Neuronal Activity in the Primate Prefrontal Cortex in the Process of Motor Selection Based on Two Behavioral Rules

EJI HOSHI, KEISETSU SHIMA, AND JUN TANJI

Introduction

It has long been thought that the lateral prefrontal cortex is involved in the executive control of behavior (Fuster 1981, 1990; Goldman-Rakic 1987a,b; Miller 1999; Passingham 1993; Petrides 1996; Watanabe 1998; Wise et al. 1996). Motor selection is an important aspect of behavioral decisions that are central to executive control of individuals. Neuronal activity in the prefrontal cortex (PF) involved in motor selection has been studied during oculomotor (Asaad et al. 1998; Funahashi et al. 1993; Hasegawa et al. 1998; Kim and Shadlen 1999; Rao et al. 1997; Wilson et al. 1993) or limb motor tasks (Boussaoud and Wise 1993a; di Pellegrino and Wise 1991; Miller et al. 1996; Niki and Tsutsui 1997; Quintana et al. 1988; Sakagami and Niki 1994a; Watanabe 1981, 1986a; White and Wise 1999). In these studies, however, the motor selection was made in response to sensory information after a single rule specified by an instruction (Asaad et al. 1998; Boussaoud and Wise 1993a; di Pellegrino and Wise 1991; Hasegawa et al. 1998; Kim and Shadlen 1999; Miller et al. 1996; Niki and Tsutsui 1997; Quintana et al. 1988; Rao et al. 1997; Watanabe 1981, 1986a; Wilson et al. 1993), or according to a set of rules determined individually by instruction signals (Funahashi et al. 1993; Sakagami and Niki 1994a; White and Wise 1999). In real life, subjects often have to make a selection by obeying more than one rule at a time when dealing with multiple factors to make an appropriate motor selection. How is it possible to select a particular motor act by promptly processing multiple factors to obey two rules? Previous studies have shown that one of the prominent roles of the PF is to store information on-line so that the information can be used for constructing future actions (Barone and Joseph 1989b; Funahashi et al. 1989, 1997; Fuster and Alexander 1971; Goldman-Rakic 1987a; Kubota and Niki 1971; Miller et al. 1996; Rainer et al. 1998a). How, then, does the PF combine current information with information that was stored in memory to find a solution to a complex problem having two rules? How do neurons in the PF deal with the old and current information, to select a future motor act under such a requirement? As a first step to answer these questions, we examined neuronal activity in the PF while animal subjects were required to briefly store one set of information and then deal with a current set of information. Their task was to combine the two sets of information to select a future movement determined by two different rules. We describe neuronal activity in detail at the time of motor selection, when the subjects had to combine the two sets of information rapidly and find the correct motor response. The three types of neuronal activity found in the present study suggest that the PF plays a role in motor selection under such behavioral demands. A preliminary account of this study appeared elsewhere (Hoshi et al. 1998a,b).

Methods

Subjects and apparatus

Two male Japanese monkeys (Macaca fuscata, 8 and 10 kg) were used in this study. They were cared for according to the Guidelines for...
Subsequently, three cue frames measuring 8 cm each trial, the screen was blank for a 3-s intertrial interval (ITI). If they released the hold button prematurely, the screen went blank until the GO signal appeared. The subjects were required to keep pressing the hold button from the initiation of a trial until the GO signal appeared. If the subject continued to press the hold button for another 1.5 s, the color of the choice cue was reversed (i.e., one of the choice cues in the standard task appeared as the sample cue, and one of the standard sample cues appeared as the choice cue). Therefore the subjects did not have to determine a target, because only one object was presented as a choice cue. All they had to do was to reach for a target determined according to the sample and choice cues displayed. The sample and choice cues were red. When the choice cue turned green, this was the signal for the animal to reach for a target determined according to the location-matching and shape-matching rules. Top: sequence for the standard task. Bottom: sequence for the control task. In some cases, the duration of the sample cue and delay were altered so that they were 1.5 and 2.5 s, respectively.

**Behavioral paradigm**

**STANDARD TASK.** We trained the two monkeys to select a target determined by two behavioral rules (Fig. 1, top row). At the start of each trial, the screen was white for a 3-s intertrial interval (ITI). Subsequently, three cue frames measuring 8 × 8 cm appeared at the top, bottom left, and bottom right of the screen. When the subject pressed the hold button for 0.5 s after their appearance, a red sample cue, either a circle or a triangle, appeared in one of the three frames (top, left, or right) for 1 s. The circle was 4.5 cm diam, and the sides of the triangle were 5 cm long. The sample cue disappeared, and only the background frame remained visible for a 3-s delay period. After this delay, a red choice cue appeared. There were two different sets of cues; each required a different task to be performed. If the choice cue was either three triangles (after a triangle sample cue) or three circles (after a circle sample cue), the subject was required to select the triangle or circle that was in the same location as the sample cue (location-matching task). On the other hand, if the choice cue was a triangle and a circle, the subject had to select the object with the same shape as the sample cue (shape-matching task). If the subject continued to press the hold button for another 1.5 s, the color of the choice cue changed from red to green. This was the GO signal and the subjects then had to release the hold button within 1.5 s and touch the correct object. If they touched the correct object, a brief tone (1,000 Hz, 150 ms) was presented, and a drop of fruit juice (0.2 ml) was given as a reward 500 ms later. When a reward was delivered, the screen went blank, and the next ITI started. If they touched an incorrect object, a 300-Hz tone signal was given for 1 s without any reward or punishment. The subjects were required to keep pressing the hold button from the initiation of a trial until the GO signal appeared. If they released the hold button prematurely, the screen went blank and the trial was aborted. Because the two tasks (shape matching and location matching) were presented pseudorandomly, the monkeys had to remember both the shape and location of the sample cue until the choice cue was presented. When the choice cue appeared, they selected the correct target by combining the memorized information (provided by the sample cue) and the current information (provided by the choice cue).

During a period when we found and recorded task-related neurons, the two choice items (circle and triangle) used for the shape-match task occupied those two locations at which the sample cue had not appeared. This was done for the purpose of reducing the number of possible combinations of the sample-cue and choice-cue. However, when out of the recording sessions, we also added trials where the two choice items did appear at locations at which the sample cue had appeared. No detectable differences in behavioral measures (i.e., reaction time and movement time) were found ascribable to this modification of the behavioral condition.

**CONTROL TASK.** In addition to this standard task, a control task (a visually guided reaching task) was also performed (Fig. 1, bottom row). In this task, the order of presentation of the sample and choice cues was reversed (i.e., one of the choice cues in the standard task appeared as the sample cue, and one of the standard sample cues appeared as the choice cue). Therefore the subjects did not have to determine a target, because only one object was presented as a choice cue. All they had to do was to reach the target indicated by the choice cue when its color changed from red to green. This task was introduced ad libitum by the experimenter. In the control task, the sample cue was presented for either 1.5 s or 1 s, whereas the choice cue was always presented for 1.5 s. The duration of the delay period was either 2.5 s (if the sample cue was presented for 1.5 s) or 3 s (if the sample cue was presented for 1 s).

**Surgery and physiological recording**

After completing the behavioral training, aseptic surgery was performed under pentobarbital sodium anesthesia (30 mg/kg im) with atropine sulfate. Antibiotics and analgesics were used to prevent postsurgical infection and pain. Stainless steel screws were implanted in the skull, to which two stainless steel pipes used to fix the head during the daily recording sessions were attached rigidly with acrylic resin. A 20 × 20 mm area of the skull was removed over the principal sulcus, and a stainless steel recording chamber (27 × 27 mm) was implanted. We recorded neuronal activity in the following order; 1)
left and 2) right hemispheres of the prefrontal cortex, and 3) the left primary motor cortex contralateral to the arm performing the task. The opening of the skull over the primary motor cortex was 15 × 15 mm.

Before starting to record neuronal activity from the prefrontal cortex, we mapped the frontal eye field (FEF) in the anterior bank of the arcuate sulcus with intracortical microstimulation (ICMS) (cf. Bruce et al. 1985). We penetrated the prefrontal cortex around the principal sulcus and inferior convexity anterior to the FEF where ICMS did not evoke eye movements with currents <50 μA. We also recorded cell activity from the arm area of the primary motor cortex, which we identified by ICMS (Sato and Tanji 1989).

Neuronal activity was recorded with glass-insulated Elgiloy microelectrodes (0.8–2.5 MΩ at 333 Hz), which were inserted through the dura matter with a hydraulic microdrive (MO-81, Narishige) while the monkeys were performing the behavioral task. The electrode tracks were perpendicular to the cortical surface. Single-unit potentials were amplified with a Multi-Channel Processor and discriminated with a Multi-Spike Detector (MCP plus 8, MSD; Alpha Omega Engineering). Behavioral events and raster displays of neuronal activity were shown on-line on a laboratory cathode ray tube screen. We carefully advanced the electrode while monitoring extracellularly recorded spikes with an oscilloscope and sound. When we encountered a new cellular spike, we immediately started on-line analysis of its activity. If any relationships to the task events were observed, we continued to record and saved the data.

Electromyographic (EMG) activity was recorded with silver wire electrodes. The EMG activity was amplified (MEG-6100, Nihon Kohden) and digitized with an A/D converter, and the digital pulses were stored in the laboratory computer. We monitored the following muscles: biceps and triceps brachii, deltoid, trapezius, supraspinatus, infraspinatus, pectoralis major, rhomboids, and the neck and paravertebral muscles. We monitored the position and movement of the eyes 100 times per second with an infrared eye-camera system (R-21C-AS, RMS).

Data analysis

For each neuron we obtained at least 12 data files that were sorted according to the sample cue patterns and type of task (6 sample cues × 2 tasks). For each data file, we generated raster and histogram displays aligned with task events. Neuronal discharges during each trial were analyzed in five task periods: 1) the control period, the last 500 ms of the intertrial interval (ITI); 2) the sample cue presentation period, 50–1,000 ms after the sample cue appeared; 3) the delay period, from the disappearance of the sample cue until the appearance of the choice cue; 4) the choice period, 0–1,500 ms after the onset of the choice cue; and 5) the motor-response period, from the appearance of the GO signal until the screen was touched. Occasionally, these time windows were modified according to response patterns of each neuron. The number of spikes during each period was normalized by the duration of each period and calculated as the spike rate, in spikes per second (spikes/s). The normalized neuronal discharges in each task period were compared with those in the control period. If the distributions of the spike rate in the two periods were significantly different (Mann-Whitney U test, significance level, P < 0.05), the neuron was judged as having task-related activity for that period. Further details of data analyses are described in RESULTS. To determine each neuron’s onset and offset times relative to a behavioral event, we made histograms (binwidth, 20 ms) that were aligned with the appearance of the sample cue or choice cue, and determined the latency on the basis of cumulative time histograms (Funahashi et al. 1991). We defined the latency with a temporal resolution of 20 ms. The reaction time (RT) was defined as the time from the appearance of the GO signal until the hold button was released. The movement time (MT) was defined as the time from the button release until the screen was touched.

Muscle activity and eye movements

The arm and shoulder muscles in both monkeys displayed movement-related activity while performing tasks, and this activity varied with the location of the target, but not with the shape or type of task. None of the sampled muscles showed detectable changes in activity during the period between the appearance of the sample cue and the onset of the GO signal. Monkeys were not required to control their gaze while performing the behavioral task. Therefore their line of sight was not fixated on any spatial location during the sample cue and delay periods. After the onset of the choice cue, they made scanning eye movements over the choice cue, and a few hundred milliseconds before the appearance of the GO signal they made saccadic eye movements toward the future target, and remained fixated on it while reaching. Examples of eye movements while performing the task are shown in Fig. 2.

Histological studies

After collecting the neuronal data and studying the effects of muscimol injection, the monkeys were deeply anesthetized with an overdose of pentobarbital sodium (50 mg/kg) and perfused through the heart with saline followed by 8% formaldehyde with 3% potassium ferrocyanide. Serial 50-μm-thick coronal sections of the brains were made with a freezing microtome, and stained with cresyl violet. The electrode tracks were reconstructed using iron deposits, which were formed by passing a positive DC current through the tips of the microelectrodes, as reference points.

RESULTS

Task performance

Both monkeys performed the behavioral task at >90% correct rate after completion of the training of 6 mo. The majority of the errors resulted from selecting incorrect objects, and about one-fifth of the errors resulted from releasing the hold button prematurely. The RT did not differ with either the shape or location of the target, nor was the RT influenced by the type of task (ANOVA, P > 0.1). The MT varied with the location of the target (in the order Top > Left > Right in both monkeys, ANOVA, P < 0.01), but did not vary with either the target shape or the type of task (ANOVA, P > 0.1). The RT and MT data were accumulated using all of the data files obtained during recording of neuronal data. Their group mean and SD are presented in Table 1.

Muscle microinjection

After recording the neuronal activity in each monkey, muscimol solution (2.0–3.0 μL) was injected into the prefrontal cortex bilaterally around the principal sulcus. We made the solution by dissolving muscimol (Sigma, St. Louis, MO) into 0.1 M phosphate buffer at a pH of 7.4 (Kurata and Hoshi 1999; Shima and Tanji 1998). The concentration of muscimol was adjusted to 5 μg/μL. A pair of thin (ID and OD: 150 and 300 μm, respectively) stainless steel tubes was inserted through the dura matter into the right and left hemispheres simultaneously with the same hydraulic microdrives used for the recording microelectrode. A millimeter scale was visible on the surface of the injection tube during insertion and indicated the distance by which the injection tube penetrated the dura. The muscimol solution was injected at a rate of 0.1 μL/min using an electronic microinjection pump (CFV-3100, Nihon Kohden). As a control, we injected 3.0 μL of saline instead of the muscimol solution, using the same procedures.

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### TABLE 1. Behavioral data for the two monkeys

<table>
<thead>
<tr>
<th>Location of the Target</th>
<th>Reaction Time</th>
<th>Movement Time</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Top</td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td><strong>Monkey 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location match</td>
<td>488 ± 111</td>
<td>481 ± 132</td>
<td>479 ± 125</td>
</tr>
<tr>
<td>Shape match</td>
<td>485 ± 121</td>
<td>480 ± 131</td>
<td>483 ± 135</td>
</tr>
<tr>
<td><strong>Monkey 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location match</td>
<td>322 ± 64</td>
<td>325 ± 87</td>
<td>325 ± 71</td>
</tr>
<tr>
<td>Shape match</td>
<td>328 ± 79</td>
<td>323 ± 83</td>
<td>327 ± 82</td>
</tr>
</tbody>
</table>

Values are means ± SD in ms.

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**FIG. 2.** Eye positions and movements monitored during the task. The 2 traces are for 2 trials with the combinations of the sample and choice cues shown in the figure. *Top traces* in each panel represent horizontal eye movements (rightward eye movements appear as an upward deflection), and the *bottom traces* represent vertical eye movements (upward movement is up). The 2 panels at the *bottom* show the data for the control task. An asterisk centered on a circle or triangle shows the target to be touched in the response period.
Neuronal database

We recorded neuronal activity from the PF in both hemispheres, and from the left primary motor cortex (MI) in the two monkeys. In monkey 1, we recorded task-related activity in 308 (right, 181; left, 127) PF and 159 MI neurons. In monkey 2, we recorded task-related activity in 390 PF (right, 168; left, 222) and 119 MI neurons. To analyze neuronal activity, we pooled the data from the two monkeys because the distributions of neurons related to the sample cue, the delay period, the choice cue, and to the motor response were similar in the two monkeys. First we describe the activity of the 698 PF neurons, then the activity of the 278 MI neurons. The number of PF neurons classified according to the task-related phase is summarized in Table 2.

Sample cue period activity

Out of the 698 PF neurons, 297 (43%) satisfied the criteria for sample cue period activity. In the behavioral task, two factors influenced the neuronal activity during the cue period: the shape and the location of the sample cue (Rainer et al. 1998b). Therefore we performed a two-factor ANOVA (2-way ANOVA, significance level, \( P < 0.05 \)) to determine the relationship of neuronal activity to the shape (circle or triangle) and location (top, left, or right) of the sample cue. From the results of the ANOVA analysis, we classified the neuronal activity into four categories, which were termed location selective, shape selective, location and shape selective, and non-selective. Location-selective neurons were only significant \((P < 0.05)\) for the location factor. Shape-selective neurons were only significant for the shape factor. Location- and shape-selective neurons were significant for both factors. Nonselective neurons were not significant \((P \geq 0.05)\) for either factor.

A location- and shape-selective neuron is shown in Fig. 3. This neuron discharged markedly only when a circle appeared as the sample cue in the left frame (shape factor, \( P < 0.001 \); location factor, \( P < 0.001 \)). A shape-selective neuron is illustrated in Fig. 4. This neuron responded briskly to the appearance of the sample cue if it was a triangle, regardless of its location (shape factor, \( P < 0.001 \); location factor, \( P > 0.6 \)). The same neuron also responded to the choice cue, but the response in that period will be explained later.

A location- and shape-selective neuron is shown in Fig. 3. In raster displays, each row represents a trial, and dots represent discharges of this neuron. The 1st, 2nd, and 3rd triangles in each row of the raster display show when the monkey pressed and released the hold button, and touched the screen, respectively. The 1st and 2nd vertical lines in the raster show when the sample cue was turned on and off, and the 3rd and 4th lines indicate the appearance of the choice cue and GO signal, respectively. Discharges are summed in perievent histograms (bin-width, 40 ms) below each raster display. The ordinate of the histograms represents the number of neuronal discharges per second (40 spikes/s). This neuron exhibited strong responses only when a circle was presented in the left frame. This neuron did not show any responses to the appearance of the choice cues.

TABLE 2. Distribution of prefrontal cortex neurons related to different phases of the task period

<table>
<thead>
<tr>
<th>Task-Related Phase</th>
<th>Monkey 1</th>
<th>Monkey 2</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample cue only</td>
<td>14</td>
<td>19</td>
<td>33</td>
</tr>
<tr>
<td>Delay only</td>
<td>10</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>Choice only</td>
<td>36</td>
<td>64</td>
<td>100</td>
</tr>
<tr>
<td>Response only</td>
<td>27</td>
<td>45</td>
<td>72</td>
</tr>
<tr>
<td>Sample cue + delay</td>
<td>0</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Sample cue + choice</td>
<td>53</td>
<td>41</td>
<td>94</td>
</tr>
<tr>
<td>Sample cue + response</td>
<td>6</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Delay + choice</td>
<td>27</td>
<td>75</td>
<td>102</td>
</tr>
<tr>
<td>Delay + response</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Choice + response</td>
<td>43</td>
<td>55</td>
<td>98</td>
</tr>
<tr>
<td>Sample cue + delay + choice</td>
<td>22</td>
<td>23</td>
<td>45</td>
</tr>
<tr>
<td>Sample cue + delay + response</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Sample cue + choice + response</td>
<td>42</td>
<td>25</td>
<td>67</td>
</tr>
<tr>
<td>Delay + choice + response</td>
<td>7</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>Sample cue + delay + choice + response</td>
<td>16</td>
<td>23</td>
<td>39</td>
</tr>
<tr>
<td>Total</td>
<td>308</td>
<td>390</td>
<td>698</td>
</tr>
</tbody>
</table>
of location-selective neuronal activity is shown in Fig. 5. This neuron showed delay period activity most markedly when the sample cue was in the right frame, regardless of its shape (shape factor, \( P > 0.8 \); location factor, \( P < 0.001 \)). On the other hand, the neuron shown in Fig. 4 exhibited selectivity for the shape and was continuously active when the sample cue was a triangle (shape factor, \( P < 0.001 \); location factor, \( P > 0.05 \)). An example of location- and shape-selective neuronal activity is presented in Fig. 6. This neuron showed the most prominent activity when a circle sample cue was presented in the top frame (shape factor, \( P = 0.001 \); location factor, \( P < 0.001 \)).

Based on the ANOVA analysis of the 226 delay-related PF neurons, 121 (54%) were judged as location-selective, 11 (5%) as shape-selective, 27 (12%) as location- and shape-selective, and 67 (30%) as nonselective.

Choice cue period activity

Of the 698 task-related PF neurons, 561 (80%) satisfied the criteria for choice cue period activity. Because the primary aim of this study was to look for neuronal activity in this period, the choice cue period was analyzed in detail. We found that two types of neuronal activity appeared newly in this period. These were classified as choice cue configuration selective, and reach-target selective (362 of the 561 choice-cue–related neurons). In addition, we found a third type of activity that appeared as a continuation of the delay period activity, and reflected information contingent on the properties of the sample cue. Therefore we defined the third type of neurons as the sample-cue–dependent (199 of the 226 delay-related neurons).

We will first deal with the choice-cue-configuration–selective and reach-target–selective neurons. Thereafter, we will describe the sample-cue–dependent activity observed in the choice cue period.

Choice cue configuration selective activity. We found that a group of PF neurons (classified as the 1st type) responded to the choice cue and exhibited activity that depended on the spatial arrangement of circles and triangles constituting the choice cue, namely, the configuration of the choice cue. We further found that the configuration-selective neurons exhibited subtypes of activity properties. The first subtype (subtype C1) of these PF neurons responded preferentially to the choice cue consisting of three circles or three triangles. A neuron that responded strongly after the appearance of a choice cue consisting of three objects is shown in Fig. 7. This neuron re-
sponded vigorously when three triangles or three circles were presented as a choice cue (Fig. 7, A–F). In contrast, when a circle and triangle were presented, it responded only modestly (G–L). The difference between the response to three objects and to a circle and a triangle was significant (ANOVA, \( P < 0.001 \)). An example of a neuron selective for the shape of the target is illustrated in Fig. 10. This neuron was more active when the target was a circle (A, C, E, G, H, and I) than when it was a triangle (B, D, F, J, K, and L; 2-way ANOVA; shape of target, \( P < 0.01 \); location of target, \( P > 0.3 \)), and its activity was enhanced in shape-matching (ANOVA, \( P < 0.001 \)). A neuron that was selective for the location and shape of the target is illustrated in Fig. 11 (2-way ANOVA; shape of target, \( P < 0.01 \); location of target, \( P < 0.001 \)).

STATISTICAL CLASSIFICATION FOR THE CONFIGURATION-SELECTIVE AND REACH-TARGET-SELECTIVE ACTIVITY. To determine systematically how many of the 362 PF neurons exhibited the two properties (configuration selectivity or reach-target selectivity), we first measured the response-onset latency of each neuron after the onset of the choice cue. The latency was measured on the basis

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**FIG. 6.** PF neuron whose delay-period activity appeared selective for both the location and shape of the sample cue. When a circle sample cue was presented in the top frame, this neuron showed marked activity that built up toward the end of the delay period. The offset time after the appearance of the choice cue was 160 ms.

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**REACH-TARGET-SELECTIVE ACTIVITY.** We found that the second type of PF neuron exhibited activity that reflected either the location or shape of the target of the forthcoming reaching movement. This means that the information about the future target was already reflected in the PF activity during the choice period, well before the GO signal that triggered the movement. A typical neuron selective for the location of the target is shown in Fig. 9. This neuron was much more active when the target was in the left frame (B, E, G, and L), regardless of the target shape (2-way ANOVA; shape of target, \( P > 0.9 \); location of target, \( P < 0.001 \)). An example of a neuron selective for the shape of the target is illustrated in Fig. 10. This neuron was more active when the target was a circle (A, C, E, G, H, and I) than when it was a triangle (B, D, F, J, K, and L; 2-way ANOVA; shape of target, \( P < 0.01 \); location of target, \( P > 0.3 \)), and its activity was enhanced in shape-matching (ANOVA, \( P < 0.001 \)). A neuron that was selective for the location and shape of the target is illustrated in Fig. 11 (2-way ANOVA; shape of target, \( P < 0.01 \); location of target, \( P < 0.001 \)).

**FIG. 7.** Discharges of a PF neuron selective for the configuration of the choice cue. It was most active in response to either 3 triangles or 3 circles (subtype C1 response). The response latency after the onset of the triple triangle cue was 100 ms (in A). In the control task, this neuron also responded to the appearance of a sample cue consisting of 3 triangles (M).
of cumulative time histogram (see METHODS). Subsequently, we set a time window for the statistical analysis of the magnitude of responses as the first 500 ms after the response-onset latency. Occasionally, the duration of the time window was modified depending on the response patterns of neurons. Then we performed both one-way ANOVA (significance level, $P < 0.05$) looking at choice cue configuration (3 circles, 3 triangles, or 3 sets of a circle and a triangle) and two-way ANOVA (significance level, $P < 0.05$) looking at the shape (circle or triangle) and location (top, right, or left) of the future target. If a neuron only showed significance for one analysis, it was classified into that group. If a neuron showed significance for both analyses, we compared the $P$ values and classified the neuron according to the smaller $P$ value. As a result, we divided the neurons into three main types. Out of the 362 neurons, 165 were classified as configuration selective, 117 as reach-target selective, and the remaining 80 as nonselective.

Subsequently, we classified the configuration-selective neurons into the three subtypes (C1, C2, and C3) based on ANOVA looking at the type of task. The subtype C1 neurons were significantly more active ($P < 0.05$) when the type of task was location matching. The subtype C2 neurons were significantly more active ($P < 0.05$) when the type of task was shape matching. The rest of the configuration-selective neurons did not exhibit significant selectivity for the type of task ($P > 0.05$) and were defined as subtype C3 neurons. As a result, of the 165 configuration-selective neurons, 23, 88, and 54 were classified as subtype C1, C2, and C3, respectively.

In the next step of analysis, we determined whether the subtype C2 configuration-selective neurons (that are more active during the shape-matching task) also contained information about where a triangle or circle in the pair was located at the top, right, or left. This analysis was performed because we noticed that a large number of subtype C2 cells exhibited selective activity depending on where the triangle (or circle) is located in the pair. A typical example of such selectivity is shown in Fig. 12, where the activity responding to the paired choice cue is most prominent if the triangle is located in the left (A–D). Accordingly, we performed ANOVA looking at the location of the circle in the pair and the location of the triangle in the pair. The neuron in Fig. 12 had significant information for the location of the triangle in the pair (ANOVA, $F$ ratio = 100.355, $P < 0.0001$) and for the circle in the pair (ANOVA, $F$ ratio = 18.599, $P < 0.0001$). The neuron in Fig. 8 also had significant information for the location of the circle (ANOVA, $P < 0.0001$) and the triangle (ANOVA, $P < 0.0001$) in the pair. To perform this ANOVA analysis, it was necessary to

![Figure 8](http://jn.physiology.org/lookup/doi/10.1152/jn.00745.2003) Discharges of a PF neuron selective for the configuration of the choice cue. It was active in response to paired circle and triangle (subtype C2 response), but not to triple objects. The response also exhibited selectivity, depending on the position of the circle. In this case, the neuron was most active when the circle was in the left frame (A–D). The response-onset latency for the trials shown in A was 140 ms.

![Figure 9](http://jn.physiology.org/lookup/doi/10.1152/jn.00745.2003) PF neuron whose activity was selective for the location of the target selected to reach. This neuron was most active when the target was in the left frame, regardless of the configuration of the choice cue (B, E, G, and I). The response-onset latency in B was 360 ms after the onset of the choice cue.
observe neuronal activity under 12 behavioral conditions having different combinations of the sample cue and paired choice cue, as in Figs. 8 and 12. We were able to do this analysis for 41 neurons among the 88 subtype C2 neurons. For the rest of 47 neurons, recordings were made for only 6 conditions (as in Fig. 4) because of the technical limitation of recording time. As for the 41 neurons analyzed, 13 had information for the location of both circle and triangle in the pair, 19 had information for the location of either the circle or triangle, and the rest of 9 did not have the information.

On the other hand, the 117 neurons categorized as reach-target selective were further classified into three groups: location selective \((P < 0.05 \text{ for the location, } P \geq 0.05 \text{ for the shape, } n = 70)\), shape selective \((P < 0.05 \text{ for the shape, } P \geq 0.05 \text{ for the location, } n = 20)\), and location and shape selective \((P < 0.05 \text{ for the shape, } P < 0.05 \text{ for the location, } n = 27)\). We then analyzed the “type of task selectivity” (cf. Hoshi et al. 1998b) for these reach-target–selective neurons. As a result of ANOVA analysis (factor, type of task), 21 of the 70 location-selective neurons, 8 of the 20 shape-selective neurons, and 11 of the 27 location- and shape-selective neurons showed the type of task selectivity.

**FIG. 10.** PF neuron whose activity was selective for the shape of the reach target. In the shape-matching task, this neuron discharged more often when the target was a circle (A, C, and E) than when it was a triangle (B, D, and F). The response-onset latency in A was 560 ms after the onset of the choice cue.

**FIG. 11.** PF neuron whose activity was selective for both the location and shape of the target. When the target was a circle, this neuron was more active when the target was in the top frame (A and G) than when the target was in the left (B and H) or right (C and I) frames. Moreover, when the target was in the top frame, this neuron was more active if it was a circle (A and G) than if it was a triangle (D and J). The response-onset latency in A was 660 ms. In the control task, this neuron was active when the target was a circle in the top frame (M), but it was not active when the circle was in the right frame (N).
The response-onset latency of the configuration-selective neurons was significantly different (ANOVA, F ratio = 73.8, P < 0.0001). To further facilitate the comparison of the temporal development of the configuration-selective, target-selective, and delay-related activity, we calculated population activity histograms for each of the three types of neurons. The population histograms were calculated separately for the location-match and shape-match trials, as shown in Fig. 13. To construct the population histogram, we added each 20-ms bin of individual histograms for individual cells of each type, after selecting histograms showing the most prominent activity increase during the delay or choice-cue period. The population histogram, then, was recalculated so as to express the activity relative to the control values (calculated during the last 500 ms of ITI). The configuration-activity histogram for the location-match was made from the subtype C1 neurons (n = 23), and the histogram for the shape-match was made from the subtype C2 neurons (n = 88). The target-selective activity histogram was made from neurons that were selective for the location of the target (n = 70). The delay-related activity histogram for the location-match was calculated from the data for location- (n = 121) and location and shape- (n = 27) selective neurons during the location-matching task, and that for the shape-match was calculated from the data for shape- (n = 11) and location and shape- (n = 27) selective neurons during the shape-matching task. For the location match task (Fig. 13, top), the delay-related activity (in green) developed gradually during the delay period, exceeded the response ratio of two at 1,140 ms before the choice cue onset, reached its peak 140 ms after its onset, and fell below the response ratio of four at 360 ms. The configuration selective activity (blue) increased sharply after the choice-cue onset, exceeded the response ratio of four at 140 ms, reached its peak at 200 ms, and fell below the response ratio of four at 640 ms. The target selective activity (red) developed immediately after the configuration-selective activity, exceeding the response ratio of two at 180 ms, and reaching its peak at 480 ms. The activity remained above the response ratio of two until 2,700 ms after the choice cue onset, that is, 1,200 ms after the onset of GO signal. For the shape match task (bottom), the delay-related activity exceeded the response ratio of two at 820 ms before the choice cue onset, reached its peak 180 ms after its onset, and fell below the response ratio of two at 520 ms. The configuration-selective activity exceeded the response ratio of four at 140 ms, reached its peak at 240 ms, and fell below the response ratio of four at 500 ms. The target selective activity exceeded the response ratio of two at 220 ms, reached its peak at 520 ms, and fell below the response ratio of two at 2,740 ms after the choice cue onset, that is, 1,240 ms after the onset of GO signal.

The population activity during both location-match and shape-match tasks indicated the following. 1) Shortly after the choice cue appeared, the configuration-selective activity developed quickly before development of the reach-target activity, while a large part of the sample cue information (delivered in the delay-period activity) was still available. 2) By the time when the target-selective neurons became maximally active, the configuration-selective activity passed its peak, and the delay-related activity was largely diminished.
Motor-response period activity

Of the 698 PF neurons, 305 (44%) satisfied the criteria for motor-response period activity. First, we applied the same analysis as for the choice period activity to characterize the neuronal activity during the motor-response period. Of the 305 motor-response–related neurons, 34 (11%) were judged choice cue configuration selective, 32 (10%) target shape selective, 105 (34%) target location selective, 45 (15%) target location and shape selective, and 89 (29%) nonselective. Next, we analyzed the extent to which the type of task (match location or shape) influenced the neuronal activity, because the type of task appeared to be one of the primary determinants of movement-related neuronal activity in the PF (Hoshi et al. 1998b). Of the 305 motor-response–related neurons, 102 were classified as “type of task selective” (ANOVA, \( P < 0.05 \)) (Hoshi et al. 1998b). We then studied the relationship between the neuronal distribution according to the classifications based on the two analyses. We found that 27 of the 34 configuration-selective neurons were also selective for the type of task. Furthermore, 15 of the 32 shape-selective neurons, 36 of the 105 location-selective neurons, 20 of the 45 neurons selective for location and shape, and 4 of 89 nonselective neurons showed selectivity for the type of task.

Neuronal activity in the primary motor cortex

We did not find any neurons that displayed significant activity changes selectively during the sample cue period in the arm area of the primary motor cortex (MI). Similarly, no MI neurons displayed delay period–specific activity. On the other hand, we found three types of neuronal activity related to arm movement during the task performance. The first type was observed in 88 of the 278 task-related neurons. They showed phasic activity when animals pressed the hold button, as shown in a typical example illustrated in Fig. 14A. Thirty-five neurons were tonically active while the hold button was pressed. As shown in Fig. 14B, the second type exhibited continuous activity during the entire time the hold button was pressed. The third type of activity was found in 233 neurons that were active during the motor-response period, like the example shown in Fig. 14C. The rasters and histograms in Fig. 14C are aligned both on the onset of the sample cue and on the hold-key release, clipped 1.5 s after the delay onset, and restarted at 3.5 s before the key release. Although the activity of the neuron in Fig. 14C was suppressed when pressing the hold button, it did not respond to the appearance of the sample cue. For the third type of neurons with movement-related activity during the motor-response period, we performed the same analysis as for the PF neurons. Of the 233 motor-response–related neurons, 131 (56%) showed location selectivity and 102 (44%) neurons were classified as nonselective. No neurons were classified as selective for the shape, location and shape, or configuration of the choice cue. Only 1 of the 131 location-selective neurons showed type-of-task selectivity.

Comparison of neuronal activity in PF and MI

We found three major differences in the activity of PF and MI neurons. First, although we found cue-, delay-, and choice-related neuronal activity in PF, we did not observe these types of activity in MI neurons. Second, a considerable proportion of MI neurons (32%) showed phasic activity when the subject pressed the hold button, and 13% of them had tonic activity while the hold button was pressed. In contrast, only 10 of 698 PF neurons (1.4%) had phasic discharges when the subject pressed the hold button, and no neurons exhibited tonic activity that continued while the button was pressed. Third, further differences were found between the PF and MI neurons that showed motor-response–related activity. Although we found PF neurons that were selective for the shape or location and shape of the target, and for the choice cue configuration, we did not find such selectivity in any MI neurons. Furthermore, in
reconstructed along electrode tracks and plotted on coronal sections of the prefrontal cortex. The recording sites for the four types of cue- and delay-period activity are displayed in Fig. 16, and the recording sites for the five types of choice- and response-period activity are shown in Fig. 17.

We then systematically studied the spatial distribution of PF neurons by counting the neurons in each category in each coronal section, and by summing the data for the four hemispheres. Because we observed task-related neurons (661 of 698 PF neurons, 94.7%) mainly in the ventral bank of the principal sulcus and inferior convexity area (vPS/IC area), we analyzed the types of activity in the vPS/IC area along the rostrocaudal axis. We measured the rostrocaudal location of recording sites of each neuron as the distance from the posterior end of the principal sulcus. The results are summarized in Fig. 18. For the neurons analyzed during all four time periods (sample cue, delay, choice cue, and reach) of the task, location-selective neurons were found significantly more posterior than shape- and location and shape–selective neurons (ANOVA, P < 0.01). For the neurons analyzed during the reach period, shape–selective neurons were found significantly more anterior than shape and location–selective neurons (ANOVA, P < 0.01). Configuration-selective neurons were located significantly more posterior than shape of target–selective neurons (ANOVA, P < 0.05), and significantly more anterior than location of target–selective neurons (ANOVA, P < 0.05).

**Muscimol microinjection into the bilateral prefrontal cortex**

In the first monkey, the muscimol solution was injected at six sites in the dorsal bank of the principal sulcus and superior convexity area (dPS/SC area) and at seven sites in the vPS/IC area. In the second monkey, the muscimol was injected at two sites in the dPS/SC area and three sites in the vPS/IC area. We always injected the solution bilaterally, into symmetrical locations in both hemispheres simultaneously. Figure 19C shows the locations of the injection sites in the left hemisphere of both monkeys.

When we injected muscimol into either the anterior or middle vPS/IC area, the error rate increased significantly in both monkeys. The performance of monkey 2 after a 3-μL injection (at site A in Fig. 19C) is shown in Fig. 19A. We counted the number of selection errors in each 20-min interval while ~50 shape-matching and location-matching trials were performed. For the shape-matching task, the number of errors increased significantly (χ² test, P < 0.01) in all intervals after 20 min. For the location-matching task, the error increased significantly (χ² test, P < 0.01) after 40 min. In contrast, when muscimol was injected into the dPS/SC area, the number of selection errors did not increase significantly. The performance of monkey 2 after a 3-μL muscimol injection into the dPS/SC area (at site B in Fig. 19C) is illustrated in Fig. 19B. The number of selection errors never differed from the value before injection (χ² test, P > 0.05).

To analyze the effects of muscimol injection systematically, we compared the number of selection errors for each task during the 30 min preceding the muscimol injection with the period from 60 to 120 min after the injection. If the number of selection errors increased significantly (χ² test, P < 0.01) for the shape-matching or location-matching tasks, or for both, we
FIG. 16. Coronal sections of the left hemisphere (monkey 2) showing the penetration tracks and recording sites of task-related neurons. A: the most anterior (11 mm anterior from the end of the principal sulcus). K: the most posterior (1 mm anterior from the end of the principal sulcus). Each section is 1 mm apart. Short bars on each side of the tracks (rightward for the cue period, leftward for the delay period) represent the recording sites of task-related neurons. Each color indicates the type of neuronal activity as indicated in the inset at the bottom right. PS, principal sulcus; AS, arcuate sulcus.

FIG. 15. Cortical surface maps showing recording sites in the PF and MI of monkeys 1 and 2. Symbols represent the recording sites where task-related neurons were (●) or were not (○) found. Asterisks indicate the penetration sites where saccades were evoked with thresholds <50 µA. The circle size represents the number of task-related neurons recorded in each penetration. PS, principal sulcus; AS, arcuate sulcus; CS, central sulcus.
defined the injection as effective for the task (or tasks). In the example shown in Fig. 19A, the number of selection errors was significantly increased in both shape matching ($\chi^2$ test, Value = 17.8, df = 1, $P < 0.001$) and location matching ($\chi^2$ test, Value = 10.8, df = 1, $P < 0.01$). The number of selection errors after the injection in the example shown in Fig. 19B was not different from the control values for either shape matching ($\chi^2$ test, Value = 1.56, df = 1, $P > 0.2$) or location matching ($\chi^2$ test, Value = 1.01, df = 1, $P > 0.3$). The RT and MT were not influenced by the injections in any cases (ANOVA, $P > 0.1$).

In summary, we found that the muscimol injections effectively increased the selection errors in the anterior and middle parts of the vPS/IC area (Fig. 19C). In contrast, we did not observe any effects after injections in the dPS/SC area or the posterior part of the vPS/IC area. The RT and MT were not influenced by muscimol injection at any sites (ANOVA, $P > 0.1$), even when the selection error increased. When 3.0 $\mu$L of saline was injected (2 dorsal and 2 lateral injection sites in monkey 1, 1 dorsal and 2 lateral sites in monkey 2), there were no changes in the number of selection errors ($\chi^2$ test, $P > 0.05$).

**DISCUSSION**

In this study we found that a variety of neuronal activity appears in the lateral prefrontal cortex that seems useful in the process of motor selection in conforming to two behavioral rules. The activity seems to play a crucial role in motor selection, because the selection was impaired by temporarily inactivating the part of the prefrontal cortex where the neuronal activity was recorded.

**Rationale for the use of this motor task**

In this study, we designed a behavioral task that required monkeys 1) to make a motor selection following two rules (the location-matching and shape-matching rules), 2) using both memorized and currently available information at the time of motor selection, and 3) to make the selection within a short period of time well in advance of executing the movement. We established an experimental model to study the role of the PF in motor selection conforming to multiple rules by meeting these requirements.
Role of PF neurons in the motor selection process

In our behavioral task, motor selection had to be made during the choice cue period. During the period of motor selection, we identified three types of neuronal activity. The first was a short-latency response to the choice cue (configuration-selective activity, with a mean response-onset time of 180 ms), and we found three subtypes within this type. The first subtype (C1) was a selective response to the choice cue of three circles or three triangles that induced monkeys to perform the location-matching task. The second subtype (C2) was a selective response to the choice cue consisting of a circle and a triangle, which required the shape-matching task. Of note, in the majority of neurons with this subtype of activity, was that the magnitude of the response depended on arrangements of the circle or triangle and had the information of where of three locations (top, left, or right) a circle or a triangle in the pair was positioned. The third subtype (C3) responded to both the three-object and paired-object choice cues. The second (reach-target–selective) type of activity appeared later in the choice period (with a mean response-onset time of 345 ms) and exhibited selectivity for the target. In the majority of cases, the activity was selective for the location of the target, whereas in the rest it was selective for the shape or both location and shape of the target. The third type of activity appeared as a continuation of the delay-related activity and reflected the sample cue, even though the activity was observed during the choice cue period. The activity reflected either the shape or location of the sample cue, or both. The time relationship observed among the three types of responses during the choice cue period (Fig. 13) seems to indicate a manner in which the motor selection process took under way in the prefrontal cortex promptly by integrating different sets of information (i.e., sample and choice cues), giving rise to new information (i.e., reach target).

Selective responses to the spatial arrangement of multiple visual objects

In this study, we found that the selective response to the choice cue is one of the prominent aspects of the visual responses of PF neurons. Of particular interest are the responses specific to the spatial positions of the circle and triangle. As seen in the example in Fig. 8, the selectivity of a subtype of responses to the choice cue depended on the relative spatial location of the two visual objects. This selectivity means that PF neurons of that type receive information about both the spatial localization and shape of visual objects, and the two sets of information converge in individual PF neurons. This supports the recent proposition that the information characterizing “where” and “what” aspects of visual objects converge at the neuronal level in the PF (Rainer et al. 1998a,b; Rao et al. 1997). Furthermore, we found novel aspects of the response of PF neurons in the visual properties of the choice.
cues: the selective responses to three objects (Fig. 7) and paired objects (Figs. 8 and 12). What is intriguing about the former is that these neurons did not respond to single or paired objects, but responded to either three circles or triangles. In the latter group, the selectivity was for a circle and a triangle. Are these responses specific for the choice period? In the control experiment, we presented either the triple or paired objects as the sample cue, which was meaningless for the selection process in the behavioral task. Nevertheless, we invariably found comparable neuronal responses, indicating that the appearance of visual objects with a specific configuration was sufficient to activate those types of neurons. These response properties indicate that the neuronal activity in the PF in response to multiple objects expresses the configuration as “triple” or “pair,” leading to the possibility that PF neurons may be involved in categorizing groups of visual objects. It seems probable that such sophisticated neuronal activity in the PF is the outcome of behavioral training that calls for the development of activity necessary to achieve a particular behavioral task.

Distribution of task-related activity within the PF

It is remarkable that the great majority of the activity related to any period in the behavioral task was found in the ventral bank of PS and IC area. There were very few task-related neurons in the dorsal bank of PS and SC area of the lateral PF. At least two factors seem to account for this distribution. First, in this study, we required monkeys to select forelimb movements, but not eye movements. In previous studies employing oculomotor control tasks (Barone and Joseph 1989b; Funahashi et al. 1989; Hasegawa et al. 1998; Rao et al. 1997), the area dorsal to the PS was invariably rich in task-related neurons. In most studies of limb motor control tasks, active spots are found in the ventrolateral PF (Boussaoud and Wise 1993b; di Pellegrino and Wise 1991, 1993; Funahashi et al. 1997; Kubota et al. 1974; Niki 1974; Niki and Watanabe 1976; Rosenkilde et al. 1981; Sakagami and Niki 1994a; Watanabe 1981). Romo et al. (1999) reported neuronal activity reflecting the frequency of mechanical vibration in the inferior convexity. However, the task-related neuronal activity has been reported in the ventral bank of PS and IC in oculomotor tasks (Funahashi et al. 1989; Hasegawa et al. 1998; Rao et al. 1997) and in the dorsal bank of PS and SC in the limb motor tasks (Quintana and Fuster 1999). Therefore the spatial segregation of oculomotor and limb-motor activity in the PF is not decisive. We need further studies in which both eye and limb movements are included in a behavioral task (Mushiake et al. 1996; Snyder et al. 1997). Second, the differences between the behavioral requirements of this study and previous studies may produce the disparate distribution of task-related neurons. Wilson et al. (1993) reported that the neurons in the dorsolateral PF (above the principal sulcus) were predominantly active during spatial delayed response tasks, whereas ventrolateral PF neurons were predominantly active during delayed response tasks requiring object identity processing. In our behavioral task, concurrent processing of shape and location information was required.

We examined the spatial distribution of location-selective and shape-selective neurons during the sample cue, delay, choice cue, and motor response periods. During all these periods, we found more location-selective neurons in the posterior part of the lateral PF, whereas more shape-selective neurons were found in the anterior part. This finding supports the concept of areal segregation of spatial and object information processing in the PF (Courtney et al. 1998; Goldman-Rakic 1988; Wilson et al. 1993). In recent reports, Rushworth and co-workers (Rushworth et al. 1997; Rushworth and Owen 1998) summarized results obtained from positron emission tomography studies of human subjects. They found that spatial tasks activated more posterior foci in the PF, whereas object (or color) detection tasks tended to activate more anterior foci. White and Wise (1999) reported that neurons in the posterior portion of the dorsolateral prefrontal cortex were more active when the subjects attended to the spatial attributes of the visual cue than when attending to nonspatial attributes, although their segregation was mainly concerned with conditional rule versus spatial rule rather than shape versus location. We examined whether the location- and shape-selective neurons were distributed differentially in the ventral bank of the principal sulcus and in the inferior convexity. We found that the distribution was not different ($\chi^2$ test, $P > 0.1$), although we must admit that had we sampled more neurons laterally in the inferior convexity area that has massive connections with inferior temporal cortices (Barbas 1988), we might have found more shape-selective neurons. A recent report revealed areal segregation of face-processing neurons in the inferior convexity (Ó Scalaidhe et al. 1997). Apparently, we sampled neurons responding to plain visual objects, and not face-selective neurons.

Comparison of our results with previous reports on the PF

Previous neurophysiological studies have established a basic principle for neuronal activity involved in coordinating sensory input with motor output that constitutes purposeful behavior in the PF of monkeys (Fuster 1998; Goldman-Rakic 1987a; Leon and Shadlen 1998; Miller 1999). Neurons respond to visual signals, which represent the identity (di Pellegrino and Wise 1991; Miller et al. 1996; Ono et al. 1984; Quintana et al. 1988; Rao et al. 1997; Rosenkilde et al. 1981; Villa and Fuster 1992; Wilson et al. 1993) or spatial location of visual objects (Battue et al. 1985; Funahashi et al. 1990). The sensory information is stored in short-term memory to be prepared for subsequent use (Funahashi et al. 1989; Fuster and Alexander 1971; Goldman-Rakic 1987a; Miller et al. 1996; Quintana and Fuster 1992; Rainer et al. 1998b; Watanabe 1981). This has been reported extensively as delay-period activity. As revealed previously in oculomotor (Boch and Goldberg 1989; Funahashi et al. 1991) and limb motor tasks (Barone and Joseph 1989a; Boussaoud et al. 1995; Carlson et al. 1997; Kubota and Funahashi 1982; Kubota and Niki 1971; Sawaguchi 1987), the PF neurons also participate in movements. Therefore most of the activity observed in our study conforms to the general participation of PF activity in sensory-motor integration (Fuster 1997; Quintana and Fuster 1999). Previous reports describe the PF activity related to the decision of whether to initiate a motor task in a GO/NO-GO task (Kubota and Komatsu 1985; Rolls et al. 1996; Sakagami and Niki 1994a,b; Thorpe et al. 1983; Watanabe 1986a,b), or related to the decision to initiate limb or saccadic eye movements (Asaad et al. 1998; Boussaoud and Wise 1993b; Hasegawa et al. 1998; Kim and Shadlen 1999;
Our study clarifies the way in which the PF neurons participate in solving a complex problem of motor selection following more than one rule. This resulted in the discovery of three types of neuronal activity during the choice period, the period critical for motor selection.

It is of interest to compare the timing of neuronal activity involved in motor decision making in previous studies and this study. Hasegawa et al. (1998) showed that information for the direction of future saccades appeared within 200 ms in their oculomotor delayed matching to a sample task. Asaad et al. (1998) reported that, as a result of learning the association between a cue and response, the neuronal activity reflecting the direction of saccades appeared at 250 ms. Kim and Shadlen (1999) found that the PF activity revealing a monkey’s decision for the direction of a forthcoming saccade appeared as early as 100–200 ms after the appearance of a visual cue. In the orbitofrontal cortex, it has been reported that the discrimination of reward/nonreward occurs mainly between 140 and 210 ms after the onset of visual stimulus (Thorpe et al. 1983). In the frontal eye field, it has been reported that target discrimination for future saccades occurs between 120 and 150 ms (Schall and Thompson 1999; Thompson et al. 1996). These values are comparable with the latency for the configuration-selective responses to the choice cue in our study. In our study, the appearance of target-selective activity came later (mean, 345 ms). This is reasonable, because in our behavioral task the animal had to determine the type of task as well as select a target.

**Contrasting neuronal activity in the prefrontal and primary motor cortex**

Our findings revealed that the properties of neuronal activity in the lateral PF differed from those in the MI in many respects. First, a variety of neuronal responses to the sample cue, either shape-selective or location-selective, appeared only in the PF and not in the MI. Second, no shape-selective or location-selective activity in the delay period appeared in the MI. Third, the responses to the choice cue categorized as configuration-specific activity were not found in the MI. Fourth, in the MI, the selectivity of activity during the movement period and in the preparatory period preceding motor initiation depended on the location of the target. Movement-related activity selective to the shape of the target was frequently found in the PF, but not found in the MI. Fifth, phasic activity related to pressing the button to initiate the behavioral task was commonly found in the MI, but only infrequently in the PF. Furthermore, tonic activity that continued the entire time the button was pressed (encompassing the sample cue, delay, and choice cue periods) was found in the MI, but not in the PF. Taken together, it appears that the neurons in the PF and MI played roles in different aspects of the behavioral task, suggesting a minor role, if any, for the MI neurons in the process of motor selection. Because the neuronal activities reflecting the serial order of stimulus presentation (Carpenter et al. 1999) or stimulus-response mapping rule (Riehle et al. 1994) have been reported in the MI, we carefully looked for the neuronal activity that may reflect the motor selection process. However, we failed to observe the selection-related activity. It seems that differences in experimental conditions or the behavioral context may explain the seemingly disparate results.

**Methodological consideration and limitations of interpretation**

In this study, monkeys were required to perform the behavioral task by controlling body movements (primarily the forelimb), but not eye movements. Thus if oculomotor tasks were imposed, the properties of neuronal activity and the spatial distribution of cue-, delay-, and choice-period activity in the PF may appear different. On the other hand, we looked for differences in eye movements during the three periods depending on whether the animals performed the shape-match or location-match task. First, we analyzed the frequency of saccades during the three periods. Subsequently, we analyzed the time period during which the gaze was on either the cued frame during the sample cue and delay periods or on the frame containing the target during the choice cue period. We did not find any differences in these values that depended on whether the task was based on shape matching or location matching. Therefore it seems reasonable to discard the possibility that the animals’ oculomotor behavior was different in any purposeful manner.

Muscimol injection into the middle and anterior parts of the ventral bank of the PS and the IC area induced impairments in the task performance. The impairments were not caused by the failure in the execution process during the motor performance because the RT and MT were unchanged. However, it was not tested whether the impairments were specific to the performance in the two-rule task or should have been observed even when the animals were performing the shape-matching task only or location-matching task only. Therefore it remains to determine whether the impairments are related to the performance of either task alone or are specific to the two-rule task.

**Concluding remarks**

We found three main types of neuronal activity in the lateral PF that seems to be instrumental in performing motor selection concurrently conforming to two behavioral rules. The neuronal activity providing the remembered information and the activity selective for the current information appear to be temporally structured in a way strategically appropriate for contributing to the activity specifying the target to be selected for future movement. These findings point to a crucial involvement of the PF in the process of motor selection complying with complex behavioral rules.

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