Effects of Local Inactivation of Monkey Medial Frontal Cortex in Learning of Sequential Procedures

KAE NAKAMURA,[1] KATSUYUKI SAKAI,[1,2] AND OKIHIDE HIKOSAKA[1]
[1]*Department of Physiology, School of Medicine, Juntendo University; and* [2]*Department of Neurology, Division of Neuroscience, Graduate School of Medicine, University of Tokyo, Tokyo 113, Japan*

Nakamura, Kae, Katsuyuki Sakai, and Okihide Hikosaka. Effects of local inactivation of monkey medial frontal cortex in learning of sequential procedures. *J. Neurophysiol.* 82: 1063–1068, 1999. To examine the role of the medial frontal cortex, supplementary motor area (SMA), and pre-SMA in the acquisition and control of sequential movements, we locally injected muscimol into 43 sites in the medial frontal cortex while monkeys (n = 2) performed a sequential button-press task. In this task, the monkey had to press two of 16 (4 × 4 matrix) buttons illuminated simultaneously in a predetermined order. A total of five pairs were presented in a fixed order for completion of a trial. To clarify the differential contribution of the medial frontal cortex for new acquisition and control of sequential movements, we used novel and learned sequences (that had been learned after extensive practice). We found that the number of errors increased for novel sequences, but not for learned sequences, after pre-SMA inactivations. A similar, but insignificant, trend was observed after SMA injections. The reaction time of button presses for both novel and learned sequences was prolonged by inactivations of both SMA and pre-SMA, with a trend for the effect to be larger for SMA inactivations. These findings suggest that the medial frontal cortex, especially pre-SMA, is related to the acquisition, rather than the storage or execution, of the correct order of button presses.

INTRODUCTION

In a previous study (Nakamura et al. 1998), we recorded neuronal activity in the medial frontal cortex while the monkey performed a sequential button press task, known as "2 × 5 task" (Hikosaka et al. 1995). Presupplementary motor area (pre-SMA) neurons were more active when the animal performed new sequences than when it performed well-learned sequences. The results suggested that the pre-SMA is related to the acquisition of new sequences rather than the storage of procedural memory. The results of our functional magnetic resonance imaging (MRI) study (Hikosaka et al. 1996) were also consistent with this hypothesis.

However, it is still possible that the pre-SMA activation could reflect activity in other structures that contain the true learning mechanism. In fact, it has been shown that multiple brain areas are activated during new learning, such as the dorsolateral prefrontal cortex, the parietal cortex (Sakai et al. 1998), the lateral premotor area, anterior cingulate area (Jenkins et al. 1994; Jueptner et al. 1997), cerebellum (Friston et al. 1992), and basal ganglia (Seitz and Roland 1992). To investigate this issue, we inactivated SMA or pre-SMA locally by injecting muscimol (a GABA agonist) while the monkey performed the sequential button press task, "2 × 5 task" (Hikosaka et al. 1995). If the medial frontal cortex is necessary for acquisition, not for performance of the learned sequences, the inactivation should impair the monkey's ability to learn new sequences but should not affect performance of well-learned sequences.

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METHODS

General procedures

We used the same two Japanese monkeys (*Macaca fuscata*) as in our recording experiment (Nakamura et al. 1998): *monkeys BO* and *GA*. The surgical procedures and the task have been described in a previous paper in detail (Hikosaka et al. 1995). Briefly, a head-holding device and a chamber for unit recording and drug injection were implanted under general anesthesia. A scleral search coil was implanted in one eye for monitoring eye position (Judge et al. 1980). All surgical and experimental protocols were approved by the Juntendo University Animal Care and Use Committee and are in accordance with the National Institutes of Health Guide for the Care and Use of Animals.

In the 2 × 5 task (Fig. 1*A*), the monkey was asked to press five pairs of buttons in the correct order. The animal began a trial by pressing the home key. Then, 2 of 16 target LEDs (4 × 4 grid) turned on simultaneously ("set"). The monkey had to press the illuminated buttons in the correct order. A total of five sets ("hyperset") were presented in a fixed order for completion of a trial. If the wrong button was pressed, the trial was aborted, and the monkey had to start the trial again by pressing the home key. Each hyperset was presented repeatedly in a block until 10–20 successful trials had been performed. The monkeys performed "new hypersets," which were experienced for the first time, and "learned hypersets," which had been practiced extensively (almost every day) and could be performed with few errors. The number of learned hypersets and the duration of practice before the inactivation experiments was: *monkey BO*, $n = 16$, >2 yr; *monkey GA*, $n = 10$, > 8 mo.

Injection procedures

The injection sites were determined to be in the pre-SMA and SMA by the results of microstimulation, unit recording using the electrode attached to the injection tube, and histology (*BO*) or MRI (*GA*) (Fig. 2). For the pre-SMA, larger currents (40–80 μA) and more pulses (40–60 pulses) were needed to evoke movements compared with the SMA (20–40 μA at 20 pulses) (Nakamura et al. 1998).

The injection device consisted of a stainless steel tube connected to a piece of a polyethylene tubing that was in turn connected to the tip of Hamilton syringe (10 μl). A tungsten microelectrode was attached to the tube's side. For each experiment, muscimol solution (5 μg/μl × 4 μl) was pressure-injected at a single site (Fig. 2).

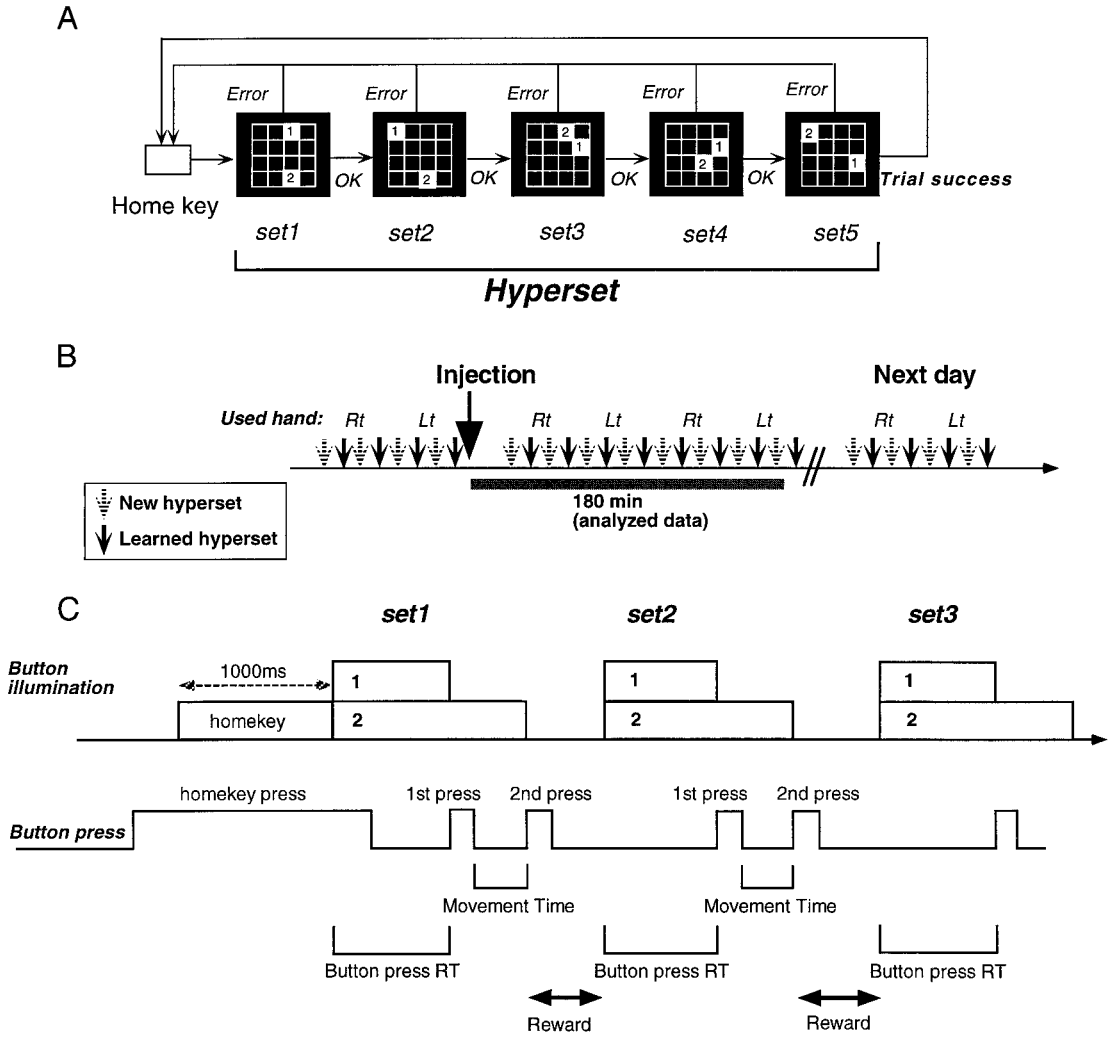


FIG. 1. *A*: 2 × 5 task. *B*: experimental procedure. Performance was ensured to be normal before and on the next day of the injection. *C*: task sequence and behavioral measures. Only the events for the 1st 3 sets are illustrated.

Before the injection, we asked the monkey to perform several learned and new hypersets to make sure that the monkey was well motivated and the performance was within the normal range. After the injection, the monkey performed 20–40 hypersets, which included both new and learned hypersets. The hand used was changed alternately every four to five hypersets (Fig. 1*B*). The postinjection data were analyzed for the period after each injection for 180 min, although we obtained the data for the period ≤200 min. We also obtained the data on the next day of the experiment to see if any effects by injection remained.

Control data were obtained when no injection was made or after saline injections. Saline injections were made at several sites (4 sites for *monkey BO*, 5 sites for *monkey GA*, Fig. 2) where the muscimol injection showed a strong effect.

Data analysis

To assess the accuracy of performance, we counted the number of errors to criterion (10 successful trials) for each block of the experiment. To assess the speed of performance, we measured, for each set, the button-press reaction time (BP-RT): the time from the stimulus onset to the pressing of the first button and the movement time (MT): the time between the releasing of the first button to the pressing of the second button (Fig. 1*C*). We also calculated the percentage of anticipatory saccades that started before the target onset and ended within the area of the first target of the next set. More frequent anticipatory saccades indicate the extent of long-term learning (Miyashita et al. 1996).

RESULTS

We injected muscimol at a single site in either the SMA or pre-SMA. All injection sites were located within the medial wall not including the upper bank of the cingulate sulcus. We infer that the spread of infused muscimol was restricted (probably within 2–3 mm in diameter) from the following results. First, we did not observe the effect when muscimol was injected outside of, but just beside the pre-SMA or SMA (sites indicated in Fig. 2, *). Second, after the experiment, we advanced the electrode attached to the injection tube deeper into the brain and observed neuronal activity at ~1.5 mm from the injection site.

Change of the number of errors

In the control condition, the number of errors for learned hypersets was much smaller than that for new hypersets (Fig. 3*A*). After muscimol injections, the number of errors for

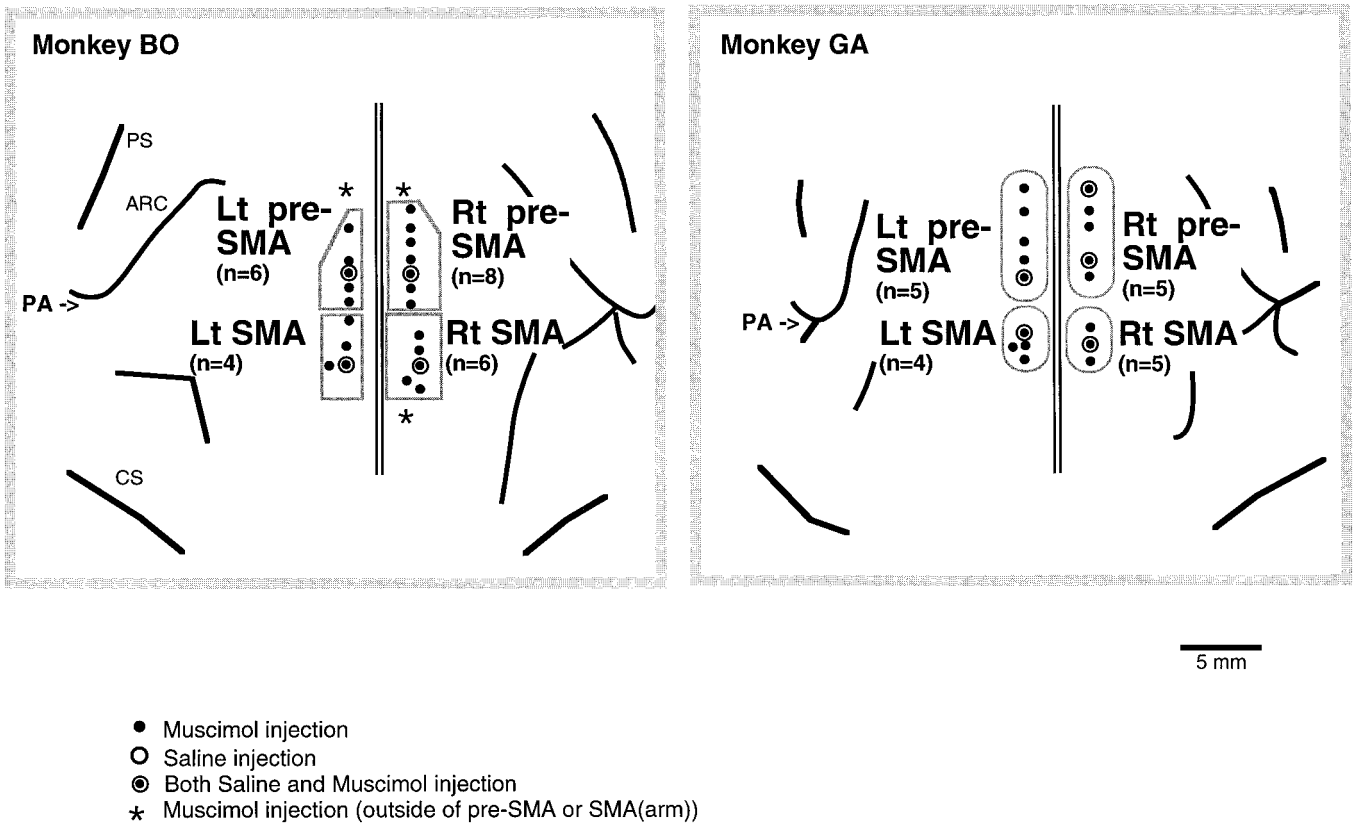


FIG. 2. Top view of the brain of 2 monkeys (anterior: *top*). ●, injection sites. For some points, the injection was performed twice. ○, sites for saline injection; *, sites of muscimol injection outside the presupplementary motor area (pre-SMA) or SMA (arm representation). PS, principal sulcus; ARC, arcuate sulcus; CS, central sulcus; PA, the genu of the arcuate sulcus. *n*, number of experiments.

learned hypersets showed no significant changes. In contrast, the number of errors for new hypersets was significantly greater by muscimol injections into the pre-SMA compared with the control condition. A similar change was observed by the SMA injections, which, however, was not statistically significant (Bonferroni/Dunn test, $P = 0.020$ for *monkey BO*, $P = 0.038$ for *monkey GA*). The results obtained from two monkeys were very similar. Although the injection was unilateral, the effect was present regardless of which hand the monkey used. The errors took the form of pressing the other lighted button; we did not see errors made by pressing a button that was not illuminated.

Figure 3*B* shows the time course of the effect on the number of errors for new hypersets. After muscimol injections in the pre-SMA, not saline injections or no-injection experiments, the number of errors for new hypersets increased within 30 min and the effect lasted for >120 min. The number of errors returned to the normal range on the next day.

Change of the kinematic parameters

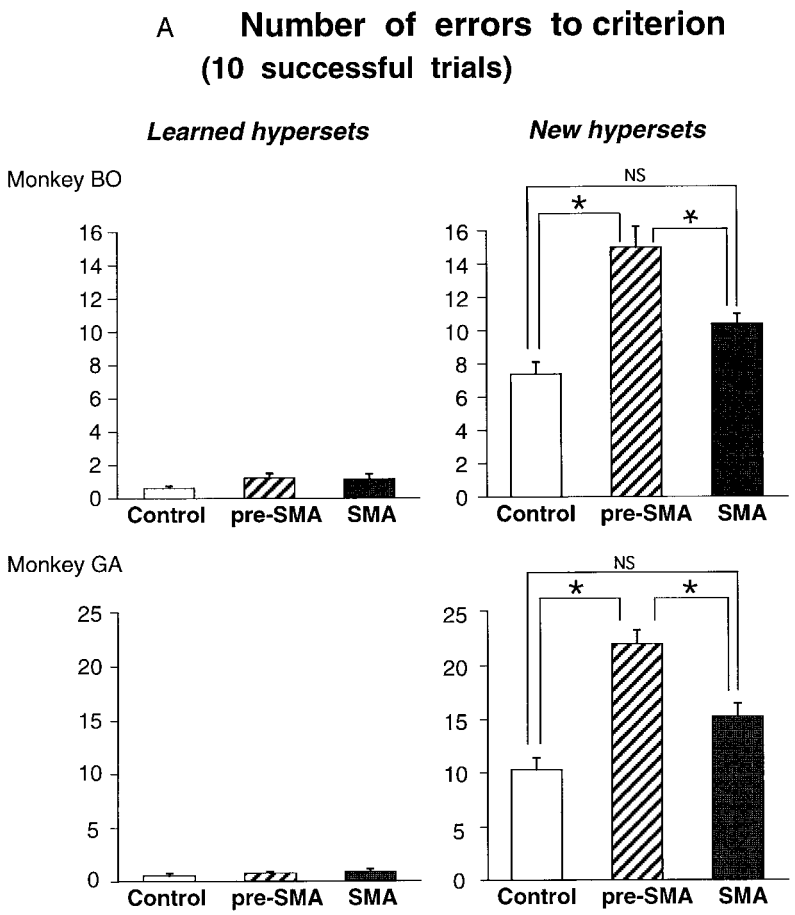
As shown in Table 1, the BP-RT increased after SMA and pre-SMA injections for both new and learned hypersets. For new hypersets, the increase in the BP-RT was significantly greater for the SMA than pre-SMA injections. For learned hypersets, we did not observe a consistent difference between SMA and pre-SMA injections. The MT became longer consistently for learned hypersets for both monkeys. The difference of MT between pre-SMA and SMA inactivations was not consistent between monkeys. The percentage of anticipatory saccades showed no change.

DISCUSSION

Effects on the performance of new sequences

The increase of errors for new hypersets, not for learned hypersets, suggests that the pre-SMA contributes to the acquisition of new sequential procedures. The SMA might play a similar role but less potently, given the similar but insignificant effects of inactivation. This result is consistent with our previous data, which show that the pre-SMA became more active during learning of new sequences than execution of well-learned sequences (Hikosaka et al. 1996; Nakamura et al. 1998; Sakai et al. 1998). However, deficient learning of new sequences could reflect interruption of several factors other than learning per se, such as novelty detection, selective attention, decision making, error correction, switching motor plan, and memory coding and retrieval (Nakamura et al. 1998).

The effect of muscimol injections was relatively modest so that the animals could reach the criterion after some errors. This modest effect may be partly due to the fact that the injection was relatively small and unilateral. Alternatively, it may indicate that the medial frontal cortex plays only a partial role in acquisition of new sequences. Studies from our laboratory have shown that other brain regions also are related to



The total number of hypersets performed

(Monkey BO)	Learned	New
Control	95	52
pre-SMA injection	194	176
SMA injection	157	131

(Monkey GA)	Learned	New
Control	33	28
pre-SMA injection	61	78
SMA injection	59	51

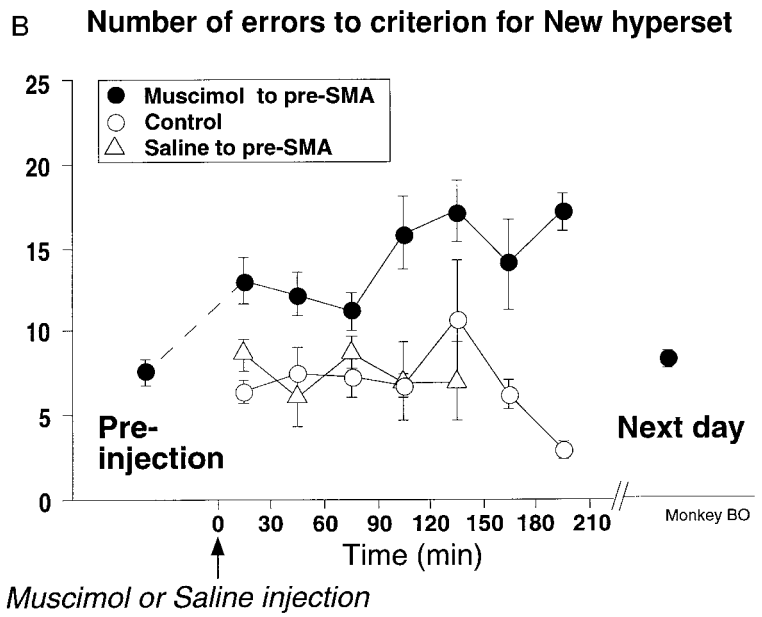


FIG. 3. *A*: number of errors before completing 10 successful trials for learned hypersets (*left*) and new hypersets (*right*). They are shown separately for control (no injection) experiments (Control), pre-SMA injections (pre-SMA), and SMA-injections (SMA). Results obtained for both hands/hemispheres are included. Analysis of variance revealed significant difference in the number of errors among the 3 conditions: Control, pre-SMA, and SMA ($P < 0.01$). Post hoc test (Bonferroni/Dunn test) was performed for each pair: $^*P < 0.01$; NS, not significant. Error bars: ±1SE. *B*: time course of the change in the number of errors before completing 10 successful trials. Data (means ± SE) were calculated for every 30 min after muscimol/saline injections. Effects of muscimol injections in the pre-SMA ($n = 14$), saline injections in the pre-SMA ($n = 2$), and control experiments ($n = 9$) are plotted separately (*monkey BO*). Preinjection: data obtained before the muscimol injection; next day: data obtained on the next day of muscimol injection.

TABLE 1. *Kinematic changes by unilateral inactivation of pre-SMA and SMA*

	Monkey BO			Monkey GA		
	Button-Press RT, ms	Movement Time, ms	Anticipatory Saccade, %	Button-Press RT, ms	Movement Time, ms	Anticipatory Saccade, %
			A. New hyperset			
Control	467.3 ± 144.3	147.2 ± 53.1	11.2 ± 10.5	516.5 ± 186.8	201.9 ± 84.0	11.8 ± 10.0
Pre-SMA	518.1 ± 173.6 (*)	162.7 ± 66.8 (*)	8.9 ± 10.2 (NS)	612.7 ± 229.4 (*)	194.2 ± 64.8 (NS)	8.6 ± 9.4 (NS)
SMA	580.8 ± 213.0 (*)	191.5 ± 81.9 (*)	8.7 ± 10.5 (NS)	651.0 ± 258.9 (*)	203.1 ± 84.8 (NS)	9.4 ± 9.3 (NS)
SMA/pre-SMA	(*)	(*)	(NS)	(*)	(*)	(NS)
			B. Learned hyperset			
Control	136.3 ± 49.6	168.6 ± 49.5	36.8 ± 21.6	182.8 ± 64.6	229.8 ± 53.9	65.5 ± 13.0
Pre-SMA	190.4 ± 78.9 (*)	194.0 ± 69.3 (*)	39.5 ± 18.0 (NS)	212.5 ± 78.4 (*)	247.2 ± 64.2 (*)	56.8 ± 15.8 (NS)
SMA	208.3 ± 77.9 (*)	216.4 ± 75.2 (*)	38.9 ± 18.5 (NS)	210.2 ± 91.5 (*)	247.6 ± 81.0 (*)	60.0 ± 15.8 (NS)
SMA/pre-SMA	(*)	(*)	(NS)	(NS)	(NS)	(NS)

Data (means ± SD) were combined for both hands/hemispheres. Analysis of variance revealed significant difference in each kinematic parameter among the three conditions, control, presupplementary motor area (pre-SMA), and SMA ($P < 0.01$). Post hoc test was performed for each pair; pre-SMA or SMA indicates the comparison with Control, SMA/pre-SMA indicates the comparison between injections in the SMA and pre-SMA. Signifances are in parentheses. RT, reaction time. *$P < 0.01$; NS, not significant.

learning of new sequences: the anterior part of the striatum (Miyachi et al. 1997), dorsolateral prefrontal cortex, precuneus, and intraparietal cortex (Sakai et al. 1998). What is unique about the pre-SMA and SMA remains unresolved.

We also observed the prolongation of the reaction time (BP-RT) for new hypersets. This effect was stronger for SMA injections in contrast to the increase in the number of errors. The prolongation of BP-RT might be due to the animal's uncertainty about the order of button presses, but this view is not consistent with the fact that the increase in the number of errors was greater by pre-SMA than SMA injections. A second possibility is the slowness of movements measured as the prolongation of movement time (MT), but this was true only for *monkey BO*. A third possibility is that the SMA, rather than the pre-SMA, is related to the acquisition of anticipatory execution of hand movements; we previously showed that the BP-RT becomes shorter with practice because the eye and hand move in an anticipatory manner (Miyashita et al. 1996).

Execution of learned sequential procedure

We found no significant increase in the number of errors for learned hypersets for either SMA or pre-SMA inactivation. This was true even though there are some cells especially in the SMA that are preferentially active for learned hypersets (Nakamura et al. 1998). However, this cannot simply be taken to indicate that the medial frontal cortex is unrelated to long-term storage of sequential procedures. That the SMA is related to sequential movements has been shown repeatedly by single-unit recording studies (Tanji and Shima. 1994) and human imaging studies (Grafton et al. 1992, 1994, 1995; Jenkins et al. 1994; Seitz and Roland 1992; van Mier et al. 1998). We consider the following possibilities to account for the discrepancy. *1*) Our localized and unilateral inactivation may have been ineffective as the intact hemisphere may have compensated for the possible deficit. In fact, previous studies have shown that bilateral lesions or inactivations disrupt the learned performance of sequence tasks (Halsband 1987; Shima and Tanji 1998). *2*) The effect of inactivation may vary with the kinds of sequential movements. In our 2×5 task, the targets of sequential movements are presented as visuospatial patterns, unlike in the previous studies (Halsband. 1987; Shima and Tanji. 1998). *3*) The performance of new hypersets requires explicit selection of the correct order similarly to those in previous studies (Barone and Joseph 1989; Kermadi and Joseph 1995; Mushiake and Strick 1993; Mushiake et al. 1991), whereas learned hypersets may be performed nearly automatically as a continuous motor trajectory. This suggests that different brain areas may mainly contribute to the performance of learned hypersets, such as the cerebellar dentate nucleus (Lu et al. 1998), posterior striatum (Miyachi et al. 1997), or M1 (Aizawa et al. 1991).

On the other hand, BP-RT for learned hypersets increased consistently for both monkeys. This might be taken to suggest that the medial frontal cortex is necessary for the learned performance in terms of quick, anticipatory execution rather than correct execution. However, this hypothesis remains to be confirmed, because the MT also was increased, indicating the slowness of movement itself.

We showed previously that the skillful performance for learned hypersets was associated with the co-occurrence of anticipatory saccades and anticipatory hand movements (Miyashita et al. 1996). The inactivation of the SMA or pre-SMA was not followed by a decrease in the occurrence of anticipatory saccades, suggesting that anticipatory saccades may be controlled by other brain areas.

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Present address of K. Nakamura: Center for the Neural Basis of Cognition, Dept. of Neuroscience, University of Pittsburgh, 115 Mellon Institute, 4400 Fifth Ave., Pittsburgh, PA 15213-2683.

Address reprint requests to: O. Hikosaka, Dept. of Physiology, School of Medicine, Juntendo University, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113, Japan.

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