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RESEARCH ARTICLE

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Characteristics of a long-term procedural skill in the monkey

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Abstract The purpose of this study was to characterize the nature and structure of procedural memory. We have previously studied the process of learning sequential behavioral procedures using monkeys. The monkey's task was to press five consecutive pairs of buttons (indicated by illumination) in the correct order for every pair, which he had to find by trial-and-error in a block of trials. The whole sequence was called a "hyperset"; each pair was called a "set". We first examined whether monkeys learned to perform a hyperset as a single sequence or learned the order of button-presses individually for each set. To answer this question, we generated hypersets that were the same as the hypersets that had been extensively learned except that the order of the sets was reversed. The performance of these "reversed hypersets" was much worse than the performance of the original learned hypersets and was similar to the performance of new hypersets, as regards both the number of errors and the performance time. The result suggests that monkeys learned a hyperset as a sequence. To examine whether the learned performance was specific to the hand used for practice, we had monkeys use the same hand throughout the long-term practice of each hyperset, and then tested the opposite hand. The performance using the opposite hand was worse than the performance using the trained hand, but was better than the performance for new hypersets. This indicates that the memory for the sequential procedure is only partially accessible to the hand that was not used for the practice.

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Introduction

Learned procedures are often composed of a sequence of movements. However, how such temporal sequences might be implemented as neural mechanisms is unclear. For example, it is still difficult to answer the following questions. What is the relationship between component procedures that make up a sequential procedure? It is likely that, before learning starts, such component procedures are generated by independent neural mechanisms. What then occurs during learning? Do the individual mechanisms become associated in a totally new mechanism, or do the individual mechanisms remain independent? One way to answer these questions, and the idea behind the present study, is to manipulate a learned procedure to see how the same subject performs the modified procedure.

In a previous study (Hikosaka et al. 1995) we investigated the process of learning of sequential procedures using monkeys. Our behavioral paradigm, which we call the "2×5 task", required monkeys to press ten LED buttons in a predetermined order. The whole sequence (called a hyperset) was composed of five components (called sets), each of which was composed of a fixed sequence of two button-presses. With repeated practice of the hyperset, monkeys' performance improved gradually in terms of the number of errors and speed. However, such skilled performance was specific to the learned sequence; the monkeys had to start over when another hyperset was introduced. By repeating this procedure, each monkey acquired a repertoire of well-learned sequences.

Let us now rephrase the original question in terms of this experimental situation. Did the monkeys learn to press the two buttons in the correct order for each set independently of other sets, or did they learn to press the ten buttons in the correct order for a whole hyperset as a unique sequence? In experiment 1 we attempted to answer this question by generating a hyperset whose component sets were identical to one of the well-learned hypersets but were arranged in the reverse order. Monkeys' performance would remain accurate if they had learned individual sets independently; their performance would deteriorate if they had learned the whole hyperset as a single unique sequence.

In experiment 2 we examined whether the learning transferred to the opposite hand that was naive to the sequence. To answer this question, we had the monkeys use the same hand for the entire practice of a given hyperset, and then got them to use the opposite hand to see how the performance changed. This is in fact a long-standing question that has been addressed by many researchers (for review see Thut et al. 1996). However, relatively few studies have been done using animal subjects.

We also think that this question is important when we ask where in the brain the memory for the sequential procedures might be stored. If we learn to perform an action using the right hand, the memory might be stored in the left hemisphere, in close relation to the motor system for individual hand movements. Then the memory might not be accessible to the motor system for the left hand. Alternatively, the memory may be independent of the motor systems, perhaps stored in both hemispheres, so that the left hand also is able to perform the same action.

Materials and methods

Experimental animals

We used four male Japanese monkeys (*Macaca fuscata*): PI (7.7 kg), BO (8.7 kg), ME (8.0 kg), and KO (8.5 kg). The present study followed the *Principles of Laboratory Animal Care* (NIH publication no. 86–23, revised 1985).

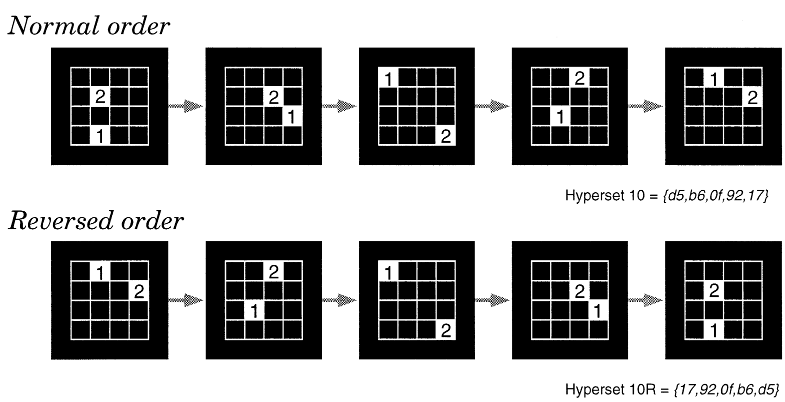

Fig. 1 Procedure of the 2×5 task: an example of learned hypersets for monkey PI in its original form (*Normal order*) and the reversed form (*Reversed order*). In each case, a pair of LED buttons was illuminated simultaneously (called a *set*) following a home-key press, and the monkey had to press the illuminated buttons in the correct order (indicated as "1" and "2"), which he had to find out by trial and error. A total of five sets (called a *hyperset*) were presented in a fixed order for completion of a trial. If the monkey pressed a wrong button, the trial was aborted and the monkey had to start from the first set. In the reversal experiment, the order of sets used for the previous practice was reversed

Apparatus and behavioral paradigm

The monkeys were trained to perform a sequential button-pressing task, called the 2×5 task. Details have been given in a previous paper (Hikosaka et al. 1995). The monkey sat in a primate chair and faced a black panel on which 16 LED buttons were mounted in a 4×4 matrix. At the bottom of the panel was another LED button that was used as a home key. The animal's head was fixed with a head holder connected to a primate chair. To make the monkey use only one hand for button-pressing, a vertical Plexiglas plate was attached to the chair in an oblique direction between the LED panel and the hand not being used. The plate could be moved to the other side of the primate chair to change which hand would be used.

Figure 1 (top, "Normal order") shows an example of the sequence of events in a single task trial. At the start of each trial, the home key was illuminated. When the animal pressed and held the home key for 500 ms, two of the 16 target LEDs were illuminated simultaneously; we called such a pair of LEDs a "set". The animal had to press the illuminated buttons in a correct (predetermined) order that he had to find out by trial and error. If successful, another pair of LEDs, i.e., a second set, was illuminated which again the monkey had to press in a predetermined order. A total of five sets were presented in a fixed order for completion of a trial; we call the five sets a "hyperset". When the animal pressed a wrong button, the trial was aborted without any reward. The animal then had to start again from the home key as a new trial. In the following analyses, a trial was classified as successful only when the animal completed the whole hyperset (five sets).

After each successful set, however, the animal was given a liquid reward. The amount of the reward increased gradually from the first to the final set (from 150 to 300 ms of reward delivery duration) so that the total amount of reward was maximized by completing all sets.

Procedures

The same hyperset was used throughout a block of experiments so that the monkey experienced the same sequences of button-presses repeatedly until he completed 20 successful trials. Some of the hypersets were chosen as *learned hypersets*. During the period of learning, the monkey performed the learned hypersets as the daily routine, one block for each hyperset. The total number of learned hypersets was 28 for monkey PI, 14 for monkey BO, 18 for monkey ME, and 21 for monkey KO. In addition to the learned hypersets, each monkey experienced one or two *new hypersets* each day. Half the learned and new hypersets were performed by the right hand, the other half by the left hand.

To examine the nature of the memory for performing individual hypersets, we changed the normal schedule of learning described above to perform the following experiments.

Experiment 1. Inter-set reversal

Experiment 1 was designed to examine whether the monkey learned to perform a hyperset as a single sequence or learned to perform consecutive sets individually. When the animal had learned the procedure for a hyperset sufficiently (experienced for more than 15 blocks), the order of the component sets was reversed (Fig. 1). Note that the order within a set remained the same.

If the animal learned the order of button-presses within individual sets (regardless of their positions in the hyperset), the reversed hyperset should still be regarded as a learned hyperset and therefore the skilled performance should not be affected. If, instead, the animal learned the sequence of the hyperset as a whole, the reversed hyperset should be regarded as a new hyperset and should disrupt the learned performance, leading to the performance being comparable to a new hyperset.

This experiment could be done only once for a given learned hyperset, because once the reversal had been experienced it was no longer a new hyperset.

Experiment 2. Use of the opposite hand

Experiment 2 was designed to examine whether the memory was specific to the hand used for practice. For this purpose, the hand used was fixed for a given hyperset during the period of learning. After the animal had learned the hyperset sufficiently, the monkey was made to use the opposite hand. If the memory for the learned hyperset was specific to the hand, the performance should deteriorate; otherwise, the performance should remain unchanged. This experiment also could be done only once for a given learned hyperset.

Data analysis

We used two parameters to assess the monkey's performance. As a measure of procedure, we counted the number of errors to reach a criterion, which was ten successful trials. Only sequence errors were included in which the monkey pressed the two illuminated buttons in a wrong order; other errors, such as pressing of a non-illuminated button, were not included. To evaluate the speed we measured performance time for a hyperset [i.e., the time between pressing the home key and the second button-press of the final (fifth) set], which was then averaged across the initial ten successful trials.

We compared the monkeys' performance for the modified hypersets (reversal or opposite hand) with their performance for learned hypersets and their performance for new hypersets. The performance for learned and new hypersets was obtained in experiments performed within the 5 days preceding the modification. Statistical comparisons were made between the different conditions for each monkey using the Mann-Whitney U-test.

Results

Experiment 1. Is the memory sequential or fragmentary?

In Fig. 2 we compare, for each monkey, the performances for learned hypersets when they were performed in the original order (stippled columns) and when they were performed in the reverse order (filled columns). In addition, the performances for new hypersets are shown (open columns) that were performed at approximately the same time as the reversal experiments.

The changes in the number of errors to criterion (completing ten successful trials) were consistent across the monkeys. Before the reversal, the numbers of errors were close to zero, indicating that the hypersets had been learned adequately. When the inter-set orders of the learned hypersets were reversed, the mean numbers of errors increased significantly in every monkey (Mann-Whitney U-test: $P<0.0001$ for every monkey). The results excluded the possibility that the monkeys had learned the order of button-presses individually for each set.

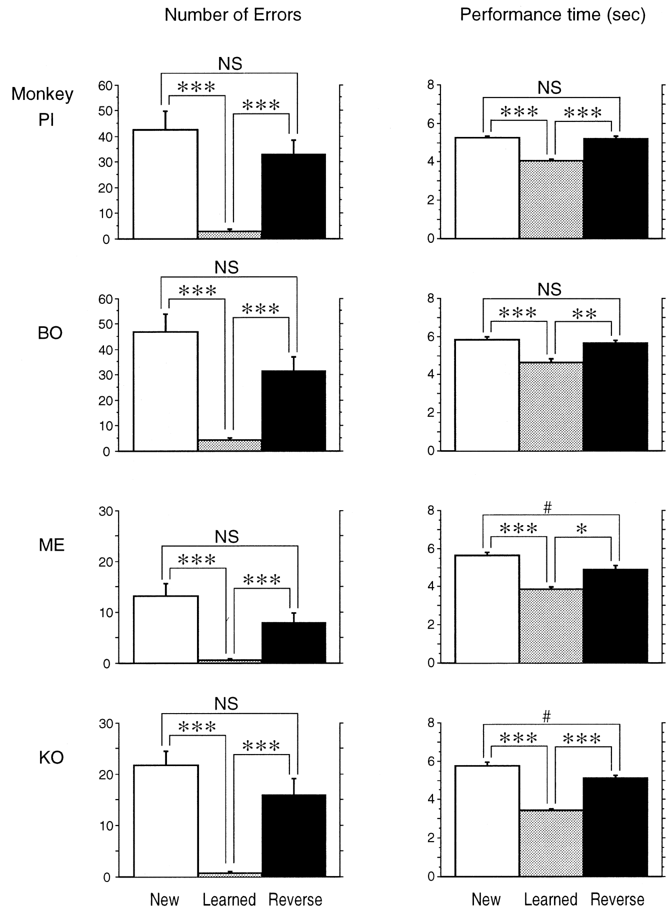

Fig. 2 Changes in the number of errors (*left*) and the performance time (*right*) caused by the reversal of learned sequences. Statistical comparisons were made between the three conditions using a Mann-Whitney U-test and the significance levels are indicated: NS, not significant; $^{\#}P<0.05$; $^{*}P<0.01$; $^{**}P<0.001$; $^{***}P<0.0001$

Figure 2 also shows that the numbers of errors for the reversed hypersets were not statistically different from those for the new hypersets in every monkey ($P>0.05$). The results suggest that the monkeys did not benefit from the correctly learned button-presses for individual sets when their orders were reversed. However, the mean number of errors for the reversed hypersets was smaller than that for the new hypersets for every monkey, though the difference was not statistically significant.

For every monkey, the performance time was increased by the reversal ($P<0.0001$ for monkey PI and KO; $P=0.0005$ for monkey BO; $P=0.0012$ for monkey ME), supporting the conclusion based on the number of errors. In two monkeys (ME and KO), the mean performance time for the reversed hypersets was significantly shorter than those for the new hypersets ($P=0.0130$ for monkey ME; $P=0.0104$ for monkey KO), while in the other two monkeys there was no such statistical difference.

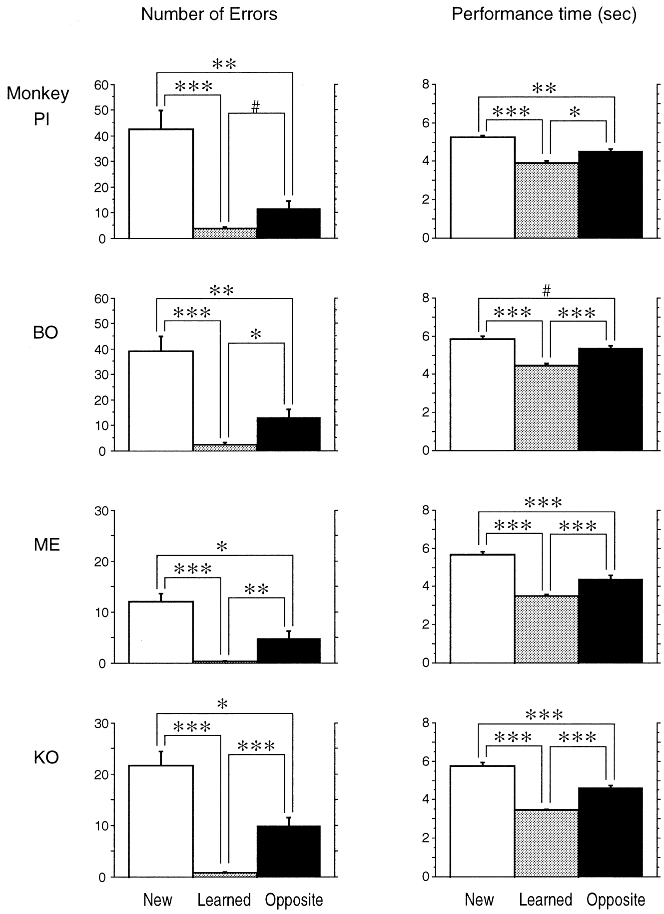

Fig. 3 Changes in the number of errors (*left*) and the performance time (*right*) caused by the use of the opposite hand for learned sequences. #$P<0.05$; *$P<0.01$; **$P<0.001$; ***$P<0.0001$

Experiment 2. Is the memory specific to the hand?

In Fig. 3 we compare, for each monkey, the performances for learned hypersets when they were performed by the hand used for the long-term practice (stippled columns) and when they were performed by the opposite (unpracticed) hand (filled columns). In addition, the performances for new hypersets are shown (open columns).

The results were consistent across the monkeys. The mean number of errors increased significantly when using the opposite hand (P=0.0450 for monkey PI; P=0.0019 for monkey BO; P=0.0006 for monkey ME; $P<0.0001$ for monkey KO). The mean performance time also increased significantly when using the opposite hand (P=0.0046 for monkey PI; P=0.0001 for monkeys BO, ME, and KO). However, the performances using the opposite hand were not as poor as for new hyperesets as regards number of errors (P=0.0008 for monkey PI; P=0.0004 for monkey BO; P=0.0017 for monkey ME; P=0.0014 for monkey KO) or performance time (P=0.0007 for monkey PI; P=0.0334 for monkey BO; $P<0.0001$ for monkeys ME and KO)

These results suggest that the memory for performing individual hypersets was partially specific to the hand used for the practice.

Discussion

Structure of procedural memory

The structure of the 2×5 task is hierarchical, the sets being subordinate to the hyperset. Therefore, the successful performance of a hyperset was definitely based on the successful performances of the component sets. However, we found that, after learning the whole hyperset, the monkey failed in individual sets when their orders were changed. More specifically, when the order of sets was reversed, the monkeys performed as though the hyperset was unfamiliar. The results suggest that what the monkeys learned was not an arbitrary set of individual stimulus-response associations, but their sequences.

However, it is still unclear whether the monkeys learned the whole sequence. Suppose a learned hyperset is composed of sets A, B, C, D, and E in this order. Set B, for example, is preceded by set A in this original hyperset, but is preceded by set C in its reversed form. Therefore, the whole sequence could be implemented by a neural mechanism that encodes individual transitions (i.e., A-B, B-C, C-D, D-E) in addition to correct orders in individual sets.

This suggestion seems relevant to previous behavioral studies (Cleeremans and McClelland 1991; Jackson et al. 1995) in which human subjects, while performing event sequences, acquire implicit knowledge on the temporal context set by previous elements of the sequence. It may also be consistent with recent physiological studies. For example, Tanji and his colleagues demonstrated that neurons in the monkey supplementary motor area may become active at a particular transition within a sequential behavior (Mushiake et al. 1990; Halsband et al. 1994; Tanji et al. 1995). Similar neurons have been found in the prefrontal cortex (Joseph and Barone 1987), caudate nucleus (Kermadi and Joseph 1995), putamen (M. Kimura, personal communication), and globus pallidus (Strick et al. 1995).

Is procedural memory lateralized?

The present study showed that the monkeys' performance for well-learned sequences that had been learned extensively using one hand transferred to the opposite hand. This was indicated by the fact that both the number of errors and the performance time for the learned hypersets performed by the opposite hand were significantly smaller than those for new hypersets (Fig. 3). However, our results also indicated that the transfer was incomplete, as the performance became significantly worse in terms of the above two parameters when the hand was switched from the learned side to the opposite side. This was obviously not because the inexperienced hand was generally less skillful, for the inexperienced hand for one hyperset was the experienced hand for another.

It has been shown repeatedly, at least in human subjects, that learning transfers across different motor systems, especially between hands (Laszlo et al. 1970; Imamizu and Shimojo 1995; Thut et al. 1996), though sometimes asymmetrically (Parlow and Kinsbourne 1989). However, it was difficult in these human studies to evaluate how complete the transfer between hands was, because obviously the preferred hand was more skillful even before learning started. We did not see such clear handedness in our monkeys; their right and left hands showed no significant asymmetry in motor skills as measured by the number of errors and the performance time, at least when the present experiments started.

Another important difference between our monkey study and human studies was the difference in the extent of learning. Our monkeys had been trained for learned sequences as everyday routines for several weeks. On the other hand, human subjects (Imamizu and Shimojo 1995; Thut et al. 1996; Parlow and Kinsbourne 1989) learned a procedure for a shorter period (i.e., within a day) before the transfer was tested. Our results of incomplete intermanual transfer at the well-learned stage might suggest that the effect of learning becomes gradually more specific to the motor system that has been used for the learning.

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