RESEARCH NOTE

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Differential activation of monkey striatal neurons in the early and late stages of procedural learning

Abstract The basal ganglia is a key structure for procedural learning. To examine in what aspects of procedural learning the basal ganglia participate, we recorded from striatal neurons (phasically active neurons) in monkeys while the animals were performing a sequential button press task (the 2×5 task) and compared the neuronal activity between two conditions: (1) while learning new sequences and (2) while executing over-learned sequences. Among 147 neurons recorded in two monkeys, 45 neurons were activated preferentially for new sequences (new-preferring neurons), 34 for over-learned sequences (learned-preferring neurons), and 68 were activated non-selectively (non-selective neurons). New-preferring neurons were more abundant in the "association" region [association striatum (AS); caudate nucleus and rostral putamen anterior to the anterior commissure], while the learned-preferring neurons were more abundant in the "sensorimotor" region [sensorimotor striatum (SM); putamen posterior to the anterior commissure]. In addition to the learning dependency, the AS and SM neurons were activated in different task periods: many AS neurons were activated during the delay period, while the SM neurons were more activated with reaching and button presses. These data, together with the data from our previous blockade study, suggest that the "association" and "sensorimotor" regions of the basal ganglia contribute preferentially to the early and late stages of procedural learning, respectively.

Keywords Basal ganglia · Putamen · Caudate · Sequential movement · Extracellular recording

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Introduction

Procedural learning is essential in human and animal life for acquiring many behavioral repertoires. Our previous studies on procedural learning in monkeys and human subjects using a sequential button press task ("2×5 task") (Hikosaka et al. 1995) have suggested that separate groups of brain structures participate in the early and late stages of procedural learning; the pre-supplementary motor area (pre-SMA) (Nakamura et al. 1998, 1999) and the dorsolateral prefrontal cortex (DLPC) (Sakai et al. 1998) in the early stage, and the cerebellar dentate nucleus in the late stage (Lu et al. 1998).

The basal ganglia is another important group of structures for procedural learning (Butters et al. 1985; Knopman and Nissen 1991; Knowlton et al. 1996). These nuclei have the potential to participate in both early and late stages of procedural learning since they are connected with most areas of the cerebral cortex, including association and sensorimotor cortices, in a topographical manner: the caudate nucleus and rostral putamen anterior to the anterior commissure ("association" striatum, AS) receive projections from the frontal association areas, while the more caudal part of the putamen ("sensorimotor" striatum, SM) receives projections from the sensorimotor cortices (Alexander et al. 1990; Parent and Hazrati 1995). Our previous study (Miyachi et al. 1997) showed that the new learning of procedures and execution of well-learned procedures were specifically impaired by blockade of the AS and the SM, respectively.

In agreement with this result, our present study shows that striatal neurons in the AS and SM were differentially

activated during performance of new and overlearned sequences.

Materials and methods

We used two male Japanese monkeys (*Macaca fuscata*): monkeys ME and KO. All surgical and experimental protocols were approved by the Juntendo University Animal Care and Use Committee and were in accordance with the *Principles of laboratory animal care* (NIH publication No. 86-23, revised 1985).

Behavioral paradigm

The monkeys were trained to perform a sequential button press task (the 2×5 task), in which the animals had to press ten buttons in a predetermined order (Fig. 1; for details, see Hikosaka et al. 1995). The animal sat facing a 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 (home key). When the animal pressed the home key and waited for 1–2 s, 2 of the 16 target LED buttons turned on simultaneously (set 1). The animal had to press the two buttons in a predetermined order, which had to be discovered by trial and error. If successful, the buttons turned off, a liquid reward was delivered, and another pair of buttons (set 2) was illuminated. A total of five sets were presented in a fixed order for the completion of a trial. This fixed sequence of five sets is called a "hyperset." If the animal pressed a wrong button, the trial was

Fig. 1 a A schematic diagram of the 2×5 task. To complete a trial, the monkey had to press ten buttons (two buttons × five sets) in a predetermined order. **b** Typical examples of a block of trials using a learned hyperset (*left*) and a new hyperset (*right*). The *abscissa* indicates how many sets were completed successfully in each trial. The trial was repeated until the monkey completed the entire hyperset (5 sets) 10 times. In the learned hyperset (*left*), the monkey completed the entire hyperset in every trial. In the new hyperset (*right*), the monkey made 12 errors (indicated by *crosses*) before completing the hyperset 10 times. The discharge rates in the first ten successful trials of each set (indicated by *shadow*) were averaged and compared between new and learned hypersets. **c** Activity of a new-preferring neuron (*left*) and a learned-preferring neuron (*right*) for two new hypersets (*top*) and two learned hypersets (*bottom*). Raster and histogram are aligned by the first button press in each set. An identical set was contained in a new hyperset and a learned hyperset for the new-preferring neuron (indicated by *boxes*), but the new-preferring neuron's activity for this set was much greater in the new hyperset
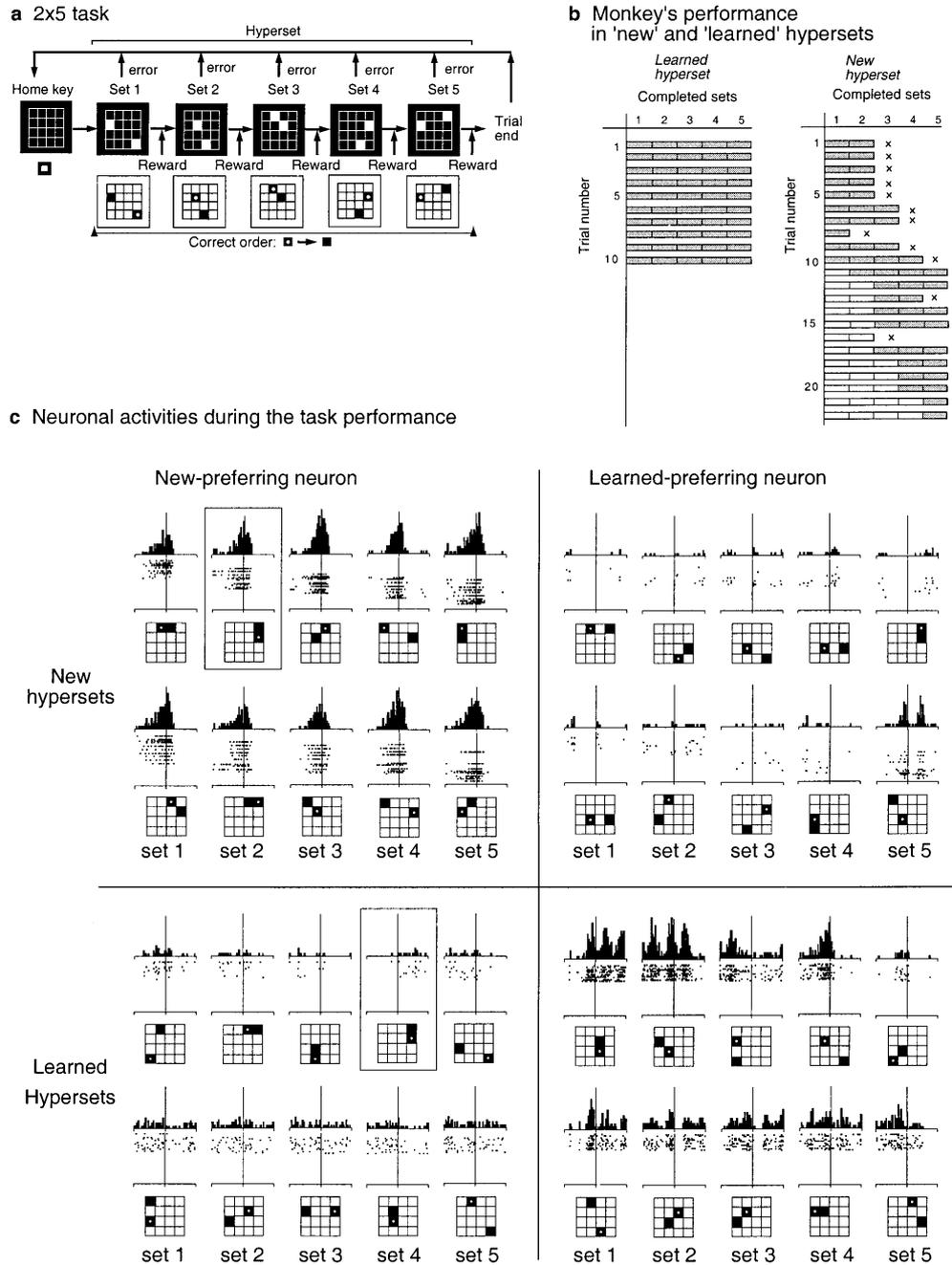
aborted and the animal had to start a new trial. The same hyperset was used throughout a block of trials until the monkey completed ten cumulative successful trials (Fig. 1b). A different hyperset was used for the next block. The monkey was forced to use only one hand during a block of trials, with the other hand blocked by an acrylic plate. Most of the new hypersets were used only once (for one block) and were never used again. Some of the hypersets (16 for ME, 19 for KO) were chosen for extensive learning. These hypersets were presented every day so that, in a few months, the monkeys could perform these "learned hypersets" very skillfully with almost no errors. In addition, the monkeys performed a few "new hypersets" on each experimental day.

Neuronal recording and data analysis

Extracellular spike activities of the phasically active neurons (presumably projection neurons) were recorded in the striatum while the monkey was performing the task with the contralateral hand. For each neuron recorded, the monkey performed at least four hypersets (two new and two learned hypersets). For each trial, the discharge rate for each successful set (from the onset of the set to the end of reward delivery) was calculated and averaged across the trials until the cumulative number of set success, but not trial success, reached ten (Fig. 1b). This calculation was done to adjust for the differences in the monkeys' familiarity with different sets (e.g., in the 11th trial of the new hyperset in Fig. 1b, set 5 was presented for the first time, while set 1 was presented for the 11th time). For each neuron, the mean discharge rates averaged for all sets were compared between new and learned hypersets using the Mann-Whitney U-test. Based on the statistical test, we classified the neurons into three types: (1) "new-preferring neurons," which were significantly more active in new hypersets than in learned hypersets, (2) "learned-preferring neurons," which were more active in learned hypersets, and (3) "non-selective neurons," which showed no significant difference between the two types of hypersets.

The neurons' activities were classified, by examination of raster and histogram displays, into eight types: tonic activity prior to the onset of set 1 (delay), phasic activity from 100 ms before to 100 ms after the target onset in any set (set on), phasic or tonic activity between the target onset and first button press (reach 1), phasic activity from 100 ms before to 100 ms after the first button press (press 1), phasic or tonic activity between first and second button press (reach 2), phasic activity from 100 ms before to 100 ms after the second button press (press 2), phasic or tonic activity after set 5 (post), and long-lasting activity extended over more than one period (multi).

Histological reconstruction

After the series of experiments, monkey KO was sacrificed, and the recording sites were reconstructed histologically. Recording sites in monkey ME were determined based on MR images. All the recording sites were divided into two regions based on the conventional classification: the AS, which includes the caudate nucleus and the putamen anterior to the anterior commissure, or the SM, which corresponds to the putamen posterior to the anterior commissure (Parent and Hazrati 1995).

Results

Many of the neurons showed differential activation for new and learned hypersets. Two examples are shown in Fig. 1c. The new-preferring neuron (left) was strongly activated while the monkey was performing new hypersets, but was inactive during the performance of learned hypersets. In contrast, the learned-preferring neuron
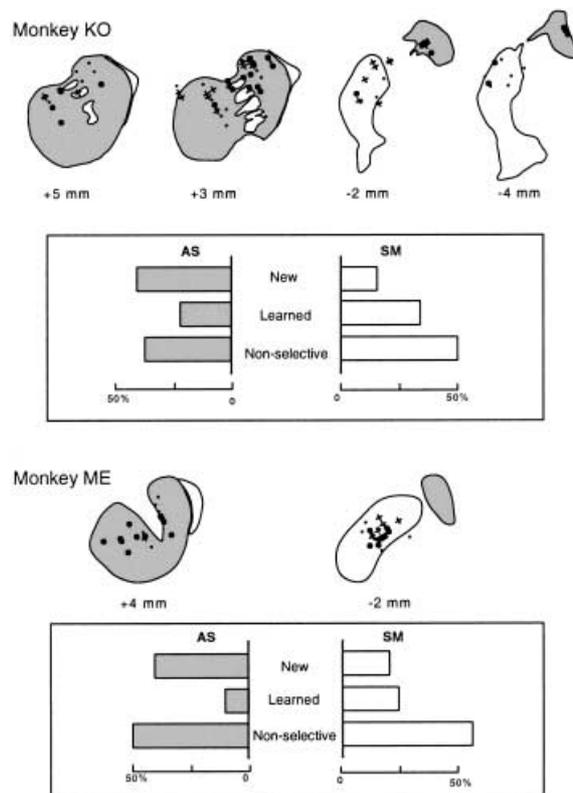

Fig. 2 Recording sites in the striatum (coronal sections), histologically reconstructed (*monkey KO*) or estimated from MR images (*monkey ME*). The anterior-posterior distances from the anterior commissure are indicated. Neurons were classified as new-preferring (*filled circles*), learned-preferring (*crosses*), or non-selective (*small dots*). The bar charts indicate the proportion of the three types of neurons, shown separately for the association striatum (*AS, left*) and the sensorimotor striatum (*SM, right*) for each monkey

(right) was rather inactive for new hypersets, while it was very active for learned hypersets. Note that the new-preferring neuron showed burst activity for set 2 of the first new hyperset but was inactive for set 4 of the first learned hyperset, even though the button combination was identical.

Among 147 (92 for monkey KO, 55 for monkey ME) neurons that were recorded and tested properly, 45 [28 (30%) for KO and 17 (31%) for ME] were new-preferring, 34 [25 (27%) for KO and 9 (16%) for ME] were learned-preferring, and 68 [39 (42%) for KO and 29 (53%) for ME] were non-differentially active for new and learned hypersets (non-selective neurons).

We found that the three types of neurons were unevenly distributed in the striatum as shown in Fig. 2. In the association part, new-preferring neurons were more abundant than learned-preferring neurons [22 (41%) to 12 (22%) in monkey KO, 12 (40%) to 3 (12%) in monkey ME]. In the sensorimotor part, there were more learned-preferring than new-preferring neurons [13 (34%) to 6 (16%) in KO, 6 (24%) to 5 (20%) in ME]. The chi-square test showed that the difference was statistically significant for both animals (chi-square $P<0.05$ for both animals).

Table 1 Number of neurons activated with the task events

		Set on	Reach1	Press1	Reach2	Press2	Post	Delay	Multi
ME	AS	4 (14%)	7 (24%)	4 (14%)	3 (10%)	3 (10%)	12 (41%)	9 (31%)	24 (83%)
	SM	4 (16%)	13 (52%)	9 (36%)	11 (44%)	9 (36%)	5 (20%)	5 (20%)	8 (32%)
KO	AS	3 (5%)	30 (53%)	8 (14%)	19 (34%)	8 (14%)	23 (41%)	29 (51%)	40 (71%)
	SM	6 (16%)	21 (56%)	10 (27%)	22 (59%)	16 (43%)	17 (46%)	9 (24%)	17 (46%)

We also found that AS neurons and SM neurons tended to be activated at different phases in task performance (Table 1). Activity related to specific movements (i.e., reaching and pressing the buttons) was more common in SM neurons, while activity in the delay period and activity extending over two or more task events (Multi) were more common in AS neurons. There was no notable difference in activity patterns between neurons of different learning dependency (new-, learned-preferring, or non-selective).

Discussion

The main finding in the present study is that the new-preferring neurons and the learned-preferring neurons were distributed differentially in the striatum: the former were found mainly in the "association" striatum (AS), whereas the latter were more abundant in the "sensori-motor" striatum (SM). This result, together with the data from our blockade study (Miyachi et al. 1997), suggests that the "association" and "sensorimotor" regions of the basal ganglia contribute preferentially to the early and late stages of procedural learning, respectively. Jueptner and Weiller (1998) reached a similar conclusion using human imaging studies. This functional segregation corresponds to the topographical distribution of the corticostriatal projections (Parent and Hazrati 1995). The caudate and rostral putamen (AS) receive cortical inputs from the DLPC, the pre-SMA, and other frontal association areas (Selemon and Goldman-Rakic 1985; Parthasarathy et al. 1992), whereas the putamen caudal to the anterior commissure (SM) receives inputs from the sensorimotor cortices including the primary motor cortex (M1) and the SMA (Takada et al. 1998). Previous studies have shown that the DLPC and the pre-SMA are related to the early stage of the learning of the 2×5 task (Nakamura et al. 1998, 1999; Sakai et al. 1998). On the other hand, more "learned-preferring" neurons were found in the SMA than in the pre-SMA (Nakamura et al. 1998). Another "sensorimotor" area that could represent movement sequences is the M1 (Karni et al. 1995; Gerloff et al. 1998; Rioult-Pedotti et al. 1998; Sanes and Donoghue 2000), although whether M1 represents the motor sequence has not been examined with the 2×5 task. These differential neuronal activities in the procedural learning among the cortical areas are very similar to the neuronal activities in the association and sensorimotor striatum in this study.

By examining neuronal activity in more detail, we found that SM neurons tended to be activated with more specific movements, while AS neurons tended to show delay or non-specific activity. This difference is similar to the difference in cortical neurons: delay activities are abundant in the DLPC and the pre-SMA neurons, while movement-related activities are more common in SMA and M1 (Funahashi et al. 1989; Mushiake et al. 1991; Matsuzaka et al. 1992).

It should be noted that the topographic segregation of new- and learned-preferring neurons was not exclusive: up to 20% of the cells recorded in the association striatum were learned-preferring, and about 10% of sensorimotor striatal neurons were new-preferring. These anomalous neurons might act as an important link between the short-term and long-term systems. The learned-preferring neurons in the association region may contribute to error correction during the execution of learned sequences. The new-preferring cells in the sensorimotor striatum may trigger the generation of long-term memory.

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