Contrasting Effects of Reward Expectation on Sensory and Motor Memories in Primate Prefrontal Neurons

The lateral prefrontal cortex (LPFC) has been implicated in working memory that guides goal-directed behavior. However, mechanism that integrates the reward value into the working memory for goal-directed behavior is not understood. To help clarify this issue, we examined the effect of reward expectation on the neuronal process in the LPFC associated with memory-based sensorimotor processing. By temporally dissociating visuospatial sensory and saccade-directional motor memories in the LPFC, we here show that reward expectation significantly enhanced the directional selectivity of sensory working memory but did not affect the directional selectivity of motor memory. The enhancement of sensory working memory in the neuronal population was sustained during the delay but extinguished soon after the motor memory appeared. These results suggest that the expectation of high reward value primarily affects the sensory working memory that may be used for behavioral guidance rather than preparation for forthcoming saccades. It thus appears that the LPFC is a neuronal substrate for working memory used to guide a reward-oriented behavior, rather than reflecting an efficient control of motor action in motivated states.

Keywords: goal-directed behavior, prefrontal cortex, reward expectation, sensorimotor transformation, working memory

Introduction

The value of a reward obtained with successful behavior is important for guiding purposeful behavior. The lateral prefrontal cortex (LPFC) has been implicated in working memory that guides goal-directed behavior. However, mechanism that integrates the reward value into the working memory for goal-directed behavior is not understood. To help clarify this issue, we examined the effect of reward expectation on the neuronal process in the LPFC associated with memory-based sensorimotor processing. By temporally dissociating visuospatial sensory and saccade-directional motor memories in the LPFC, we here show that reward expectation significantly enhanced the directional selectivity of sensory working memory but did not affect the directional selectivity of motor memory. The enhancement of sensory working memory in the neuronal population was sustained during the delay but extinguished soon after the motor memory appeared. These results suggest that the expectation of high reward value primarily affects the sensory working memory that may be used for behavioral guidance rather than preparation for forthcoming saccades. It thus appears that the LPFC is a neuronal substrate for working memory used to guide a reward-oriented behavior, rather than reflecting an efficient control of motor action in motivated states.

Keywords: goal-directed behavior, prefrontal cortex, reward expectation, sensorimotor transformation, working memory

Materials and Methods

Subjects

Two Macaca fuscata monkeys (a male, weighing ~8 kg, and a female, weighing ~6 kg) were used in the experiments. All the experimental procedures were conducted in accordance with the Guide for Care and Use of Laboratory Animals of United States National Institute of Health and the guidelines of our Institute, and were approved by animal care committee of our Institute. The monkeys were habituated before training to a monkey chair, and then preliminary surgery was performed under deep sodium pentobarbital anaesthesia (25 mg/kg, i.v.) and aseptic conditions. The skull was partly exposed, and two head-holding devices were implanted and held by dental acrylic. Small stainless-steel bolts for ground connections were anchored to the skull and fixed with dental acrylic. Antibiotics were injected intramuscularly on the day of surgery and daily thereafter for one week to prevent infection.

Task Procedures

The monkeys were trained to perform a variant of an antisaccade task (Fig. 1A). The task started when the monkey gazed at a central fixation point (a white circle, 0.5° in diameter). After the 1.5 s fixation period, a visual cue (either a yellow triangle or cyan circle; 1° in diameter) was presented randomly, either to the right or left (18° from the fixation point), for 0.8 s. Each cue was associated with a size of reward (Fig. 1C). In block 1, the yellow triangle indicated a large reward and the cyan circle indicated a small reward. The cue-reward sizes were reversed in block 2. The cue was then extinguished and the first delay period (delay 1) of 2 s followed. After this delay, the rule cue was superimposed over the central fixation point for 0.6 s. The rule cue was either a white

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of gaze was within 8° of the circular window around the target, a white square (0.9° by 0.9°) appeared at the target location. The monkey was required to fixate on this confirmation cue for 1 s, and a water reward was delivered depending on the reward condition (the large reward was ~0.3 ml; the small reward was ~0.01 ml). The contingency of cue and reward size was alternated blockwise after recording 13 trials at least for every task condition.

**Task Procedure for the Probe Test**
Blocks 1 and 2 of the antisaccade task were different in their cue-reward contingencies. However, because these contingencies are not necessary information for monkeys in performing the task, we could not check whether the monkey really noticed the block change only by the antisaccade task. To confirm this, a probe test was randomly inserted in the task (Fig. 1D). This test began when the monkey fixated on a central fixation point (a white square; 1° by 1°). Two cue stimuli (the same yellow triangle and cyan circle shown in the antisaccade task) were simultaneously presented to the right and left (or left and right) peripheral locations. After the 0.8 s cue period, the peripheral cues were extinguished and a 2 s delay period followed. When the central fixation point was turned off, the monkey was free to choose either cue as a target by making a saccade. After the saccade, the selected target reappeared at the target location as a confirmation cue. After a 1 s confirmation period, water reward was delivered in an amount depending on the reward condition of the selected cue.

If the monkey correctly notices the reward amount associated with the stimulus, it should select a large-reward target consistently during the block. During the recording session, we examined whether the monkey consistently selected the large reward target. Both monkeys had a tendency to change the target immediately after the block alternation. Once they noticed that the reward amount was changed (i.e. reduced), they changed the target in the next probe test. After they had changed their target, they had a tendency to choose the large-reward target consistently during the block (with a >95% rate for both monkeys). If the monkey chose the large-reward target three consecutive times, we considered that it had notice the block change at the first selection of the large-reward target, and used the neuronal data after the first selection of the target. By these probe tests, we confirmed that the monkeys could change their association immediately after the block alternation, and that they could keep the correct cue-reward association during the trials.

**Neuronal Recording**
Upon the completion of training, a recording chamber was fixed to the skull of each monkey. Under sodium pentobarbital anesthesia (~25 mg/kg i.v.) and aseptic conditions, an oval opening was made in the skull, exposing the dura matter overlaying the frontal cortex, and a stainless steel cylinder was implanted using dental acrylic. Prophylactic antibiotics were injected i.m. on the day of surgery and daily thereafter for 1 week. The recording and control system consisted of an infrared eye-motion camera (R-21C-A, RMS, Hiroasaki, Japan), two networked personal computers and other peripheral equipment. The eye positions from the camera were converted to digital signals at 250 Hz. One computer controlled the tasks and the other monitored neuronal activity, eye position, and task events.

The activity of single neurons was recorded with glass-insulated elongated microelectrodes (impedance, 0.3 – 1.5 MΩ), using conventional electrophysiological techniques, as previously described (Tsujimoto and Sawaguchi, 2004). Microelectrodes were inserted vertically with a pulse motor-driven micromanipulator (MO-81, Narishige, Tokyo), and in the plane of the cortex with a plastic grid attached to the cylinder. Data collection began as soon as an advancing electrode recorded well-isolated activity of one or more neurons. This activity was not pre-screened for task-related responses. Neuronal activity was digitized with a Multi-Spike Detector (Alpha-Omega Engineering, Israel), stored in the data collection computer, and later analyzed offline. Neuronal activity, eye positions and task sequences were simultaneously recorded on digital audiotape (PC-208 M recorder, Sony, Tokyo).

We recorded from neurons in the LPFC rostral to the frontal eye field (FEF); most were located in the caudal half of areas 46 and 8a (Fig. 3). To determine whether the recording electrode was in the FEF, we applied intracortical microstimulation (ICMS) through the recording electrode (22 cathodal pulses of 0.3 ms duration at 333 Hz, with currents up to...
100 µA). When eye movements were elicited by ICMS at current intensities of <100 µA, the site was considered to be within the high threshold region of the FEF (Bruce et al., 1985). Recordings from these sites were excluded from this study.

**Data analyses**

**Behavioral Analysis**

Error trials consisted of fixation breaks (premature terminations by cessation of fixation), wrong saccades (saccadic eye movements opposite to the correct target in the go period) and others (including omissions of the go signal, saccades to non-target and so on). We calculated the success rates based on correct and wrong saccades for each combination of saccade rules and reward conditions, so that we could test whether the intended saccade directions were affected by either the saccade rule or the reward size. Fixation break was defined as the gaze shift that left from the central fixation window before the offset of the fixation spot. Reaction time was defined as the delay from the offset of the fixation spot to the moment when the eye position left from the central fixation window. In calculating the behavioral data (success rate, mean of saccade onset and fixation break rate) for whole sessions (Fig. 2), we first calculate the means for one daily session, separately for saccade rules and reward conditions, and then averaged them across the session. We compared these data across sessions by paired t-tests to clarify the effect of reward condition and saccade rule on the behavioral changes.

**Neuronal Classification**

The mean discharge rate in each task period was compared with that in the pre-cue ‘control’ period (the 1 s duration before the cue onset) to examine whether the neuron showed significant task-related activities. If the mean discharge rate in a given period was significantly different from that in the control period (Mann-Whitney U-test, \( P < 0.05 \)), the neuron was considered to show task-related activity in that period. The directional selectivity (i.e. the selectivity of the mean discharge rate for a cue location or a saccade direction) was examined for a delay neuron that showed a significant increase in activity during delay 1 or delay 2, compared with the pre-cue control period activity.

Before examining the effect of reward condition on the directional selectivity, we removed cells whose activities varied as a function of the visual attributes of the cue. We performed a two-way analysis of variance (ANOVA) on the activities during delays 1 and 2 (cue location versus reward condition during delay 1; saccade direction versus reward condition during delay 2), separately for each block. We collected neurons that showed significant dependence (\( P < 0.05 \)) on the reward condition (either main or interaction effect) and checked their preference for large or small reward condition (i.e. whether the activity is high in the large or small reward condition) in each block. If the activity showed a significant dependence on reward condition in both blocks but the preference was different between blocks, we considered that the neuron responded to the visual attributes of the cue rather than the reward condition. These cue-discriminative neurons were excluded from the results.

**Effects of Reward Conditions on Delay-period Activities**

After excluding cue-discriminative neurons, the delay-period activities were analyzed without discriminating two blocks. Effects of cue location and reward condition on the delay-period activities were classified by a two-way ANOVA with significance accepted at \( P < 0.05 \) (Table 1). The mean activities during delay 1 were analyzed with the test for cue location versus reward. If the activity during delay 1 was significantly affected only by cue location, the activity was classified as CO type. If the activity was significantly affected by cue location and reward (i.e. main effect of cue and main effect of reward) or by their interaction (i.e. interaction effect of cue and reward), the activity was classified as CR type. If the CR-type activity was higher in the large reward condition, it was classified as CR(+) type. If the activity was higher in the small reward condition, it was classified as CR(-) type. Similarly, the effects of saccade direction and reward condition on the activity during delay 2 were classified as well. The mean activity during delay 2 was analyzed with the test for saccade direction versus reward. If the activity during delay 2 was affected significantly only by saccade direction, the activity was classified as SO type. If the activity was significantly affected by both saccade direction and reward or by their interaction, the activity was classified as SR type. If the SR-type activities were higher and lower in the large reward condition, they were classified as SR(+) and SR(-) types, respectively.

**Classification of Directional Delay Neurons**

Based on the above classification of delay-period activities, we classified neurons as follows. In this study, we focus on the effect of reward condition on the directional delay-period activity, which has been considered to be involved in mnemonic process (Funahashi et al., 1989), including memory-based sensorimotor transformation. If the activity during delay 1 of a neuron showed a significant dependence on cue location (i.e. if the activity was of CO, CR(+) or CR(-) type), we considered that the neuron showed ‘cue-directional’ activity. Similarly, if the activity during delay 2 of a neuron showed a significant dependence on the saccade direction (i.e. if the activity was of SO, SR(+) or SR(-) type), we considered that the neuron showed ‘saccade-directional’ activity. If a neuron showed saccade-directional activity without saccade-directional activity, the neuron was classified as C type. If a neuron showed saccade-directional activity without cue-directional activity, the neuron was classified as S type. If a neuron showed both cue-directional and saccade-directional activities, the neuron was classified as CS type. When a neuron showed larger mean discharge rate for a cue location than the other in the large reward condition, the cue location was defined as the ‘preferred cue location’ of the neuron. When a neuron

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**Figure 2. Effects of reward conditions on behavior.** (A) Correct performance rate. The height of each bar indicates the mean of correct performance rates across all recording sessions, obtained from each monkey in large reward (gray) and small reward (white) conditions. Each value was obtained by first computing the mean for each daily session and then averaging these. Error bars indicate the standard error of the mean (SEM) across sessions. (B) The means of saccade onset for large and small rewards were computed in the same way. (C) The rate of fixation breaks during delay periods. An asterisk indicates that the value was significantly different between the two reward conditions (paired t-test, \( P < 0.05 \)).
showed larger mean discharge rate for a saccade direction than the other in the large reward condition, the saccade direction was defined as the 'preferred saccade direction' of the neuron.

**Population Activity**
In order to calculate the population activity (shown in Figs 5A, 6A and 8A), we collected spike density function (SDF) of each neuron. In calculating the SDF for a single neuron, spike trains were first convolved with a Gaussian function (SD = 40 ms), and then averaged over trials. In averaging, we collected all the trials that contributed to each task period of the SDF. Because the saccade rules were not determined before the rule-cue onset in this task, we did not discriminate the trials by saccade rules before the rule-cue onset. For example, in the prosaccade condition, both pro- and antisaccade trials contributed to the SDF before the rule-cue onset, but only prosaccade trials contributed to the SDF after that. For confirmation, we calculated the SDF by discriminating by saccade rules also before the rule, but there was no difference except for a clearer time course in an original one. After averaging over trials, the SDF was normalized by the mean firing rate during the pre-cue control period (1 s duration before the cue onset), to exclude the effect of baseline activity. Even if the activity was not normalized, we confirmed quite similar temporal patterns and effects of reward condition. Based on the normalized SDF of each neuron, the population activity was obtained by averaging the SDFs over collected neurons.

**Directional Index**
To quantify the difference in activities between preferred and non-preferred directions for each neuron, we used 'directional index' (DI) (Zhang and Barash, 2004). In calculating the DI, we used the SDF, which is obtained by convolving spike trains with a Gaussian function (SD = 60 ms). The DI of C- or CS-type neuron was obtained by subtracting the SDF for the nonpreferred cue location from that for the preferred cue location. Similarly, the DI of S-type neuron was obtained by subtracting the SDF for the nonpreferred saccade direction from that for the preferred direction.

To describe the time course of directional selectivity in population, we used the population mean of the DI (mean DI), illustrated by black and gray lines in Figures 5B, 7B and 8B. The mean DI was calculated by averaging the DIs over collected neurons. Assuming that the distribution forms a Gaussian, we used the 95% confidence interval as the criteria of statistical significance of the mean DI. The confidence interval was calculated for each 20 ms bin. If the lower bound of the confidence interval was larger than zero, the mean DI was considered to be significantly larger than zero. If the upper bound of the confidence interval was smaller than zero, the mean DI was considered to be significantly smaller than zero.

Further, to characterize the time course of the effect of reward conditions, we used the population mean of the difference between DI for large and DI for small reward conditions (mean difference of DI), illustrated by red and blue lines in Figures 5B, 7B and 8B. In calculating this first we collected the difference of DIs for each neuron, which is obtained by subtracting the DI for the small reward from that for the large reward condition. We then averaged them over collected neurons. We also used the 95% confidence interval of the mean as the criteria for statistical significance. The confidence interval was calculated for each 20 ms bin. If the confidence interval was smaller or larger than zero, the difference was considered to be significant.

In the statistical test used for the mean DI and the mean difference of DIs, neurons were the unit of observation. Therefore, the disparity between delay 1 and 2 in the numbers of trials, which contributed to calculating each SDF, did not impact on the degree of freedom and the magnitude of the population mean. We also performed statistical tests based on the SDFs which were discriminated by saccade rules also before the rule onset, and confirmed similar results. Further, because the tuning indexes (TI; explained later) for cue location and saccade direction had small differences in the number of trials, similar results obtained from the analyses of DI and TI suggest no impact in disparity in the number of trials.

**Tuning Indexes for the Lateral Distribution**
The ipsilateral and contralateral distributions for cue- and saccade-directional activities were quantified by tuning indexes (TI), which were calculated for each neuron (Fig. 9). The tuning index for cue location (Tlc) was calculated using the following equation: 

\[
Tlc = \frac{Rlc - Rli}{Rlc + Rli}
\]

where \(R_{lc}\) and \(R_{li}\) indicate the mean discharge rates of a neuron during delay 1, when the cue was presented contralateral to the recording site (\(R_{lc}\)), or ipsilateral to the recording site (\(R_{li}\)) respectively. Similarly, the tuning index for saccade direction (Tks) was calculated using the following equation:

\[
Tks = \frac{Rsc - Rsi}{Rsc + Rsi}
\]

where \(R_{sc}\) and \(R_{si}\) indicate the mean discharge rates of a neuron during delay 2 when the next saccade direction was contralateral to the recording site (\(R_{sc}\)), or ipsilateral to the recording site (\(R_{si}\)) respectively. Tlc indicates the difference between the activities during delay 1 for contralateral and ipsilateral cue presentation to the recording site. Similarly, Tks indicates the difference between the activities during delay 2 for contralateral and ipsilateral saccades.

**Histology**
After the experiments were completed, the monkeys were deeply anesthetized with an overdose of sodium pentobarbital and perfused with 0.9% saline, followed by 10% formalin. The brain was removed and photographed, and then cortical surface was examined to detect the penetration points. The points were distributed throughout the caudal half of the periprincipal sulcal area. Cortical distributions of the recorded neurons were illustrated in Figure 3.

**Results**

**Behavioral Performance**
Behavior was analyzed using data of all recording sessions (Fig. 2). Error trials contained errors in saccade direction (trial error; Fig. 2A), fixation errors before the fixation-point termination (fixation break; Fig. 2C) and others (including omission and saccade to non-target location). Our results showed that the behaviors were mainly affected by reward conditions. The
correct performance rate for large rewards was significantly greater than that for small rewards (paired t-test, \( P < 0.05; \) Fig. 2A). The rate of fixation breaks (\( P < 0.05; \) Fig. 2C) and mean of saccade latencies (\( P < 0.05; \) Fig. 2B) were significantly less than those for large rewards. Pro- and antisaccade conditions were not significantly correlated with the success rates or saccade onsets in either monkey (paired t-test, \( P > 0.05; \) Fig. 2A–C). The results indicate that, although the animals were free to ignore the reward conditions, their behavior was systematically influenced by the reward size. On the other hand, the saccade rules did not significantly affect behavior.

**Classification of Directional Delay Neurons**

We recorded activity from 896 LPFC neurons. Of these, 874 neurons showed task-related activity during at least one task period. Forty-two of these neurons changed their directional preference, depending on the visual attributes of cues rather than the reward conditions, across the two trial blocks. Because we were interested in the effects of reward condition, these cue-discriminative neurons were excluded from this study, and we used remaining 832 neurons for following classification.

We focused on the effect of the reward condition on the directional activity during the delay periods, because the delay-period activity has been considered to reflect memory processes (Fuster, 1973; Funahashi et al., 1989) including memory-based sensorimotor transformation. The activity in 668 of the 832 neurons showed a significant increase during delay 1 or delay 2, or both, compared with the pre-cue control activity (the 1 s duration before the cue onset). Using data of these neurons, we tested the effect of cue location and reward conditions on the activity during delay 1 (two-way ANOVA; cue location versus reward condition), and the effect of saccade direction and reward conditions on the activity during delay 2 (two-way ANOVA; saccade direction versus reward condition).

To dissociate the sensory and motor memory processes, we classified the delay neurons according to their directional selectivities of delay-period activities. When the activity during delay 1 of a neuron significantly differed with the cue location (main or interaction effect of cue location; \( P < 0.05 \)), we considered that the neuron showed ‘cue-directional’ activity. When the activity during delay 2 of a neuron significantly depended on the saccade direction (main or interaction effect of saccade direction; \( P < 0.05 \)), we considered that the neuron showed ‘saccade-directional’ activity. Neurons were classified into three distinct groups: C-type neurons (117/249, 47.0%), which showed cue-directional activity without saccade-directional activity; S-type neurons (65/279, 26.1%), which showed saccade-directional activity without cue-directional activity; and CS-type neurons (67/279, 26.9%), which showed both cue- and saccade-directional activities.

The recording sites and distributions of C-, CS- and S-type neurons are shown in Figure 3A. These three types were broadly distributed through the LPFC, but there were asymmetrical tendencies in their distributions. To examine whether the rostrocaudal or dorsoventral distributions of three types were significantly different each other, we performed chi-square tests between distributions for each combination of three types. The rostrocaudal distribution of CS type was significantly different from that of C type (\( P < 0.001 \)) and that of S type (\( P < 0.05 \)). The dorsoventral distribution of C type was significantly different from that of S type (\( P < 0.05 \)) and that of CS type (\( P < 0.05 \)). Therefore, C- and CS-type neurons tended to be at the different part of the LPFC. C-type neurons tended to be at the rostral part of the LPFC, ~7.5 mm from the rostral edge of the FEF, and CS-type neurons tended to be at the middle part of the LPFC, ~3 mm from the FEF. Because of this inhomogeneous distribution, each process of memory-based sensorimotor transformation

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**Figure 3.** Cortical distribution. Black dots show the recording sites. An x marks a frontal eye field (FEF) track identified by microstimulation. The principal sulcus (PS) and arcuate sulcus (AS) are drawn on the maps. A summary of the rostrocaudal and dorsoventral distributions of three types of neurons is shown in histograms. Cortical distributions for both the right and left hemispheres in two monkeys are shown in one diagram. (A) Cortical distribution of C-, CS- and S-type neurons. The sizes of blue, green and red circles indicate the numbers of C-, CS- and S-type neurons recorded at each site, respectively. Inset figure indicates the results of chi-square tests between distributions of each type. Upper triangular part of the inset figure indicates the results of rostrocaudal distribution, and lower triangular part indicates dorsoventral distribution. (B) Cortical distribution of reward effects. The sizes of blue, red and light gray circles indicate the numbers of neurons whose activities were enhanced, reduced and had no effect by large reward, respectively.

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(e.g. sensory memory and transformation) seems to be carried out in a distributed cluster of neurons in the LPFC. On the other hand, the cortical distribution of neurons that showed reward effects (Fig. 3B) has little tendency towards local concentration. Only one significant but weak difference was found between dorsoventral distributions of enhancement (R+) and no effect (No) \((P = 0.04)\). Accordingly, the reward effects (e.g. enhancement and depression) seem to be almost homogeneously observed throughout the LPFC.

**Influence of Reward Conditions on C- and S-Type Neurons**

An example of the activity of a C-type neuron is illustrated in Figure 4. This neuron showed a gradual increase in activity during delay 1 when the cue was presented to the right side and the reward was large (an interaction effect between cue location and reward condition; \(P < 0.05\)). The increased activity reached its peak and then rapidly declined to the baseline level during the rule-cue period.

The directional selectivities of cue-directional activities in individual neurons were widely distributed (Fig. 9A,B and 10A), but a clear effect of reward expectation was observed in the population activity of C-type neurons (Fig. 5A). The population activity showed a sustained increase during delay 1, particularly with large rewards. To quantify the degree to which directional activities were affected by reward size, the population mean of directional index (DI), which indicates the difference between cue- and saccade-directional activity of an individual neuron (Zhang and Barash, 2004), was calculated (Fig. 5B). Although the mean DI of the C-type population was significantly larger than zero for both large and small rewards all through delay 1, the mean DI for large reward was significantly larger than that for small reward also through delay 1.

In contrast to C-type neurons, little effect of reward condition was observed in the population activity of S type (Fig. 6A), although the activities of some S-type neurons were affected by the reward conditions (Figs 9C,D and 10B). Also the mean DI was little affected by reward conditions in both pro- and antisaccade conditions (Fig. 6B). Accordingly, we consider that the reward size did not significantly differentiate the saccade-directional activity in neuronal population.

**Influence of Reward Conditions on CS-type Neurons**

The different effects of rewards on the cue- and saccade-directional activities were also observed within a single neuron. Figure 7 shows an example of the activity of a CS-type neuron. During delay 1, this neuron showed a significant increase in activity when the cue was presented to the right side and the reward was large (an interaction effect between cue location and reward condition; \(P < 0.05\)). During delay 2, this neuron showed a significant increase in activity for rightward saccades, but the activity was not significantly affected by reward conditions (main effect of saccade direction; \(P < 0.05\)). Thus, the effect of reward size on the delay-period activity of this neuron was changed before and after the rule-cue presentation.

The overall effects of reward size on the activities of CS-type neurons were examined based on their population activity (Fig. 8A). However, because the preferred directions of CS-type neurons were not necessarily the same between delay 1 and 2, the classification should be depended on whether the activity is reversed or sustained across the two delay periods. When the preferred cue location and saccade direction were the same (e.g. the neurons shown in Fig. 7), the neurons were classified as 'same-directional' neurons. When the preferred directions were opposite, they were classified as 'opposite-directional' neurons. Of CS-type neurons, more than half (46/67, 68.7%) were same-directional and the rest (21/67, 31.3%) were opposite-directional. Except for the difference in directional preferences, the basic properties of the same- and opposite-directional neurons were similar. In the prosaccade condition, the activity of the same-directional neurons was sustained through delays 1 and 2. In the antisaccade condition, the activity was reversed; the increased activity during delay 1 decreased rapidly during the rule-cue period, whereas the low activity during delay 1 increased during the rule-cue period. On the other hand, the activity of the opposite-directional neurons was sustained in the antisaccade condition and reversed in the prosaccade condition.

To summarize the temporal activity pattern of all the CS-type neurons, we gathered the data of CS-type neuron by calculating the population activity according to whether the neurons reversed ('reversing condition') or sustained ('nonreversing condition') their activities across rule-cue period (Fig. 8B). The population activity of CS-type neurons revealed a clear difference in the effect of reward size between the cue- and saccade-directional activities (Fig. 8A). The reward size significantly affected the activity in delay 1, but not in delay 2. The mean DI of the CS-type neurons is shown in Figure 8B. Large rewards increased the mean DI during delay 1 significantly, but they increased little during delay 2. Accordingly, the reward expectation somewhat selectively and persistently enhanced the selectivity of cue-directional activity of CS-type neurons at the population level during delay 1.

**Tuning Indexes for Ipsi- and Contralateral Distribution**

In order to characterize how the directional selectivity of each neuron is distributed, the tuning indexes for lateral distribution were calculated for each neuron, separately for cue location \((T_{CI})\) and saccade direction \((T_{SA})\). Although the tuning indexes of single neurons were so widely distributed that the three types of neurons could not be clearly separated by the distribution, statistical tendencies were found in tuning indexes of three neuronal groups.

The mean \(T_{CI}\) for C-type neurons (Fig. 9A,B) was significantly greater than zero for large rewards \((\mu = 0.087, \sigma^2 = 0.022\); one-sample \(t\)-test, \(t = 6.07, P < 0.001\)) and small rewards \((\mu = 0.038, \sigma^2 = 0.013; t = 3.55, P < 0.001\)). The mean values were significantly different between the two reward conditions (paired \(t\)-test, \(t = 3.46, P < 0.001\)). The mean \(T_{SA}\) had a statistical tendency to be contralateral to the recording sites, and the values were enhanced by large reward. The mean \(T_{SA}\) for S-type neurons (Fig. 9C,D) was significantly larger than zero in both the large \((\mu = 0.067, \sigma^2 = 0.023; t = 4.10, P < 0.001\)) and small \((\mu = 0.077, \sigma^2 = 0.022; t = 4.69, P < 0.001\)) reward conditions, but not significantly different between reward conditions (paired \(t\)-test, \(t = 0.62, P > 0.05\)). Thus, the mean \(T_{CI}\) for S-type neurons had a statistical tendency to be contralateral to the recording sites, and the values were not affected by reward conditions. The mean \(T_{CI}\) for CS-type neurons (Fig. 9E,F) was significantly larger than zero in both the large \((\mu = 0.137, \sigma^2 = 0.021; t = 10.5, P < 0.001\)) and small \((\mu = 0.078, \sigma^2 = 0.017; t = 6.66, P < 0.001\)) reward conditions. The mean values were
significantly different between reward conditions (paired t-test, \( t = 4.51, P < 0.001 \)). The mean \( T_{IC} \) for CS-type neurons was significantly larger than zero in both the large (\( \mu = 0.128, \sigma^2 = 0.040; t = 7.11, P < 0.001 \)) and small (\( \mu = 0.113, \sigma^2 = 0.024; t = 8.12, P < 0.001 \)) reward conditions. However, the mean \( T_{IS} \) values were not significantly different between reward conditions (paired t-test, \( t = 1.08, P > 0.05 \)).

### Overall Effect of Reward Condition

The effects of reward condition on tuning indexes of each neuron were not uniform. To characterize the effect of reward condition on the tuning indexes, we plotted the \( T_{IC} \) and \( T_{IS} \) along with the large and small reward conditions. In Figure 10A, the covariance eclipse, indicating the covariance of distribution for \( T_{IC} \), was deviated toward rightward from diagonal line. The mean \( T_{IC} \) in the large reward condition is significantly larger than that in the small reward condition (paired t-test, \( P < 0.001 \)). These results indicate that the \( T_{IC} \) had a tendency to be enhanced by a large reward toward contralateral, and then in population the distribution of the \( T_{IC} \) appears to be skewed to contralateral.

On the other hand, in Figure 10B, the major axis of the eclipse for the \( T_{IS} \) is almost on the diagonal line. The mean \( T_{IS} \) were not significantly changed at all (paired t-test, \( P > 0.05 \)). These results indicate that the mean \( T_{IS} \) had a tendency to show similar values in two reward conditions and then in neuronal population the \( T_{IS} \) does not appear to be changed because of a balance of enhancement and depression.

Figure 11 summarizes the effect of reward condition on the cue-directional and saccade-directional activities. Directional indexes for cue location and saccade direction were averaged over neuron and averaged over time. The temporal average of the mean DI for cue location became significantly larger than zero both in the small (\( \mu = 0.025, \sigma^2 = 0.012; t = 3.16, P < 0.001 \)) and large reward conditions (\( \mu = 0.085, \sigma^2 = 0.020; t = 8.73, P < 0.001 \)), and their values were further significantly different for the two conditions (paired t-test, \( t = 6.06, P < 0.001 \)). On the other hand, the temporal average of the mean DI for saccade direction did not differ according to reward size (paired t-test, \( t = 0.20, P > 0.05 \)), although their values were significantly larger than zero in both the small reward (\( \mu = 0.110, \sigma^2 = 0.028; t = 7.82, P < 0.001 \)) and large reward conditions (\( \mu = 0.113, \sigma^2 = 0.027; t = 8.18, P < 0.001 \)). These results suggest a selective
Discussion

Neurons in the lateral prefrontal cortex (LPFC) exhibit sustained activity during the delay period, and have been implicated in working memory (Goldman-Rakic, 1995; Fuster, 2001). To characterize the mnemonic process that guides a goal-directed behavior, we temporally separated the delay-period activities that reflect memory of cue location and memory of saccade direction. Because the delay-period activities for sensory cue are sustained until rule-cue presentation, they are thought to reflect online working memory (Sawaguchi and Yamane, 1999), which is used to guide saccade direction. Because represented information is rapidly transformed to saccade direction after the rule-cue period, the delay-period activities for saccade direction are involved in memory and/or preparation of forthcoming saccades (Takeda and Funahashi, 2004). To investigate the effect of reward expectation on the mnemonic processes to guide a goal-directed behavior, we examined the effect of reward size on these delay-period activities. We classified the neurons according to their directional preferences for cue location and saccade direction to dissociate the sensory and motor memory processes, and then compared the influence of reward size on these mnemonic activities. We found that reward expectation primarily enhanced the neuronal process for memory of sensory cue location used for behavioral guidance, but did not affect the memory process for saccade direction, which appears after response selection has taken place in the LPFC.

Improvement of Action Control in the Large-reward Condition

A central feature of motivated behavior is that motivational state facilitates the motor response that leads to appetitive behavior (Stellar and Stellar, 1985; Roesch and Olson, 2003, 2004). In accordance with this nature, behavioral performance in our data was significantly affected by reward size. Monkeys tended to make more correct responses, and respond faster, for the larger reward. Further, the relatively larger numbers of enhanced cue-directional activity in neuronal population by large reward expectation.
fixation breaks with the small reward than with the large reward observed in both monkeys suggest that reward expectation is important for initiating and maintaining this goal-directed behavior. Therefore, the reward expectation seems to be important not only for an efficient control of action but also for initiating and maintaining a goal-directed behavior.

Antisaccade paradigm has been used to test the ability of suppression of saccades to the peripheral cues. In the antisaccades without delay, it has been reported that a certain number of erroneous saccades were directed to the presented peripheral cues (Hallett, 1978; Krappmann et al., 1998). Because of this, the peripheral cue presentation has been considered to automatically induce a saccade toward it. Moreover, to perform a correct antisaccades, suppression of the saccade towards it seems to be necessary (Everling and Fischer, 1998). Conversely, in our data, the behavioral performance did not show any specific changes for the antisaccade situations. Therefore, a possible mechanism of automatic induction (and suppression) of saccades by peripheral cue presentation is not likely to affect the saccades observed in the go period. Accordingly, we consider that the saccades observed in the go period in our task were not affected by peripheral cue presentation, and that the major factor affecting the behavior (including success rate, reaction time, and fixation break) was the size of the reward instead of it.

Sensorimotor Processing in the LPFC

The LPFC has been considered as one of the centers for memory-based sensorimotor processing (Kim and Shadlen, 1999; Quintana and Fuster, 1999; Hoshi et al., 2000; Constantinidis et al., 2001; Tanji and Hoshi, 2001; Takeda and Funahashi, 2002, 2004). Recent studies on nonhuman primates have found that the activities of LPFC neurons are correlated with both task performance and sensory stimulus strength (Kim and Shadlen, 1999; Constantinidis et al., 2001), suggesting that the sensory representation for LPFC neurons may predict subsequent behavioral actions. We have previously demonstrated that a subset of LPFC neurons shifted their mnemonic representation from sensation (Sawaguchi and Yamane, 1999) to motion (Hasegawa et al., 1998) soon after the behavioral rule was presented in a task like the one used in this study (Amemori and Sawaguchi, 2003). CS-type neurons showed both the cue- and saccade-directional activities, and the representation shifted rapidly during the rule-cue period. Because of this rapid

Figure 6. Effects of reward condition on S-type neurons (n = 65). The format and abbreviations are the same as in Figure 5. (A) Population activity of S-type neurons. (B) Population mean DI for S-type neurons. The mean DI of S-type neurons did not differ with the reward conditions during delay 2.
shifting, these neurons appear to link retrospective sensory memory with prospective motor commands. These findings appear to implicate the LPFC in the transformation of sensory memory into motor commands, a memory-based sensorimotor transformation.

**Motivation Enhances the Memory for Decision**
The sustained activities of C- and CS-type neurons during delay 1 suggest that these neurons are involved in active maintenance of visuospatial sensory information, i.e. working memory (Funahashi et al., 1989; Goldman-Rakic, 1995; Sawaguchi and Yamane, 1999), that is used for guiding a saccade command (Hasegawa et al., 1998; Takeda and Funahashi, 2004). As we have seen in Figures 5 and 8, the directional activities for cue location were significantly enhanced by large rewards. Working memory, which is used for behavioral organization, may be especially affected by reward through the neuronal mechanisms located in the LPFC. Further, because the reward effect was sustained through delay 1, it thus appears that reward information as well as sensory information is actively maintained through delay 1 to affect a process in the rule-cue period. Our task paradigm changed cue-reward contingencies by blocks. However, most directional delay neurons did not change their activities with changes in the visual properties of cues, but with changes in reward size (Watanabe, 1996, 1998; Leon and Shadlen, 1999; Kobayashi et al., 2002; Watanabe et al., 2002). Accordingly, the reward information in the LPFC is likely to be integrated with visuospatial information and sustained until the saccade direction is determined.

**Motivation Little Affects the Motor Memory/Preparation**
The saccade-directional activities of S- and CS-type neurons rapidly increased soon after the rule-cue presentation. Because this activity was sustained during delay 2, it is likely that these neurons are involved in memory, or in preparing for the forthcoming saccade, or both. Because the behavioral parameters of saccades were significantly improved by the large rewards, the neuronal processes for motor memory or preparation for saccades in the LPFC might also be affected by the reward. Indeed, recent studies using a delayed-saccade task suggest that the activities in the caudate nucleus and premotor cortex reflect the facilitation of saccadic eye movements in the rewarded condition (Lauwereyns et al., 2002; Roesch and Olson, 2004). It is thus critical to determine whether the reward-dependent delay-period activities may reflect the efficient control of motor actions. In contrast to the results shown in the premotor cortex (Roesch and Olson, 2003, 2004), this study shows that in the neuronal process of the LPFC for motor memory or preparation for saccades are not so dependent on the reward that we can detect at the population level. Further,
Figure 8. Effects of reward condition on CS-type neurons (n = 67). The activities were classified according to whether the increased activities were sustained (nonreversing condition) or reversed (reversing condition) across the delay 1 and delay 2 periods. The other formats and abbreviations are the same as in Figure 5. (A) Population activity of CS-type neurons. The directional activity for cue location was sustained and enhanced by large reward. After the rule-cue period, the differences in the directional activities between reward conditions were rapidly reduced. (B) Population mean DI for CS-type neurons. Enhancement of DI by reward expectation was sustained through delay 1 but not observed during delay 2.
Figure 9. Distribution of the tuning indexes. Tuning indexes for cue location ($T_c$) and saccade direction ($T_s$) for each neuron were calculated to examine the lateral distributions of cue- and saccade-directional activity. A summary of distributions of $T_c$ and $T_s$ is shown in histograms. Dotted horizontal and vertical lines indicate the means of $T_s$ and $T_c$, respectively. Gray and black lines indicate the means of small reward and large reward conditions, respectively. To compare the distributions between reward conditions, covariance ellipses for both reward conditions were added. Solid ellipse indicates the covariance of the distribution, and dotted indicates that in the other reward condition. (A) C-type neurons in the small reward condition. Gray dotted lines indicate the means of $T_c$ and $T_s$ in the small reward condition, and black dotted lines indicate the means in the large reward condition. (B) C-type neurons in the large reward condition. The mean of $T_c$ was contralaterally deviated, and the deviation was greater for the large reward. (C) S-type neurons in the small reward. (D) S-type neurons in the large reward condition. The mean of $T_s$ was contralaterally deviated but the deviation was not different for the two reward conditions. (E) CS-type neurons in the small reward condition. (F) CS-type neurons in the large reward condition. $T_c$ was contralaterally deviated, and the deviation was greater for large rewards. The $T_s$ mean was contralaterally deviated but the deviation was not different for the two reward conditions.
not a simple reflection of a facilitation of motor control. Instead, it appears that the reward expectation in the LPFC is systematically involved in a memory process that guides a motor command. Contrasting effects of reward expectation to sensory and motor memories support the notion that the LPFC is involved in integration of reward information into goal-directed behavior rather than in the efficient control of saccadic eye movement.

In addition to the explanation that the reward expectation selectively affects the sensory working memory in the LPFC, there seem to be at least one alternative explanation. In a memory-based sensorimotor transformation, the temporal order of sensory and motor memories is needed to be fixed. Therefore, we could not exclude completely the alternative hypothesis that the effect of reward expectation on the neural activity in the LPFC may decreases with time. However, we also observed that the enhancement by large reward was sustained during delay 1 and rapidly disappeared after the rule-cue presentation. Therefore, the integrated representation of reward and sensory memory in the LPFC is not a temporal and gradual change of reward effect. Instead, the reward information seems to be systematically involved in derivation of a motor command from working memory.

The LPFC is considered one of the critical nodes for memory-based sensorimotor transformation because it integrates multimodal sensory information and it generates divergent motor command (Tanji and Hoshi, 2001; Takeda and Funahashi, 2004). The value of reward associated with a successful behavior appears to be important for this goal-directed action (Dickinson and Balleine, 1994). Our results suggest that reward expectation significantly enhances the neuronal process for sensory working memory but not for motor memory or preparation for movement in the LPFC. It thus appears that the LPFC is a neuronal substrate for the organization of reward-oriented behavior rather than for the motivational control of motor actions.

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Notes
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