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RAPID PUBLICATION

Activation of Human Presupplementary Motor Area in Learning of Sequential Procedures: A Functional MRI Study

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SUMMARY AND CONCLUSIONS

1. Using functional magnetic resonance imaging, we investigated the neural correlates of sequential procedural learning. During the test scans the subjects learned a new sequence (position or color) of button presses; during the control scans they pressed the buttons in any order. The comparison of the test and control scans was expected to reveal the neural activities related to learning, not sensory-motor processes.

2. We found that a localized area in what we regard to be the human homologue of the presupplementary motor area (pre-SMA) was particularly active for learning of new sequential procedures (either position or color sequences), not movements per se.

3. In contrast, the SMA proper (posterior to pre-SMA) was active for the performance of sequential movements, not learning. This was shown in another paradigm in which the subjects pressed the buttons in any order in the test scans and just watched the sequence in the control scans.

4. The learning-related pre-SMA region, which was consistent across different experiments in single subjects, was identified on only one side in each subject.

INTRODUCTION

The role of the supplementary motor area (SMA) in the control of sequential movements has been demonstrated by studies in which trained monkeys (Halsband et al. 1994; Mushiake et al. 1991; Tanji and Shima 1994) and human subjects (Lang et al. 1990; Roland et al. 1980; Shibasaki et al. 1993) were used. It remains unclear, however, how sequential movements might be acquired.

Although previously regarded as a single area, the SMA is now divided into at least two functional subdivisions, SMA proper posteriorly and pre-SMA anteriorly (Luppino et al. 1991; Rizzolatti et al. 1990; Tanji 1994). Unlike the SMA, the pre-SMA has few connections to the spinal cord or to the primary motor cortex (Dum and Strick 1991) and instead receives inputs from the prefrontal cortex (Luppino et al. 1993). Neurons in the pre-SMA frequently show preparatory activity before a forthcoming movement, whereas neurons in the SMA are more likely to show phasic activities locked with individual movements (Matsuzaka et al. 1992). These results raised the possibility that the pre-SMA and SMA play differential roles in learning of sequential movements.

To test this hypothesis, we utilized the sequential button press task that was originally developed for monkeys (Hikosaka et al. 1995). The task, however, required sensory-motor processes in addition to the presumed learning processes. To differentiate between the learning-related activities and the movement-related activities, we performed two kinds of experiments with the use of *1*) a learning-versus-pseudolearning paradigm and *2*) a perform-versus-watch paradigm. These paradigms were designed to extract the learning-related activities and the movement-related activities, respectively, by comparing the test and control conditions.

METHODS

Design of functional magnetic resonance imaging experiments

Eight normal right-handed subjects participated in this study. Experiments were performed with the use of a 1.5-T whole body scanner (Siemens Vision) with a circular polarized head coil. With the use of a multislice T2-weighted gradient echo sequence (FLASH: TR 90 ms, TE 56 ms, flip angle 20°, 64 × 128 matrix, field of view 200 mm, 5 slices, slice thickness 4 mm, scan time 6 s per slice), functional images were obtained in transverse or sagittal planes. The data were analyzed by pixel-to-pixel Student's paired *t*-test such that the sequential control-test pairs were compared. In the following *t*-test images thus calculated, we show the brain areas that showed test-control differences (e.g., learning-related activation or suppression) more significant than 0.1%.

In an experimental session, the control scan and the test scan were alternately repeated, 12 times each. Before each scan the subjects were instructed as to which kind of task was to be performed (e.g., whether or not to learn). The rate of button presses was paced by sound, at 1 Hz for a pair of button presses (see below). One scan lasted 30 s. In some experiments, the experimental session was repeated two or three times while the subject was learning to perform a single sequence.

During the experiment, the subject's head was fixed by adjusting the coil's restraint cushions as tightly as possible without causing discomfort. In addition, vertical head movements were restrained by taping down the head. The stability of the head was checked after the experiment by displaying individual functional images sequentially to detect slight changes in their positions. If any motion was detected, the data were discarded.

Learning procedure: 2 × 10 sequence task

The learning task was to press buttons in the correct order, which the subjects had to find by trial and error (Fig. 1). The subjects lay in the supine position in the magnetic resonance scanner and held a plate on which four button switches were arranged in a 2 × 2 matrix, each button to be pressed by the corresponding one of four fingers (index and middle fingers of the right and left hands). Through a mirror, the subjects saw four white rectangles on a screen in which two circles appeared in different colors (of 4 possible colors). The subjects had to press the two buttons corre-

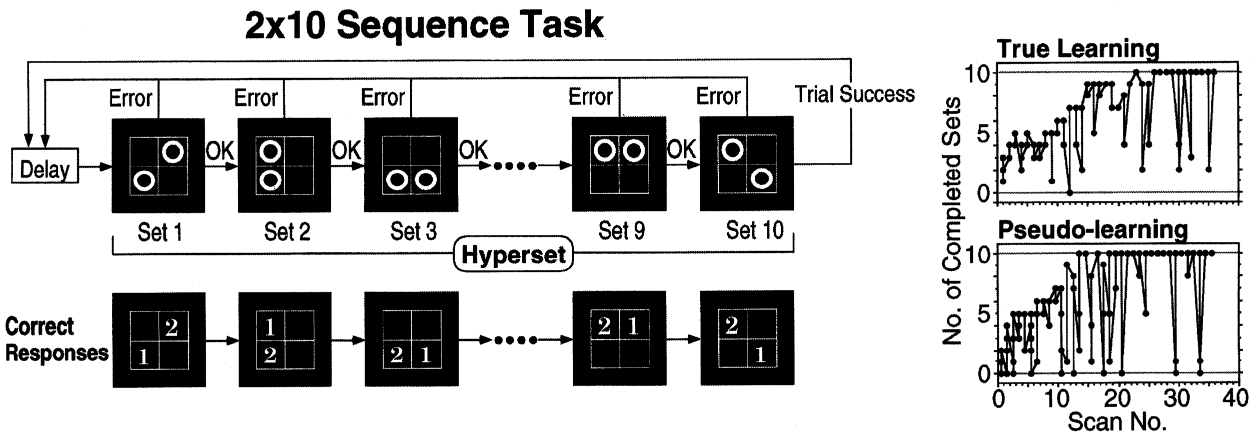

FIG. 1. Experimental paradigm to extract learning-related brain activities. *Right*: examples of the learning process for a single experiment, for the true learning (*top*) and for the pseudolearning trials (*bottom*). The number of completed sets is plotted against the scan number. In this experiment, 3 sessions (i.e., 12 × 3 scans for each condition) were performed in series while the subject was learning a single sequence.

sponding to the positions of the displayed circles (set 1) in the correct order (determined by the computer). The subjects had to find the correct order by trial and error. If the subjects were successful, another pair of circles (set 2) appeared and the subjects again had to press the appropriate buttons in the correct order. In this way, a total of 10 sets was presented in a fixed order for completion of a trial. The whole sequence was called a ''hyperset.'' If the subjects pressed a wrong button, the trial was aborted and the subjects had to start a new trial from the first set. For experienced subjects, we used 3 × 10 task in which the subjects had to press three buttons in the correct order for each set.

Because there was no general rule to find out the correct order, the subjects had to learn the whole hyperset as a single unique sequence. Thus the number of hypersets that can be generated is practically unlimited (Hikosaka et al. 1995). Furthermore, the same stimulus set could be used either as a position sequence or as a color sequence. In the case of position sequence learning, the correct order of button presses, which the subjects had to learn, was determined by the positions of the displayed circles, independent of their colors. For color sequence learning, the correct order of button presses depended on the colors of circles, independent of their positions.

Procedure to extract learning-related activities: learning versus pseudo-learning

To extract the brain activity related to learning, not sensory-motor-processes, we devised a ''learning-versus-pseudolearning'' paradigm in which learning and pseudolearning were used as the test and control tasks, respectively. In the pseudolearning task, unlike in the learning task described above, both the order of sets and the correct order of button presses were randomized for each trial; thus the subjects experienced the same trial-and-error sensory-motor processes, but learned nothing at all (and were so instructed that it was no use trying to learn). To mimic the learning process, however, the probability of success for each set was increased by a small amount each time the subjects completed the set successfully. Consequently, the number of completed sets increased gradually as trials went on, in much the same way as the subjects actually learned the hyperset (Fig. 1, *right*).

Procedure to extract movement-related activities: perform-versus-watch paradigm

To extract movement-related activities unrelated to learning, we devised a ''perform-versus-watch'' paradigm. The perform task, used as the test task, was similar to the pseudolearning task, except that button presses of any order were accepted as successful. The order of sets was randomly determined for each trial. The subjects were required to press the two corresponding buttons in any order; pressing noncorresponding buttons was not allowed. The watch task, used as the control task, was the same as the perform task, except that the computer performed the task; the subjects just watched as the stimuli turned on and off. The same hyperset was used for both tasks, and therefore the subjects watched the same visual stimuli turning on and off at the same rate. There was nothing to learn, because the buttons could be pressed in any order.

RESULTS

In preliminary experiments, using the learning-versus-pseudolearning paradigm, we obtained *t* test images of transverse slices. We found learning-related activation in the mesial frontal cortex, mesial parietal cortex (precuneus), dorsolateral parietal cortex (especially around the intraparietal sulcus), dorsolateral prefrontal cortex, and cerebellar cortex.

In this study, we concentrate on the activation in the mesial frontal cortex, because it showed the most consistent activation across subjects and experiments. Figure 2 shows areas with learning-related activation in three different experiments that were obtained in a single subject. The mesial surface of the right frontal cortex is enlarged to show the surrounding structures. The first two experiments were performed on different days with the use of different position sequences (2 × 10 and 3 × 10 versions). The third experiment was performed on the same day as the second one, but with the use of a color sequence (2 × 10).

Common to these experiments was an area showing consistent learning-related activation, which was located slightly anteriorly to the anterior commissure. By comparing our data with previous positron emission tomography studies (Deiber et al. 1991; Stephen et al. 1995), we identified this area as part of the pre-SMA, which was originally characterized in the monkey (Tanji 1994). There were additional active sites in the neighborhood, but they were not consistent between the experiments.

To reveal the relationship of the learning-specific pre-SMA region with the SMA proper, we used the perform-versus-watch paradigm to extract the movement-related ac-

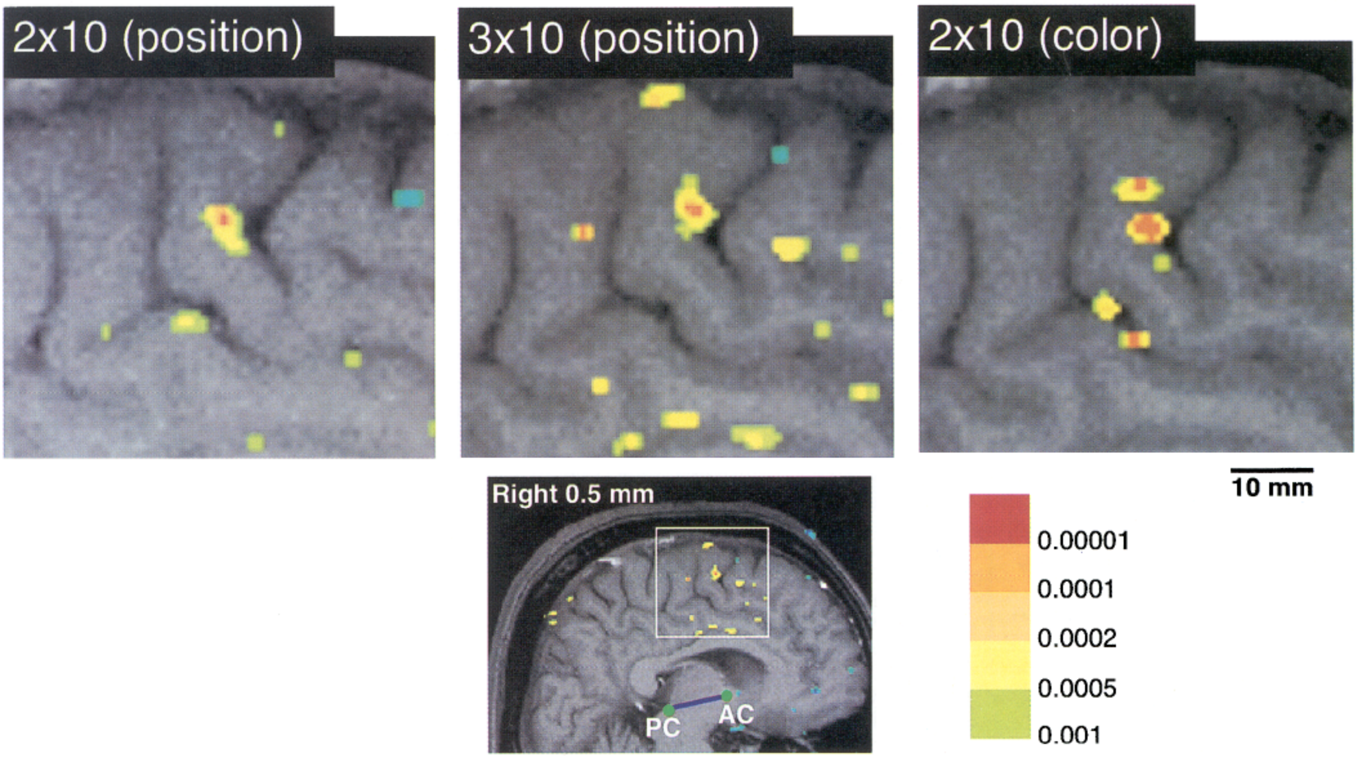

FIG. 2. Consistent activation of presupplementary motor area (SMA) during learning of position and color sequences. The SMA/pre-SMA region (white rectangle in the inset at *bottom*) is enlarged for each of 3 experiments. From left to right are shown the data obtained during learning of a 2 × 10 position 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* at *bottom*. The levels of significance for the *t* test images are color-coded according to the legend at *bottom right*.

tivities while eliminating other factors, especially learning and memory.

In the experiment shown in Fig. 3, we first asked the subject to perform the learning-versus-pseudolearning paradigm; the experiment revealed a focus of activation that we regarded to be in the pre-SMA (Fig. 3, *top*, ○). We then asked the same subject to perform the perform-versus-watch paradigm using the same 3 × 10 hyperset; the experiment revealed a focus of activation that we regarded to be in the SMA (Fig. 3, *top*, △).

Differential activation of the pre-SMA and SMA is clearly seen in the graphs in Fig. 3 showing the cumulative sum of percent signal changes at the two regions of interest for each of the two paradigms. In the learning-versus-pseudolearning paradigm (Fig. 3, *left*), the pre-SMA showed fairly consistent activation, whereas the SMA proper showed no net activation. In contrast, in the perform-versus-watch paradigm (Fig. 3, *right*), the SMA showed consistent activation, whereas the pre-SMA showed little activation.

The subject's performance is shown below for comparison for each paradigm. The cumulative sum of the pre-SMA activation was similar to the improvement of the performance, suggesting that the pre-SMA is particularly active during learning or acquisition of new sequences. These results suggest that the pre-SMA is related to learning, not movements, whereas the SMA proper is related to movements, not learning.

Among eight subjects who participated in this experiment, six subjects showed a dominant focus of learning-related activation in the mesial frontal cortex on one side. The learning-related focus was invariably anterior to the anterior commissure (i.e., in the pre-SMA), although the configuration of the mesial cortex varied between the subjects.

Three subjects repeated the experiment more than three times. The learning-related activation was observed consistently at the same pre-SMA region, again on only one side for each of the three subjects [on the right side in the 1st (male) subject and on the left side in the 2nd (female) and 3rd (male) subjects]. On the other hand, the movement-related activation in the SMA was observed on both sides in each subject.

DISCUSSION

We have shown that a small area in the human pre-SMA is particularly active during learning of new sequential procedures, whether they are position sequences or color sequences. Our data further suggest that the learning-related function may be lateralized, but that laterality may not be determined by sex or handedness.

However, we do not know, on the basis of this experiment, how critical the pre-SMA activation is for learning of sequences. The question can be answered by animal studies in which monkeys perform essentially the same task (Hikosaka et al. 1995). Indeed, Miyashita et al. (1995) have shown that many neurons in the monkey pre-SMA became

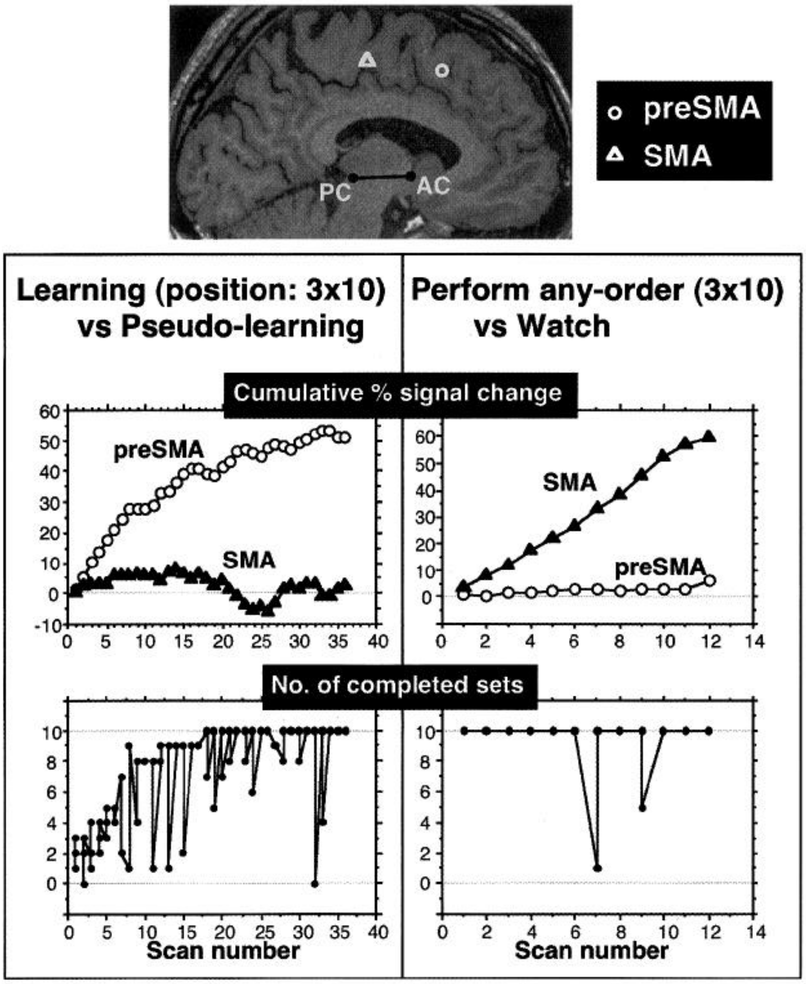

FIG. 3. Differential activation of pre-SMA and SMA. In the magnetic resonance image at the *top* are shown the main locus of learning-related activity (○) in the pre-SMA and the main locus of movement-related activity in the SMA (△) obtained in a single subject. These activities were revealed, respectively, by the learning-vs.-pseudolearning paradigm and the perform-vs.-watch paradigm. In the graphs at *bottom* are shown, across the consecutive magnetic resonance scans, the cumulative sums of the learning-related activity (*left*) and the cumulative sums of the movement-related activity (*right*) for each of the pre-SMA and SMA sites. For comparison are shown the subject's performance (number of completed sets) in these experiments (only for the test tasks). Cumulative % signal change: the percent signal change was essentially the test-minus-control signal intensity at the pre-SMA sites or the SMA site. It was calculated for each test scan by subtracting the average of the signal intensities in the preceding and following control scans from the signal intensity at the given test scan. In this graph are shown the cumulative sum across consecutive scans. Three sessions were performed in series in the learning-vs.-pseudolearning paradigm; 1 session for the perform-vs.-watch paradigm.

active in the initial phase of learning and that the functional blockade of this region disrupted the monkey's ability to learn new sequences (unpublished observation).

Positron emission tomography studies have indicated that the SMA becomes more active as motor performances improve with practice (Grafton et al. 1992; Jenkins et al. 1994). This is opposite to what we observed in the pre-SMA in the present study. No human study, to our knowledge, has indicated specifically the role of the pre-SMA in procedural or motor learning, but it has been shown that the region corresponding to our pre-SMA is activated when higher-order aspects of motor control are required (summarized by Picard and Strick 1996).

This in turn raises a further question on the function of the pre-SMA: is the pre-SMA activation specific to learning of sequences? Or is it related to working memory or attention, which might itself be unrelated to sequence? Relevant to this question is the hypothesis that the anterior cingulate cortex, which is close to the pre-SMA, is related to volitional control of attention (Posner and Petersen 1990). We now try to dissociate these functions by modifying the behavioral paradigms.

Finally, further studies are necessary to better understand how the pre-SMA works as a part of a larger neural system underlying sequential procedural learning (Grafton et al. 1995; Jenkins et al. 1994), including the motor cortex (Karni et al. 1995; Pascual-Leone et al. 1995), basal ganglia (Kermadi and Joseph 1995; Mushiake and Strick 1996), and cerebellum (Jenkins et al. 1994; Pascual-Leone et al. 1993; Seitz and Roland 1992).

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