, variance 3,592; contralateral hand, variance 6,128). In the counterclockwise sequences, in which the initial saccades and arm movements went toward the ipsilateral hemifield, the variance was 2,935 for the ipsilateral arm and 3,896 for the contralateral arm. The lowest variance in relative timing of saccade onset and hand release occurred when the monkey used the ipsilateral arm and performed the counterclockwise sequence, pressing the button in the ipsilateral half of extrapersonal space.

Unilateral dopamine depletion and change in reaction times during sequence retrieval and relearning

To examine the effects of dopamine depletion on the retrieval of learned sequences and on the relearning of sequences, we measured initial and serial reaction times in monkey GN, which received MPTP after learning the one- to four-push tasks. Figure 17, A and B, illustrates the monkey’s performance before MPTP was given, after he had mastered the one-, two-, and three-push tasks and was learning the four-push task. Figure 17, C and D, illustrates the changes in reaction times as the monkey was tested on the four-push task after MPTP had been infused into the left striatum. The serial reaction times shown are for movements from the second to the third button.

During monkey GN’s original training on the four-push task before MPTP infusion, his serial reaction times from the second to the third button showed relatively small but significant shortenings for both the right arm and left arm tasks for both the clockwise and counterclockwise sequences (Table 3). The monkey’s performance showed an arm asymmetry, however, because in the clockwise sequence, the reaction times were shorter for the right than for the left arm (Fig. 17A), whereas in the counterclockwise sequence, the reaction times were shorter for the left than for the right arm (Fig. 17B). The initial reaction times were shorter for the left arm for both directions of movement during the initial 4 days of training, but the difference became insignificant at the later stages of training (Table 3). These directional differences may have reflected a behavioral habit acquired during training.

During retraining on the four-push task after unilateral infusion of MPTP, no significant difference was observed between the initial reaction times from the first to the second button in the ipsilateral hand task and those in the contralateral hand task (Table 3). The serial reaction times from the second to the third button for the ipsilateral hand were short and stable for both the clockwise and counterclockwise sequences (Fig. 17, C and D). The serial reaction times during the initial relearning in the clockwise sequence (Fig. 17C, day 1–day 4, average 133.2 ± 42.0 ms) were slightly longer than those during the late stage of original learning (Fig. 17A, day 10–15, average 113.6 ± 29.8 ms). Contralateral to the side of MPTP infusion, serial reaction times during the first 4 days of retraining were longer than those of ipsilateral arm task, with one exception (day 4 for the clockwise sequence, Fig. 17C, day 4 for the counterclockwise sequence, Fig. 17D). In the clockwise sequence, the serial reaction times for movements with the contralateral arm (day 1–4, average 210.5 ± 100.3 ms, Table 3) were much longer than the stable serial reaction times before MPTP (day 10–15, average 85.8 ± 16.0 ms, Table 3). Similarly, in the counterclockwise sequence, the serial reaction times for movements with the contralateral arm (day 1–4, average 140.7 ± 71.4 ms, Table 3) were longer than the stable serial reaction times before MPTP (day 10–15, average 92.2 ± 18.6 ms, Table 3). On the other hand, the long serial reaction times for the contralateral arm diminished significantly through ~2 weeks of retraining, and the difference in the reaction times between the ipsilateral and contralateral arm became much smaller not only in the clockwise sequence (Fig. 17C) but also in the counterclockwise sequence (Fig. 17D).

**DISCUSSION**

Our findings strongly suggest that the ability of monkeys to learn and to retrieve sequential motor tasks depends on the striatum and an intact nigrostriatal dopamine system. Our evidence for a role of the basal ganglia in procedural learning is in good accord with evidence for procedural learning defects in patients with Parkinson’s disease (Knowlton et al. 1996; Pascual-Leone et al. 1993; Saint-Cyr et al. 1988) and in rodents with lesions of the caudoputamen (McDonald and White 1993). Our evidence further explicitly implicates the striatum and its nigral afferents in the development of predictive movement sequences involving changes in coordination of orofacial and eye-hand movements and efficient linkage of succeeding movements to make whole goal-oriented action sequences. Our results provide the first demonstration of spe-
specific defects in learning and memory of motor sequences induced by selective lesion of the nigrostriatal dopamine system.

The strategy of making unilateral MPTP lesions and comparing performance with the limbs ipsilateral and contralateral to the lesion allowed us to study lesion-induced deficits (contralateral) in relation to control performance (ipsilateral) in the same animal. Much evidence favors a predominant representation of contralateral body parts in the striatum and globus pallidus, with some exception for the face (Alexander and DeLong 1985b; Flaherty and Graybiel 1993; Kimura 1990; Ohno and Tsubokawa 1987; Schneider and Lidsky 1981). Some ipsilateral representation also exists, however (Alexander and DeLong 1985a; Filion et al. 1988; Flaherty and Graybiel 1993; Merchant et al. 1997; Schneider and Lidsky 1981; West et al. 1990), so that the control (ipsilateral) side was probably not normal. We emphasize that the comparisons we made indicate the performance of the limbs with relatively intact (ipsilateral) and relatively deficient (contralateral) nigrostriatal control.

Deficits in motor sequence learning are induced by nigrostriatal dopamine depletion

The monkeys in our experiments exhibited defects in performing sequential motor tasks after MPTP infusion when they used the arm contralateral to the side of infusion. Monkey KE, while he learned the button push task after MPTP infusion, showed a progressive shortening in serial reaction times characterizing movements with his ipsilateral arm, but he failed to show this shortening when using his contralateral arm. Moreover, the pushes that monkey KE made with his contralateral arm remained discrete movements and failed to acquire the overlapped time trajectories that characterized his ipsilateral pushes. Monkey GN, which learned the push task before MPTP infusion, showed similar defects in the contralateral arm task early during retraining after the MPTP infusion but he recovered. Together, our results thus point to participation of the striatum and its nigrostriatal afferents both in the acquisition and the retrieval of movement sequences.

In the intact, well-trained monkey, serial reaction times were significantly longer when the sequences of button pushes were randomized than when they were fixed (monkey TZ, Fig. 5). This suggests that the movements from the second to the third button were not guided by the visual stimuli (button 2 light off and button 3 light on) but were performed in a predictive, preprogrammed manner. By contrast, the movements from the first to the second button were by the nature of the task necessarily guided by the visual cue. The overlapped time trajectories of serial button pushes observed for monkey KE’s ipsilateral arm movements would not have been possible if each movement were guided by external visual cue, but would have been possible if the movements were performed in a predictive, preprogrammed manner. Predictive, preprogrammed movements can be accomplished through acquisition of a strategy to perform multiple movements as a sequence of
movements. Specifically what is needed is a procedure to link effectively a single movement to the next movement. When monkey KE performed the serial button-push tasks using the arm ipsilateral to the dopamine depletion, consistent eye-hand coordination was observed. A saccadic eye movement directed to the next target button lead the next button press consistently by ca. 120 ms (Fig. 16). In sharp contrast, saccades did not consistently lead button presses made with the arm contralateral to dopamine depletion. Although eye-hand coordination may not be essential for the execution of sequential movements, it is a mechanism that would allow linking one movement to the next movement. Similarly, when monkey KE started to learn the three-push task after he had learned the two-push task, he could, with the ipsilateral arm, smoothly discard a strategy for arm and orofacial movements that had been appropriate in the already learned two-push task and develop a new strategy for the three-push task. With the contralateral arm, however, the monkey persisted in using the previously learned motor strategy for a long period of time and tried to lick at the second push, for which no reward was delivered (Figs. 13–15). Previous observations have suggested that disruption of neural activity within the basal ganglia re-

![Image](https://example.com/image.png)

**FIG. 17.** Serial reaction times from the 2nd to the 3rd button for monkey GN before and after MPTP infusion. A and B: performance of the 4-push task after monkey had learned the 1-, 2-, and 3-push tasks but before MPTP infusion. C and D: relearning after MPTP infusion. *, significant difference between values for ipsilateral and contralateral arms at $P < 0.0001$ (2-tailed $t$-test).

### Table 3. Initial and serial reaction times in monkey GN

<table>
<thead>
<tr>
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<th>Initial</th>
<th></th>
<th>Serial</th>
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<tbody>
<tr>
<td></td>
<td>Clockwise</td>
<td>Counterclockwise</td>
<td>Clockwise</td>
<td>Counterclockwise</td>
</tr>
<tr>
<td><strong>Before MPTP</strong></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Right hand</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 1–4</td>
<td>495.5 ± 68.6</td>
<td>423.6 ± 37.1</td>
<td>125.7 ± 33.2</td>
<td>169.1 ± 33.0</td>
</tr>
<tr>
<td>Days 10–15</td>
<td>322.7 ± 53.9</td>
<td>320.9 ± 53.2</td>
<td>85.8 ± 16.0†</td>
<td>92.2 ± 18.6†</td>
</tr>
<tr>
<td>Left hand</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days 1–4</td>
<td>381.8 ± 57.4*</td>
<td>364.7 ± 58.1*</td>
<td>165.4 ± 58.1</td>
<td>100.1 ± 36.7</td>
</tr>
<tr>
<td>Days 10–15</td>
<td>321.0 ± 64.0</td>
<td>311.8 ± 62.1</td>
<td>113.6 ± 29.8†</td>
<td>83.8 ± 15.9†</td>
</tr>
<tr>
<td><strong>After MPTP</strong></td>
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<tr>
<td>Right (Contralateral) hand</td>
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<tr>
<td>Days 1–4</td>
<td>323.8 ± 72.4</td>
<td>344.9 ± 95.2</td>
<td>210.5 ± 100.3</td>
<td>140.7 ± 71.4</td>
</tr>
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<td>Days 10–15</td>
<td>335.7 ± 63.9</td>
<td>382.3 ± 80.4</td>
<td>127.0 ± 20.1†</td>
<td>108.8 ± 26.5†</td>
</tr>
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<td>Left (Ipsilateral) hand</td>
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<td></td>
</tr>
<tr>
<td>Days 1–4</td>
<td>330.8 ± 67.5</td>
<td>380.0 ± 82.1</td>
<td>133.2 ± 42.0</td>
<td>72.4 ± 13.8</td>
</tr>
<tr>
<td>Days 10–15</td>
<td>326.5 ± 41.2</td>
<td>361.6 ± 63.9</td>
<td>114.5 ± 34.0†</td>
<td>91.3 ± 14.2†</td>
</tr>
</tbody>
</table>

Values are means and SD of initial and serial reaction times in milliseconds from the first to the second and second to the third button, respectively, for the first 15 days of learning four-push tasks in monkey GN. MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. † Significant difference between the serial reaction times during days 1–4 and days 10–15 for the same hand and same direction ($P < 0.0001$, 2-tailed $t$-test).
results in selective learning deficits in tasks requiring switching between response strategies (Phillips and Carr 1987). We suggest that part of the difficulty in ‘‘switching’’ is due to a decreased capacity to link single movements to the next in a movement sequence in a predictive, preprogrammed manner.

The task switch trials that we carried out in monkey KE provided critical evidence that an intact nigrostriatal system is necessary for learning to perform motor sequences in a predictive, preprogrammed manner. Monkey KE, which learned the push button task for the first time after MPTP infusion, continued to push the unilluminated third button after the unexpected switch to the two-push task when he used his ipsilateral arm, and the automatically executed pushes of the third button had time trajectories very similar to those of the three-push task. This pattern suggests that, with the arm contralateral to the (relatively) intact nigrostriatal system, the monkey predictively retrieved learned programs for the three-push task in spite of an auditory cue informing him that reward would come at button 2 and in spite of the visual cue to move to button 3. When the same monkey used the arm contralateral to the side of dopamine depletion, there was a total absence of automatic execution of button 3 pushes. This behavior suggests that, in the absence of an intact nigrostriatal system, the monkey failed to link together the sequential button-push movements. This interpretation would be in accord with the suggestion that striatal processing helps to ‘‘chunk’’ sequential acts into performance units (Graybiel 1998).

Alternative deficits could have accounted for the lack of development of automatically initiated pushes with dopamine depletion. One is that there may have been a motor imbalance between the two arms or a sensory imbalance between the sides ipsilateral and contralateral to the dopamine depletion. It has been demonstrated that neurons in the internal segment of the primate globus pallidus (GPi) increase their discharge frequency after systemic MPTP injection and increase their sensory responsiveness (Filion et al. 1988; Miller and DeLong 1986). Because GPi inhibits the motor thalamus, this suggests that there would be an increase in inhibitory outputs of the basal ganglia to limb movement mechanisms, resulting in slowness of movement of the contralateral limbs. Defects in eye movements similarly could reflect abnormalities in the inhibitory pathways from the substantia nigra pars reticulata to the thalamus and the superior colliculus (Chevalier and Deniau 1990; Hikosaka and Wurtz 1985). The performance deficits could also result from imbalanced sensory processing or attention to the two sides. Sensory events from the side opposite the striatal dopamine depletion might be so poorly processed or neglected that movements toward the contralateral side would become suppressed as suggested by Miyashita et al. (1995).

However, we do not think that the slowness of movement, poor ability to perform motor tasks in general or sensory neglect were the principal reasons for the deficits in motor sequence learning that we found when monkey KE used his contralateral arm. The nigrostriatal dopamine depletion did not prolong the initial reaction times. In fact, the initial reaction times for monkey KE actually shortened as he learned the two-push task. These observations are consistent with previous observations in patients with Parkinson’s disease (Harrington and Haaland 1991; Jahanshahi et al. 1992). By contrast, the dopamine depletion did prolong the serial reaction times from the second to the third button. These results indicate that the process of initiation of visually guided movement of the contralateral arm was not impaired. Rather, the predictive, preprogrammed movements linking the second to the third push were specifically impaired when he used the arm contralateral to prior dopamine depletion. This is in accord with previous studies showing not only defects in simple and choice reaction times but clear defects in serial reaction times in patients with Parkinson’s disease (Evarts et al. 1981; Jackson et al. 1995; Jahanshahi et al. 1992; Pascual-Leone et al. 1993). The increase in the serial reaction times from the push of the second button to the release of the same button to move to the third button clearly would have given monkey KE more time to notice the changed cues in the switch trials performed with his contralateral arm, and this could have led him to stop at the second button, in contrast to his performance with the ipsilateral hand. However, the remarkable contralateral arm deficits occurred not only in the sequences in which the eye and arm moved toward the side ipsilateral to dopamine depletion but also in the sequences in which they moved toward the side contralateral to dopamine depletion. Thus contralateral neglect does not adequately account for the defects observed. We favor the view that monkey KE’s performance deficits reflected impairment of a mechanism to link one movement to the next effectively in a predictive, preprogrammed manner. This interpretation fits with the other defects that this monkey demonstrated in reprogramming orofacial-hand and eye-hand strategies.

Our findings for monkey GN, which learned the task before MPTP infusion and then relearned it, also support this view. Monkey GN did make automatic extra pushes when he used the arm contralateral to the dopamine depletion (data not shown), suggesting that he had learned the sequence of movements as a single motor program. However, the monkey made far fewer automatic extra pushes with the contralateral arm. Monkey GN had a larger zone of dopamine depletion than monkey KE, and interpretation of data for this monkey was complicated by the fact that he had deficits in contralaterally directed eye movements as well as limb movements. Nevertheless, his initial reaction times were much less impaired than his serial reaction times, suggesting that he had adequate movement and sensory attention for the task when using his contralateral arm. Taken together, our findings in the two monkeys support the view that the striatum and its nigrostriatal dopamine innervation are necessary for both the acquisition and the retrieval of programs for sequential movements. Striatal neurons in monkeys trained to perform sequential button push tasks have been shown to fire specifically in relation to particular phases of sequential push button tasks (Kermadi and Joseph 1995; Kimura et al. 1992). Such neurons, and the nigrostriatal modulation of their activity, could be part of the striatal mechanism for coding sequence information.

Basal ganglia are involved in the acquisition, retrieval, and relearning of programs for sequential motor tasks

Imaging and electrophysiological recording studies suggest that several parts of the motor/premotor cortex are activated during motor learning tasks. In humans, functional magnetic resonance imaging (fMRI) studies indicate that primary motor cortex (Karni et al. 1995, 1998) and the pre-SMA (Boecker et al. 1998; Hikosaka et al. 1996; Sakai et al. 1998) are specifi-
cally activated during the performance of motor learning tasks. In monkeys, neuronal activity in the pre-SMA (Matsuzaka and Tanji 1996; Shima et al. 1996), premotor cortex (Mitz et al. 1991), and supplementary eye field (Chen and Wise 1995a,b, 1996) changes when monkeys switch from one motor sequence to another or acquire new sensory-motor associations. In all of these recording studies, the modifications of cortical neuron activity occurred in the first several trials after conditions were changed but then disappeared. By contrast, we have found prolonged changes in neural activity in the striatum during behavioral conditioning (Aosaki et al. 1994b; Jog et al. 1997; Kubota et al. 1998). The recording evidence for the striatum and our present findings suggest that the basal ganglia are involved in learning that develops more slowly and implicitly as sequential motor tasks are acquired.

Increasing evidence suggests that there are multiple mechanisms for motor learning, involving early and late stages of learning and interactions between procedural (implicit) and declarative (explicit) learning and memory systems. Karni et al. (1995, 1998) have shown that there are slow and fast phases of learning sequential finger movements in the human and have documented involvement of primary motor cortex in both. Other imaging studies indicate a shift from anterior frontal to posterior frontal and parietal cortical activation as learning shifts from declarative to procedural stages (Jueptner et al. 1997; Peterson et al. 1998; Sakai et al. 1998). At the behavioral level, it has been suggested that the acquisition of declarative (explicit) knowledge hastens the acquisition of procedural (implicit) knowledge through repeated practice (Brooks 1986; Pascual-Leone et al. 1993). Parkinson’s patients, with depleted levels of striatal dopamine, have been shown to be capable of learning and transferring rule or schema-based representations in an explicit learning format, based on the observations on serial reaction time tasks (Dominy and Jeannerod 1997; Dominy et al. 1997). Our evidence suggests that the striatum and nigrostriatal dopamine system are essential elements of the memory network underlying the acquisition and retrieval of motor programs to perform sequential motor actions in a predictive manner in an implicit learning format.

In previous work, we found that the responses of striatal tonically active neurons acquired during sensory-motor conditioning almost completely disappear when the nigrostriatal dopamine system is depleted by the same intrastratal MPTP infusion method used here (Aosaki et al. 1994a). On the basis of this evidence, we proposed that the nigrostriatal dopamine system is responsible for maintaining the acquired neuronal activity in the striatum and for retrieving learned activity from long-term memory storage (Graybiel et al. 1994). Consistent with this view is the observation by Schultz and his colleagues that dopamine-containing neurons may report an error in the prediction of reward during learning (Hollerman and Schultz 1998; Schultz et al. 1997). These characteristics support the hypothesis of a reinforcement learning role for the midbrain dopamine system (Barto 1994; Houk et al. 1994). Interestingly, our results show that nigrostriatal dopamine depletion results in deficits in learning new motor sequence tasks and in retrieving previously learned motor sequences but spares the ability to relearn postoperatively sequential motor tasks originally learned before the nigrostriatal dopamine depletion. This suggests a highly differentiated involvement of the striatum and its nigrostriatal system in motor learning.

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MATSUMOTO, KANAWA, MAKI, GRAYBIE, AND KIMURA


