

Neuronal Activity in the Supplementary and Presupplementary Motor Areas for Temporal Organization of Multiple Movements

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Shima, Keietsu and Jun Tanji. Neuronal activity in the supplementary and presupplementary motor areas for temporal organization of multiple movements. *J Neurophysiol* 84: 2148–2160, 2000. To study how neurons in the medial motor areas participate in performing sequential multiple movements that are individually separated in time, we analyzed neuronal activity in the supplementary (SMA) and presupplementary (pre-SMA) motor areas. Monkeys were trained to perform three different movements separated by waiting times, in four or six different orders. Initially each series of movements was learned during five trials guided by visual signals that indicated the correct movements. The monkeys subsequently executed the three movements in the memorized order without the visual signals. Three types of neuronal activity were of particular interest; these appeared to be crucially involved in sequencing the multiple motor tasks in different orders. First, we found activity changes that were selective for a particular sequence of the three movements that the monkeys were prepared to perform. The sequence-selective activity ceased when the monkeys initiated the first movement. Second, we found interval-selective activity that appeared in the interval between one particular movement and the next. Third, we found neuronal activity representing the rank order of three movements arranged chronologically; that is, the activity differed selectively in the process of preparing the first, second, or third movements in individual trials. The interval-selective activity was more prevalent in the SMA, whereas the rank-order selective activity was more frequently recorded in the pre-SMA. These results suggest how neurons in the SMA and pre-SMA are involved in sequencing multiple movements over time.

INTRODUCTION

In a recent report, we presented evidence to show that both the supplementary motor area (SMA) and the presupplementary motor area (pre-SMA) in the cerebral cortex are crucially involved in sequencing multiple movements over time (Shima and Tanji 1998). In that study, monkeys were required to arrange in time the performance of three different arm movements that were individually initiated after variable intervals. Inactivating either the SMA or pre-SMA by muscimol injection greatly impaired the ability to perform the three movements in the correct order. What was striking was the impairment of the temporal arrangement, despite the ability to perform the three movements without any problems when individually guided with visual instructions. The findings prompted us to investigate how single cells in the two areas

take part in the temporal organization of multiple movements. It was also of interest to compare the neuronal activity in the SMA and pre-SMA concerning the sequencing behavior. Although these areas are known to have very different anatomical connections with the thalamus (Matelli and Luppino 1996) and other cortical areas (Luppino et al. 1993; Picard and Strick 1996), few physiological studies have looked for neuronal activities that are suggestive of functional differences (Matsuzaka and Tanji 1996; Matsuzaka et al. 1992; Nakamura et al. 1998). In this paper, we report a variety of neuronal activities that seem to be associated with retrieving information about the correct sequence of movements to be selected, processing the sequence information to arrange movements in the correct temporal order, and preparing each movement. Furthermore we show that the distribution of neurons with the different properties differs in the SMA and pre-SMA. A preliminary account of this study has appeared elsewhere (Shima et al. 1996; Tanji and Shima 1994).

METHODS

Behavioral procedures

We trained three monkeys (*Macaca fuscata*) to perform the motor task described in the following text. The animals were cared for in accordance with the National Institutes of Health's *Guide for the Care and Use of Laboratory Animals* and *Guidelines for Institutional Animal Care and Use* published by our Institute. Two of the three monkeys used in this study were also used to examine the effects of muscimol injection into the SMA and pre-SMA (Shima and Tanji 1998). We trained the animals to perform three movements (push, pull, or turn a manipulandum) in four (the 1st and 2nd monkeys) or six (the 3rd monkey) different orders with their right arms. The monkeys sat in a primate chair and were required to place the manipulandum in the neutral position and wait 2.5–4.5 s for the first movement-triggering signal (a high-pitched tone). When the animal performed the first movement, a mechanical device returned the manipulandum to the neutral position. While keeping the manipulandum in this position, the animal had to wait 1–1.4 s for each of the second and third movement-triggering signals. A series of three correct movements was rewarded with applesauce 500 ms after completing the last movement. The average interval between motor sequences was 9 s. To teach the monkey the sequence, the correct movement was initially indicated with green (turn), red (push), or yellow (pull) lights. The lights came on individually at the time for each movement together

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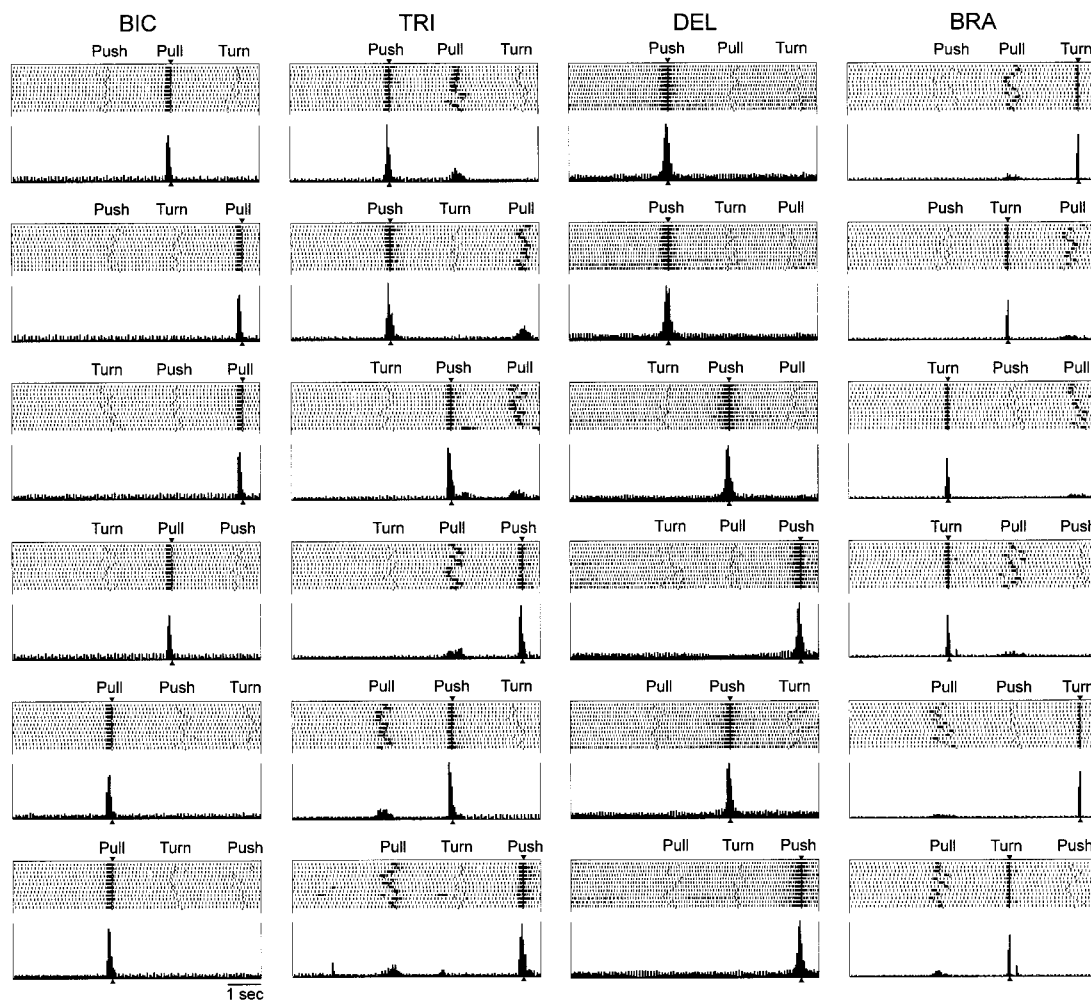


FIG. 1. Activity in 4 forearm muscles while performing 3 different movements in 6 different orders, under memory guidance. The electromyograms, digitized by an A/D converter, are displayed as rasters, with each row representing individual trials that involved performing 3 sequential movements. Peri-event histograms under each raster display the sum of muscle activity in 40-ms bins. Rasters and histograms are aligned on the onset of movement that pulls [for biceps brachii (BIC)], pushes [for triceps brachii (TRI) and deltoideus (DEL)], or turns [for brachioradialis (BRA)] the handle. Small crosses in each raster display indicate the movement onset.

with the movement-triggering tone signal. The animal had to learn the correct sequence during five visually guided trials, after which the sequential motor task was performed from memory. For the memory-guided trials, the tone signal was given as the movement trigger without any lights. After completing the memorized sequential task six times, the lights flashed randomly for 2 s signaling the end of the current sequence and the beginning of the next. There was then a waiting period of 2.5–4.5 s before the next trial began. Thus a particular sequence of movements was performed in blocks. Each block consisted of 11 trials for a given sequence of the three movements, 5 trials with visual guidance and 6 with no visual cues, followed by the next block with a different sequence of movements. The order in which different sequences were used was varied unpredictably. At least two blocks of trials for each sequence of movements were included in the data file while recording from each individual cell.

Electromyographic (EMG) recordings were made from muscles in the digits, wrist, elbow, shoulder, neck, chest, abdomen, thigh, and paravertebral area using Teflon-coated silver-wire electrodes. EMG analysis showed that the activity of the forelimb muscles changed briefly during the execution of individual movements but not during the period when the animals were waiting for the next movement-triggering signal. The EMG data were digitized and quantitatively

analyzed in a similar manner to the neuronal data. Although the area examined did not include the supplementary eye field (Mushiake et al. 1996; Schlag and Schlag-Rey 1987), vertical and horizontal eye movements were also monitored electrooculographically with a resolution of 2° at 20° from the primary position of the eye.

Surgery and neuronal recording

After completing the behavioral training, an acrylic recording chamber (40 × 30 mm) and head-fixation bolts were implanted on the skull under aseptic conditions. Markers indicating reference points using Horsley-Clarke's stereotactic coordinates were also placed on the skull. Anesthesia was induced with ketamine hydrochloride (8 mg/kg im) plus atropine sulfate, followed by pentobarbital sodium (30 mg/kg im). Antibiotics and analgesics were used to prevent postsurgical infection and pain, respectively. Standard electrophysiological techniques for single-cell recording were used to record from the left pre-SMA, SMA, and primary motor cortex (Matsuzaka and Tanji 1996; Shima et al. 1991). After complete recovery from the surgery, neuronal activity was recorded in the medial part of the frontal cortex using glass-insulated Elgiloy microelectrodes, which were inserted through the dura. The same microelectrodes were used for intracortical microstimulation (ICMS). Each ICMS consisted of a train of

either 11 or 22 cathodal pulses of 0.2-ms duration at 333 Hz in the range of 10–50 μ A. First, we mapped the medial frontal cortex to identify the SMA and rostrally adjacent pre-SMA physiologically by examining neuronal responses to somatosensory and visual stimuli and by observing movements evoked with the ICMS. Neuronal responses were observed when the monkey was sitting quietly in the monkey chair. We examined cutaneous responses by brushing parts of the body with a camel hair brush and by manipulating limb joints. We also used flashing lights and moving objects (pieces of food, tools, and small items in the laboratory) to examine visual responses. We used previously established criteria to differentiate the SMA and pre-SMA (Matsuzaka et al. 1992) based on neuronal responses and the effects of ICMS. After mapping these regions, we recorded neuronal activity while the monkey performed the trained motor tasks. Detailed data analysis was made only for neurons recorded from the forelimb area. When we recorded from portions of the cortex where ICMS evoked face movements or axial body movements, we analyzed the neuronal activity but excluded it from the data file used for subsequent analysis.

Collection and analysis of neuronal data

We first performed real-time analysis of the neuronal activity by constructing peri-event raster displays of neuronal discharges aligned to the onset of the first, second, or third movement. If the activity appeared related to any aspect of the behavioral events by visual inspection, the neuronal data were stored in a microcomputer for off-line analysis. For quantitative analysis, we distinguished the following periods: the control period (the last 500 ms of the inter-trial interval), the preparatory period before initiating the first movement (2,000 ms preceding the onset of the first movement-trigger signal), the first peri-movement period (starting 300 ms before onset of the first movement and ending 100 ms after the movement onset), the first interval between movements (900 ms preceding the second trigger signal), the second peri-movement period (starting 300 ms before onset of the second movement and ending 100 ms after the movement onset), the second interval between movements (900 ms preceding the third trigger signal), the third peri-movement period (starting 300 ms before onset of the third movement and ending 100 ms after the movement onset), and the postmovement period (500 ms after completion of the third movement).

The number of spikes during each period was normalized by the duration of each period and calculated as the spike rate in spikes per second. The normalized neuronal discharges in each task period were compared with those in the control period. If the distributions of the spike rate in the two periods were significantly different (Mann-Whitney *U* test, significant level, $P < 0.05$), the neuron was judged as having task-related activity for that period. Activity during the peri-movement period was defined as movement-related activity. To determine each neuron's onset time of activity relative to a behavioral event, we made histograms (binwidth, 20 ms) that were aligned with the appearance of the trigger signals or movement onsets. The lead time or latency of neuronal activity was determined from cumulative time histograms, with a temporal resolution of 20 ms. We defined the onset time as the first bin in the cumulative histogram whose bin count deviated more than 3 SD from the mean count in the control period (Davey et al. 1986). We performed three two-way ANOVAs looking at the relationship of neuronal activity to the task sequence, to the rank

TABLE 1. Database of neurons for this study

	SMA	Pre-SMA
<i>Monkey 1</i>	243	111
<i>Monkey 2</i>	137	144
<i>Monkey 3</i>	142	250
Total	522	505

SMA, supplementary motor area.

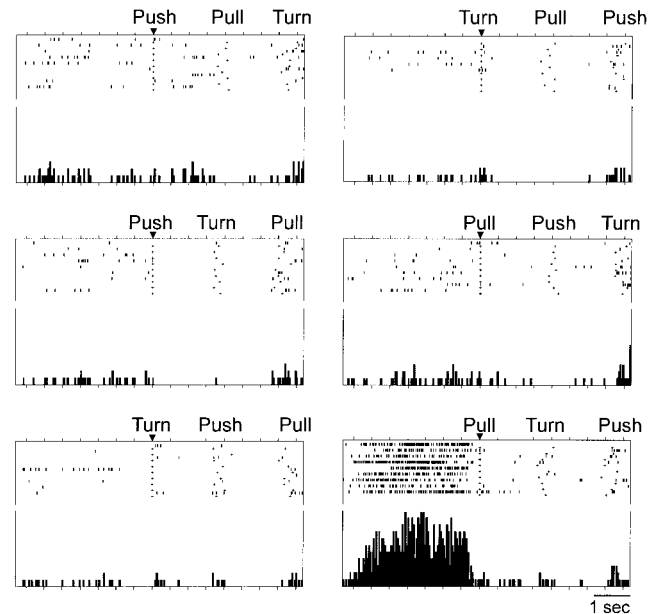


FIG. 2. Activity of a neuron in the presupplementary motor area (pre-SMA) exhibiting preferential activity to a specific order of 3 movements performed without sensory guidance. This neuron is active during the waiting period before initiating the 1st movement but only if the sequence of upcoming movements is in the order pull, turn, and push (*bottom right*). In the raster displays, each row represents a trial; dots, individual discharges of this neuron; +, the onset of movement. The histograms sum discharges over 9 trials. The binwidth for the display is 40 ms. \blacktriangle , the start of the first movement.

order among the three motor tasks (1st, 2nd, or 3rd among the 3), and to the forthcoming movement. That is, we tested for relatedness to three variables ($\alpha = 0.01$): sequence \times rank order, sequence \times movement, and rank order \times movement. The factor of sequence included six levels (for each of the 6 different sequences of 3 movements). For the first and second monkeys, the factor of sequence included four levels, while for the third monkey the factor included six levels. The factor of rank order included three levels (for 1st, 2nd, and 3rd movements), and the factor of movement included three levels (for push, pull, and turn). Where appropriate, individual groups were compared pairwise directly using Tukey's test.

Histology

After collecting the neuronal data, the monkeys were deeply anesthetized with pentobarbital (50 mg/kg im) and perfused through the heart with saline. This was followed by a fixative, containing 3.7% formaldehyde in 0.1 M phosphate buffer at pH 7.4 and then 10 and 20% sucrose solutions in the same buffer. After marking the location of the recording chamber at known electrode coordinates, the brain was removed from the skull and photographed. Then it was sectioned at 50- μ m intervals in the frontal plane on a freezing microtome for histological reconstruction of the neuronal recording sites, with ref-

TABLE 2. Selectivity of sequence-selective neurons to different sequences

	<i>Monkeys 1 and 2</i>		<i>Monkey 3</i>	
	SMA	Pre-SMA	SMA	Pre-SMA
One-sequence selective	16	15	10	11
Two-sequence selective	2	4	2	2
Three-sequence selective	0	0	1	2

Monkeys 1 and 2 performed four sequences and *monkey 3* performed six.

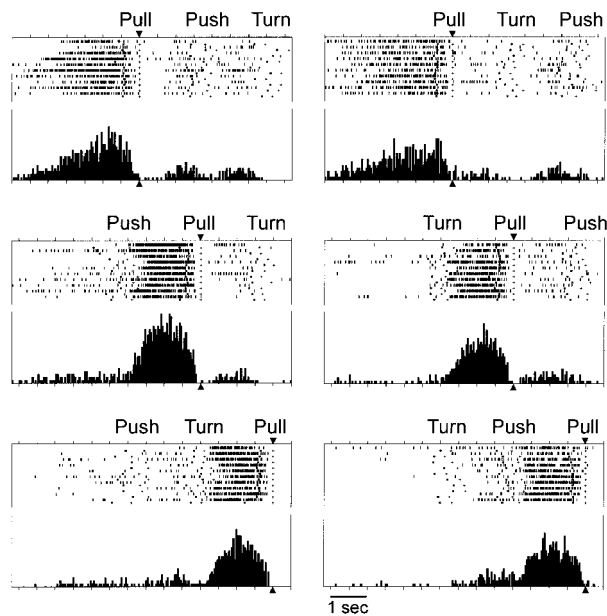


FIG. 3. Discharges of a pre-SMA neuron whose activity increased while the animal was waiting to initiate the next movement. In this example, the activity is selective to the waiting period preceding the initiation of the pull movement. The display format is the same as in Fig. 2, except for an additional display showing the occurrence of signals that triggered each movement (■).

erence to cortical markings made by passing a current through the recording electrodes strong enough to produce iron deposits (a charge of at least 300 μC).

RESULTS

Behavioral and electromyography data

The behavioral data for this experiment was reported previously (Shima and Tanji 1998). Briefly, success rates for the trials under visual guidance in the three monkeys were between

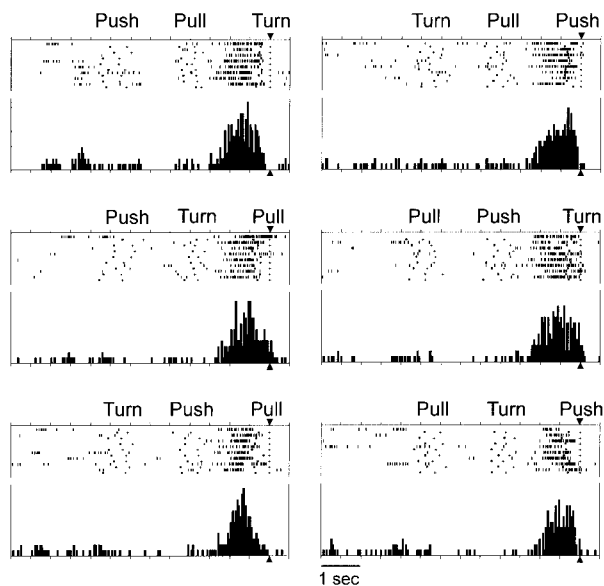


FIG. 4. Discharges of a pre-SMA neuron whose activity increased while the monkey was preparing to initiate the 3rd movement, irrespective of the type of movement. The display format is the same as in Fig. 3.

98 and 99%. When the three movements were performed sequentially by memory, the success rates for completing the correct sequence of movements were 95 ± 3 , 96 ± 4 , and $98 \pm 2\%$ (means \pm SD) for the first, second, and third monkeys, respectively. The EMG analysis revealed that activity in the forelimb muscles increased phasically with execution of the three movements, but no consistent changes in activity were detectable during the waiting periods. As a result of extensive behavioral training (more than 12 mo), the phasic movement-related activity of all the muscles examined did not vary significantly with the temporal sequence of the three movements ($P > 0.1$ by ANOVA). None of the movement-related activity recorded in any muscle exhibited any effect of the numerical order (1st, 2nd, or 3rd) of movement in the sequence ($P > 0.1$ by ANOVA). Representative muscle activity in four forelimb muscles is shown in Fig. 1. We confirmed that the occurrence of saccades was not related to any of the events in the behavioral task by analyzing the electrooculogram.

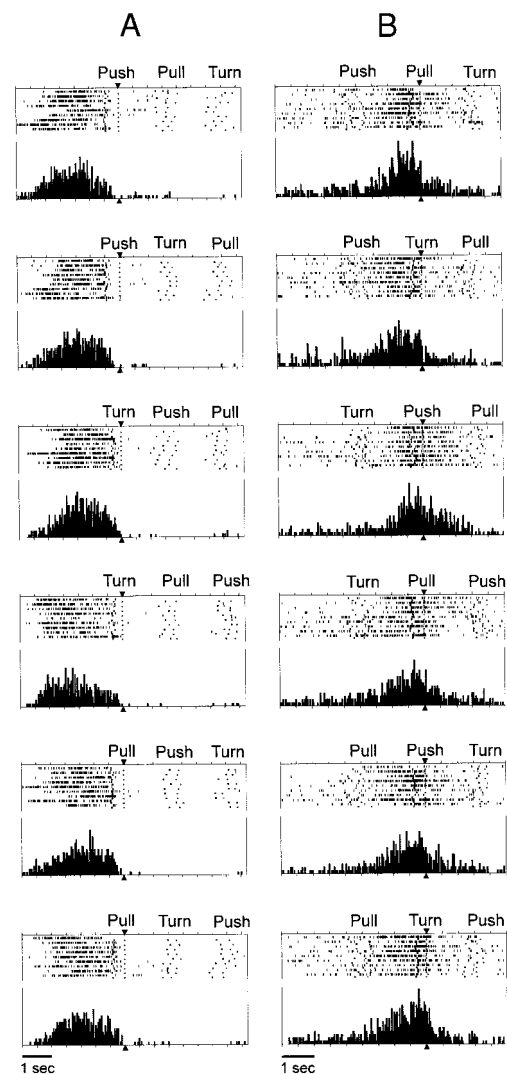


FIG. 5. A: discharges of a pre-SMA neuron whose activity was most prominent during the preparatory period before initiating the 1st movement. B: in this pre-SMA neuron, activity was most prominent during the preparatory period before the 2nd movement.

TABLE 3. Selectivity of rank-order selective neurons to the first, second, or third preparatory period

Order	SMA	Pre-SMA
First	68	115
Second	7	24
Third	32	41
First and second	2	10
Second and third	4	10
First and third	0	4
Total	113	204

Neuronal activity

We analyzed the activity of 1,027 task-related neurons recorded from the SMA and pre-SMA in the three monkeys (Table 1). Five different types of neuronal activity that seemed relevant to the sequential performance of the three movements were observed during performance of the sequencing task from memory. We describe these and then briefly present some properties of the neuronal activity when the motor performance was guided by visual signals.

Preparatory activity appearing selectively before initiation of the first of the three movements

The first of the five types of activity changes during the remembered sequence task was observed before initiation of the first movement in individual trials. The changes in neuronal activity exhibited selectivity to either the sequence of the three movements or to the next movement to be performed. The activity changes started 880–3,860 ms (with a median of 1,960 ms) before the first movement-trigger signal. We observed two subtypes of preparatory activity. The first subtype was preparatory activity that was selectively observed before the initiation of a particular sequence of three movements. The second subtype of activity was selective to the occurrence of the first movement in the sequence.

SEQUENCE-SELECTIVE ACTIVITY. Figure 2 shows a typical example of sequence-selective activity. The neuronal activity recorded in the SMA only increased before the animal initiated the first movement, pull, when the second movement was turn and the third movement was push. Selective activity was not observed when the first movement (pull) was followed by push and then turn. The preparatory activity of 31 SMA and 35 pre-SMA neurons (6 and 7% of the total task-related neurons in the 2 areas) exhibited significant dependence ($P < 0.01$) on the sequence and not on the first movement. We subsequently performed Tukey's test to look for a relationship between preparatory activity and individual sequences. The selectivity of each neuron to the motor sequence was remarkable. A majority of neurons (84% of SMA and 76% of pre-SMA sequence-selective neurons) exhibited preparatory activity selective to only one of the four or six sequences, just as in the example shown in Fig. 2. Four SMA and six pre-SMA neurons exhibited selectivity to two sequences, and one SMA and two pre-SMA neurons showed selectivity to three sequences (Table 2). To test whether the sequence-selective activity was contingent on the performance of three sequential movements, we performed a control experiment for 21 of the 65 sequence-selective neurons. In the control experiment, we incorporated trials requiring either a sequence of two movements or a single

movement. In all of the control experiments, we found that the sequence-selective preparatory activity was either abolished or diminished when one or two movements in the sequence (in which the neuron was active) were eliminated.

MOVEMENT-SELECTIVE PREPARATORY ACTIVITY. Sixty-six SMA (13% of 522) and 63 pre-SMA neurons (12% of 505) belonged to this subtype of preparatory activity that was selective for the forthcoming movement. For these neurons, ANOVA revealed the dependence of the activity on the movement immediately following the preparatory period ($P < 0.01$). An example of a pre-SMA neuron whose activity is selective for the preparatory period before initiating the pull movement is shown in Fig. 3. Tukey's test revealed the selectivity of individual neurons to each of the possible next movements. For 54 SMA and 49 pre-SMA neurons, the activity was selective for one of the three movements as in the example in Fig. 3. For the remaining neurons (12 SMA and 14 pre-SMA), the activity of each neuron was selective for two of the three movements.

Rank order-selective preparatory activity

The second type of activity, classified as *rank-order selective*, exhibited preferential activity during the first, second, or

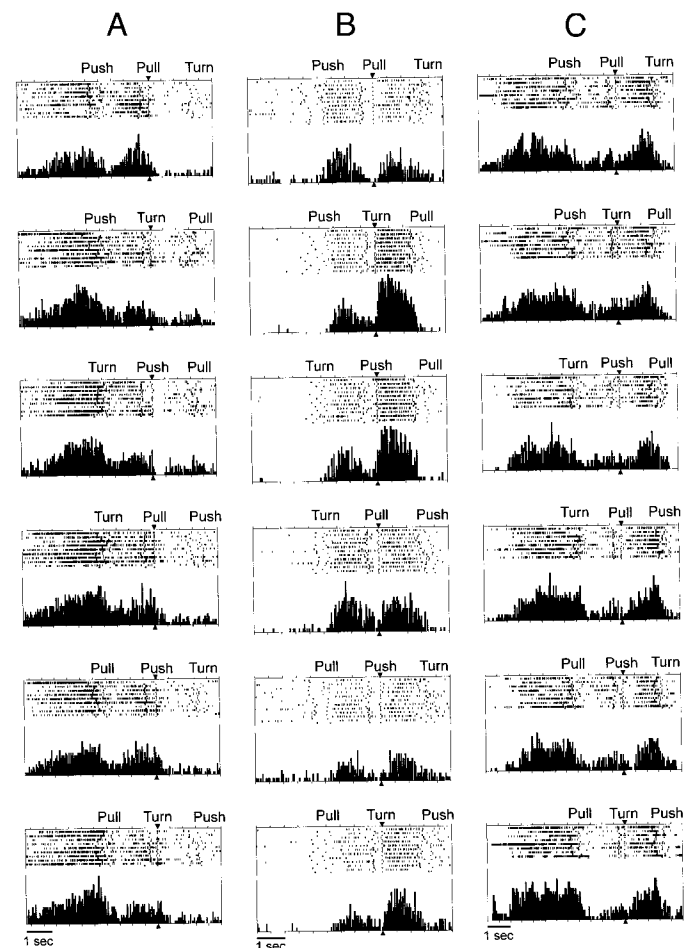


FIG. 6. Discharges of 3 pre-SMA neurons exhibiting increased activity during more than 1 preparatory period. A: activity was prominent during the 1st and 2nd preparatory periods. B: activity increased during the 2nd and 3rd preparatory periods. C: activity increased during the 1st and 3rd preparatory periods.

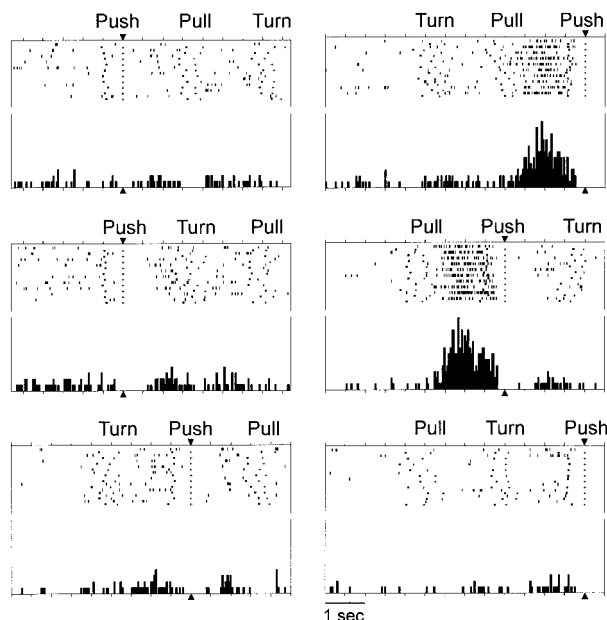


FIG. 7. Discharges of an SMA neuron whose activity increased selectively during the interval after completion of 1 particular movement, pull, and before the initiation of another, push.

third preparatory period in individual trials regardless of the sequence of the three movements. An example of rank order-selective preparatory activity is shown in Fig. 4. This pre-SMA neuron was active only during the third preparatory period, irrespective of which movements to follow the preparatory period. When the number of movements to be performed during individual trials was reduced to two (2-movement sequence), the preparatory activity disappeared entirely. In both the SMA and pre-SMA, a majority of neurons of this type were preferentially active during one of the three preparatory periods. Examples of two pre-SMA cells exhibiting prominent activity during the first and second preparatory periods are shown in Fig. 5, *A* and *B*, respectively. A minority of neurons were preferentially more active in two of the three preparatory periods (Table 3). An example of a pre-SMA neuron exhibiting preparatory activity during both the first and second preparatory periods is shown in Fig. 6*A*. In the example shown in Fig.

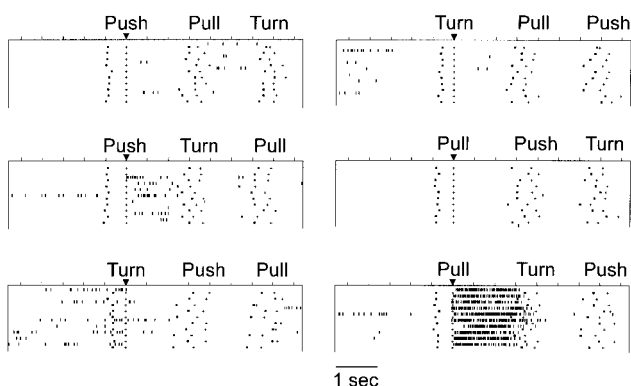


FIG. 8. Discharges of a pre-SMA neuron whose activity increased selectively during the waiting period after completion of a pull movement and before the initiation of a turn movement. The interval-selective activity was observed only when the pull movement was the 1st and the turn movement was the 2nd movement (*bottom right*). The activity increase was not observed when the pull-turn interval came as the interval between the 2nd and 3rd movements (*top left*).

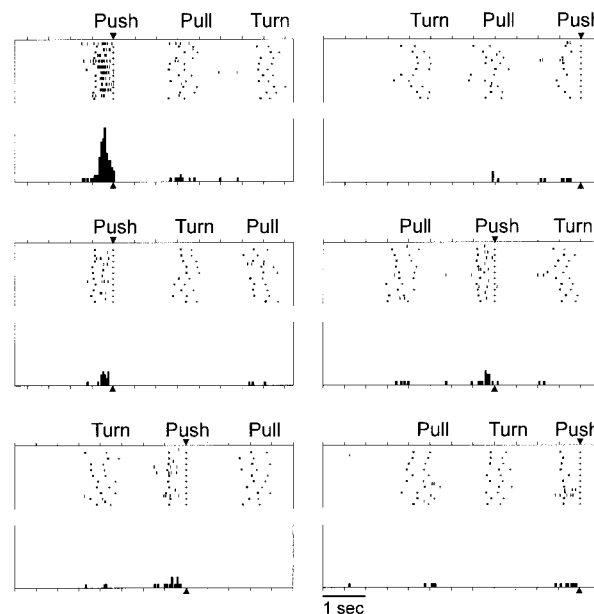


FIG. 9. Discharges of an SMA neuron whose activity increased immediately before initiation of push movement. The movement-related activity, however, was conditional. The activity preceding the push movement was prominent only when the sequence of the 3 movements was push, pull, and turn.

6*B*, the neuronal activity was preferential for the second and third preparatory periods, whereas for the neuron shown in Fig. 6*C*; the preference was for the first and third preparatory periods. Overall, rank-order selective neurons were more prevalent in the pre-SMA (204/505, 40%) than in the SMA (113/522, 22%). The difference in the rate of occurrence of this type of neuron in the two areas was significant at $P < 0.001$ (χ^2 test).

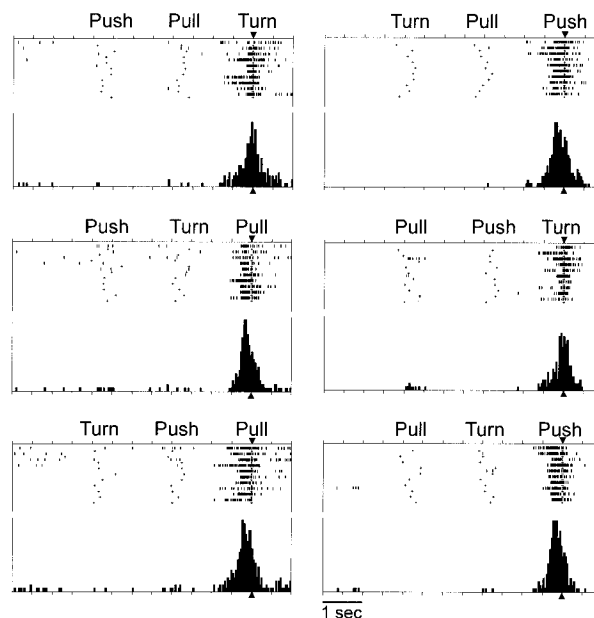


FIG. 10. Discharges of a pre-SMA neuron whose activity increased during and before initiation of the 3rd movement, regardless of the movement (push, turn, or pull).

Interval-selective activity

The third type of activity was observed selectively during the intervals between the occurrence of the three movements. The most prominent property of this type of activity (found in 38 and 23% of task-related neurons in the SMA and pre-SMA) was a preference for an interval between one particular movement and another. That is, the activity depended on which movements preceded and followed the interval. In the SMA neuron shown in Fig. 7, activity was most prominent following the execution of pull and before the execution of push. In 42 SMA and 30 pre-SMA neurons, we found interval-selective activity in the intervals preceding both the second and third of three movements. For these neurons, the magnitudes of activity change were no different whether the activity preceded the second or third movement. Therefore these neurons (23% of the interval-selective neurons) were not rank-order selective. In some of the interval-selective activity, however, we found that the activity depended on the rank order of the intervals ($P < 0.01$, Mann-Whitney U test). In 51 SMA and 43 pre-SMA neurons (30%), interval-selective activity was found only or predominantly in the interval preceding the second movement, as in the example shown in Fig. 8. In this example, neuronal activity increased immediately after the animal performed the first movement, pull. The activity lasted until the trigger signal to initiate the second movement, turn. When pull and turn were the second and third movements (Fig. 8, *top left*), the activity increase was not observed. In another group of

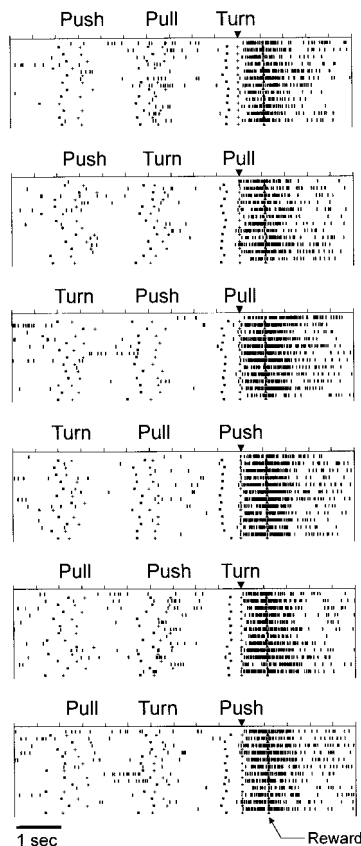


FIG. 11. Discharges of a pre-SMA neuron whose activity increased after the initiation of the third movement, regardless of the sequence of the 3 movements.

TABLE 4. Distribution of neurons exhibiting relationships to various aspects of the sequencing task relying on memorized information

	SMA	Pre-SMA
Sequence-selective preparatory (SSP) only	18/(31)	28/(35)
Movement-selective preparatory (MSP) only	33/(66)	41/(63)
Rank-order-selective preparatory (RS) only	71/(113)	132/(204)
Interval-selective (IS) only	149/(199)	78/(118)
Sequence-selective movement-related (Ms) only	18/(24)	29/(47)
Rank-order-selective movement-related (Mr) only	26/(39)	61/(88)
Nonselective movement-related (M) only	52/(112)	5/(9)
Post third-movement (Post) only	3/(5)	16/(21)
SSP + RS	1	3
SSP + IS	8	2
SSP + Ms	2	1
SSP + Mr	0	1
SSP + M	2	0
MSP + RS	4	9
MSP + IS	6	0
MSP + Ms	1	5
MSP + Mr	0	3
MSP + M	19	1
RS + IS	12	22
RS + Ms	1	6
RS + Mr	10	17
RS + M	11	3
RS + Post	2	5
IS + Ms	2	6
IS + Mr	3	3
IS + M	16	0
MSP + IS + RS	1	4
MSP + IS + M	2	0
RS + IS + Mr	0	3

* Numbers in parentheses indicate numbers of neurons exhibiting multiple relationships.

neurons (26 SMA and 21 pre-SMA, 15%), the interval-selective activity was observed only or predominantly between the second and third movements and not between the first and second movements. This analysis of the dependence of rank-order selectivity on the rank order of movements was not possible for 83 SMA and 25 pre-SMA neurons recorded from the first and second monkeys because the selective interval appeared only once in the four sequences performed. Overall 199 SMA neurons and 118 pre-SMA neurons exhibited interval-selective activity (significantly different distribution, $P < 0.001$ by χ^2 test).

Movement-related activity

The fourth type of activity observed during the memory-guided motor task was activity during and immediately preceding the onset of individual movements (see METHODS for the definition of movement-related activity). We classified movement-related activity into three subtypes, depending on selectivity for either the sequence of movements or the rank order of the three movements.

SEQUENCE-SELECTIVE MOVEMENT-RELATED ACTIVITY. Twenty-six SMA and 47 pre-SMA neurons (5 and 9% of task-related neurons in each area) exhibited movement-related activity whose magnitudes differed significantly ($P < 0.01$) depending on the sequence of the three movements. In the example shown in Fig. 9, the activity observed immediately preceding the push

movement was strongest when the sequence was push-pull-turn. Of the 73 sequence-selective neurons, the movement-related activity was significantly greater in one sequence for 34 neurons, in two sequences for 24 neurons, and in more than 3 sequences for 15 neurons.

RANK-ORDER-SELECTIVE MOVEMENT-RELATED ACTIVITY. For 39 SMA (7%) and 88 pre-SMA (17%) neurons, the movement-related activity differed significantly depending on the rank-order of the movement performed. In the pre-SMA neuron shown in Fig. 10, activity was most marked when the animal performed the third movement, regardless of the movement performed. For 65 of the 127 rank-order selective neurons, activity was not selective for the type of movement (push, pull, or turn), as in Fig. 10. For the remaining 62 neurons, the order-selective activity was also selective for the type of movement.

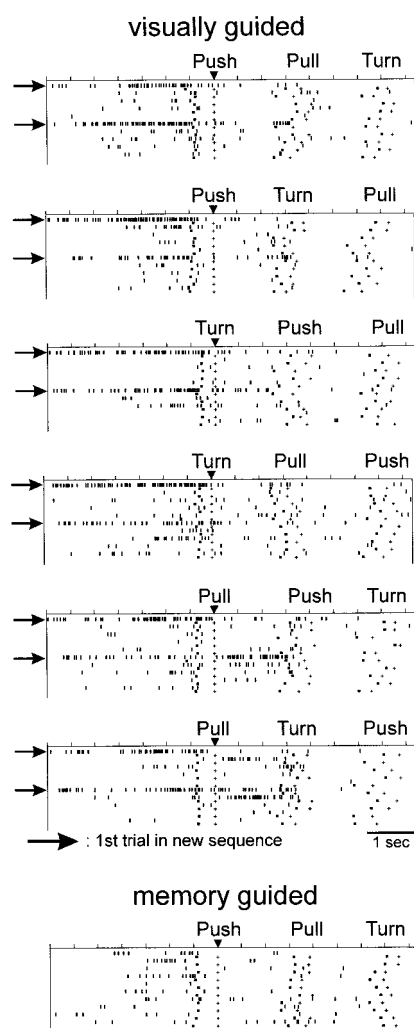


FIG. 12. Activity of a pre-SMA neuron exhibiting discharge increase primarily at the 1st trial in individual sequences of visually guided 3 movements. The activity starts each time the animal is prepared to start a new sequence, regardless of the order of appearance of the 3 movements. The activity increase is not observed when the sequential task is guided by memory (*bottom*). Each horizontal arrow denotes the beginning of the first trial in a series of trials with each sequence of the 3 movements (5 visual trials followed by 6 memory-guided trials). Occurrences of blocks of trials with each sequence were intermixed during actual task performance, but trial blocks with the same sequence are displayed together.

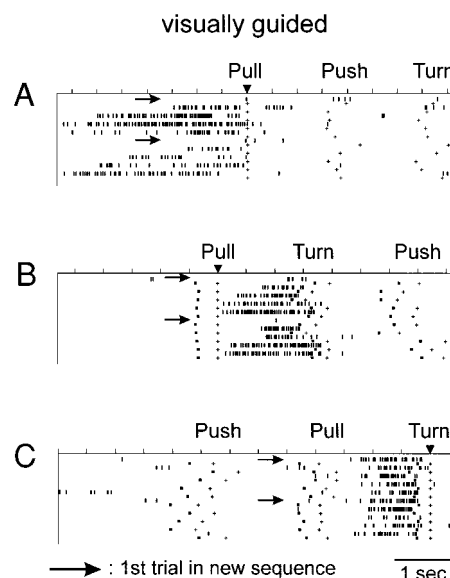


FIG. 13. Discharges of 3 types of neurons during performance of visually guided movements. *A*: activity of the sequence-selective, preparatory neuron (the same neuron as shown in Fig. 2) at the performance of 3 movements in the order of pull, push, and turn, under visual guidance. The activity, not observed at the 1st trials (\rightarrow), develops gradually in later trials. *B*: activity of the interval-selective neuron shown in Fig. 8 during performance of the sequence pull, turn, and push, under visual guidance. The interval selective activity develops when the same sequence is repeated. *C*: activity of the rank-order selective neuron (the same neuron as shown in Fig. 4) during performance of 3 visually guided movements.

NONSELECTIVE MOVEMENT-RELATED ACTIVITY. For 102 SMA neurons (20% of the task-related neurons), the movement-related activity was influenced by neither the sequence nor the rank order of the motor task. Of these, 27 neurons were active with one of the three movements, whereas 33 and 42 neurons were active with two and three movements, respectively. In contrast, in the pre-SMA, we found only nine neurons (2%) exhibiting nonselective movement-related activity. Thus the great majority of movement-related activity in the pre-SMA showed selectivity for either the sequence or rank order.

Activity appearing after the third movement

The fifth type of activity appeared after initiation of the third movement at the completion of the motor task. This activity, found in 5 SMA (1%) and 21 pre-SMA (4%) neurons, lasted for 360–2,860 ms (with a mean of 1,540 ms) and decayed before the initiation of the next trial. Like the typical example shown in Fig. 11, the activity was not selective for the sequence of movements. We did not observe this type of activity in the face area, although we did observe neuronal activity in that area related to orofacial, food-taking movements that came after the reward. Therefore the post-third movement activity was specific to the forearm area.

Neurons exhibiting relationships to more than one aspect of the behavioral task

We found that some neurons in both the SMA and pre-SMA were active in relation to multiple aspects of the behavioral

factors during performance of the memory-based sequencing task. We summarize the distribution of neurons exhibiting relationships to one or multiple aspects of the behavioral task in Table 4.

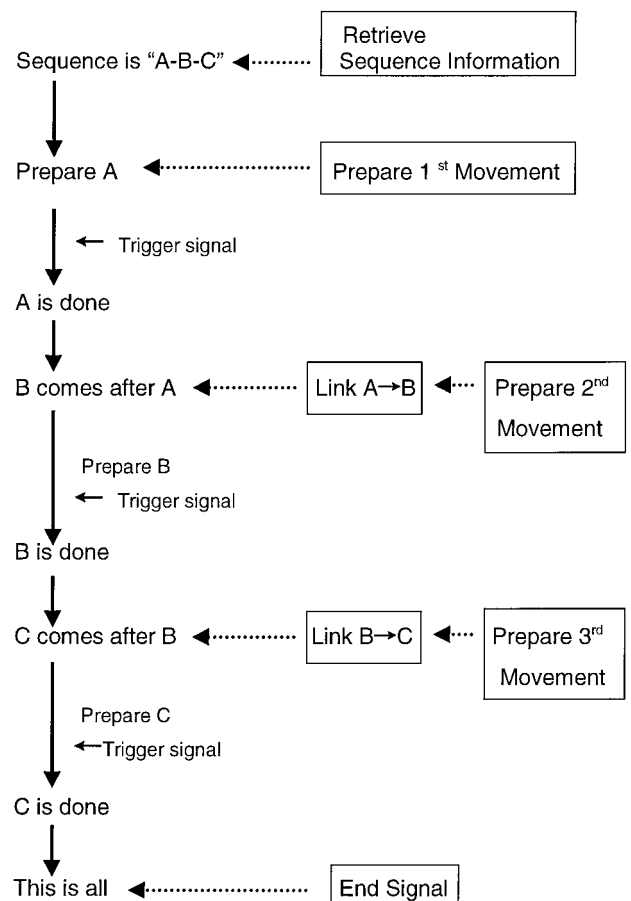
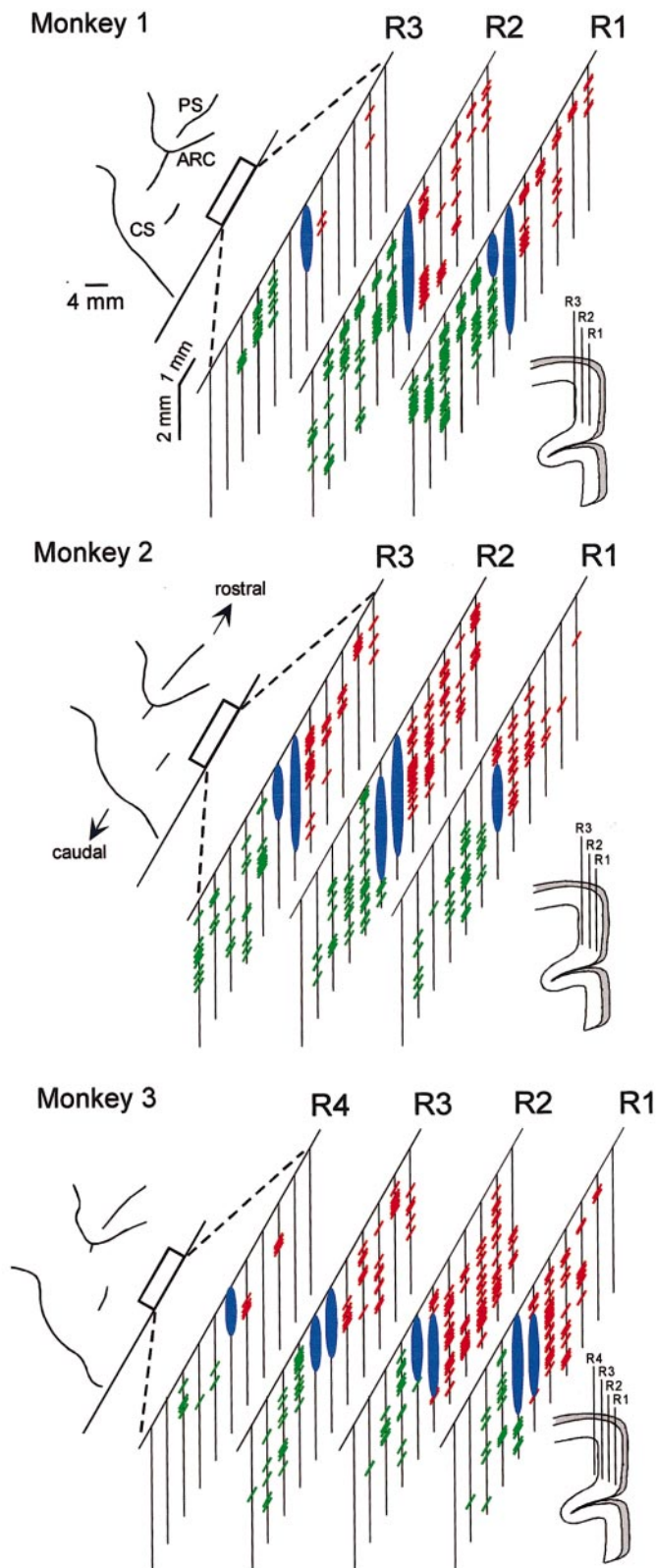


FIG. 15. Processes and necessary actions for the orderly performance of 3 movements separated by time intervals, when the sequence is in the order of A, B, and C. The information for the correct sequence should lead to a chain of events that gives rise to orderly delivery of output signals appropriate for commanding 3 movements in the correct sequence. The timing of the initiation of each movement is externally determined with a trigger signal.

Neuronal activity during performance of three movements under visual guidance

Since the primary aim of this report is to describe the neuronal activity that occurred while performing three sequential movements based on memorized information, we only briefly describe the neuronal activity observed when the three movements were guided with visual signals.

SELECTIVE ACTIVITY AT THE INITIATION OF A NEW SEQUENCE. We found that in 89 pre-SMA neurons (18% of task-related neurons), activity increased profoundly when the animal was

FIG. 14. Spatial location of the recording sites in the medial frontal cortex of the 1st (top), 2nd, and 3rd (bottom) monkeys. *Left*: a surface view of the frontal cortex showing the rostrocaudal and mediolateral extent of recording sites (rectangles) relative to cortical sulci. *Right*: 3-dimensional reconstruction of the medial wall of the left frontal cortex (extending rostrocaudally from a to b in the surface map) surveyed with microelectrode penetrations, arranged in 3 or 4 mediolateral rows (R1–R3 or R1–R4, each separated by 1 mm). Blue spindles denote portions of the cortex where the face is represented [where intracortical microstimulation (ICMS) evokes orofacial movements and neurons respond to somatosensory stimulation of the orofacial region or are active with facial movements]. Red and green bars denote the depth at which task-related neurons were recorded at electrode penetrations in the forelimb part of the pre-SMA and SMA, respectively. Each region is defined according to previously established criteria (Matsuzaka et al. 1991). CS, central sulcus; ARC, arcuate sulcus; PS, principal sulcus.

required to perform the first trial in any new sequence. A typical example of this type of neuronal activity is shown in Fig. 12. The activity increased selectively when the animal was required to abandon a currently correct sequence and acquire the next sequence, while receiving visual signals. This activity was suggested to take part in updating the sequence information in our previous report (Shima et al. 1996). In the SMA, on the other hand, this type of activity occurred much less frequently, being found in only 11 neurons (2%). The distribution of neurons in the two areas was significantly different ($P < 0.001$, χ^2 test).

ABSENCE OF PREPARATORY ACTIVITY AND INTERVAL-SELECTIVE ACTIVITY. When the animals initiated a new sequence out of the six sequences, they had no knowledge of the correct sequence to perform, which they had to learn while receiving the three visual signals. At this stage of the visually guided task performance, we did not observe the two types of preparatory activity described in the preceding text (sequence-selective and movement-selective). However, when the animals repeated the same sequence under visual guidance, the preparatory activity developed gradually, as shown in Fig. 13A. Thus it appeared that the preparatory activity grew in parallel with the repetition of the visually guided trials when the animals learned the correct sequence. Similarly, when the animals began to learn and anticipate the sequence during repetitions of visually guided trials with a particular sequence, the interval selective activity developed gradually, as shown in Fig. 13B. On the other hand, we did observe rank-order-selective activity and movement-related activity during the visually guided trials even at the first trials. An example of the activity related to the third rank order (the same neuron as shown in Fig. 4) during the visually guided task performance is shown in Fig. 13C.

Recording sites of task-related neurons

The recording sites of neurons described in this study are illustrated in Fig. 14. They were recorded in the forelimb part of either the pre-SMA or SMA, as defined using the criteria described previously (Matsuzaka et al. 1992). We tried to find differences in the distribution of different types of neurons (such as preparatory or movement-related) within either the pre-SMA or SMA but failed to find any trends for selective distribution of any type of neuron according to the depth of the recording site or location along the rostrocaudal or mediolateral axis.

DISCUSSION

In this study, we found that neurons in both the SMA and pre-SMA exhibited profound activity changes at a number of different phases in the behavioral task not merely in relation to the execution of individual movements. In both areas, activity appeared at time intervals that were strategically important for planning the sequence of movements and preparing forthcoming movements based on memorized information about the sequence of three movements. First, we observed two different types of activity well before the initiation of the first movement. The sequence-selective activity, reflecting a particular sequence of three movements, seems to be used in retrieving information about the temporal order of motor events and planning the behavioral sequence. On the other hand, the

preparatory activity for the next movement seems to reflect a process of preparing to initiate the first movement. Second, we observed activity that indicated the rank order of movements to be performed, reflecting which of the first, second, or third movements the animal was preparing to perform. Third, we found activity during a specific interval between the occurrence of one particular movement and another. The results of our previous study of inactivating the SMA and pre-SMA (Shima and Tanji 1998) suggest that the neuronal activities observed in this study play a number of crucial roles in organizing multiple movements in the correct temporal order. We present a hypothesis of how each type of neuronal activity described is used to accomplish the sequencing task.

Hypothetical use of neuronal activity to arrange multiple movements in the correct temporal order

Let us consider the possibility that a neural structure involving the SMA and pre-SMA serves to provide the signals necessary to perform a sequence of movements A, B, and C, in that order (Fig. 15 shows the process and necessary actions). What kinds of neural elements, with what properties, will provide the necessary signals? First, we need an element that retrieves and transmits the information that the currently required sequence is A-B-C. The sequence-selective preparatory activity found in the SMA and pre-SMA serves as such an element. The other type of preparatory activity found in this study was selective for the forthcoming movement. This activity seems to aid the preparatory process for the first movement, A. When the trigger signal appears after the preparatory process, it is then easy to start the first movement, A. When movement A is accomplished, the sequence information carried in the sequence-selective preparatory neurons halts, as exemplified by the neuronal activity shown in Fig. 2. What mechanisms tell that the next movement is movement B and retain that information during the waiting period until the signal to start the second movement? This requires an element that carries information that movement B occurs after movement A. We propose that the interval-selective neuronal activity observed serves to link the occurrence of B after A because the activity starts after movement A and ends before the initiation of movement B. This tonic signal is useful for feeding information to the next element in the preparatory process before initiating the next movement B. Alternatively, the tonic signal may continuously activate an element that, after receiving external signals to trigger a movement, produces a signal that will command an output signal to initiate movement B. When movement B is complete, the next step is to connect the occurrence of movement C after movement B; this can be done by a linking element that is activated after movement B and inactivated at the initiation of movement C (see Fig. 15). This linking element feeds a tonic input to the next element that acts to command the output to initiate movement C. When the third movement is complete, an element that becomes active after the third movement (as we observed predominantly in the pre-SMA) may signify an end signal, reporting that the task has been accomplished.

If this hypothetical process is correct, then it follows that the linking elements described should be involved in the process in the following way. To perform sequence A-B-C, two elements linking $A \rightarrow B$ and $B \rightarrow C$ should be activated. Then, it must

be determined when to use these two elements. The linking element $A \rightarrow B$ should come into play after the first movement in the sequence ends and long before the initiation of the second movement. The linking element $B \rightarrow C$ should come into play after the second movement in the sequence ends and long before the initiation of the third movement. This requires information about which of the three movements in the sequence is next. The large number of rank-order selective neurons found in this study could provide this information. If the neural system has a way of telling whether to prepare the second or third movement, the linking elements $A \rightarrow B$ and $B \rightarrow C$ would be incorporated at the appropriate times. In other words, the rank-order selective elements serve to regulate the linking-element information. In this study, we observed three types of interval-selective activity. This activity appeared either nonselectively before the second and third movements or selectively before the second or third movements (Figs. 7 and 8). These neurons are candidates as the linking elements that act before and after receiving regulatory action from the rank-order elements.

Figure 16 summarizes our hypothesis on the use of the neural elements with the properties observed in this study. It illustrates the general scheme for the actions of the elements that are assembled to produce the signals necessary to perform the three movements in the order of A, B, and C. We realize, however, that much study must be done to determine whether the neural structures in the SMA and pre-SMA actually operate in the proposed fashion. In the future, we also need to study the participation of other cortical and subcortical structures in the temporal organization of multiple movements.

Implications for functional differences between the SMA and pre-SMA

Although both the SMA and pre-SMA are crucial to performing this behavioral task and both areas have a wealth of neuronal activity relevant to task performance, we observed some differences in the proportions of different categories of neurons present in the two areas. We found that interval-selective activity was more prevalent in the SMA, whereas rank-order-selective preparatory and movement-related activity were more prevalent in the pre-SMA. The two different categories of sequence-related activity reflect two fundamentally different aspects of performing the behavior. The interval-selective activity links the occurrence of two different movements and, therefore, determines the order of the component movements in the sequence (relational order). On the other hand, the rank-order-selective activity specifies the ordinal position of the three movements (numerical order). Clower and Alexander (1998) reported a clear numerical order effect in spatially tuned neuronal activity in the SMA and pre-SMA. They analyzed neuronal activity while monkeys sequentially positioned a cursor on a video display by moving a joystick with the hand and found that about 40% of neuronal activity related to spatial variables of the motor task (origin, direction, and endpoint) were influenced by numerical order of motor components. They also found that the numerical order representation was significantly more prevalent in the pre-SMA than in the SMA. It is conjectured that numerical order representation may be more abstract in nature than representation of the relational order of component movements. Picard and Strick

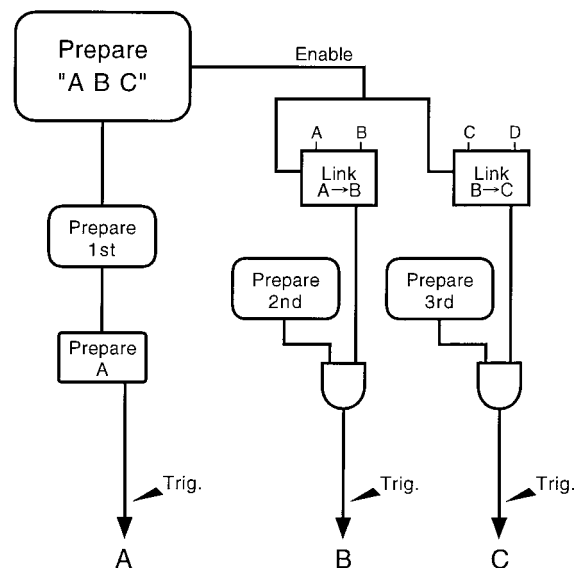


FIG. 16. A diagram illustrating a hypothetical network connecting the neural elements found in the present study. If the preparatory elements delivering signals with 4 different properties feed output to linking elements and output elements in the way drawn, then they will contribute to the automatic delivery of output signals in the order of A, B, and C.

(1996) hypothesized that the pre-SMA is more involved than the SMA in motor tasks requiring higher-order aspects of motor control as suggested in studies of human brain activation (Deiber et al. 1991; Zatorre et al. 1992) and in our early studies of subhuman primates (Matsuzaka and Tanji 1996; Matsuzaka et al. 1992; Tanji 1994). Our present findings are in line with this view.

In this study, we found another aspect of the differences in the activity properties of SMA and pre-SMA neurons, that is, in the properties of movement-related neurons. In the SMA, we found that 61% of the movement-related neurons exhibited selectivity to the type of movement performed (push, pull, or turn) regardless of the temporal sequence of the three movements or the numerical order of the movement among the three. Neuronal activity of this type is useful for specifying or commanding the motor output to perform a particular movement. Remarkably, this type of movement-specific activity was rare in the pre-SMA (6% of movement-related neurons). Most of the movement-related neurons in the pre-SMA reflect the occurrence of a particular movement in a particular sequence or reflect the numerical order of the movement to be performed. Their activity seems to be more useful for monitoring the occurrence of motor events in the context of temporal structuring of multiple movements.

Furthermore, we found that activity occurring shortly after the third movement in each trial was more frequent in the pre-SMA. Was this activity related to orofacial movements involved in consuming the reward? This seems unlikely because we did not find such activity in the face representation area of the SMA. Instead we found that reward-consumption-related activity occurred in the face representation area after the reward was delivered. Although some of the post third-movement activity may be related to expectation of the reward, we think that this activity may signal the end of a sequence of three movements. Such a signal may be a part of the internal feedback providing information on the accomplishment of a

series of temporally structured motor behaviors. If this view is correct, it follows that the pre-SMA is more involved in internal monitoring of the temporal sequence of behavioral events.

Relevance of this study to previous reports

In our previous reports (Mushiake et al. 1990, 1991), we described how neurons in the cortical area traditionally defined as the SMA exhibit activity that is selective for the temporal order of sequential movements. This observation lends support to the view that the SMA plays a role in organizing movements that are performed in succession. The involvement of the SMA in sequential movements, originally suggested in clinical reports on human subjects with lesions in the medial frontal cortex (Laplane et al. 1977; Luria 1966), has been the subject of studies in both monkeys (Brinkman 1984; Chen et al. 1995; Mushiake et al. 1991; Nakamura et al. 1998; Thaler et al. 1995) and humans (Boecker et al. 1998; Gerloff et al. 1997; Grafton et al. 1995; Hikosaka et al. 1996; Jenkins et al. 1994; Lang et al. 1994; Muri et al. 1995; Roland et al. 1980; Sadato et al. 1996; Sakai et al. 1998). Although other cortical and subcortical structures also participate in controlling sequential movements (Aldridge and Berridge 1998; Grafton et al. 1998; Hikosaka et al. 1999; Kermadi and Joseph 1995; Mushiake and Strick 1995), the importance of the SMA (and the subsequently defined pre-SMA) in sequential movements seems to be established (Tanji 1996).

The focus in these previous reports was the study of the mechanisms that control the sequential performance of continuous movements with multiple components. It is important to note that in these reports, control of a spatial sequence was the central factor. Our study dealt with a different aspect of sequential motor behavior. The issue was how to arrange a sequence of multiple movements that are performed separately with a variable time interval between each movement. Our previous report demonstrated that both the SMA and pre-SMA are necessary for this type of behavioral control, and this study suggests a number of ways in which individual neurons in the two areas participate in sequencing multiple movements. We obtained evidence that information about the exact sequence to be performed is reflected in the activity of SMA and pre-SMA neurons. Where do they obtain the sequence information? Anatomically, the pre-SMA receives corticocortical inputs from the prefrontal cortex and other areas (Bates and Goldman-Rakic 1993; Luppino et al. 1993). Barone and Joseph (1989) trained monkeys in a delayed spatial-sequencing task. They found that a group of neurons in the dorsolateral prefrontal cortex showed activity that reflected the order of illumination of three targets in individual sequences. It is possible that the dorsolateral prefrontal cortex may contain the sequence of future motor events, even if the spatial factor involved in the behavioral task is minimal, as in our study. Therefore the dorsolateral prefrontal cortex is a potential source for the sequence-selective preparatory activity described in the preceding text. On the other hand, both the SMA and pre-SMA receive afferent projections via the thalamus from both the basal ganglia and the cerebellum (Inase et al. 1996; Matelli and Luppino 1996; Rouiller et al. 1994; Sakai et al. 1996, 2000). It is possible that the processes leading to sequential delivery of commands for different movements may involve the participation of cortico-basal ganglia loops (Alexander et al. 1986;

Matsumoto et al. 1999) and cortico-cerebellar loops (Schell and Strick 1984).

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