

# Participation of the Primate Presupplementary Motor Area in Sequencing Multiple Saccades

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Submitted 12 December 2003; accepted in final form 21 February 2004

**Isoda, Masaki and Jun Tanji.** Participation of the primate presupplementary motor area in sequencing multiple saccades. *J Neurophysiol* 92: 653–659, 2004. First published February 25, 2004; 10.1152/jn.01201.2003. The aim of this study was to investigate whether, and how, the presupplementary motor area (pre-SMA) is involved in the organization of oculomotor sequence. We trained two monkeys to perform three center-out saccades in six different orders. Each sequence consisted of a block of eight trials, initially with visual instruction (4 trials) and then from memory (4 trials). During memory-guided performance of sequential saccades, ~75% of task-related neuronal activity was selective for, or influenced by, the numerical position of saccades within each sequence (rank order). Neurons tuned for the direction of saccades were in the minority. We also found that 22% of sampled neurons increased their activity preferentially at a transitional period when monkeys were in the process of renewal of required saccade sequences. These data indicate that the pre-SMA is involved in the organization of *oculomotor* sequence, particularly in representing rank-order information and in updating sequence information. Together with previous reports on the participation of the pre-SMA in sequencing of multiple arm movements, we propose here that this area may contribute to cognitive aspects of sequential behavioral control, in an effector-independent manner.

## INTRODUCTION

When engaged in behavior with distinct elementary actions, the order must be correct, otherwise, the behavioral outcome would be maladaptive. Much of the work has been dedicated to discover how the elements of action sequence are chronologically organized and how different areas of the brain are coordinated to regulate the serial order of elementary actions (see Tanji 2001 for review). As to the cortical motor areas of primates, recent studies have emphasized the importance of medial frontal areas in sequencing of multiple actions: the supplementary motor area (SMA) and presupplementary motor area (pre-SMA) for limb-motor sequence (Clower and Alexander 1998; Mushiake et al. 1991; Shima and Tanji 2000; Tanji and Shima 1994) and the supplementary eye field (SEF) for oculomotor sequence (Isoda and Tanji 2002, 2003; Lu et al. 2002).

Among these motor areas, the pre-SMA seems unique in terms of its lack of connectivity with primary motor regions. Specifically, the SMA but not the pre-SMA is directly connected to the primary motor cortex (Lu et al. 1994; Luppino et al. 1990, 1993; Matsuzaka et al. 1992; Tokuno and Tanji 1993) and to the spinal cord (Dum and Strick 1991, 1996; He et al. 1995). The SEF but not the pre-SMA sends their axons directly

to the frontal eye field (Bates and Goldman-Rakic 1993; Huerta et al. 1987), superior colliculus, and brain stem oculomotor center (Fries 1985; Hartmann-von Monakow et al. 1979; Huerta and Kaas 1990; Shook et al. 1988, 1990). Instead, the pre-SMA is richly interconnected with the prefrontal cortex (Bates and Goldman-Rakic 1993; Lu et al. 1994; Luppino et al. 1990, 1993). These anatomical features suggest that the pre-SMA is involved in more cognitive processes subserving voluntary motor control, regardless of effector of movements (eyes or arms), rather than being associated with motor execution itself (Picard and Strick 2001). Supporting this view, recent imaging studies on human subjects have revealed the involvement of the pre-SMA in associative sensory-motor behavior and/or learning of sequential motor responses with eyes (Curtis and D'Esposito 2003; Kawashima et al. 1998; Merriam et al. 2001) or hands (Hikosaka et al. 1996; Kurata et al. 2000; Sakai et al. 1999). Moreover, and more specifically, as many as 36% of neurons in the pre-SMA fired nonselectively when the monkey captured a target with a reach, saccade, or combined saccade-reach (Fujii et al. 2002). Having considered the involvement of the pre-SMA in sequencing forelimb movements and its possible effector-nonselectivity, we hypothesized that the pre-SMA also plays an important role in the organization of saccade sequences. To test this hypothesis and also to specify neural signals that pre-SMA neurons exhibit preferentially for temporal behavioral control, we analyzed single-neuron activity while monkeys were performing three saccades in sequence that were instructed and memorized.

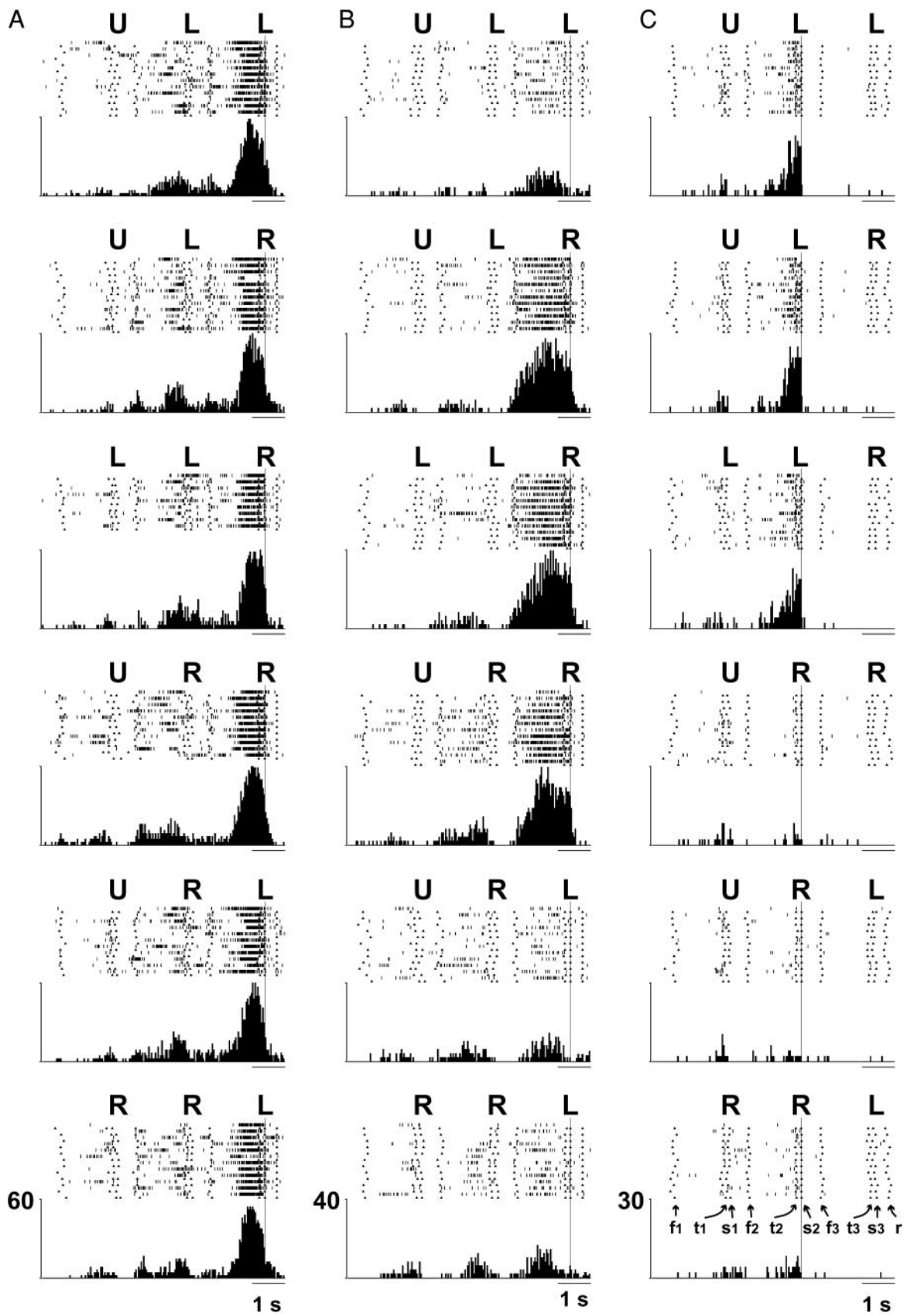
## METHODS

### *Subjects and apparatus*

We used two male Japanese monkeys (*Macaca fuscata*, 5–6 kg). During experimental sessions, each monkey was seated in a primate chair and faced a display monitor on which three peripheral lights (red, 0.5° diam) were illuminated above (U), to the left (L), and to the right (R) of center (12° each). The experiments were performed while the monkey's head and arms were restrained, and its eye movements were monitored using an infrared monitor system (R-21C-A, RMS, Hirosaki, Japan); sampling was at 250 Hz. All surgical and experimental protocols were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the guidelines for Institutional Animal Care and Use published by our institute.

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### Behavioral procedures

We used a behavioral task that has been described previously (Isoda and Tanji 2003). The monkeys were trained to execute three saccades to the peripheral lights (center-out saccade) in six different orders. The correct sequence was instructed during visually guided sequence trials. Before each center-out saccade, the monkey fixated on a central, white fixation point (FP, 0.5° diam) for a variable interval (1,450–1,600 ms). After this, the central FP was extinguished (the “go” signal), and simultaneously, one of the three peripheral lights turned white, thereby indicating a saccade target. If the animal acquired the target within 500 ms and maintained his gaze on it for another 350 ms, the saccade target returned to red, a high-pitch tone sounded, and the central FP was illuminated again. The animal was then required to capture the FP within 1,000 ms with re-fixation eye movements to attain central fixation prior to the subsequent center-out saccade. In this manner, the monkey made second and third center-out saccades, each following a period of central fixation (1,450–1,600 ms). A series of three correct saccades was rewarded after the third target-fixation period. When performing a center-out saccade, the monkey was required to acquire a target with a single saccade; an initial saccade to an incorrect target that was rapidly followed by a second saccade to a correct target was defined as an error. For capturing the central FP (re-fixation eye movements), however, the monkey was allowed to make either single or multiple saccades with variable trajectories. After the completion of four trials under visual guidance, the monkey was required to repeat the same sequence of three center-out saccades based on memory (memory-guided trials). The temporal sequence of events in the memory-guided trials was the same as in the visually guided trials, except that the three potential saccade targets remained red throughout the trial, compelling the monkey to choose the correct target solely from memory. After the monkey accomplished four trials from memory, flashing lights (duration 700 ms) signaled both the end of the current sequence and the beginning of the next, followed by an intertrial interval (ITI). The ITI was fixed at 2.5 s for every trial. Thus each sequence was performed in blocks, each of which comprised of four visually guided trials followed by four memory-guided trials. Six different sequences were presented in a pseudo-random order.

### Neuronal recording

Recordings were made from three hemispheres of the pre-SMA with glass-insulated Elgiloy microelectrodes (1.0–2.5 MΩ at 333 Hz). Each neuron was tested for at least two blocks of trials for each sequence. The localization of cortical sites was based on physiological criteria established previously (Matsuzaka et al. 1992). In short, we first mapped the SMA by observing somatosensory responses and effects of intracortical microstimulation (ICMS, a train of 12–42 cathodal pulses of 0.2-ms duration at 333 Hz) (Asanuma and Sakata 1967). Then the pre-SMA was identified just rostrally to the face representation of the SMA, which was confirmed by magnetic resonance imaging (OPART 3D-System, Toshiba, Tokyo, Japan). An area that was determined to be the pre-SMA was not overlapped with the SEF, which was also mapped in the same monkeys (Isoda and Tanji 2003).

### Data analysis

The first step of analysis was aimed at determining the relationship of neuronal activity to saccade direction, the numerical position of saccades in each sequence (rank), and sequence of three center-out saccades during memory-guided trials. We did not analyze re-fixation eye movements because they were not memory-based and frequently contaminated with saccades of variable trajectories. We set two task epochs for the analysis: preparatory and presaccadic intervals. The preparatory activity was averaged across an 800-ms interval starting 900 ms before go signals (disappearance of the central FP). The presaccadic activity was summed over a 200-ms interval preceding the onset of the saccade. If the number of discharges during the test periods was different from that during a control period (a 500-ms interval prior to trial onset as a default time window) in at least one sequence (Mann-Whitney *U* test,  $\alpha = 0.05$ ), the neuron was judged to be task-related for that interval and used for further analysis. We then analyzed the effects of saccade direction, sequence of saccades, and saccade rank in a sequence on neuronal activity. We were unable to apply a three-way ANOVA to our data set because we used a limited number of saccade sequences for technical reasons imposed by experimental practice; 6 of 27 sequences theoretically possible were used (Fig. 1). We, therefore took two separate measures. First, we analyzed the effects of saccade direction and saccade rank in a sequence on neuronal activity by employing a two-way ANOVA ( $\alpha = 0.01$ ). The rank factor included three levels (1st, 2nd, and 3rd), but the direction factor included two levels (rightward and leftward), because upward saccades appeared only at the first saccade (Fig. 1). Second, we studied possible relationship of neuronal activity to sequence of saccades. For this purpose, we applied a one-way ANOVA ( $\alpha = 0.01$ ) to five saccade categories, each of which comprised saccades of equal direction and rank (Isoda and Tanji 2002; their Fig. 1B). This analysis may not be a theoretically legitimate statistical method and was taken as a practical way of finding possible relationship of neuronal activity to sequences. However, significant ANOVA outcomes indicate that neuronal activity preceding a particular saccade (categorized with a direction and rank) is influenced by which saccade precedes or follows in a sequence. This analysis was considered meaningful because such neuronal activity could be used to discriminate different sequences. Significant ANOVA outcomes were further analyzed with Fisher's least significant difference (LSD) post hoc test for multiple comparisons ( $\alpha = 0.05$ ).

We quantified selectivity for the direction and rank of saccades using the following equations: Direction index =  $(Fpd - Fnpd)/(Fpd + Fnpd)$ ; Rank index =  $(Fpr - Fnpr)/(Fpr + Fnpr)$ . In these equations, *Fpd* and *Fnpd* refer to the firing rate preceding saccades in the preferred and nonpreferred directions. *Fpr* and *Fnpr* refer to the firing rate for saccades in the preferred and nonpreferred ranks. We also quantified the selectivity for the sequence, despite limitations for interpretation stated above. Sequence index =  $(Fps - Fnps)/(Fps + Fnps)$ . In this formula, *Fps* and *Fnps* refer to the firing rate for saccades in sequences in which neurons were most and least active. The sequence index was calculated for each of the five saccade categories described above, each of which was treated as a separate case.

In a previous study, we found that neuronal activity in the pre-SMA was profoundly influenced by trial numbers within a trial block

FIG. 1. Examples of neuronal activity representative of presupplementary motor area (pre-SMA) during memory-guided trials. In the raster displays, each row represents a single trial, and thin vertical bars represent individual neuronal discharges. The 10 small triangles in each row indicate the occurrence of behavioral events, as denoted at the bottom panel in C (f1, onset of 1st central fixation; t1, 1st saccade trigger; s1, onset of 1st center-out saccade; f2, onset of 2nd central fixation; t2, 2nd saccade trigger; s2, onset of 2nd saccade; f3, initiation of 3rd central fixation; t3, 3rd saccade trigger; s3, onset of 3rd saccade; r, reward). Raster displays and histograms (40-ms bins) are aligned with the onset of the 3rd saccade in A and B, and with the onset of the 2nd saccade in C, which are indicated by the solid vertical lines. U, upward saccade; L, leftward saccade; R, rightward saccade. Time scales (s) and spike rates (sp/s) are indicated in the bottom panels. A: discharges selective only for the rank order (3rd saccades). B and C: discharges selective for the combination of rank and direction (3rd rightward saccades in B and 2nd leftward saccades in C).

(Shima et al. 1996). To investigate whether the first-trial unique activity observed in a limb-motor sequence task will also appear in the present oculomotor task, we analyzed the relationship of neuronal activity to trial numbers within a trial block ( $n = 8$ ). For this analysis, we used all neurons sampled in the pre-SMA ( $n = 250$ ) and examined their activity during four task epochs, specifically a 2,000-ms interval preceding trial onset and the three presaccadic intervals. We conducted a one-way ANOVA on neuronal activity in each interval ( $\alpha = 0.01$ ). Where appropriate, Fisher's LSD test was carried out to determine whether the selectivity was determined by individual trial numbers ( $\alpha = 0.05$ ).

RESULTS

*Relationship of neuronal activity to direction, rank, and sequence of three center-out saccades guided by memory*

A total of 250 pre-SMA neurons were studied during performance of the task. We found that, for the majority of pre-SMA neurons, activity during both preparatory and presaccadic periods was selective for, or influenced by, the rank order of three saccades, whereas only a minor part of neuronal activity was selective for saccade direction or sequence. ANOVA revealed that 78% of preparatory activity ( $n = 139$ ) and 75% of presaccadic activity ( $n = 135$ ) were influenced by the rank order (Table 1). Of these, about two-thirds were selective for rank only (Fig. 1A; Table 1), and the remaining one-third was selective for both rank and direction (Fig. 1, B and C; Table 1). Most of these neurons showed the same rank-selective and/or direction-selective activity during performance of the visually guided saccade sequence. Across the population, pre-SMA neurons covered the entire numerical position in the rank, although the third rank was most preferred (Fig. 2, A and B). A scatter plot revealed that the rank index, as a population, was much greater than the direction index ( $P < 0.001$ , Mann-Whitney  $U$  test) for both the preparatory (Fig. 2C) and presaccadic intervals (Fig. 2D). This difference in distribution between the two indices ( $P < 0.001$ , Kolmogorov-Smirnov test) is more directly shown with a cumulative histogram in Fig. 2E (blue line vs. red line). The distribution of each index was also different between the two intervals ( $P < 0.05$ , Kolmogorov-Smirnov test; Fig. 2E); the index during the presaccadic interval (solid line) was larger than that during the preparatory interval (dotted line) both for direction (blue line) and rank (red line). In contrast to the abundance of rank signals, sequence-selective activity was much less frequent in the pre-SMA (Table 1). The distribution of the sequence index (Fig. 2F) indicates that the tuning strength of pre-SMA neurons was generally weak for saccade sequences. Like the direction and rank indices, however, the sequence index during the

TABLE 1. *Distribution of neuronal selectivity during preparatory and presaccadic intervals*

Task periods	Selectivity				Total Task-Related Neurons
	Direction	Rank	Rank and Direction	Sequence	
Preparatory	12 (7)	96 (54)	43 (24)	16 (9)	179
Presaccadic	5 (3)	89 (50)	46 (26)	10 (6)	179

Values denote the number of neurons. Values in parentheses are expressed in percentages.

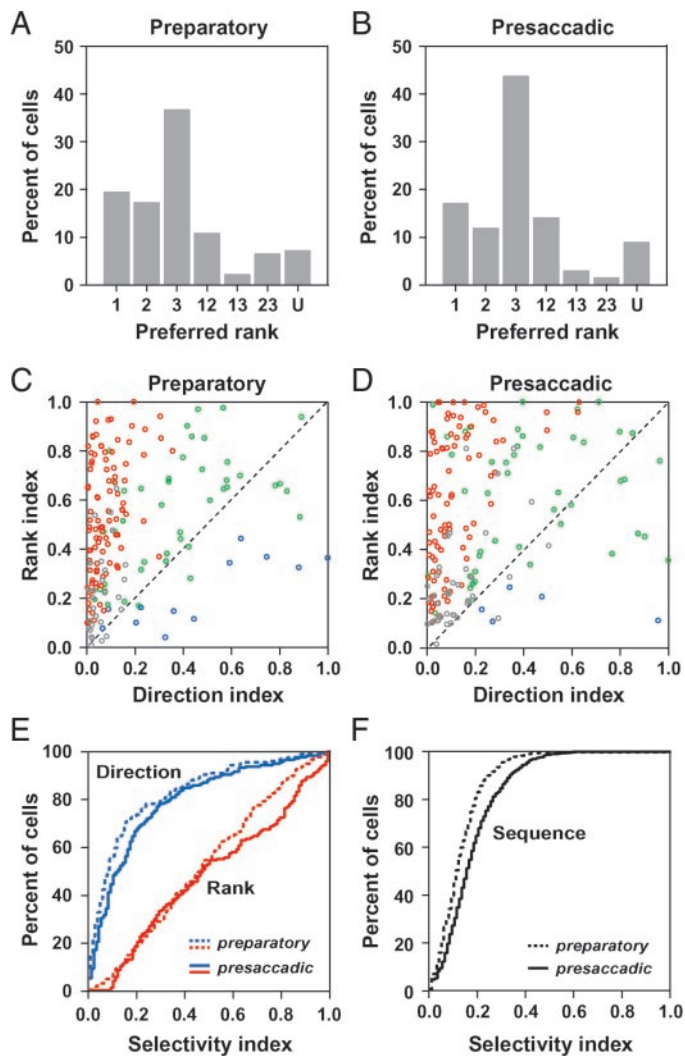


FIG. 2. Quantitative analyses of direction-, rank-, and sequence-selective activity. A and B: distribution of preferred ranks in the preparatory (A) and presaccadic periods (B). 1, 1st; 2, 2nd; 3, 3rd; 12, 1st and 2nd; 13, 1st and 3rd; 23, 2nd and 3rd; U, unclassified. C and D: scatter plots of the rank (vertical axis) vs. direction index (horizontal axis) for individual neurons that exhibited significant activity modulation during the preparatory (C) and presaccadic periods (D). Blue circles denote direction-selective neurons; red circles denote rank-selective neurons, while green circles are for combination-selective neurons and gray circles are for nonselective neurons. The dotted lines (45°) indicate where the 2 indices are equal. E and F: cumulative distributions of direction (E, blue line), rank (E, red line), and sequence indices (F) for the preparatory (dotted line) and presaccadic intervals (solid line).

presaccadic interval was greater than that during the preparatory interval ( $P < 0.001$ ; Fig. 2F, solid line vs. dotted line).

To determine whether the selectivity for rank and sequence could be accounted for by differences in kinematic saccade parameters (reaction times, movement times, peak saccade velocity), we carried out an analysis of covariance (ANCOVA) using each of these parameters as a covariate. None of the parameters co-varied with either rank or sequence for all but one case.

*Neuronal activity at the transition of sequences*

We found that 22% (55/250) of all sampled neurons in the pre-SMA exhibited an increase of activity during the *first* trial

within a trial block, regardless of the order of saccades. Figure 3A shows a typical example in which the activity preceding trial onset increased markedly during the first trial (V1), irrespective of the order of the three saccades (Fig. 3A, top). The increase of the firing rate, however, became less noticeable soon thereafter (Fig. 3A, V2-M1). The transition-selective activity appeared prior to trial initiation, like the neuron in Fig. 3A, in 55% of transition-selective neurons. In the remaining

cases, however, the activity was apparent during the presaccadic interval (38%) or during both periods (7%; Fig. 3B, top). For presaccade-period active neurons, the first-trial-selective activity appeared either at one specific position of saccades in each sequence or in more than one position (Fig. 3B, bottom). None of the transition-selective activity could be ascribed to the difference in kinematic saccade parameters within a trial block (ANCOVA).

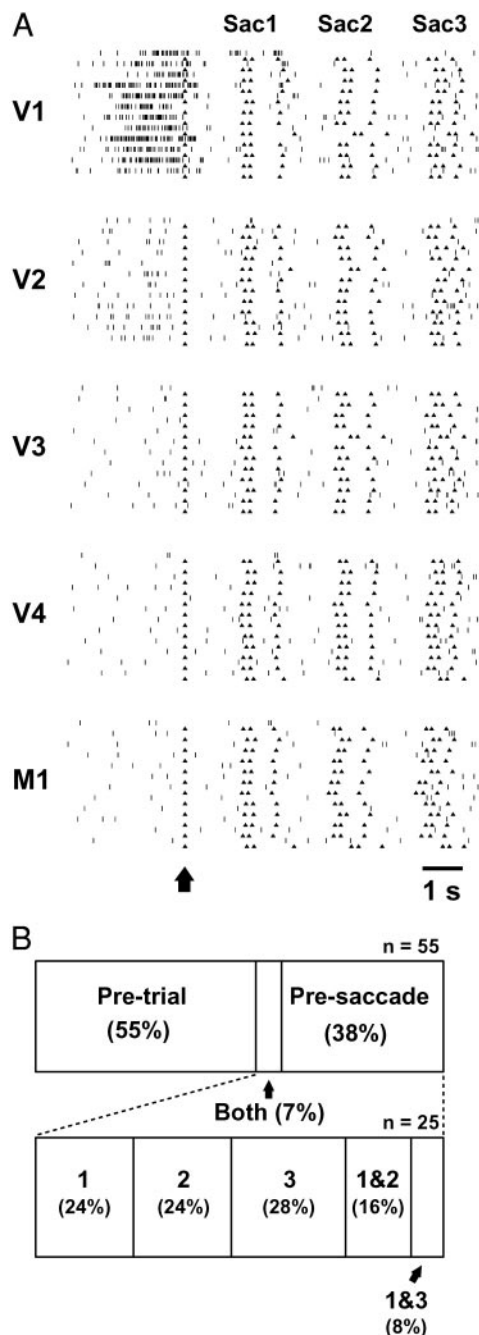


FIG. 3. Discharges of the transition-selective neuronal activity. *A*: trials during performance of 6 different saccade orders were combined together and rearranged based on the trial number within a trial block. *Sac I*, 1st saccade; *Sac II*, 2nd saccade; *Sac III*, 3rd saccade. V1–V4, 1st through 4th trial under visual guidance; M1, memory-guided 1st trial. Raster displays are aligned with trial onset, which is indicated by a solid arrow. Other conventions are as in Fig. 1. *B*: classification of the transition-selective activity according to the task phase (top) and to the preferred numerical position of saccades (bottom).

## DISCUSSION

This study has investigated how single neurons in the primate pre-SMA are involved in the temporal organization of multiple saccades, focusing on activity properties during the preparatory and presaccadic intervals. We found that 1) pre-SMA activity was predominantly determined by the numerical position of saccades within a sequence, and 2) 22% of pre-SMA neurons increased their firing preferentially at the transition of each sequence. In contrast, neurons encoding saccade directions with no rank effects, and neurons that were selective for the specific order of three saccades were much less frequently found.

Roesch and Olson (2003) recently reported that reward-dependent modulation of motor-related signals is prevalent in several cortical motor areas, probably including the pre-SMA (their SMAr). Since, in our task, the monkey was rewarded only after the third saccade, and ‘third’ was preferred by approximately one-third of order-selective neurons, the rank-selective activity of these neurons might reflect the monkey’s expectation of reward rather than the rank-order information per se. In fact, other researchers focused on how the expectation of reward affects neural signals in the ventral striatum (Shidara et al. 1998) and anterior cingulate area (Shidara and Richmond 2002). Although we cannot rule out this possibility, the reward expectation hypothesis cannot account directly for other neurons preferring the first or second rank. An interesting possibility is that the monkeys might have been using the reward-timing information to construct the rank-order information by scaling in time the closeness of each saccade to the reward occurrence.

The transition-selective activity appeared relevant to the period when the monkey was required to prepare for switching from one sequence to another. In other words, during that time, the monkey needed to discard the current information about saccade sequence and prepare for the next task performance. This type of activity was recently observed in the SEF under the same experimental conditions, and the functional significance was extensively discussed (Isoda and Tanji 2003). Although the nonrandom nature of the six sequences used in this study could produce an anticipatory bias to the up-target appearance (a 67% probability) before the presentation of the first target in a new sequence, and this up-target bias might yield the transition-selective activity preceding the trial onset and first saccade, this bias hypothesis is still insufficient to explain the transition-selective activity prior to the second and third saccades. Therefore applying the same logic here (Isoda and Tanji 2003), we propose that the transition-selective activity in the pre-SMA is also related to the underlying process of updating motor plans for the organization of saccade sequence.

The present finding is, in part, related to a report by Sommer and Tehovnik (1999), who studied the effects of inactivating

the monkey dorsomedial frontal cortex (DMFC) on double-step saccade performance. They showed that the injection of lidocaine into the DMFC induced a severe impairment in the generation of sequential double-step saccades but not of single saccades, suggesting an important contribution of the DMFC to the control of sequential saccades. Although the DMFC is an area that encompasses a large expanse of dorsomedial regions in the frontal cortex, including the SEF, pre-SMA, SMA, and dorsal premotor area (see, Tehovnik 1995 for review), it seems likely that some of their injection sites were within the pre-SMA (Sommer and Tehovnik 1999; their Fig. 1B). If this is the case, their findings and ours reinforce the argument that the pre-SMA is crucially involved in the temporal organization of multiple saccades. Further studies are necessary to better understand how the pre-SMA participates in controlling oculomotor behavior, as a part of the neuronal network including the cerebral cortex, basal ganglia, and cerebellum.

This study in monkeys further raises the issue of the existence of the presupplementary eye field (pre-SEF) in humans as a distinct motor area. The human homologue of the SEF is presumed to lie along the interhemispheric surface in the anterior part of the SMA and posterior to the VCA line (Grosbras et al. 1999; Luna et al. 1998), which is considered as the posterior limit of the pre-SMA (Picard and Strick 1996; Stephan et al. 1995). In addition to a focus in the traditional SEF, another activation site was identified in its rostral part while the subjects were performing oculomotor sequence tasks (Grosbras et al. 2001; Heide et al. 2001; Petit et al. 1996). This rostral part was preferentially active during the performance of a prelearned memorized sequence (Petit et al. 1996), during the execution of newly learned sequences (Grosbras et al. 2001), and when performing memorized triple-step saccades (Heide et al. 2001). This cortical region was referred to as the pre-SEF by these authors in analogy with SMA/pre-SMA distinction for a skeletomotor system of primates. Because the activity focus was centered rostrally to the VCA line, it seems possible, or even probable at present, to assign the active area to the pre-SMA. This notion is consistent with the equivocal somatotopy in the pre-SMA (Picard and Strick 1996).

The data obtained in this study to examine the pre-SMA can be directly compared with those examining the SEF of the same monkeys (Isoda and Tanji 2002, 2003). Although both areas contained a variety of neuronal activity that seemed useful to construct multiple saccades in sequence, some noticeable differences were found between our pre-SMA and SEF populations. Specifically, the proportion of rank-selective neurons was significantly higher in the pre-SMA ( $P < 0.001$ ,  $\chi^2$  test), whereas sequence-selective ( $P < 0.001$ ), rank-and-direction-selective ( $P < 0.01$ ), and direction-selective neurons ( $P < 0.001$ ) were more prevalent in the SEF. In accordance with this difference in the distribution, the rank index was significantly larger for pre-SMA neurons ( $P < 0.001$ , Mann-Whitney  $U$  test). In contrast, the direction index and sequence index were significantly greater for SEF neurons ( $P < 0.001$ ). These findings could be interpreted that the pre-SMA is involved primarily in monitoring or determining when to move the eyes in a sequence (ordinal position), whereas the SEF is involved more in determining where to move the eyes and in what order. The rank-order signals that are abundantly represented in the pre-SMA may be transmitted to the SEF, where the signals could be used to decode saccade sequences (Isoda and Tanji

2003). This signal might be transmitted by way of the anterior cingulate area (Hatanaka et al. 2003; Huerta and Kaas 1990; Luppino et al. 1993; Wang et al. 2001), because anterior cingulate neurons are also found to encode serial order of sequence components (Procyk and Joseph 2001; Procyk et al. 2000). On the other hand, we found a similarity between the pre-SMA and SEF. Both of the two areas exhibited transition-selective activity in a similar proportion ( $P > 0.3$ ). In this respect, the two areas were strikingly contrasted with the FEF where only 4% of all sampled neurons were active at the renewal of the saccade sequences (Isoda and Tanji 2003). Although simple dichotomy would not be acceptable, our findings on the area-selective distribution of the transition-selective activity appear to be in accord with a view pointing to functional differentiation between the medial versus lateral cortical motor areas, which has been proposed by several investigators (e.g., Coe et al. 2002; Mushiaki et al. 1991; Schall et al. 2002).

In previous studies that contrasted neuronal activity in the pre-SMA and SMA, it was shown that, for sequential organization of multiple *forelimb movements*, the pre-SMA is more specialized in the representation of rank-order information (Clower and Alexander 1998; Shima and Tanji 2000) and in the updating of sequence plans (Shima et al. 1996). The present findings are consistent with these reports despite the difference in the relevant effector. Since, for most cases, the rank-order activity was independent of the direction of saccade (Table 1) or the type of forelimb movements (Shima and Tanji 2000), the activity specifying the rank seems abstract in nature as a task-regulating signal. Also abstract in nature is the transition-selective activity because it consistently appeared regardless of the order of actions (Fig. 3; see also Shima et al. 1996). These arguments lead to a proposal that the pre-SMA is involved in higher-order aspects of behavioral control, presumably in an effector-independent manner. The lack of clear-cut somatotopy and the existence of connections with the prefrontal cortex, but not with the primary motor regions, provide further support for the above proposal. From a functional standpoint, therefore, the pre-SMA is more akin to the prefrontal cortex than either to oculomotor or limb-motor areas (cf. Picard and Strick 2001).

#### ACKNOWLEDGMENTS

We thank M. Kurama and Y. Takahashi for technical assistance.

#### GRANTS

This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas (C)—Advanced Brain Science Project—from the Ministry of Education, Culture, Sports, Science and Technology of Japan and by the Cooperation Research Program of Primate Research Institute of Kyoto University.

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