Population Vector Analysis of Primate Prefrontal Activity during Spatial Working Memory

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Population vectors were used to examine information represented by a population of prefrontal activity and its temporal change during spatial working memory processes while monkeys performed ODR and R-ODR tasks. In the ODR task, monkeys made a saccade to the cue location after the delay, whereas in the R-ODR task, they made a saccade 90° clockwise from the cue location. We first constructed population vectors using cue- and response-period activity. The directions of population vectors were similar to the cue directions and the saccade target directions, respectively, indicating that population vectors correctly represented information regarding directions of visual cues and saccade targets. We then calculated population vectors during a 250 ms time-window from the cue presentation to the end of the response period. In the ODR task, all population vectors were directed toward the cue direction. However, in the R-ODR task, the population vector gradually rotated during the delay period from the cue direction to the saccade direction. These results indicate that spatial information represented by a population of prefrontal activity can be shown as the direction of the population vector and that its temporal change during spatial working memory tasks can be depicted as the temporal change of the vector's direction.

Keywords: information processing, oculomotor-delayed response, population vector, prefrontal cortex, rhesus monkey, spatial working memory

Introduction

Working memory is a dynamic neuronal system for manipulating and integrating information as well as a temporary storage of information (Baddeley, 1986; Funahashi and Kubota, 1994; Miyake and Shah, 1999; Funahashi, 2001). Since higher cognitive functions such as language comprehension, decision-making and reasoning require working memory, understanding the neuronal mechanism of working memory is crucial for understanding the neuronal mechanisms of these cognitive functions. The prefrontal (PF) cortex has been considered to play an important role in working memory (Goldman-Rakic, 1987; Petrides, 1994; Fuster, 1997). Neurophysiological studies using spatial working memory tasks have revealed that many PF neurons exhibit tonic activation during the delay period (delay-period activity; Fuster, 1973; Niki, 1974; Niki and Watanabe, 1976; Kojima and Goldman-Rakic, 1982, 1984; Funahashi et al., 1989, 1993; Wilson et al., 1993; Rao et al., 1997; Rainer et al., 1998). This delay-period activity has been considered to be a neuronal correlate of a temporary active storage mechanism in working memory processes (Goldman-Rakic, 1987, 1995, 1996; Funahashi and Kubota, 1994; Fuster, 1997).

Although we now have a better understanding of the temporary storage mechanism of information, the neuronal mechanism for manipulating and integrating information is still unclear. To understand the mechanism for information processing, we first need to know what information neuronal activity represents and then how the information represented by neuronal activity changes along a trial. In our previous study (Takeda and Funahashi, 2002a), we examined what information each task-related activity represented using two spatial working memory tasks (ODR and R-ODR tasks). As a result, all cue-period activity and 86% of delay-period activity represented the location of the visual cue, whereas the remaining 13% of the latter represented the direction of the saccade. In addition, ~60% of response-period activity represented the direction of the saccade, whereas the remaining 40% represented the location of the visual cue. We now know what information each task-related activity represents while the monkeys perform ODR tasks. Therefore, in the present experiment, we examined how the information represented by neuronal activities changes along ODR trials.

A particular piece of information (e.g. a spatial position of a visual cue) is apparently encoded by a population of prefrontal neurons. For example, a large number of prefrontal neurons responded repeatedly when the visual cue was presented at the same position in the visual field. However, the best responded position, the spatial tuning, the magnitude of the activation and its temporal pattern are all different from neuron to neuron (Funahashi et al., 1989, 1990, 1991; Takeda and Funahashi, 2002a). In spite of these differences, the activities of all these neurons encode the position where the visual cue is presented in the visual field. Therefore, to understand how information is processed in the PF cortex, we need to consider not only the information that each single-neuron activity represents but also the information that a population of PF activity represents and the temporal change of the information along the task performance.

In the present study, we selected a population vector analysis to examine information represented by a population of PF activity and its temporal change during ODR performances. The population vector analysis has been proposed and advanced by Georgopoulos and others (Georgopoulos et al., 1983, 1986, 1988, 1989, 1993; Georgopoulos, 1988; Kettner et al., 1988; Schwartz et al., 1988; Lurito et al., 1991; Smyrnis et al., 1992). Population vectors calculated by a population of motor cortical activity have been shown to predict movement directions during arm-reaching behavior in three-dimensional space (Georgopoulos et al., 1986, 1988; Kettner et al., 1988; Schwartz et al., 1988) as well as on a two-dimensional plane (Georgopoulos et al., 1983). Schwartz (1993, 1994) and Schwartz and Moran (1999) showed that population vectors predict movement trajectories while monkeys drew sinusoidal or more complex curves by hand. Population vectors calcu-

lated by a population of motor cortical activity have also been shown to depict temporal changes of internally represented information during motor preparation and programming for arm-reaching movements (Georgopoulos *et al.*, 1989; Smyrnis *et al.*, 1992; Ashe *et al.*, 1993). In addition, Kruse *et al.* (2002) showed that population vectors calculated by a population of MT neurons can predict the direction of the stimulus movement during a visually guided tracking task. In our ODR tasks, four or eight radial directions were used for depicting visual cue locations and saccade directions. Most of the task-related activities observed in the PF cortex were directionally tuned.

These results indicate that the population vector analysis could be a suitable method for visualizing information represented by a population of PF activity and its temporal change during ODR and R-ODR performances. Therefore, we applied a population vector analysis to examine information represented by a population of PF activity during spatial working memory processes. In the present experiment, we recorded single-neuron activity from the PF cortex one-at-a-time. Therefore, the present data do not reflect the activity of simultaneously recorded ensembles of neurons in the PF cortex. A preliminary report of this study has been published in abstract form (Takeda and Funahashi, 2000, 2002b).

Materials and Methods

Subjects and Apparatus
Two rhesus monkeys (monkey M, 3.6 kg; monkey N, 3.8 kg) used in this study were the same as those used in our previous study (Takeda and Funahashi, 2002a). The experimental apparatus, surgical procedures and histological examinations have been described in detail previously (Takeda and Funahashi, 2002a). In brief, during training and recording sessions, the monkey sat in a primate chair in a dark sound-attenuated room. The monkey's head was fixed by a head-restraining instrument. The monkey faced a 21 in. color TV monitor (E77F; Nanao), on which a fixation point and visual cues were presented. The monkey's eye positions were monitored by the magnetic search coil technique (Robinson, 1963). Two laboratory computers (PC-386GE and PC-486HX; Epson) presented visual stimuli on the monitor, recorded neuronal activity and monitored eye movements. All experiments were conducted according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. This experiment was approved by the Animal Research Committee at the Faculty of Integrated Human Studies, Kyoto University.

Behavioral Tasks
In the present experiment, we used two spatial working memory tasks (ODR and R-ODR tasks). In the ODR task, the monkey was required to make a saccade after a 3 s delay to the location where a visual cue had been presented. The temporal sequence of this task is illustrated in Figure 1*A* (top). After a 5 s intertrial interval (ITI), a fixation point (FP; a white circle, 0.5° in visual angle) was presented at the center of the monitor. If the monkey continued to look at FP for 1 s (fixation period), a visual cue (a white circle, 1° in visual angle) was presented for 0.5 s at one of the eight predetermined locations around FP (Fig. 1*B*, left). The monkey was required to maintain fixation at FP throughout the 0.5 s cue period and subsequent 3 s delay period. At the end of the delay period, FP was extinguished. This was the 'go' signal for the monkey to make a saccade within 0.35 s (response period) to the location where the visual cue had been presented. If the monkey performed a correct eye movement, it was rewarded with a drop (~0.2 ml) of water.
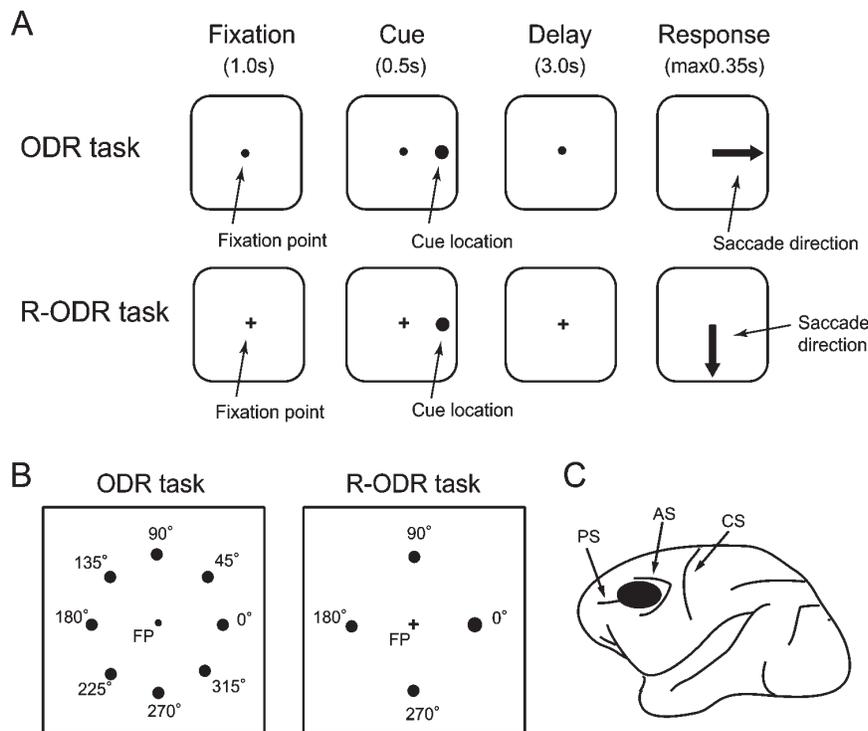
Figure 1. Behavioral tasks and recording area. (*A*) Temporal sequence of task events in two oculomotor delayed-response (ODR) tasks. Top: standard oculomotor delayed-response (ODR) task. The monkey was required to make a saccade to the location where the visual cue had been presented. Bottom: rotatory oculomotor delayed-response (R-ODR) task. The monkey was required to make a saccade 90° clockwise from the location where the visual cue had been presented. (*B*) Locations of the visual cue in the ODR task (left) and R-ODR task (right). FP, fixation point. The eccentricity of cue locations was 17°. (*C*) Recording area. Neuronal activity recorded from the cortex within and surrounding the principal sulcus. PS, principal sulcus; AS, arcuate sulcus; CS, central sulcus.

In the R-ODR task, the monkey was required to make a saccade 90° clockwise from the location where the visual cue had been presented. The temporal sequence of this task is illustrated in Figure 1*A* (bottom). After a 5 s ITI, FP (a white 'plus' character, 0.5° in visual angle) was presented at the center of the monitor. If the monkey continued to look at FP for 1 s (fixation period), the visual cue (a white circle, 1° in visual angle) was presented for 0.5 s at one of the four predetermined locations around FP (Fig. 1*B*, right). The monkey was required to maintain fixation at FP throughout the 0.5 s cue period and subsequent 3 s delay period. At the end of the delay period, FP was extinguished. This was the 'go' signal for the monkey to make a saccade within 0.35 s (response period) to the 90° clockwise location from where the visual cue had been presented. If the monkey performed a correct eye movement, it was rewarded by a drop (~0.2 ml) of water.

To estimate whether the monkey's eye movement was correct, we set a square window (4–6° in visual angle) around the target location and considered that the monkey performed a correct saccade if its eye position fell within this window. If the monkey broke fixation during the cue or delay period, if it failed to perform a saccade within the 0.35 s response period, or if the eye movement did not fall within the correct window, the trial was immediately aborted without a reward and the next trial began.

Recording Single-neuron Activity

We recorded single-neuron activity from the cortex within and surrounding the principal sulcus while the monkeys performed both tasks. In the present experiment, we recorded single-neuron activity from the PF cortex one-at-a-time. Therefore, the present data do not reflect the activity of simultaneously recorded ensembles of neurons in the PF cortex. In the recording sessions, the monkey performed each task in blocks of 100–150 trials. Single-neuron activity was recorded by glass-coated elgiloy microelectrodes (0.5–1.5 MΩ at 1 kHz). Raw activity was amplified using an amplifier (DAM80; World Precision Instruments) and monitored on an oscilloscope (SS-7802; Iwatsu Electronics). At the same time, we isolated single-neuron activity using a window discriminator (DIS-1; BAK Electronics) and monitored its output on an oscilloscope. The outputs of the window discriminator were input to the computer (PC-386GE; Epson) and stored together with task events on magnetic media. We also stored raw neuron activity, task events and horizontal and vertical eye movements on magnetic tape using a data recorder (PC-108M; Sony Precision Technology).

Definition of Task-related Activity

To examine whether a recorded single-neuron activity was task-related, we first made rasters and histograms triggered at three alignment points (the onset of the delay period, the end of the delay period and the onset of reward delivery) for each cue condition in both tasks. We then performed a statistical analysis to identify the characteristics of task-related activity. For cue-period activity, we calculated the mean discharge rate during a 300 ms period (from 100 to 400 ms after the onset of the visual cue) for each cue condition. If the mean discharge rate during the cue period differed significantly from the mean discharge rate during the 1 s fixation period by Mann–Whitney U-test ($P < 0.05$), we considered that the neuron exhibited cue-period activity. Similarly, for delay and response-period activity, we calculated the mean discharge rate during the 3 s delay period for each cue condition and the mean discharge rate during a 300 ms response period (150 ms before and after the period when the peak activity was observed) for each cue condition. If the mean discharge rate during either the delay period or the response period differed significantly from the mean discharge rate during the 1 s fixation period by Mann–Whitney U-test ($P < 0.05$), we considered that the neuron exhibited delay-period activity or response-period activity, respectively. These statistical tests were run independently for each task-related activity.

Directional Tuning of Task-related Activity

We examined whether task-related activity exhibited directional tuning or not. First, the preferred direction of task-related activity was estimated using a standard directional statistic, 'mean direction'

(Mardia, 1972). In this method, the discharge rate during each trial at each cue condition was depicted as a vector (trial vector); its direction corresponds to the cue direction and its length corresponds to the discharge rate. These trial vectors were calculated for all trials of all cue conditions using a particular task-related activity. Then, we obtained a resultant vector by summing up all these trial vectors. As a result, the resultant vector shows the direction where the activity was maximally excited. Therefore, the direction of the resultant vector was tentatively considered the preferred direction of this task-related activity.

We then tested the statistical significance of the observed directional tuning of task-related activity by using a statistical bootstrapping technique (Lurito *et al.*, 1991; Smyrnis *et al.*, 1992; Constantinidis *et al.*, 2001b; Kruse *et al.*, 2002). We first calculated the mean resultant vector R_0, which was obtained by dividing the length of the resultant vector by the sum of the lengths of all trial vectors. Then, we shuffled trial vectors against cue conditions, assigned each trial vector to a randomly selected cue condition and calculated the mean resultant vector R_i using shuffled trial vectors. This procedure was repeated 1000 times and the lengths of the 1000 mean resultant vectors (R_1 – R_{1000}) obtained by shuffled trial vectors were rank ordered. If the length of the mean resultant vector R_0 was greater than the 95th percentile in the distribution of mean resultant vectors R_i calculated by shuffled trial vectors, this task-related activity was considered to be directionally tuned and the direction of the mean resultant vector R_0 was considered the preferred direction of its activity. In this analysis, if a neuron exhibited significant activation only at one cue direction and was silent at all other cue directions, the length of the mean resultant vector R_0 was always the same as the mean resultant vector R_i obtained by any combinations of shuffled trial vectors. Therefore, this neuron would be mistakenly classified as having no tuning. However, all neurons analyzed in the present study had at least spontaneous activity at any task period in any cue direction. We found no such special case in the present analysis.

Calculation of the Population Vector

The neuronal population vector in a particular cue condition was the weighted sum of cell vectors that were calculated from the individual neuron's activity. A cell vector was calculated from each neuron's activity under a particular cue condition. The direction of the cell vector represents the preferred direction and the length of the cell vector represents the magnitude of the activity under a particular cue condition. The preferred direction to calculate the cell vector was determined from the activity recorded in the ODR task using the procedure described above.

The population vector **P** of population size N under a particular cue condition is calculated as

$$\mathbf{P} = \sum W_i^* \cdot C_i$$

where C_i is the preferred direction of the ith cell and W_i is a weighted value of ith cell's activity. W_i is calculated as

$$W_i = d_i - a_i$$

where d_i is a square-root transformed value of the mean discharge rate of the ith cell during a particular period (e.g. the cue period) and a_i is a square-root transformed value of the mean discharge rate of the ith cell during the 1 s fixation period.

Population Averaged Histograms of PF Activity

To examine the temporal pattern of PF neurons' delay-period activity along one trial, we constructed population averaged histograms of delay-period activity. In our previous study (Takeda and Funahashi, 2002a), we found two kinds of delay-period activity in the PF cortex; one encoding the location of the visual cue (D*cue*) and the other encoding the direction of the saccade (D*sac*). Base on this result, we constructed two population averaged histograms using D*cue* activity and D*sac* activity. We first selected neuronal activity at the best cue condition that the neuron exhibited the maximum delay-period activity, and calculated Z-scores along a trial from the means and the standard deviations of the discharge rates during 20 ms time window

which moved from the beginning of the fixation period to 1s after the reward delivery. Then, we constructed population averaged histograms by calculating mean *Z*-scores for all neurons having D*cue* activity and all neurons having D*sac* activity. The constructed population histograms were smoothened by convolving with a Gaussian function (sigma = 3).

Results

Database

We recorded the activity of 304 neurons in two monkeys from the cortex within and surrounding the principal sulcus. In the present experiment, we recorded single-neuron activity from the PF cortex one-at-a-time. Most of these task-related neurons were recorded from the middle and posterior part of the principal sulcal area (Fig. 1*C*). The results of histological examination have been described previously (Takeda and Funahashi, 2002a). Of 304 neurons recorded, 210 neurons exhibited task-related activity (73 were recorded from the left hemisphere of monkey M, 100 and 37 were recorded from the left hemisphere and the right hemisphere of monkey N, respectively). Among them, 121 neurons exhibited task-related activity during both tasks, 37 exhibited task-related activity only during the ODR task and 53 exhibited task-related activity only during R-ODR task. Among task-related activity, response-period activity can be classified into either pre-saccadic activity or post-saccadic activity. In our database, ~90% of response-period activity was post-saccadic. Since most of both pre- and post-saccadic activities exhibited directional selectivity and since characteristics of directional selectivity of post-saccadic activity were similar to those of pre-saccadic activity, we considered both pre- and post-saccadic activities a single category (response-period activity) in the present study. Task-related activity and its directional selectivity are summarized in Table 1.

Among the 158 neurons exhibiting task-related activity during ODR performances, 102 neurons had only one type of task-related activity (22 had cue-period activity, 31 had delay-period activity and 49 had response-period activity). However, the remaining 56 neurons exhibited two or more types of task-related activity. Based on which types of task-related activity the neuron exhibited, we classified 158 task-related neurons into seven groups (Fig. 2). The neurons having only cue-period, delay-period, or response-period activity were classified as C, D, or R groups, respectively (Fig. 2, left). The neurons having both cue- and delay-period activities were classified as CD group, those having both delay- and response-period activities were classified as DR group and those having both cue- and response-period activities were classified as CR group (Fig. 2, middle). The neurons having all three types of task-related activities were classified as the CDR group (Fig. 2, right). The result of this classification is summarized in a Venn diagram in Figure 2.

Population Vectors Calculated by Cue- and Response-period Activity

We first calculated population vectors using a population of cue-period activity and response-period activity during the ODR task to confirm that population vectors calculated using these activities correctly represent the directions of visual cues and the directions of saccade targets, respectively.

Of 158 neurons that exhibited task-related activity during the ODR task, 56 exhibited directional cue-period activity (Table 1). For this analysis, cue-period activity was defined as the mean discharge rate during the 300 ms period from 100 to 400 ms after the onset of the visual cue. Figure 3*A* shows the distributions of the preferred directions for these 56 cue-period activities. Preferred directions were distributed evenly around FP and did not show any statistically significant directional bias (Rayleigh test for uniformity, *P* > 0.1). Figure 3*B* shows population vectors (thick lines) and cell vectors (thin lines) calculated using a population of cue-period activity for each cue condition. The population vector in each cue condition was in a direction similar to that of the visual cue. The differences between the directions of population vectors and the directions of visual cues were distributed between 0.4° and 34.2° (mean = 16.2°). Because cue-period activity has been shown to represent visual information received by PF neurons (Funahashi *et al.*, 1990; Takeda and Funahashi, 2002a), this result indicates that population vectors calculated using cue-period activity correctly represent visual information encoded by a population of PF neurons.

Of the 158 neurons that exhibited task-related activity during the ODR task, 84 exhibited directional response-period activity (Table 1). For this analysis, response-period activity was defined as the mean discharge rate during the 300 ms period from 200 to 500 ms after the presentation of the 'go' signal. Figure 3*C* shows the distributions of the preferred directions for these 84 response-period activities. Preferred directions were distributed in all directions around FP and did not show any statistically significant directional bias (Rayleigh test for uniformity, *P* > 0.1). Figure 3*D* shows population vectors (thick lines) and cell vectors (thin lines) calculated from a population of response-period activity for each cue condition. The population vector in each cue condition was in a direction similar to that of the saccade target. The differences between the directions of population vectors and the directions of the saccade targets were distributed between 0.5° and 21.8° (mean = 8.8°). Because most of the response-period activity has been shown to represent saccade information in the ODR task (Takeda and Funahashi, 2002a), these results indicate that population vectors calculated using response-period activity correctly represent saccade information encoded by a population of PF neurons.

Table 1
Directional selectivity of task-related activity in each task condition

Task-related activity	ODR task	R-ODR task	Both tasks
Cue-period activity	59	57	43
Directional	56	54	41
Omni-directional	3	3	2
Delay-period activity	72	88	60
Directional	68	83	56
Omni-directional	4	5	4
Response-period activity	99	110	70
Directional	84	90	57
Omni-directional	15	20	13
Total	158	174	121

Because many neurons exhibited task-related activity at more than one task event, the total of each task-related activity exceeds the total number of neurons.
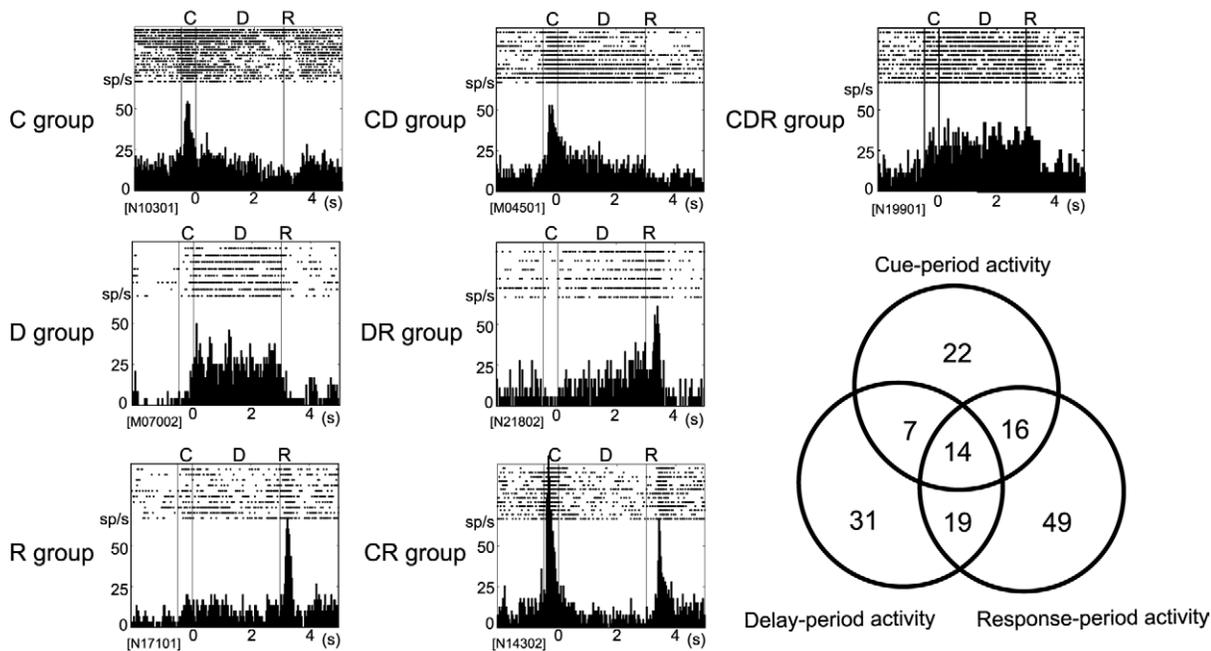
Figure 2. Examples of the activities for seven groups of PF neurons in the ODR task. The histogram bin width was 30 ms. The delay length was 3 s. C, cue period; D, delay period; R, response period. The Venn diagram indicates numbers of neurons classified into each group in the ODR task.

Determination of Overall Preferred Direction of Each Neuron

The directions of population vectors calculated from a population of cue-period activity represented the directions of the visual cues, while those calculated from a population of response-period activity represented the directions of the saccade targets. These results indicate that a population vector analysis is a suitable method to visualize information represented by a population of PF neurons and its temporal change during the delay period of ODR and R-ODR tasks. To examine the temporal change of represented information during the delay period, we need to calculate population vectors along a trial sequence. Population vectors are calculated by using cell vectors. To calculate a cell vector, it is necessary to determine the preferred direction of the neuron's activity. A basic assumption for calculating multiple population vectors along a trial is that the preferred direction of a neuron's task-related activity is maintained along the trial as well as even during different task conditions. This has been confirmed for each task-related activity (Funahashi *et al.*, 1990, 1991; Takeda and Funahashi, 2002a). However, many PF neurons exhibit two or more task-related activities. Therefore, to calculate multiple population vectors along a trial sequence, it is necessary to determine one overall preferred direction across different task-related activities for each neuron.

As is shown in Figure 2, PF neurons having task-related activities were classified into seven groups based on the combination of task-related activities. Among these groups, C, D and R groups showed only one type of task-related activity. Therefore, the preferred directions of cue-, delay- and response-period activity can be used as the overall preferred direction for neurons in C, D and R groups, respectively. On the other hand, 56 neurons (CD, DR, CR and CDR groups) showed two

or more types of task-related activities. To determine these neurons' overall preferred directions, we compared the preferred directions among cue-, delay- and response-period activities. Figure 4 shows scattergrams of the preferred directions between different task-related activities in each group of neurons. In the CD and CDR groups, the preferred directions of cue-period activity were almost identical to the preferred directions of delay-period activity (CD group, correlation coefficient, $r = 0.949$, $P < 0.001$; CDR group, correlation coefficient, $r = 0.985$, $P < 0.001$). The similarity of the preferred directions between cue- and delay-period activity was further confirmed by Wilcoxon signed-rank test (CD group, $P = 0.18$, $n = 7$; CDR group, $P = 0.42$, $n = 12$). In the CR group, the preferred directions of cue-period activity were again almost identical to those of response-period activity (correlation coefficient, $r = 0.970$, $P < 0.001$). The similarity of the preferred directions between cue- and response-period activity was confirmed also by Wilcoxon signed-rank test ($P = 0.22$, $n = 13$). In the DR and CDR groups, the preferred directions of delay-period activity were different from those of response-period activity in 11 neurons. However, in the remaining 18 neurons, the preferred directions of delay-period activity were almost identical to those of response-period activity (DR group, correlation coefficient, $r = 0.982$, $P < 0.001$; CDR group, correlation coefficient, $r = 0.904$, $P < 0.01$). The similarity of the preferred directions between delay- and response-period activity was again confirmed by Wilcoxon signed-rank test (DR group, $P = 0.53$, $n = 12$; CDR group, $P = 0.75$, $n = 6$). Based on these results, we decided to use the preferred direction of the cue-period activity as the neuron's overall preferred direction in CD, CDR, and CR groups and the preferred direction of response-period activity as the neuron's overall preferred direction in the DR group.

ODR task

Cue-period activity

Response-period activity

A

Distribution of
preferred directions

C

(n=56)

(n=84)

B

Population vector

D
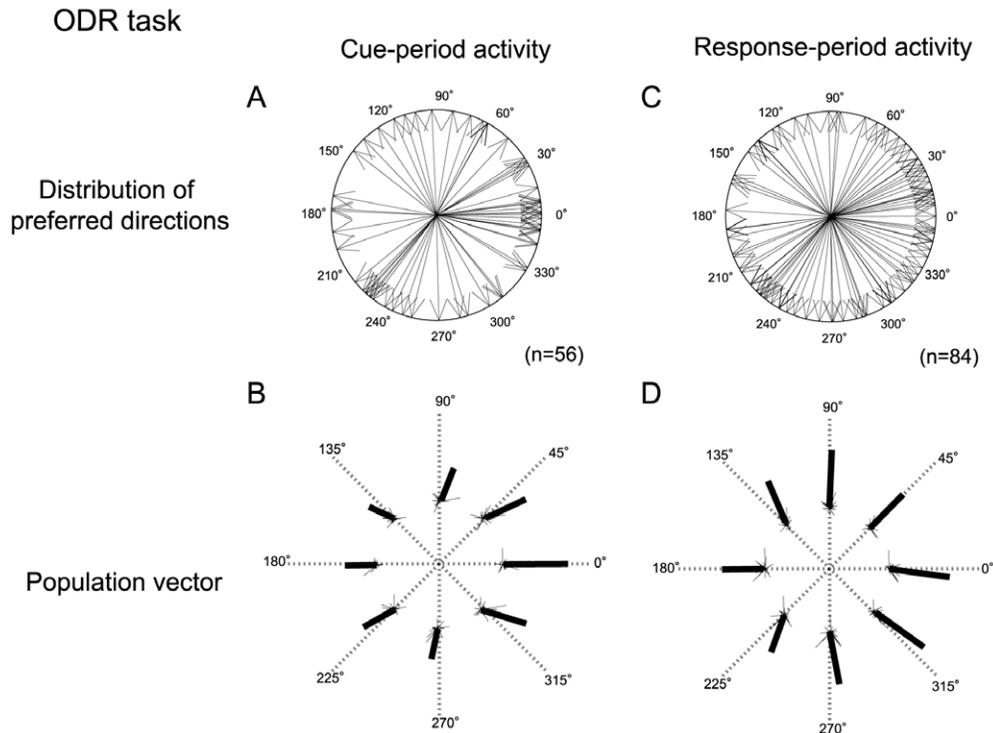
Figure 3. Distributions of preferred directions and population vectors calculated by cue- and response-period activities. (*A*) Distribution of preferred directions of 56 directional cue-period activities during the ODR task. No statistically significant directional bias was observed. (*B*) Cell vectors (thin lines) and population vectors (thick lines) calculated by cue-period activities in all cue conditions during the ODR task. The directions of population vectors were 359.6° for 0° condition, 25.0° for 45° condition, 68.0° for 90° condition, 155.1° for 135° condition, 181.1° for 180° condition, 207.0° for 225° condition, 256.3° for 270° condition and 349.2° for 315° condition. (*C*) Distribution of preferred directions of 84 directional response-period activities during the ODR task. No statistically significant directional bias was observed. (*D*) Cell vectors (thin lines) and population vectors (thick lines) calculated by response-period activities in each cue condition during the ODR task. The directions of population vectors were 352.1° for 0° condition, 45.5° for 45° condition, 87.5° for 90° condition, 113.2° for 135° condition, 180.6° for 180° condition, 252.8° for 225° condition, 280.8° for 270°° condition and 324.3° for 315° condition.

Temporal Change of Population Vectors during the ODR Task

To examine temporal change of population vectors along a trial sequence of the ODR task, we used the activity recorded from 121 neurons in both tasks. To calculate the population vectors, we set a 250 ms time-window along a trial and calculated the mean discharge rate during this time window. We moved this time window in 50 ms time-steps from the cue onset to the end of the response period and calculated a population vector at each time-step. As a result, we obtained 75 population vectors from the cue onset until the end of the response period for each cue condition. Figure 5*A* is an example of these population vectors at the 180° cue condition of the ODR task. Most of the population vectors showed similar directions as that of the visual cue. This tendency was maintained throughout the delay period. The result that most population vectors were directed toward the cue direction during the delay period was observed across all cue conditions. To confirm this observation, we examined mean differences between the direction of the population vector and the direction of the visual cue across all cue conditions for each time window. Figure 5*B* shows the temporal change of the mean differences between the direction of the population vector and the direction of the visual cue across all cue conditions. The mean differences were close to 0° across all periods. This result indicates that the same directional information is maintained along the delay period in each cue condition by a population of

PF neurons. In the ODR task, the monkeys were required to retain information regarding either the direction of the visual cue or the direction of the saccade during the delay period. In addition, the direction of the visual cue and the direction of the saccade were the same. Therefore, population vectors calculated by a population of PF activity during ODR performances correctly show that the directional information is maintained during the delay period by a population of PF neuron activity.

Temporal Change of Population Vectors during the R-ODR Task

In the R-ODR task, the direction of the saccade is 90° clockwise from the cue location. Therefore, it is expected that the directional information represented by a population of PF activity will change along a trial sequence of the R-ODR task. To examine information represented by a population of PF activity during the delay period of the R-ODR task, we performed the same analysis as in the ODR task. The preferred direction of each neuron was determined from the activity observed in the ODR task. Figure 6*A* is an example of the temporal change of the population vectors during the delay period at the 180° cue condition of the R-ODR task. The population vector gradually rotated during the delay period from the direction of the visual cue to the direction of the saccade target. This rotation began ~2 s after the start of the delay period. The population vector continuously rotated until it was in a direction similar to that of the saccade target. A rotation of the population vector during
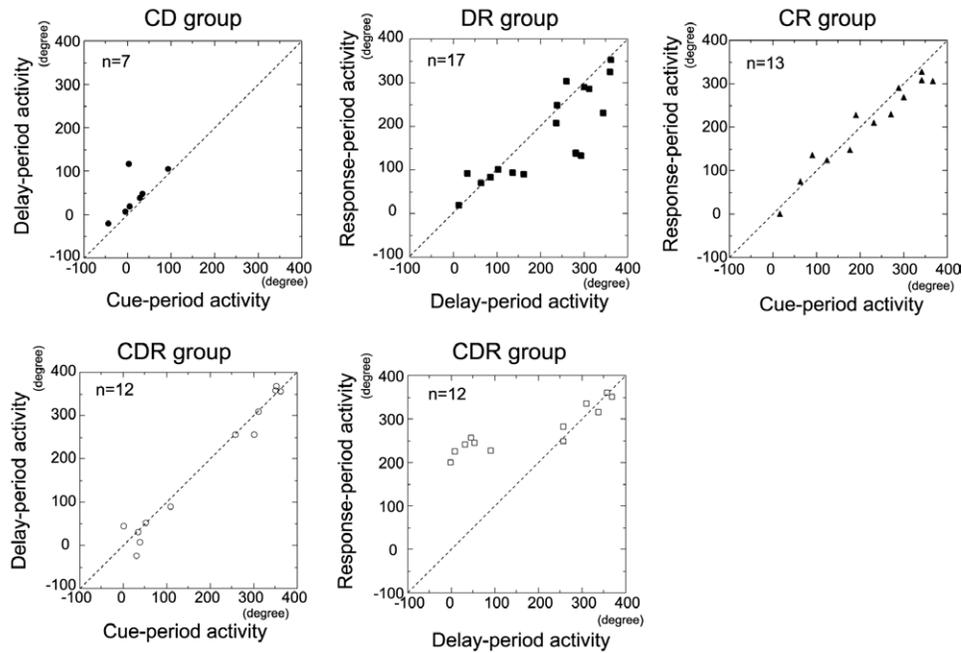
Figure 4. Scattergrams comparing the preferred directions between different task-related activities in CD, DR, CR and CDR groups. In the CD and CDR groups, preferred directions of cue-period activities were almost the same as those of delay-period activities (CD group, $r = 0.949$, $P < 0.001$; CDR group, $r = 0.985$, $P < 0.001$). In the CR group, preferred directions of cue-period activities were almost the same as those of response-period activities (CR group, $r = 0.970$, $P < 0.001$). In most neurons of the DR and CDR groups, preferred directions of delay-period activities were almost the same as those of the response-period activities (DR group, $r = 0.982$, $P < 0.001$; CDR group, $r = 0.904$, $P < 0.01$).
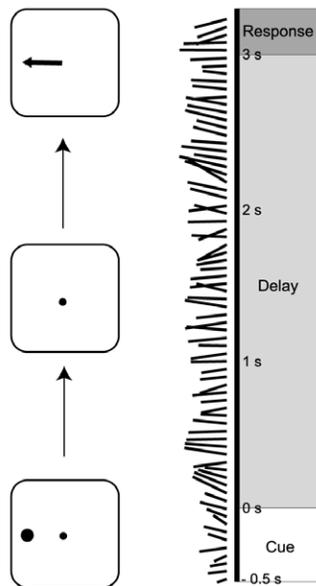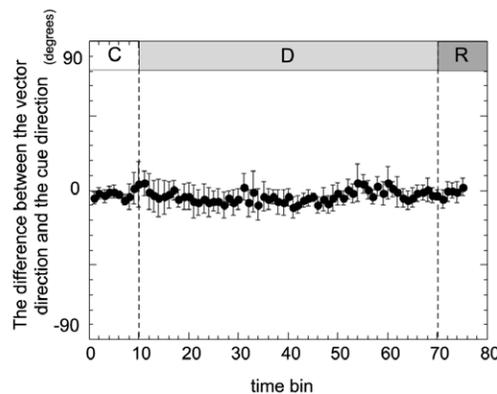
Figure 5. (*A*) Temporal change of population vectors along the 180° trial of the ODR task. All population vectors were in the same direction as the visual cue. Population vectors were calculated using the activities of 121 neurons during the 250 ms time-window that was moved in 50 ms time-steps from the onset of the visual cue until the end of the response period. (*B*) Temporal changes of the difference between the directions of population vectors and the cue directions in the ODR task. Each data point represents the mean difference and its standard error between the direction of the population vector and the cue direction. The mean differences were close to 0° throughout the delay period.

the delay period was observed in all cue conditions of the R-ODR task. Figure 6*B* shows the temporal change of the mean differences between the directions of the population vectors and the directions of the visual cue across all cue conditions of the R-ODR task. The mean differences gradually changed from close to 0° to almost 90° during the delay period. The vector began to rotate at around bin number 50 (~2 s after delay onset and 1 s before the Go signal) and rotation was maintained at
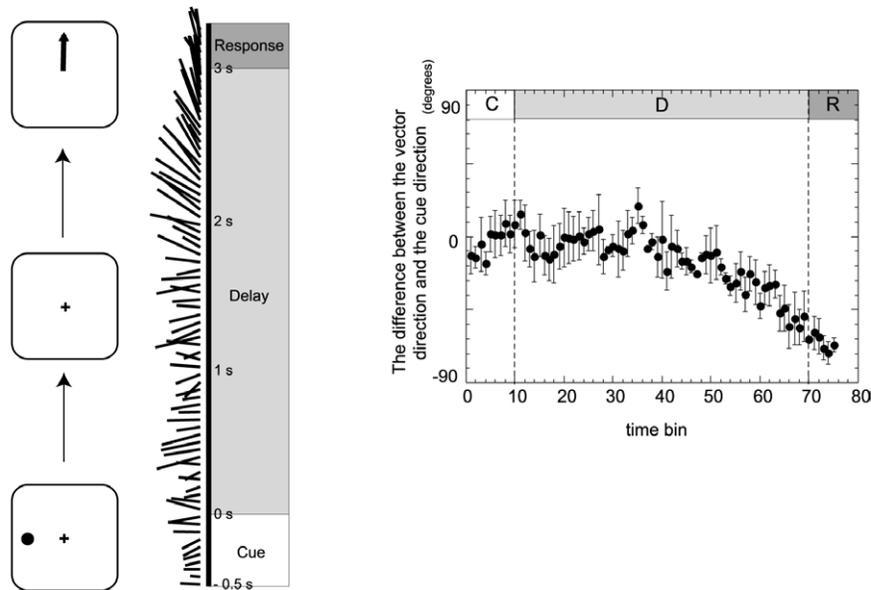
Figure 6. (*A*) Temporal change of population vectors along the 180° trial of the R-ODR task. The population vectors were calculated using the activity of 121 neurons during the 250 ms time-window that was moved in 50 ms time-steps from the onset of the visual cue until the end of the response period. (*B*) Temporal changes of the difference between the directions of population vectors and the cue directions in the R-ODR task. Each data point represents the mean difference and its standard error between the direction of the population vector and the cue direction. The mean differences changed gradually from close to 0° to almost 90° during the delay period.

a constant speed (~90°/s) until the vector was in a direction similar to that of the saccade target. These results indicate that directional information represented by a population of PF activity changed from visual input to motor output during the delay period of the R-ODR task and that the temporal changes of directional information represented by a population of PF neuron activity can be shown as the rotation of population vectors.

Discussion

Information Represented by a Population of PF Activity
In the present study, we examined information represented by a population of PF neuron activity and its temporal change during ODR and R-ODR tasks using population vector analysis. Population vectors obtained by a population of cue- and response-period activity were directed toward the direction of the visual cue and the direction of the saccade target, respectively, indicating that population vectors obtained by PF visual and oculomotor activities correctly represent information regarding the directions of the visual cues and the directions of the saccade targets, respectively. We also examined temporal changes of information represented by a population of PF neuron activity during the delay period. In the ODR task, all population vectors calculated along the trial sequence showed the same direction as the direction of the visual cue, whereas, in the R-ODR task, population vectors gradually rotated during the delay period from a direction similar to the cue direction to a direction similar to the saccade target. These results indicate that the temporal change of the information represented by PF neurons during ODR performances can be shown as a temporal change of directions of population vectors obtained by a population of PF neuron activity.

Population Vector Analysis
In this study, we applied the population vector analysis to examine PF mechanisms for manipulating and processing information in relation to spatial working memory processes. There are several reasons why we selected the population vector analysis to examine PF activity. First, we used an oculomotor version of the delayed-response task (ODR and R-ODR tasks), in which the monkey maintained gazing at the central FP and four or eight visual cues were presented radially at a constant eccentricity around FP. Therefore, the location of each visual cue can be depicted as a vector originating at the central FP. Secondly, most of task-related activity observed during ODR and R-ODR performances showed directional selectivity. We could also construct a tuning curve of task-related activity using a Gaussian function to obtain the maximally activated direction (the preferred direction) and the magnitude of the discharge rate at this direction. Therefore, using these values, we could draw a vector showing tuning characteristics of each task-related activity. Thirdly, in ODR and R-ODR tasks, the monkeys performed saccadic eye movements from the central fixation point to peripheral targets as response behaviors. Usually both the saccade target and the saccade direction are depicted as vectors in oculomotor experiments (Bruce and Goldberg, 1985a,b; Goldberg and Bruce, 1990; Russo and Bruce, 1993, 1996). In addition, tuning characteristics of oculomotor activity (e.g. the spatial position of the response field) have often been depicted as vectors (Russo and Bruce, 1996). Therefore, not only the direction of the saccade target and the saccade direction, but also tuning characteristics of oculomotor activity observed in the PF cortex could be depicted as vectors originated at the central FP. Fourthly, Georgopoulos and others originally introduced the population vector analysis for predicting movement directions during arm-reaching behavior on a two-dimensional plane (Georgopoulos *et al.*, 1983) as well as in three-dimensional

space (Georgopoulos *et al.*, 1986, 1988; Kettner *et al.*, 1988; Schwartz *et al.*, 1988). Subsequently, this analysis was applied to depict temporal changes of internally represented information during motor preparation and programming for arm-reaching movements (Georgopoulos *et al.*, 1989; Smyrnis *et al.*, 1992; Ashe *et al.*, 1993). And recently, Kruse *et al.* (2002) applied this method to a population of MT neuron activity to predict the direction of the stimulus movement during a visually guided tracking task. For these reasons, we found it advantageous to apply the population vector analysis to a population of PF activity.

In both the ODR and R-ODR tasks, the monkeys were required to maintain fixation at a central FP from the beginning of the trial until the end of the 3 s delay period. During this period, the monkeys needed to obtain information regarding the visual cue location, maintain this information, prepare motor programming during the delay period and then initiate motor responses. Both the direction of the visual cue and the direction of the saccade can be depicted as vectors. Therefore, it is expected that spatial information represented by a population of PF neuron activity and its temporal change along the trial sequence can be drawn using population vectors calculated by a population of PF activity.

Population Vectors Calculated from Cue- and Response-period Activity

In our previous study, we showed that all cue-period activity encoded the location of the visual cue (Takeda and Funahashi, 2002a). Similarly, Funahashi *et al.* (1990) showed that preferred directions of cue-period activity in the ODR task were the same as those of visual responses in a visual probe task and that there was no significant difference in the magnitude of activity between cue-period activity in the ODR task and visual responses in the visual probe task for the same neuron. These results indicate that cue-period activity corresponds to the visual response of PF neurons. Based on these observations, we first evaluated whether population vectors calculated by a population of cue-period activity correctly represent the direction of the visual cues. As is shown in Figure 3*B*, the directions of population vectors calculated by a population of cue-period activity were almost identical to the directions of the visual cue. The mean difference between the directions of population vectors and the directions of the visual cue across all cue conditions was only 16.2° in the ODR task. This result indicates that population vectors calculated by a population of cue-period activity accurately represent the directions of the visual cue.

Response-period activity consists of two kinds of saccade-related activities (pre- and post-saccadic activity) and most response-period activity in PFC has been shown to be post-saccadic (Funahashi *et al.*, 1990; Takeda and Funahashi, 2002a). In addition, ~60% of response-period activity encoded the direction of the saccade, whereas the remaining 35% encoded the location of the visual cue (Takeda and Funahashi, 2002a). Therefore, although most of the response-period activity was post-saccadic, we can conclude that a majority of response-period activity is related to the direction of the saccade. Based on these observations, we also evaluated whether population vectors calculated by a population of response-period activity correctly represent the directions of the saccade targets. As is shown in Figure 3*D*, the directions of population vectors calculated by a population of response-

period activity were almost the same as the directions of the saccade targets. The mean difference between the directions of the population vectors and the directions of the saccade targets was only 8.8° in the ODR task. This result also indicates that population vectors calculated by a population of response-period activity accurately represent the saccade targets. Therefore, we can conclude that the information represented by a population of PF neuron activity can be drawn by a population vector calculated by these activities.

Population Vectors during the Delay Period

Population vectors obtained during the delay period of the ODR task were directed toward the direction of the visual cue across all cue conditions. Because the direction of the visual cue and the direction of the saccade were the same in the ODR task, this result indicates that population vectors correctly represent information maintained during the delay period by a population of PF neuron activity. A similar observation has been reported by Smyrnis *et al.* (1992). They recorded single-neuron activity from the motor cortex while monkeys performed memory-guided arm reaching movements to peripheral targets after a 450–750 ms delay. Using all recorded single-neuron activity, they calculated population vectors for every 20 ms during the delay period. They found that all population vectors obtained during the delay period were directed toward the target direction and that the lengths of population vectors were longer in the memory-guided condition than in a visually guided control condition. These results indicate that a population of PF neurons as well as a population of motor cortical neurons maintains directional information during the delay period in a similar manner and that this can be seen as the population vectors directing toward the target direction.

We also calculated population vectors during the delay period of the R-ODR task and found that the population vectors rotated gradually during the delay period from the direction of the visual cue to the direction of the saccade. Since the saccade direction was 90° clockwise from the direction of the visual cue in this task, this result indicates that information represented by a population of PF activity changes gradually from visual information to motor information during the delay period. A similar rotation of population vectors during the pre-movement period has been reported by Georgopoulos *et al.* (1989). They recorded neuronal activity from the motor cortex while monkeys performed a rotation task, in which monkeys were required to make arm-reaching movements 90° counter-clockwise from the visual cue direction immediately after the presentation of the visual cue. They calculated population vectors every 10 ms from the onset of the visual cue until 100 ms after the movement initiation and found that the population vector rotated gradually counterclockwise from the direction of the visual cue to the direction of the movement at an average rate of 732° per second. Based on these results, they suggested that the motor cortex is related to a cognitive process such as mental rotation, and that the internal process related to such process can be visualized by the temporal change of population vectors (Georgopoulos *et al.*, 1989; Lurito *et al.*, 1991). Similarly, Ashe *et al.* (1993) recorded neuronal activity from the motor cortex while monkeys performed a memorized movement task, in which they were required to make arm movements with an orthogonal bend (first upward then leftward) after a 600–700 ms waiting period. They calculated population vectors every 10 ms during the

waiting period and found that population vectors first directed toward the direction similar to the second movement (leftward) and then rotated back to the direction similar to the first movement (upward). This result indicates that neuronal processes retrieving the motor sequence during the waiting period can be shown by the temporal change of population vectors constructed by a population of motor cortical activities.

Our present results, together with those of Georgopoulos *et al.* (1989) and Ashe *et al.* (1993), indicate that population vector analysis is an adequate method for visualizing internally operating neuronal processes related to such cognitive functions as spatial working memory. Particularly, temporal change of the direction as well as the amplitude of population vectors might be effective indicators in examining internally operating neuronal processes. Fuster (1997) has proposed the mediation of cross-temporal contingency as an important function of the PF cortex. He considered the delay period of the task as the period for the cross-temporal bridging of sensory-motor information, which is a dynamic process of internal transfer as well as a process of cross-temporal matching. The present result that population vectors rotate gradually from a sensory information domain to a motor information domain during the delay period supports his notion that the PF cortex plays a significant role for mediating the cross-temporal contingency.

Neural Mechanism of the Temporal Change of Represented Information during the Delay Period

Figure 7*A* shows the temporal change of population vector directions in the ODR and R-ODR tasks. Directions of population vectors were maintained along a trial in the ODR task, while the population vector rotated gradually during the delay period from the direction of the visual cue to the direction of the saccade target in the R-ODR task. The rotation started ~2 s after the initiation of the delay period and maintained rotation at a constant speed until the population vector directed toward the direction of the saccade target. What is the neuronal mechanism in the PF cortex responsible for the rotation of population vectors during the delay period?

In our previous study (Takeda and Funahashi, 2002a), we reported the delay-period activity encoding the location of the visual cue (D*cue*) and the delay-period activity encoding the direction of the saccade (D*sac*). In the present study, we constructed the population histogram using these two types of delay-period activity at the preferred direction in each neuron. Figure 7*B* shows these population histograms in the R-ODR task. D*cue* activity showed tonic sustained excitation throughout the delay period, whereas D*sac* activity showed a gradual increase of activation during the delay period. Initially, the magnitudes of both D*cue* and D*sac* activities were almost the same. However, the magnitude of D*sac* activity became greater than the magnitude of D*cue* activity at ~2 s after the start of the delay period. The timing when D*sac* activity became greater than D*cue* activity coincided with the beginning of the rotation of population vectors. Furthermore, the population vector rotated at a constant speed until it pointed at the same direction as the saccade target. Similarly, the magnitude of D*sac* activity increased monotonously until the saccade initiation. These results suggest that the rotation of the population vectors observed during the delay period is closely related to an increase of the population of activated neurons encoding

A: Temporal change of population vector directions
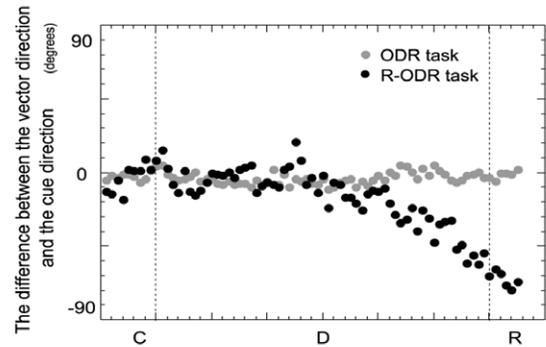
B: Population histograms of delay-period activity in R-ODR task
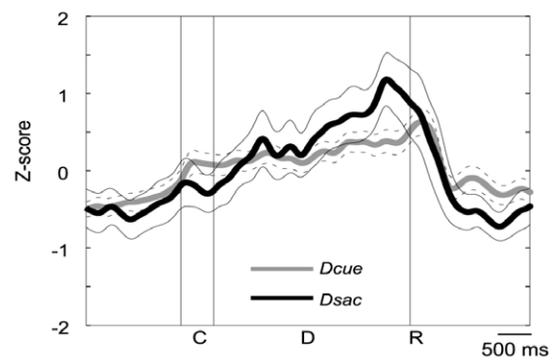
Figure 7. (*A*) Temporal change of the differences between the directions of population vectors and the cue directions in ODR and R-ODR tasks. The same data is shown in Figures 5*B* and 6*B*. (*B*) Population averaged histograms constructed by neuronal activities for all neurons having delay-period activity encoding the location of the visual cue (D*cue*) and all neurons having delay-period activity encoding the direction of the saccade (D*sac*) in R-ODR condition. The thin dotted line indicates the standard error of D*cue* activity and the thin solid line indicates the standard error of D*sac* activity. C, cue period; D, delay period; R, response period. The length of the delay period was 3s.

saccade directions as well as an increase of the magnitude of excitation of these neurons. Although it is not known how motor information is constructed using visual inputs in the PF cortex, functional interactions among prefrontal neurons with different task-related activities (Funahashi and Inoue, 2000; Constantinidis *et al.*, 2001a) and the temporal modulation of functional connectivity (Vaadia *et al.*, 1995; Funahashi, 2001) have been reported in PFC neurons. Therefore, these interactions may play an important role in constructing motor information from visual inputs in PFC.

Notes

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