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Characteristics of sequential movements during early learning period in monkeys

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Abstract We previously demonstrated that the organization of a learned sequential movement, after long-term practice, is based on the entire sequence and that the information pertaining to the sequence is largely specific to the hand used for practice. However, it remained unknown whether these characteristics are present from the beginning of learning. To answer the question, we examined the performance of four monkeys for the same sequential procedure in the early stage of learning. The monkeys' task was to press five consecutive pairs of buttons (which were illuminated), in a correct order for every pair, which they had to find by trial-and-error during a block of trials. We first examined whether the memory of a sequential procedure that was learned once was specific to the hand used for practice. The second time that the monkeys attempted to learn a novel sequence, they were required to use either the same hand they used the first time or the opposite hand. The number of errors decreased to a similar degree in the same-hand condition and in the opposite-hand condition. The performance time decreased in the same-hand condition, but not in the opposite-hand condition. The results suggest that, in the

early stage of learning, memory of the correct performance of a sequential procedure is not specific to the hand originally used to perform the sequence (unlike the well-learned stage, where the transfer was incomplete), whereas memory of the fast performance of a sequential procedure is relatively specific to the hand used for practice (like the well-learned stage). We then examined whether memory of a sequential procedure depends on the entire sequence, not individual stimulus sets. For the second learning block, we had the monkey learn the sequence in the same or reversed order. In the reversed order, the order within each set was identical, but the order of sets was reversed. The number of errors decreased in both the same-order and reversed-order conditions to a similar degree for two out of four monkeys; the decrease was larger in the same-order condition for the other two monkeys. For all monkeys, the performance time decreased in the same-order condition, but not in the reversed-order condition. The results suggest that the memory structure for correct performance varies among monkeys in the early stage of learning (unlike the well-learned stage, where the memory of individual sets was consistently absent). On the other hand, memory of the fast performance of a sequential procedure is relatively specific to the learned order used for practice (like the well-learned stage).

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Introduction

Repeated practice is necessary to acquire a procedural (or motor) skill. Earlier psychological studies have noted and emphasized a transition of learning processes from the early stage to the late stage during skill learning (Anderson 1982; Fitts 1964; Fitts and Posner 1967), which is often characterized as a transition from controlled to automatic processes (Schneider and Shiffrin 1977). For example, it may at first be extremely difficult to learn to

type, but after 1 year of training, it will be almost automatic to type your own name.

Such changes in movement organizations during the course of learning suggest that multiple mechanisms contribute to the learning process. Behavioral studies on human subjects have provided data consistent with this idea (Curran and Keele 1993; Ghilardi et al. 1995; Lai and Shea 1998; Shadmehr and Brashers-Krug 1997). For example, using a serial reaction-time task in a dual-task condition, Curran and Keele (1993) suggested that two learning mechanisms (attentional and non-attentional mechanisms) operate independently in sequential learning. It was suggested that underlying learning of sequential key strokes at a predetermined rhythm are two independent learning mechanisms, one for rhythm and the other for movement parameter (Lai and Shea 1998; Lai et al. 1999; Wulf et al. 1993, 1994). A mechanism for speed of performance and a mechanism for accuracy appear to be differentially involved in learning of an inverted-reversed printing task (Parlow and Kinsbourne 1989).

Experiments using intermanual and intramanual transfer have also proven useful for investigating the nature of learning mechanisms (Bray 1928; Cook 1933; Imamizu and Shimojo 1995; Imamizu et al. 1998; Keele et al. 1995; Parlow and Dewey 1991; Thut et al. 1996). Using a sequential key-press task, Keele et al. (1995) found that changing the effector (from all of the digits to the index finger) for the key press did not affect learning, suggesting that the knowledge of sequential order is important for learning. Similarly, Parlow and Dewey (1991) reported that, after learning a sequence of key presses with one hand, subjects could perform the sequence with the untrained hand. In addition, in a visuo-motor adaptation task under computer-generated rotation of visual feedback, the learning effect was transferred to the untrained hand (Cunningham and Welch 1994; Imamizu and Shimojo 1995). These studies suggest that, in the early learning period, procedural (motor) learning depends more on mechanisms processing correct selection of movements than on mechanisms related to motor execution.

These human behavioral studies have been supplemented by many recent brain-imaging studies showing that multiple brain regions were activated at different stages of learning. The existing literature demonstrates an involvement of both the cortical and subcortical structures during the early acquisition period of a movement trajectory (Seitz et al. 1994), maze tracing (Petersen et al. 1998), finger/arm movement sequences (Doyon et al. 1996; Grafton et al. 1995; Honda et al. 1998; Jueptner et al. 1997; Jueptner and Weiller 1998; Karni et al. 1998; Petersen et al. 1998), and verbal production (Petersen et al. 1998). However, the mechanisms by which these neural structures subserve such learning remain unknown.

In contrast to the wealth of data on human subjects, few studies have been done on animal subjects to investigate the mechanism of learning sequential movements. Application of a sequence-learning task to animal subjects is crucial for better understanding the underlying

neural mechanisms, because we can then study the mechanism at the single-neuron level. For this purpose, we developed a sequence learning task, called the "2×5 task" (Hikosaka et al. 1995), which requires monkeys to press ten LED buttons in a predetermined order. The ten LED-button presses were accomplished by successful completion of five component sets, each of which consisted of a two-button press sequence. An advantage of the 2×5 task is that a virtually unlimited number of new sequences can be generated and, at the same time, a group of sequences can be used for long-term learning. Consequently, a given monkey at a given experimental period had a repertoire of sequences, whose learning stages varied from completely new to well-learned (e.g., practice >1 year). This is an ideal situation for studying the neural mechanisms involved during different learning stages.

For the analysis of the 2×5 task, we used two parameters to assess learning: (1) number of errors to criterion, and (2) performance time. We found that these two parameters changed with different time courses during learning of a given sequence: the number of trials decreased more quickly than the performance time. The decrease in the performance time, rather than the number of trials, was correlated with the emergence of anticipatory eye and hand movements (Miyashita et al. 1996). These data suggest that procedural (motor) learning occurs in at least two different aspects: the correct selection of targets reflected by the number of errors and the speed of performance.

In our earlier behavioral study (Rand et al. 1998), we examined the characteristics of procedural memory in its late (well-learned) stage using two kinds of manipulation: opposite-hand and reversed-order experiments. In the opposite-hand experiment, we had the monkey learn a particular sequence for a long time using one hand and, then, had the monkey use the opposite hand. Both the number of errors and the performance time increased. In the reversed-order experiment, we reversed the order of component target sets, but not the order within each set. Again, the values of these parameters increased. These results suggested that procedural memory is relatively specific to the hand used for practice (effector-specific) and specific to the order of sequence (order-specific).

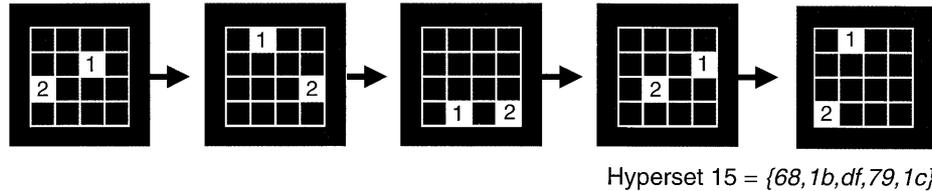
The present study examined whether these characteristics are present from the beginning of learning. If not, it would suggest that the characteristics of procedural memory change with practice. And if the change occurs differentially for these parameters, it would suggest that the correct selection and the speed of performance are acquired differentially with different time courses. These are, in fact, what we found in the present study.

Materials and methods

Experimental animals

We used five male Japanese monkeys (*Macaca fuscata*): monkey PI (7.7 kg), BO (8.7 kg), ME (8.0 kg), KO (8.5 kg), and GA (5.5 kg).

Normal Order



Reversed Order

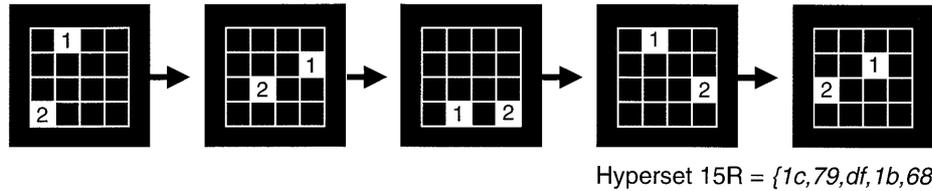


Fig. 1 Procedure of the 2×5 task: an example of learned hypersets for monkey PI in its original form (*Normal order*) and the reversed form (*Reversed order*). In each case, a pair of LED buttons was simultaneously illuminated (called the “set”), following a home-key press, and the monkey had to press the illuminated buttons in a 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

The monkeys were kept in individual primate cages in an air-conditioned room, where food was always available. At the beginning of each experimental session, they were carried to the experimental room in a primate chair. The monkeys were given restricted amount of fluid during training and recording periods. Their health condition, including factors such as body weight and appetite, was checked daily. Supplementary water and fruit were provided daily. All surgical and experimental protocols were approved by the Jun-tendo University Animal Care and Use Committee and are in accordance with the National Institutes of Health Guide for the Care and Use of Animals.

Apparatus and behavioral paradigm

The monkeys were trained to perform a sequential button-pressing task, called the 2×5 task. Details were described in a previous paper (Hikosaka et al. 1995). Each monkey sat in a primate chair facing a black panel, on which 16 LED buttons were mounted in a 4×4 matrix. The animal’s head was fixed with a head holder connected to the primate chair. At the bottom of the panel was another LED button that 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 in an oblique direction between the plate and the hand not being used. To change the hand for use, the plate was shifted to the other side.

Figure 1 (top, “Normal order”) shows an example of a button-press sequence in a single-task trial. At the start of a trial, the home key was turned on. When the animal pressed the home key for 500 ms, two of the 16 target LEDs turned on simultaneously; this is called 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, these LEDs turned off as they were pressed and another pair of LEDs, a second set, was illuminated, which the monkey had to press again in a predetermined order. A total of five sets was presented in a fixed order for completion of a trial; they are called a “hyperset”. When the animal pressed a wrong button, all LED buttons were illuminated briefly with an

unpleasant beep sound, and the trial was aborted without any reward. The animal then had to start over again from the home key to begin a new trial. It should be emphasized that, in the following analyses, a trial was determined to be successful only when the animal completed the entire hyperset (five sets).

Experimental procedures

To examine the characteristics of procedural memory, we modified the procedure of performance in two ways: (1) opposite hand, and (2) reversed order (Rand et al. 1998). In the present study, we focused on the early stage of learning. For this purpose, we compared the performance for a given hyperset between the first and second blocks of learning. For a test group of hypersets, the second learning block was tested in the modified way (opposite hand or reversed order); for a control group of hypersets, the second learning block was tested in the same way as the first learning block. The first and second learning blocks, respectively were tested on two consecutive days.

The data for the early stage of learning were then compared with the data for the well-learned stage, which were taken from the same data source used in the previous study (Rand et al. 1998). For this purpose, the monkeys practiced a group of hypersets daily for more than 15 days. The performance for such well-learned hypersets was very skillful and nearly error-free with anticipatory hand and eye movements (Miyashita et al. 1996). We then modified the performance procedure (opposite hand or reversed order) to examine the characteristics of procedural memory in its well-learned stage. In the following, the detailed procedures for the opposite-hand experiment and for the reversed-order experiment are shown.

Experiment 1: use of the opposite hand

This experiment was designed to examine whether memory was specific to the hand used for practice. For the early-stage examination, we had the monkey perform new hypersets twice on two consecutive days. On each experimental day, the monkey performed four new hypersets (first learning block) and four learned hypersets (second learning block) (Fig. 2A). The four hypersets for the second learning block were the hypersets that the monkey experienced for the first time on the previous day. Two of the hypersets for the second learning block were tested with the hand same as in the first learning block (i.e., right→right, left→left); the other two with the opposite hand (i.e., right→left, left→right). We compared the performance for a given hyperset between the first and second blocks of learning. If the memory of the hyperset was specific to the hand, the performance should not improve in the opposite-hand condition; otherwise, the performance should improve in either case.

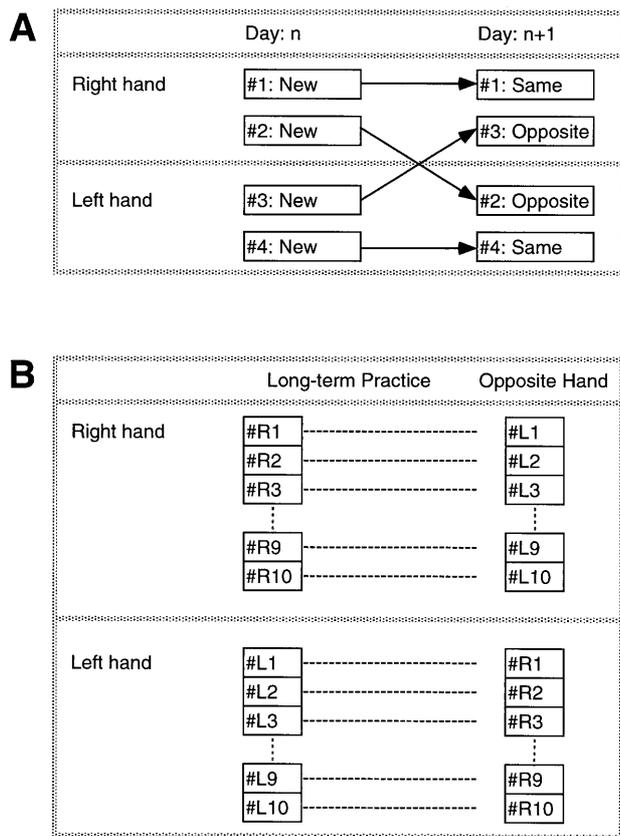


Fig. 2 Experimental schedule for the use of the opposite hand for the early stage (**A**) and the well-learned stage (**B**). For the early-stage examination (**A**), the monkey performed eight hypersets on each experimental day: four for the first learning block and four for the second learning block. For simplicity, **A** shows only four hypersets that the monkey learned for the first time on day “ n ” and for the second time on day “ $n+1$ ”. For the second learning block, two of the hypersets were performed with the same hand (right->right, left->left) and the other two with the opposite hand (right->left, left->right). For the well-learned-stage examination (**B**), the monkey performed about ten hypersets with the right hand and another ten with the left hand everyday for more than 15 days and, then, was required to perform the same hypersets with the hand opposite to that used for the long-term practice. Different hypersets were used for the early stage and the well-learned stage

For the well-learned stage, we had each monkey learn two sets of hypersets for a long time (>15 days), one using the right hand and the other using the left hand all the time. We then switched the schedule, so that the monkey performed the well-learned hypersets with the opposite hand (Fig. 2B). If the memory for the learned hyperset was specific to the hand, the performance should be no better than for new hypersets; otherwise, the performance should remain unchanged.

Experiment 2: inter-set reversal

This experiment was designed to examine whether the monkeys learned to perform a hyperset as a whole sequence or as individual sets. For the early stage-examination, we had the monkey perform new hypersets twice on two consecutive days, similarly to the opposite-hand experiment (Fig. 3A). Two hypersets for the second learning block were tested in the reversed order; the other two in the same order. The hand used was unchanged between the first and second learning blocks. For the reversal, the order of the component sets

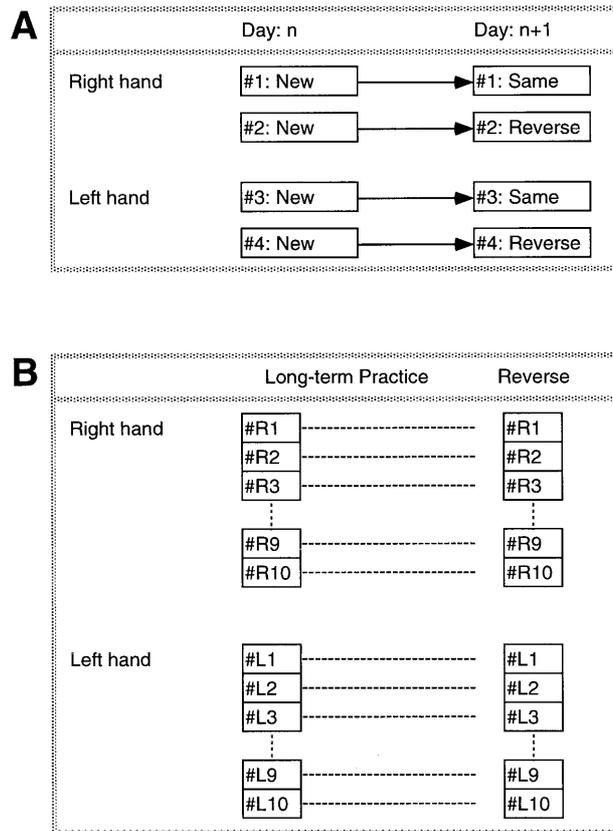


Fig. 3 Experimental schedule for the inter-set reversal for the early stage (**A**) and the well-learned stage (**B**). Same format as in Fig. 2

was reversed while the order within a set remained the same (Fig. 1; bottom, “Reversed Order”). We compared the performance for a given hyperset between the first and second blocks of learning. If the memory for the hyperset was specific to the order of an entire sequence, the performance should not improve in the reversed-order condition; otherwise, the performance should improve in either case.

For the well-learned stage, we had each monkey learn a set of hypersets for a long time (>15 days). We then switched the schedule so that the monkey performed the well-learned hypersets in the reversed order (Fig. 3B).

Data analysis

We used two parameters to assess the monkeys’ performance: number of errors and performance time. As a measure of correct selection, we counted the number of errors to reach a criterion, which was ten successful trials. Only sequence errors were included in which the monkeys pressed the two illuminated buttons in a wrong order; other errors, such as pressing a non-illuminated button, were very rare and, if any, were not included. To evaluate performance speed, we measured a performance time [i.e., the time from the home-key press to the second button press of the final (5th) set], which was then averaged across the initial ten successful trials.

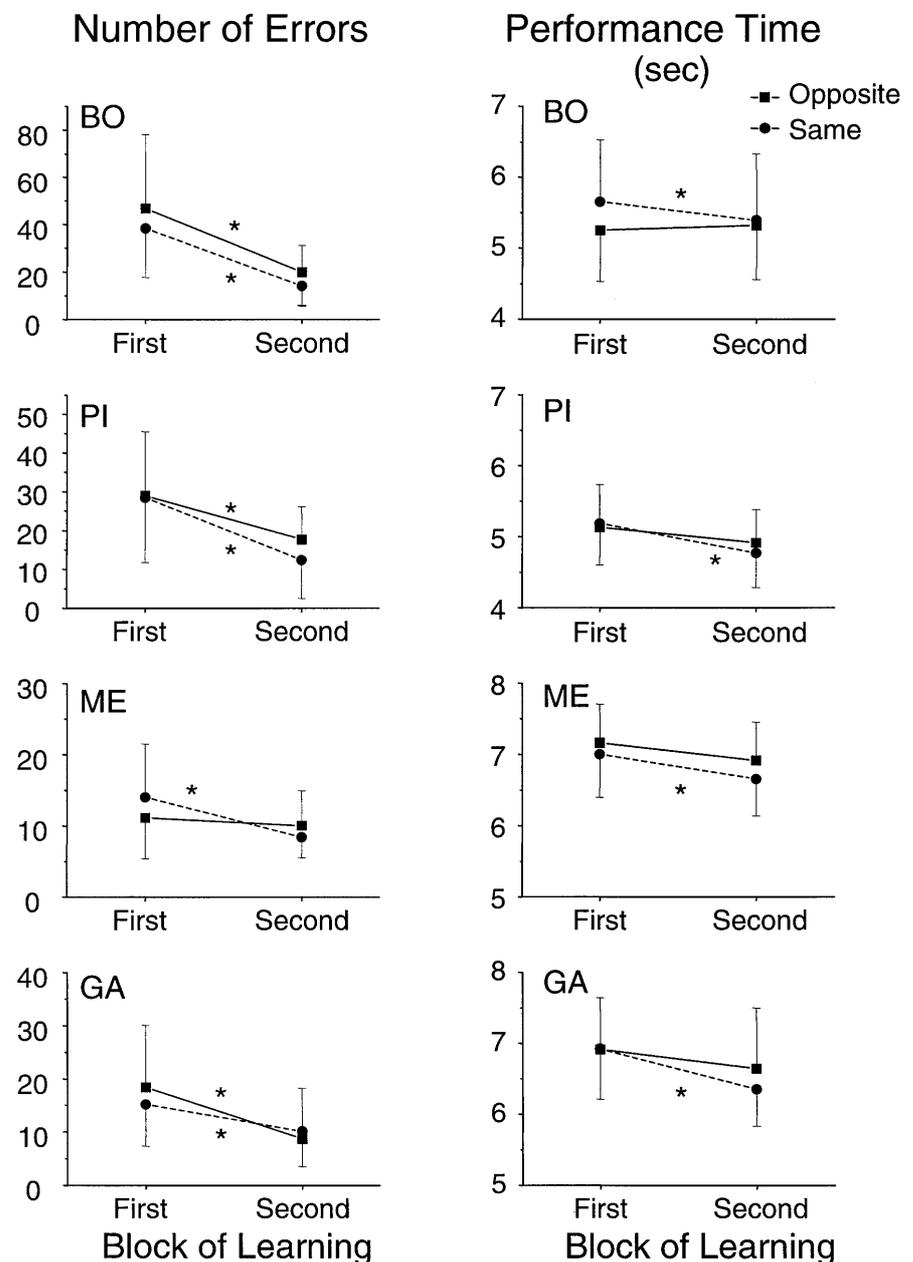
Statistical analysis was performed in two stages for each of the experimental modifications (opposite hand and sequence reversal):

1. Characterization of early learning stage. In order to examine whether the effect of learning was present in the early stage in the original and modified conditions, we performed statistical analysis on these parameters for each monkey between the first and second learning blocks by using the Wilcoxon matched-pairs signed-ranks test.

2. Comparison between the early and late learning stages. In order to examine whether the one-time learning and the long-term learning were differentially affected by the experimental modifications, we first calculated the “difference score” for each of these parameters in each stage. The difference score indicates the benefit of learning (pre-learning value minus post-learning value). The score was measured in the two conditions: the original and modified conditions. For the early stage of learning, the difference score was simply the value in the first learning block (pre-learning) minus the value in the second learning block (post-learning). For the late stage of learning, the pre-learning value was the average value across different new hypersets, because the learning performance, irrespective of specific sequences, improved with long-term practice of the 2×5 task (Hikosaka et al. 1995) and, therefore, the pairwise comparison of pre-post learning values for the same well-learned hypersets would give a misleading result. Accordingly, the difference score was the average value of the new hypersets performed within the 5 days preceding the modification (pre-learning) minus the value for each well-learned hyperset (post-learning).

We then compared the mean difference scores between the two conditions: (1) same versus opposite hand for the opposite-hand experiment; (2) same versus reversed order for the inter-set-reversal experiment. For each monkey, the mean value for all hypersets was calculated for each of the original (i.e., same) and modified (i.e., opposite or reversed) conditions. Then, these means for individual monkeys were used for group comparison between the two conditions by using the paired *t*-test, which allows a pair-wise comparison of data with a small sample size, such as $n=4$ in this study. To ensure the validity of *t*-test results, the Levene test was used to determine if variances between the two compared conditions were equal. In addition, the effect size was calculated to assess the magnitude of differences between the two conditions: absolute effect size = $|\text{mean}(\text{original condition}) - \text{mean}(\text{modified condition})| / \text{SD}(\text{original condition})$. The significance level used for discussion of all data was $P < 0.05$.

Fig. 4 The effect of using the opposite hand on the number of errors (*left*) and the performance time (*right*) in the early learning stage. Data are shown separately for four monkeys (*BO*, *PI*, *ME*, and *GA*). In the opposite-hand experiment (*solid lines*), the monkeys used one hand on the first day and the opposite hand on the second. In the control experiment (*dashed lines*), the monkeys used the same hand on both learning days. Mean values are plotted against the number of learning blocks. *Starred values* indicate that the means for the first and second learning blocks were significantly different (Wilcoxon matched-pairs signed-ranks test: $P < 0.05$). *Error bars* represent the standard deviations



Results

Experiment 1: is memory specific to the hand?

We used monkeys BO, PI, ME, and GA for this part of experiment. Figure 4 shows the number of errors and performance time for each monkey in the opposite-hand experiment (solid lines) and the control (same-hand) experiment (dashed lines) for the early-learning stage. For the control experiment, the number of errors decreased significantly for all four monkeys (Fig. 4, left) [Wilcoxon matched-pairs signed-ranks test; BO: $T(10)=0$, $P<0.01$; PI: $T(41)=60.0$, $P<0.01$; ME: $T(20)=26.0$, $P<0.01$; GA: $T(24)=73.5$, $P<0.05$]. Similar to the control experiment, for the opposite hand experiment, the number of errors decreased significantly for monkeys BO, PI, and GA, but not for ME [BO: $T(10)=7.5$, $P<0.05$; PI: $T(21)=33.0$; $P<0.01$; GA: $T(24)=10.5$, $P<0.01$; ME: $T(19)=62.0$, $P>0.05$]. In contrast, the performance time (Fig. 4, right)

did not decrease in the opposite-hand experiment in all four monkeys [BO: $T(10)=23.0$; PI: $T(21)=69.0$; ME: $T(19)=63.0$; GA: $T(24)=106.0$; $P>0.05$ for all monkeys], unlike the control experiment [BO: $T(10)=6.0$, $P<0.05$; PI: $T(41)=122.0$, $P<0.01$; GA: $T(24)=36.0$, $P<0.01$; ME: $T(20)=52.0$, $P<0.05$].

We then compared these data with the late stage of learning using the difference score (i.e., magnitude of learning effect) (Fig. 5). Table 1 shows mean difference scores of all monkeys for the early and late learning stages. For the number of errors, the difference scores for the opposite hand and the same hand were not different in the early stage (t -test: $P>0.05$) (Fig. 5A), but were different in the well-learned stage ($P<0.01$) (Fig. 5C). For the performance time, the difference scores were different both in the early stage ($P<0.05$) (Fig. 5B) and in the well-learned stage ($P<0.01$) (Fig. 5D). Where differences were present, the difference score was greater in the control (same-hand) experiment than in the opposite-

Fig. 5 The difference score for the number of errors (A and C) and for the performance time (B and D) for the control (same) and the opposite hand (opposite) experiments. Values from the early learning stage are presented in A and B, and those from the well learned stage in C and D. Starred values indicate that the means of all four monkeys were significantly different between the control and opposite hand experiments (t -test: $P<0.05$)

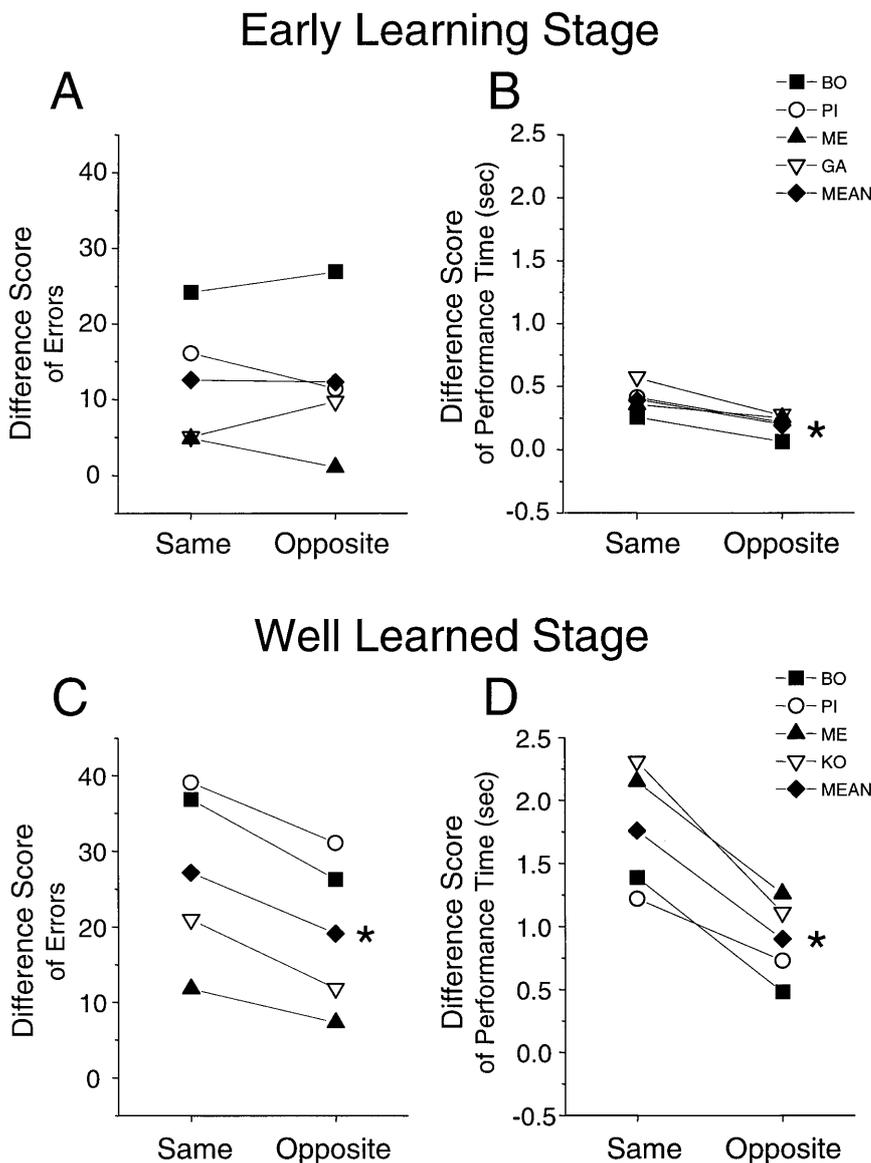


Fig. 6 The effect of the sequence reversal on the number of errors (*left*) and performance time (*right*) in the early learning stage. Data are shown separately for four monkeys (*BO*, *PI*, *ME*, and *KO*). In the reversal experiment (*solid lines*), the monkey learned the original hypersets in the first learning block and the reversed hypersets in the second learning block. In the control experiment (*dashed lines*), they learned the same hypersets twice in both learning blocks. *Starred values* indicate that the means for the first and second learning blocks were significantly different (Wilcoxon matched-pairs signed-ranks test: $P < 0.05$). The *error bars* represent the standard deviations

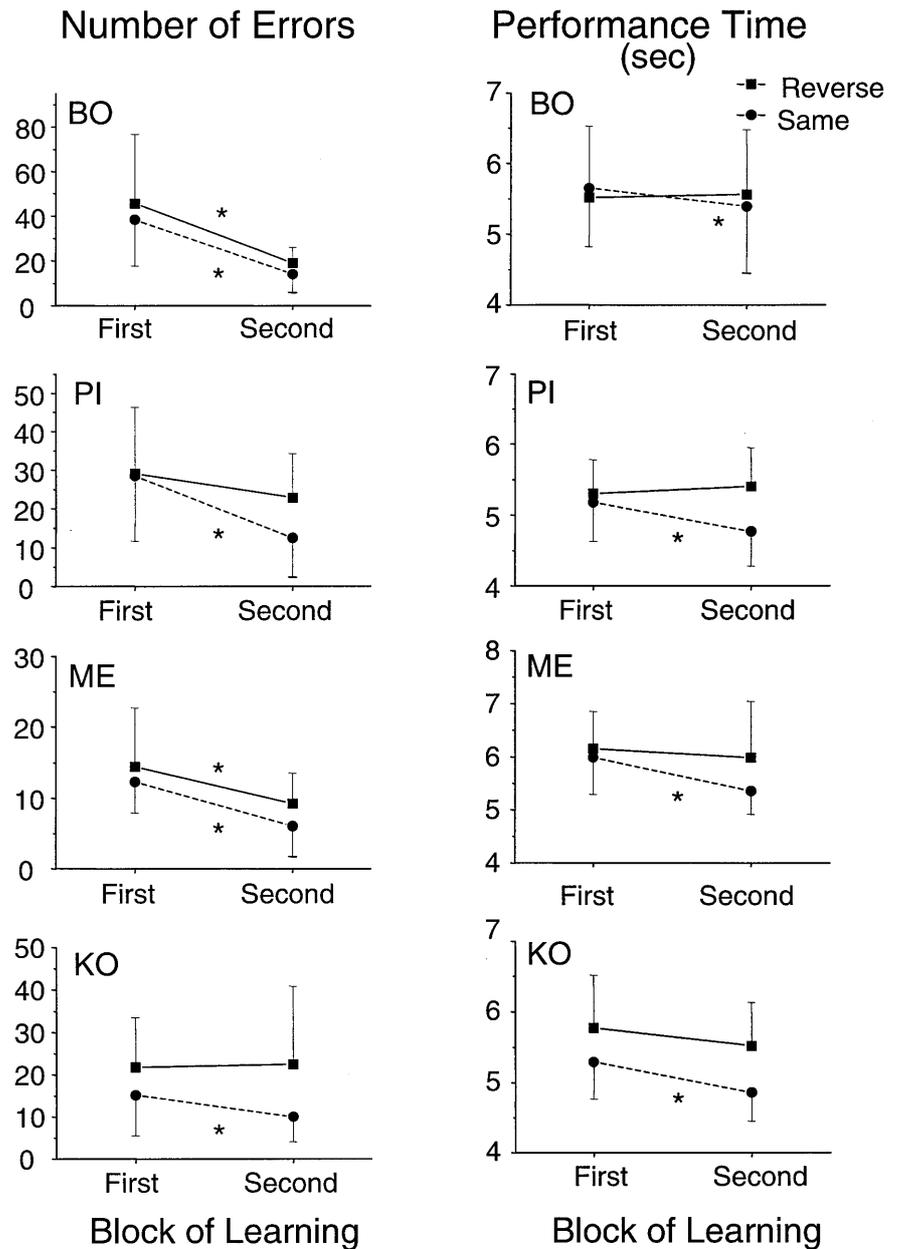
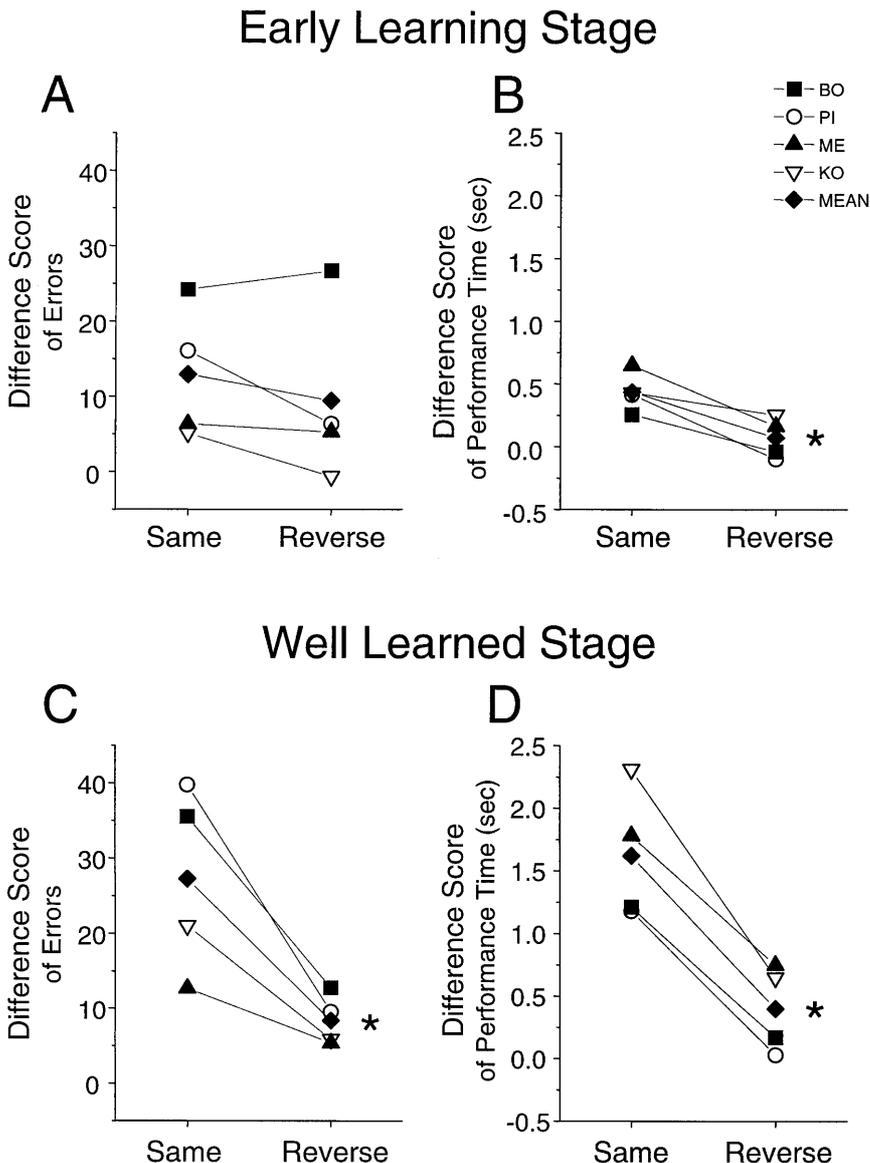


Table 1 Magnitude of learning from pre-learning to post-learning (difference score) for the same- and opposite-hand conditions in the early learning stage and the well-learned stage ($n=4$)

	Same-hand condition	Opposite-hand condition	Levene-test comparison of variances	<i>t</i> -test comparison	Effect size
Early learning stage					
Number of errors	12.6±9.4	12.3±10.8	$F=0.05$ (n.s.)	$t=0.17$ (n.s.)	0.03
Performance time (s)	0.40±0.13	0.17±0.16	$F=0.27$ (n.s.)	$t=4.46^*$	1.77
Well-learned stage					
Number of errors	27.2±13.0	19.1±11.4	$F=0.28$ (n.s.)	$t=6.22^{**}$	0.62
Performance time (s)	1.77±0.54	0.90±0.36	$F=5.12$ (n.s.)	$t=5.98^{**}$	1.61

Mean (\pm SD) difference scores; Levene-test and *t*-test comparisons are made between conditions; n.s. = not significant; absolute effect size = $|\text{mean (same)} - \text{mean (opposite)}| / |\text{SD (same)}|$; * $P < 0.05$; ** $P < 0.01$

Fig. 7 The difference score for the number of errors (A and C) and for the performance time (B and D) for the control (*same*) and the reversal (*reverse*) experiments. Values from the early learning stage are presented in A and B and from the well-learned stage in C and D. *Starred values* indicate that the means of all four monkeys were significantly different between the control and reversal experiments (*t*-test: $P < 0.05$)



hand experiment. The magnitude of these differences was also expressed as the effect size (Table 1; see Materials and methods). For the number of errors, the effect size in the early learning stage (0.03) was well below that what Cohen (1988) identified as a small effect size (0.2). Thus, there was no meaningful difference between the same- and opposite-hand experiments. In contrast, the effect size in the well-learned stage (0.62) was between moderate (0.5) and large (0.8) (Cohen 1988). For the performance time, the effect sizes were very large both in the early learning stage and the well-learned stage, showing that there were meaningful differences between the same- and opposite-hand experiments.

Experiment 2: is memory sequential or fragmentary?

We used monkeys BO, PI, ME, and KO for this part of experiment. Figure 6 shows the number of errors and per-

formance time for each monkey in the reversed-order experiment (solid lines) and the control (same-order) experiment (dashed lines). For the control experiment, the number of errors decreased significantly for all four monkeys (Fig. 6, left) [Wilcoxon matched-pairs signed-ranks test; BO: $T(10)=0$, $P < 0.01$; PI: $T(41)=60.0$, $P < 0.01$; ME: $T(10)=0$, $P < 0.01$; KO: $T(18)=30.0$, $P < 0.05$]. For the reversed-order experiment, the number of errors decreased significantly for monkeys BO and ME [BO: $T(9)=1.0$, $P < 0.01$; ME: $T(13)=12.0$, $P < 0.05$], but remained unchanged for monkeys PI and KO [PI: $T(19)=62.5$, $P > 0.05$; KO: $T(18)=79.0$, $P > 0.05$].

For the effect of learning in terms of speed, the performance time (Fig. 6, right) did not decrease in the reversed-order experiment in all four monkeys [BO: $T(9)=20.0$; PI: $T(20)=76.5$; ME: $T(13)=23.0$; KO: $T(18)=60.0$; $P > 0.05$ for all monkeys], unlike the control experiment [BO: $T(10)=6.0$, $P < 0.05$; PI: $T(41)=122.0$, $P < 0.01$; ME: $T(10)=7.0$, $P < 0.05$; KO: $T(18)=25.0$, $P < 0.01$].

Table 2 Magnitude of learning from pre-learning to post-learning (difference score) for the same and reversed order conditions in the early learning stage and the well-learned stage ($n=4$)

	Same-order condition	Reverse-order condition	Levene-test comparison of variances	<i>t</i> -test comparison	Effect size
Early learning stage					
Number of errors	12.9±9.0	9.25±12.0	$F=0.17$ (n.s.)	$t=1.32$ (n.s.)	0.41
Performance time (s)	0.41±0.17	0.07±0.16	$F=0.09$ (n.s.)	$t=5.19^*$	2.0
Well-learned stage					
Number of errors	27.3±12.6	8.4±3.5	$F=14.19^{**}$	$t=3.83^{**}$	1.50
Performance time (s)	1.62±0.54	0.40±0.35	$F=1.20$ (n.s.)	$t=8.18^{**}$	2.26

Mean (\pm SD) difference scores; Levene-test and *t*-test comparisons are made between conditions; n.s. = not significant; absolute effect size = |mean (same) – mean (reverse)|/|SD (same)|; * $P<0.05$; ** $P<0.01$

We then compared these data with the late stage of learning using the difference score (i.e., magnitude of learning effect) (Fig. 7). Table 2 shows mean difference scores of all monkeys for the early and late learning stages. For the number of errors, the difference score was not different in the early stage (*t*-test: $P>0.05$) (Fig. 7A), but was different in the well-learned stage ($P<0.05$) (Fig. 7C). For the performance time, the difference score was different both in the early stage ($P<0.01$) (Fig. 7B) and in the well-learned stage ($P<0.01$) (Fig. 7D). Where differences were present, the difference score was greater in the control (same-order) experiment than in the reversed-order experiment. In addition, the effect size for the difference score of errors was between small and moderate for the early learning stage, and very large for the well-learned stage (Table 2). The effect size for the difference score of performance time was very large both for the early learning stage and for the well-learned stage.

Discussion

Is procedural memory lateralized?

When the monkeys, using the same hand, performed the same sequences (hypersets) for the second time, the performance improved consistently in terms of correct selection (measured by the number of errors) and speed (measured by the performance time). The result confirms our previous finding (Hikosaka et al. 1995) and is evidence for learning in its early stage.

A key finding in the present study was a dissociation between selection and speed. When the hand was switched between the first and second learning blocks, the number of errors, not the performance time, decreased (Fig. 4). The magnitude of learning in the opposite-hand experiment was similar to that in the same-hand experiment for the error measure (Fig. 5A), but it was significantly reduced for the performance time measure (Fig. 5B). These results suggest that, in the early stage of learning, memory for correct selection transfers to the opposite (unpracticed) hand, while memory for speed is accessible only to the hand used for practice.

Interestingly, the pattern of memory dissociation changed in the late (well-learned) stage. For both the error and the performance time measures, the magnitude of learning in the opposite-hand experiment was clearly reduced compared with that in the same-hand experiment (Fig. 5C and D). These data suggest that both memory for selection as well as memory for speed became relatively specific to the performing hand, thereby reducing the intermanual transfer of memory.

These results support the notion that there are at least two types of memory for the performance of the 2×5 task: one related to the correct selection and the other to the speed of performance (Hikosaka et al. 1995, 1999a). Hikosaka et al. (1995) showed that, during the course of learning over several months, the decrease in the number of errors occurred earlier than the decrease in the performance time. Furthermore, when well-learned sequences were retested after 6 months of no practice, the memory for speed was preserved while the memory for correct selection was abolished. In addition to our previous findings, the present data provides further evidence that the pattern of memory dissociation between the selection and speed alters during the time course of learning from the early learning stage to the well-learned stage.

In some related studies in which human subjects learned a ratio of each segment duration within a multiple key-stroke sequence, the memory for the correct selection of required timing (measured by relative timing error) were separated from the memory for movement parameter (measured by absolute timing error) during the early learning stage of these movements (Lai and Shea 1998; Lai et al. 1999; Wulf et al. 1994; see also Wulf et al. 1993). Interestingly, Lai et al. (1999) further indicated that establishing memory for correct selection is a requisite for establishing memory for movement parameter.

The present data imply that two types of memory (correct selection and performance speed) are subserved by separate neural mechanisms. Based on this assumption, we propose the following hypothesis (Table 3). The memory mechanism for correct selection is not specific to the body parts used for practice (i.e., not effector-specific), while the memory mechanism for performance

Table 3 Two kinds of memory

	Memory for correct selection	Memory for performance speed
Effector-specific?	No	Yes
Order-specific?	No	Yes
Early stage?	Strong	Weak
Late stage?	Weak	Strong
Long-term retention?	No	Yes
Implication	What to do	How to do
Brain areas	Pre-SMA; Ant. Striatum	Cerebellum; Post. Striatum

speed is effector-specific. These two mechanisms work concurrently in acquisition of sequential procedures, but with differential emphasis, depending on learning stages. A main stage-dependent change would be that the subject depends gradually less on the mechanism for selection. In the early stage of learning, the number of errors decreased even when the unpracticed hand was used because the improvement depends on the memory mechanism for correct selection (which is not effector-specific), while the performance time decreased only for the practiced hand because the improvement depends on the memory mechanisms for performance speed (which is effector-specific). In the late (well-learned) stage of learning, the subject now heavily depended on the memory for performance speed, so that the unpracticed hand was unable to perform the sequence correctly. The memory for performance speed may be more robust so that, after a long absence of practice, the correct selection may be lost while the performance speed is maintained.

Consistent with this hypothesis, a series of studies from our laboratory has indicated that different brain regions are critically related to different learning stages and, therefore, different aspects of learning. The medial frontal cortex, especially the presupplementary motor area (pre-SMA), and the striatum, especially its anterior part, are related to learning of correct selection. This was shown by three lines of observations using the 2×5 task: (1) neurons in the pre-SMA (Nakamura et al. 1998) and the anterior striatum (Miyachi et al. 1997b) tend to be more active in the early learning stage; (2) reversible inactivation of the pre-SMA (Nakamura et al. 1999) or the anterior striatum (Miyachi et al. 1997a) leads to deficits in correct selection only in the early learning stage; and (3) these effects are present in a non-effector-specific manner. Functional magnetic-resonance imaging (fMRI) studies on human subjects support this conclusion (Hikosaka et al. 1996) and provide further evidence that the dorso-lateral prefrontal cortex, possibly together with parietal cortical areas, contribute to the early stage of learning (Sakai et al. 1998). On the other hand, the posterior striatum and the cerebellar dentate nucleus are related to learning of movement parameters. This was shown again by several lines of observations: (1) neurons in the posterior striatum tend to be more active in the late learning stage (Miyachi et al. 1997b); (2) revers-

ible inactivation of the posterior striatum (Miyachi et al. 1997a) and the cerebellar dentate nucleus (Lu et al. 1998) leads to deficits in the late learning stage; and (3) the effect on the dentate nucleus is present in a effector-specific manner.

Structure of procedural memory

Procedural skill is usually composed of a sequence of elementary actions. Earlier psychological studies have noted that a main effect of procedural learning is to compile the elementary actions into a unique chain (Anderson 1982). Underlying this suggestion is that memory for the whole sequence is created with practice. But it was unclear when and how the chaining occurs.

The results of the reversed-order experiment provide a suggestion to this issue. When the order of sequence was reversed between the first and second learning blocks, the number of errors decreased in two monkeys (Fig. 6), suggesting that the structure of memory may be based on individual component sets. On the other hand, the other two showed no change, suggesting memory structure is based on a whole sequence. Group data reflected this behavioral variability among the four monkeys. The magnitude of learning effects (difference score) between the same-order experiment and the reversed-order experiment (Fig. 7) was not statistically different in terms of the number of errors. However, there was some meaningful difference between the two experiments, since the effect size was between small and moderate. Thus, it appears that the structure of memory varies among monkeys in the early stage of learning: some are based on individual component sets and some are based on a whole sequence.

In contrast, the results of performance time were consistent among monkeys. The performance time of the reversed-order experiment showed no change (Fig. 6) from the first learning block to the second. Similarly, the magnitude of learning effects between the same-order experiment and the reversed-order experiment was significantly different (Fig. 7). These results suggest that the effect of learning on speed is based on the entire sequence in the early stage of learning. Thus, the memory for performance speed is specific to the order.

We have suggested that, in the late (well-learned) stage of learning, the subject is more dependent on the memory for performance speed than for correct selection of response. Consistent with this idea, the difference score in the reversed-order experiment was smaller than that in the same-order experiment, not only for the performance time, but also for the number of errors (Fig. 7C and D). In other words, the structure of memory was altered from the early stage to the well-learned stage. With practice, the arbitrary structure of memory, which varies among monkeys, was consistently replaced with the dominant memory controlling an entire sequence of a hyperset. Evidence consistent with our data has been shown in well-learned human behaviors, such as typing

of words (see Keele 1981; Rosenbaum 1991; Stelmach and Diggles 1982).

Conclusion

In summary, we suggest that there are two types of memory that support learning of sequential procedures. The first type of memory is assessed by the number of errors (memory for correct selection), while the second type is assessed by the performance time (memory for performance speed). They would help mastering skills in two aspects: (1) what to do and (2) how to do. This classification of memory is supported by their different characteristics: (1) the memory for correct selection is not specific to the hand used for practice and not always specific to the order of sequence; (2) the memory for performance speed is specific to the hand used for practice and specific to the order of sequence. These memory mechanisms work concurrently in the early stage of learning, but, with long-term practice, the memory for performance speed becomes a dominant determinant of performance (Hikosaka et al. 1999b). This hypothesis is consistent with the results of physiological experiments using the same 2×5 task, which suggest separate brain regions corresponding to the two types of memory.

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