Exp Brain Res (1997) 115:1–5

RESEARCH ARTICLE

Shigehiro Miyachi · Okihide Hikosaka
Kae Miyashita · Zoltán Kárádi · Miya Kato Rand

Differential roles of monkey striatum in learning of sequential hand movement

Received: 20 July 1996 / Accepted: 13 November 1996

Abstract To study the role of the basal ganglia in learning of sequential movements, we trained two monkeys to perform a sequential button-press task (2×5 task). This task enabled us to examine the process of learning new sequences as well as the execution of well-learned sequences repeatedly. We injected muscimol (a GABA agonist) into different parts of the striatum to inactivate the local neural activity reversibly. The learning of new sequences became deficient after injections in the anterior caudate and putamen, but not the middle-posterior putamen. The execution of well-learned sequences was disrupted after injections in the middle-posterior putamen and, less severely, after injections in the anterior caudate/putamen. These results suggest that the anterior and posterior portions of the striatum participate in different aspects of learning of sequential movements.

Key words Procedural learning · Basal ganglia · Caudate · Putamen · Muscimol · Monkey

Introduction

The role of the basal ganglia in learning has been suggested by recent experimental studies. They include classical conditioning (Graybiel et al. 1994), reinforcement learning (Dunnett and Iversen 1982; Schultz and Romo 1990; Robbins and Everitt 1996), and procedural or implicit learning (Butters et al. 1985; Squire 1986; Saint-Cyr et al. 1988; Knopman and Nissen 1991; Knowlton et al. 1996). Cellular mechanisms have been proposed that might underlie these types of learning (Calabresi et al. 1996). Such experimental findings have stimulated theoretical approaches to formulate functional models of the basal ganglia (Dominey et al. 1995; Hikosaka 1994; Houk, et al. 1995).

We have been studying learning of sequential hand movements by training monkeys on a sequential button-press task, called the "2×5 task" (Hikosaka et al. 1995a). Our previous behavioral analysis suggested that learning proceeded at different levels: short-term sequence-selective, long-term sequence-selective, and long-term sequence-unselective processes. As the learning of a given sequence reached the long-term stage, the performance became extremely skillful, with anticipatory eye and hand movements, but on the other hand became less flexible (e.g., less likely to be transferred to the other hand; Miyashita et al. 1996). These results suggested that the neural systems necessary for short-term learning of new sequences (learning mechanism) may be separate from the neural systems for the storage or retrieval of long-term memories (memory mechanism).

This hypothesis led us to a prediction that can be tested experimentally: a selective lesion of the learning mechanism would produce a difficulty in learning of new sequences, while leaving the performance of well-learned sequences intact; a selective lesion of the memory mechanism would produce a difficulty in performing well-learned sequences, while leaving the new learning intact.

The 2×5 task was ideal for this experiment because we can generate a practically unlimited number of new sequences. Instead of lesioning, we injected muscimol (a GABA agonist) to block the local neural activity reversibly. These experimental procedures allowed us to examine the functions of different parts of the basal ganglia repeatedly in the same animal.

S. Miyachi (✉)[1] · O. Hikosaka[1] · K. Miyashita[1] · Z. Kárádi[2]
M. K. Rand[3]
Laboratory of Neural Control, National Institute for Physiological Sciences, Okazaki 444, Japan

Present address:
[1] Department of Physiology, Juntendo University, School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113, Japan; Fax: 81-3-3813-4954, e-mail: miyachi@med.juntendo.ac.jp
[2] Institute of Physiology, Pécs University Medical School, Szigeti Street 12, Pécs, H-7643, Hungary
[3] Motor Control Laboratory, Arizona State University, Tempe, AZ 85287-0404, USA

Materials and methods

This study was performed using two male Japanese monkeys (*Macaca fuscata*): monkey PI and monkey ME (8–9 kg). They were kept in individual primate cages in an air-conditioned room where food was always available. They were carried to the experimental room in a primate chair before each experimental session. The monkeys were given a restricted amount of fluid during training and experiments. Their health conditions such as body weight and appetite were checked daily. Supplementary water and fruit were provided daily. Animal care and experiments were in accordance with *Principles of laboratory animal care* (NIH publication No. 86–23, revised 1985).

Surgical procedures

A head holder and a chamber for unit recording and drug injection were implanted under surgical procedures. The monkey was sedated with ketamine (4.6–6.0 mg/kg) and xylazine (1.8–2.4 mg/kg) intramuscularly and then general anesthesia was induced by intravenous injection of pentobarbital sodium (4.5–6.0 mg/kg per hour). Surgical procedures were conducted in aseptic conditions in an operating room.

For muscimol injection, Teflon-made guide tubes (diameter 0.85 mm) were implanted toward the striatum and fixed to the skull using dental acrylic resin. The operation was performed under anesthesia with ketamine and xylazine. The locations of the guide tubes were determined on the basis of magnetic resonance (MR) images (Hitachi Laboratory MRIS, 2.11 T), using the procedure described by Kato et al. (1995), and were confirmed by single-unit recording. The guide tubes were directed to the striatum (caudate and putamen, left and right) at three anteroposterior levels (6 mm anterior, 2 mm anterior, and 2 mm posterior to the anterior commissure). The results of the unit recording were also used to determine the depths of muscimol injections.

Apparatus

The monkey sat in a primate chair facing a black panel on which 16 light-emitting diode (LED) buttons were mounted in a 4×4 matrix. At the bottom of the panel was another LED button, which was used as a home key. To have the monkey use only one hand for button press, a vertical Plexiglas plate was attached to the chair between the panel and the hand not being used. To change the hand for use, the plate was placed on the other side. The animal's head was fixed with a head holder connected to the primate chair.

Behavioral paradigm

As described in detail in a previous paper (Hikosaka et al. 1995a), the monkeys were trained to perform a sequential button-press task, called the 2×5 task. Figure 1A shows an example of the sequence of events in a single task trial. At the start of a trial, the home key turned on. When the animal pressed the home key for 500 ms, two of the 16 target LED buttons turned on simultaneously, which we call "set". The animal had to press the illuminated buttons in the correct (predetermined) order, which he had to find out by trial and error. If successful, the illumination of the buttons turned off and another pair of LED buttons, a second set, was illuminated, which the monkey had to press again in the predetermined order. A total of five sets were presented in a fixed order for completion of a trial, which we call "hyperset". When the animal pressed a wrong button in any set, the trial was aborted and the animal then had to start again from the home key as a new trial. The same hyperset was used throughout a block of experiments until the monkey completed ten cumulative, successful trials (Fig. 1B); a different hyperset was used for the next block. After each successful set, a fluid reward was delivered. To encourage the monkey to complete the whole hyperset, the amount of reward was increased gradually from the first to the final set.

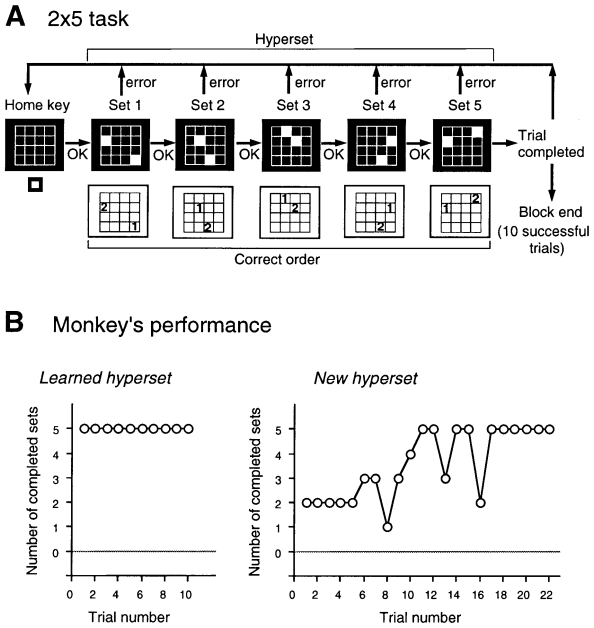

Fig. 1 **A** An example of "hyperset" in the 2×5 task. To complete a trial, the monkey had to press ten buttons (two buttons × five sets) in a correct (predetermined) order. **B** An example of a block of practice trials using a learned hyperset (*left*) and a new hyperset (*right*; monkey ME). The numbers of completed sets (*ordinate*) are shown for consecutive trials (*abscissa*). In the learned hyperset, the monkey completed the block (ten successful trials) with no error. In the new hyperset, the same monkey made errors initially at the third or fourth set, but the number of completed sets increased gradually. Thus, the monkey made 12 errors before completing the block. Before the injection experiments, the monkeys had performed learned hypersets almost every day for more than 3 months. New hypersets were used only once (for one block) and had never been used previously

Experimental procedures

In order to examine the mechanism of long-term storage of procedural memories and their retrieval, it was crucial for the monkeys to have acquired long-term memories for sequential movements before the injection experiments. For this purpose, we chose some hypersets as "learned hypersets" such that the monkeys performed these hypersets almost every day for more than 3 months (number of learned hypersets: 28 for monkey PI, 16 for monkey ME). In consequence, the monkeys performed the learned hypersets very skillfully, with few errors (Fig. 1B, left). In daily training sessions, the monkeys performed 20–40 hypersets containing the learned hypersets and, in addition, "new hypersets", which the monkey experienced for the first time. Note that as many new hypersets as necessary could be generated by having the computer generate random numbers. For each new hyperset, the monkeys learned the correct sequence by trial and error, which was quite different from the learned hypersets (Fig. 1B, right).

We injected muscimol solution in saline (5 μg/μl, except for one case using 10 μg/μl) to block the neural activities. Usually multiple injections of muscimol were administered (bilateral, two or four sites). The solution of 1 μl was injected for each site at a rate of 0.2 μl/min.

After the muscimol injection (within 200 min), we collected data by asking the monkey to perform learned and new hypersets

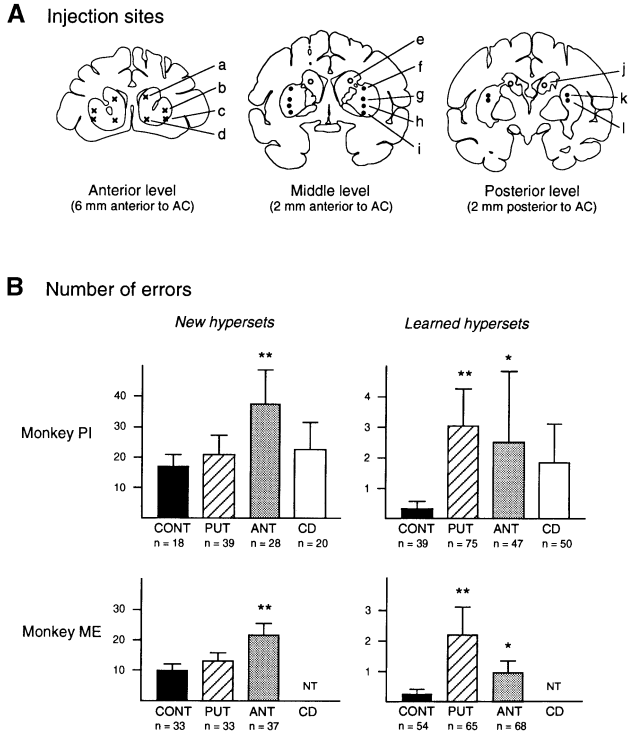

Fig. 2 **A** Sites of muscimol injection in monkey PI at three anteroposterior levels, which were 6 mm anterior, 2 mm anterior, and 2 mm posterior to the anterior commissure (*AC*). A total of 23 experiments (muscimol injections) were carried out. Injections were bilateral for all experiments with the following combinations: *a* ($n = 4$), *b* ($n = 1$), *a* and *b* ($n = 1$), *c* ($n = 1$), *d* ($n = 1$), *f* ($n = 1$), *g* ($n = 3$), *i* ($n = 1$), *g* and *k* ($n = 2$), *h* and *l* ($n = 1$), *e* ($n = 2$), *j* ($n = 3$), *e* and *j* ($n = 2$), where *n* is the number of experiments). These injection sites were classified into three groups: anterior caudate and putamen (*ANT*; *crosses*); middle and posterior putamen (*PUT*; *filled circles*); middle and posterior caudate (*CD*; *open circles*). Injections in monkey ME were made at analogous positions: a and b ($n = 4$), g and l ($n = 4$), as determined by MRI inspection. **B** Averaged numbers of errors (mean, SD) for each of the three muscimol injection groups (two groups for monkey ME) and a saline injection group (*Cont*). The data were calculated for learned hypersets and new hypersets separately. Each of the post-muscimol data were compared with the postsaline data (Mann-Whitney *U*-test); significant differences are indicated by $^{**}P < 0.005$ and $^{*}P < 0.05$. Note that the scale of the ordinate is different between new hypersets and learned hypersets. (*n* number of blocks, *NT* not tested)

as in the daily training session. The number of hypersets examined was usually 20–30 for learned hypersets and 5–10 for new hypersets, depending on the efficiency of the monkey's performance.

For control experiments, the same amount of saline was injected at the same sites as used for the muscimol injections (a and b, g and k, or g in Fig. 2A). There was no indication that the monkeys' performances changed after any of the saline injections. Therefore, the data for all of the saline injection experiments were grouped and used as control data.

Data analysis

The muscimol injections were classified into three groups based on their locations: (1) anterior caudate and putamen, (2) middle-posterior putamen, and (3) middle-posterior caudate. This classification was substantiated by the within-group similarities of the injection effects.

The parameter we analyzed in the present study was the number of errors before the monkey completed ten cumulative, successful trials. Only the errors due to pressing the buttons in the incorrect order were included; other types of errors such as pressing of a non-illuminated button were excluded. For each of the three groups of injection, the numbers of errors obtained for different hypersets were averaged separately for learned hypersets and new hypersets. The averaged value for each group was then compared with the averaged value obtained in the control (saline) injections using the Mann-Whitney *U*-test.

Histological reconstruction

One of the monkeys (monkey PI) was killed at the end of the series of experiments under deep pentobarbital anesthesia and was perfused with formaldehyde through the heart. The brain was blocked for coronal sectioning, fixed in formaldehyde, and dehydrated in aqueous sucrose solution. Frozen sections (50 μm thick) were made and stained with cresyl violet, and the tracks of injection tubes were reconstructed. Injection sites were estimated based on the tracks and electrophysiological mapping (see Fig. 2A). The results were consistent with the estimation based on MRI inspection.

Results

In the control experiments in which saline was injected into the striatum, the mean number of errors before completing ten cumulative, successful trials was much smaller for learned hypersets than that for new hypersets (mean, 0.3 and 16.9 for monkey PI; 0.3 and 10.0 for monkey ME, respectively).

The number of errors for new hypersets increased significantly after muscimol injections into the anterior caudate and putamen (mean, 37.4 for monkey PI, $P<0.005$; 21.6 for monkey ME, $P<0.005$), but not after injections into the middle-posterior putamen (mean, 20.9 for monkey PI, $P>0.05$; 13.0 for monkey ME, $P>0.05$).

The number of errors for learned hypersets increased after anterior caudate and putamen and middle-posterior putamen injections, but the deficits were more severe for the latter (mean, 3.0 for monkey PI, $P<0.005$; 2.2 for monkey ME, $P<0.005$) than for the former (mean, 2.5 for monkey PI, $P<0.05$; 1.0 for monkey ME, $P<0.05$).

These results suggest that the anterior caudate/putamen preferentially contributes to the process of learning (learning mechanism) although it contributes, to some degree, to the execution of well-learned sequences (memory mechanism). In contrast, the middle-posterior putamen participates in the memory mechanism, but not in the learning mechanism.

The blockade of middle and posterior caudate (tested in monkey PI) produced no significant changes in the number of errors for learned hypersets and new hypersets.

Discussion

When a new hyperset was presented, the monkey had to find out the correct sequence by trial and error. With re-

peated practice of particular hypersets for several weeks, the monkeys' performance for these hypersets became very skillful and the skill was retained for a long time without further practice (Hikosaka et al. 1996a; Miyashita et al. 1996). Therefore it is reasonable to suppose that there would be different mechanisms for learning of new sequences and for execution of well-learned sequences.

We actually found that the local functional blockade of different portions of the monkey striatum induced different effects on learning and execution of sequential movements: performances in learning of new hypersets became deficient only after the blockade of the anterior striatum, whereas the execution of learned hypersets became deficient after the blockade of the middle-posterior putamen and, to a lesser degree, after the blockade of the anterior striatum.

These results suggest that different subdivisions of the striatum contribute to different aspects of learning and execution of sequential movements. This view is consistent with anatomical studies suggesting that there are multiple separated subchannels within the basal ganglia-thalamo-cortical system (Selemon and Goldman-Rakic 1985; Alexander and Crutcher 1990; Hoover and Strick 1993; Parent and Hazrati 1995).

The anterior part of the striatum receives massive projections from the dorsolateral prefrontal cortex, which is heavily related to spatial working memory (Sawaguchi and Goldman-Rakic 1991; Funahashi et al. 1993). This is the kind of memory that is required when the monkey tries to learn new sequences (Hikosaka et al. 1995b). The anterior striatum also receives dense projections from the presupplementary motor area (pre-SMA; Parthasarathy et al. 1992) where we found neurons that were preferentially activated during learning of new hypersets (Miyashita et al. 1995). It is reasonable therefore that the monkey had difficulty in learning new sequences when the anterior striatum was functionally blocked.

The modest increase in the number of errors for learned hypersets by the blockade of the anterior striatum might also be explained by the deficient spatial working memory, because any errors in learned hypersets, though rare, would not be corrected without working memory.

The blockade of the middle-posterior putamen led to the deteriorated performances for learned hypersets. It is well known that this part of the putamen receives inputs mainly from the sensory-motor cortical areas, including the premotor and supplementary motor areas as well as the primary motor area (Percheron et al. 1984; Hoover and Strick 1993; Strick et al. 1995). One possibility is that the long-term memory of sequential procedures is stored somewhere in these cortical areas and is sent to the putamen for the learned sequences to be executed.

However, the effects of muscimol injections in the striatum were generally modest. Thus, the performances after the blockade of the middle-posterior putamen were still much better than those for new hypersets. Likewise, the monkeys were still able to learn new sequences, though more slowly, after the blockade of anterior striatum. Therefore, it seems likely that, although different parts of the striatum may play differential roles in learning and memory for sequential movements, their contributions are not exclusive but partial, in that other brain areas also participate in these processes (Miyashita et al. 1995; Hikosaka et al. 1996b).

In conclusion, by combination of a new learning task (2×5 task) and the reversible blockade of neural activities with muscimol, we revealed the differential roles of the subdivisions of the monkey striatum in learning and memory for sequential movements: (1) the anterior caudate and putamen takes part in the mechanism that is necessary for the learning of new sequences and, to a lesser degree, for the execution of well-learned sequences; (2) the middle-posterior putamen is preferentially related to the long-term storage or retrieval of memory for sequential movements.

Acknowledgements We are grateful to Dr. M. Kato for designing the computer programs. We thank M. Togawa, M. Yoshitomo for technical assistance. This study was supported by Grant-in-Aid for Scientific Research on priority areas from The Ministry of Education, Science, Culture and Sports of Japan and the Uehara Memorial Foundation.

References

Alexander GE, Crutcher MD (1990) Functional architecture of basal ganglia circuits: neural substrates of parallel processing. Trends Neurosci 13: 266–271

Butters N, Wolfe J, Martone M, Granholm E, Cermak LS (1985) Memory disorders associated with Huntington's disease: verbal recall, verbal recognition and procedural memory. Neuropsychologia 23: 729–743

Calabresi P, Pisani A, Mercuri NB, Bernardi G (1996) The corticostriatal projection: from synaptic plasticity to dysfunctions of the basal ganglia. Trends Neurosci 19: 19–24

Dominey P, Arbib M, Joseph J-P (1995) A model of cortico-striatal plasticity for learning oculomotor associations and sequences. J Cogn Neurosci 7(3): 311–336

Dunnett SB, Iversen SD (1982) Neurotoxic lesions of ventrolateral but not anteromedial neostriatum in rats impair differential reinforcement of low rates (DRL) performance. Behav Brain Res 6: 213–226

Funahashi S, Bruce CJ, Goldman-Rakic PS (1993) Dorsolateral prefrontal lesions and oculomotor delayed-response performance: evidence for mnemonic "Scotomas". J Neurosci 13: 1479–1497

Graybiel AM, Aosaki T, Flaherty A, Kimura M (1994) The basal ganglia and adaptive motor control. Science 265: 1826–1831

Hikosaka O (1994) Role of basal ganglia in control of innate movements, learned behavior and cognition – a hypothesis. In: Percheron G, McKenzie JS, Feger J (eds) New ideas and data on structure and function. (The basal ganglia, vol IV) Plenum Press, New York, pp 589–596

Hikosaka O, Rand MK, Miyachi S, Miyashita K (1995a) Learning of sequential movements in the monkey – Process of learning and retention of memory. J Neurophysiol 74: 1652–1661

Hikosaka O, Rand MK, Miyachi S, Miyashita K (1995b) Procedural learning in the monkey. In: Kimura M, Graybiel AM (eds) Functions of the cortico-basal ganglia loop. Springer, Tokyo, pp 18–30

Hikosaka O, Miyachi S, Miyashita K, Rand MK (1996a) Learning of sequential procedures in monkeys. In: Bloedel JR, Ebner TJ, Wise SP (eds) The acquisition of motor behavior in vertebrates. MIT Press, Cambridge, Mass, pp 303–317

Hikosaka O, Sakai K, Miyauchi S, Takino R, Sasaki Y, Pütz B (1996b) Activation of human pre-SMA and precuneus in learning of sequentail procedures. Neurosci Res [Suppl] 20: S243

Hoover JE, Strick PL (1993) Multiple output channels in the basal ganglia. Science 259: 819–821

Houk JC, Adams JL, Barto A (1995) A model of how the basal ganglia generate and use neural signals that predict reinforcement. In: Houk JC, Davis JL, Beiser DG (eds) Models of information processing in the basal ganglia. MIT Press, Cambridge, Mass, pp 249–270

Kato M, Miyashita N, Hikosaka O, Matsumura M, Usui S, Kori A (1995) Eye movements in monkeys with local dopamine depletion in the caudate nucleus. I. Deficits in spontaneous saccades. J Neurosci 15: 912–927

Knopman D, Nissen MJ (1991) Procedural learning is impaired in Huntington's disease: evidence from the serial reaction time task. Neuropsychologia 29: 245–254

Knowlton BJ, Mangels JA, Squire LR (1996) A neostriatal habit learning system in humans. Science 273: 1399–1402

Miyashita K, Hikosaka O, Lu X, Miyachi S (1995) Neuronal activities in medial premotor area of monkey during learning of sequential movements. Soc Neurosci Abstr 21: 1928

Miyashita K, Rand MK, Miyachi S, Hikosaka O (1996) Anticipatory saccades in sequential procedural learning in monkeys. J Neurophysiol 76: 1361–1366

Parent A, Hazrati LN (1995) Functional anatomy of the basal ganglia. I. The cortico-basal ganglia-thalamo-cortical loop. Brain Res Rev 20: 91–127

Parthasarathy HB, Schall JD, Graybiel AM (1992) Distributed but convergent ordering of corticostriatal projections: analysis of the frontal eye field and the supplementary eye field in the macaque monkey. J Neurosci 12: 4468–4488

Percheron G, Yelnik J, François C (1984) A Golgi analysis of the primate globus pallidus. III. Spatial organization of the striato-pallidal complex. J Comp Neurol 227: 214–227

Robbins TW, Everitt BJ (1996) Neurobehavioural mechanisms of reward and motivation. Curr Opin Neurobiol 6: 228–236

Saint-Cyr JA, Taylor AE, Lang AE (1988) Procedural learning and neostriatal dysfunction in man. Brain 111: 941–959

Sawaguchi T, Goldman-Rakic PS (1991) D1 dopamine receptors in prefrontal cortex: involvement in working memory. Science 251: 947–950

Schultz W, Romo R (1990) Dopamine neurons of the monkey midbrain: contingencies of responses to stimuli eliciting immediate behavioral reactions. J Neurophysiol 63: 607–624

Selemon LD, Goldman-Rakic PS (1985) Longitudinal topography and interdigitation of corticostriatal projections in the rhesus monkey. J Neurosci 5: 776–794

Squire LR (1986) Mechanisms of memory. Science 232: 1612–1619

Strick PL, Dum RP, Mushiake H (1995) Basal ganglia "loops" with the cerebral cortex. In: Kimura M, Graybiel AM (eds) Functions of the cortico-basal ganglia loop. Springer, Tokyo, pp 106–124