Contrasting Neuronal Activity in the Supplementary and Frontal Eye Fields During Temporal Organization of Multiple Saccades

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Submitted 14 April 2003; accepted in final form 5 August 2003

INTRODUCTION

Clarifying mechanisms that enable multiple actions to be performed in an orderly manner is a central issue in the neural control of motor behavior (Lashley 1951; Rosenbaum 1991; Zingale and Kowler 1987). When organisms act to achieve an objective, they usually need to execute more than one action. Several individual actions need to be organized for purposeful behavior, and the incorrect ordering of actions can lead to disastrous consequences. Therefore the organization of a series of actions into an appropriate temporal order is particularly important in the voluntary control of behavior (Tanji 2001). In the present study, we addressed the cortical control of saccade sequences as an experimental model with which to elucidate the role of the cerebral cortex in organizing behavioral sequences.

Among the multiple motor areas within the cerebral cortex, two principal oculomotor fields have been established (Schall 1985, 1987), namely the frontal eye field (FEF) (Bizzi 1967; Bruce and Goldberg 1985; Stanton et al. 1989), located laterally, and the supplementary eye field (SEF) (Schlag and Schlag-Rey 1985, 1987), located medially. Although these two areas share several anatomical connections (Huerta and Kaas 1990; Huerta et al. 1987; Parthasarathy et al. 1992; Shook et al. 1990, 1991; Stanton et al. 1988a, b) and exhibit similar physiological properties (Bruce and Goldberg 1985; Russo and Bruce 2000; Schall 1991a, b; Schlag and Schlag-Rey 1987), some functional aspects are known to differ between the two areas. Based largely on studies examining effects of electrical stimulation, it has been proposed that the FEF codes saccades in an oculo-centric coordinate system, whereas the SEF codes saccades in a craniocentric coordinate system (Bon and Lucchetti 1992; Mann et al. 1988; Mitz and Godschalk 1989; Schall 1991b; Tehovnik and Lee 1993; but see Russo and Bruce 1993, 1996). On the other hand, it is also hypothesized that SEF neurons code saccades with reference to an object-centered coordinate (Olson and Gettner 1995, 1999), although FEF neurons have not yet been tested under the same experimental condition. Other intriguing hypotheses propose that the SEF is more involved in the learning of stimulus-saccade associations (Chen and Wise 1995b), eye-hand coordination (Mushiake et al. 1996), anti-saccade production (Everling and Munoz 2000; Schlag-Rey et al. 1997), decision-making saccade processes (Coe et al. 2002), and supervisory control of sensorimotor processes (Hanes et al. 1998; Schall et al. 2002; Stuphorn et al. 2000).

Although we have now some knowledge about aspects of functional differences between the two areas, little is known about how differently these areas take part in organizing the temporal sequence of saccades (Isoda and Tanji 2002; Lu et al. 2002). Having considered previous reports indicating the involvement of medial cortical motor areas in sequential control of limb movements in both humans (Dick et al. 1986; Gaymard et al. 1990; Hikosaka et al. 1996; Kawashima et al. 1998; Muri et al. 1995; Petit et al. 1996; Roland et al. 1980; Shibasaki et al. 1993) and nonhuman primates (Clover and Alexander 1998; Mushiake et al. 1990, 1991; Nakamura et al. 1998, 1999; Schiller and Chou 1998; Shima and Tanji 1998, 2000; Tanji and Shima 1994), we hypothesized that neuronal activity in the SEF, more than the FEF, represents signals that are relevant to the temporal structuring of multiple saccades. To test this...
Subjects and apparatus

We used two male Japanese monkeys (Macaca fuscata, 5–6 kg). The monkeys were kept in individual primate cages in an air-conditioned room with food available ad libitum. They were given restricted amount of fluid during periods of training and recording. Their body weight and appetite were checked daily, and supplementary water and fruit were provided as appropriate. During experimental sessions, each monkey was seated in a primate chair and placed in a sound-attenuated room and faced a display monitor on which three peripheral lights in different orders. The correct sequence was indicated during visually guided trials (Fig. 1). Before each saccade, the monkey fixated on a central, white fixation point (FP, 0.5° diam) for 1,450–1,600 ms. When the FP was extinguished (the go signal), one of three red peripheral lights turned white, thereby indicating a saccade target. If the animal acquired the target within 500 ms and maintained fixation on it for ≥350 ms, the target color was changed to red, a high-pitch tone sounded, and the central FP was illuminated again. The animal was then required to capture the central FP within 1,000 ms (re-fixation). For capturing the FP, monkeys were allowed to make either single or multiple saccades with variable trajectories. In the same way, a period of central fixation was followed by the second and third saccade triggers. A series of correct saccades was rewarded with a drop of juice after the third target-trigger, and two head holders on the skull under deep pentobarbital sodium anesthesia. 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.

Behavioral procedures

We used a behavioral task that has been described previously (Isoda and Tanji 2002). Using operant conditioning with positive reinforcement, monkeys were trained to perform three saccades to the peripheral lights in six different orders. The correct sequence was indicated during visually guided trials (Fig. 1). Before each saccade, the monkey fixated on a central, white fixation point (FP, 0.5° diam) for 1,450–1,600 ms. When the FP was extinguished (the go signal), one of three red peripheral lights turned white, thereby indicating a saccade target. If the animal acquired the target within 500 ms and maintained fixation on it for ≥350 ms, the target color was changed to red, a high-pitch tone sounded, and the central FP was illuminated again. The animal was then required to capture the central FP within 1,000 ms (re-fixation). For capturing the FP, monkeys were allowed to make either single or multiple saccades with variable trajectories. In the same way, a period of central fixation was followed by the second and third saccade triggers. A series of correct saccades was rewarded with a drop of juice after the third target-trigger, and two head holders on the skull under deep pentobarbital sodium anesthesia. 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.

Training procedures

The monkeys were first trained to generate a single saccade to one of the three peripheral lights within 300 ms after the central FP was turned off. Saccades to each direction were repeated in a block of eight trials: four trials under visual guidance and then four trials from memory. When the performance of each center-out saccade was stable after 2 wk of training, the monkey learned to make two center-out saccades in a correct order with an intervening re-fixation eye move-
ments to capture the FP (within 1,000 ms), followed by a period of central fixation. The order of two center-out saccades employed was U-L, U-R, R-L, and L-R; each order was repeated in blocks. In this task phase, the time restriction to acquire peripheral targets was relaxed to 500 ms, but the reaction time of saccades remained mostly within 250 ms. When performing the center-out saccades, the monkeys were required to acquire a correct target with a single saccade. Turn-around saccades were not allowed even if the correct target was acquired within 500 ms. This strict restriction was not applied to eye movements to recapture the central fixation target. The monkeys had only to come back to the FP within 1,000 ms, and multiple saccades with a variety of trajectories were allowed. The monkeys acquired stable performance of this two center-out saccade task after about 1 mo of training. Thereafter, the final stage of task training that required three center-out saccades was introduced. It took 5–6 mo for both monkeys to become proficient in performing three saccades in six different orders that were selected based on memory.

Neuronal recording

Neuronal activity was recorded with glass-insulated Eldigio micro-electrodes (1.0–2.5 MΩ at 333 Hz). An electrode was inserted through the dura using a hydraulic microdrive (MO-951S; Narishige, Tokyo, Japan). Neuronal activity was amplified and band-pass filtered (1–6 kHz) and single-unit potentials were discriminated using a template-matching algorithm (MCP-Plus and MSD; Alpha Omega Engineering, Nazareth, Israel). Behavioral events and raster displays of action potentials were displayed on-line and data were stored in a microcomputer for analysis off-line.

Recording sites

We recorded neuronal activity in the SEF and FEF. The localization of cortical sites was based on physiological criteria established previously (Bruce and Goldberg 1985; Bruce et al. 1985; Mushiake et al. 1996; Russo and Bruce 1996; Schlag and Schlag-Rey 1987). The SEF was defined as a cortical area in the dorsomedial convexity immediately lateral to the presupplementary motor area (pre-SMA) (Matsu-zaka et al. 1992; Picard and Strick 1996), in which saccadic eye movements were elicited at a low threshold (<50 μA) of intracortical microstimulation (ICMS, a train of 12–50 cathodal pulses of 0.2-ms duration at 333 Hz). The FEF was defined as an area in the anterior bank of the arcuate sulcus in which low-threshold ICMS evoked saccades. The recording sites were confirmed by magnetic resonance imaging (OPART 3D-System; Toshiba, Tokyo, Japan).

Data analysis

Our initial aim was to determine the relationship of presaccadic activity to the direction, rank, and sequence of three center-out saccades during trials guided by memory. Eye movements required to bring the gaze back to the central FP was not included in this analysis because the re-fixation eye movements were not memory based and frequently contaminated with saccades having variable trajectories. For this purpose of analysis, we selected neurons that had task-related presaccadic activity. If the number of discharges during a presaccadic period (200–0 ms before saccade onset) was significantly different from that during a control period (the last 500 ms of ITI as a default time window) for at least one sequence (Mann-Whitney U test, α = 0.05), the neuron was judged to be active during the presaccadic period. The selection of the time window for presaccadic activity was based on our finding that most part of phasic activity preceding saccades fell on that interval. Furthermore, we obtained similar results by selecting the interval of 100–0 ms preceding saccade initiation for the quantitative analysis. The time window of 200–0 ms was comparable to previous studies (Chen and Wise 1995a; Mushiake et al. 1996; Schlag-Rey et al. 1997). The selection of the last ITI for the control period, instead of a fixation epoch after the start of each trial, was based on the following reasons. Because each sequence of saccades was performed in blocks, the monkey had prior knowledge about which sequence was currently required before the initiation of the first saccade. This “cognitive set” occasionally led to changes in neuronal activity during the epoch of central fixation but not during ITI. Therefore we considered that ITI was more suitable as a control period. A separate analysis revealed that neuronal activity during the 500-ms ITI was not significantly influenced by the metrics of the first saccade or the sequence of saccades, lending support to the choice of the ITI period for the control.

Because we used only six different sequences out of all of the possible 27, we could not apply three-way ANOVA. Instead, we first performed two-way ANOVA for relatedness to the direction and rank (α = 0.01). The rank factor included three levels (1st, 2nd, and 3rd) and the direction factor included two levels (rightward and leftward), because upward saccades appeared only as the first saccade (see Fig. 1). In the next step of the analysis, we examined the effect of saccade sequences on neuronal activity. For this analysis, we selected five subgroups of data, each of which comprised saccades of equal direction and rank (see saccade category in Fig. 4). We then applied one-way ANOVA to each saccade category (α = 0.01). Significant ANOVA outcomes were further analyzed with Fisher’s least significant difference (LSD) post hoc test for multiple comparisons (α = 0.05).

We quantified selectivity for the direction, rank and sequence of saccades using the following equations: direction index = [(Fpd − Fnpd)/(Fpd + Fnpd)]; rank index = [(Fpr − Fnpr)/(Fpr + Fnpr)]; and sequence index = [(Fps − Fnps)/(Fps + Fnps)].

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 rank. Fps and Fnps refer to the firing rate for saccades in sequences in which neurons were most and least active. A sequence index was calculated for each of the five saccade categories described in the preceding text, each of which was treated as a separate case.

A separate analysis was carried out to detect selectivity in neuronal activity for trial numbers within each trial block (n = 8). For this analysis, we used all neurons sampled in the SEF (n = 443) and FEF (n = 259) and examined their activity during four epochs, specifically the 2,000 ms preceding the trial start (onset of the fixation period) and the three presaccadic periods. We performed one-way ANOVA on neuronal activity in each trial epoch to examine the effect of trial number (α = 0.01). Where appropriate, Fisher’s LSD test was carried out to determine whether selectivity was determined by individual trial numbers (α = 0.05).

RESULTS

Neuronal activity preceding memory-guided sequential saccades

We studied 443 neurons in the SEF and 259 in the FEF that were isolated for long enough to perform a statistical analysis. Among these cells, 323 SEF and 167 FEF neurons exhibited presaccadic activity. We found a striking difference in the characteristics of presaccadic activity during the performance of memory-guided sequential tasks (Fig. 1). For half of the FEF neurons, presaccadic activity was direction selective, irrespective of whether a saccade was first, second, or third in a sequence of three saccades. By contrast, for the majority of SEF neurons, presaccadic activity was selective for, or influenced by, the position of a saccade within a sequence. Based on two-way ANOVA (see METHODS), presaccadic activity was classified into three major types according to whether activity was selective for saccade direction, the numerical position of a 5-6 mo.
saccade within each sequence (rank), or a combination of direction and rank. The first type of activity was observed in 81 (49%) FEF and 60 (19%) SEF neurons. A typical example is shown in Fig. 2A, in which the presaccadic activity of an FEF neuron appears to be selective for the leftward saccade, irrespective of rank. The second type of activity was observed in 58 (18%) SEF and 8 (5%) FEF neurons in which presaccadic activity depended only on the saccade rank, regardless of direction. In an example of an SEF neuron exhibiting this type of activity (Fig. 2B), the activity was most prominent when the monkey made the first saccade, irrespective of the saccade direction. When the monkey made a second or third saccade, the neuron was much less active. The third type of activity was observed in 123 (38%) SEF and 25 (15%) FEF neurons in which presaccadic activity depended on both the direction and rank or the interaction between these two factors. Neuronal activity of this type is shown in Fig. 2C, where a neuron exhibited presaccadic activity that was preferential for both the direction (leftward saccades) and rank (2nd saccades), being most active before the second, leftward saccades. When we compared the distribution of each type of activity between the two cortical fields, we found that direction-selective neurons were significantly more prevalent in the FEF (P < 0.001, χ² test), whereas rank-selective neurons and combination-selective neurons were observed more frequently in the SEF (P < 0.001, χ² test).

To quantify the selectivity of individual cells, we computed an index for direction selectivity and rank selectivity for presaccadic activity (see METHODS). A scatterplot of the direction selectivity index versus the rank selectivity index (Fig. 3A) revealed that the selectivity of SEF neurons was greater for rank than for direction (P < 0.001, Mann-Whitney U test), although both indices exhibited considerable variability. By contrast, for FEF neurons, selectivity of FEF neurons was greater for direction than rank (P < 0.001, Mann-Whitney U test). To compare the two indices in each of the two cortical areas more directly, we constructed cumulative frequency histograms (Fig. 3B). The rank index was significantly larger for

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**FIG. 2.** Direction-, rank-, and combination-selective neuronal activity during performance guided by memory. A: discharges of a direction-selective FEF neuron. B: a rank-selective SEF neuron. C: a combination-selective SEF neuron. In the raster displays, each row represents a trial and thin vertical bars represent individual cellular discharges. Ten small squares in each row indicate the time of occurrences of a behavioral event, as denoted at the 4th panel from top in A. 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. The raster displays and histograms (40-ms bins) are aligned with the onset of the 2nd saccade in A and C and with the onset of the 1st saccade in B, as indicated by the filled triangles. U, upward saccade; L, leftward saccade; R, rightward saccade. Timescale (s) and spike rate (spikes/s) are indicated (bottom).
respectively. In Fig. 4.

The proportion of sequence-selective neurons was significantly greater in the SEF than in the FEF ($P < 0.001$, Kolmogorov-Smirnov test). We found that the sequence selectivity appeared at different phases of the saccade sequence in the SEF and FEF. In the SEF, sequence selectivity preceding the first saccades was more prevalent than sequence selectivity preceding the third saccades (Fig. 5B). By contrast, in the FEF, sequence selectivity was more prevalent for the third saccade rather than the first saccade. The distribution of the frequency of sequence selectivity preceding the first and third saccades differed significantly between the two areas ($P < 0.001$, $\chi^2$ test).

To determine whether the selectivity of presaccadic activity for rank and sequence could be ascribed to differences in kinematic saccade parameters (reaction times, movement times, and peak saccade velocity), we carried out an analysis of covariance (ANCOVA) using each of these parameters as a covariate. None of the saccade parameters covaried with either rank or sequence in 97% of cases.

FIG. 3. Comparison of direction and rank indices for SEF and FEF neurons. A: scatterplot of the rank index (vertical axis) vs. the direction index (horizontal axis) for SEF (left) and FEF (right) neurons that exhibited significant activity modulation during the behavioral task. The broken line (45°) indicates where the 2 indices are equal. B: cumulative frequency histograms illustrating the distribution of the rank index (left) and direction index (right). The rank index was much greater for SEF neurons (black line), whereas the direction index was greater for FEF neurons (gray line), with significant differences in the distribution of each index ($P < 0.001$ for rank and $P < 0.05$ for direction). Cumulative frequency of 1.0 equals to 100% of population exhibiting presaccadic activity ($n = 323$ for SEF, $n = 167$ for FEF).
Neuronal activity at the transition of sequences

In the present study, each sequence of saccades was performed in blocks of eight trials: four trials under visual guidance followed by four trials from memory (Fig. 1). After the eighth trial, the monkey was required to prepare for switching from one sequence to another. During this period, we found that a discrete set of neurons exhibited a profound increase in activity as in an example shown in Fig. 6A. When the monkey performed a U-L-L sequence under visual guidance, neurons within this set exhibited a gradual increase in activity toward the initiation of the first trial (arrows in Fig. 6A, top). In later trials, however, the increase in activity was less marked and had disappeared by the time the sequential performance was based on memory (Fig. 6A, bottom). This trend was observed every time the saccade sequence was updated. For example, neuronal activity also increased before the onset of the first trial in the visually guided U-L-R sequence (Fig. 6A, 2nd panel) but became less noticeable soon thereafter. To demonstrate selectivity for the first trial more clearly, we rearranged the same data set according to the trial number within a block, irrespective of the saccade sequence. As is apparent in Fig. 6B, the increase in activity occurred primarily in the first trial of each sequence. The modulation at sequence transitions started 1.6 s before the start of a trial (900 ms after the end of the light flash that signaled a transition) and peaked near the initiation of the trial. This time course suggests that such selective activity was prospective for the new sequence rather than a direct response to the visual flash. We observed this type of neuronal activity in 112 SEF and 9 FEF neurons (25.3 and 3.5% of all neurons recorded in isolation, respectively). The proportion of neurons exhibiting first-trial selectivity was significantly higher for the

**FIG. 4.** Sequence selectivity during performance guided by memory. A: sequence-selective activity preceding the 1st saccade observed in an SEF neuron. Presaccadic activity was most prominent for the U-R-L sequence. The raster diagrams and histograms are aligned with the onset of the 1st saccade. B and C: 2 examples of sequence-selective activity preceding the 2nd saccade directed either leftward (B, SEF neuron) or rightward (C, SEF neuron). D and E: 2 examples of sequence-selective activity preceding the 3rd saccade, directed leftward (D, FEF neuron) or rightward (E, SEF neuron). In B–E, histograms for data from 1,500 ms before to 300 ms after the onset of each saccade (A). Other conventions are as in Fig. 2.

**Neuronal activity at the transition of sequences**

In the present study, each sequence of saccades was performed in blocks of eight trials: four trials under visual guidance followed by four trials from memory (Fig. 1). After the eighth trial, the monkey was required to prepare for switching from one sequence to another. During this period, we found that a discrete set of neurons exhibited a profound increase in activity as in an example shown in Fig. 6A. When the monkey performed a U-L-L sequence under visual guidance, neurons within this set exhibited a gradual increase in activity toward the initiation of the first trial (arrows in Fig. 6A, top). In later

**FIG. 5.** Comparison of sequence selectivity in the SEF and FEF. A: cumulative frequency histogram for the sequence index. The sequence index was calculated for each of the 5 saccade categories using all neurons found to be active prior to the saccade. The distribution of the sequence index differed between the 2 areas, being significantly greater for neurons in the SEF ($P < 0.001$). B: proportions of sequence-selective activity (percent relative frequency) preceding the 1st and 3rd saccades for all significant sequence-selective cases in the SEF (■) and FEF (□). For the SEF, $n = 84$. For the FEF, $n = 22$. Before calculating the relative frequency, the number of selective cases that appeared in each rank was normalized to the number of sequences examined, i.e., by dividing by 4 for the 1st saccade and by 6 for the 2nd and 3rd saccades.
SEF as compared with the FEF (P < 0.001, χ² test). Because the monkey was not required to control its gaze during the ITI before the trial onset (before the initiation of gaze fixation), we analyzed the frequency, direction and amplitude of saccades during this period (i.e., in the 2,000-ms period prior to the start of the trial). These parameters did not differ according to the number of trials within a visual block.

In approximately half of the neurons that exhibited first-trial selectivity, an increase in activity was observed before the initiation of the trial (Table 1) as for the cell shown in Fig. 6. In other cells, selectivity was observed during the presaccadic period. For such cells, the novel sequence-selective activity appeared to either precede a saccade at one specific position within the sequence (68%) or in more than one position (32%). Figure 7 illustrates an example in which first-trial selectivity was observed largely in the first of three saccades. ANCOVA revealed that such first-trial selectivity during presaccadic periods could not be ascribed to differences in kinematic saccade parameters within a block.

**Cortical localization of neuronal activity belonging to different types**

In the present study, we recorded neuronal activity in three hemispheres of SEF and two hemispheres of FEF, using two monkeys. Figure 8A shows surface reconstructions of the penetration sites of monkey 1 from which saccadic eye movements could be evoked by low-threshold ICMS (50 μA). Saccades were elicited primarily in a small region of the dorsomedial

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**TABLE 1. Distribution of transition-period-selective activity**

<table>
<thead>
<tr>
<th></th>
<th>Task Epochs</th>
<th>Total Number Tested</th>
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<tbody>
<tr>
<td></td>
<td>ITI</td>
<td>Presaccadic period</td>
</tr>
<tr>
<td></td>
<td>49 (11.1)</td>
<td>52 (11.7)</td>
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<tr>
<td></td>
<td>6 (2.3)</td>
<td>2 (0.8)</td>
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Values denote number of neurons. Parentheses enclose percentages. SEF and FEF, supplementary and frontal eye fields, respectively; ITI, intertrial interval.
convexity of the frontal lobe (SEF) and in the anterior bank of the arcuate sulcus (FEF). To examine whether activity properties of any types were localized to a particular cortical portion within the SEF and FEF, we analyzed the distribution of each type of neuronal activity within each area (Fig. 8B). Although rank-selective, sequence-selective, and transition-selective activities were encountered significantly more rostrally than other types of activity in the right SEF of monkey 1 ($P < 0.01$, FIG. 7. Preferential activity at the transition of sequences (presaccadic periods). The presaccadic activity of this SEF neuron occurred almost exclusively during the 1st trial in the visually guided performance. Raster displays and histograms are aligned with the onset of the 1st saccade. Other conventions are as in Fig. 6.

FIG. 8. Cortical surface maps of monkey 1. A: ●, points of electrode entry from which saccadic eye movements were evoked by low-threshold ($<50 \mu A$) intracortical microstimulation. PS, principal sulcus; ARC, arcuate sulcus; CS, central sulcus. B: recording sites of neurons exhibiting five different properties in the FEF and SEF. Each horizontal row of rectangles corresponds to the 3 rectangles shown in A. The number of task-related neurons with different properties was plotted separately at each penetration sites. Each circle’s size is proportional to the number of neurons, selective for saccade direction (D), direction and rank (DR), rank (R), sequence (S), and sequence transition (T).
Kruskal-Wallis test; Fig. 8B), we could not find significant differences in distribution in the left SEF or in the FEF. For monkey 2, the difference in distribution was not observed in either area.

**D I S C U S S I O N**

Contrasting neuronal activity in the two oculomotor areas preceding memory-guided sequential saccades and their functional significance

Our results revealed that the FEF and SEF exhibit a variety of neuronal activity that appeared to be useful for the correct performance of memorized saccade sequences. In addition, the distribution of each type of activity differed among neurons in the two fields. Sequence-, rank-, and combination-selective (direction and rank selective) activities were more prevalent in the SEF than in the FEF. As a population, selectivity for the rank and sequence was expressed to a greater degree in neurons in the SEF than in the FEF. By contrast, direction selectivity was more prevalent in the FEF and direction selectivity was the dominant signal encoded by FEF neurons. Direction-selective activity would appear to be useful for encoding a forthcoming saccade at the behavioral stage of output signaling, but in itself contains no information about the time structure of multiple saccades. By contrast, other types of activity appear to be relevant to the temporal sequencing of saccades. Rank-selective activity allows the discrimination of the numerical position of saccades within a sequence, specifying which of the first, second, or third saccades a subject should perform. This type of activity was independent of saccade direction and, therefore more abstract in nature as a control signal. Combination-selective activity, which was selective for both the saccade direction and rank, appears to be useful for specifying direction-selective saccades at a specific rank within a sequence. Because saccades of equal direction in the task used in the present study appeared twice in each of the four sequences (specifically, U-L-L, L-L-R, U-R-R, and R-R-L), activity that distinguished the same direction of saccades seems to be a component of a task-regulating signal. Furthermore, sequence-selective activity, which was more prevalent in the SEF, would appear to be useful for structuring the temporal order of the three saccades as a sequence.

Apart from the difference in the distribution of neuronal activity and the magnitude of selectivity, we found a difference in the sequence selectivity between the two areas. In the SEF, sequence selectivity tended to appear earlier in a sequence, whereas in the FEF it appeared later. As is illustrated in Fig. 5B, for SEF neurons, sequence selectivity preceding the first saccades was more prevalent than selectivity preceding the third saccades. By contrast, for FEF neurons, selectivity was more prevalent in the period preceding the third saccades rather than the first. How might this finding be interpreted? The selective activity preceding the first saccades was selective for the sequence of upcoming saccades, whereas the activity preceding the third saccades was specific for the sequence of saccades that had already been performed. It is possible that SEF neurons are more involved in prospective coding of the saccade sequence, whereas FEF neurons are influenced more by what the animal has already performed. However, such a prospective/retrospective coding hypothesis requires further investigation, because we used a limited number of sequences in this study (6 of 27 possible sequences).

**Functional significance of the transition-selective activity in the SEF**

During visually guided task performance, we observed another type of neuronal activity that characterized SEF neurons. Approximately one-quarter of neurons sampled in the SEF exhibited activity selectively at the transition of sequences (i.e., selectively for the first trial in any sequence). Can this activity be ascribed to factors such as level of arousal, general attention or the task difficulty? The following findings argue against this possibility. First, first-trial selectivity was much less frequent in the FEF. Second, the reaction times at individual saccades were unrelated to the magnitude of transition-selective activity. Finally, neither the reaction times nor the error rates for the first visual trials were different from those of other trials ($P > 0.05$). Consequently, other behavioral factors should be considered in trying to explain transition-selective activity. In the behavioral task used in this study, the monkeys learned six sequences extensively and were completely familiar with the task. Therefore it seems reasonable to assume that sequential saccade performances were based on information that had been acquired and stored, which we shall tentatively refer to as a “motor plan.” During trials guided by memory, the task performance seemed to be based on motor plans for individual sequences. After the completion of the final memory-guided trial, the monkey had to discard the current motor plan and then call for the next motor plan. It was at this particular point in the task performance that selective neuronal activity was observed only in the SEF. It is plausible, therefore that activity at the transition of sequences reflects the process of updating motor plans to organize the sequence of a subsequent saccade. This activity is likely to be the oculomotor equivalent of the activity observed in the presupplementary motor area, which is selective for the renewal of limb motor sequences (Shima et al. 1996).

**Relevance of the transition-selective activity to previous studies**

Some aspects of cellular activity in the medial cortical areas reported previously resemble the characteristic activities observed in the present study: learning-selective activity in the SEF (Chen and Wise 1995a), preference for a new behavioral set in the pre-SMA (Nakamura et al. 1998), and search-related activity in the anterior cingulate cortex (Procyk et al. 2000). Learning-selective activity was observed while monkeys learned the correct association of novel visual stimuli and the direction of saccades (Chen and Wise 1995a). Activity associated with preference for a new behavioral set was observed while subjects acquired novel visuo-skeletonmotor associations to construct a spatial pattern (Nakamura et al. 1998). Search-related activity was observed during the period in which monkeys were required to find the correct spatial-temporal pattern for combined eye-arm movements (Procyk et al. 2000). It is important to note that the activity in each of these reports was observed during the acquisition of novel associations between visual stimuli and motor responses through trial-and-error exploration. Although entirely new association was not required in Procyk’s task, the trial-and-error process was a necessary prerequisite for search-prefering cells to become active. In our study, by contrast, neither novel associations nor trial-and-error
processes were involved in the behavioral task. For both animals in the present study, all saccade sequences were sufficiently familiar to have become a behavioral routine through extensive training. In addition, the correct sequence was initially prompted by means of visual cues and was not acquired by searching. Taken together, we interpret these results to mean that the sequence-transition selective activity observed in the present study differs from the activities reported previously despite their apparent resemblance.

Methodological consideration and limitation of interpretation

The essence of the oculomotor task in this study was to select the direction of center-out saccades and perform three saccades in a correct sequence based on memorized information. In the process of behavioral training, care was taken to establish that the metrics of individual saccades and their latencies did not differ depending on the temporal sequence of saccades. In contrast to strictly controlled saccades to the three peripheral targets, no strict requirements were imposed for eye movements that brought the gaze back to the central FP. Instead, re-fixation movements often included two saccades with a corrective-saccade component or turn-around saccades accompanied by eye blinks. Thus quantitative analysis was made for the three center-out saccades but not for saccades at the time of re-fixation. We found that neuronal activity accompanying the re-fixation process was not apparent, as exemplified in Fig. 2, or highly variable. The above behavioral factors (nondemanding task requirements and variable metrics) seem to account for the paucity of neuronal activity during the period of re-fixation eye movements.

On the other hand, we should discuss a few issues with regard to limitation of interpretation of the present results due to the design of the behavioral task employed in this study. The first concerns the nonrandom nature of the six sequences of saccades. Among six sequences employed in this study, four sequences started with the UP target. Thus prior to the first target appearance in a new sequence, there was a 67% probability of UP target appearance. If the SEF has preferential access to probability signals, then the bias to the upward target could produce the increase in activity at the transition of sequences preceding the start of the first trial. In fact, Coe et al. (2002) showed that, using a free-choice saccade task, SEF activity preferentially reflected anticipatory bias of subsequent saccade-target. This interpretation, however, could not account for all the mechanisms underlying the transition-selective activity during ITI because the modulation of the activity at the transition was observed regardless of gaze direction during ITI, whereas anticipatory bias reported by Coe et al. was receptive-field bound. Accessibility of SEF neurons to probability signals per se, like midbrain dopamine neurons (Fiorillo et al. 2003), remains to be studied.

Second, we relied on three possible saccade directions (up, left, and right) to determine directional preference of neuronal activity. Considering the small number of directions to analyze the directional preference, it seems likely that the number of directionally tuned neurons was underrepresented, especially in the FEF. It is possible that the difference in the distribution between the two areas for the direction index (Fig. 3B, right) might actually be greater than that shown.

Hypothetical operation of neural elements in the SEF and FEF for orderly arrangement of three memorized saccades

We now propose a hypothesis to explain how the variety of neuronal activity observed in the SEF and FEF might be coordinated to provide the signals that are required to perform three saccades in an orderly manner. Let us consider the sequence U-L-L as an example (Fig. 9A). First, the subject needs to be informed that the sequence that is currently required is U-L-L; sequence-selective activity could serve to retrieve and carry such information. If the sequence information is combined with information about rank to indicate that the first saccade is required, then the information about saccade direction would be provided, in this case, an upward saccade. During this decoding process, the combination of information about direction and rank could signal that an upward saccade should be executed first (combination-selective activity). After the first saccade has been correctly performed, a signal specifying a leftward saccade could be provided by combining the sequence and rank (specifying the second saccade) signals. In this decoding process, the signal specifying a leftward saccade in the second rank of the sequence could be generated. Then after, a signal for the third (leftward) saccade could be generated and an analogous manner to complete the U-L-L-L sequence. Once one block of the U-L-L sequence has been accomplished, transition-selective activity could serve to discard the current sequence and prepare the next motor plan, thereby enabling a smooth and nonpersistent transition to the next sequence. We are aware that the processes illustrated in Fig. 9A might be too

FIG. 9. A diagram showing hypothetical operation of neural elements for sequential saccades and their distribution. A: hypothetical processes for the orderly specification of saccades, involving neural elements observed in the present study. An example is shown in which the correct order of 3 saccades is upward (U), leftward (L), and then leftward (L). 1st, 2nd, and 3rd refer to the saccade order. B: histograms showing the distribution (percent) of each category of neuronal activity observed in the SEF (left) and FEF (right).
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DISCLOSURES

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

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


