Influences of Rewarding and Aversive Outcomes on Activity in Macaque Lateral Prefrontal Cortex

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Summary

Both appetitive and aversive outcomes can reinforce animal behavior. It is not clear, however, whether the opposing kinds of reinforcers are processed by specific or common neural mechanisms. To investigate this issue, we studied macaque monkeys that performed a memory-guided saccade task for three different outcomes, namely delivery of liquid reward, avoidance of air puff, and feedback sound only. Animals performed the task best in rewarded trials, intermediately in aversive trials, and worst in sound-only trials. Most task-related activity in lateral prefrontal cortex was differentially influenced by the reinforcers. Aversive avoidance had clear effects on some prefrontal neurons, although the effects of rewards were more common. We also observed neurons modulated by both positive and negative reinforcers, reflecting reinforcement or attentional processes. Our results demonstrate that information about positive and negative reinforcers is processed differentially in prefrontal cortex, which could contribute to the role of this structure in goal-directed behavior.

Introduction

Obtaining food and avoiding damage are essential abilities for animals to survive in the wild. In experimental settings, such abilities can be tested with well-controlled appetitive and aversive events. Animals will increase the probability of making a specific response if the response is contingently followed by a reward such as juice (positive reinforcement). The probability of making a specific response will also increase if an aversive event such as an air puff can be contingently avoided by the response (negative reinforcement). However, our knowledge of how neurons distinguish the valence of stimuli and how information is used to approach appetitive events and to avoid aversive events is still incomplete. A pioneering study by Thorpe et al. (1983) reported that neurons in the orbitofrontal cortex discriminate visual stimuli associated with appetitive (fruit juice) and aversive (hypertonic saline) stimuli in a visual discrimination go/no-go task with reversal. Nishijo et al. (1988) reported that neurons in amygdala are excited by conditioned stimuli (CS) associated with affectively significant stimuli (e.g., juice and electric shock) more strongly than CS associated with neutral stimuli, but they were not selective for either positive or negative valence of the associated stimuli. Roitman et al. (2005) studied nucleus accumbens neurons in a Pavlovian conditioning paradigm using appetitive (sucrose) and aversive (quinine) stimuli. A large proportion of cue-responsive neurons showed differential responses to sucrose-associated and quinine-associated cues, the responses being usually greater for the sucrose cues. Some functional imaging studies mapped brain areas dissociating valence and intensity of gustatory stimuli (Small et al., 2003) and odors (Anderson et al., 2003). For instance, there was a double dissociation between the amygdala, which responded to intensity irrespective of valence, and the orbitofrontal cortex, which responded to affective valence irrespective of intensity.

The lateral prefrontal cortex (LPFC) is known to be involved in reward processing (Watanabe, 1996; Leon and Shadlen, 1999; Kobayashi et al., 2002; Roesch and Olson, 2003). Since the LPFC also plays a crucial role in working memory, it is of particular interest to investigate how reward information influences LPFC activity in working memory tasks. Monkey single-unit studies showed that the LPFC processes information regarding reward quality (e.g., raisin and cabbage; Watanabe, 1996), quantity (large versus small; Leon and Shadlen, 1999; Roesch and Olson, 2003), and availability (present versus absent; Kobayashi et al., 2002) as predicted by visual cues while monkeys performed delayed response tasks. However, these experiments investigated only reward as behavioral outcome, and it remains unknown to what extent LPFC neurons are influenced by aversive events, which constitute another major class of reinforcers, that determine goal-directed behavior.

We aimed to study the extent to which punishers might have an influence on prefrontal function and distinguish reinforcement-related processes by valence. We recorded the activity of single prefrontal neurons while two monkeys performed a memory-guided saccade task with three trial types involving a liquid reward, the active avoidance of an aversive air puff, and a simple feedback sound (Figures 1A and 1B).

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A

B

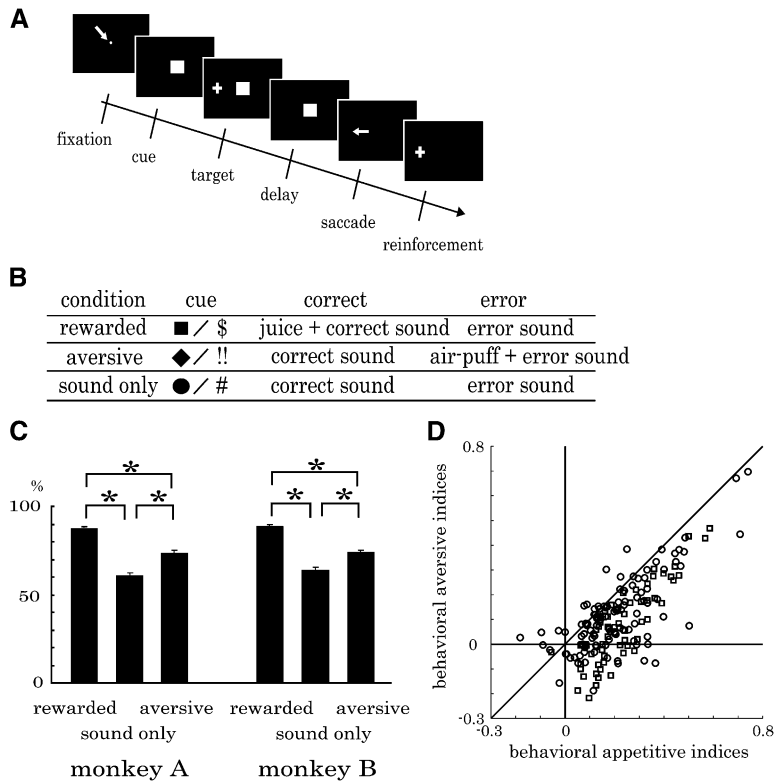| condition	cue	correct	error
rewarded	■ / $	juice + correct sound	error sound
aversive	◆ / !!	correct sound	air-puff + error sound
sound only	● / #	correct sound	error sound

C

D

Figure 1. Behavioral Task and Performance

(A) Memory-guided saccade task with differential reinforcement. A trial was initiated when the subject fixated a point presented at the center of the monitor. After 500 ms, a cue appeared at the center to indicate the outcome. After 500–1000 ms, a peripheral target flashed briefly (200 ms) at one of two possible positions, indicating the future target for the saccade. After a delay period (1–2 s), the central cue disappeared, which signaled the subject to make a saccade to the previously instructed target.

(B) The three trial types with their specific instruction cues. The two cue sets show the geometric figures and typographic characters used with monkey B. Correctly performed rewarded trials were followed by liquid reward. Correct aversive trials were followed by air puff avoidance. Correct behavioral responses were followed by a high-pitched sound, and incorrect behavioral responses were followed by a low-pitched sound in all trial types.

(C) Correct performance rate in each trial type. In both monkeys, correct performance rate was highest in rewarded trials, intermediate in aversive trials, and lowest in sound-only trials. Error bars indicate standard error of the means (SE); *statistical significance at $p < 0.01$ by Scheffé test.

(D) Behavioral performance indices. The indices reflect the change of correct performance rate in rewarded (abscissa) and aversive (ordinate) trials compared to sound-only trials. Each symbol shows average performance in one trial block (circle, monkey A, square, monkey B). Most plots fall below the oblique line for both monkeys, indicating that positive reinforcers improved performance more than negative reinforcer.

Results

Behavior

Rates of correct performance differed depending on behavioral outcomes in both monkeys (Figure 1C; one-way ANOVA, $p < 0.01$). Correct performance rate was highest in rewarded trials, intermediate in aversive trials, and lowest in sound-only trials ($p < 0.01$ for all comparisons, post hoc Scheffé test). The behavioral indices quantifying performance increases in rewarded and aversive over sound-only trials were mostly positive and larger for rewarded than aversive trials (Figure 1D). The result indicates stronger reinforcing effects of liquid reward compared with air puff.

Animals committed fixation break errors during the cue period (monkey A, 27.4%; monkey B, 20.3%; percentage of the whole errors of each animal), target period (monkey A, 3.9%; monkey B, 31.1%), and delay period (monkey A, 47.7%; monkey B, 44.3%). They made incorrect saccades at different rates (monkey A, 20.0%; monkey B, 4.4%). Fixation break and saccade errors during cue and saccade periods were most frequent in sound-only trials, intermediate in aversive trials, and least frequent in rewarded trials in both monkeys ($p < 0.01$, Scheffé test).

Peak velocity of saccades in correct trials also differed depending on behavioral outcomes ($p < 0.01$,

one-way ANOVA). Post hoc analysis revealed that saccadic velocity was significantly faster in rewarded trials (monkey A, 416.7°/s ± 7.0°/s; monkey B, 538.3 ± 4.4; mean ± SE), compared to the other two trial types (aversive: monkey A, 351.6°/s ± 5.4°/s; monkey B, 473.6°/s ± 4.2°/s; sound only: monkey A, 365.1°/s ± 6.5°/s; monkey B, 500.0°/s ± 4.0°/s) ($p < 0.01$, Scheffé test). Saccade latency did not vary depending on behavioral outcomes in either monkey and ranged from 204 to 228 ms in means ($p > 0.05$; one-way ANOVA).

Neuronal Database

We recorded the activity of 214 neurons from three hemispheres of two monkeys (monkey A, 119 neurons; monkey B, 95 neurons). We analyzed neuronal activity separately in cue, target, delay, and saccade periods. Activity was classified with one-way ANOVA ($p < 0.01$) and post hoc Scheffé tests ($p < 0.05$) (Table 1; see Experimental Procedures for classification criterion). Hereafter, we use the term "response" to refer to the average activity of one neuron in one time window in a specific trial type.

Preferential Modulation of Neuronal Activity in Rewarded Trials

Behavioral outcomes influenced the activity of LPFC neurons in various ways. The majority of neurons

Table 1. Number of Neurons that Were Modulated by Behavioral Outcomes in Each Task Period

	Direction of Modulation	Cue	Target	Delay	Saccade	Total
Reward preferential	increase	4 [0]	12 [3]	13 [1]	15 [4]	52 (24.3%)
	decrease	3 [1]	10 [0]	12 [3]	8 [2]	
Aversive preferential	increase	1 [1]	4 [1]	5 [2]	4 [0]	12 (5.6%)
	decrease	1 [1]	1 [0]	1 [0]	0 [0]	
Valence-independent	both increase	3 [0]	2 [0]	2 [0]	3 [0]	23 (10.7%)
	both decrease	3 [0]	5 [0]	5 [0]	5 [0]	
Bivalent		1 [0]	3 [0]	1 [0]	0 [0]	5 (2.3%)

Numbers in brackets indicate the number of neurons whose activity was explained by both behavioral outcomes and saccade metrics (valence + motor coding) by a regression analysis (see main text and Experimental Procedures). The rightmost column indicates the number of neurons for which activity is classified to each type in at least one time window.

changed activity only in rewarded trials. An example is shown in Figure 2A. The activity of this neuron built up after presentation of the cue on the left and was maintained during the delay period. Delay activity was significantly higher in rewarded trials compared to the other two types of trials [reinforcer: $F_{(2,53)} = 7.47$, $p < 0.01$; target position: $F_{(1,53)} = 31.85$, $p < 0.001$].

In some neurons, the modulation by expected reward consisted of a decrease rather than increase in activity, as shown in Figure 2B. This neuron showed a sustained decrease in activity during the delay in rewarded trials. Activity was similar in aversive and sound-only trials [reinforcer: $F_{(2,40)} = 11.2$, $p < 0.001$].

Preferential Modulation of Neuronal Activity in Aversive Trials
Some neurons changed activity only when the monkey performed saccades to avoid the air puff. A typical example is shown in Figure 3. This neuron showed significant sustained activity during the delay period only in
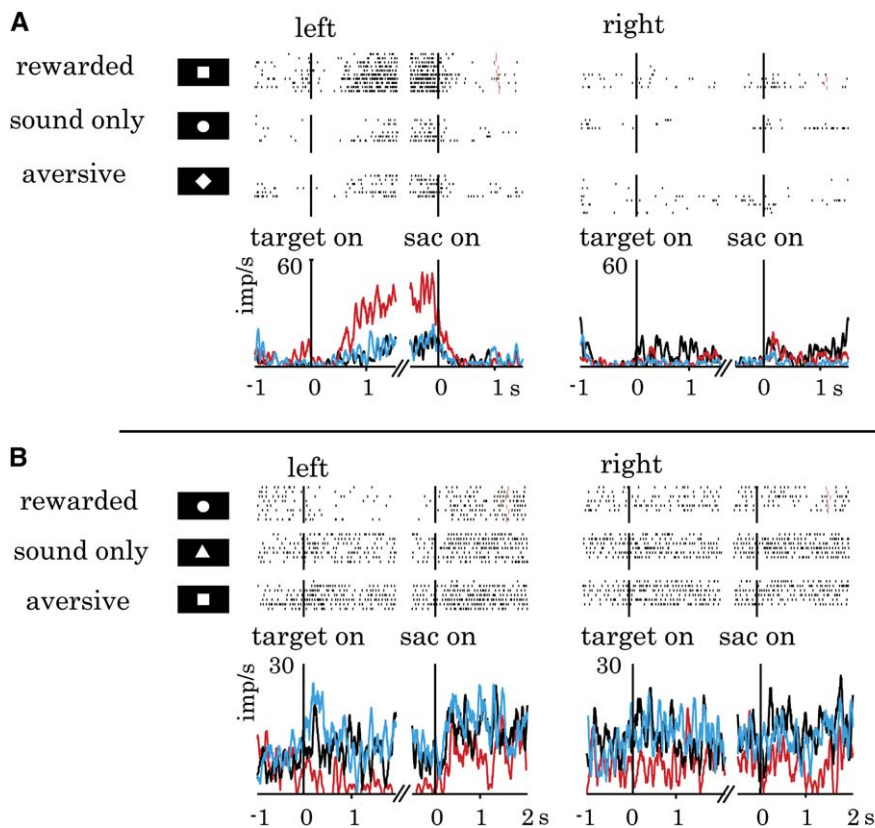

Figure 2. Example Neurons with Preferential Changes in Rewarded Trials

(A) Activity from a single neuron that increased in rewarded trials (monkey B, right hemisphere). Rastergrams are shown separately for each target position and each reinforcement type. For each rastergram, the sequence of trials runs from top to bottom. The schema on the left indicates the cue indicating the behavioral outcome. Vertical lines in the rastergram indicate the time of target onset (left) and saccade onset (right). Red tick mark indicates the onset of reward delivery (in rewarded trials). Black tick marks show times of neuronal impulses. Histograms at bottom compare mean discharge rates (red line, rewarded trials; black line, sound-only trials; blue line, aversive trials). Correct performance rates in this particular block were 86.4%, 80.0%, and 64.7% in rewarded, aversive, and sound-only trials, respectively. Only correctly performed trials are displayed.

(B) Activity of a single neuron that decreased in rewarded trials (monkey A, right hemisphere). Correct performance rates in this particular block were 100.0%, 82.3%, and 70.3% in rewarded, aversive, and sound-only trials, respectively.
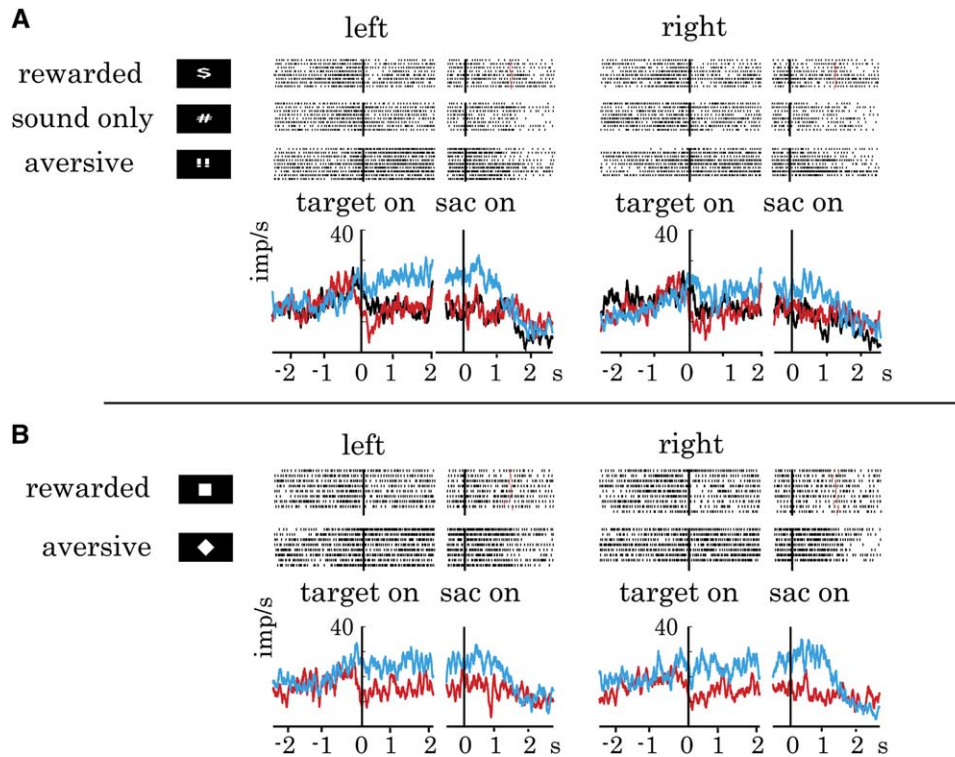
Figure 3. An Example Neuron with Preferential Changes in Aversive Trials

(A) Three trial types were given randomly, instructed by typographic characters (shown to the left). Activity in delay and saccade periods was higher in aversive trials than in the other two types of trial. Correct performance rates in this particular block were 100%, 61.1%, and 43.8% in rewarded, aversive, and sound-only trials, respectively.

(B) Activity of the same neuron as in (A), using the alternative cue set. Only rewarded and aversive trials were tested in this block. The activity in the delay and saccade periods was higher in aversive trials than in rewarded trials. The format is the same as in Figure 2.

aversive trials to the left, but not in rewarded and sound-only trials (Figure 3A) [reinforcer: $F_{(2,43)}$ = 22.13, p < 0.001; target position: $F_{(1,43)}$ = 2.72, p = 0.10; interaction: $F_{(2,43)}$ = 2.79, p = 0.07]. A similar result was obtained with a second set of visual cues (Figure 3B), suggesting that the differential activity was due to the different outcomes rather than the visual properties of the cue.

Dissociable Effects of Appetitive and Aversive Outcomes on LPFC Neurons

The examples in Figures 2 and 3 suggest that appetitive and aversive outcomes influenced separate groups of neurons. Among the 214 neurons investigated, 52 neurons (24.3%) showed activity preferentially modulated in rewarded trials (p < 0.05, Scheffé test; Table 1). Responses of these neurons either increased (Figure 4A) or decreased (Figure 4B) in rewarded trials as compared with the other two types of trials. Among 52 reward-preferential neurons, 18 (34.6%) showed spatial preference to the target position (p < 0.01, two-way ANOVA reinforcer × target position).

In analogy to behavioral performance changes, we used neuronal indices to quantify the changes in neuronal activity, both for rewarded and aversive trials compared to sound-only trials. Preferential changes in rewarded trials produced positive or negative appetitive indices (average 0.37 ± 0.16 for increases and −0.46 ± 0.20 for decreases) without deviations from zero in aversive indices (Figure 4C).

To distinguish between reward association and visual cue properties, we tested 22 of 52 reward-preferential neurons with alternative cue sets in separate trial blocks. Reward preferential modulation was generally reproduced with alternative cue sets, with similar neuronal indices (average appetitive indices were 0.31 ± 0.30 for increases and −0.36 ± 0.28 for decreases; see Figure S1A in the Supplemental Data available with this article online). The result indicates that these neurons were selective for reward outcome, not visual properties of the cues.

Although we used physically identical reinforcers in every trial, correct performance rate fluctuated across blocks of trials (Figure 1D). This indicates that the behavioral impact of reinforcer was different in each block. We investigated whether reward-preferential activity changed depending on the impact of the reward on correct performance rate but found no significant correlation between them: how much neuronal activity changed, as measured by neuronal indices, did not depend on how much correct performance rate improved, as measured by behavioral indices, in either rewarded or aversive trials (see Figures S1B and S1C and Table S1). We also found that different behavioral outcomes after correct and incorrect behavioral responses did not influence the activity of reward-preferential neurons: pooled activity of reward-preferential neurons (30 for increases and 22 for decreases) did not change significantly between correct and incorrect trials in aversive and
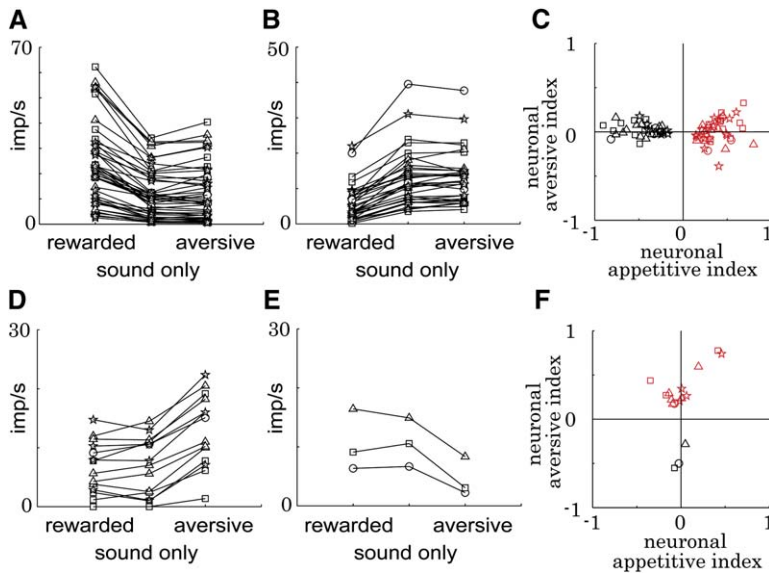
Figure 4. Activity of Neurons Related to Outcome Valence

(A and B) Preferential increase (A) or decrease (B) of mean discharge rate in rewarded compared to sound-only trials (44 responses for increases and 33 responses for decreases). Each line corresponds to single-unit activity in a specific task period. These neurons did not change discharge rate between aversive and sound-only trials significantly ($p > 0.05$, Scheffé test).

(C) Index plot of neurons whose activity changed preferentially in rewarded trials. Neuronal indices reflect changes of neuronal activity in rewarded and aversive trials compared to sound-only trials. Increases and decreases of neuronal responses in rewarded trials are indicated by positive (red marks, right) and negative indices (black marks, left).

(D and E) Preferential increase (D) or decrease (E) of mean discharge rate in aversive trials compared to sound-only trials (14 responses for increases and 3 responses for decreases). Activity was averaged across the two target positions.

(F) Index plot of activity that preferentially changed in aversive trials. Preferential increases and decreases of neuronal responses in aversive trials are indicated by positive (red marks) and negative (black marks) indices. Symbols indicate the different task periods of neuronal changes (circle, cue period; square, target period; triangle, delay period; asterisk, saccade period).

sound-only trials during cue, target, and delay periods ($p > 0.05$, Student's t test; see Figure S2). The result indicates that whether monkeys made correct or incorrect responses could not be predicted by neuronal activity of reward-preferential neurons.

We found only a few prefrontal neurons whose activity showed preferential changes in aversive trials (12/214 neurons, 5.6%; Table 1). Their responses either increased (Figure 4D) or decreased (Figure 4E) in aversive trials as compared with the other two trial types. Preferential changes in aversive trials produced positive or negative aversive indices (Figure 4F; average 0.41 ± 0.26 for increases for increases and −0.44 ± 0.14 for decreases).

It is possible that preferential activations in aversive trials were less common than in appetitive trials because the effect of the air puff on behavior might have been weaker than the effect of liquid reward (Figures 1C and 1D). However, we found that the preferential modulations in aversive trials were still much less common than preferential modulations in rewarded trials in 94 trial blocks in which the effects of appetitive and aversive outcomes on behavior were not different ($p > 0.05$, chi-square test; Table S1). This result confirms the relative insensitivity of prefrontal neurons to aversive outcomes as compared to reward.

Very few neurons showed opposite changes between appetitive and aversive outcomes (5/214 neurons, 2.3%; bivalent type in Table 1). Neuronal responses increased in appetitive trials and decreased in aversive trials as compared with sound-only trials, or vice versa (see Figure S1D).

Valence-Independent Modulation of Task-Related Activity

In some neurons, responses changed in the same direction for appetitive and aversive outcomes. In the neuron shown in Figure 5, the cue response was higher in both rewarded and aversive trials compared to sound-only trials [reinforcer: $F_{(2,57)} = 8.07$, $p < 0.001$]. Furthermore, the activity of this neuron appeared to correlate with the monkey's behavior, as correct performance rate and cue response were highest in rewarded trials (mean discharge rate, 55.5 impulses/s; correct rate, 95.5%), intermediate in aversive trials (46.9 impulses/s; 68.4%), and lowest in sound-only trials (33.2 impulses/s; 47.8%).

In total, 23 out of 214 task-related neurons (10.7%) changed responses in both rewarded and aversive trials in the same direction at least in one task period (Table 1). The change consisted of either significant increase (Figure 6A) or decrease (Figure 6B) in both rewarded and aversive trials, as compared to sound-only trials. Most of these neurons did not change responses by target position (main effect of target position $p > 0.01$ in 22/23 neurons; interaction between reinforcer and target position $p > 0.01$ in 20/23 neurons). The scatter plot in Figure 6C indicates that responses of valence-independent neurons were modulated more strongly in rewarded than aversive trials (appetitive indices larger than aversive indices; $p < 0.001$, paired Student's t test).

We further examined the influence of behavioral performance on valence-independent activity. Block-by-block variations of correct performance rate were quantified for each reinforcer by using behavioral indices. Changes of neuronal activity in each reinforcer condition were quantified by neuronal indices. If behavioral performance was related to valence-independent activity, behavioral and neuronal indices might show correlation. Figure 6D shows the relationship between behavioral and neuronal indices of valence-independent responses (filled symbols, appetitive indices; open symbols, aversive indices; since these responses were modulated independent of outcome valence, both appetitive and
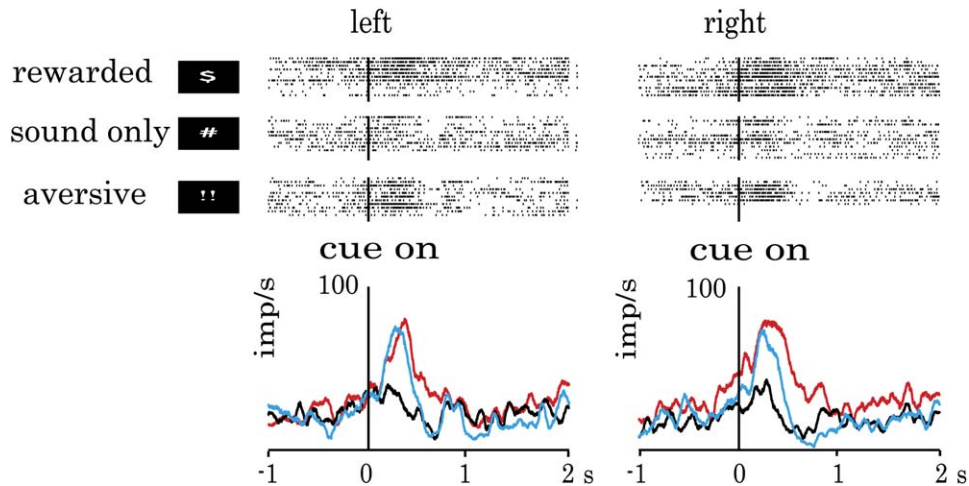
Figure 5. Valence-Independent Modulation of Task-Related Prefrontal Activity

The activity of this neuron increased in both rewarded and aversive trials compared to sound-only trials (monkey B, right hemisphere). The format is the same as in Figure 2, except activity is aligned at cue onset (vertical line). Only correct trials are displayed.

aversive indices are collapsed). We found that neuronal indices tended to be larger when behavioral indices were larger (correlation coefficient: 0.30, p = 0.02, Pearson's correlation test). The result indicates that the better monkeys performed rewarded and aversive trials as compared with sound-only trials, the more the activity of

Figure 6. Activity of Neuronal Populations Changed in Both Rewarded and Aversive Trials

(A and B) Common increases (A) or decreases (B) of mean discharge rate in rewarded and aversive trials (10 responses for increases and 18 responses for decreases).

(C) An index plot of neuronal appetitive index versus neuronal aversive index from responses shown in (A) (red) and (B) (black). The blue line shows the first component from principal component analysis.

(D) Index plots of behavioral indices versus neuronal indices. Neuronal appetitive indices (filled symbols) and neuronal aversive indices (open symbols) for increases (red) and decreases (black) are plotted against behavioral appetitive and aversive indices, respectively. Ellipse, 2SD distribution contour. To evaluate the absolute magnitude of response modulation, the sign of neuronal indices for response decreases is reversed.

valence-independent neurons changed. The influence of behavioral performance on neuronal activity was also confirmed by comparing activity between correct and incorrect trials (Figure S3). When monkeys performed the task correctly, population activity of valence-independent neurons was graded in rewarded, aversive, and sound-only trials in this order (n = 10; Figure S3A) or in the reversed order (n = 13; Figure S3C) during target and delay periods (p < 0.05, Scheffé test). When monkeys performed the task incorrectly, however, valence-independent activity did not change significantly between aversive and sound-only trials in any task period (p > 0.05, Scheffé test).

In sum, valence-independent neurons changed activity depending on block-by-block variations of correct performance rate and whether monkeys performed each trial correctly or not. The results suggest that these neurons were sensitive to the impact of reinforcer on behavior independent of its valence.
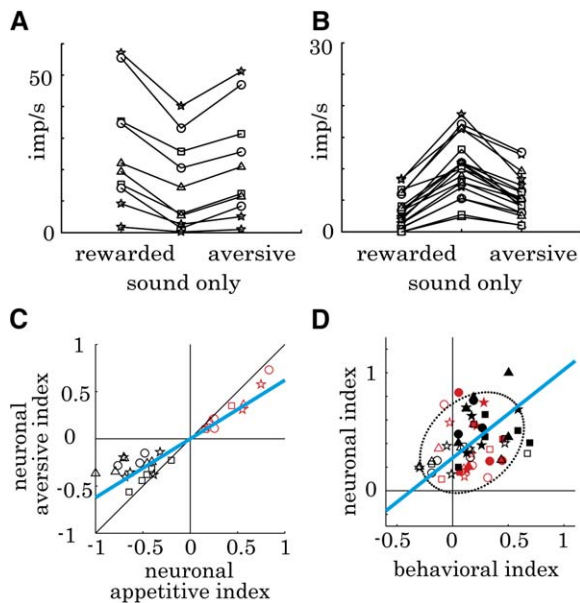
Correlations between Reinforcement and Saccade Metrics

As saccadic velocity varied between the different reinforcers (see above), the question arose whether the observed neuronal differences might simply reflect the saccadic differences. We performed multiple linear regressions and correlated neuronal responses with behavioral outcomes (valence coding), saccade kinematics (motor coding), and valence and saccade kinematics combined (valence + motor coding) (nested F test, p < 0.05). We found that most responses correlated with valence. Only a small number of responses correlated significantly with saccade kinematics and values combined, and only insignificant correlations were seen with saccade kinematics (Table 1).

Anatomical Distribution

Recording positions were confirmed by in vivo MRI and postmortem histology. The four types of responses, namely reward-preferential, aversive-preferential, valence-independent, and bivalent types, were observed widely in the LPFC, both ventral and dorsal to

the principal sulcus, and no clear topographical segregation was observed within the LPFC (see Figure S4).

Discussion

This study shows that a sizeable fraction of prefrontal neurons distinguish between rewarding and aversive outcomes during the performance of a typical prefrontal spatial delayed response task. Most valence-discriminating neurons were sensitive to rewards, whereas only a small fraction of neurons were preferentially modulated in aversive avoidance trials. A subpopulation of prefrontal neurons showed similar influences of appetitive and aversive outcomes that correlated with changes in behavioral performance, indicating a close relationship to behavior rather than outcome valence per se. Taken together, the data suggest that dorsolateral prefrontal neurons are more sensitive to rewarding than aversive outcomes, and that only parts of this differential sensitivity are due to the direct effects of the reinforcers on behavior.

Reinforcement Effects on Behavior
Performance rates of both monkeys differed in the three reinforcer conditions, which suggested that they discriminated the visual instruction cues (Figure 1C). Furthermore, significantly better performance in rewarded compared to sound-only trials indicates a positive motivational effect of liquid reward. Monkeys also performed aversive trials significantly better than sound-only trials. This is important because behavioral outcomes in aversive trials and sound-only trials were physically identical when the trials were performed correctly, namely a high-pitched sound. The result indicates that the cues in aversive trials had a stronger motivational effect than the cues in sound-only trials.

Valence-Dependent Activity in LPFC
The most common observation was a change in neuronal responses in rewarded trials, whereas much fewer neurons changed responses preferentially in aversive trials (Table 1). It is not likely that these changes were induced by the physical properties of the instruction cue, as similar changes occurred when the outcome was indicated by the alternative cue set (Figure 3 and Figure S1A). Neither could the changes be explained by kinematic aspects of the saccades; although the peak velocity of the saccade changed systematically depending on the outcome, the saccade kinematic, which varied in every trial, rarely accounted for trial-by-trial variations in neuronal responses (Table 1). Therefore, this type of neuronal responses appears to reflect the valence of behavioral outcomes, rather than the visual instruction cue or the planned action.

The fraction of reward-preferential responses in our study (24.3% of task-related activity) is consistent with previous studies on the same area (Kobayashi et al., 2002) and larger than the fractions in studies that varied the magnitude of reward (Leon and Shadlen, 1999; Roesch and Olson, 2003).

Other brain areas also show valence-dependent activity changes while monkeys perform operant tasks. Striatal neurons and orbitofrontal neurons differentiate appetitive from aversive outcomes (Thorpe et al., 1983; Ravel et al., 2003; Yamada et al., 2004; Roesch and Olson, 2004; Roitman et al., 2005). Neurons in anterior cingulate cortex are differentially activated in lever pressing for reward or shock avoidance (Nishijo et al., 1997; Koyama et al., 2001). Studies in rodents suggested an involvement of the amygdala in learning associations between stimuli and aversive outcomes: rats with amygdala lesions show impairments in aversive association learning, but no effect of lesions on appetitive association learning (Cahill and McGaugh, 1990); infusion of muscimol in the amygdala of rats prevents fear conditioning (Muller et al., 1997); amygdala neurons show activation while rats are learning to avoid aversive outcomes (Schoenbaum et al., 1998). Amygdala neurons in monkeys respond to both positive and negative reinforcing stimuli (Paton et al., 2006). Our results suggest that reward takes stronger precedence over aversive processes in LPFC compared to these other structures.

It should be noted, however, that several interpretations are possible for the asymmetric influences of appetitive and aversive outcomes in the LPFC. We might explain the asymmetry by different impacts of appetitive and aversive outcomes on behavior: in general, reward improved behavioral performance more than the aversive outcome. Thus, the less common processing of aversive information in the LPFC might be due to a weaker impact of the air puff on behavior. However, we found the effects of reinforcers on reward-preferential responses to be independent of the effects on correct performance (Figures S1B and S1C; and Table S1). Also, previous studies reported that a substantial fraction of striatal TANs was even modulated by air puffs (103 kPa, 100 ms, Ravel et al., 2003; 179 kPa, 30 ms, Yamada et al., 2004) that were weaker than used in the current experiment (200 kPa, 100 ms). Another possibility is that neurons did not respond in aversive trials because monkeys experienced air puffs less frequently than liquid reward. However, we observed the predominance of reward-preferential responses even when correct performance rate in aversive trials was low and monkeys experienced air puffs in many trials (cf. blocks with low behavioral aversive indices in Figure S1C). These considerations suggest that our findings of dissociable neuronal responses in rewarded and aversive trials may not be explained by an asymmetry in the behavioral impact of reinforcers, cue salience, or frequency of reinforcer delivery.

A second explanation for the asymmetric processing is that behavioral reactions in rewarded and aversive trials are qualitatively different and mediated by separate neural circuits. The literature provides evidence that behavioral reactions differ fundamentally when appetitive outcomes are compared with aversive outcomes: in operant conditioning, positive reinforcers increase the probability of responses on which they are contingent, whereas negative reinforcers by definition produce a decrease (Thorndike, 1911); learning to avoid aversive outcomes is more resistant to extinction than learning to obtain appetitive outcomes (Solomon and Wynne, 1953, 1954); and finally, the learning speed in avoidance trials is slow when the required action is not consistent with the subject's innate repertoire of defensive behavior (Bolles, 1970; Fanselow, 1997). In our experiments, training for the aversive condition took considerably

longer than training for the rewarded condition, because the monkeys often broke eye fixation during the delay period, perhaps as a part of defensive behavior. To enable differential behavior in events of different valence, the brain might have developed separate neural circuits, and the LPFC appears to be predominantly involved in appetitive processing.

Although we believe that our results reflect functional asymmetry of appetitive and aversive information processing in the LPFC, future investigations are needed to clarify this issue, for example, using different kinds of aversive stimuli, including gustatory stimuli and electric shock.

Valence-Independent Activity in LPFC

We found that some neurons changed responses in both rewarded and aversive trials in the same direction (Figures 5 and 6). The effects are not likely to be attributed to motor intention or motor preparation, because the variance of firing rates was not explained by saccade parameters in the regression analysis (Table 1). Correlations between neuronal responses and behavioral responses suggest that valence-independent responses may have reflected the behavioral impact of the reinforcers (Figure 6D and Figure S3). The rather limited number of these neurons in the LPFC suggests that the strong influences of reinforcers on behavioral reactions are mediated further downstream, perhaps in the premotor cortex (Roesch and Olson, 2004).

Neural Structures Processing Valence-Dependent and Valence-Independent Information

Dissociable effects of appetitive and aversive outcomes on LPFC neurons support the notion that the two kinds of information are processed separately in the LPFC. Several brain areas are known to process valence-dependent information and possibly transfer the information to the LPFC. Midbrain dopamine neurons, which respond preferentially to rewards (Mirenowicz and Schultz, 1996), send projections to many cortical areas, including LPFC. The orbitofrontal cortex, which represents appetitive and aversive information (Gottfried et al., 2002; Zald et al., 2002; Anderson et al., 2003; Small et al., 2003), has reciprocal corticocortical projections with LPFC.

The origin of valence-independent signals in LPFC is less clear. The ascending monoaminergic and cholinergic systems play crucial roles in the effects of stressors, motivation, arousal, and attention on behavior (Steriade and Buzsaki, 1990). For instance, changes in the activity of locus coeruleus neurons correlate closely with fluctuations in behavioral performance in a visual discrimination task (Usher et al., 1999). Lesions in the nucleus basalis of Meynert have profound effects on task performance due to attentional dysfunction (Muir et al., 1994). These findings suggest the ascending monoaminergic and cholinergic systems as possible origins of the valence-independent signals in the LPFC.

A network of attention has been proposed that comprises frontal, parietal, and thalamic structures, all of which are influenced by reticular and noradrenergic modulatory systems (Mesulam, 1981; Posner and Petersen, 1990). The dopamine system may also modulate the cortical activity (Lewis et al., 1987; Sawaguchi et al., 1990). Therefore, the cerebral cortices may potentially receive multiple influences from various neuromodulatory systems, including monoaminergic and cholinergic systems. Modulatory effects on the cerebral cortex may also be realized through corticocortical interconnections and basal ganglia-thalamocortical circuits. Characterizing the roles of the prefrontal cortex in attentional and motivational control is particularly important because the prefrontal cortex is thought to provide top-down control over posterior visual areas (Luck, 1995; Desimone and Duncan, 1995; Posner and Dehaene, 1994). Our results suggest that the LPFC may provide appetitive signals to posterior visual areas when the subject performs a task to obtain reward. Although reward-related activity is reported in the perirhinal cortex (Liu and Richmond, 2000) and lateral intraparietal area (LIP) (Platt and Glimcher, 1999), and a recent study suggested independent influence of reward and attention on LIP neurons (Bendiksby and Platt, 2006), further study is necessary to substantiate this hypothesis using paradigms in which reward can be dissociated from punishment, arousal, and attention.

Experimental Procedures

Subjects and Surgery

We used two adult male Japanese monkeys (*Macaca fuscata*), monkey A and monkey B, weighing 9 kg and 8 kg, respectively. Surgical procedures are described in detail in a previous paper (Kobayashi et al., 2002). Before the recording experiments started, we implanted a head holder, a chamber for unit recording, and a scleral search coil under general anesthesia. A rectangular recording chamber (antero-posterior, 42 mm; lateral, 30 mm; depth, 10 mm) was placed over the LPFC. The position of the recording chamber was verified with magnetic resonance imaging (Siemens, Sonata 1.5T). All surgical experimental protocols were approved by the Tamagawa University Animal Care and Use Committee and were in accordance with the US National Institutes of Health Guide for Care and Use of Animals.

Behavioral Paradigm

The monkey sat in a primate chair inside a completely enclosed sound-attenuated room with its head fixed. Visual stimuli were presented on a computer display placed at 70.5 cm in front of the monkey. The monkey performed a memory-guided saccade task, with different reinforcers (Figures 1A and 1B). A trial started with the onset of a central fixation spot (0.21° in visual angle). After the monkey gazed at the fixation spot for 500 ms, the fixation spot changed into a picture that was associated with a particular reinforcer. After 500–1000 ms, a peripheral target (0.53°) appeared for 200 ms randomly at one of two positions. We searched the response field with a target at the top, bottom, left, and right of the visual field (6.5°). When the neuron showed spatial preference, we selected the preferred position and the diagonally opposite position. When the neuron did not show any spatial preference, we used left and right as target positions. The monkey had to remember the target position during the delay period of random duration between 1 s and 2 s. The disappearance of the figure at the center after the delay period signaled to the monkey that it should make a saccade to the previously cued position within 800 ms.

We used three trial types with different outcomes, liquid rewarded trials, aversive air puff avoidance trials, and sound-only trials. The monkey was presented with a tone (1500 Hz rectangular waveform) in all correctly performed trials. The tone was accompanied by a drop of liquid in rewarded trials, whereas correct aversive and sound-only trials terminated without a liquid reward. Incorrect behavioral responses in all trial types were followed by an auditory tone (800 Hz rectangular waveform). Aversive error trials resulted in a stream of compressed air (200 kPa, 100 ms duration) directed toward the face from 10 cm distance. Reinforcement in correct trials was given 700 ms after correct saccade, and reinforcement in

incorrect trials was given 700 ms after making an incorrect behavioral response. Errors in all trial types led to trial repetition.

We used two sets of visual stimuli to indicate the same outcomes, a set of geometric pictures and a set of typographic characters. For monkey B, for instance, a black square and "$" were associated with the rewarded condition, a black diamond and "!!" were associated with the aversive condition, and a black circle and "#" were associated with the sound-only condition (Figure 1B). The same set of stimuli was used in monkey A. Stimulus-reinforcer mapping was counterbalanced across the two monkeys.

Recording Procedures

Conventional extracellular single-unit recording was conducted using tungsten electrodes (1–5 MΩ; measured at 1 kHz; Frederick Haer, ME). The electrode was inserted into the brain through a stainless steel guide tube (diameter, 0.8 mm). Microelectrodes were advanced vertically to the cortical surface, using a micromanipulator (MO-95-S, Narishige, Tokyo). The action potentials were amplified, filtered (500 Hz–2 kHz), and matched online with a unique waveform to select out a single unit (Alpha Omega Engineering, Nazareth). A personal computer generated raster displays of neuronal activity online. The times of discharge occurrences were stored with a time resolution of 1 ms. Eye positions were recorded at 500 Hz and stored during each block of trials, using the magnetic search-coil technique (MEL-25, Enzanshi-Kogyo, Tokyo; Robinson, 1963; Judge et al., 1980).

Data Collection and Analyses

We evaluated the effects of appetitive and aversive outcomes on behavioral performance by behavioral indices that reflect how much the correct performance rate improved in rewarded and aversive trials as compared with sound-only trials. Behavioral indices are defined as follows:

$$\text{Behavioral appetitive index} = \frac{Prew - Psou}{Prew + Psou} \quad (1)$$

$$\text{Behavioral aversive index} = \frac{Pave - Psou}{Pave + Psou} \quad (2)$$

where $Prew$, $Pave$, and $Psou$ designate correct performance rates in rewarded, aversive, and sound-only trials, respectively.

We analyzed neuronal activity in the cue period (0 ms–300 ms after cue onset), the target period (0 ms–300 ms after target onset), the delay period (300 ms–900 ms after target onset), and the saccade period (−300 ms–200 ms from saccade onset). We use the term "response" to refer to the average activity of one neuron in one time window within a trial of a specific type. Responses were classified according to the three reinforcement conditions (one-way ANOVA). Responses that showed significance (p < 0.01) in one-way ANOVA were subjected to post hoc Scheffé tests (p < 0.05). Responses were compared (1) between rewarded and sound-only trials, (2) between aversive and sound-only trials, and (3) between rewarded and aversive trials. If (1) and (3) were significant and (2) was not significant, the responses were classified as preferentially modulated in rewarded trials. If (2) and (3) were significant and (1) was not significant, the responses were classified as preferentially modulated in aversive trials. If (1) and (2) were significant, the responses were classified as modulated in both rewarded and aversive trials. Of these, if a response was modulated in the same direction in both rewarded and aversive trials, it was classified as valence independent; if a response was modulated in the opposite direction, it was classified as bivalent. The number of each type of neuron was counted as sum of neurons that show each type of modulation at least in one task period.

Responses were also analyzed by two-way ANOVA (reinforcer × target position). Responses that showed a significant main effect for the target position and/or a significant interaction effect were regarded as having a spatial preference to the target.

For the quantitative analysis of single-neuron activity, we used only correctly performed trials. The effects of appetitive and aversive outcomes on neuronal discharge rate were quantified by the following indices:

$$\text{Neuronal appetitive index} = \frac{Drew - Dsou}{Drew + Dsou} \quad (3)$$

$$\text{Neuronal aversive index} = \frac{Dave - Dsou}{Dave + Dsou} \quad (4)$$

where $Drew$, $Dave$, and $Dsou$ designate mean discharge rates in rewarded, aversive, and sound-only trials, respectively.

To examine whether neuronal activity continued to depend on the type of behavioral outcome when the effects of saccade metrics were factored out, we performed a multivariate regression analysis, fitting three models:

(1) $Y = a_0 + a_1 \times peakV + a_2 \times RT + a_3 \times amp$
(2) $Y = a_0 + a_1 \times val$
(3) $Y = a_0 + a_1 \times peakV + a_2 \times RT + a_3 \times amp + a_4 \times val$

where Y is the discharge rate in each task period, $peakV$ is peak saccade velocity, RT is saccade latency, amp is saccade amplitude, a_0 is a constant, and a_1, a_2, a_3, and a_4 are coefficients. The variable val was set to 0 or 1 for trials in sound-only or rewarded trials, respectively. The variable val for aversive trials was set to optimize the fitting of model 2 by the least square method. Trials with different target positions were collapsed in this analysis. If neuronal activity represents saccade parameters rather than outcomes, activity would fit well into model 1, and model 3 would not improve the fitting (motor coding). If neuronal activity represents outcomes, not saccade parameters, activity would fit well into model 2, and model 3 would not improve the fitting (valence coding). Significantly better fitting in model 3 compared with models 1 and 2 might suggest that neuronal activity represents both motor and behavioral outcomes (motor + valence coding). We compared models 1 and 2 to model 3 using a nested F test (cf. Roesch and Olson, 2003).

Supplemental Data

The Supplemental Data include Supplemental Experimental Procedures, four supplemental figures, and one supplemental table and can be found with this article online at http://www.neuron.org/cgi/content/full/51/6/861/DC1/.

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