Differential Roles of the Frontal Cortex, Basal Ganglia, and Cerebellum in Visuomotor Sequence Learning

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TWO TYPES OF MEMORY

Memory can be divided into two categories: memory of events or facts, and memory for skills and rules. These are often called declarative memory and procedural memory (Squire, 1986; Tulving, 1985), or they are often characterized as "what" memory and "how" memory. An accumulation of declarative memories will comprise knowledge; an accumulation of procedural memories will comprise intelligence.

This classification is based largely on the fact that amnesic patients, who cannot remember daily episodes, can still learn new behavioral procedures (Cohen and Squire, 1980; Mishkin and Appenzeller, 1987). Because we know that the medial temporal areas including the hippocampus are crucial for declarative memory, we naturally assume that neural systems for procedural memory should be somewhere outside the medial temporal areas.

Which brain areas are responsible for holding procedural memory, then? The answer is still far out of reach, partly because procedural memory is difficult to examine experimentally. Procedural memory cannot be acquired just by showing something to the subject; the subject must perform the procedure repeatedly. The presence of procedural memory cannot be indicated by just a yes-or-no answer; it can be expressed only by performing the procedure.

Our research on procedural learning and memory is an attempt to break through such difficulties. For this purpose, we devised a sequential button press task in which the subjects learn new visuomotor sequences and perform well-learned sequences (Hikosaka et al., 1995).

BEHAVIORAL ANALYSIS OF VISUOMOTOR SEQUENCE LEARNING

In front of a subject, in this case a Japanese monkey, is a panel on which 16 LED buttons are arranged in a 4×4 matrix (Fig. 1A). Beneath the panel is another button called the "home key." If the monkey presses the home key,

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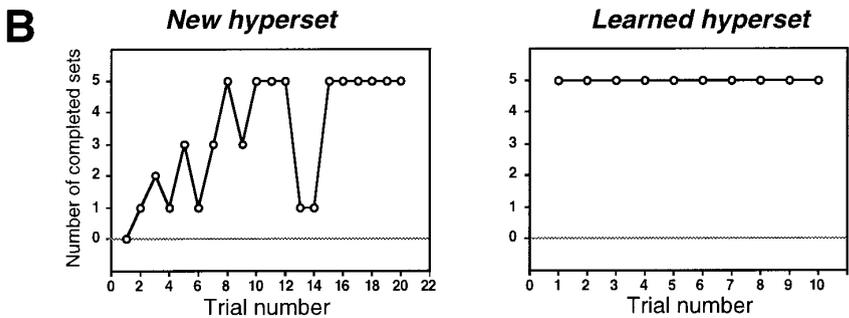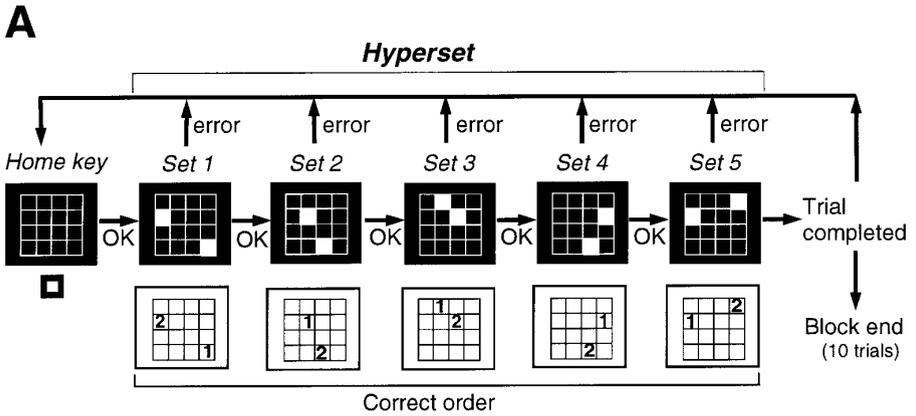
FIG. 1. Procedure of 2×5 visuomotor sequence task for monkeys. (A) An example of a hyperset in the 2×5 task. To complete a trial, the monkey had to press 10 buttons (2 buttons $\times$ 5 sets) in the correct (predetermined) order. (B) An example of a block of practice trials using a new hyperset (left) and a learned hyperset (right). The numbers of completed sets (ordinate) are shown for consecutive trials (abscissa). In the learned hyperset, the monkey completed the block (10 successful trials) with no errors. In the new hyperset, the same monkey made errors initially in the first or second set, but the number of completed sets increased gradually.

2 of the 16 LED buttons are illuminated simultaneously. The monkey has to press them in the correct (predetermined) order which he must determine by trial and error. This is called a "set." If successful, another pair of LEDs, a second set, is illuminated, which again the monkey must press in a predetermined order. A total of 5 sets is presented in a fixed order for completion of a trial, which we call a "hyperset." If the monkey presses a wrong button, the trial is aborted and the monkey must start again from the home key as a new trial. One block of experiments is terminated when the monkey has performed 10 or 20 trials successfully for a particular hyperset, and another hyperset is used for another block of experiments.

A major advantage of the 2×5 task is that nearly an infinite number of new hypersets can be generated. Some of the hypersets were chosen as learned hypersets and were examined daily. In addition to the learned hypersets, the monkey experienced many new hypersets, each of which was learned just once (one block).

Figure 1B shows the performance for one monkey of a new hyperset and a learned hyperset. For each trial from the beginning of the experiment, the

number of completed sets is plotted. For the new hyperset (Fig. 1B, left), the monkey tried to determine the correct order of button presses for consecutive sets. As a consequence, the number of completed sets increased gradually, and at the 10th trial and thereafter he became fairly confident in performing the whole hyperset successfully. It took only 3 min or so for the monkey to acquire knowledge of the sequence. We usually terminated the experimental block when the monkey completed the whole hyperset 10 or 20 times. However, the speed of performance was still decreasing toward the end of the experimental block.

For the learned hyperset (Fig. 1B, right), the monkey usually made no errors and performance time was close to the minimum level which was considerably shorter than that for the new hyperset. However, such excellent procedural skill was acquired with long-term practice. The number of trials to criterion decreased rapidly over the first few days and decreased more gradually afterward until about day 30, approaching the minimum value of 10. The total performance time decreased more gradually and kept shortening even after day 30.

Excellent skill was also evident in eye movements (Miyashita et al., 1996). Initially, the saccade toward the correct target occurred after illumination of the targets (visually guided saccade). After sufficient learning, the saccade tended to occur before the target illumination (anticipatory saccade). This was true only for the hyperset that had been learned. The likelihood of anticipatory saccade increased gradually over 20–30 days of practice of the particular hyperset. The time course was similar to that for the hand sequence.

Another feature of skill learning is that the skill is maintained for a long time even after practice has stopped. This was true for our monkeys' ability to perform learned sequences (Hikosaka et al., 1996a). Our monkeys had the opportunity to take a rest for 6 months, thus forgetting about the 2×5 task. We found that their performance for the learned hypersets was nearly perfect after the rest period. The memories for individual sequences were maintained for a long time without practice—not just 1 motor sequence but 14 different sequences.

These behavioral experiments suggested that the memory for visuomotor sequences may change considerably in nature and that the brain areas responsible may also change during long-term practice. This consideration led us to generate a working hypothesis suggesting that the mechanism for learning new sequences (learning mechanism) and the mechanism for performing learned sequences (memory mechanism) are independent in the brain.

According to this hypothesis, neurons belonging to the learning mechanism should be active during learning of new sequences, whereas neurons belonging to the memory mechanism should be active during execution of learned sequences. The 2×5 task was ideal for testing this prediction because it allowed us to examine both new and learned sequences whenever a single neuron was recorded. Thus, nearly as many new sequences as possible could be generated (by generating random numbers) while the monkey had learned a set of sequences with everyday practice.

Where in the brain are these mechanisms located? How do they operate and interact? We considered three areas that might be responsible for learning-related processes: frontal cerebral cortex, basal ganglia, and cerebellum. First,

we will concentrate on the data for the medial frontal cortex, in monkeys and then in humans.

ROLE OF MEDIAL PREMOTOR CORTEX

Premotor cortical areas are composed of multiple functional zones. The supplementary motor area (SMA) is of particular interest: there is converging evidence that the SMA is related to complex or sequential movements (Tanji, 1994). Recent anatomical and physiological studies indicated that the classical SMA is subdivided into two distinct areas, the presupplementary motor area (pre-SMA), located rostrally, and the SMA proper (SMA), located caudally (Matsuzaka et al., 1992; Matelli et al., 1991).

We found that neurons in the pre-SMA and neurons in the SMA were quite different in terms of visuomotor sequence learning (Miyashita et al., 1997). Pre-SMA neurons were particularly interesting in that they discriminated between learned sequences and new sequences, as shown in Fig. 2. We asked the monkey to perform four learned hypersets and four new hypersets while we were recording from the same pre-SMA neuron. This typical pre-SMA neuron was nearly silent when the monkey performed well-learned sequences, whereas it was very active when the monkey was learning new sequences. For the new hypersets, the neuron was active before the first button press for every set, though the activity varied with different sets. For the learned hypersets, the neuron fired only at the very beginning: before the first button press for the first set of the first trial.

The preference for new sequences was fairly common among neurons in the pre-SMA. A considerable portion of pre-SMA neurons were more active for new sequences than for learned sequences; neurons preferential for learned sequences were rare. In contrast, most SMA neurons showed no preferential activity; if anything, most of them were preferentially active for learned sequences.

These results suggested that pre-SMA neurons may be related to the learning of new sequential procedures, not storage or retrieval of long-term procedural memories. The function of the SMA in learning was still unclear after these experiments were completed.

However, the single-unit recording study cannot prove that the pre-SMA is necessary for learning of new visuomotor sequences. The pre-SMA activation could be a by-product of the true learning mechanism which may reside somewhere outside the pre-SMA.

A method to solve this issue would be to remove the pre-SMA and see whether new learning is impaired. However, such a lesion would make repeated experiments impossible, which was undesirable for our study. We made, instead, "reversible lesions" by injecting a small amount of muscimol, a GABA agonist (Miyashita et al., 1996), according to the method of Hikosaka and Wurtz (1985).

After the injection we asked the monkey to perform learned hypersets and new hypersets over a period of 3 h. Learned hypersets were chosen from the monkey's skill repertoire of about 20 hypersets. New hypersets were generated by asking the computer to generate random numbers. The test for each hyperset was usually completed within 5 min. The hand used for the performance
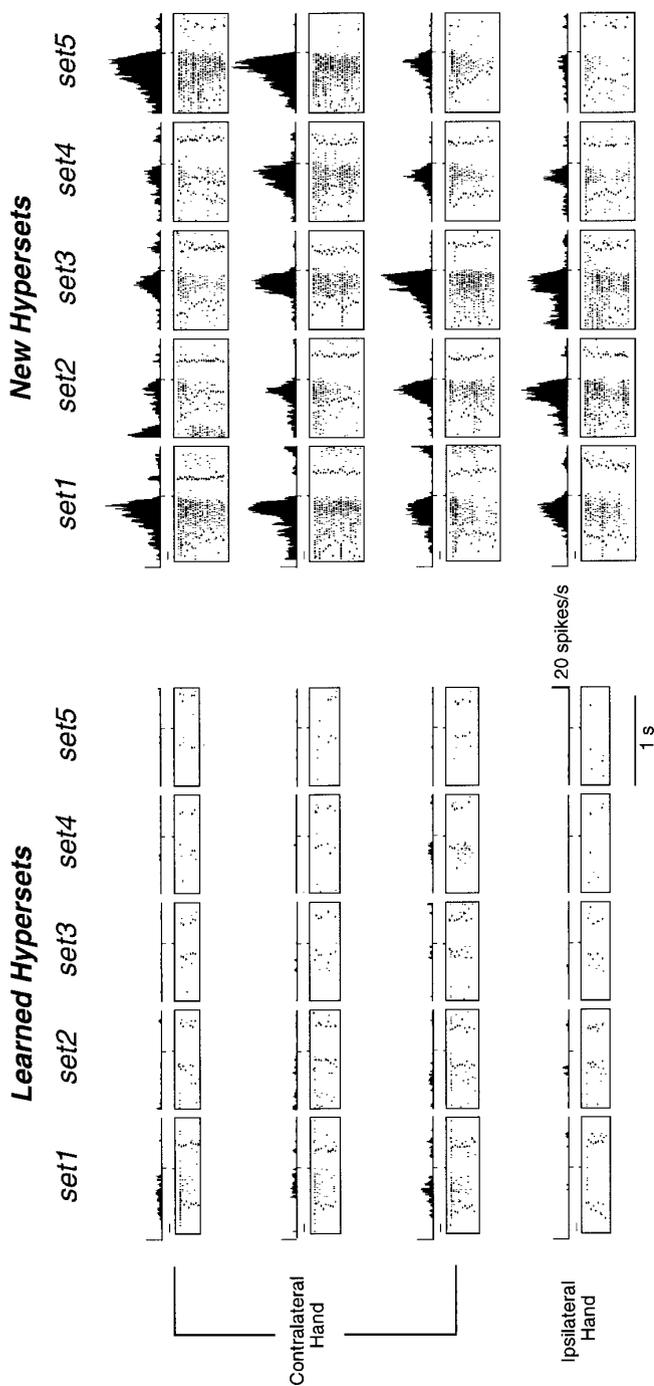
FIG. 2. Selectivity of pre-SMA cell activity for new hypersets. Activity of a pre-SMA cell for four learned hypersets (left) and four new hypersets (right). The spike activities, shown by rasters and histograms, were aligned at the time when the monkey pressed the first button for each set. Only activities for correctly executed trials are shown, with the first trial at the top and the last at the bottom. Inverted triangles in the raster indicate other task-related events (stimulus onset and second button press). The top three rows indicate the data when the monkey used the hand contralateral to the recording site; the bottom row indicates the data when the ipsilateral hand was used.
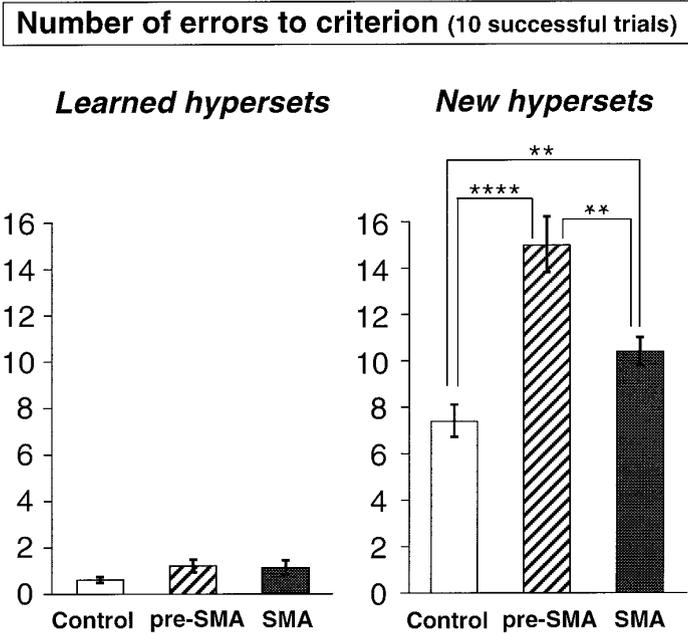
FIG. 3. Effects of muscimol injection in SMA and pre-SMA: number of errors before completing 10 successful trials for learned hypersets (left) and new hypersets (right). The data obtained from one monkey are shown separately for control (white bars), muscimol injections in the pre-SMA (striped bars), and muscimol injections in the SMA (black bars). Results obtained for both hands/hemispheres are included. Compared with the control, the number of errors for new hypersets increased clearly with pre-SMA injections and less so with SMA injections. Data were analyzed using the Mann–Whitney U test (**$p < .01$; ****$p < .0001$). Error bars indicate ±1 SE.

was changed every four hypersets or so to see the laterality of the effects of muscimol.

Figure 3 shows the average number of errors that occurred before 10 successful trials were completed, shown separately for learned hypersets and for new hypersets in three conditions: control saline injections, muscimol injections into the pre-SMA, and muscimol injections into the SMA.

For the control condition, the number of errors was very low, about 0.5 for learned hypersets, while it was about 7.5 for new hypersets (as expected). After muscimol injections into the pre-SMA, the number of errors increased highly significantly for new hypersets, but not for learned hypersets. The data strongly support the hypothesis that the pre-SMA is part of the learning mechanism, that is to say, the pre-SMA is used for learning of new sequences, but is not used for storage of long-term memory or its retrieval.

Muscimol injection into the SMA also led to an increase in the number of errors for new hypersets, but not for learned hypersets; however, the effect was less clear than for the pre-SMA.

Different lines of experiments—single-unit recording, fMRI, and muscimol inactivation—fit together very well to suggest the unique role of the pre-SMA in learning of new procedures. We expected that procedural memories are stored in the SMA, but the experiments we have done so far did not support this idea.
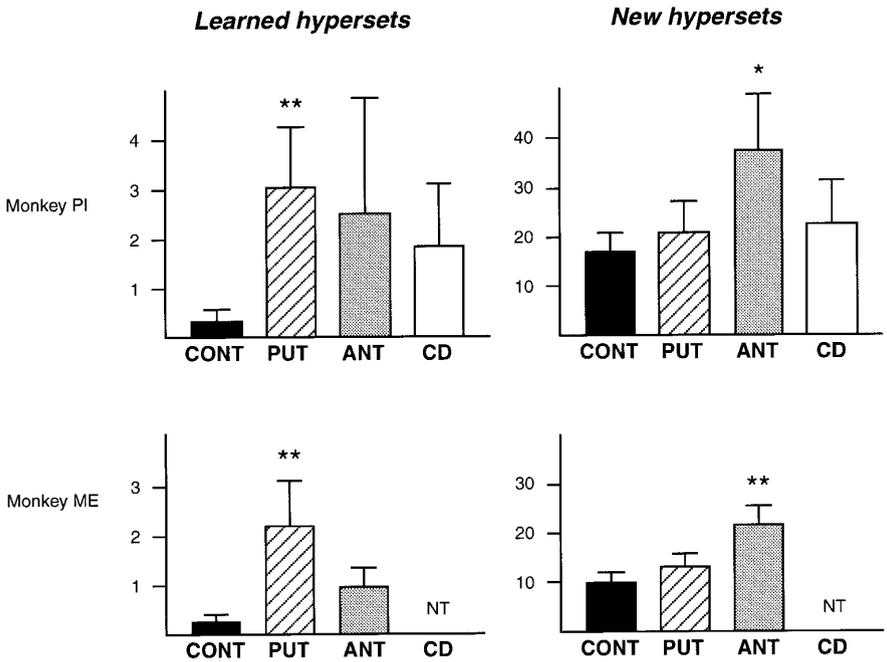
FIG. 4. Effects of muscimol injection in striatum: number of errors before completing 10 successful trials for learned hypersets (left) and new hypersets (right) for each of the three muscimol injection groups (anterior caudate and putamen, ANT; middle and posterior putamen, PUT; middle and posterior caudate, CD) and a saline injection group (CONT). Each of the postmuscimol data sets was compared with the postsaline data set using the Mann–Whitney U test (*$p < .05$; **$p < .005$). NT, not tested. Note that the scale of the ordinate is different between new hypersets and learned hypersets.

ROLE OF BASAL GANGLIA

We then decided to extend our survey to the cerebellum and the basal ganglia, the areas that have been considered to be crucial for voluntary movements. We first injected muscimol at different parts of the striatum (Miyachi et al., 1997). According to the muscimol effects, we classified the areas into three groups: group 1, anterior striatum including the head of the caudate and the anterior part of the putamen; group 2, middle and posterior parts of the putamen; group 3, middle and posterior parts of the caudate.

The results were heterogeneous among the three groups (Fig. 4). By muscimol injections into the anterior striatum, the number of errors increased for new hypersets, but not for learned hypersets, suggesting that the anterior striatum is related to learning of new sequences. By injections into the middle-posterior putamen, the number of errors increased for the learned hypersets, but not for new hypersets, suggesting that the middle-posterior putamen is related to the storage or retrieval of long-term memories. The function of the middle-posterior caudate in learning was unclear.

ROLE OF CEREBELLUM

Our targets in the cerebellum were the cerebellar nuclei, through which cerebellar outputs are sent out. We implanted a guide tube directed at the
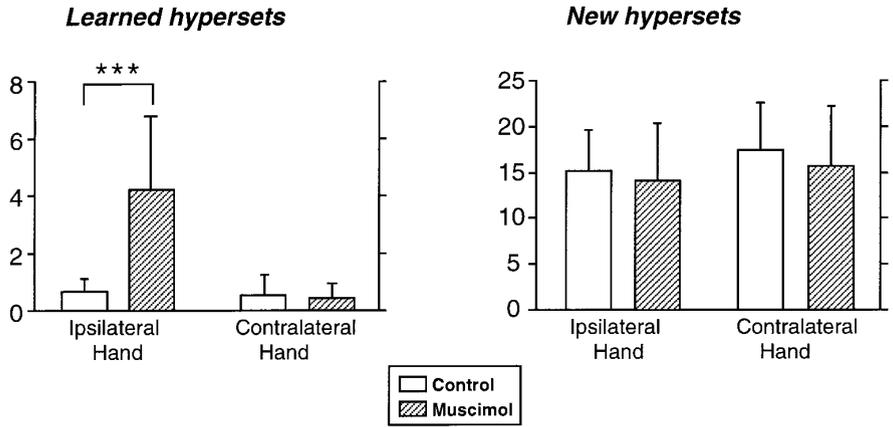
Learned hypersets   **New hypersets**

FIG. 5. Effects of muscimol injection in cerebellar dentate nucleus. The number of errors before completing 10 successful trials (mean and SD) is compared between the control experiments (saline injections) (open column) and the muscimol injections (hatched column), separately for kinds of hypersets (learned vs new), and the hand used (ipsi- vs contralateral to the injection). The number of errors for learned hypersets increased significantly when performed by the ipsilateral hand (***$p < .001$), but not the contralateral hand ($p > .05$). In contrast, the number of errors for new hypersets showed no change ($p > .05$).

cerebellar dentate nucleus. Through the guide tube, we first recorded from single neurons in the dentate nucleus which were related to the performance of the 2×5 task. We then injected a small amount of muscimol at the location where the task-related neurons were recorded (Lu et al., in press).

The mean numbers of errors were compared between the saline control condition and the muscimol condition, separately for learned hypersets and for new hypersets and separately for the ipsilateral hand and the contralateral hand (Fig. 5).

The muscimol effect was present selectively but consistently for different hemispheres. That is, the number of errors increased significantly for learned hypersets only when the hand ipsilateral to the injection was used. No change was observed for new hypersets.

These data suggest that the cerebellar dentate nucleus is used for the storage or retrieval of long-term procedural memories, but not for learning of new sequences. The data stand in sharp contrast with the data for the pre-SMA and SMA.

Of course, we cannot exclude the possibility that different parts of the cerebellum are involved in the learning of new procedures. In addition, the numbers of errors for learned hypersets after the dentate nucleus inactivation were by no means comparable with the number of errors for new hypersets.

A confounding factor we were concerned about was movement deficits which would be caused by the inactivation of the dentate nucleus. The movement time actually increased by muscimol injections only for the ipsilateral hand. The effect was observed for both learned and new hypersets. This result raised the possibility that the increase in the number of errors was due to the movement deficit. However, our data did not support such a possibility because the numbers of errors and the movement times for individual data showed no significant correlation. Thus, the increase in the number of errors was limited

to the muscimol injections in the dorsal part of the dentate nucleus, whereas the increase in the movement time was more evident by injections in the ventral part.

ROLE OF CEREBRAL CORTEX—HUMAN STUDY

We showed that the neurons in the presupplementary motor area (pre-SMA) were activated preferentially for new sequences and the activity decreased each time the monkey acquired a new motor sequence (Miyashita et al., 1997). However, it was difficult to compare the activities between different areas simultaneously. Imaging studies should be advantageous in this respect. We thus decided to apply the same task to human subjects using the functional magnetic resonance imaging (fMRI) technique (Hikosaka et al., 1996b; Sakai et al., 1998).

The subject lay in the supine position in the MRI scanner and held a plate with four button switches. Through a mirror, the subject saw four white rectangles on a screen in which two circles appeared in different colors. The task was the same as that used for the monkeys with some modifications. For each pair of targets, or each set, the subject had to press the corresponding buttons in the correct order which he had to find by trial and error.

The whole process, however, includes various processes including visual and motor processes in addition to the learning process. In order to cancel out the brain activation related to such nonlearning processes, we used a control task in which the same stimuli were presented but the subject was allowed to press the corresponding buttons in any order.

Figure 6 shows areas with learning-related activation in three different experiments which were obtained for a single subject (Hikosaka et al., 1996b). Common to these experiments was an area showing consistent learning-related activation which was located slightly anterior to the anterior commissure (AC). By comparing our data with previous PET studies (Picard and Strick, 1997), we identified this area as part of the pre-SMA. There were additional active sites in the neighborhood, but they were not consistent between the experiments.

In the next study, we obtained multiple MR images to examine the learning-related activation of the whole frontal, parietal, and occipital cortices (Sakai et al., 1998). We found four cortical regions that were consistently related to visuomotor sequence learning: dorso-lateral prefrontal cortex (DLPFC), pre-SMA, precuneus (medial parietal cortex), and intra-parietal sulcus (IPS).

We further found that the time courses of activation were different among these regions (Fig. 7). Thus, the prefrontal activation and pre-SMA activa-

FIG. 6. Human pre-SMA activation during visuomotor sequence learning. Consistent activation of pre-SMA during learning of position and color sequences. The SMA/pre-SMA region (white rectangle in the inset figure below) is enlarged for each of three experiments. From left to right are shown the data obtained during learning of a 2×10 position sequence, a 3×10 position sequence, and a 2×10 color sequence. The subject experienced a new hyperset for each experiment. Common to these data was a local activation that was slightly anterior to the anterior commissure (AC) relative to the axis connecting the AC and PC (posterior commissure), as shown in the inset. The levels of significance for the t test images are color-coded according to the legend at the bottom right.
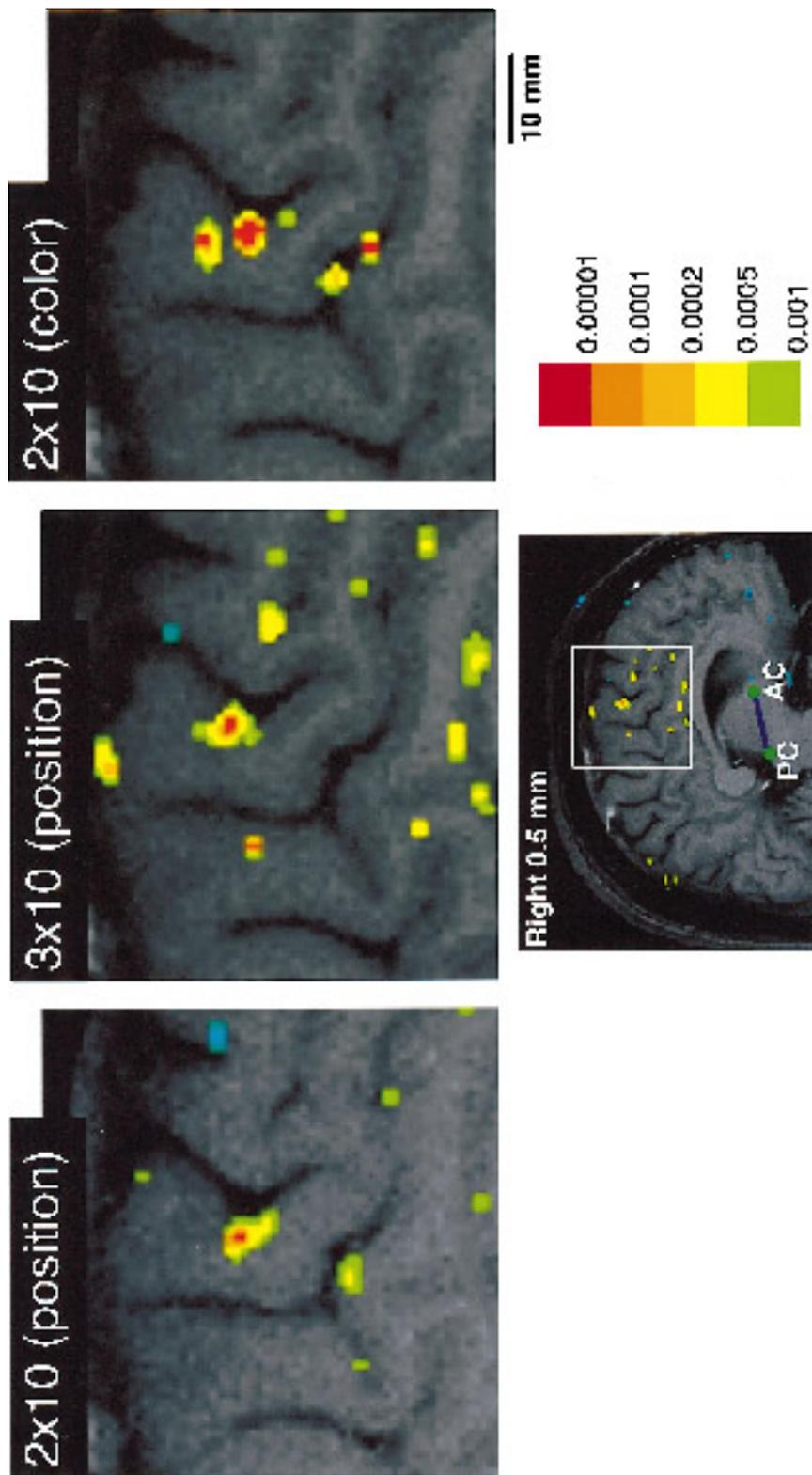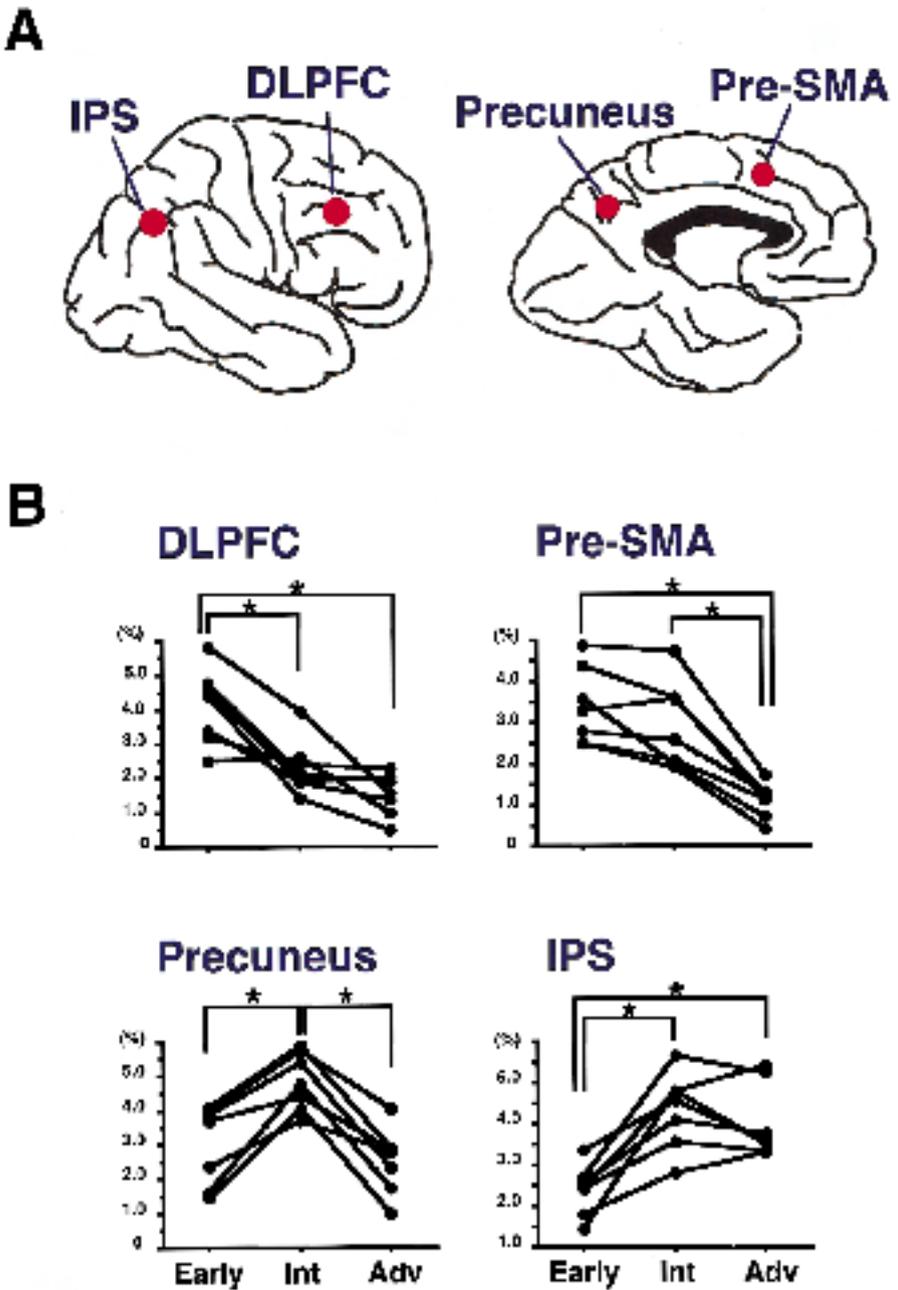
FIG. 7. Correlation of cortical activation with learning stages. (A) Four cortical areas related to learning of visuomotor sequences: dorsolateral prefrontal cortex (DLPFC), presupplementary motor area (pre-SMA), precuneus, and intraparietal sulcus (IPS). (B) For each cortical area, data obtained from six subjects are shown for the three learning stages: early (left), intermediate (center), and advanced (right) stages. Paired comparisons were made between the learning stages using the Wilcoxon signed rank test; significant differences ($p < .05$) are indicated by asterisks.

tion were robust in the early phase of learning and faded as the subject learned the sequence; the precuneus activation and intraparietal activation were initially low and became robust at the intermediate and advanced stages. The initial frontal activation was followed by parietal activation, as if the information was transmitted via the frontoparietal connections.

As far as the pre-SMA is concerned, we see a remarkable similarity between single-unit data in monkeys and fMRI data in humans. These results strongly suggested that the pre-SMA, perhaps together with prefrontal cortex, is related to learning of new sequences.

CONCLUSION

Studies in our laboratory using the 2×5 task have shown that different regions in the cerebral cortex and the basal ganglia contribute either to new learning or to long-term storage of visuomotor sequences. The dynamics of information processing along the long-term learning of visuomotor sequences can be summarized as follows. In the early stage of visuomotor sequence learning, as in our 2×5 task, the prefrontal cortex and pre-SMA together with the anterior part of the basal ganglia would act to initiate learning. In the intermediate stage, the sequence information would then be stored in the parietal cortex (perhaps as a visuomotor sequence). After long-term practice, the motor sequence is now produced by the neural mechanism including the cerebellar dentate nucleus and the posterior part of the basal ganglia, so that performance becomes automatic.

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