

Effect of Expected Reward Magnitude on the Response of Neurons in the Dorsolateral Prefrontal Cortex of the Macaque

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Summary

The dorsolateral prefrontal cortex plays a critical role in guiding actions that ensue seconds after an instruction. We recorded from neurons in area 46 and the frontal eye field (FEF) while monkeys performed a memory-guided eye movement task. A visual cue signaled whether a small or large liquid reward would accompany a correct response. Many neurons in area 46 responded more when the monkey expected a larger reward. Reward-related enhancement was evident throughout the memory period and was most pronounced when the remembered target appeared in the neuron's response field. Enhancement was not present in the FEF. The mixture of neural signals representing spatial working memory and reward expectation appears to be a distinct feature of area 46.

Introduction

The dorsolateral prefrontal cortex is thought to play a role in guiding behavior that does not ensue immediately but is to be enacted seconds after the acquisition of a sensory instruction (Jacobsen, 1935; Fuster, 1989). During this time gap, termed an "instructed delay," neurons in the principal sulcus and its adjacent gyri exhibit sustained discharge, which is thought to provide the neural substrate for short-term (working) memory or movement preparation (reviewed by Fuster, 1985; Goldman-Rakic, 1987; Fuster, 1995). In a well-studied example, many neurons have been shown to respond between the brief presentation of a visual target and an eye movement made seconds later to its remembered location (Bruce and Goldberg, 1985; Funahashi et al., 1989; Funahashi et al., 1991). The sustained response is usually restricted to target positions in a limited region of the visual field, termed the neural response field, and is thus thought to encode the remembered location or, more generally, the temporal linkage between visual instruction and eye movement response (Levy and Goldman-Rakic, 1999; Miller, 1999; Quintana and Fuster, 1999; Rainer et al., 1999).

While the mechanism underlying such sustained activity is largely unknown, several studies indicate that dopamine may play an important role (Sawaguchi et al., 1990; Sawaguchi and Goldman-Rakic, 1994; Williams and Goldman-Rakic, 1995). The dorsolateral prefrontal cortex receives a rich dopaminergic input from neurons

in the ventral tegmentum and substantia nigra (Ilinsky et al., 1985; Berger et al., 1991; Williams and Goldman-Rakic, 1993; Haber and Fudge, 1997), which have recently been shown to discharge in response to rewarding stimuli (Schultz, 1998). These observations raise the possibility that within the prefrontal cortex, neural signals related to the memory of spatial locations might interact with signals related to reward.

The purpose of the present study is to determine whether the sustained (delay period) activity of neurons in the prefrontal cortex is affected by the magnitude of expected reward. We trained rhesus monkeys to perform memory-guided saccades under conditions in which they were led to expect a large or small water reward upon successful execution of the task. We compared the neural responses associated with different reward expectations but the same memory demand and the same behavioral response. Many neurons in area 46 exhibited stronger responses when the monkey expected the larger reward. The enhanced neural activity occurred throughout the memory period and was greatest when the monkeys made memory-guided saccades to a restricted portion of the visual field. Such reward-related modulation of neural activity was conspicuously absent in the neighboring frontal eye field (FEF).

Results

Effect of Reward Expectation on Behavior

Two rhesus monkeys performed a working memory task in which they were required to remember the location of a briefly lit target and to shift their gaze to its location upon extinction of the fixation point (Figure 1). During the task, a change in the color of the fixation point indicated whether the monkey would receive a small or large reward at the end of the trial. A total of 20,660 trials were obtained in the course of studying 125 neurons. Of these, the monkeys completed 17,322 trials (84%) by making a saccadic eye movement to the location of the remembered target. Most of the unsuccessful trials were attributed not to incorrect responses but to broken fixation (2,685 of 3,338 [80%]). Interestingly, the monkeys broke fixation nearly twice as often after the fixation color signaled a small reward (odds ratio 1.88; 95% confidence interval [CI] = 1.73–2.05; $p < 0.0001$, χ^2 test). This behavior was detrimental because the monkeys received no reward for unsuccessful trials and would ultimately complete an equal number of trials ending in large and small reward. The observation indicates, however, that the monkeys were in some sense aware of the reward contingencies associated with the task.

Even among the successfully completed trials, we observed subtle differences in the saccadic eye movements that were associated with large and small reward. On trials ending in a large reward, saccadic latencies were 2.4% longer (CI = 1.8%–3.0%), the amplitudes were 2.1% shorter (CI = 1.4%–2.8%), peak velocities were 2.7% faster (CI = 1.9%–3.6%), and accuracy (the reciprocal of the distance between the final saccadic endpoint and the location of the spatial cue) was 0.23%

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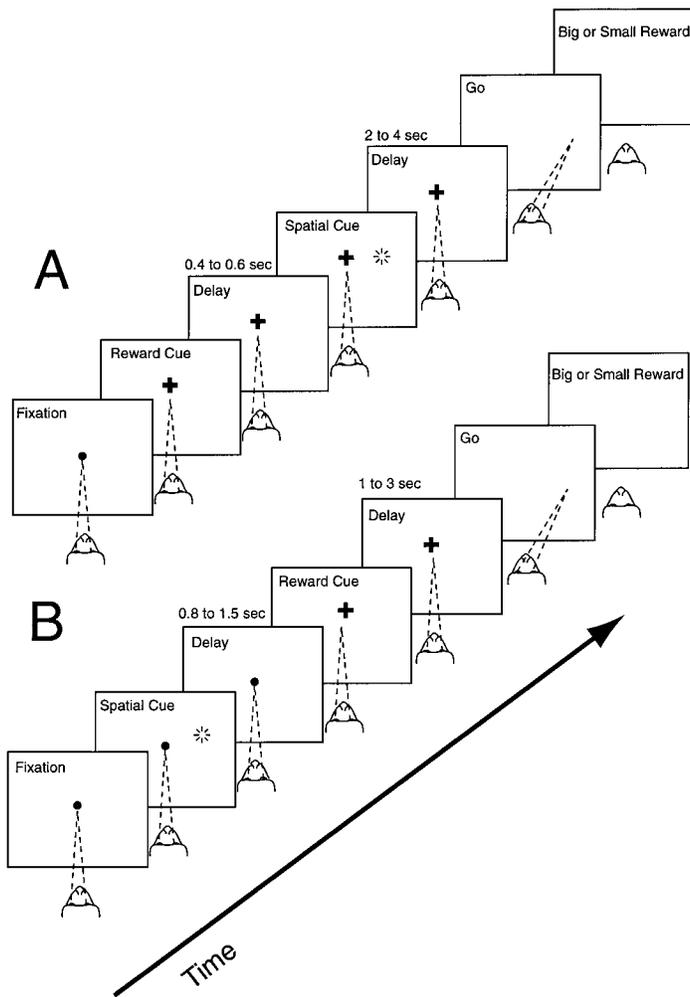


Figure 1. Variable-Reward Memory Saccade Task

The monkey held its fixation on a central point. A peripheral target appeared briefly that served as the spatial/memory cue. After a variable delay period, the fixation point was extinguished, signaling the monkey to make a rapid eye movement to the location of the remembered target. The monkey received a liquid reward for making an eye movement to the correct position. The size of this reward was indicated by a change in the color of the fixation point (reward cue), which occurred during the trial according to one of two sequences.

(A) On half of the trials, the monkey was informed of the size of the reward before the appearance of the spatial/memory cue. (B) On the other half of the trials, the reward cue appeared after the spatial/memory cue, during the memory period. The total time for the two sequences was approximately equal, on average. The memory period (time from spatial cue to extinction of the fixation point) was 1–4 s. The location of the spatial cue and the reward size were determined by random selection on each trial.

smaller (CI = 0.14%–0.32%; all comparisons significant by *t* tests, $p < 0.0001$). These differences were subtle, but they provide additional evidence that the monkeys' behavior was influenced by the color–reward contingencies.

Effect of Reward Expectation on Neural Response

We recorded from 125 neurons in the dorsolateral prefrontal cortex (Figure 2): 34 from the FEF and 91 from the banks and gyri of the principal sulcus (Walker area 46). We studied neurons that responded during the delay period of the memory saccade task in a spatially selective manner (see Experimental Procedures), thereby allowing us to identify a memory response field (RF). The monkey performed a memory saccade task using two to four target locations, one of which was in the neuron's RF. At each target location, half of the trials were associated with a large or small reward.

Larger Reward Was Associated with Enhanced Neural Activity in Area 46

Figure 3 shows data obtained from a neuron in area 46 during the memory saccade task. The neuron emitted a sustained volley of action potentials when the spatial memory cue appeared 13° to the right of the fixation

point. In addition to this spatial selectivity, the response was enhanced on trials in which the monkey was cued to expect the larger reward. This is perhaps clearest on the trials in which the reward cue preceded the appearance of the spatial cue in the neuron's RF (Figures 3A and 3B). For both reward sizes, the response increased when the target appeared, but this response was sustained at a greater level if the color of the fixation point had cued a larger reward (compare Figures 3A and 3B). The average response during the delay period was 48.5 ± 2.8 spikes/s when the expected reward was big, compared to 30.3 ± 2.9 spikes/s when the expected reward was small ($p < 0.0002$, *t* test).

Comparable enhancement was evident on the trials in which the spatial cue preceded the reward cue. For example, in Figures 3E and 3F, there was a consistent increase in the spike rate in the ~0.5 s after the spatial cue was flashed in the neuron's RF. Then, when the fixation point changed color to indicate the size of the reward, the sustained discharge underwent additional modulation. On big-reward trials (Figure 3F), the discharge increased slightly and remained elevated for the duration of the memory period (41.1 ± 2.7 spikes/s). On small-reward trials (Figure 3E), there was an abrupt decrease in the spike rate ~200 ms after the reward

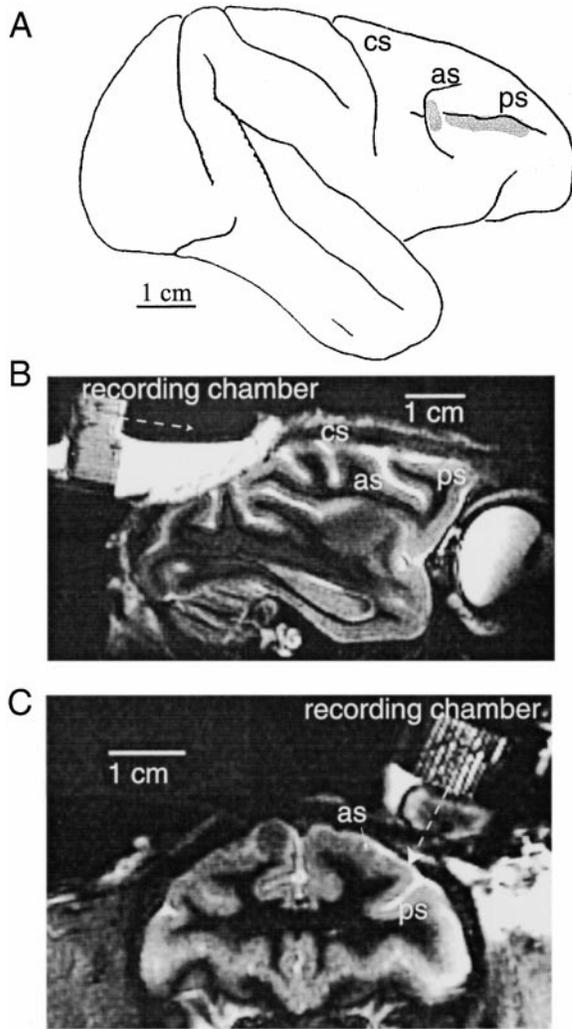


Figure 2. Location of Recording Sites

(A) Schematic diagram of the rhesus monkey brain. Shading demarcates the location of neurons described in this report, determined from MRIs (fast spin-echo, short T1, inversion-recovery sequence; slice thickness and spacing, 1.5 mm).

(B) Representative MRI slice from monkey H. The sagittal image, 16.5 mm lateral to midline, shows the center of the recording chamber. The electrode grid is at the back of the chamber, which was filled with saline (white meniscus below the arrow). The dashed arrow shows an approximate trajectory of an electrode that entered the brain near the central sulcus. The angle of the recording chamber enabled electrode penetrations along the full extent of the principal sulcus. Actual reconstruction of the penetration was facilitated by registration of MRI with electrophysiological landmarks.

(C) Representative MRI slice from monkey I. The coronal image, taken 31 mm anterior to interaural canals, shows the saline-filled recording cylinder centered over the principal sulcus. Upper and lower rami of the arcuate sulcus are just visible. The approximate trajectory of an electrode into the upper bank of the principal sulcus is shown (arrow). Abbreviations: as, arcuate sulcus; cs, central sulcus; ps, principal sulcus.

cue (arrow). Yet, despite the decrease, the average sustained activity remained above baseline (27.3 ± 2.4 spikes/s), consistent with the fact that the monkey successfully completed the memory saccade to the neuron's RF. For the combined data from the two sequences

of cues depicted in the left half of Figure 3, the delay period activity was 1.56 times larger when the monkey expected a big reward. We term this value the enhancement ratio (ER).

When the monkey made memory-guided saccades to targets that appeared outside the RF, the neuron responded weakly during the memory period and reward-related enhancement was less clear. Consider the trials in which the spatial cue preceded the reward cue (Figures 3G and 3H). When the spatial cue appeared, the response remained at background level or declined slightly. Then, shortly after the color of the fixation point signaled a small reward, there was further attenuation of the response (Figure 3G), but this decline was subtle and variable. For the combined data from the two sequences of cues depicted in the right half of Figure 3, the mean spike rate during the memory period was 7.4 ± 0.82 for the small reward trials, compared to 9.8 ± 0.91 for the large reward trials (Figures 3C, 3D, 3G, and 3H; ER = 1.32; $p = 0.056$, *t* test). The reward-related enhancement was thus weak at best when the remembered target appeared outside the neuron's RF. As shown below, this pattern of spatial selectivity was a common finding in area 46.

There is another interesting feature of the data depicted in Figure 3. Notice that the enhancement seen in this neuron is not an immediate consequence of the reward cue itself. On trials in which the reward cue preceded the saccade target, the neural responses were not distinguishable until after the spatial cue appeared. In the epoch between the reward cue and spatial cue (Figures 3A–3D, epoch between inverted triangles and vertical line), there is no modulation with reward size (12.4 ± 1.6 spikes/s versus 10.1 ± 1.4 spikes/s for big and small reward trials, respectively; $p = 0.29$). The reward cue was not sufficient to affect the response on its own but appears instead to modulate the sustained (mnemonic) response. For this neuron, reward-related enhancement occurred selectively during the memory period preceding eye movements to the neuron's RF. As shown below, this was the dominant pattern of enhancement among neurons in area 46.

We also encountered neurons that modulated their response shortly after the reward cue appeared, more or less independently of when and where the spatial cue was presented. In contrast with the previous example, the neuron depicted in Figure 4 modulated its response just after the fixation point signaled the reward size. This is best appreciated by inspecting the upper rasters of Figures 4A–4D during the short epoch between the reward cue (vertical line) and spatial cue (triangles). In this ~ 0.5 s interval, the mean response was 20.4 ± 1.6 spikes/s after the "big reward" cue, compared to 11.3 ± 1.8 spikes/s after the "small reward" cue ($p < 0.001$). Because reward size affected the neural response before the appearance of the spatial cue, it comes as no surprise that reward-related enhancement for the remainder of the memory period did not depend on the location of the remembered target. Although the neuron depicted in Figure 4 responded weakly when the spatial cue appeared outside the RF (up and to the right), the most profound attenuation in response was apparent when the reward size was small. The mean spike rate during the memory period was 3.4 ± 0.4 for

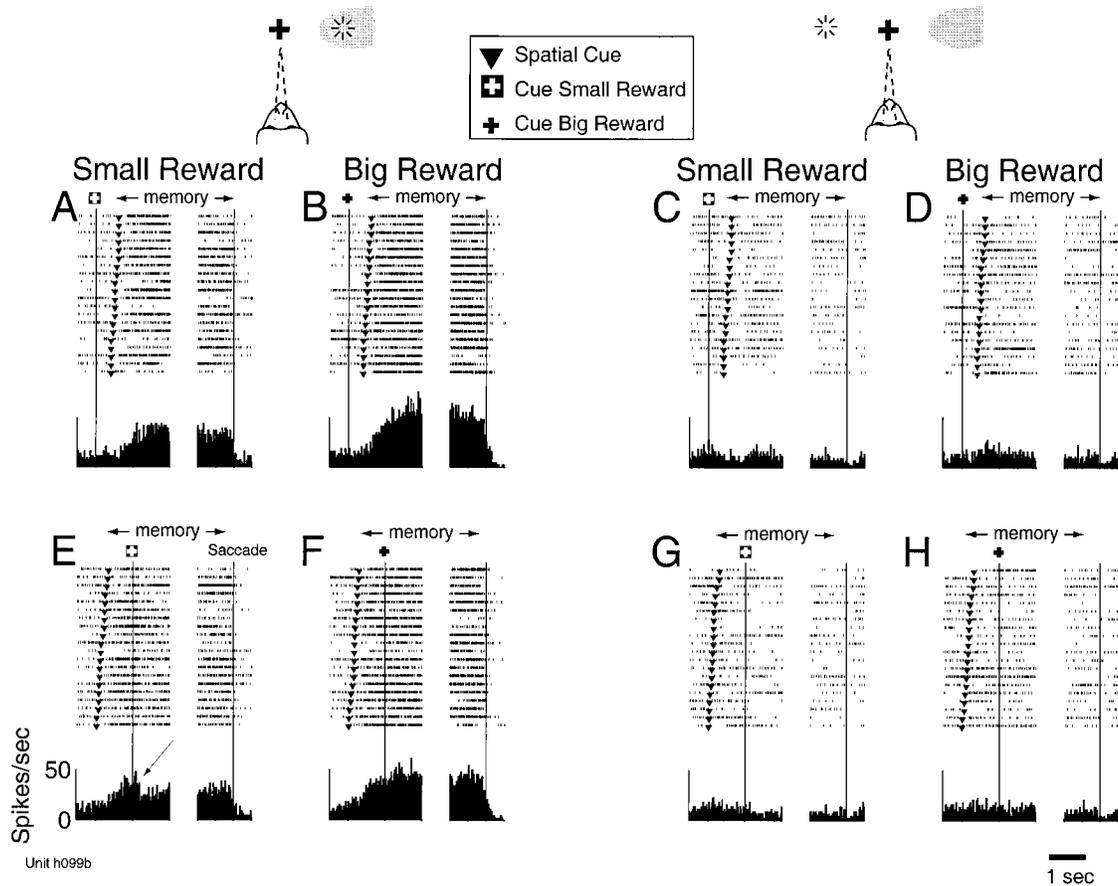


Figure 3. Response of Neuron in Area 46 during the Variable Reward Task

The location of the neuron's response field in relation to the fixation spot is shown in gray. Time axes are broken to align the responses to the onset of the reward cue and the initiation of the monkey's saccade (vertical lines). The spatial/memory cue appeared at the time indicated by the triangle, either inside (A, B, E, and F) or outside (C, D, G, and H) the response field of the neuron.

(Upper row of axes, A–D) The color of the fixation point indicated the size of the expected reward before the appearance of the spatial/memory cue.

(Lower row of axes, E–H) The reward size was cued after the appearance of the target, during the memory period. This neuron showed an enhanced response during the delay period when the monkey expected the large reward. The effect is clearest for memory-guided saccades to the neuron's response field.

the small reward trials, compared to 5.8 ± 0.6 for the large reward trials (Figures 4C, 4D, 4G, and 4H; ER = 1.7; $p < 0.002$). When the spatial cue appeared inside the RF (down and left), the neuron responded well, but the activity was maximal when the larger reward was expected (Figures 4A, 4B, 4E, 4F; ER = 1.62; $p < 10^{-5}$).

To quantify the reward-related enhancement across the population of neurons in area 46, we compared each neuron's response during the memory period on trials in which the monkey expected the large and small reward. The comparison is conveniently summarized by the enhancement ratio (ER; see Experimental Procedures). We computed ERs separately for trials in which the monkey made memory saccades into and away from the RF. An ER greater than 1 indicates enhanced delay period activity when the monkey expected the larger reward, whereas an ER less than 1 would indicate the reverse; an ER equal to 1 would imply that there is no effect of reward expectation.

Enhancement ratios for all 91 area 46 neurons are shown in Figure 5. Across the population, the geometric

mean ER was 1.06 (CI = 1.02–1.11) when the monkey made memory-guided saccades into the RF. Although small overall, the enhancement was significant over the population ($p < 0.002$, $H_0: \log(\text{ER}) = 0$, t test; $p < 10^{-8}$, F test, see Equation 2). Moreover, 13 of the 15 neurons with statistically significant modulation of neural activity exhibited ERs greater than unity (Figure 5, upper shaded histograms). It is unclear whether these neurons comprise a distinct subset of the population or are simply the more reliable examples of the unimodal distribution depicted in Figure 5.

When the spatial cue appeared outside the neuron's RF, the enhancement was less consistent, as in the examples above. The geometric mean ER was 1.01 but did not represent a significant departure from unity (CI = 0.94–1.09; $p = 0.75$, t test; $p = 0.073$, F test). We were thus unable to demonstrate a consistent pattern of enhancement for memory-guided saccades to locations outside the neural RF. There nevertheless appear to be neurons, like the one shown in Figure 4, that exhibit similar enhancement regardless of the direction of the

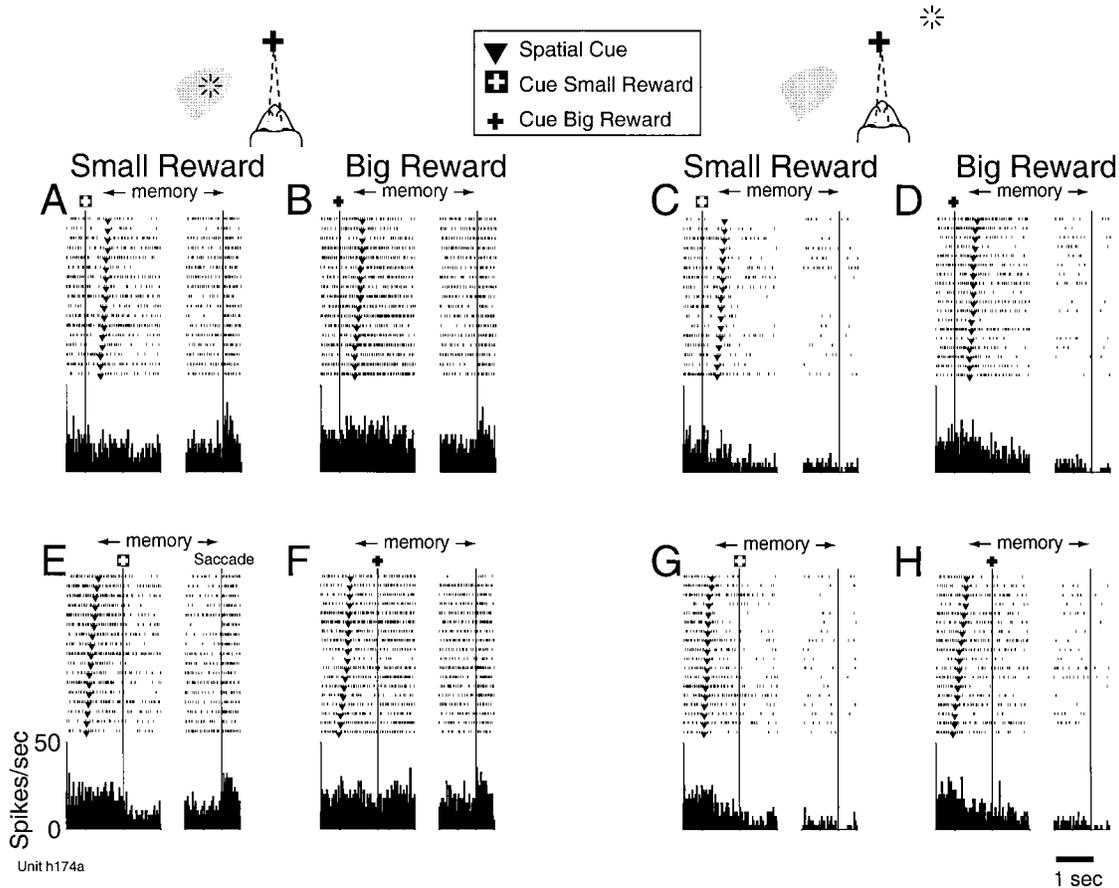


Figure 4. Response of a Second Neuron in Area 46 during the Variable Reward Task

The layout is the same as in Figure 3. A decline in the response occurred shortly after the reward cue signaled the small reward. Differences in response associated with expectation of large and small reward is apparent for both target locations and for both sequences (upper and lower rows).

ensuing eye movement. This is supported by the weak correlation between ERs, which is evident in the scatter plot ($r_{\log(\text{ER})} = 0.44$, $\text{CI} = 0.26\text{--}0.59$; $p < 0.00001$, Fisher z transform).

Enhancement Was Absent in the FEF

We obtained data from 34 neurons in the FEF that would be classified as visuo-movement or fixation cells (Bruce and Goldberg, 1985). Although these neurons responded similarly to the neurons in area 46 on memory-guided eye movement tasks, we failed to observe reward-related enhancement during the delay period. Only 4 of 34 neurons exhibited statistically significant differences in delay period activity on big- and small-reward trials ($p < 0.05$, t test), and these were as likely to show depression ($\text{ER} < 1$) as enhancement ($\text{ER} > 1$) with expectation of the larger reward. Across the population of FEF neurons tested, the responses on “big” and “small” reward trials were nearly identical. The geometric mean of the ER was 1.01 ($\text{CI} = 0.96\text{--}1.07$) for memory-guided saccades to the RF and 1.01 ($\text{CI} = 0.85\text{--}1.20$) for eye movements away from the RF. Neither of the population means depart significantly from a ratio of 1 ($p = 0.65$ and 0.91 , respectively). A possible concern is that the absence of reward-related enhancement in

the FEF might be a consequence of the smaller number of neurons recorded, leading to a type II statistical error. However, a comparison of enhancement in the FEF and area 46 was significant when analyzed by a two-way ANOVA ($p < 0.01$; memory-guided saccades to the RF), implying that the difference between these brain regions cannot be attributed to a lack of statistical power.

Time Course of Reward Enhancement

To examine the time course of the reward-related modulation in area 46, we combined data from those neurons that exhibited statistically significant reward enhancement on memory saccades to the RF (13 of the 15 neurons depicted by the shaded upper histograms in Figure 5). The responses from each neuron were normalized to the mean delay period activity using all trials in which the spatial cue appeared in the neuron’s RF, regardless of reward size (see Experimental Procedures). The curves shown in Figure 6 represent the running mean of the normalized responses from the 13 neurons on big- and small-reward trials. By selecting neurons with clear enhancement, we have ensured that the response will tend to be larger than average on big-reward trials. The question we wish to address is over what interval this difference is detectable.

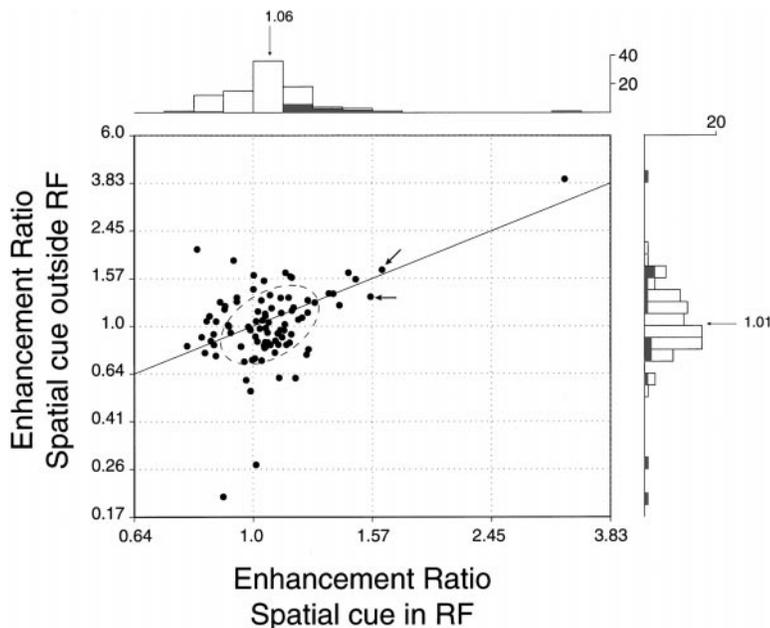


Figure 5. Distribution of Enhancement Ratios for 91 Neurons in Area 46

The enhancement ratio (ER) compares the delay period response on trials in which the monkey is cued to expect a large versus a small reward. Values greater than 1 correspond to increases in neuronal responses when the large reward was expected. The scatter plot shows ERs computed separately for trials in which the spatial/memory cue appeared inside and outside the neural response field. Arrows below and above the diagonal line of equality denote units illustrated in Figures 3 and 4, respectively. Horizontal histograms, spatial cue in the RF; vertical histograms, spatial cue outside the RF. Arrows on histograms indicate geometric means. Shaded histograms indicate units with significantly different firing rates on small and large reward trials (two-tailed t test, $p < 0.05$). Dashed ellipse is the bivariate normal approximation to the data (unit standard deviation contour line). The orientation of the ellipse demonstrates a weak positive correlation between the ERs (see text for details).

Figure 6 shows that the size of the expected reward influenced the neural activity from about 200 ms after the beginning of the delay period until the time of the eye movement response. The difference in response associated with the expectation of large and small rewards was most pronounced in the first second of the memory period, but it was clearly present until the initiation of the monkeys' eye movement response.

Interestingly, enhancement was not present before the memory period. Recall that on half of the trials, the reward size was indicated ~ 0.5 s before the appearance of the spatial memory cue (Figure 1A). Figure 7A plots the averaged normalized responses obtained in this epoch for the same 13 neurons depicted in the Figure 6. There was little difference in neural activity during this period. (The example in Figure 4 [1 of the 13 neurons

included in this analysis] was therefore exceptional in this regard.) On the other hand, when the spatial cue appeared first (Figure 1B), enhancement was detectable 250 ms after the reward cue was presented. This suggests that reward-related enhancement observed in area 46 may be specifically related to spatial memory or the maintenance of sustained activity during the delay period.

Alternative Explanations for Enhancement

Our results indicate that the expectation of reward size can influence the activity of neurons in area 46 during performance of the memory saccade task. In what follows, we consider two possible alternative explanations for this phenomenon.

First, it is possible that the color of the reward cue,

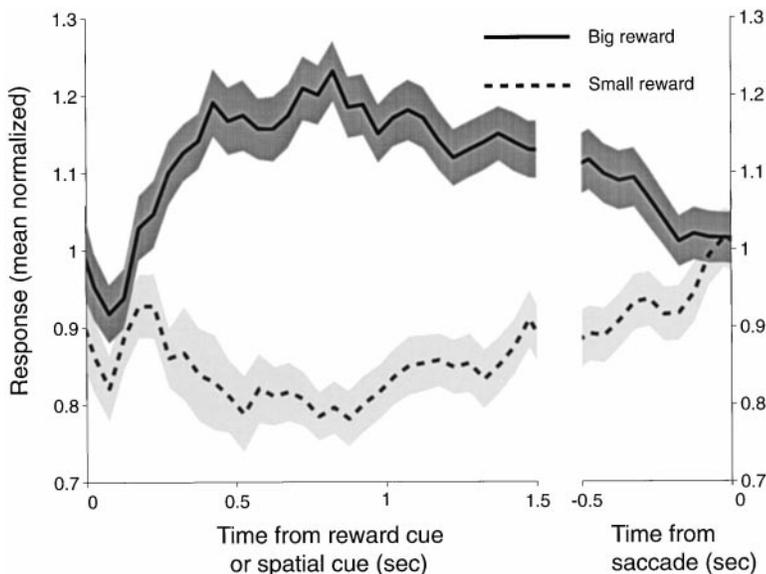


Figure 6. Time Course of Reward-Related Enhancement

Curves depict the averaged normalized response from 13 neurons that exhibited statistically significant reward-related enhancement on memory-guided saccades into the neural response field. Solid and dashed curves compare the average response on large and small reward trials, respectively. A value of 1 on the ordinate represents the mean spike rate, regardless of reward size. The curves and standard error (shading) depict the running mean as a function of time, using an epoch width of 150 ms. The time axis is broken to align responses either to the initiation of the saccadic eye movement or to the beginning of the period in which the monkey was instructed of both the reward size and the spatial cue.

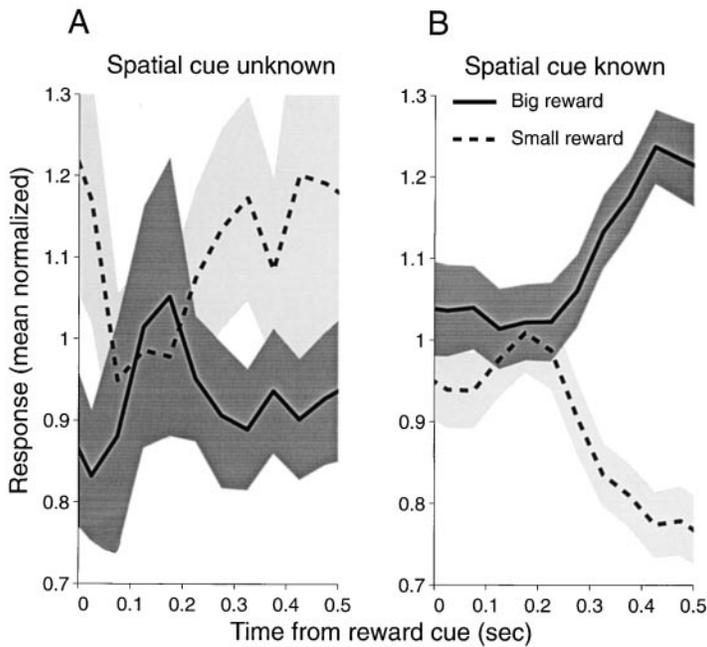


Figure 7. Context Dependence of Reward-Related Enhancement

Both panels plot the averaged normalized responses from the same neurons depicted in Figure 6 during the 500 ms following the reward cue. Running means and standard errors are shown as in Figure 6.

(A) Trial sequence as depicted in Figure 1A: the reward size was indicated before the monkey had seen the spatial/memory cue. Across the 500 ms epoch, the mean normalized response was 0.96 ± 0.09 and 1.04 ± 0.08 for big and small reward, respectively ($p = 0.53$, t test).

(B) The reward size was indicated after the monkey had seen the spatial/memory cue (sequence in Figure 1B). The normalized means were 1.10 ± 0.03 and 0.90 ± 0.03 ($p < 0.0001$).

rather than the magnitude of reward it signified, affected the response. To test this, 27 area 46 neurons were recorded in an additional memory saccade task in which we reversed the association of reward size with fixation point color. In the first block of trials, we used the color-reward combination that the monkey had learned. As shown in Figure 8, these neurons exhibited enhanced responses in association with this standard color-reward association (geometric mean ER = 1.10; CI = 1.04–1.17; $p < 0.003$). We then conducted a second block of trials in which we reversed the association between color and reward. The reward-related enhancement in this second block was diminished (geometric mean ER = 1.01; CI = 0.93–1.10; $p = 0.83$), consistent

with a disruption of the color-reward association. More importantly, however, the neurons failed to show a consistent preference for the color of the fixation point.

If reward-related enhancement were in fact a color preference in disguise, then the association between response and reward size should have reversed in the second block. Indeed, despite the overall reduction in reward-related enhancement, individual neurons exhibited some consistency in their response to expected reward under the two configurations, as evidenced by the weak positive correlation in the scatter plot ($r = 0.35$; CI = -0.033 – 0.65 ; $p = 0.07$, Fisher z) and the tendency for individual neurons with significant enhancement (shaded upper histograms) to retain positive ERs in the

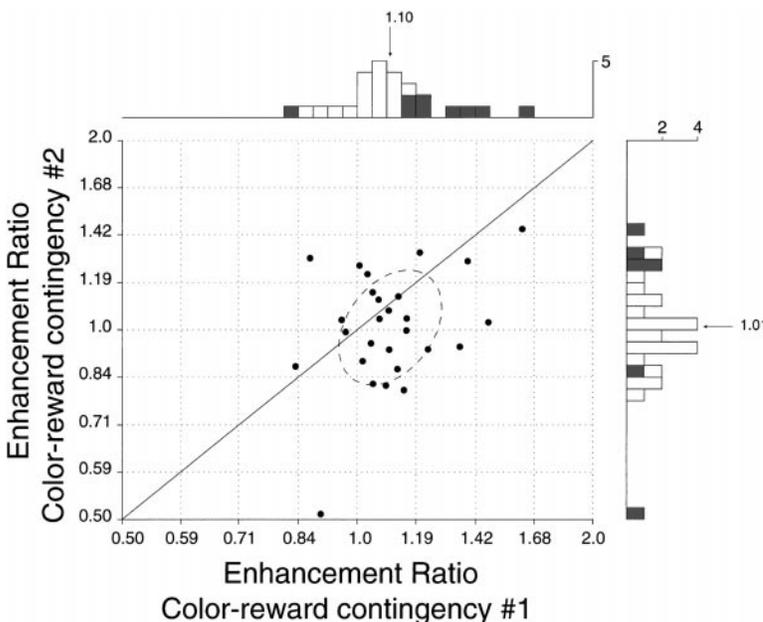


Figure 8. Enhancement Ratios before and after Reversing the Association between Color and Reward Size

The ER for the trained association is shown along the horizontal axis. The ER for the new (reversed) association is shown along the vertical axis. In both cases, the ER is computed with respect to the actual reward size. If the enhancement is actually a response to the color cue, then the reversal should change the ER to its reciprocal value. This would be evident as a negative correlation in the scatter plot. The principal components ellipse (dashed) describes a weak positive correlation between the two conditions (see text). Arrows indicate geometric means.

second block. Across the population of 27 neurons, reward size was found to influence the neural response significantly when analyzed against a possible color confounder ($p < 10^{-6}$, two-way ANOVA as described in Experimental Procedures, Equation 3, model $R^2 = 0.81$). These observations, along with the findings that enhancement generally occurred only after the spatial cue appeared in the RF (Figures 5 and 7), allow us to reject the idea that color is responsible for the response modulation that we observed.

A second possibility is that response enhancement might simply reflect a difference in the way the monkey performed the task when the larger reward was expected. Recall that saccadic eye movements on small- and large-reward trials differed in latency, amplitude, peak velocity, and accuracy. We therefore evaluated the possibility that such factors played a confounding role. We performed multiple least-squares regression in which we modeled the memory period response on each trial as a linear combination of eye movement descriptors and reward size:

$$z = \beta_0 + \beta_1 \text{LAT} + \beta_2 \text{ACC} + \beta_3 \text{AMP} + \beta_4 \text{VMAX} + \beta_5 \text{REW} \quad (1)$$

To combine data from many neurons, we first normalized the response for each neuron to its mean response during the delay period, using all the trials employing the same memory target. The first four independent variables (uppercase) were obtained from eye trace records as described in Experimental Procedures. These values were also normalized before combining across experiments. The last variable, REW, is 1 or 0 for large and small reward trials, respectively. To test whether expected reward size affects the neural response in a manner that is not accounted for by variation in eye movements, we compared fits with $\beta_5 = 0$ or free, and applied the principle of extra sum of squares (Draper and Smith, 1966). We found that incorporating reward size into the model resulted in a significant improvement in the regression fit ($p < 10^{-5}$). This implies that variations in the monkey eye's movements do not account for the enhanced responses of neurons in area 46 when the monkeys expected the larger reward. We obtained the same result when we applied this analysis to the subset of neurons analyzed in Figures 6 and 7 ($p < 10^{-6}$). It is also worth noting that the same analysis failed to uncover enhancement when the monkey made eye movements away from the RF or among the neurons in the FEF.

Discussion

Neurons near the principal sulcus are known to exhibit sustained discharge on delayed-response tasks (reviewed by Fuster, 1989; Miller, 1999). In oculomotor delayed-response tasks, this sustained response is often selective for a remembered target location, consistent with a role for these neurons in working spatial memory or movement preparation (Goldman-Rakic, 1987; Funahashi et al., 1989; Funahashi et al., 1991). We have shown that many of these neurons also modulate their response in a manner that reflects the magnitude of expected

reward. This modulation was induced by indicating the size of the reward that would be provided upon successful completion of a memory-guided saccadic eye movement. The study design allowed us to compare neural responses associated with different expected reward size under conditions in which the monkey performed the same behavior in a highly stereotyped fashion. Other than a change in color of the fixation point, the visual stimuli and the difficulty of the task (memory burden) were identical on trials in which the monkey was led to expect a small or large reward. The monkey made very similar eye movements under the two reward contingencies, and any small differences were measured and incorporated in the data analysis. Thus, we are confident that the difference in activity seen on small- and large-reward trials is a direct reflection of the magnitude of the expected reward.

The reward-related enhancement of neural activity was largely restricted to trials in which the monkey made eye movements into the neuron's response field and was mainly expressed during the memory period of the task. On trials in which the reward cue preceded the spatial (memory) cue, the enhancement typically occurred only after the spatial requirements of the task were specified. These observations suggest that reward expectation might play a role in modulating the sustained activity of neurons in area 46. Such selectivity also argues against the possibility that the enhancement could be due to mechanisms related to the monkey's state of arousal that would affect the overall neural discharge. This possibility seems all the more unlikely because we failed to observe reward-related enhancement among neurons in the FEF of the same monkeys. Mechanisms related to arousal and excitability, such as changes in blood pressure or $p\text{CO}_2$, would be expected to affect both regions of the dorsolateral prefrontal cortex.

A limitation of the present study is that we used only two reward sizes. With only two sizes, we are unable to determine whether the magnitude of expected reward is represented parametrically in area 46. Our informal observations with different quantities suggest that it is not, but studies from other laboratories using different food types raise the possibility of a graded representation of reward size (Watanabe, 1996). The limited range of reward size in our study might also explain the rather modest effects we observed. Previous studies of reward-related activity in area 46 compared the response on trials in which some reward is given to those in which there is no reward at all (Niki and Watanabe, 1979; Inoue et al., 1985; Watanabe, 1990; Watanabe, 1992). Examples of neural responses in these conditions show enhancement similar to our better cases, but these reports do not include population data for comparison. Watanabe (1996) reported sizable differences in responses using a variety of food rewards that differed in preference, but the rewards also varied in type (e.g., apple, raisins, etc.), making it difficult to quantify the relative magnitudes of the different rewards.

Neurons in area 46 have access to several sources of information about reward magnitude in our task. For example, there are reciprocal connections with the lateral intraparietal area (LIP; Andersen et al., 1985; Cavada and Goldman-Rakic, 1989), which has recently been

shown to contain neurons that encode expected payoff in a delayed eye movement task (Platt and Glimcher, 1999). Area 46 also makes reciprocal connections to the orbitofrontal cortex (Pandya et al., 1971; Kawamura and Naito, 1984; Selemon and Goldman-Rakic, 1988), which is thought to play a role in motivational control and the processing of reward (Iversen and Mishkin, 1970; Dias et al., 1996; Rolls, 1996). Many neurons in orbitofrontal cortex reflect the monkey's relative preference for food and liquid reward, and a small fraction exhibit modulation during delayed-response tasks (Tremblay and Schultz, 1999). In contrast with area 46, however, reward-related activity in orbitofrontal cortex appears to be unrelated to specific spatial demands of the task, and it is not temporally restricted to the memory/delay period.

In the caudate nucleus of the basal ganglia, expectation of reward can influence the neural response so profoundly (Hikosaka et al., 1989) as to override spatial (or movement) selectivity (Kawagoe et al., 1998). Reward-predicting neurons in the caudate nucleus could influence neurons in the prefrontal cortex indirectly, through the substantia nigra pars reticulata and medio-dorsal nucleus of the thalamus (Goldman-Rakic and Porrino, 1985; Hikosaka and Wurtz, 1989). The latter structure is believed to play a role in mediating reward reinforcement (Robertson, 1989; Gaffan and Murray, 1990), presumably owing to input from the amygdala. Area 46 receives only sparse input from the amygdala itself (Jacobson and Trojanowski, 1975).

Finally, the dorsolateral prefrontal cortex also receives a rich dopaminergic input from neurons in the ventral tegmental area and the substantia nigra pars compacta (Ilinsky et al., 1985; Berger et al., 1991; Williams and Goldman-Rakic, 1993; Haber and Fudge, 1997). This projection may be relevant to the reward enhancement observed in area 46. Dopamine neurons are modulated by stimuli that predict reward (Schultz et al., 1993; Hollerman et al., 1998; Schultz, 1998), and dopamine itself has been shown to modulate the memory-related activity of neurons in area 46 (Sawaguchi and Goldman-Rakic, 1994; Williams and Goldman-Rakic, 1995). This explanation is not entirely satisfactory, however, because the FEF also receives a rich dopaminergic projection from the same midbrain structures (Williams and Goldman-Rakic, 1993), yet it showed no enhancement. The effect of dopamine on sustained activity in the FEF has not been studied, but if the dopaminergic input to area 46 is responsible for the reward-related enhancement that we observed, then our findings would suggest that dopamine is unlikely to affect the sustained activity of FEF neurons. Further experiments will be required to test this idea.

A number of experiments have demonstrated that the activity of dorsolateral prefrontal cortex neurons are predictive of an animal's decision in behavioral choice tasks (Fuster, 1989; Rao et al., 1997; Asaad et al., 1998; Hasegawa et al., 1998; Leon and Shadlen, 1998; Kim and Shadlen, 1999). These neurons may therefore comprise a substrate whose role it is to transform a sensory cue into a behavioral action. If this hypothesis is correct, then it is quite reasonable to expect these same neurons to be modulated by the magnitude of an expected reward, as this represents a crucial variable in decisions

made by animals exhibiting optimal choice behavior (Leon and Gallistel, 1998; Platt and Glimcher, 1999).

Experimental Procedures

Recording

Two adult rhesus monkeys (monkey I, male, 8.0 kg; monkey H, female, 4.7 kg) were implanted with an eye coil, head holding device, and recording cylinder suitable for magnetic resonance imaging (MRI) (Crist Instrument, Damascus, Maryland). We used two approaches to the principal sulcus. In monkey H, the cylinder was placed over the parietal lobe in the sagittal plane of the FEF and principal sulcus (Figure 2B). Microelectrodes were advanced through a stainless steel guide tube into the brain and then passed through the arcuate sulcus to make tangential penetrations parallel to the principal sulcus. In monkey I, the recording cylinder was placed over the arcuate sulcus and the posterior third of the principal sulcus (Figure 2C). Sturdy tungsten/glass microelectrodes punctured the dura mater to reach the cortex.

We used standard methods for single-unit extracellular recording, as previously described (Kim and Shadlen, 1999). Single units were isolated using a dual voltage-time window discriminator (Bak Electronics, Germantown, MD). The time of action potentials were marked as events with 1 ms precision and stored to disk for offline analysis. Horizontal and vertical eye position was measured with a scleral search coil (C-N-C Engineering) and stored to disk (250 Hz per channel) for offline analysis. All procedures and treatment were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and approved by the University of Washington Animal Care Committee.

Identification of Recording Sites

We recorded from 91 neurons located in the banks and neighboring gyri of the principal sulcus (Walker area 46) and 34 neurons in the FEF (area 8Ac and area 45) of two rhesus monkeys. To identify recording sites, electrode penetrations were registered with MRIs (e.g., Figures 2B and 2C). In addition, we identified the FEF by eliciting saccades using the microstimulation protocol of Bruce et al. (1985). The FEF was clearly distinguished by the capacity to elicit stereotyped (fixed vector) saccadic eye movements with stimulating current of $<50 \mu\text{A}$. Histological confirmation of recording sites is not available since both monkeys are alive and participating in other studies. Combining measurements from MRI and electrophysiological landmarks, however, we are confident that the neurons were located in the regions of cortex denoted by the shading in Figure 2A.

Behavioral Tasks and Neuron Selection

During the initial screening of neurons, monkeys performed a memory saccade task. The monkey fixated a small red dot (fixation point) at the center of the computer monitor (Power Macintosh 7500 running MATLAB and using the extensions provided by the high-level Psychophysics Toolbox and low-level VideoToolbox; Brainard, 1997; Pelli, 1997). A red spot, which subtended $\sim 1/3^\circ$ visual angle, appeared for 200 ms at a random location in the visual field. The monkey was required to maintain its gaze within 1.5° of the fixation point for a variable memory period (1–2 s), which ended with extinction of the fixation point ("go" signal). The monkey was required to make a saccadic eye movement to the location of the remembered target within 500 ms of fixation point offset, and received a water reward if the saccade was accurate to within 4° – 6° (depending on eccentricity). The monkeys always received a single reward during this screening procedure, equivalent to the "small" reward during data acquisition.

We used the memory saccade task to identify neurons that responded in a sustained fashion during the delay period preceding saccades to a restricted region of the visual field, termed the neural RF. Some neurons also responded transiently at the onset of an eye movement or to the appearance of the spatial cue, but this was not a criterion for their selection. Neurons selected for further study were tested in a variant of the memory saccade task in which we varied the size of the water reward.

In the variable-reward memory saccade task (Figure 1), a visual

cue indicated whether the monkey would obtain a small or large reward upon successful execution of a memory-guided eye movement. The task differed in two ways from the memory saccade task used for screening and initial response characterization. First, the spatial cue appeared at 1 of only 2–8 possible locations (2 for most experiments); one of the locations was in the neuron's RF. Second, on each trial the color of the fixation point changed from red (CIE coordinates, $xy = 0.63, 0.34; 7.6 \text{ cd/m}^2$) to either green ($xy = 0.30, 0.61; 21.7 \text{ cd/m}^2$) or white ($xy = 0.29, 0.29; 32.7 \text{ cd/m}^2$) to indicate whether the reward would consist of one or three squirts of water (0.11 or 0.33 ml for monkey H; 0.15 or 0.45 ml for monkey I). We refer to the change in the color of the fixation point as the "reward cue." On half of the trials the reward cue preceded the spatial cue by a variable duration (median duration = 500 ms; Figure 1A), and on the other half the reward cue was presented during the memory period, a variable time after the spatial cue (median duration = 1000 ms; Figure 1B).

Analysis of Neuronal Responses

All physiological data reported in this paper were acquired from trials in which the monkeys successfully completed the memory saccade task. We compared neural activity on trials ending in small and large reward, focusing mainly on the period after the monkey had seen both the spatial/memory cue and the reward cue. Unless otherwise noted, the average spike rate was computed in an epoch beginning 300 ms after the onset of the spatial cue or reward cue, whichever occurred last, and ending with the offset of the fixation point ("go" signal). The epoch was chosen to exclude transient visual responses to the saccade targets and any presaccadic burst activity. Analysis of neural discharge in other epochs is noted clearly in the main text.

For single neurons, we compared the mean spike rates on small- and large-reward trials using the Student's *t* test. We also computed a single ratio between the means of the responses obtained on large- and small-reward trials, which we refer to as the ER. When combining several measurements of the ER, we used the geometric mean and performed all statistical tests on the logarithms of the individual ERs.

A more sensitive test for the influence of reward size across the population of neurons relies on an ANOVA model in which the response is modeled as a function of reward size and neuron identity:

$$y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \dots + \beta_{N-1}x_{N-1} + \beta_R\text{REW} + \epsilon, \quad (2)$$

where y is the measured spike rate on each trial, β_i represents the fitted coefficients, x_k represents dummy variables that identify the neuron ($x_k = 1$ if neuron = k and 0 otherwise), and ϵ is random error, which is assumed to be Gaussian. The null hypothesis is that $\beta_R = 0$ (i.e., reward size does not affect the response), which we tested by forming an *F* statistic using the principle of extra sum of squares (Draper and Smith, 1966). We refer to this test as an *F* test in the text. For the combined data in Figure 8, we analyzed the possibility that the color of the reward cue acted as a confounder to account for the variation in response as a function of reward size. This entails adding another categorical term for color to the ANOVA model

$$y = \beta_0 + \beta_1x_1 + \beta_2x_2 + \dots + \beta_{N-1}x_{N-1} + \beta_R\text{REW} + \beta_C\text{COLOR} + \epsilon \quad (3)$$

and again examining the consequences of removing REW from the model.

To study the time course of the response, we combined data from several neurons after applying a normalization procedure. For each neuron and for each trial, we computed the spike rate in 50 ms epochs relative to a common event (e.g., onset of the reward cue). We then divided these values by the average spike rate computed as a function of time using all trials that shared the same memory cue, irrespective of reward size. The procedure yields a time course of the response relative to the running mean, which is suitable for averaging across experiments.

Analysis of Behavioral Responses

We characterized the monkeys' propensity to break fixation more often after the small reward was cued by computing the odds ratio

of a broken fixation:

$$\text{OR} = \frac{N(\text{broken fix, small rew})N(\text{completed trials, big rew})}{N(\text{completed trials, small rew})N(\text{broken fix, big rew})} \quad (4)$$

where $N()$ is the number of observations. Confidence intervals were estimated using the Woolf procedure (Rosner, 1995). The null hypothesis, $\text{OR} = 1$, was evaluated by χ^2 test with Yates correction.

We measured properties of the monkeys' eye movements on the successfully completed trials. Four descriptors of each saccadic eye movement were extracted: its latency (LAT), amplitude (AMP), peak velocity (VMAX), and accuracy (ACC; the reciprocal of the distance between the saccadic endpoint and the location of the spatial cue). These values were normalized with respect to the mean for all saccades to the same memory target. Across experiments, we then compared the effect of small and large reward on each saccade descriptor. We also examined the effect of saccadic variability on neural response. As a caveat to the population analysis described in the Results (near Equation 1), we examined each neuron's response as a function of each of the saccade descriptors. We evaluated—but failed to support—the possibility that variation in a saccade descriptor could have opposite effects on different neurons, thereby canceling each other across the population.

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References

- Andersen, R.A., Asanuma, C., and Cowan, W.M. (1985). Callosal and prefrontal associational projecting cell populations in area 7a of the macaque monkey: a study using retrogradely transported fluorescent dyes. *J. Comp. Neurol.* **232**, 443–455.
- Asaad, W., Rainer, G., and Miller, E. (1998). Neural activity in the primate prefrontal cortex during associative learning. *Neuron* **21**, 1399–1407.
- Berger, B., Gaspar, P., and Verney, C. (1991). Dopaminergic innervation of the cerebral cortex: unexpected differences between rodents and primates. *Trends Neurosci.* **14**, 21–27.
- Brainard, D.H. (1997). The Psychophysics Toolbox. *Spat. Vision* **10**, 443–446.
- Bruce, C.J., and Goldberg, M.E. (1985). Primate frontal eye fields. I. Single neurons discharging before saccades. *J. Neurophysiol.* **53**, 603–635.
- Bruce, C.J., Goldberg, M.E., Bushnell, M.C., and Stanton, G.B. (1985). Primate frontal eye fields. II. Physiological and anatomical correlates of electrically evoked eye movements. *J. Neurophysiol.* **54**, 714–734.
- Cavada, C., and Goldman-Rakic, P. (1989). Posterior parietal cortex in rhesus monkey. II. Evidence for segregated corticocortical networks linking sensory and limbic areas in the frontal lobe. *J. Comp. Neurol.* **287**, 422–445.
- Dias, R., Robbins, T., and Roberts, A. (1996). Dissociation in prefrontal cortex of affective and attentional shifts. *Nature* **380**, 69–72.
- Draper, N., and Smith, H. (1966). *Applied Regression Analysis*, Second Edition (New York: John Wiley and Sons).
- Funahashi, S., Bruce, C., and Goldman-Rakic, P. (1989). Mnemonic coding of visual space in the monkey's dorsolateral prefrontal cortex. *J. Neurophysiol.* **61**, 331–349.
- Funahashi, S., Bruce, C., and Goldman-Rakic, P. (1991). Neuronal activity related to saccadic eye movements in the monkey's dorsolateral prefrontal cortex. *J. Neurophysiol.* **65**, 1464–1483.
- Fuster, J. (1985). The prefrontal cortex and temporal integration. In *Cerebral Cortex*, A. Peters and E. Jones, eds. (New York: Plenum), pp. 151–177.

- Fuster, J. (1989). *The Prefrontal Cortex* (New York: Raven).
- Fuster, J. (1995). Memory in the Cerebral Cortex: An Empirical Approach to Neural Networks in the Human and Nonhuman Primate (Cambridge, MA: MIT Press).
- Gaffan, D., and Murray, E. (1990). Amygdalar interaction with the mediodorsal nucleus of the thalamus and the ventromedial prefrontal cortex in stimulus-reward associative learning in the monkey. *J. Neurosci.* *10*, 3479–3493.
- Goldman-Rakic, P. (1987). Circuitry of primate prefrontal cortex and regulation of behavior by representational memory. In *Handbook of Physiology, Section I: The Nervous System, F. Plum, ed.* (Bethesda, MD: American Physiological Society), pp. 373–417.
- Goldman-Rakic, P., and Porrino, L. (1985). The primate mediodorsal (MD) nucleus and its projection to the frontal lobe. *J. Comp. Neurol.* *242*, 535–560.
- Haber, S., and Fudge, J. (1997). The primate substantia nigra and VTA: integrative circuitry and function. *Crit. Rev. Neurobiol.* *11*, 323–342.
- Hasegawa, R., Sawaguchi, T., and Kubota, K. (1998). Monkey prefrontal neuronal activity coding the forthcoming saccade in an oculomotor delayed matching-to-sample task. *J. Neurophysiol.* *79*, 322–334.
- Hikosaka, O., and Wurtz, R.H. (1989). The basal ganglia. In *The Neurobiology of Saccadic Eye Movements*, R.H. Wurtz and M.E. Goldberg, eds. (Amsterdam: Elsevier), pp. 257–281.
- Hikosaka, O., Sakamoto, M., and Usui, S. (1989). Functional properties of monkey caudate neurons. III. Activities related to expectation of target and reward. *J. Neurophysiol.* *61*, 814–832.
- Hollerman, J.R., Tremblay, L., and Schultz, W. (1998). Influence of reward expectation on behavior-related neuronal activity in primate striatum. *J. Neurophysiol.* *80*, 947–963.
- Ilinsky, I., Jouandet, M., and Goldman-Rakic, P. (1985). Organization of the nigrothalamocortical system in the rhesus monkey. *J. Comp. Neurol.* *236*, 315–330.
- Inoue, M., Oomura, Y., Aou, S., Nishino, H., and Sikdar, S. (1985). Reward related neuronal activity in monkey dorsolateral prefrontal cortex during feeding behavior. *Brain Res.* *326*, 307–312.
- Iversen, S., and Mishkin, M. (1970). Perseverative interference in monkeys following selective lesions of the inferior prefrontal convexity. *Exp. Brain Res.* *11*, 376–386.
- Jacobsen, C.F. (1935). Functions of frontal association area in primates. *Arch. Neurol. Psychiatry* *33*, 558–569.
- Jacobson, S., and Trojanowski, J. (1975). Amygdaloid projections to prefrontal granular cortex in rhesus monkey demonstrated with horseradish peroxidase. *Brain Res.* *100*, 132–139.
- Kawagoe, R., Takikawa, Y., and Hikosaka, O. (1998). Expectation of reward modulates cognitive signals in the basal ganglia. *Nat. Neurosci.* *1*, 411–416.
- Kawamura, K., and Naito, J. (1984). Corticocortical projections to the prefrontal cortex in the rhesus monkey investigated with horseradish peroxidase techniques. *Neurosci Res.* *1*, 89–103.
- Kim, J.-N., and Shadlen, M.N. (1999). Neural correlates of a decision in the dorsolateral prefrontal cortex of the macaque. *Nat. Neurosci.* *2*, 176–185.
- Leon, M., and Gallistel, C. (1998). Self-stimulating rats combine subjective reward magnitude and subjective reward rate multiplicatively. *J. Exp. Psychol. Anim. Behav. Process.* *24*, 265–277.
- Leon, M.I., and Shadlen, M.N. (1998). Exploring the neurophysiology of decisions. *Neuron* *21*, 669–672.
- Levy, R., and Goldman-Rakic, P. (1999). Association of storage and processing functions in the dorsolateral prefrontal cortex of the nonhuman primate. *J. Neurosci.* *19*, 5149–5158.
- Miller, E.K. (1999). The prefrontal cortex: complex neural properties for complex behavior. *Neuron* *22*, 15–17.
- Niki, H., and Watanabe, M. (1979). Prefrontal and cingulate unit activity during timing behavior in the monkey. *Brain Res.* *171*, 213–224.
- Pandya, D., Dye, P., and Butters, N. (1971). Efferent cortico-cortical projections of the prefrontal cortex in the rhesus monkey. *Brain Res.* *31*, 35–46.
- Pelli, D.G. (1997). The VideoToolbox software for visual psychophysics: transforming numbers into movies. *Spat. Vision* *10*, 437–442.
- Platt, M.L., and Glimcher, P.W. (1999). Neural correlates of decision variables in parietal cortex. *Nature* *400*, 233–238.
- Quintana, J., and Fuster, J. (1999). From perception to action: temporal integrative functions of prefrontal and parietal neurons. *Cereb. Cortex* *9*, 213–221.
- Rainer, G., Rao, S., and Miller, E. (1999). Prospective coding for objects in primate prefrontal cortex. *J. Neurosci.* *19*, 5493–5505.
- Rao, S.C., Rainer, G., and Miller, E.K. (1997). Integration of what and where in the primate prefrontal cortex. *Science* *276*, 821–824.
- Robertson, A. (1989). Multiple reward systems and the prefrontal cortex. *Neurosci. Biobehav. Rev.* *13*, 163–170.
- Rolls, E. (1996). The orbitofrontal cortex. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* *351*, 1433–1443.
- Rosner, B. (1995). *Fundamentals of Biostatistics, Fourth Edition* (Belmont, CA: Duxbury Press).
- Sawaguchi, T., and Goldman-Rakic, P. (1994). The role of D1-dopamine receptor in working memory: local injections of dopamine antagonists into the prefrontal cortex of rhesus monkeys performing an oculomotor delayed-response task. *J. Neurophysiol.* *71*, 515–528.
- Sawaguchi, T., Matsumura, M., and Kubota, K. (1990). Effects of dopamine antagonists on neuronal activity related to a delayed response task in monkey prefrontal cortex. *J. Neurophysiol.* *63*, 1401–1412.
- Schultz, W. (1998). Predictive reward signal of dopamine neurons. *J. Neurophysiol.* *80*, 1–27.
- Schultz, W., Apicella, P., and Ljungberg, T. (1993). Responses of monkey dopamine neurons to reward and conditioned stimuli during successive steps of learning a delayed response task. *J. Neurosci.* *13*, 900–913.
- Selemon, L., and Goldman-Rakic, P. (1988). Common cortical and subcortical targets of the dorsolateral prefrontal and posterior parietal cortices in the rhesus monkey: evidence for a distributed neural network subserving spatially guided behavior. *J. Neurosci.* *8*, 4049–4068.
- Tremblay, L., and Schultz, W. (1999). Relative reward preference in primate orbitofrontal cortex. *Nature* *398*, 704–708.
- Watanabe, M. (1990). Prefrontal unit activity during associative learning in the monkey. *Exp. Brain Res.* *80*, 296–309.
- Watanabe, M. (1992). Frontal units of the monkey coding the associative significance of visual and auditory stimuli. *Exp. Brain Res.* *89*, 233–247.
- Watanabe, M. (1996). Reward expectancy in primate prefrontal neurons. *Nature* *382*, 629–632.
- Williams, S., and Goldman-Rakic, P. (1993). Characterization of the dopaminergic innervation of the primate frontal cortex using a dopamine-specific antibody. *Cereb. Cortex* *3*, 199–222.
- Williams, G.V., and Goldman-Rakic, P.S. (1995). Modulation of memory fields by dopamine D1 receptors in prefrontal cortex. *Nature* *376*, 572–575.