Activity in the Lateral Prefrontal Cortex Reflects Multiple Steps of Future Events in Action Plans

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Summary

To achieve a behavioral goal in a complex environment, we must plan multiple steps of motor behavior. On planning a series of actions, we anticipate future events that will occur as a result of each action and mentally organize the temporal sequence of events. To investigate the involvement of the lateral prefrontal cortex (PFC) in such multistep planning, we examined neuronal activity in the PFC of monkeys performing a maze task that required the planning of stepwise cursor movements to reach a goal. During the preparatory period, PFC neurons reflected each of *all* forthcoming cursor movements, rather than arm movements. In contrast, in the primary motor cortex, most neuronal activity reflected arm movements but little of cursor movements during the preparatory period, as well as during movement execution. Our data suggest that the PFC is involved primarily in planning multiple future events that occur as a consequence of behavioral actions.

Introduction

It has long been thought that the lateral prefrontal cortex (PFC) is involved in the executive control of behavior (Goldman-Rakic, 1987; Fuster, 1990; Passingham, 1993; Petrides, 1996; Wise et al., 1996; Watanabe, 1996; Kim and Shadlen, 1999; Miller, 1999; Hoshi and Tanji, 2004). Planning future actions to achieve a goal is an important aspect of executive control, and the PFC has been inferred to represent behavioral goals (Fuster, 1997; Leon and Shadlen, 1999; Saito et al., 2005). To achieve a behavioral goal in daily life, we often need to plan multiple steps of motor behavior that involve selection of a series of actions. The question arises: how are individual neurons within the PFC involved in the plan-

ning of multistep behaviors? More specifically, does the activity of PFC neurons during the process of planning reflect the multiple movements required during future actions or the individual future events that occur as a result of the actions? To answer this question, we examined neuronal activity in the dorsolateral PFC of monkeys that performed a path-planning task that required multiple stepwise movements of a cursor within a maze to reach a goal.

Figures 1A and 1C show the sequence and timing of events for each trial in our behavioral task. As the monkeys placed their wrists in neutral positions, a start display appeared, followed by a goal display. When the start display changed its color after delay periods, the monkeys initiated the first movement. Thereafter, they performed the second and third movements, each after a delay period, to capture the goal. Our previous behavioral study demonstrated that this task provides a behavioral model in which monkeys were able to plan multiple steps of actions in advance to avoid obstacles and achieve a goal (Mushiake et al., 2001). In the present study, we dissociated the actions (arm movements) from the events that occur as a result of the actions (motions of a cursor in a visual display) by incorporating several different assignments that defined the relationship between arm movements and cursor movements (Figure 1B). We used two sets of goals in the maze in which pathways were partially blocked in a variable manner (Figure 2A). We analyzed neuronal activity in the dorsolateral PFC (Figure 2B) during a preparatory period that preceded the GO signal for the first movement.

Here we report that neuronal activity in the PFC predominantly reflected multiple future events that would ensue as a result of planned actions, rather than the arm movements required to perform these actions, in contrast to activity in the primary motor cortex that primarily reflected movements of the arm.

Results

Overall Proportion of Neurons with Identified Properties

We trained animals to be able to reach final goals at a success rate averaging more than 85%. It took about a year for the animals to perform at this level. During recording sessions, the success rates for the two animals were 93.1% and 89.4% (not significantly different). We found that 470 of 1020 neurons sampled in the PFC exhibited activity during the delay period that differed significantly from activity during the control period (p < 0.01, Wilcoxon's signed-ranks test). For these 470 neurons, we performed linear regression analysis to elucidate the relationships among the four behavioral variables described in the Experimental Procedures. This analysis revealed that the activity of 176 PFC neurons that exhibited preparatory activity was related to the position of the final goal, while the activity of 88 neurons was related to the path block; the activity of 31 neurons was related to both the goal position and path block (Table 1). The properties of these neurons will be
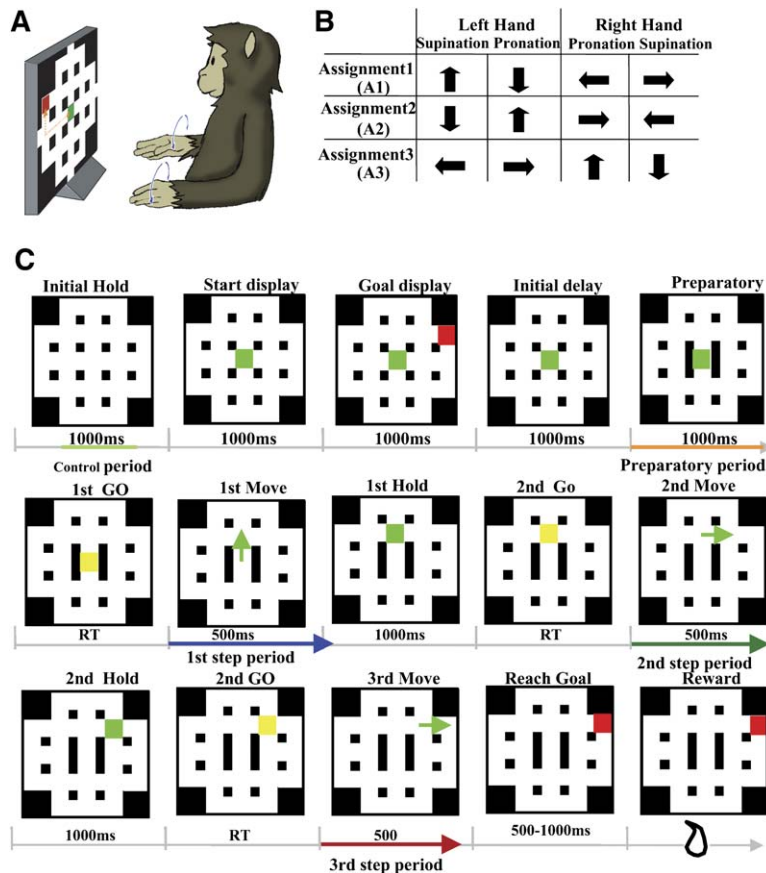
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Figure 1. Behavioral Conditions and Task Sequence

(A) Experimental setup.

(B) Directions of cursor movements (arrows) assigned to supination (SUP) or pronation (PRO) of either arm. Arm movements were assigned to each cursor movement in three different ways. A1–3 represents arm-cursor assignments 1–3.

(C) Temporal sequence of events during the behavioral task. The behavioral sequence is depicted from the top left to the bottom right. Each panel represents a maze that was displayed on a computer monitor. Green and red squares denote the start position and the location of the final goal, respectively. Yellow squares represent a signal for the initiation of movement (GO signal). Green arrows indicate the directions of cursor movements.

described in detail in a separate paper (also see the Supplemental Data available online). The activity of 210 neurons was found to be related to the motion of the cursor. In contrast, the activity of only 27 PFC neurons was related to arm movements.

Among the remaining neurons that were not significantly modulated during the preparatory period, 267 neurons were active during the movement execution period. We found that 236 out of the 267 neurons were related to the cursor motion, whereas 31 neurons were related to arm movements. We also found that a group of PFC neurons responded to the goal display (n = 61). Others were active after reaching the final goal (n = 81) or after the reward delivery (n = 93).

Preparatory Activity that Reflected Cursor Movement
For the 210 PFC neurons for which activity during the preparatory period was related to cursor movement, we used linear regression analysis to further examine the relationship between the activity of these neurons and the first, second, and third cursor movements. We found that 111 PFC neurons were selective for the first of the three cursor movements. A representative example of such activity is presented in Figure 3A. In this example, neuronal activity during the delay period was significantly greater (p < 0.01) when the monkey was preparing to move the cursor to the goal using three steps, including R-R-U, R-U-R, R-R-D, and R-D-R (R, U, and D denote cursor movements directed toward the right, up, and down, respectively), but not with the

other steps shown in the figure. The common factor among these steps was that the cursor movement during the first step was directed to the right. Of 111 neurons with first cursor movement-related activity, activity was selective for either rightward (n = 31), leftward (n = 24), upward (n = 26), or downward (n = 30) motion. An alternative explanation for the apparent selectivity of movement direction is that activity was related to the intended location of the cursor after the first cursor movement (to the right, left, top, or bottom of the start position). In this study, no attempts were made to differentiate between these two possibilities.

For 87 of the 210 PFC neurons with preparatory activity, we found that there was selectivity for the second of the three steps of the planned cursor movement. An example of such selectivity is presented in Figure 3B. In this example, neuronal activity was observed selectively when the planned three-step cursor movements were U-L-U and D-L-D (L denotes a cursor movement directed to the left). Thus, the neuron in Figure 3B exhibited selectivity for leftward motion of the intended second movement. In 95 of the 210 PFC neurons with preparatory activity, activity was selective for the third of the three steps of the planned cursor movements. An example of such selectivity is presented in Figure 3C, which illustrates that neuronal activity was selective for the steps U-U-L and D-D-L, i.e., this neuron exhibited selectivity for leftward motion of the intended third movement. These findings indicate that each of the three cursor movements (first, second, and third) that

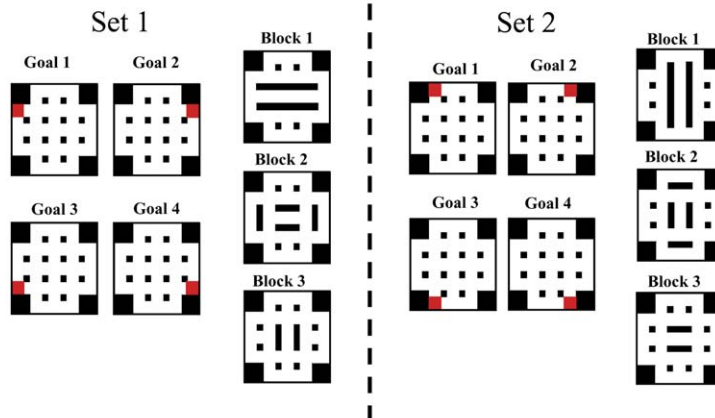## A Goals and Path Blocks used in a Recording Session

Figure 2. Goals and Path Blocks Used in a Recording Session and Cortical Surface Maps of Recording Sites

(A) Two sets of behavioral conditions for the choice of goal positions (Goals 1–4) and path blocks (Blocks 1–4). Either set 1 or set 2 or both were used while recording the activity of individual prefrontal cortex (PFC) neurons.

(B) Recording sites corresponding to the locations of neurons that exhibited selectivity for cursor movement. The number of neurons selective for the first, second, or third cursor movement is plotted separately for each penetration site. The size of the circle is proportional to the number of neurons. PS, principal sulcus; ARC, arcuate sulcus.

B Locations of Cursor-motion Selective Neurons
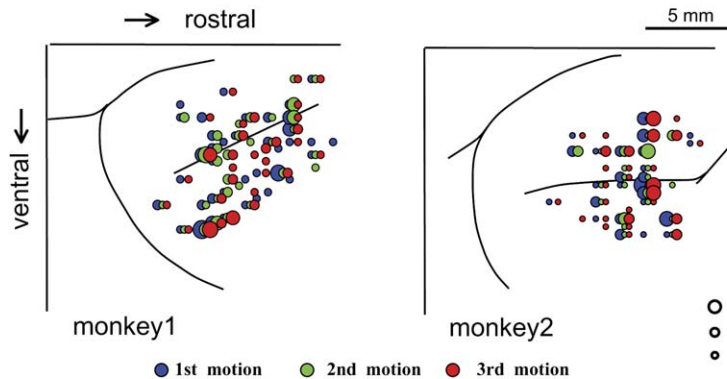
were planned before initiation of the first cursor movement were represented amply by the activity of PFC neurons during the delay period that preceded movement. The recording sites at which the aforementioned neurons were located are presented in Figure 2B. The neurons were distributed widely throughout the banks of the principal sulcus and the dorsal and ventral surface of the periprincipal region. There did not appear to be any tendency for PFC neurons with selective activity to be located within specific regions.

Correspondence between Preparatory Activity and Activity during the Motor Execution Period
A majority of the neurons for which activity was related to the preparatory period (described above) did not exhibit activity during the period in which the three movement steps were executed (motor execution period). Nevertheless, activity in 37% of the preparatory period-related neurons was also detectable during the motor execution period. We examined the relationship between the activity of these neurons and the three

directions of cursor movement by analyzing neuronal activity during the first, second, and third movements with the same linear regression analysis used to analyze activity during the preparatory period ($p < 0.01$). Among the 111 neurons that exhibited selectivity for the first cursor movement during the preparatory period, 48 neurons (43%) also exhibited selectivity for the first cursor movement during the first movement. Activity of a representative example of such a neuron is presented as perievent spike density histograms in the left panel of Figure 4A. The four histograms show the time course of activity for each of the trials beginning with the first step of cursor movement directed to the right (R-x-x), left (L-x-x), up (U-x-x), or down (D-x-x). Only the histogram representing activity during the first cursor movement to the right (R-x-x) exhibited prominent peaks during the preparatory period and the first movement. To quantify the amount of information contained in timevarying neuronal activity, we calculated the predictive information (Is) carried by the occurrence of spikes by quantifying the decrease in entropy in the cursor

Table 1. Classification of the Activity of Preparatory Prefrontal Cortex Neurons and Primary Cortex Neurons

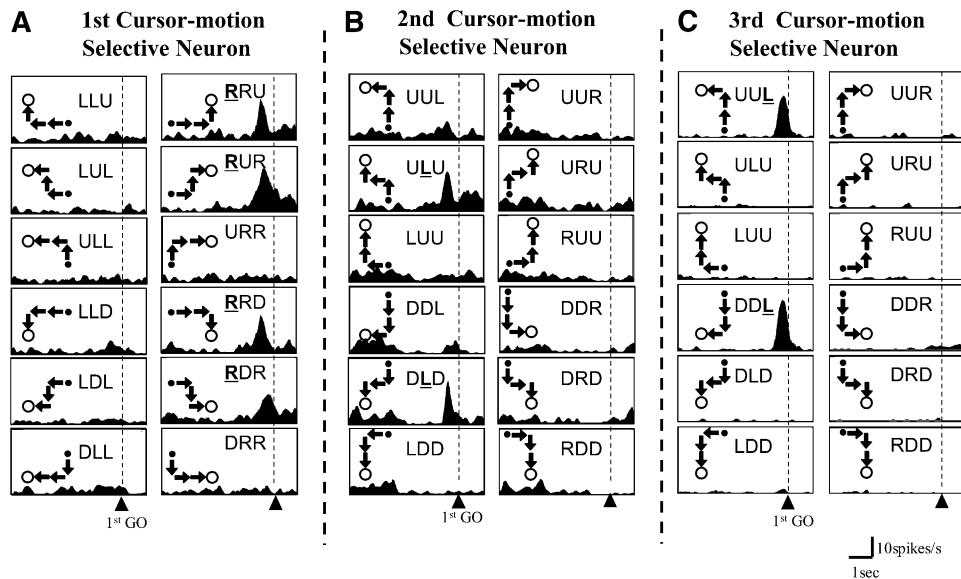| Area	Total	Cursor-Movement Related	Arm-Movement Related	Goal-Location Related	Path-Block Related	Goal and Block Related
PF	470	210	27	176	88	31
MI	72	5	60	3	4	0

Figure 3. Preparatory Activity of Three Examples of PFC Neurons

Each panel contains a perievent spike-density histogram sorted according to the sequence of the three cursor movements required to reach the final goal. The histograms are aligned to the appearance of the first GO signal (filled triangles). (A) First-cursor-selective neuron. This neuron was selectively active during the preparatory periods that preceded sequences of cursor movements that included a rightward movement in the first step, i.e., rightward (R)-R-upward (U), R-U-R, R-downward (D)-R, and R-R-D. The vertical bar at bottom right denotes 10 spikes/s.

(B) Second-cursor-selective neuron. This neuron was active prior to the initiation of sequences of cursor movements that included a leftward cursor movement in the second step, i.e., U-leftward (L)-U and D-L-D.

(C) Third-cursor-selective neuron. This neuron was active prior to the initiation of sequences of cursor movements that included a leftward cursor movement in the third step, i.e., U-U-L and D-D-L.

direction as described in the Experimental Procedures. The amount of information that predicted the first movement (blue trace in the right panel in Figure 4A) exceeded statistical significance (p < 0.01) during the preparatory period and also during the first movement.

Next, we analyzed the activity of 87 neurons that exhibited selectivity for the second cursor movement during the preparatory period. Among these neurons, 27 (31%) also exhibited selectivity for the second movement. Figure 4B illustrates an example of such second-cursor-movement-selective activity. In Figure 4B, the spike density histogram for trials that included leftward cursor movement during the second step (x-L-x) exhibited two prominent peaks that corresponded to the preparatory period and the second movement. Quantitative analysis of information predicted by time-varying neuronal activity (right panel of Figure 4B) revealed two prominent peaks that predicted the second cursor movement (green trace) during the preparatory period and during the second movement. A similar analysis of 95 neurons that exhibited selectivity for the third cursor movement during the preparatory period revealed that 34 neurons (36%) also exhibited selectivity for the third movement during the execution period. An example of neuronal activity that exhibited such selectivity is presented in Figure 4C. The spike density histogram illustrates that activity for those trials that included leftward cursor movement during the third step (x-x-L in the left panel) exhibited two prominent peaks during the preparatory period and third movement. Activity in this neuron strongly predicted the third movement during the preparatory period and the third movement (right panel in Figure 4C).

To quantify the correlation between the magnitudes of information that predicted the first cursor movement during the preparatory period and during the execution of the first movement, a cross-correlation coefficient was calculated for the 111 neurons that exhibited selectivity for the first cursor movement during the preparatory period. As illustrated in the left panel of Figure 5B, in most of these neurons, direction selectivity during the preparatory period was correlated positively (p < 0.01) with selectivity during the first movement. Similarly, in most of the 87 second-movement-selective neurons, direction selectivity during the preparatory period correlated positively (p < 0.01) with selectivity during the second movement (Figure 5B, middle panel). In most of the 95 third-movement-selective neurons, direction selectivity during the preparatory period correlated positively (p < 0.01) with selectivity during the third movement (Figure 5B, right panel).

Population Analysis

To what extent does the entire population of preparatory period-related PFC neurons reflect cursor movement or arm movement? What is the time course of information that reflects cursor movement? To answer these questions, we used the perievent spike-density data for all of the 470 preparatory period-related neurons to calculate the bin-by-bin average of information (It) that predicted the first, second, and third cursor movements. The results of this analysis are presented in Figure 5, in which the time-varying magnitude of quantified information is plotted. As illustrated in Figure 5A (upper panel), each of the three cursor movements was represented during the delay period, while information that reflected

A   **1st Cursor-motion Selective Neuron**

B   **2nd Cursor-motion Selective Neuron**

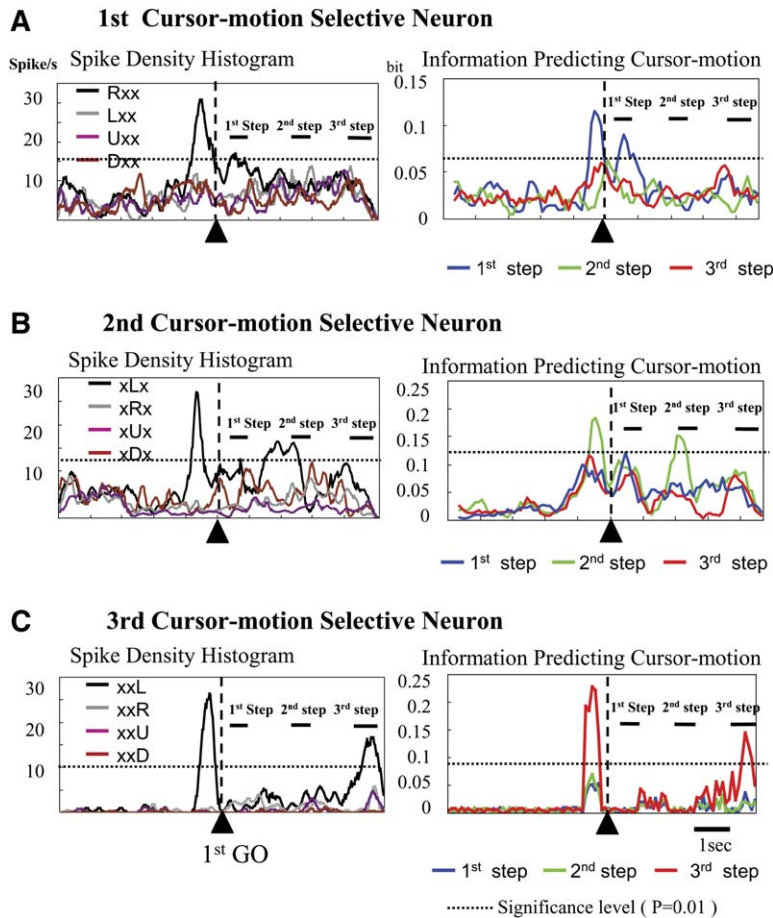**C**   **3rd Cursor-motion Selective Neuron**

Figure 4. Three Examples of PFC Neurons Exhibiting Selectivity for Cursor Movement during Both the Preparatory and Execution Period

In (A–C), the left column presents spike-density histograms, while the right column presents neuronal information predictive of cursor motions. The histograms, aligned to the appearance of the first GO signal (filled triangles), are sorted according to the direction of the first (A), second (B), or third (C) cursor movement. In the right column, magnitudes of information predictive of the first (blue), second (green), and third (red) cursor movement, carried by the activity of the neurons in (A)–(C), are plotted in 100 ms bins. (A) First-cursor-motion-selective neuron. This neuron exhibited an increase in activity when the first movement was in the rightward direction.
(B) Second-cursor-motion-selective neuron. This neuron exhibited an increase in activity when the second movement was in the leftward direction.
(C) Third-cursor-motion-selective neuron. This neuron exhibited an increase in activity when the third movement was in the leftward direction. Information was carried predominantly during the preparatory period and also during the first (A), second (B), or third (C) movement period.

the first, second, and third cursor movements also appeared during and immediately before the first, second, and third movements, in that order. In contrast, information that predicted the first, second, and third arm movement remained at low levels throughout the task and never reached statistical significance (p > 0.01) (Figure 5A, lower panel). We performed an additional analysis by aligning the population data on both the second and third GO signals, and presented the data with six panels in Figure S2A. As a result of this analysis, we confirmed our point that, for PFC neurons, information predicting cursor motion exceeded a statistically significant level (p < 0.01), whereas information predicting arm movement did not.

Contrasting Neuronal Activity in the Primary Motor Cortex
For the purpose of comparison, we also examined neuronal activity in the MI. In the arm area of MI, we obtained 72 neurons that were defined as preparatory related (Table 1) and 55 neurons that were defined as nonpreparatory related. All of the preparatory MI neurons were active during both preparatory and execution periods. Of these, 60 neurons were found to be arm-movement related. A typical example of such MI neurons is presented in Figure 6A. That neuron was active during the preparatory period when the first arm movement was left pronation (panel in the top left). In addition, the same neuron was active during the movement period for left

pronation, regardless of whether that movement appeared in the first, second, or third movement step. For this neuron, information predicting the cursor movement was negligible, in sharp contrast to the richness of information predicting arm movement (right panels in Figure 6A). Results of population analysis for the 72 MI neurons are presented in Figure 6B, where it is apparent that neuronal activity in the MI predicted each of the three arm movements, whereas information predicting the cursor movement did not reach a statistically significant level. We confirmed our findings by aligning the data to the second GO (left panels in Figure S2B) and to the third GO (right panels) signals.

Relationship between Neuronal Activity and Eye Positions and Movements
We examined whether any of the neuronal activity described above might be related to eye positions or eye movements during the preparatory and execution periods. We performed a multiple regression analysis using four sets of regressors, namely the vertical and horizontal eye positions and the vertical and horizontal components of saccades (see Experimental Procedures). Figures 7A–7C illustrate representative results of such an analysis of the activity of the three neurons, for which activity is presented in Figures 4A–4C. In Figure 7, we plotted the regression coefficients for the four regressors sequentially across time in 50 ms bins after normalizing these values to T values that corresponded

A   Population Analysis for PF Neurons

(i) Information Predicting Cursor-motion

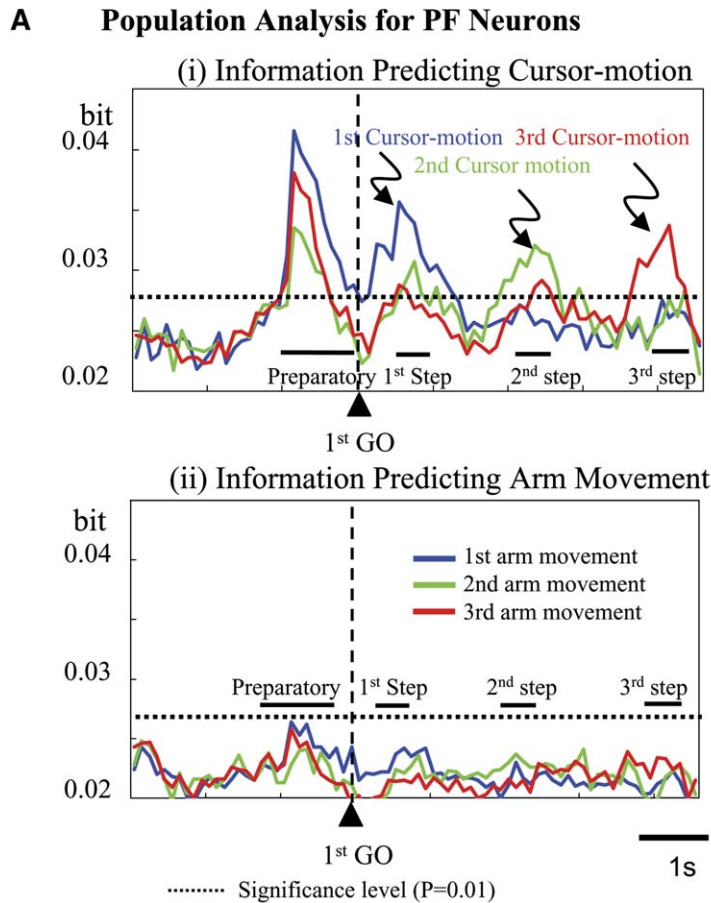### (ii) Information Predicting Arm Movement

Figure 5. Correspondence between Preparatory Activity and Movement-Period Activity

(A) Population analysis of PFC neurons. (Ai) Time course of information that predicted the first (plotted in blue), second (green), and third (red) cursor movements. The magnitude of information predictive of cursor movement carried by neuronal activity (spikes) was calculated as described in the Experimental Procedures and is plotted in consecutive 50 ms bins. Values obtained from all preparatory period-related PFC neurons are averaged. Data are aligned to the onset of the first GO signal. Information that predicted each of the three cursor movements was most prominent (peak) during the preparatory period, but there was an additional peak both prior to and during the execution of each movement. (Aii) Time course of information that predicted each of the three arm movements. The magnitude of information that predicted each arm movement did not reach a statistical significance level of 0.01.

(B) Distribution of cross-correlation coefficients. Data are calculated for all cursor-movement-selective PFC neurons, for comparison of direction selectivity during the preparatory and motor execution periods. The ordinate indicates the relative frequency distribution of the cross-correlations.

B   Cross-Correlation Analysis for Direction Selectivity
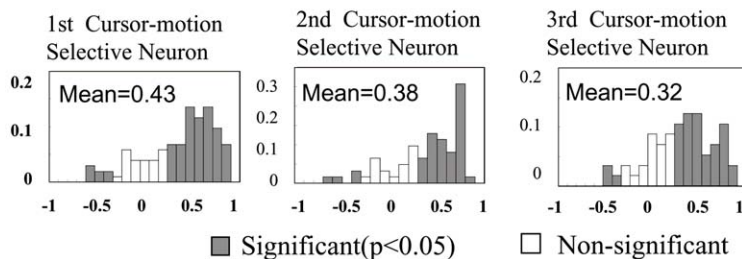
to a significance level of 0.01. As illustrated in Figures 7A–7C, none of these three neurons exhibited activity that was significantly related to eye position or eye movement during the preparatory period or during the execution of movements. We subsequently extended our analysis to all 470 neurons that exhibited preparatory activity. Figure 7D illustrates sequential plots of time-varying mean values of the regression coefficients normalized to the T value. In Figure 7D, the regression coefficients remained well below statistical significance (1.0) during all of the periods that were analyzed, which indicated that neuronal activity was not significantly related to eye position or eye movements. Examination of data for individual neurons revealed that eight neurons exhibited activity that appeared to be related to eye movements during the goal display period, but no neurons exhibited activity that was related to eye position

or eye movements during the preparatory period or during the execution of movements.

Discussion

We found that neurons in the lateral PFC exhibited substantial changes in activity during a preparatory period in which monkeys were required to plan multiple steps of motor behavior. Neuronal activity during the preparatory period predominantly reflected intended (future) movements of a cursor along a particular path within a maze to reach an intended goal. All cursor movements that had to be prepared (the first, second, and third of three movements) to reach the goal were reflected by the activity of the PFC neurons. In contrast, very few PFC neurons (9%) reflected the intended arm movements during the preparatory period.

A Analysis for a MI neuron

(i) Spike Density Histogram

Ls-X-X
Lp-X-X
Rp-X-X
Rs-X-X

X-Ls-X
X-Lp-X
X-Rp-X
X-Rs-X

X-X-Ls
X-X-Lp
X-X-Rp
X-X-Rs

Ls:Left supination
Lp:Left pronation
Rp:Right pronation
Rs:Right supination

1st GO

10spikes/s

1s

(ii) Information Predicting Arm-Movement

bit

1st movement 2nd movement 3rd movement

0.4

Preparatory 1st Step 2nd step 3rd step

(iii) Information Predicting Cursor-motion

bit

0.4

1st Cursor-motion
2nd Cursor-motion
3rd Cursor-motion

Preparatory 1st Step 2nd step 3rd step

1st GO
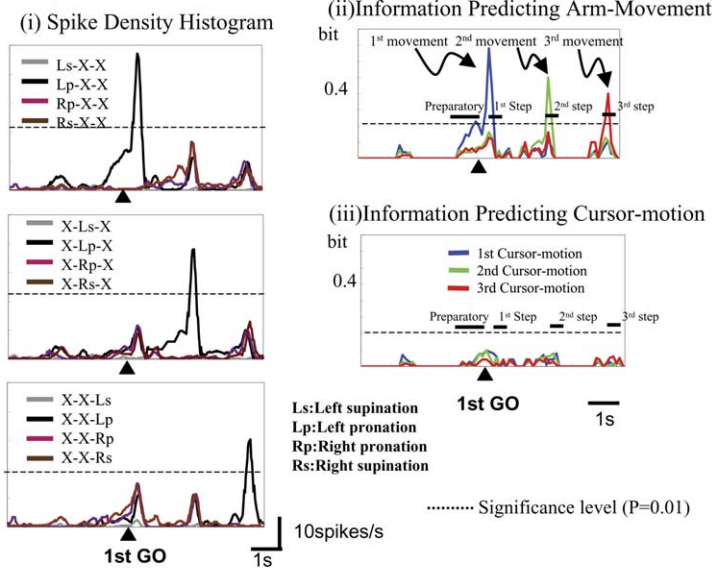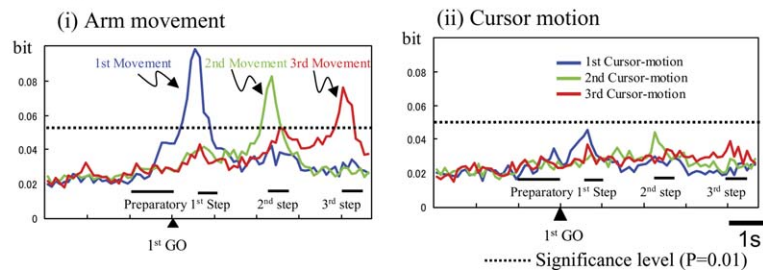
1s

·········· Significance level ($P=0.01$)

Figure 6. Analysis of Neuronal Activity in the Primary Motor Cortex

(A) Analysis for a typical M1 neuron. In this example, neuronal activity increased preceding the execution of left pronation, regardless of whether that particular movement appeared in the first, second, or third step. In the spike-density histograms (left panel), neuronal activity is sorted by the first (top), second (middle), and third (bottom) arm movement. In the right panel, neuronal information predicting arm movement (Aii) or cursor motion (Aiii) is plotted in consecutive 100 ms bins.
(B) Population analysis for MI neurons. Activity in this area clearly predicted the occurrence of the first, second, and third stepwise movements of the arm, and activity peaked at the onset of each of the three movements. However, neuronal activity that was predictive of cursor movements remained below the level of statistical significance.

B Population analysis for MI neurons

(i) Arm movement

bit

1st Movement 2nd Movement 3rd Movement

0.08

0.06

0.04

0.02

0

Preparatory 1st Step 2nd step 3rd step

1st GO

(ii) Cursor motion

bit

1st Cursor-motion
2nd Cursor-motion
3rd Cursor-motion

0.08

0.06

0.04

0.02

0

Preparatory 1st Step 2nd step 3rd step

1st GO

1s

·········· Significance level ($P=0.01$)

Properties of Neuronal Activity during the Preparatory Period

During the preparatory period, the monkeys were required to generate the information needed to move the cursor to a predetermined goal using three stepwise movements of the cursor. Among the numerous possible sources of information the monkeys may have used, the cursor motion appeared to be reflected in the activity of PFC neurons, i.e., the monkeys planned forthcoming behavior (at least in part) in terms of step-by-step movement of the cursor toward the goal. For the neuronal activity that reflected the first cursor movement, it is possible to interpret the activity as specifying either the direction of the first cursor movement or the position at which the cursor was to be located after the first movement. Moreover, for some neurons (n = 87), activity was selective for the direction of the second cursor movement. It is theoretically possible to plan the location of the cursor to be achieved with the second movement. However, not many neurons exhibited activity that reflected the location of the cursor in the second step (see the Supplemental Data). Remarkably, as many as 44% of neurons that exhibited preparatory activity were selective for the third cursor movement during the preparatory period that preceded the presentation of the first GO signal. For these neurons, selectivity was for the direction of the cursor movement that was

to occur during the third step. In contrast to the abundance of neuronal activity that reflected cursor movement, activity that reflected arm movements was not apparent among PFC neurons. This implies that the motion of the cursor itself, rather than arm movement, was primarily processed within the PFC for the purpose of planning motor behavior. We also analyzed 127 neurons in the primary motor cortex of the same monkeys. We found that the activity of these neurons primarily reflected arm movements. In sharp contrast to PFC neurons, activity of MI neurons primarily reflected arm movements. Population analysis also revealed that the representation of cursor motions remained nonsignificant.

It is of interest that, during the preparatory period, the neuronal responses that encode each cursor movement occurred simultaneously, not in the order of the first, second, and third motion of the cursor. This finding could imply that the animals were engaged in simultaneous planning of cursor movements, while planning the sequence of their occurrences separately. An alternative explanation may be that the monkeys were engaged in planning the sequence in a retrograde order (starting from the last motion to capture the goal, followed by the second and then the first motion), in conjunction with a sequence planning with an anterograde order. Further works are necessary to reveal the nature of the sequential planning.

A

B

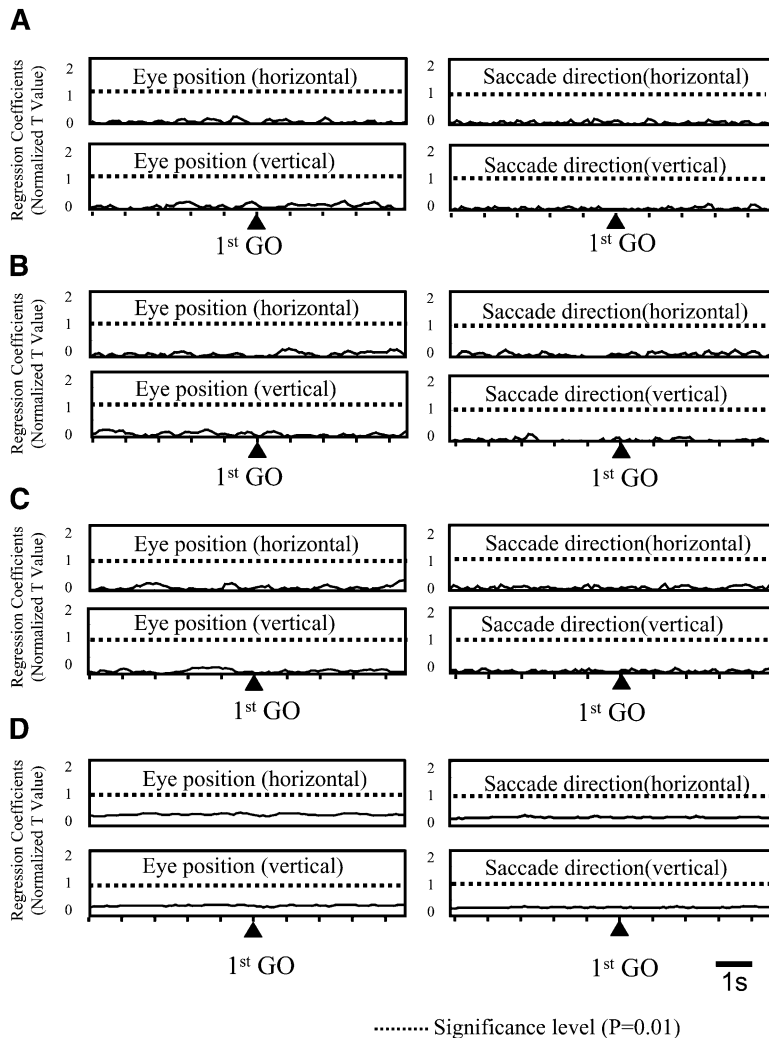**C**

D

·········· Significance level (P=0.01)

Figure 7. Regression Analysis of the Correlation between PFC Neuronal Activity and Oculomotor Parameters

(A–C) Examples of analysis of the three PFC neurons for which activity was presented in Figures 6A–6C. The four oculomotor parameters were vertical and horizontal eye positions and vertical and horizontal components of saccades. Each panel shows a sequential plot (50 ms bins) of regression coefficients for the four regressors that were normalized to T values that corresponded to a significance level of 0.01. Data are aligned to the onset of the first GO signal.

(D) Population analysis. Regression analysis of the correlation between the activity of 470 PFC neurons that exhibited preparatory period-related activity and the four oculomotor parameters plotted as in (A)–(C).

Relationship between the Present Study and Previous Studies of the Lateral Prefrontal Cortex

Although considerable interest has been directed at the behavioral aspects of the acquisition, retention, and modulation of sensory information, which is often referred to as "working memory" (Petrides, 1996; Niki and Watanabe, 1976; Goldman-Rakic, 1987; Romo et al., 1999; Constantinidis et al., 2001), the role of the lateral PFC in planning behavior has also been the focus of much research (Boussaoud and Wise, 1993; Fuster, 1997; Fuster et al., 2000; Hasegawa et al., 1998; Kim and Shadlen, 1999; Jenkins et al., 2000; Rowe et al., 2000; Tanji and Hoshi, 2001; Takeda and Funahashi, 2002; Fukushima et al., 2004). However, little is known about which elements of behavior are planned within the PFC, because few studies have examined the exact nature of planned behavioral factors. Previous studies have revealed that behavioral factors other than the parameters used to specify movement itself influence planning-related activity in the PFC (Hasegawa et al., 2000; Nieder et al., 2002; Fujii and Graybiel, 2003; Ninokura et al., 2003). Behavioral rules and task requirements profoundly affect neuronal activity (Hoshi et al., 1998, 2000; White and Wise, 1999; Asaad et al., 2000; Wallis and Miller, 2003), as do reward conditions (Watanabe,

1996; Leon and Shadlen, 1999). In our previous report, we took up the issue of goal planning in the PFC (Saito et al., 2005), clarifying the role of PFC neurons in representing behavioral goals. For the attainment of a behavioral goal, it is crucial to decide how to achieve the goal-oriented behavior by planning multiple actions. The present study deals with this issue of action-selection planning; how do PFC neurons take part in planning a series of actions? Averbeck et al. (2002) used a behavioral task that required monkeys to draw geometric figures to demonstrate that the serial elements in a sequence of movements were represented by changes in the activity in the PFC that occurred prior to the execution of movements. The Averbeck et al. (2002) report suggested that there was parallel processing of planned serial movements. However, the planned serial elements could be either arm movements or the trajectories of the segments of the geometric figure that was to be drawn, because these two factors were not dissociated in the Averbeck et al. (2002) experiment. In the present study, we used the planning of multiple movements that were to be executed in discrete intervals rather than serial or continuous movements and employed an experimental paradigm in which planned cursor movements and executed movements were dissociated. Our findings

reveal that the motion of an object to be generated as a consequence of planned action is the behavioral factor that is reflected by PFC neuronal activity, and that information for multiple action consequences is processed simultaneously during planning.

Based on the current analyses, it appears that PFC exhibits greater task-related information during the preparatory than movement periods and that PFC "reactivates" the representation of the remaining sequence at each step. There also appears to be greater information about the first and last steps than the middle step, consistent with the U-shaped serial position curve observed in previous behavioral (Kesner and Novak, 1982) and single-unit (Averbeck et al., 2002) studies of sequential tasks.

Implications for the Role of the Prefrontal Cortex in Planning Multistep Behavior

When we plan multiple steps of actions in daily life, we usually do so by consciously arranging future events that we expect to occur as the consequence of actions in a particular temporal order; we rarely consider the temporal sequence of motor actions themselves. The properties of PFC neurons that we observed in the present study are compatible with behavioral planning based on future events. If we assume that planning for multiple movements in monkeys is analogous to that in humans, it follows that PFC neurons in the monkey brain process information for future events in a prospective manner to generate action plans based on a series of events during the course of reaching a behavioral goal. Our findings on the MI indicate that neuronal activity reflects movements to be performed, fulfilling the requirement for the motor command. In future studies, it would be of great interest to investigate where in the brain the information for future events is transformed into information for the generation of motor commands.

Experimental Procedures

Subjects and Behavioral Task

We trained two monkeys (*Macaca fuscata*) to perform a path-planning task that required the planning of multiple cursor movements to reach preinstructed goals. Animals were cared for in accordance with the Guiding Principles for the Care and Use of Laboratory Animals of the National Institutes of Health and the Guidelines for Institutional Animal Care and Use published by our institute.

During the experiment, an animal was seated in a primate chair that faced a computer monitor on which a checkerboard-like maze was displayed (Figure 1A). A single trial of the path-planning task comprised the following series of events (Figure 1C). A trial commenced when the animal grasped two manipulanda (one provided for each arm) and held them in a holding position. After 1 s, a green cursor appeared at the center of the maze. One second later, a red square indicated the position of an initial goal within the maze for 1 s. After a delay period (1 s), between two and six of the maze paths were blocked, as illustrated in Figure 2. After another delay period (1 s), the cursor color was changed from green to yellow; this was the first GO signal. Upon presentation of the GO signal, the monkey was required to move the cursor to the position of the goal in a step-by-step manner by operating the two manipulanda through supination or pronation of the wrists. The monkey was required to initiate the first movement within 500 ms. When the cursor was moved to the next position, the cursor color was changed from yellow to green. After a hold period (1 s), the cursor color was changed from green to yellow, which represented the next GO signal. The monkey was again required to move the cursor within 500 ms. The monkeys were allowed to move the cursor one step

in any direction, except when the path was blocked. If the cursor was moved successfully to the location of the goal, the monkey was rewarded with fruit juice.

In this study, we used two sets of goal positions and path blocks (set 1 and 2), as illustrated in Figure 2. Each set comprised four goals and three combinations of path blocks. We recorded the activity of individual PFC neurons during sessions with either set 1 or 2, or during both sets. At least three movements of the cursor were required to reach the goals in both sets. We only analyzed neuronal activity during trials in which the animals were able to reach the goal in three steps. For example, leftward, leftward, and upward movement (LLU) of the cursor was required to reach goal 1 in set 1 if block 1 was presented (Figure 2); LUL was required for block 2, and ULL was required for block 3. The animals were allowed to move the cursor along any path within the maze to reach the goal. To dissociate arm movements from the direction in which the cursor moved, we trained the monkeys to perform the path-planning task with three different arm-cursor assignments (see Figure 1B). The arm-cursor assignment was changed between blocks of 24 or 48 trials.

Surgical and Recording Methods

We used conventional electrophysiological techniques to obtain in vivo single-cell recordings (Mushiake et al., 1991). After completion of the initial behavioral training, an acrylic recording chamber was attached to the skull of the monkey under aseptic conditions using pentobarbital sodium anesthesia (30 mg/kg, i.m.) with atropine sulfate. Antibiotics and analgesia were used to prevent postsurgical infection and pain, respectively. Neuronal activity was recorded using glass-insulated Elgiloy microelectrodes (0.8–1.5 MOhm at 333 Hz). The electrodes were inserted through the dura mater using a hydraulic microdrive (Narishige MO-81; Tokyo, Japan) and a remotely controlled electrode-positioning system (Alpha Omega Engineering, Nazareth, Israel). Neuronal activity was discriminated using spike-sorting software (Alpha Omega Engineering) based on template matching. We monitored eye positions and eye movements with an infrared corneal reflection monitoring system. Discriminated unit activities were stored with a record of behavioral events, eye positions, and electromyography data on a computer hard drive. The same computer was used to control the task parameters. Cortical sulci and recording locations were identified using a magnetic resonance imaging scanner (OPART 3D-System; Toshiba, Tokyo, Japan). Prior to recording neuronal activity in the PFC, we first determined the location of the frontal eye field using intracortical microstimulation (Bruce and Goldberg, 1985). The recording sites covered the expanse of the PFC that extended 11 mm rostrocaudally to where intracortical microstimulation with currents less than 80 μA did not evoke saccades. We sampled neuronal activity from the dorsolateral PFC rostral to the frontal eye field, including the banks of the principal sulcus and the adjacent cortical convexity (see Figure 2B). For the purpose of comparison, we also recorded from the arm area of the M1 in the anterior bank of the precentral surface and its anteriorly adjacent part of the precentral cortex, identified with intracortical microstimulation (Sato and Tanji, 1989).

Analysis of Single-Unit Activity

Recordings of neuronal activity were sorted according to the task conditions and were displayed online as raster plots and perievent histograms. Our database included neurons from which activity was recorded during more than two blocks of trials for each arm-cursor assignment. We defined four task periods: the preparatory period (1 s prior to the first GO signal); the first movement (500 ms following the onset of the first cursor movement); the second movement (500 ms following the onset of the second cursor movement); and the third movement (500 ms following the onset of the third cursor movement). Neuronal activity (discharge rate) during the preparatory period that was significantly different (Wilcoxon's signed-ranks test; $\alpha = 0.05$) from that recorded during a control period (500 ms during the initial hold period, starting 300 ms after the onset) was defined as preparatory activity. In this report, we only describe cells that exhibited significant activity during the preparatory period.

To assess how parameters such as goal position, path block, and individual cursor and arm movements were related to the activity of

PFC neurons, we performed multiple linear regression analysis using the following equation:

$$\text{Firing rate} = \beta_0 + \beta_1 \times (\text{goal}) + \beta_2 \times (\text{block}) + \beta_3 \times (\text{cursor direction}) + \beta_4 \times (\text{arm movement}). \quad (1)$$

In Equation (1), β_0 is the intercept and β_1, β_2, β_3, and β_4 are the coefficients. The first categorical factor was the goal position with four levels (goal 1–4 in set 1 or 2; see Figure 2). The second categorical factor was the path block with three levels (path block 1–3 in set 1 or 2; see Figure 2). The third categorical factor was cursor direction with four levels (up, down, left, right, regardless of the order of appearance). The fourth categorical factor was arm movement with four levels (left supination, left pronation, right pronation, right supination, regardless of the order of appearance) . The categorical regressors indicated in the parentheses were entered into the analysis as dummy variables based on the statistical method, as appeared in Applied Regression Analysis by Draper and Smith (Draper and Smith, 1998). We calculated the probability (p) that each coefficient of dummy variables equaled zero. If any of the probability of each coefficient of dummy variables for each categorical factor is less than 0.01 , the neuronal activity was accepted as reflecting each factor.

For neurons that exhibited activity that reflected the direction of cursor movement, we analyzed how preparatory activity reflected the first, second, or third movement of the cursor. To assess the extent to which each cursor movement was represented by the activity of the population of PFC neurons, we carried out a linear regression analysis of neuronal activity using the following equation:

$$\text{Firing rate} = \beta_0 + \beta_1 \times (\text{first cursor movement}) + \beta_2 \times (\text{second cursor movement}) + \beta_3 \times (\text{third cursor movement}). \quad (2)$$

In Equation (2), β_0 is the intercept and β_{1-3} are coefficients. The regressors in the parentheses were entered into the analysis as dummy variables. The categorical factors for cursor movement were upward, downward, leftward, and rightward. We calculated the probability (p) that each coefficient of dummy variables equaled zero. If any of the probability of each coefficient of dummy variables for each categorical factor is less than 0.01, the neuronal activity was accepted as reflecting each factor.

For neurons that exhibited activity that reflected arm movement, we analyzed how preparatory activity reflected the first, second, or third arm movement. To assess the extent to which arm movement was represented by the activity of the population of PFC neurons, we performed a linear regression of neuronal activity using the following equation:

$$\text{Firing rate} = \beta_0 + \beta_4 \times (\text{first arm movement}) + \beta_5 \times (\text{second arm movement}) + \beta_6 \times (\text{third arm movement}). \quad (3)$$

In Equation (3), β_0 is the intercept and β_{4-6} are coefficients. The categorical factors for arm movement were left supination, left pronation, right pronation, and right supination. The regressors indicated in the parentheses were entered into the analysis as dummy variables. We calculated the probability (p) that each coefficient of dummy variables equaled zero. If any of the probability of each coefficient of dummy variables for each categorical factor is less than 0.01, the neuronal activity was accepted as reflecting each factor.

Regression and probability analyses were performed using commercially available software (MATLAB 6.5; MathWorks, Natick, MA). The typical amount of single-unit response variance explained by their basic regression model is about 0.7. Furthermore, we calculated the *variance inflation factors* (VIF) to examine the possible existence of multicollinearity for multiple factors. We did this analysis to check the possibility that the behavioral strategies adopted by the monkeys led to a bias in the number of times the combinations of the factors occur together (Draper and Smith, 1998). We confirmed that the VIF was small enough (<2) to rule out the behavioral bias concerned.

We also made correlation analyses to quantify the correlation between the magnitudes of information that predicted the first cursor movement during the preparatory period and during the execution of the first movement. We calculated correlation coefficients between the firing rates for four directions and for goals during the preparatory period and those during the movement period of each step of the cursor movement. We performed the test of significance for correlation coefficients.

Quantification of Information Carried by Neuronal Activity

To evaluate the extent to which PFC neuronal activity predicted information associated with cursor movements, we calculated the predictive information (Is) carried by the occurrence of spikes by quantifying the decrease in entropy in the cursor directions as follows (cf. Kitazawa et al., 1998):

$$\text{Is} = \sum - (\text{ni}/\text{N})\log_2(\text{ni}/\text{N}) - \sum - (\text{mi}/\text{N})\log_2(\text{mi}/\text{N}). \quad (4)$$

In Equation (4), N is the total number of trials, n is the number of trials for each direction of cursor movement (i = 1, 2, 3, and 4), and m is the number of trials for each direction during which a spike occurred within a given time window. We calculated the predictive information (Ins) carried by the absence of spikes by quantifying the decrease in entropy in the cursor directions as follows:

$$\text{Ins} = \sum - (\text{ni}/\text{N})\log_2(\text{ni}/\text{N}) - \sum - ((\text{ni} - \text{mi})/(\text{N} - \text{M}))\log_2((\text{nI} - \text{mi})/(\text{N} - \text{M})). \quad (5)$$

In Equation (5), M is the total number of trials during which a spike occurred within a given time window.

By solving Equations (4) and (5), we calculated the total predictive information (It) as follows:

$$\text{It} = p \times \text{Is} + (1 - p) \times \text{Ins}. \quad (6)$$

In Equation (6), p is the probability of spike occurrence during the time window under consideration. We used a time window of 100 ms to analyze the occurrence of spikes recorded from PFC neurons. If the total predictive information was significantly larger (Wilcoxon signed-ranks test, <0.05) than that during a control period (500 ms in Initial-Hold period, starting 300 ms after its onset), we defined the regression coefficients as significantly deviated from those of control period.

Statistical Analysis of Eye Movements

Although the monkeys were not required to control their gaze while performing the task, we nevertheless analyzed eye positions and movements during the task. We used a multiple regression analysis to estimate how task-related neuronal activity was related to eye positions and saccades. We calculated the mean firing rate, mean eye position, and vertical and horizontal components of saccades in 50-ms bins for each trial. We used the following linear model to express neuronal activity:

$$\text{Firing rate} = \beta_0 + \beta_1 \times (\text{horizontal eye position}) + \beta_2 \times (\text{vertical eye position}) + \beta_3 \times (\text{horizontal saccade vector}) + \beta_4 \times (\text{vertical saccade vector}). \quad (7)$$

In Equation (7), β_0 is the intercept and β_{1-4} are coefficients. To evaluate the relationships between neuronal activity and each factor, we calculated the T value of the regression coefficient using the corresponding bin-by-bin data for neuronal activity and eye position/saccade metrics. The T value was normalized to the T value that corresponded to a significance level of 0.01.

Supplemental Data

The Supplemental Data for this article can be found online at http://www.neuron.org/cgi/content/full/50/4/631/DC1/.

Acknowledgments

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