Cellular Activity in the Supplementary Eye Field During Sequential Performance of Multiple Saccades

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Received 22 April 2002; accepted in final form 8 August 2002

Isoda, Masaki and Jun Tanji. Cellular activity in the supplementary eye field during sequential performance of multiple saccades. J Neurophysiol 88: 3541–3545, 2002; 10.1152/jn.00299.2002. To investigate how single neurons in the supplementary eye field (SEF) participate in sequential performance of multiple saccades, we analyzed presaccadic activity while monkeys were performing three saccades in six different orders from memory. The saccades in each sequence were separated by a fixation period and initiated from the same fixation point with intervening return saccades. We found that the majority of the presaccadic activity of the SEF neurons differed significantly depending on the numerical position of saccades in each sequence (rank order). This rank-order selectivity was found in parallel with the selectivity for the sequence of three saccades. Our data suggest a role for SEF neurons in the coding of temporally ordered saccadic eye movements.

INTRODUCTION

When performing purposeful motor behavior, it is usually necessary to execute multiple movements in the correct temporal order. Studies on nonhuman primates have revealed that medial motor areas in the frontal cortex, i.e., the presupplementary (pre-SMA) and supplementary (SMA) motor areas, are crucial for the temporal organization of voluntary arm movements (Tanji 2001; Tanji and Shima 1994). With regard to oculomotor control regions, clinical reports (Gaymard et al. 1990; Muri et al. 1995) and functional brain imaging studies (Petit et al. 1996) on human subjects have pointed to the importance of the supplementary eye field (SEF) in the temporal control of saccades. However, the question of how single neurons in the SEF take part in the temporal organization of multiple saccades remains unanswered, since few single-neuron studies have been carried out. Very recently, Lu et al. (2002) recorded neurons in the SEF of monkeys performing an oculomotor sequence task. They found three major types of neuronal activity selective for 1) the saccade direction, 2) the combination of a saccade target and distractor, and 3) the sequence within which the target/distractor combination appeared. They reported, however, that the selectivity for the numerical order in each saccade sequence (rank-order selectivity) was not observed. For the temporal structuring of motor behavior, information on the rank order in a series of saccades is strategically important to be combined with information on the sequence itself. In fact, many neurons in the pre-SMA and SMA show preferential activity for a particular rank order during performance of sequential arm movements (Clower and Alexander 1998; Shima and Tanji 2000). Here we present evidence that the rank order, indeed, was reflected in the presaccadic activity of the majority of SEF neurons, apart from the selectivity for the sequence that was also observed in some SEF neurons.

METHODS

We trained two monkeys (Macaca fuscata) to perform three saccades in six different orders. The animals were cared for according to the Guide for the Care and Use of Laboratory Animals (National Institutes of Health). The monkeys sat in a primate chair with their heads restrained, facing a display monitor in which three red targets (top, left, and right) were illuminated. Each monkey was initially taught the correct sequence during a visually guided sequence task (Fig. 1A, left). Before each saccade, the monkey was required to fixate on a central, white fixation point (FP) for 1,450–1,600 ms. When the FP was extinguished (the GO signal), one of three targets turned white, thereby indicating a saccade target. If the animal acquired the target within 500 ms and maintained fixation for 350 ms, the target turned red, a high-pitch tone sounded, and the central FP was turned on again. The animal was then required to make a return saccade within 1,000 ms. Similarly, the monkeys fixated for 1,450–1,600 ms before the second and third saccade triggers. A series of three correct saccades was rewarded with a drop of juice after the third target fixation period was completed. During the visually guided sequence task, the animals were required to learn the correct sequence, after which the task was performed solely from memory (the memory-guided sequence task, Fig. 1A, right). In this situation, the correct target remained red when the FP was turned off. After the memory-guided sequence task was completed, flashing lights signaled both the end of the current sequence and the beginning of the next sequence. Thus a particular sequence of saccades was performed in blocks, each consisting of four trials of the visually guided sequence task and four trials of the memory-guided sequence task. Six different sequences were presented in a pseudorandom order (Fig. 1B). At least two blocks of trials for each sequence were included in a data file of recordings from individual cells.

We used conventional electrophysiological techniques for single-cell recordings (Matsuzaka et al. 1992). To localize the SEF, we applied intracortical microstimulation (ICMS, 10–50 cathodal pulses of 0.2-ms duration at 333 Hz in the range of 10–80 μA). Eye position...
was sampled at 200 Hz with an infrared monitor system. The recording sites were confirmed using MRI.

In this report, we focused on activity during the presaccadic period in the memory-guided sequence task. If the number of discharges during the presaccadic period (200–0 ms before saccade onset) was significantly different from that during the control period (the last 500 ms of the intertrial interval as a default time window) in at least one sequence, as assessed by the Mann–Whitney U test (\(P < 0.05\)), then the neuron was judged to be task related and was used for further analysis.

We performed a two-way ANOVA to determine the relationship of presaccadic activity to the forthcoming saccade direction and the rank order (first, second, or third in each sequence). For the purpose of this analysis, we used only the activity of the rightward (R) and leftward saccades (L), because the upward saccade (U) was not included for the second and third rank of each sequence, as shown in Fig. 1B. Note that the exclusion of the data for upward saccades from this analysis produces an underestimation of the effect of direction and rank order.

To determine the relationship between presaccadic activity and the sequence, we selected five groups of data sets in which the direction and rank were matched (Fig. 1B). This was due to the fact that the rank order and direction of saccades were critical factors in determining the magnitude of presaccadic activity for the majority of the SEF neurons, as described below. We performed a one-way ANOVA within each group to determine the relationship between presaccadic activity and sequence. The significance level for ANOVA was set at \(P < 0.01\) and was followed by the Fisher LSD posthoc test for multiple comparisons (\(P < 0.05\)).

**RESULTS**

The SEF was defined as the contiguous cortical area whose boundary was not more than 1 mm from the low-threshold (<50 μA) saccade-evoking sites. Using these criteria, the SEF was located typically in a small region of the dorsomedial convexity of the frontal lobe and did not include penetration sites that evoked limb movements. The localization of the SEF in this study was in accordance with previous reports (Mu-
We analyzed the presaccadic activity of 300 task-related neurons (73.7% of all neurons) that were recorded from three hemispheres of two monkeys as they performed the memory-guided sequence task. Apart from a group of neurons that showed only a directional selectivity (45/300, 15%), we observed three classes of cellular activity with apparent relevance to the sequencing of saccades:

1) activity that is selective only for the rank order,
2) activity that is selective both for the rank order and the direction, and
3) activity that is selective for the sequence.

These three types of cellular activity are the subject of this report.

A first type of activity was observed in 55 neurons (18.3% of task-related neurons). Figure 2 shows an example of cells in which presaccadic activity depends only on the rank order. The discharge rate was particularly high for the third saccade, regardless of its direction. During the visually guided sequence of saccades, this group of cells also showed rank-order selectivity and were spatially nonselective. The detailed account of subtle differences in activity during the visually guided versus memory-guided tasks will be the subject of a separate paper.

A second type of activity was observed in 112 neurons (37.3% of task-related neurons) in which activity differed significantly depending both on the rank order and the direction, and on the interaction between these two factors. In an example shown in Fig. 3A, presaccadic activity was observed before the rightward saccade, but the magnitude was particularly high when the animal performed the third saccade. This neuron was much less active for the first and second rightward saccades. Table 1 shows the distribution of the rank-order effect.

A third type of activity was observed in 68 neurons (22.8% of task-related neurons) in which presaccadic activity differed significantly depending on the sequence of the three saccades. An example cell of this type is shown in Fig. 3B. This neuron was active before the first saccade when the sequence was U – R – R (Fig. 3B, right). It seems unlikely that the activity of this neuron encodes a presaccadic signal for the upward saccade or a signal for the first saccade, because the cell was not active when the animal performed the U – L – L sequence (Fig. 3B, left). At the first trial of the visually guided sequence task, this type of cells did not show the sequence-selective activity, because the monkeys did not know the correct sequence to be performed. As the animal repeated the same sequence under visual guidance, activity developed gradually. Therefore this type of SEF neuron appears to signal from memory the specific order of multiple saccades rather than an individual forthcoming saccade.

To examine whether the activity that is selective for the rank order and the sequence was related to differences in the way the saccade was executed, we conducted analysis of covariance (ANCOVA) with saccade parameters (reaction times, movement times, peak saccade velocity, and saccade trajectory) as covariates. ANCOVA revealed that the saccade parameters did not co-vary with either the rank order or the sequence in 97% of cases.
DISCUSSION

In the present study, we found three types of cellular activity in the SEF that were not simply related to the direction of individual forthcoming saccades but seemed relevant to the sequential organization of multiple saccades. These activity changes were not likely to be ascribable to any aspect of the saccade itself (ANOVA).

Using behavioral tasks involving arm or combined eye–arm movements, rank-order effects on movement-related activity have been described in the SMA and pre-SMA (Clower and Alexander 1998; Shima and Tanji 2000), the anterior cingulate cortex (Procyk et al. 2000), the caudate nucleus (Kermadi and Joseph 1995), and the pallidum (Mushiake and Strick 1995). Here we show that the SEF also encodes the rank order of sequence components during a multiple saccade task. Some neurons appear to signal the serial positions of saccades within a sequence (e.g., the 3rd saccade irrespective of its direction). Other neurons appear to signal the specific direction of saccades and their serial position (e.g., the rightward saccade as the 3rd saccade). Here, one might argue that the rank-order selective neurons may all be defined as sequence selective, if the definition of sequence selectivity is expanded to include more than one sequence. Cells in which activity depends both on the rank and the direction may be explained by such interpretation. However, cells that are selective only for the rank (the first type in our study) cannot be interpreted as being sequence selective, because these cells did not show the difference in activity depending on the sequence. This group of cells exhibited comparable magnitude of activity in all sequences. Therefore these cells are sequence nonselective and yet rank-order selective.

Lu et al. (2002) were the first to record neuronal activity in the SEF during an oculomotor sequence task. They reported that many SEF neurons showed activity changes depending on the sequence context (their “S activity”). The existence of sequence-selective activity in their report is basically in accord with our present findings on sequence-selective neurons, albeit with some differences in statistical definition. What may appear as a discrepancy between their report and ours is the occurrence of rank-order selectivity in the SEF. They reported that they did not find “such numerical order selective activity as reported in the SMA and pre-SMA.” On the other hand, they also discussed as follows: “The selectivity of S (sequence-selective) activity could depend on the numerical order. S activity could be generated if C (combination of target/distractor) activity is conditioned by the numerical order selective activity, except for some cases in which this explanation is unlikely.” Thus the discrepancy may not be fundamental. More interestingly, the two reports may be interpreted from a different perspective. The behavioral tasks employed in Lu’s study and in ours differ in significant ways. In Lu’s task, the spatial patterns of visual signals (for a saccade target and a distractor) changed from trial to trial, whereas in our task the spatial patterns remained the same in a trial block. Thus the spatial pattern (for the combination of target/distractor) was crucially important in Lu’s task, whereas the monkey may have relied heavily on the rank order as an essential cue for the correct performance of our task. The behavioral differences could

<table>
<thead>
<tr>
<th>Classification of rank-order selective neurons</th>
<th>Cell Count</th>
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<tbody>
<tr>
<td>Type A (M1 &gt; M2 = M3)</td>
<td></td>
</tr>
<tr>
<td>1st &gt; 2nd = 3rd</td>
<td>26</td>
</tr>
<tr>
<td>2nd &gt; 1st = 3rd</td>
<td>24</td>
</tr>
<tr>
<td>3rd &gt; 1st = 2nd</td>
<td>32</td>
</tr>
<tr>
<td>Type B (M1 = M2 &gt; M3)</td>
<td></td>
</tr>
<tr>
<td>1st = 2nd &gt; 3rd</td>
<td>23</td>
</tr>
<tr>
<td>1st = 3rd &gt; 2nd</td>
<td>5</td>
</tr>
<tr>
<td>2nd = 3rd &gt; 1st</td>
<td>9</td>
</tr>
<tr>
<td>Type C (M1 &gt; M2 &gt; M3)</td>
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</tr>
<tr>
<td>1st &gt; 2nd &gt; 3rd</td>
<td>6</td>
</tr>
<tr>
<td>1st &gt; 3rd &gt; 2nd</td>
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<tr>
<td>2nd &gt; 1st &gt; 3rd</td>
<td>1</td>
</tr>
<tr>
<td>2nd &gt; 3rd &gt; 1st</td>
<td>3</td>
</tr>
<tr>
<td>3rd &gt; 1st &gt; 2nd</td>
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<td>3rd &gt; 2nd &gt; 1st</td>
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<tr>
<td>Unclassified</td>
<td>26</td>
</tr>
<tr>
<td>Total</td>
<td>167</td>
</tr>
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M1 for the highest, M2 for the median, and M3 for the lowest mean firing rate. Unclassified corresponds to neurons that could not be classified into categories above, where M1 > M3, M1 = M2, and M2 = M3.
explain divergent properties of neuronal activity in the two reports. If this hypothesis of relative importance of the cues is correct, then the two reports suggest plastic changes of activity in the SEF depending on behavioral conditions, endorsing previous reports on functional plasticity in cortical motor areas (Aizawa et al. 1993; Chen and Wise 1996; Mann et al. 1988).

Finally, we propose that the cellular activity we found in this study provides a means by which the SEF is involved in the temporal sequencing of multiple saccades. To organize multiple saccades in a specific temporal order from memory, information about the rank order in a series of saccades is strategically important to be combined with the information about the sequence itself. Thus it is important to make a clear distinction between the information about the rank order and the sequence, because each represents a different aspect of information necessary for the temporal structuring of motor behavior.

We thank M. Kurama and Y. Takahashi for technical assistance. This work was supported in part 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, Japan.

REFERENCES