Monkey Prefrontal Neuronal Activity Coding the Forthcoming Saccade in an Oculomotor Delayed Matching-to-Sample Task

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Hasegawa, Ryohei, Toshiyuki Sawaguchi, and Kisou Kubota. Monkey prefrontal neuronal activity coding the forthcoming saccade in an oculomotor delayed matching-to-sample task. J. Neurophysiol. 79: 322–333, 1998. To determine the role of the dorsolateral prefrontal cortex (PFC) in the selection of memory-guided saccadic eye movements, we recorded the activities of PFC neurons while macaque monkeys performed an oculomotor delayed matching-to-sample task. The task was designed to dissociate motor factors from visual factors in the selection and retention of the direction of the forthcoming saccade during delay periods after the visual cue but before the GO signal was presented. While the monkey fixated on a central fixation spot (FX period, 1 s), a sample cue (1 of 4 geometric figures) and a matching cue composed of two geometric figures were presented in succession (SC and MC periods, respectively, 0.5 s) with a brief delay (D1 period, 1 or 1.5 s). After another delay (D2 period, 1.5 s), the monkey made a saccade (GO period, <0.5 s) toward one of four locations (the goal) that had been indicated by the combination of the sample and matching cues in the MC period. We recorded the activities of 224 neurons in the periprincipal sulcal area of 3 hemispheres of 2 monkeys. Sixty-five neurons (29%) showed a significant increase in activity during the D2 period. Some of these also responded during other phases of the task (SC period, n=32; D1, 22; MC, 53; GO, 47). Some of the activity during the D2 (52/65, 80%) and GO (40/47, 85%) periods was associated with the direction of the forthcoming saccade (“direction selective”). Although most MC-period activities of D2 neurons were direction selective (38/53, 73%), a fraction of them (14/38) was also affected by both saccade direction and matching cue pattern. To compare quantitatively the contribution of motor (saccade direction) and visual (matching-cue pattern) factors to the activity of D2 neurons, we calculated directional and visual dependency indices (DDI and VDI) for each of the three periods (MC, D2, and GO). In both the D2 and GO periods, D2 neurons with high DDI values and low VDI values predominated. In the MC period, however, there was no significant difference between the distributions of DDI and VDI values. These findings suggest that PFC neurons store the direction of memory-guided saccades during a delay period before eye movement and that the same neurons may be involved in the decision-making process that underlies the selection of the saccade direction during the MC period.

INTRODUCTION

Activities of neurons in the primate prefrontal cortex (PFC) are related to memory-guided behavior (cf. Funahashi and Kubota 1995; Goldman-Rakic 1995). For example, in delayed-response tasks, which require spatial short-term memory (Butters et al. 1971; Jacobsen 1936), PFC neurons show sustained activity during the delay period when a monkey remembers the spatial location of a visual cue that guides an appropriate motor response (Fuster 1973; Fuster and Alexander 1971; Kubota et al. 1974; Niki 1974). These studies have revealed that delay neurons often show differential activity for cue/goal location. Such activities are considered to hold spatial information for a while. Furthermore, recent studies have shown the PFC also plays a role in the retention of nonspatial information such as form or color of object (Miller et al. 1996; Rao et al. 1997; Wilson et al. 1993). These studies have established the general idea that the PFC provides the neural basis of both spatial and nonspatial working memory, which is a kind of short-term memory necessary in cognitive tasks (Baddeley 1992).

In addition to its role in memory, the PFC appears to be related to other cognitive or behavioral aspects such as the visual perception or recognition (Boch and Goldberg 1989; Funahashi et al. 1990; Fuster et al. 1982; Ito 1982; Kubota et al. 1974; Mikami et al. 1982; Pigarev et al. 1979; Rosen-kilde et al. 1981; Suzuki and Azuma 1983; Yamatani et al. 1990) and the execution of directional movement (Kojima et al. 1981; Kojima and Goldman-Rakic 1982; Kubota et al. 1974; Kubota and Funahashi 1982; Niki 1974; Niki and Watanabe 1976). Furthermore, visual responses have often been reported to differ depending on the type of motor response made by the monkey at the end of the trial (Komatsu 1982; Quintana et al. 1988; Sagakami and Niki 1994; Wata-nabe 1986; Yajeya et al. 1988). These results suggest that the PFC is involved not only in memory processes but also in more global processes necessary for memory-guided behavior including decision-making processes that underlie the selection of forthcoming movements.

We hypothesized that neurons of the dorsolateral PFC play a role in both determining and retaining the direction of movements guided by visual memory. To test this hypothesis, we adopted an oculomotor paradigm, because of its advantage in behavioral control and monitoring. In delayed saccade tasks, many PFC neurons show directional responses analogous to those observed in manual delayed-response paradigms (Funahashi et al. 1989). This directional activity has been reported during delay, cue, and response periods (Boch and Goldberg 1989; Funahashi et al. 1989–1991; Joseph and Barone 1987; Kojima 1980). Furthermore, PFC neurons respond to peripheral visual stimuli that guide the direction of the saccade (Boch and Goldberg 1989; Bruce and Goldberg 1985). These reports suggest that the PFC plays a role in the initiation of voluntary eye movements, in addition to its role in the initiation of manual movements. We added to the conventional delayed matching paradigm a second delay between the presentation of the comparison.
stimuli and the choice response. Compared with the conventional delayed matching-to-sample task, our task can examine neural components of the decision process, because the motor instruction period was temporally separated from the execution process by introduction of the second delay.

We report here that the activity of PFC neurons during a presaccadic delay period depends on the direction of the forthcoming saccade and arises as early as the cue period in which decision-making for movement selection may occur. Preliminary reports of some of these data have appeared elsewhere (Hasegawa et al., 1994, 1995).

**Methods**

**Subjects and behavioral procedures**

Two young male rhesus monkeys (*Macaca mulatta, 3–4 yr old, 4–5 kg, KU and SE*) were used. The animals were housed in individual cages and supplied with food ad libitum. On training days, the monkeys’ water intake was restricted, and they instead obtained water as a reward for task performance. At least once a week, they received water ad libitum. The monkeys were treated in accordance with the ‘‘Guidelines for the Care and Use of Laboratory Animals’’ of the National Institutes of Health (1985) and the ‘‘Guide for Care and Use of Laboratory Primates’’ published by our Institute (1986). For the first 2 wk, the monkeys were trained to sit in a primate chair and to perform a simple lever-pressing task. Preliminary surgery was then performed under pentobarbital sodium anesthesia (25 mg/kg iv). During this surgery, a portion of the skull was exposed, and two head-holding devices (stainless steel pipes, 8 mm ID) were implanted using stainless steel bolts (1.5 mm diam). All pipes and bolts were completely surrounded by dental acrylic cement.

After this preliminary surgery and training, the monkeys were trained to perform an oculomotor delayed matching-to-sample (ODM) task. The monkey sat in a primate chair with its head fixed, facing a 21-in. monitor (PC-TV471; NEC, Tokyo) positioned 42 cm from the eyes. Eye position was monitored by an infrared eye-camera system (R-21-C-AC and R-221-C-AS; RMS, Hirokaki, Japan) with a 4-ms sampling rate. Each trial in the ODM task consisted of seven periods: fixation, sample cue, first delay, matching cue, second delay, response, and reward or time out after errors. The task (Fig. 1A) was controlled by a personal computer (PC9801FA; NEC, Tokyo).

Each trial began when the monkey pressed a hold-lever with the hand contralateral to the recorded hemisphere. The other hand was restrained. The monkey had to keep the lever down during the entire trial. After a waiting period of 0.8 s, a fixation spot (white square, 1° by 1°) appeared in the center of the screen. After the monkey had fixated on this spot for 1 s (FX period), a sample cue (within 3° diam) was presented for 0.5 s over the fixation spot (SC period). The sample cue was one of four colored figures: green cross, green triangle, red cross, or red triangle. The SC period was followed by the first delay period (D1 period, 1.5 s for *monkey KU* or 1 s for *monkey SE*). The sample cue was extinguished and the fixation spot reappeared. Next, the matching cue (MC) was presented for 0.5 s. The matching cue consisted of two different figures that appeared at two of four locations (15° above and 15° below, or 15° to the right and 15° to the left of the fixation spot). These stimuli were selected from among the four figures used as sample cues. Of the matching cue, one figure (‘‘target’’) was always identical to the sample, whereas the other (‘‘distractor’’) differed from the sample in both color and shape. The MC period was followed by the second delay period (D2 period, 1.5 s); the matching cue was extinguished while the fixation spot remained on the monitor. At the onset of the response period (GO period), the fixation spot was extinguished and as the go signal four white squares (1° by 1°) appeared simultaneously at peripheral locations (15° above, 15° below, 15° to the right, 15° to the left of the extinguished fixation spot). The correct response was to make a saccade eye movement to the location where the target had been presented during the MC period. To be counted as correct, the eye movement had to fall inside a circular window (6° diam) around the target within 0.5 s of the onset of the GO period and be maintained at that location for a while (0.5 s for *monkey KU* or 0.25 s for *monkey SE*). A correct response was rewarded with a drop of water (~0.1 ml). If the monkey failed to maintain fixation within the 6°-diam window around the fixation spot before the GO period, the trial was aborted without reward, and the monkey had to press the lever again to initiate a new trial. After daily training for 6 mo, the monkeys reached a criterion of 80% correct responses for >3 consecutive days, and kept performing 500–1,500 trials in each day. There were 16 possible pattern combinations of the 3 figures used as sample and matching cue (Fig. 1B). On each trial, the combination was chosen pseudorandomly so that the monkey did not know the correct saccade direction until the MC period.

We examined performance accuracy during recording trials for 30 representative sessions, which were randomly sampled from initial to last recording session (averaged trial number was 591 ± 174, mean ± SD). Oculomotor performances of the two monkeys were fairly stable throughout the recording sessions. The mean latencies for saccadic eye movement from the onset of the GO period were 163 ± 9.6 ms for *KU* and 194 ± 17.7 ms for *SE* (mean ± SD across sessions). The endpoint of the saccade fell around the center of the target (a 15° saccade from the fixation point to the center of the matching cue) with 2.8° SD for *monkey KU* and 1.6° SD for *monkey SE*.

In some trials, the monkeys made an error by a fixation break before the GO period or a wrong choice for saccade location in the GO period. Mean correct percent was 86 ± 8% for *monkey KU* and 77 ± 15% for *monkey SE*. Most incorrect trials (~60% of all errors for both monkeys) were due to a saccade toward the distractor in the GO period. On such trials, there was no tendency for stimulus or direction preference. The remaining incorrect trials were due to interruption of fixation of the central spot before the GO period. The chosen figure was correct for more than one-half of the errors produced by breaking the fixation.

**Recording of single neuron activity**

Surgery was performed under pentobarbital sodium anesthesia (25 mg/kg iv) to implant a cylinder for electrophysiological recording. An oval opening (20 ± 5 mm) was made in the skull covering the dorsolateral PFC to expose the dura, and an oval stainless steel cylinder (20 × 40 mm) was fixed on the skull with dental acrylic cement. During recording sessions, the activity of single neurons was recorded with glass-coated elygol microelectrodes (1–5 MΩ measured at 60 Hz) (Suzuki and Azuma 1979) while the monkeys performed the ODM task. Electrodes were advanced vertically to the cortical surface, using a pulse motor-driven micromanipulator (MO-81; Narishige, Tokyo). A plastic grid with numerous small holes (0.7 mm ID, 1.5 mm apart; Nakazawa, Tokyo) was attached to the cylinder to provide a coordinate frame for vertical electrode penetration (Crist et al. 1988). The electrical signals were amplified by a custom-made amplifier and processed by a window discriminator (DIS-1; BAK Electronics, Germantown, MD), the output of which was stored in a data-collection computer (PC9821AS; NEC, Tokyo) with a 1-ms sampling rate. Rasters and histograms of neuronal activity were generated online. For each neuron, an average of 5–10 trials was performed for each of the 16 combinations of the figure presented as sample and matching cue (see Fig. 1B). More than 80 correct trials (>20 min) were usually required to collect data for each neuron. Event
FIG. 1. Oculomotor delayed matching-to-sample (ODM) task. A: temporal sequence of a representative trial. While the monkey fixated on the central fixation spot (FX period), a single sample cue and the matching cue composed of 2 figures were presented successively (SC and MC periods, respectively) with a short interval period between them (D1 period). After another delay period (D2 period), the monkey made a saccade (in the GO period) toward the location that had been indicated by the relation between the sample and matching cue in the MC period. The monkey remembered the direction of the required saccade during the D2 period. The duration of each period is indicated under the panel. B: the 16 possible combinations of sample and matching cue figures. As shown, different matching figure combinations could indicate the same saccade direction, and the identical matching figure could indicate different saccade directions. Arrows represent saccade directions. The circled numbers (1–8) and letters (L, R, U, or D) represent the pattern of the matching cue and the indicated saccade direction.

signals (changes of task period), and the horizontal and vertical coordinates of eye position were digitized by the data-collection computer and stored on magneto-optical (MO) diskettes with an MO disk drive (MK230E; Mitsubishi, Tokyo). Analog data were recorded on DAT tape using a digital audio tape recorder (PC-108 M; Sony, Tokyo) and later digitized for off-line analyses. In total, we collected and analyzed the responses of 224 PFC neurons. In this report, we concentrate on the results of the 65 neurons that were active during the D2 period, because sustained premovement activity has been documented as a characteristic aspect of the PFC, especially in relation to memory-guided behavior.

To estimate the physiological location of the frontal eye field (FEF) (cf., Bruce et al. 1985), we applied intracortical microstimulation (ICMS, a train of 11 cathodal pulses of 300-μs duration at a frequency of 333 Hz) through the recording electrodes using a constant-current stimulator (SEN-7203, Nihon Kohden, Tokyo). This was done at the end of the daily recording session, particularly for recording sites in the prearcuate area. While the monkey fixated on a spot in the center of the monitor, stimulation was applied at 0.5–1 Hz at various depths from the surface of the cortex (from 0 to 10,000 μm, in steps of 250 μm). When eye movements were induced at a current intensity of <40 μA, the site was considered to be within the FEF. Some neuronal activities related to the task were recorded in the FEF, but they are not documented here, because data obtained from the FEF were too small to compare with those from the periprincipal sulcal area. At sites where task-related activity was observed, marks were made with electrolytic lesions (5–10 μA of direct cathodal current for 30 s) to confirm the recording site histologically.

Data analysis

We generated rasters and averaged histograms (binwidth, 20 ms) of neuronal activity, aligned with respect to each task event (onset and offset of sample and matching cue, onset of GO signal, onset and end of saccadic eye movement, and reward presentation). To detect significant changes in activity during the D2 period, we compared the distribution of spike counts from histogram bins in the D2 period to the distribution in a 0.5-s control period that immediately preceded the onset of the sample cue. If the two distributions were significantly different (Mann-Whitney U test, \(P < 0.05\)), the change in activity of the neuron was considered to be significant during the D2 period. Because the D2 period occurs after presentation of the matching cue and before movement execution, D2-period activity may be influenced by at least two factors: the matching-cue pattern and the correct or anticipated saccade direction. Hence we performed a two-factor analysis of variance (ANOVA) to detect the involvement of motor and/or visual factors in the activity for individual neurons. On the basis of the ANOVA results, we categorized D2 neurons as “directional,” “visuo-directional,” “visual,” or “nonselective.” For directional neurons, there is a statistically significant \((P < 0.05)\) effect for saccade
Histology

Table 1 summarizes the changes in the activities of 65 saline and 10% formalin. The brains were removed, photographed, and then sectioned coronally into 100-μm serial sections. The sections were stained with conventional cresyl violet.

The sites, at which D2 neurons were recorded in the PFC, were projected onto the cortical surface of unfolded prefrontal cortical maps (open circle in Fig. 2), which were drawn as in previous studies (Arikuni et al. 1988; Kubota 1996). To unfold the depths of the principal sulcus, the cortical surface between medial and lateral banks of the principal sulcus was extended medially and laterally in each section, based on lateral and medial surface points and the bottom point of the principal sulcus. Most of the sites where D2 neurons were recorded from the two monkeys (KU: left, 39; right, 20; SE: left, 6) were located in the caudal half of the principal sulcal area, which was identified cytoarchitecturally as areas 46 and 8 (Walker 1940). At some sites in the prearcuate area, saccade eye movement was evoked by ICMS (cross symbol in Fig. 2). These sites considered to be the FEF (shaded area in Fig. 2). As shown, D2-period activities recorded from both monkeys were not in the FEF.

RESULTS

General

While the monkeys were performing the ODM task, the activities of a total of 224 neurons were sampled from the dorsolateral prefrontal cortex (PFC), especially the periprincipal sulcal area (monkey KU, n = 205; monkey SE, n = 19). Of these 224 neurons, 145 showed activities (monkey KU, n = 128; monkey SE, n = 17) that were related to one or more events of the task (MC, D2, or GO periods). And, among this set of 145 task-related neurons, 65 (45%; monkey KU, n = 59; monkey SE, n = 6) showed a significant increase in activity during the D2 period (“D2-period activity”). Most of the D2 neurons (n = 59, 91%) also showed significant activity in the task periods immediately before or after the delay period (MC and D2 periods: 12 neurons, 18%; D2 and GO periods: 6 neurons, 9%; MC, D2, and GO: 41 neurons, 63%). Although many D2 neurons also showed a statistically significant increase in activity during the SC (49%, 32/65) and/or D1 (34%, 22/65) periods, their activity changes relative to the control period were usually slight and did not depend on the physical properties of the sample cue; only a few neurons showed a significant change in activity specific to the sample cue during the SC (3/32) and D1 (2/22) periods. The remaining 80 task-related neurons showed significant activity only in the MC period (n = 33), only in the GO period (n = 16), or in both the MC and GO periods (n = 31).

Activity of PFC neurons were categorized as “directional selective” or “directional nonselective.” The former was further categorized into directional or visuo-directional, whereas the latter was categorized as visual or nonselective. These activity types were determined by which factor (motor or visual) was related to the activity. Directional or visual means that activities were affected only by the motor or visual factor, respectively. Visuo-directional activities were affected both by motor and visual factors.

Table 1 summarizes the changes in the activities of 65 D2 neurons. During the D2 period, most D2 neurons (total, 58/65, 89%) changed their activities depending on the forthcoming saccade direction (directional selective). Of them, 52 activities were affected by saccade direction (directional), whereas only 6 were affected both by saccade direc-
FIG. 2. Distribution of the recording sites of the prefrontal neurons active during the D2 period of the oculomotor delayed matching-to-sample task of 2 monkeys (KU and SE). The principal sulcus (PS) was opened as described previously (Arikuni et al. 1988). Open circles represent the sites in which D2 neurons were recorded. Cross symbols indicate a site where intracortical microstimulation (ICMS) evoked an eye movement. Shaded area is the estimated frontal eye field from ICMS. PS, principal sulcus; AS, arcuate sulcus.

TABLE 1. Characteristics of D2 neurons, significantly activated during MC, D2, and/or GO periods for monkeys KU and SE

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<th>MC</th>
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<th>GO</th>
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<tr>
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<td>KU (L)</td>
<td>KU (R)</td>
<td>SE (L)</td>
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<tr>
<td>Directional selective</td>
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<td>12</td>
<td>2</td>
</tr>
<tr>
<td>Directional</td>
<td>13</td>
<td>10</td>
<td>2</td>
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<tr>
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<tr>
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<tr>
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<td>4</td>
<td>1</td>
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<tr>
<td>Total</td>
<td>33</td>
<td>17</td>
<td>3</td>
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Numbers in parentheses are percentages. D2, 2nd delay period; MC, matching cue; L, left hemisphere; R, right hemisphere.
Examples of D2-period activity

We analyzed PFC neurons during the ODM task for all 16 possible combinations of 4 sample cues and 4 horizontal or vertical locations of the matching-cue figures (see displays in Fig. 1B). Figure 3 shows an example of the activity of a PFC neuron that showed higher activity during the D2 period. This neuron was also activated in the SC, D1, MC, and GO periods. In the SC period, in which the sample cue was presented at the fixation point, a slight increase in activity was observed compared with the control period (7.6 spikes/s). However, the mean discharge rates in the SC period for the four sample cue patterns (in reference to Fig. 1B: 1L, 4R, 5U, and 8D, green cross, 12.3 spikes/s; 2L, 3R, 6U, and 7D, green triangle, 10.2 spikes/s; 2R, 3L, 6D, and 7U, red cross, 13.1 spikes/s; 1R, 4L, 5D, and 8U, red triangle, 13.7 spikes/s) were not significantly different. We referred to such an increase in activity, which was independent of the physical features of the stimuli, as nonselective. Similar nonselective activity was observed in the subsequent D1 period in which the fixation spot was replaced by the sample (green triangle, 9.4 spikes/s; green triangle, 8.5 spikes/s; red cross, 10.5 spikes/s; red triangle, 10.9 spikes/s). During the MC period, the activity increased considerably at ~100 ms after the onset of the matching cue for downward trials (5D, 6D, 7D, and 8D) and was followed by sustained activity throughout the subsequent D2 period while the monkey fixated on the central spot. This neuron showed a significant increase in activity, with a maximal rate in downward trials throughout the D2 period. This sustained activity lasted until the GO period, i.e., until 20 ms after the onset of the saccade, which was made 160 ms after the onset of the GO signal. There was no increase in activity for upward trials (5U, 6U, 7U, and 8U). However, some increases were observed for leftward and rightward trials during the D2 period, although their rates were not as high as those in downward trials. Thus this neuron maintained high activity from the MC to the GO period that depended on the direction of the forthcoming saccade or on the location of the target.

Figure 4 shows the activity of another PFC neuron, which was influenced not only by the anticipated saccade direction but also by the pattern of the matching cue. This neuron showed a slight increase in all conditions during the D1 period (5.4–8.1 spikes/s) compared with the control fixation period. After such a nonselective activation, this neuron responded more strongly to a particular pattern of the matching cue (1R) and sustained this activity during the D2 period. Responses to three other patterns of the matching cue (2R, 3R, and 4R), which instructed the monkey to saccade in the same rightward direction, were smaller than those to 1R. During the D2 period, the difference in the magnitudes

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**FIG. 3.** Example of the activity of a prefrontal neuron during the ODM task. A raster display and averaged histograms of the spikes are shown for all 16 combinations of sample and matching figures. There were 8 horizontal combinations, consisting of 4 patterns for the matching cue (1–4) by 2 directions for the saccade (L and R), and 8 vertical combinations, consisting of 4 patterns (5–8) by 2 directions (U and D). This neuron, recorded in the left hemisphere, showed sustained activity before downward saccades (5D, 6D, 7D, and 8D) throughout the D2 period. SC, sample cue period; D1, 1st delay period; MC, matching cue period; D2, 2nd delay period; GO, response period.
FIG. 4. Prefrontal cortex (PFC) neuronal activity with visual influence in the MC period. This neuron, recorded in the left hemisphere, showed transient activity during the MC period for some combinations of sample and matching figures. This activity was sustained during the D2 period after presentation of matching cues that indicated a rightward saccade (1R, 2R, 3R, and 4R). Abbreviations are the same as those in Fig. 3.

of the activities became smaller than that in the MC period. This neuron also showed a similar response to matching cue 1L, which had the same pattern as 1R but instructed a saccade in the opposite direction. After presenting the 1L stimulus, the activity was smaller than that after 1R. This direction-dependent D2-period activity was also observed between upward (5U–8U) and downward (5D–8D) trials. Thus, in this neuron, D2-period activity mainly depended on the subsequent saccade direction, while MC-period activity was more affected by the pattern of the matching cue.

Quantitative analysis of D2 neurons

To reveal the relative involvement of motor and/or visual factors, we performed a quantitative analysis on the effects of differences in the physical properties of the matching-cue pattern and saccade direction on neuronal activity. Typical changes of the two neurons illustrated in Figs. 3 and 4 are shown in Figs. 5 and 6. Thus Fig. 5A compares the mean discharge rates in the MC, D2, and GO periods for the PFC neuron illustrated in Fig. 3. During the D2 period, the average discharge rates in downward trials (19.8–24.0 spikes/s) were significantly [F(1, 62) = 251.5, P < 0.01] higher than those in upward trials (8.0–11.5 spikes/s), but the effects of the matching-cue pattern were not significant. Thus the D2-period activity of this neuron was considered directional. We designed this kind of activity (i.e., coding forthcoming saccade direction) as ‘directional activity.’ Similar results were obtained in 52 D2 neurons.

In this neuron, MC- and GO-period activities were also directional, but some MC-period activities depended on the matching-cue pattern. Figure 6A shows the effect of the matching-cue pattern on the MC-period activity of the neuron illustrated in Fig. 4. Although the activity of this neuron was affected by the forthcoming saccade direction in the MC period [F(1,48) = 20.4; P < 0.01], the magnitude of the MC-period activity was also significantly different for the matching cue [F(3,48) = 3.9; P < 0.05]. Thus both the matching-cue pattern and the forthcoming saccade direction were significant factors in MC-period activity. And therefore we designated such activities as visuo-directional.

Of 53 D2 neurons with MC-period activity, 14 showed visuo-directional activity, although 25 showed directional activity in this period, as this neuron.

Distribution of the maximal direction

Because the activities of most D2 neurons depended on the direction of the forthcoming saccade, we determined for each neuron the directions that showed the highest activation. We averaged discharge rates for each direction regard-
we calculated in each neuron the values of the directional and the visual pattern of the matching cue. For this purpose, paired the effects of the direction of the forthcoming saccade and the visual pattern of the matching cue. We compared directional and visual dependency indexes to determine how much D2 neurons were influenced by the direction of the matching cue (\textit{i.e.,} direction only) and the visual pattern of the matching cue. These indexes range from 0 to 1. When the Max (maximal) direction and the Opposite values have similar magnitudes, the DDI value is close to zero. When the Max direction is twice the Opposite value, the DDI value is 0.33.

When the Max direction is 3 times the Opposite value, the DDI value is 0.5. The same relationships apply to the VDI value. Figure 7 shows the distributions of the two indexes for all of the D2 neurons (\(n = 65\)), where VDI is plotted against DDI. For MC-period activity, plots were scattered along a 45° slope. Although 60% of the plots (32/53) were below the 45° slope, there was no significant difference in the number of plots above and below this line \(\chi^2 = 2.28, \ P > 0.05\), not significant]. In contrast, most of the plots for D2-period activity (75%, 49/65) were below the 45° slope \(\chi^2 = 16.75, \ P < 0.01\), indicating that DDI was higher than VDI for D2-period activity in most D2 neurons. This tendency was highly significant in the GO period; i.e., >80% of the plots (83%, 39/47) were below the 45° slope \(\chi^2 = 33.72, \ P < 0.01\). Thus DDI values significantly increased as the trial proceeded. In Fig. 8A, distributions of DDI for MC- and D2-period activities are illustrated for D2 neurons with MC-period activity (\(n = 53\)). As shown by distributions and medians (arrows), DDI was significantly greater in the D2 period (0.28 ± 0.16; median, 0.24) than in the MC period (0.22 ± 0.13; median, 0.19; \(t = 2.47, \ P < 0.05\), paired \(t\)-test). Figure 8B shows the distributions of DDI for D2- and GO-period activities of D2 neurons with GO-period activity (\(n = 47\)). DDI was greater in the GO period (0.37 ± 0.20; median, 0.32) than in the D2 period (0.28 ± 0.16; median, 0.23) \(t = 3.24, \ P < 0.01\), paired \(t\)-test]. Thus DDI gradually increased from the MC period to the GO period.

Temporal change of D2-period activity

As shown above, most of the D2-period activity continues from the MC period to the GO period. To examine the temporal change of D2 neurons in the ODM task, we summed 45 directional D2 neuron activities with a DDI value >0.2. These activities were averaged with a 100-ms timescale.

Comparison of directional and visual dependency indexes

To examine what kind of information the PFC neurons coded from visual instruction until motor execution, we compared the effects of the direction of the forthcoming saccade and the visual pattern of the matching cue. For this purpose, we calculated in each neuron the values of the directional dependency index (DDI) and visual dependency index (VDI) for MC-, D2-, and GO-period activities to determine how much D2 neurons were influenced by the direction of the forthcoming saccade and the visual pattern of the matching cue. These indexes range from 0 to 1. When the Max (maximal) direction and the Opposite values have similar magnitudes, the DDI value is close to zero. When the Max direction is twice the Opposite value, the DDI value is 0.33.

When the Max direction is 3 times the Opposite value, the DDI value is 0.5. The same relationships apply to the VDI value. Figure 7 shows the distributions of the two indexes for all of the D2 neurons (\(n = 65\)), where VDI is plotted against DDI. For MC-period activity, plots were scattered along a 45° slope. Although 60% of the plots (32/53) were below the 45° slope, there was no significant difference in the number of plots above and below this line \(\chi^2 = 2.28, \ P > 0.05\), not significant]. In contrast, most of the plots for D2-period activity (75%, 49/65) were below the 45° slope \(\chi^2 = 16.75, \ P < 0.01\), indicating that DDI was higher than VDI for D2-period activity in most D2 neurons. This tendency was highly significant in the GO period; i.e., >80% of the plots (83%, 39/47) were below the 45° slope \(\chi^2 = 33.72, \ P < 0.01\). Thus DDI values significantly increased as the trial proceeded. In Fig. 8A, distributions of DDI for MC- and D2-period activities are illustrated for D2 neurons with MC-period activity (\(n = 53\)). As shown by distributions and medians (arrows), DDI was significantly greater in the D2 period (0.28 ± 0.16; median, 0.24) than in the MC period (0.22 ± 0.13; median, 0.19; \(t = 2.47, \ P < 0.05\), paired \(t\)-test). Figure 8B shows the distributions of DDI for D2- and GO-period activities of D2 neurons with GO-period activity (\(n = 47\)). DDI was greater in the GO period (0.37 ± 0.20; median, 0.32) than in the D2 period (0.28 ± 0.16; median, 0.23) \(t = 3.24, \ P < 0.01\), paired \(t\)-test]. Thus DDI gradually increased from the MC period to the GO period.

Temporal change of D2-period activity

As shown above, most of the D2-period activity continues from the MC period to the GO period. To examine the temporal change of D2 neurons in the ODM task, we summed 45 directional D2 neuron activities with a DDI value >0.2. These activities were averaged with a 100-ms timescale.

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As shown above, most of the D2-period activity continues from the MC period to the GO period. To examine the temporal change of D2 neurons in the ODM task, we summed 45 directional D2 neuron activities with a DDI value >0.2. These activities were averaged with a 100-ms timescale.
FIG. 7. Scattergrams of visual dependency index (VDI) against directional dependency index (DDI) for 65 D2 neurons in the MC, D2, and GO periods. VDIs were plotted against DDIs in each period. Most of the plots fell below the 45° slope in the D2 and GO periods. In the MC period, the plots were widely scattered around the 45° slope.

binwidth ranging from 500 ms before the MC period to 500 ms after the GO period. As shown in Fig. 9, an activity for maximal direction suddenly began at the first bin (0–100 ms) of the MC period and peaked at the second bin (100–200 ms). Then the activity gradually decreased, although it remained at a higher rate throughout the D2 period than that in the control period. Activity for the opposite direction also showed similar changes in the MC period, although its magnitude was smaller than that of the maximal direction. And it was depressed during the later phase of the D2 period. A two-factor ANOVA revealed that there was a significant effect for “saccade direction” [F(1,44) = 80.55, P < 0.01] and significant interaction between saccade direction and “time course” [F(29,1276) = 6.19, P < 0.01]. A post hoc test showed that the difference between activities for maximal and opposite directions was statistically significant (evaluated P < 0.05) from the second bin of the MC period (100–200 ms) to the third bin of the GO period (200–300 ms). Thus sustained and differential activity of the D2 neuron reached the maximal activity at the early phase of the MC period and gradually decreased, although it was sustained across the D2 period to the GO period.

DISCUSSION

To examine the involvement of the prefrontal cortex (PFC) in the selection of directional movement in visual memory-guided behavior, we recorded neuronal activity from the dorsolateral PFC of two rhesus monkeys while they performed an ODM task. The ODM task separated the effect of the motor factor from that of the visual factor on neuronal activity during the D2 period between the goal-instruction cue (MC) and response (GO) periods. Most D2-period activities were dependent on the direction of the forthcoming saccade even in the MC period. These activities will lead to saccadic eye movements guided by visual and mnemonic information.

Oculomotor delayed matching-to-sample task

The task we adopted was an ocular version of a delayed matching-to-sample task with an additional response delay. Delayed matching-to-sample tasks conventionally consist of three periods (i.e., sample cue, delay, and matching periods) and have been often used for the study of nonspatial visual memory (Fuster and Jervey 1982; Kubota et al. 1980; Mikami and Kubota 1980; Mishkin and Manning 1978; Passingham 1975; Quintana and Fuster 1993). To examine how delay period activity of PFC neurons is differentially related to the visual stimuli, decision making, and motor execution, we introduced a delay between the goal instruction signal and the response to the goal. In a delayed matching paradigm, movement direction is determined not by a single

FIG. 8. Temporal change in the distribution of DDI values. A: the distribution of DDI values in 53 D2 neurons with MC-period activity. DDI was higher in the D2 period (0.28 ± 0.16; median, 0.24) than in the MC period (0.22 ± 0.13; median, 0.19). B: the distribution of DDI values in 47 D2 neurons with GO-period activity. DDI was higher in the GO period (0.37 ± 0.20; median, 0.32) than in the D2 period (0.28 ± 0.16; median, 0.23; t = 3.24, P < 0.01, paired t-test). Arrows indicate the bin that includes the median.
visual stimulus but by a combination of visual stimuli via a delay. Furthermore, motor instruction (MC period) and its execution (GO period) are temporally separated by the second delay (D2 period). So, we thought this task would allow us to dissociate PFC activity concerning the motor aspect for the upcoming eye movement from that related to visual discrimination or motor execution. However, it took much longer time (about half a year) to teach the monkeys to learn our new task than it does to teach the usual delayed matching-to-sample task to monkeys in a similar situation.

**D2-period activity**

In our matching-to-sample task, the D2 period separated motor execution during the response (GO) period from the instruction of it during the cue (MC) period. Some behavioral studies with modified versions of the delayed matching-to-sample task (delayed symbolic matching-to-sample task or pair association task) showed data indicating a strategy in which animals were considered to recall paired stimuli or responses during a delay period (Gaffan 1977; Roitblat 1980). Miyashita and his coworkers reported activity of the primate inferotemporal cortex, which was related to pair recall (Miyashita 1988; Naya et al. 1996). In addition, Li et al. (1993) suggest that many neurons in the inferotemporal cortex code not only the sensory features but also the familiarity of the stimuli. In our task, the correct response was operationally defined as moving the eye to the location of the previously presented target (matching location of stimuli). Monkeys may possibly perform the task by remembering the 16 possible combinations of sample-matching pairs and response directions individually. Because we were not able to find any pattern-specific or matching cue-specific neuronal activity during the D2 period, we thought that the performance of the monkeys was not based on retention of a given sequence of task patterns but on retention of the location of the target stimulus. However, the learning process of matching-to-sample by monkeys still remains an important issue to be studied (Iversen et al. 1986).

Previous studies using tasks with delays showed involvement of a visual factor in the dorsolateral PFC for memory in the delay period (Funahashi et al. 1993; Kubota et al. 1980; Niki and Watanabe 1976). For example, Funahashi et al. (1993) examined the property of principal sulcus neurons by comparing their activities in the oculomotor delayed response task to those in the delayed anti-saccade task. The majority of PFC neurons (59%) were stimulus dependent, although some also depended on saccade direction (25%). An earlier study with a conditional discrimination task also reported similar results (Niki and Watanabe 1976). In the D2 period of our task, we did not detect the strong effect of a visual factor. There may be several reasons for this discrepancy. The delayed matching-to-sample task has the advantage that any of the peripheral stimuli that constitute the matching cue can become the choice target depending on the preceding sample stimulus. That is, the same matching cue (both in retinal location and in physical property) will instruct different behavioral meaning (instruction of movement) depending on the sample cue. However, in the delayed response task, the location of instruction cues is closely connected with response. Therefore absence of an overt spatial visual cue instructing the motor response may induce much activation of the PFC in preparation of future action. Furthermore, the recorded area may be also the reason. In previous studies, recorded areas were anterior and/or lateral to ours, that is, the caudal part of the periprincipal sulcal area (Walker’s area 46) and its immediately adjacent cortex (area 8). Wilson et al. (1993) compared the activities of delay neurons in the periprincipal area (the dorsal PFC) with the inferior convexity area (the ventral PFC) and found that neurons in the dorsal PFC are more related to response execution, whereas neurons in the ventral PFC are more related to the visual stimulus; however, the number of neurons examined was small, and histological data were not shown. Miller et al. (1996) reported that ventral PFC neurons represent visual working memory of the sample stimulus. This area has been suggested to be a possible neural substrate for the mnemonic processing of visual objects (Kowalska et al. 1991; Passingham 1975; Quintana and Fuster 1993; Stamm 1973) and appears to share similar properties with the inferotemporal cortex and temporal pole (e.g., Miller et al. 1991; Nakamura and Kubota 1995). Contrary to the study of Wilson et al. (1993), Rao et al. (1997) recently reported that PFC neurons, even in the dorsal PFC, were related to the memory of both visual object and spatial location in a delayed matching-to-sample task with a second delay task similar to our task. To examine whether or not the PFC has domains dealing with different memories, further studies should be done in a multitask situation especially including delayed matching-to-sample and delayed response tasks.

The dorsolateral PFC is connected to the premotor cortex and the FEF (Barbas and Pandya 1989; Picard and Strick 1996; Watanabe-Sawaguchi et al. 1991). The FEF is involved especially in the generation of visually and memory-guided saccades (Bruce and Goldberg 1985; Bruce et al. 1985). The PFC may functionally interact with these areas to produce motor information. Premovement activation has been found in the premotor cortex (Kubota and Funahashi 1982; Kurata 1993; Kurata and Wise 1988). Such activity is considered as ‘‘set’’ related in preparation for the upcoming manual movement. With regard to eye movements, some FEF neurons show similar activity before saccadic eye
movement (Bruce and Goldberg 1985). D2-period activity in our study resembles this premovement activity, and it may reflect either the target location or the direction of the forthcoming movement. Boussaoud and Wise (1993) showed that many premotor neurons reflect the motor significance of stimuli. They also attempted to distinguish stimulus effects from motor effects and found that premotor neurons more preferentially respond to motor instruction cues than to attentional or mnemonic cues. These results are consistent with ours regarding the weak effect on D2-period activity and small population of sample cue-related neurons. However, Boussaoud and Wise (1993) also reported that PFC neurons showed stronger activity for attentional/ mnemonic cues. That result is not consistent with ours but may reflect either the difference between hand or eye movements in their study and our study, respectively, or the difference between the stimuli used.

Most D2-period activities were accompanied by MC-period activities. Like D2-period activities, MC-period activities were predominantly dependent on the forthcoming saccade direction. However, the visual pattern of the matching cue (i.e., visual factor) also influenced MC-period activity, indicating that visual information is processed even by delay neurons. The target location or the direction of the forthcoming saccade appears to be determined during the MC period based on the combination of sample and matching cue. Some studies reported that animals tend to use “prospective coding” (retention of information necessary for future action) rather than “retrospective coding” (retention of information for past events regarding future action) (Gaffan 1977; Roitblat 1980). We therefore propose that MC-period activity in PFC neurons may represent a transformation from visual to motor information necessary for the control of the forthcoming saccade.

D2 neurons shared a property with “visuokinetic” neurons, which were previously examined with delayed-response tasks in the PFC (Kubota et al. 1974). Most of these neurons showed activity not only during the delay period but also during cue and response periods. Although such visuokinetic neurons may be involved in choosing the correct location of the response, the fact that the cue location is the same as the response location makes it difficult to separate the effects of these factors on cue-period activity. Our study emphasizes that some delay neurons in the PFC may be related also to the process of determining the direction of the forthcoming saccade in the initial phase of activation.

Decision and memory process in the PFC

When we think of the cognitive processes necessary to perform memory-based saccade, as seen in our task, the dorsolateral PFC appears to be involved at least in the decision, retention, and execution processes. Based on the mnemonic information, the monkey would select the target location, retrieve the memory how to perform the saccade, decide parameters such as the direction and amplitude of the eye movement, and remember or prepare them at least until performing the selected response. These processes may occur successively from the MC to D2 period. Certainly, directional delay-period activity that we found may be related to the motor representation in the PFC. However, we were not able to abstract the activity specific to the decision process. In addition, other brain areas such as the FEF and the temporal or parietal lobe may be also involved in any aspect of decision process. Therefore further studies should be done in multiple areas with the task that has a clear decision phase.

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