Neuronal Activity Dependent on Anticipated and Elapsed Delay in Macaque

Prefrontal Cortex, Frontal and Supplementary Eye Fields and Premotor Cortex

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Running head: Activity Related to Delay Length in Frontal Cortex

Abstract: 245 words

Text: 75 pages

4 tables

20 figures

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ABSTRACT

In macaque monkeys performing a memory guided saccade task for a reward of variable size, neuronal activity in several areas of frontal cortex is stronger when the monkey anticipates a larger reward. This effect might depend on either the size or the value of the reward. To distinguish between these possibilities, we recorded from neurons in frontal cortex while controlling value through a manipulation of time rather than amount. A cue presented at the beginning of each trial, predicted the length of the delay during which the monkey would have to maintain fixation prior to performing a saccade and receiving a reward of fixed size. Predicting a short delay had effects closely similar to those of predicting a large reward: (1) monkeys were more motivated when working for a reward at short delay, (2) neurons tended to fire more strongly before a short delay, (3) individual neurons firing more strongly before a short delay tended also to fire more strongly before a large reward, and (4) the tendency to fire more strongly before a short delay was far more pronounced in premotor areas caudal to the arcuate sulcus than in association areas rostral to it. The association areas, in contrast, were marked by a tendency for neurons to fire more strongly at the end of the long delay. We conclude that predicting a short delay, like predicting a large reward, induces an enhancement of neuronal activity related to motivational modulation of the monkey’s preparatory state.
INTRODUCTION

The anticipation of reward is thought to lead to motivated behavior through a series of steps originating in the limbic system and terminating in the motor system (Hikosaka et al. 2000; Kalivas and Nakamura 1999; Mogenson et al. 1980; Ono et al. 2000). The limbic system is crucial for the initial stage of evaluating rewards (Roesch and Olson 2004; Tremblay and Schultz 1999, 2000). The transition from evaluating a reward to initiating action in pursuit of it is thought to depend on structures interposed functionally between the limbic system and motor system. Areas that may play a role in this transition include the dorsolateral prefrontal cortex (PFC), the frontal eye field (FEF), the supplementary eye field (SEF), the premotor cortex (PM) and the supplementary motor area (SMA). Neuronal activity in all of these areas is influenced both by the nature of the action the monkey is planning and by the value of the reward to which it will lead (Coe et al. 2002; Hikosaka et al. 1989; Hikosaka and Watanabe 2000; Kobayashi et al. 2002; Leon and Shadlen 1999; Matsumoto et al. 2003; Roesch and Olson 2003; Wallis and Miller 2003; Watanabe 1990, 1992, 1996; Watanabe et al. 2002). Thus it makes sense to think of them as a potential watershed between limbic evaluative functions and motor output.

The PFC, FEF, SEF, PM and SMA are thought to serve a variety of functions with cognitive, oculomotor and skeletomotor components. It would be surprising, for that reason, if reward-related activity took the same form or had the same significance in all of them. To test for possible differences among these areas with respect to the nature of reward-related activity, we recently carried out a comparative study in which we recorded from neurons in all of them while monkeys performed a memory guided saccade task in which a cue presented early in each trial indicated whether the reward delivered upon successful completion of the trial would be large or small (Roesch and Olson, 2003). We found that the tendency for neurons to fire more strongly
when a large reward was expected increased markedly as the recording site moved posteriorly (from PFC to FEF to PM in the lateral frontal lobe and from SEF to SMA in the medial frontal lobe).

The pattern of inter-areal differences observed in the previous study was of interest because it cast light on the possible significance of reward-related activity. This might either (1) represent the value of the anticipated reward in the service of an economic decision process or (2) reflect motivational modulation of the state of motor preparation, motor output, arousal or attention. To distinguish definitively between these possibilities is not possible in experiments that manipulate only the value of the predicted reward because, under this manipulation, perceived value and motivational state are correlated (Maunsell, 2004; Olson and Roesch, 2004). Nevertheless, in light of the fact that reward-related activity was strongest by far in PM and SMA, which is to say in motor areas, the second interpretation, based on motivational modulation of the monkey’s preparatory state, carries greatest weight.

We were concerned that these results might be specific to manipulations of value based on reward size. To address this issue, we devised an alternate approach in which the size of the reward was the same on every trial but the monkey was informed by an early cue whether the delay before delivery of the reward would be long or short. It is well known that inserting a longer anticipated delay before an anticipated reward reduces its perceived value, a phenomenon known as time-discounting (Lowenstein and Elster, 1992). We report here that varying the delay before delivery of a constant reward had very much the same effect on neuronal activity as varying the size of a reward delivered at a constant delay. In particular, the tendency for neurons to fire more strongly following a cue that predicted a short delay was much more robust in areas
behind the arcuate sulcus (FEF/PM, PM and SMAr) than in areas in front of it (PFC, FEF and SEF).
METHODS

Subjects

Four adult male rhesus monkeys were used (Macaca mulatta; laboratory designations N, P, A and F). Experimental procedures were approved by the Carnegie Mellon University Animal Care and Use Committee and were in compliance with the guidelines set forth in the United States Public Health Service Guide for the Care and Use of Laboratory Animals.

Preparatory Surgery

At the outset of the training period, each monkey underwent sterile surgery under general anesthesia maintained with isofluorane inhalation. The top of the skull was exposed, bone screws were inserted around the perimeter of the exposed area, a continuous cap of rapidly hardening acrylic was laid down so as to cover the skull and embed the heads of the screws, a head-restraint bar was embedded in the cap, and scleral search coils were implanted on the eyes, with the leads directed subcutaneously to plugs on the acrylic cap (Robinson 1963). Following initial training, recording chambers were implanted into the acrylic. At each selected site, a 2-cm-diameter disk of acrylic and skull was removed. A cylindrical recording chamber was cemented into the hole with its base just above the exposed dural membrane.

Chambers were placed either at a medial location (over SEF and SMAr) or at a lateral location (over PFC, FEF, FEF/PM, and PM). Recording was carried out from a medial chamber in monkeys A, N and F, a left lateral chamber in monkey P and a right lateral chamber in monkeys F and N. The medial chambers placed over SEF and SMAr were centered on the midline of the brain approximately 21 mm anterior to the Horsley-Clarke interaural plane. The
lateral chambers placed over PFC, FEF and PM were centered approximately at anterior 23 mm and lateral 23 mm.

**Memory Guided Saccade Task**

The aim of this task was to allow initial characterization of the spatial selectivity of each neuron. The monkeys performed memory guided saccades to six targets forming a hexagonal array at an eccentricity of 10° (Fig. 1A). Each trial began with the monkey's fixating a central spot. Five hundred ms after attainment of fixation, the six targets appeared. After an additional 300 ms a cue was presented for 250 ms in superimposition on one of the targets. Following a random delay in the range of 500 to 1000 ms, the fixation spot was extinguished, whereupon the monkey had to make a saccade directly to the previously cued target. Trials involving the six targets were interleaved in random order subject to the constraint that each block of six successful trials had to contain one trial involving each target. Testing continued until it was possible to identify the target eliciting maximal activity. Subsequent testing in other tasks involved this target and the one diametrically opposite with respect to the fixation point (Fig. 1A: 1 and 1' or 2 and 2' or 3 and 3').

**Variable-Delay Task**

The monkeys performed a memory guided saccade task in which a cue presented early in the trial predicted a short (500 ms) or a long (2500 ms) delay period. Essential features of the task are summarized in Fig. 1. Each trial began with onset of a central fixation spot (Fig. 1B1). At a time 50 ms after attainment of fixation, the spot was transformed to a cue the shape and color of which signified the length of the upcoming delay period (Fig. 1C1). After 400 ms two targets
appeared (Fig. 1D1) at diametrically opposed locations. A directional cue identical to the fixation cue except in size was then presented for 250 ms in superimposition on one of the targets (Fig. 1E1). After a 500 ms (or 2500 ms) delay period (Fig. 1F1), the fixation spot was extinguished (Fig. 1G1), whereupon the monkey was required to make a saccade directly to the previously cued target (Fig. 1H1) and to maintain fixation on it for 300-450 ms after saccade completion until delivery of a juice reward (Fig. 1I1). The intertrial interval after a correct trial was set to 1000 ms whereas, after an error, either a fixation break or of a wrong choice, it was set to 5000 ms. There were four conditions representing all possible combinations of delay length (short or long) and direction (preferred or antipreferred). The conditions were interleaved in random order subject to the constraint that one trial conforming to each condition had to be completed successfully before initiation of the next block of trials. Due to this constraint, no long-term advantage attached to breaking fixation on an undesirable (long-delay) trial. The rejected condition would simply be presented repeatedly until a trial was successfully completed. To prevent confounding activity related to delay length with selectivity for the visual properties of the cues, the cue convention was reversed after each block of 40 successful trials. The collection of data from a given neuron commonly continued until 80 trials had been completed successfully.

**Stimuli in the Variable-Delay Task**

The fixation spot was a $0.38^\circ$ white square presented at the center of the screen. Targets were $0.38^\circ$ white squares presented $10^\circ$ from central fixation. The central delay cues, which spanned $0.96^\circ$, were a red square and a blue circle. The directional cue shared all of the properties of the foveal delay cue with the exception that it spanned $1.32^\circ$. The background of
the display had a luminance of 1.5 cd/m$^2$, and CIE x and y chromaticity coefficients of 0.26 and 0.26. White stimuli had a luminance of 126.5 cd/m$^2$, and CIE x and y chromaticity coefficients of 0.28 and 0.32. Red stimuli had a luminance of 112.5 cd/m$^2$, and CIE x and y chromaticity coefficients of 0.27 and 0.61. Blue stimuli had a luminance of 110.2 cd/m$^2$, and CIE x and y chromaticity coefficients of 0.15 and 0.17.

Variable-Reward Task

Many of the neurons studied in the context of the variable-delay task were also studied in the context of the variable-reward task. In the variable reward task, the delay was fixed at 1500 ms, while the cue at the beginning of the trial predicted a big (0.3 cc) or small (0.1 cc) juice reward. Essential features of the task are summarized in Fig. 1. For further details of task design, see Roesch and Olson (2003). Data collected in the context of the variable-reward task were considered in a previous paper concerned with that task (Roesch and Olson 2003). Here, we consider data from the variable-reward task solely in connection with the question whether neurons sensitive to delay length were also sensitive to reward size.

Order of Tasks

Neuronal activity was first monitored in the context of the memory guided saccade task with reward size and delay length fixed and with targets at six locations spaced at 60° intervals around fixation (Fig. 1A). Any neuron appearing to exhibit task-related activity in this task was selected for study in the variable-delay task and the variable-reward task. In these tasks, the possible target locations were confined to the neuron’s preferred direction (as determined in the memory guided saccade task) and the opposite direction. The order in which the two tasks were
run alternated across sessions. Some neurons were studied in the context of only one of the tasks because recording instability or satiation of the monkey prevented running both.

**Single-Neuron Recording**

At the beginning of each day's session, a varnish-coated tungsten microelectrode with an initial impedance of several megohms at 1 KHz (Frederick Haer & Co., Bowdoinham, ME) was advanced vertically through the dura into the immediately underlying cortex. The dura was debrided at intervals commonly spanning a few months so as to ensure penetrability by the electrode. The electrode could be placed reproducibly at points forming a square grid with 1 mm spacing (Crist et al. 1988). The action potentials of a single neuron were isolated from the multineuronal trace by means of an on-line spike-sorting system using a template matching algorithm (Signal Processing Systems, Prospect, Australia). The spike-sorting system, on detection of an action potential, generated a pulse the time of which was stored with 1 ms resolution.

**Electromyographic Measurements**

Adhesive surface electrodes were placed on the shaved skin overlying the right splenius capitus and masseter muscles. The voltage threshold was set as low as possible subject to the constraint that the voltage did not cross threshold at rest. Muscle activity was stored as time-marked records of threshold crossings. From these, we constructed histograms representing the mean instantaneous threshold-crossing rate as a function of time during the trial under each condition.
Experimental Control and Data Collection

All aspects of the behavioral experiment, including presentation of stimuli, monitoring of eye movements, monitoring of neuronal activity, and delivery of reward, were under the control of a pentium-based computer running Cortex software provided by R. Desimone, Laboratory of Neuropsychology, National Institute of Mental Health. Eye position was monitored by means of a scleral search coil system (Riverbend Instruments, Inc., Birmingham, AL). The X and Y coordinates of eye position were stored with 4 ms resolution. Stimuli generated by an active matrix LCD projector were rear-projected on a frontoparallel screen 25 cm from the monkey's eyes.

Analysis of the Dependence of Behavior on Delay Length

We used paired t-tests to compare, across sessions, the session means of the following measures obtained on short-delay vs. long-delay trials: reaction time, error rate and fixation-break rate. Reaction time was defined as the delay from offset of the fixation spot to the moment when the eye left the central fixation window. Error rate was defined as the number of trials on which a saccade was directed to the wrong target expressed as a percentage of all trials on which a saccade was directed to either target. Fixation-break rate was defined as the percentage of all trials on which the eye left the central fixation window at any time prior to offset of the fixation spot.

Analysis of the Dependence of Firing Rate on Task Factors

We employed two-factor ANOVAs to analyze the dependence of the firing rate of each neuron on delay length and response direction. We independently analyzed data from seven trial
epochs: (1) from delay cue onset to directional cue onset (700 ms), (2) from onset to offset of the directional cue (250 ms), (3) 250 ms beginning with directional cue offset, (4) 250 ms prior to fixation spot offset, (5) 200 ms prior to saccade initiation, (6) from saccade onset to 100 ms following saccade completion, (7) 100 ms before to 100 ms after initiation of reward delivery. In all tests, the criterion for statistical significance was taken as \( p \leq 0.05 \).

Assessing Contribution of Reaction Time to Activity Related to Delay Length

To determine whether neuronal activity continued to depend on delay length when the effects of behavioral reaction time were factored out, we performed a multivariate regression analysis, fitting three models:

1. \( Y = a_0 + a_1 RT \)
2. \( Y = a_0 + a_2 DELAY \)
3. \( Y = a_0 + a_1 RT + a_2 DELAY \)

where \( Y \) was the firing rate measured from onset of the delay cue to offset of the fixation spot and RT was the behavioral reaction time. The variable DELAY was set to 1 or 0 for trials with short or long delays respectively. To determine whether adding the variable DELAY produced a significant improvement in performance, we compared model 3 to model 1. To determine whether adding the variable RT produced a significant improvement, we compared model 3 to model 2. Significance was assessed with an F-test using:

\[
F_{k,m-(n+k)} = \frac{\left( SS_{red} - SS_{full} \right) k}{SS_{full}} \frac{m-(n+k)}{m-(n+k)}
\]
where $k = 1$ was the difference in degrees of freedom between the two models, $n = 1$ was the number of neurons and $m$ was the number of trials on which the analysis was based. $SS_{\text{full}}$ and $SS_{\text{red}}$ were the residual sums of squares resulting when the data were fitted with the full model (model 1) and the reduced model (model 2 or 3) respectively. The criterion for statistical significance was taken as $p \leq 0.05$.

**Localization of Recording Sites**

To characterize the location of the recording sites relative to gross anatomical landmarks, we projected the sites onto structural MR images (Fig. 2). The images were collected by use of a Brüker 4.7 T magnet in which the anesthetized monkey was supported by an MR-compatible stereotaxic device. Fiducial marks made visible by means of a contrast agent included the centers of the ear bars and selected locations inside the recording chamber. Frontoparallel slices of 2 mm thickness spanning the entire brain were collected. In addition, slices of 2 mm thickness were collected parallel to the cortical surface underlying each lateral chamber. To determine the location of recording sites relative to functional divisions of cortex, we mapped out regions under each chamber from which motoric responses (eye, face and limb movements) could be elicited at low threshold ($\leq 40 \mu A$) by electrical microstimulation (1.65 ms biphasic pulses delivered through the recording microelectrode at a frequency of 300 Hz in trains 200 ms long).

We assigned recording sites to six areas according to the following criteria (Fig. 2). Dorsolateral prefrontal cortex (PFC): a region in front of the arcuate sulcus and surrounding and within the principal sulcus in which microstimulation did not elicit movements. Frontal eye field (FEF): a region rostral to and in the anterior bank of the arcuate sulcus in which microstimulation
elicited saccades and not movements of the face or limbs. Recording sites in the FEF were all within 4 mm of the cortical surface at locations where microstimulation at the corresponding depth elicited eye movements. Premotor cortex (PM): a region caudal to the arcuate sulcus in which microstimulation elicited face or limb movements and not saccades. Transitional cortex (FEF/PM): a region immediately caudal to the arcuate sulcus and rostral to the pure face-limb zone in which electrical stimulation elicited both eye movements and movements of the face or limbs. Recording sites in FEF/PM were all within 4 mm of the cortical surface at locations where microstimulation at the corresponding depth elicited eye and face-limb movements. On the grounds of its location behind the arcuate sulcus, this cortex belongs to the premotor area. However, we have designated it as an independent zone with the possibility in mind that its distinct traits, as revealed by electrical stimulation, might be accompanied by some differential form of sensitivity to delay-predicting cues. The finding of an oculomotor representation in PM is not without precedent (Fujii et al. 1998, 2000). Supplementary eye field (SEF): a region, located rostral to the genu of the arcuate sulcus and extending 2-5 mm from the hemispheric midline, in which microstimulation elicited saccades. Rostral supplementary motor area (SMAr): a region immediately caudal to the SEF in which microstimulation elicited movements of the face and limbs.

RESULTS

Overview

In the following sections, we will describe the impact of delay length on behavior and neuronal activity in PFC, FEF, FEF/PM, PM, SEF and SMAr. At each stage of analysis, we will
consider (first) effects related to *anticipated* delay and (second) effects related to *elapsed* delay. We will examine the impact of anticipated delay by comparing short-delay to long-delay trials during epochs aligned on the delay-predicting cue and preceding the moment at which, in short-delay trials, the signal to respond was given. We will examine the impact of elapsed delay by comparing short-delay to long-delay trials during epochs near in time to the response and aligned on response initiation. The analyses concerned with anticipated and elapsed delay involve comparing roughly identical periods of neuronal activity, as observed on short-delay trials, to non-overlapping early and late periods of activity, as observed on long-delay trials. Thus the two analyses are not entirely independent. However, as will be seen, they reveal qualitatively different effects.

**Behavior: Anticipated Delay**

To analyze the impact of anticipated delay on behavior we assessed how fixation breaks were distributed across time during the early part of the trial under short- vs. long-delay conditions. The results, shown in Fig. 3C, indicate (a) that fixation breaks were more frequent under long-than under short-delay conditions and (b) that the tendency to break fixation declined over the course of the trial under both conditions. To determine whether the impact of anticipated delay length was significant, we compared the fixation-break frequencies (number of trials prematurely terminated by cessation of fixation expressed as a percentage of all trials) observed under short- and long-delay conditions in each monkey during the first 1000 ms beginning at the presentation of the delay-cue (Fig. 3D). This analysis epoch spans a period in which equivalent events occurred at equivalent times on short- and long-delay trials, with the only difference between them due to anticipation. The tendency for fixation breaks to occur more often in anticipation of
a long delay was present and significant in every monkey (two-tailed paired t-test, \( p < 0.05 \)) and was highly significant in data collapsed across monkeys (\( p < 0.001 \)). We conclude that the monkeys were less motivated to perform the task when anticipating a long as compared to a short delay.

**Behavior: Elapsed Delay**

We will refer to effects occurring at the end of the trial (and revealed by comparing measures from short- and long-delay trials taken around the time of the behavioral response) as related to “elapsed delay.” This is an expositional tool. It does not imply that the effects were a direct consequence of elapsed delay. There is no sure way of establishing whether effects present at the time of the response depended on the monkey’s experiencing the antecedent delay or, alternatively, were due to the monkey’s having been put by the delay cue into a state which persisted until the end of the trial. With this qualification, we note that behavioral measures taken at the end of the trial were sensitive to the duration of the antecedent delay. This was evident in two behavioral measures computed for every neuronal data collection session. The error rate (percentage of trials when the incorrect target was selected relative to all trials when one target or the other was selected) was lower on short-delay (0.6%) than on long-delay (2.0%) trials (Fig. 2A). This trend was present in data from every monkey, achieving significance (two-tailed paired t-test, \( p < 0.05 \)) in three out of four of them (Fig. 2D, and was highly significant in data collapsed across all monkeys (\( p < 0.0001 \)). The average behavioral reaction time was faster on short-delay (233 ms) than on long-delay (248 ms) trials (Fig. 2B). This trend was present and achieved significance (two-tailed paired t-test, \( p < 0.05 \)) in three out of four monkeys (Fig. 2D), and was highly significant in data collapsed across all monkeys (\( p < 0.0001 \)). These results
indicate that monkeys were in a state of heightened preparation (reflected by a simultaneous improvement of accuracy and speed) following a short as compared to long delay.

Neuronal Data Analysis: Anticipated Delay

To determine whether neuronal activity was influenced by the length of the anticipated delay, we compared neuronal activity occurring prior to the imperative command (offset of the fixation spot) on short-delay trials to neuronal activity occurring during the identical period (at the end of which the fixation spot remained on) on long-delay trials. Neurons in many frontal areas exhibited activity related to the length of the expected delay. This activity commonly took the form of a main effect (with the net firing rate higher or lower on short-delay trials) and less frequently took the form of an interaction effect (with the strength of the directional signal stronger or weaker on short-delay trials). For the neurons illustrated in Fig. 4A-B, the net firing rate during the “anticipated-delay” comparison period (time-locked to delay-cue onset and highlighted in yellow) was higher on short-delay trials (top row for each neuron) than on long-delay trials (bottom row for each neuron).

In considering results from each cortical area, we will characterize the impact of anticipated delay on firing rate by proceeding through three steps. 1) Population histograms. The aim of this step is to indicate qualitatively how the length of predicted delay affected the population firing rate and the population directional signal. Population averaging, although informative, could lead to an underestimate of the strength of delay-selective activity if opposite signals carried by different neurons canceled out at the stage of averaging. The next steps of analysis address this issue. 2) Individual neurons by epoch. The aim of this step is to indicate whether effects evident at the level of the population were statistically significant at the level of
individual neurons during successive epochs of the analysis period. We will assess how many neurons showed significant increases or decreases in firing rate on short-delay as compared to long-delay trials, and how many showed significant increases or decreases in the strength of the directional signal. The epochs of interest are: (I) from delay cue onset to directional cue onset (700 ms), (II) from onset to offset of the directional cue (250 ms) and (III) 250 ms beginning with directional cue offset. 3) **Individual neurons across a long anticipatory epoch.** To complement the analysis by short epochs, which gives good temporal resolution but low statistical sensitivity, we will also characterize the delay-related activity of each neuron by considering its firing rate during the period between onset of the delay-cue and a point in time 250 ms after offset of the directional cue. This statistically robust but time-insensitive step will provide a single measure for each neuron to be used in comparing across areas the percentage of neurons sensitive to anticipated delay.

**Neuronal Data Analysis: Elapsed Delay**

Upon examination of histograms representing the activity of single neurons, it was evident that the firing rate at the end of the delay period, around the time of the saccade (epoch highlighted in green in Fig. 4), could differ according to whether the antecedent delay had been long or short. The neuron of Fig. 4A clearly fired more strongly at the end of a short than at the end of a long delay. In contrast, on trials requiring a response in the preferred (leftward) direction, the neuron of Fig. 4B fired more strongly at the end of a long delay. To analyze the nature and rate of incidence of effects dependent on elapsed delay, we will proceed through three steps of analysis. 1) **Population histograms.** These depict activity during a 1500 ms epoch beginning 500 ms before saccade initiation (righthand column in Fig’s. 5-9). The beginning of
this epoch coincides with a point in time approximately 250 ms after offset of the directional cue on short-delay trials and 2250 ms after offset of the directional cue on long-delay trials.  2) Individual neurons by epoch. We will consider whether firing rate depended on delay length or its interaction with response direction during late epochs of the trial, including epoch IV (250 ms prior to fixation spot offset), V (200 ms prior to saccade initiation), VI (from saccade onset to 100 ms following saccade completion) and VII (from 100 ms before to 100 ms after initiation of reward delivery).  3) Individual neurons across a long pre-movement epoch. In order to obtain one robust statistical measure for each neuron, so as to facilitate comparison across areas, we will determine whether the firing rate of each neuron depended significantly on delay length or its interaction with response direction during a long epoch beginning 250 ms before the imperative cue (offset of the fixation spot) and ending with saccade initiation.

Prefrontal Cortex (PFC): Anticipated Delay

Population. We collected data from 204 neurons in the PFC of two monkeys (Table 1). As a basis for qualitative assessment of the effect of anticipated delay on the activity of these neurons, we constructed population curves representing firing rate as a function of time under the four trial conditions (Fig. 5A). Population histograms shown to the left (Fig. 5A1) represent activity subject to influence by the duration of the anticipated delay. In these displays, aligned on cue onset, thick and thin lines represent population activity on trials requiring responses in the preferred and antipreferred directions respectively. Neuronal activity was strongly affected by response direction as indicated by the consistent elevation of thick above thin lines following appearance of the directional cue. Effects of anticipated delay length would be manifest as differences in firing rate between trials in which the response direction (indicated by line
thickness) was the same but expected delay length (indicated by color) was different. It is evident from the close coincidence of red and blue curves that the impact of anticipated delay on firing rate was weak. To characterize the time-course of activity modulated by anticipated delay length, we computed independently indices reflecting (1) the impact of expected delay length on net firing rate independent of direction and (2) the impact of expected delay length on the strength of the directional signal. The impact on net firing rate was measured with an index representing the average amount by which the firing rate increased under the short-delay condition. It was computed as \((SP + SA - LP - LA)/2\), where \(SP\) was the firing rate under the short-delay, preferred-direction condition, \(LA\) was the firing rate under the long-delay, antiprefered-direction condition, and so on. The impact of anticipated delay length on firing rate was variable over time and negligible in strength (Fig. 5B1). The effect of delay on the directional signal was represented by an index that corresponded to the average amount by which short-delay caused the difference in firing rate between preferred-direction and antiprefered-direction trials to increase. It was computed as \((SP - SA – LP + LA)/2\). This index hovered around zero prior to the directional signal and then became slightly positive (Fig. 5D1).

**Individual neurons by epoch.** To determine whether effects present in the population were also observable at the level of individual neurons, we analyzed data from each neuron during three trial epochs (I-III) defined in methods and depicted along the time-line at the base of Fig. 5E1. For each epoch, we carried out an ANOVA with firing rate as the dependent variable and with delay length and response direction as factors. **Main effect of delay.** Counts of neurons exhibiting a significant main effect of delay length on firing rate are shown in Fig. 5C1, where blue (or red) symbols represent the percentage of cases in which firing was increased (or decreased) for short compared to long delay trials. During each epoch (I-III), the full count of
neurons exhibiting a main effect of delay (whether this took the form of significantly stronger or significantly weaker firing under the short-delay as compared to the long-delay condition) was significantly in excess of the frequency (0.05) expected by chance from type 1 errors (chi-squared test, $p < 0.05$). This observation can be reconciled with the observation that there was little effect of delay on the average activity of the neuronal population (as indicated in the population histogram) by noting that neurons increasing and decreasing their firing rate on short-delay trials were equally common, so that the effects canceled at the population level. During no epoch was there a significant difference in number between neurons firing more strongly and those firing more weakly before a short delay (chi-squared test, $p > 0.05$). Interaction between delay and direction. Counts of neurons exhibiting a significant interaction effect are shown in Fig. 5E1, where blue (or red) symbols represent the percentage of cases in which the directional signal was stronger (or weaker) on short-delay trials. Counts during epoch I necessarily represent type 1 errors because it was only after this epoch that the directional cue appeared. During epoch III, the proportion of neurons exhibiting an interaction effect was significantly in excess of the frequency expected by chance (chi-squared test, $p < 0.05$). During no epoch was there a significant difference in number between neurons exhibiting stronger direction selectivity and those exhibiting weaker direction selectivity before a short delay (chi-squared test, $p > 0.05$).

Individual neurons across a long anticipatory epoch. To generate for each neuron a single statistical measure of the impact of predicted delay length on the neuronal firing rate, we carried out an ANOVA using, as the dependent variable, the mean firing rate across the entire period from onset of the delay cue to a point in time 250 ms after offset of the directional cue, taking as factors both expected delay length and instructed response direction. The results are presented in
Table 1 and Fig. 11. **Main effect of delay.** There was a significant main effect of expected delay in 10% of PFC neurons. This percentage was significantly in excess of that expected by chance (chi-squared test, $p < 0.05$). The difference in frequency between neurons firing more strongly and those firing more weakly before a short delay was not significant (chi-squared test, $p > 0.05$).

**Interaction between delay and direction.** Interaction effects were no more common than expected by chance (chi-squared test, $p > 0.05$). The difference in frequency between cases in which direction selectivity was stronger before a short delay and those in which it was weaker was not significant (chi-squared test, $p > 0.05$). These results did not differ significantly between monkeys.

**Summary.** Among PFC neurons, the length of the anticipated delay exerted a subtle influence on firing rate. A few neurons fired more strongly and a few more weakly in anticipation of a short delay.

**Prefrontal Cortex (PFC): Elapsed Delay**

**Population.** In curves representing the population firing rate as a function of time, it is evident that activity was enhanced after a long delay as compared to a short delay (Fig. 5A2). This was true both when the response was in the neuron’s preferred direction (the thick red curve lies above thick blue curve) and when it was in the opposite direction (the thin red curve lies above thin blue curve). The enhancement was present before saccade initiation but was most marked following the saccade (downward directed red regions in the difference histogram of Fig. 5B). In contrast to the impact of elapsed delay on net firing rate, there was almost no effect on the strength of the directional signal (Fig. 5D2).
Individual neurons by epoch. The results, presented in Fig. 5C2 and Fig. 5E2, can be summarized in the following terms. **Main effect of delay.** During each epoch (IV-VII), the proportion of neurons exhibiting a main effect was significantly in excess of the frequency expected by chance (chi-squared test, $p < 0.05$). During epochs IV and VI, the preponderance of neurons that fired more strongly after a long delay was significant (chi-squared test, $p < 0.05$).

**Interaction between delay and direction.** During epochs IV, VI and VII, the proportion of neurons exhibiting an interaction effect was significantly in excess of the frequency expected by chance (chi-squared test, $p < 0.05$). During no epoch was there a significant difference in number between neurons exhibiting stronger direction selectivity and those exhibiting weaker direction selectivity after a long delay (chi-squared test, $p > 0.05$).

Individual neurons across a long pre-movement epoch. The results, presented in Table 3 and Fig. 17, can be summarized in the following terms. **Main effect of delay.** There was a significant main effect of expected delay in 24% of PFC neurons. This percentage was significantly in excess of that expected by chance (chi-squared test, $p < 0.05$). The preponderance of neurons firing more strongly after a long delay was significant (chi-squared test, $p < 0.05$). **Interaction between delay and direction.** Interaction effects were significantly more common than expected by chance (chi-squared test, $p < 0.05$). The preponderance of neurons in which the directional signal was stronger after a long delay was significant (chi-squared test, $p < 0.05$). These results did not differ significantly between monkeys.

**Summary.** Among PFC neurons, the length of the elapsed delay exerted a marked influence on firing rate. A majority of delay-sensitive neurons fired more strongly after a long delay.

**Frontal Eye Field (FEF): Anticipated Delay**
Population. We collected data from 124 neurons in the FEF of three monkeys (Table 1). Curves representing the activity of this population as a function of time during the trial indicate that the level of anticipated delay exerted only minor effects on neuronal activity early in the trial (Fig. 6A1).

Individual neurons by epoch. The results, presented in Fig. 6C1 and Fig. 6E1, can be summarized in the following terms. **Main effect of delay.** During each epoch (I-III), the proportion of neurons exhibiting a main effect was significantly in excess of the frequency expected by chance (chi-squared test, p < 0.05). During no epoch was there a significant difference in number between neurons firing more strongly and those firing more weakly before a short delay (chi-squared test, p > 0.05). **Interaction between delay and direction.** During no epoch was the proportion of neurons exhibiting an interaction effect significantly in excess of the frequency expected by chance (chi-squared test, p > 0.05). During no epoch was there a significant difference in number between neurons exhibiting stronger direction selectivity and those exhibiting weaker direction selectivity before a short delay (chi-squared test, p > 0.05).

Individual neurons across a long anticipatory epoch. The results, presented in Table 1 and Fig. 11, can be summarized in the following terms. **Main effect of delay.** There was a significant main effect of expected delay in 19% of FEF neurons. This percentage was significantly in excess of that expected by chance (chi-squared test, p < 0.05). The difference in frequency between neurons firing more strongly and those firing more weakly before a short delay was not significant (chi-squared test, p > 0.05). **Interaction between delay and direction.** Interaction effects were no more common than expected by chance (chi-squared test, p > 0.05). The difference in frequency between cases in which direction selectivity was stronger before a
short delay and those in which it was weaker was not significant (chi-squared test, p > 0.05).

These results did not differ significantly between monkeys.

**Summary.** Among FEF neurons, the length of the anticipated delay exerted a moderate influence on firing rate. Some neurons fired more strongly and some more weakly in anticipation of a short delay.

**Frontal Eye Field (FEF): Elapsed Delay**

**Population.** Population activity was enhanced after a long delay as compared to a short delay (Fig. 6A2). The time-course of the enhancement is summarized in Fig. 6B2. There was no consistent effect of elapsed delay on the strength of the directional signal (Fig. 6D2).

**Individual neurons by epoch.** The results, presented in Fig. 6C2 and Fig. 6E2, can be summarized in the following terms. **Main effect of delay.** During each epoch (IV-VII), the proportion of neurons exhibiting a main effect was significantly in excess of the frequency expected by chance (chi-squared test, p < 0.05). During epochs IV and VI, the preponderance of neurons that fired more strongly after a long delay was significant (chi-squared test, p < 0.05).

**Interaction between delay and direction.** During epochs IV-VI, the proportion of neurons exhibiting an interaction effect was significantly in excess of the frequency expected by chance (chi-squared test, p < 0.05). During no epoch was there a significant difference in number between neurons exhibiting stronger direction selectivity and those exhibiting weaker direction selectivity after a long delay (chi-squared test, p > 0.05).

**Individual neurons across a long pre-movement epoch.** The results, presented in Table 3 and Fig. 17, can be summarized in the following terms. **Main effect of delay.** There was a significant main effect of expected delay in 43% of FEF neurons. This percentage was
significantly in excess of that expected by chance (chi-squared test, p < 0.05). The preponderance of neurons firing more strongly after a long delay was significant (chi-squared test, p < 0.05). Interaction between delay and direction. Interaction effects were significantly more common than expected by chance (chi-squared test, p < 0.05). The difference in number between neurons exhibiting stronger and weaker direction selectivity after a long delay was not significant (chi-squared test, p > 0.05). These results did not differ significantly between monkeys.

Summary. Among FEF neurons, the length of the elapsed delay exerted a marked influence on firing rate. A majority of delay-sensitive neurons fired more strongly after a long delay.

**FEF/PM: Anticipated Delay**

Population. We collected data from 34 neurons in FEF/PM of two monkeys (Table 1). Curves representing the activity of this population as a function of time during the trial indicate that the length of anticipated delay exerted a strong effect on neuronal activity (Fig. 7A1). The net firing rate was elevated shortly after the presentation of cues predicting short delays and the elevation persisted throughout the delay period (Fig. 7B1).

Individual neurons by epoch. The results, presented in Fig. 7C1 and Fig. 7E1, can be summarized in the following terms. Main effect of delay. During each epoch (I-III), the proportion of neurons exhibiting a main effect was significantly in excess of the frequency expected by chance (chi-squared test, p < 0.05). During each epoch, the preponderance of neurons firing more strongly before a short delay was significant (chi-squared test, p < 0.05). Interaction between delay and direction. During no epoch was the proportion of neurons exhibiting an interaction effect significantly in excess of the frequency expected by chance (chi-
squared test, p > 0.05). During no epoch was there a significant difference in number between neurons exhibiting stronger direction selectivity and those exhibiting weaker direction selectivity before a short delay (chi-squared test, p > 0.05).

**Individual neurons across a long anticipatory epoch.** The results, presented in Table 1 and Fig. 11, can be summarized in the following terms. **Main effect of delay.** There was a significant main effect of expected delay in 24% of FEF/PM neurons. This percentage was significantly in excess of that expected by chance (chi-squared test, p < 0.05). The preponderance of neurons firing more strongly before a short delay was significant (chi-squared test, p < 0.05). **Interaction between delay and direction.** Interaction effects were no more common than expected by chance (chi-squared test, p > 0.05). The difference in frequency between cases in which direction selectivity was stronger before a short delay and those in which it was weaker was not significant (chi-squared test, p > 0.05). These results did not differ significantly between monkeys.

**Summary.** Among FEF/PM neurons, the length of the anticipated delay exerted a marked influence on firing rate. Most delay-sensitive neurons fired more strongly in anticipation of a short delay.

**FEF/PM: Elapsed Delay**

**Population.** Population activity was markedly enhanced toward the end of a short delay as compared to a long delay (Fig. 7A2). The sign of the effect reversed after saccade completion, as indicated by the transition from an upward directed blue region to a downward directed red region in Fig 7B2. There was an apparent slight tendency for the directional signal to be stronger after a short delay (Fig. 7D2).
Individual neurons by epoch. The results, presented in Fig. 7C2 and Fig. 7E2, can be summarized in the following terms. **Main effect of delay.** During epochs IV-VI, the proportion of neurons exhibiting a main effect was significantly in excess of the frequency expected by chance (chi-squared test, $p < 0.05$) and the preponderance of neurons that fired more strongly after a short delay was significant (chi-squared test, $p < 0.05$). During epoch VII, neurons that fired more strongly after a long delay were significantly preponderant. **Interaction between delay and direction.** During epoch IV, the proportion of neurons exhibiting an interaction effect was significantly in excess of the frequency expected by chance (chi-squared test, $p < 0.05$). During no epoch was there a significant difference in number between neurons exhibiting stronger direction selectivity and those exhibiting weaker direction selectivity after a long delay (chi-squared test, $p > 0.05$).

Individual neurons across a long pre-movement epoch. The results, presented in Table 3 and Fig. 17, can be summarized in the following terms. **Main effect of delay.** There was a significant main effect of elapsed delay in 38% of FEF/PM neurons. This percentage was significantly in excess of that expected by chance (chi-squared test, $p < 0.05$). The preponderance of neurons firing more strongly after a short delay was significant (chi-squared test, $p < 0.05$). **Interaction between delay and direction.** Interaction effects were no more common than expected by chance (chi-squared test, $p > 0.05$). There was no difference in number between neurons exhibiting stronger and weaker direction selectivity after a long delay. These results did not differ significantly between monkeys.

**Summary.** Among FEF/PM neurons, the length of the elapsed delay exerted a marked influence on firing rate. Before and during the saccade, a majority of delay-sensitive neurons
fired more strongly after a short delay. During a period beginning after the saccade and extending through reward delivery, this pattern was reversed.

**Premotor Cortex (PM): Anticipated Delay**

**Population.** We collected data from 76 neurons in PM of two monkeys (Table 1). Curves representing the activity of this population as a function of time during the trial indicate that the length of the anticipated delay exerted a strong effect on neuronal activity (Fig. 8A1). The net firing rate was sharply elevated throughout the period following presentation of the short-delay cue (Fig. 8B1). The strength of the directional signal was also moderately elevated (Fig. 8D1).

**Individual neurons by epoch.** The results, presented in Fig. 8C1 and Fig. 8E1, can be summarized in the following terms. **Main effect of delay.** During each epoch (I-III), the proportion of neurons exhibiting a main effect was significantly in excess of the frequency expected by chance (chi-squared test, \( p < 0.05 \)). During each epoch, the preponderance of neurons firing more strongly before a short delay was significant (chi-squared test, \( p < 0.05 \)).

**Interaction between delay and direction.** During no epoch was the proportion of neurons exhibiting an interaction effect significantly in excess of the frequency expected by chance (chi-squared test, \( p > 0.05 \)). During no epoch was there a significant difference in number between neurons exhibiting stronger direction selectivity and those exhibiting weaker direction selectivity before a short delay (chi-squared test, \( p > 0.05 \)).

**Individual neurons across a long anticipatory epoch.** The results, presented in Table 1 and Fig. 11, can be summarized in the following terms. **Main effect of delay.** There was a significant main effect of expected delay in 28% of PM neurons. This percentage was significantly in excess of that expected by chance (chi-squared test, \( p < 0.05 \)). The
preponderance of neurons firing more strongly before a short delay was significant (chi-squared test, p < 0.05). Interaction between delay and direction. Interaction effects were no more common than expected by chance (chi-squared test, p > 0.05). The difference in frequency between cases in which direction selectivity was stronger before a short delay and those in which it was weaker was not significant (chi-squared test, p > 0.05). These results did not differ significantly between monkeys.

Summary. Among PM neurons, the length of the anticipated delay exerted a dramatic influence on firing rate. Most delay-sensitive neurons fired more strongly in anticipation of a short delay.

**Premotor Cortex (PM): Elapsed Delay**

**Population.** Population activity was markedly enhanced toward the end of a short delay as compared to a long delay (Fig. 8A2). There was an apparent slight tendency for the directional signal to be stronger after a short delay (Fig. 8D2).

**Individual neurons by epoch.** The results, presented in Fig. 8C2 and Fig. 8E2, can be summarized in the following terms. **Main effect of delay.** During each epoch (IV-VII), the proportion of neurons exhibiting a main effect was significantly in excess of the frequency expected by chance (chi-squared test, p < 0.05). During epochs IV and V, the preponderance of neurons that fired more strongly after a short delay was significant (chi-squared test, p < 0.05).

**Interaction between delay and direction.** During epoch V, the proportion of neurons exhibiting an interaction effect was significantly in excess of the frequency expected by chance (chi-squared test, p < 0.05). During no epoch was there a significant difference in number between
neurons exhibiting stronger direction selectivity and those exhibiting weaker direction selectivity after a long delay (chi-squared test, p > 0.05).

Individual neurons across a long pre-movement epoch. The results, presented in Table 3 and Fig. 17, can be summarized in the following terms. **Main effect of delay.** There was a significant main effect of elapsed delay in 42% of PM neurons. This percentage was significantly in excess of that expected by chance (chi-squared test, p < 0.05). The preponderance of neurons firing more strongly after a short delay was significant (chi-squared test, p < 0.05). **Interaction between delay and direction.** Interaction effects were no more common than expected by chance (chi-squared test, p > 0.05). There was no difference in number between neurons exhibiting stronger and weaker direction selectivity after a long delay. These results did not differ significantly between monkeys.

**Summary.** Among PM neurons, the length of the elapsed delay exerted a marked influence on firing rate. During the period leading up to the saccade, a majority of delay-sensitive neurons fired more strongly after a short delay.

**Supplementary Eye Field (SEF): Anticipated Delay**

**Population.** We collected data from 147 neurons in the SEF of two monkeys (Table 1). Curves representing the activity of this population as a function of time during the trial indicate that the length of anticipated delay had only a minor impact on neuronal activity (Fig. 9A1-B1).

**Individual neurons by epoch.** The results, presented in Fig. 9C1 and Fig. 9E1, can be summarized in the following terms. **Main effect of delay.** During epoch II, the proportion of neurons exhibiting a main effect was significantly in excess of the frequency expected by chance (chi-squared test, p < 0.05). During no epoch was there a significant difference in number
between neurons firing more strongly and those firing more weakly before a short delay (chi-squared test, $p > 0.05$). **Interaction between delay and direction.** During no epoch was the proportion of neurons exhibiting an interaction effect significantly in excess of the frequency expected by chance (chi-squared test, $p > 0.05$). During no epoch was there a significant difference in number between neurons exhibiting stronger direction selectivity and those exhibiting weaker direction selectivity before a short delay (chi-squared test, $p > 0.05$).

**Individual neurons across a long anticipatory epoch.** The results, presented in Table 1 and Fig. 11, can be summarized in the following terms. **Main effect of delay.** There was a significant main effect of expected delay in 12% of SEF neurons. This percentage was significantly in excess of that expected by chance (chi-squared test, $p < 0.05$). The difference in frequency between neurons firing more strongly and those firing more weakly before a short delay was not significant (chi-squared test, $p > 0.05$). **Interaction between delay and direction.** Interaction effects were no more common than expected by chance (chi-squared test, $p > 0.05$). The difference in frequency between cases in which direction selectivity was stronger before a short delay and those in which it was weaker was not significant (chi-squared test, $p > 0.05$). These results did not differ significantly between monkeys.

**Summary.** Among SEF neurons, the length of the anticipated delay exerted a very weak influence on firing rate. A few neurons fired more strongly and a few more weakly in anticipation of a short delay.

**Supplementary Eye Field (SEF): Elapsed Delay**

**Population.** Population activity was enhanced after a long delay as compared to a short delay (Fig. 9A2). The enhancement was present during the late phase of the delay period and carried
over into the saccadic period (Fig. 9B2). There was little or no apparent effect on the strength of the directional signal (Fig. 9D2).

**Individual neurons by epoch.** The results, presented in Fig. 9C2 and Fig. 9E2, can be summarized in the following terms. **Main effect of delay.** During each epoch (IV-VII), the proportion of neurons exhibiting a main effect was significantly in excess of the frequency expected by chance (chi-squared test, $p < 0.05$). During epochs IV-VI, the preponderance of neurons that fired more strongly after a long delay was significant (chi-squared test, $p < 0.05$).

**Interaction between delay and direction.** During epoch IV, the proportion of neurons exhibiting an interaction effect was significantly in excess of the frequency expected by chance (chi-squared test, $p < 0.05$). During no epoch was there a significant difference in number between neurons exhibiting stronger direction selectivity and those exhibiting weaker direction selectivity after a long delay (chi-squared test, $p > 0.05$).

**Individual neurons across a long pre-movement epoch.** The results, presented in Table 3 and Fig. 17, can be summarized in the following terms. **Main effect of delay.** There was a significant main effect of expected delay in 20% of SEF neurons. This percentage was significantly in excess of that expected by chance (chi-squared test, $p < 0.05$). The preponderance of neurons firing more strongly after a long delay was significant (chi-squared test, $p < 0.05$). **Interaction between delay and direction.** Interaction effects were significantly more common than expected by chance (chi-squared test, $p < 0.05$). The difference in number between neurons exhibiting stronger and weaker direction selectivity after a long delay was not significant (chi-squared test, $p > 0.05$). These results did not differ significantly between monkeys.
Summary. Among SEF neurons, the length of the elapsed delay exerted a marked influence on firing rate. A majority of delay-sensitive neurons fired more strongly after a long delay.

Rostral Supplementary Motor Area (SMAr): Anticipated Delay

Population. We collected data from 84 neurons in the SMAr of one monkey (Table 1). Curves representing the activity of this population as a function of time during the trial indicate that the length of the anticipated delay exerted a marked effect on neuronal activity (Fig. 10A1). There was a substantial increase of firing rate beginning shortly after the delay-cue on short-delay trials (Fig. 10B1).

Individual neurons by epoch. The results, presented in Fig. 10C1 and Fig. 10E1, can be summarized in the following terms. Main effect of delay. During each epoch (I-III), the proportion of neurons exhibiting a main effect was significantly in excess of the frequency expected by chance (chi-squared test, p < 0.05). During each epoch, the preponderance of neurons firing more strongly before a short delay was significant (chi-squared test, p < 0.05). Interaction between delay and direction. During epoch III, the proportion of neurons exhibiting an interaction effect was significantly in excess of the frequency expected by chance (chi-squared test, p < 0.05). During no epoch was there a significant difference in number between neurons exhibiting stronger direction selectivity and those exhibiting weaker direction selectivity before a short delay (chi-squared test, p > 0.05).

Individual neurons across a long anticipatory epoch. The results, presented in Table 1 and Fig. 11, can be summarized in the following terms. Main effect of delay. There was a significant main effect of expected delay in 45% of SMAr neurons. This percentage was significantly in excess of that expected by chance (chi-squared test, p < 0.05). The
preponderance of neurons firing more strongly before a short delay was significant (chi-squared test, \( p < 0.05 \)). Interaction between delay and direction. Interaction effects were no more common than expected by chance (chi-squared test, \( p > 0.05 \)). The difference in frequency between cases in which direction selectivity was stronger before a short delay and those in which it was weaker was not significant (chi-squared test, \( p > 0.05 \)). These results did not differ significantly between monkeys.

**Summary.** Among SMAr neurons, the length of the anticipated delay exerted a dramatic influence on firing rate. Most delay-sensitive neurons fired more strongly in anticipation of a short delay.

**Rostral Supplementary Motor Area (SMAr): Elapsed Delay**

**Population.** Population activity was markedly enhanced toward the end of a short delay as compared to a long delay (Fig. 10A2). However, as the time for the saccade approached and passed, there was a complex series of reversals in the pattern of dependence of firing rate on antecedent delay length (Fig. 10B2). There was no consistent relation between delay length and the strength of the directional signal (Fig. 10D2).

**Individual neurons by epoch.** The results, presented in Fig. 10C2 and Fig. 10E2, can be summarized in the following terms. Main effect of delay. During epochs IV-VI, the proportion of neurons exhibiting a main effect was significantly in excess of the frequency expected by chance (chi-squared test, \( p < 0.05 \)). During epochs IV and VI, the preponderance of neurons that fired more strongly after a short (or long) delay was significant (chi-squared test, \( p < 0.05 \)). Interaction between delay and direction. During no epoch was the proportion of neurons exhibiting an interaction effect significantly in excess of the frequency expected by chance (chi-
squared test, \( p > 0.05 \)). During no epoch was there a significant difference in number between neurons exhibiting stronger direction selectivity and those exhibiting weaker direction selectivity after a long delay (chi-squared test, \( p > 0.05 \)).

**Individual neurons across a long pre-movement epoch.** The results, presented in Table 3 and Fig. 17, can be summarized in the following terms. **Main effect of delay.** There was a significant main effect of expected delay in 32% of SMAr neurons. This percentage was significantly in excess of that expected by chance (chi-squared test, \( p < 0.05 \)). The preponderance of neurons firing more strongly after a short delay was not significant (chi-squared test, \( p > 0.05 \)). **Interaction between delay and direction.** Interaction effects were no more common than expected by chance (chi-squared test, \( p > 0.05 \)). The difference in number between neurons exhibiting stronger and weaker direction selectivity after a long delay was not significant (chi-squared test, \( p > 0.05 \)). These results did not differ significantly between monkeys.

**Summary.** Among SMAr neurons, the length of the elapsed delay exerted a mixed influence on firing rate. During the period leading up to the saccade, a majority of delay-sensitive neurons fired more strongly after a short delay. This pattern reversed, however, in the perisaccadic period.

**Selectivity for Anticipated Delay: Comparison among Areas**

To determine whether the impact of anticipated delay length varied systematically across areas, we carried out area-to-area comparisons based on counts of neurons in which firing depended significantly on expected delay length, direction and a delay x direction interaction
Several systematic inter-areal differences were clearly evident upon comparison.

Enhancement in anticipation of short delay. Neurons firing significantly more strongly under the short-delay condition became steadily more frequent with progress in a posteriorward direction across the cortical surface (Fig. 11A, blue bars). This was true in both the lateral frontal lobe (PFC < FEF < FEF/PM < PM) and the medial frontal lobe (SEF < SMAr). Most pairwise inter-areal differences in the frequency of neurons exhibiting stronger firing on short-delay trials were highly significant (Table 2).

Direction selectivity. Neurons exhibiting a statistically significant dependence of firing rate on response direction (blue and red bars in Fig. 11B) were more numerous in the eye fields (FEF and SEF) than in other areas. The frequency of such neurons (as measured by a chi-squared test) was significantly higher in the FEF than in the PFC (p < 0.01) and PM (p < 0.05) and was significantly higher in the SEF than in the PFC (p < .001), PM (p < 0.0001) and SMAr (p < 0.01).

Impact of anticipated delay on directional signals. Neurons exhibiting a statistically significant dependence of firing rate on the interaction between delay length and response direction were rare in all areas. The frequency with which these neurons were observed in the total sample (0.048) was in fact no greater than the expected rate of type 1 errors (0.05).

Selectivity for Anticipated Delay: Impact of Reversing the Cue-Delay Associations

At the end of every 40 successful trials in the variable-delay task, the cue previously associated with short delay became associated with long delay and vice versa. Consequently, in each 80-trial data collection session, there was one block conforming to each cue convention.
This manipulation allowed us to consider the influence of anticipated delay length on neuronal activity independently of any selectivity neurons may have possessed for the visual attributes of the stimuli. However, it may have resulted in an attenuation of activity related to the anticipated delay. This would be true if it took monkeys many trials to adjust their expectations after each switch. We addressed this concern by asking how long it took monkeys to adjust to the new cue-delay contingencies.

We first assessed the impact of the switch in cue-significance on the behavioral reaction time. To achieve power in this analysis, we combined reaction time measures across all data collection sessions in all monkeys, considering only those trials that the monkey completed successfully. The results are shown in Fig. 12A. This plots, as a function of trial number, the reaction time to the pre-switch short-delay cue minus the reaction time to the pre-switch long-delay cue. The values were negative before the switch because the monkeys responded more swiftly on trials in which there was a short delay. The values became positive after the switch for the same reason. The index was not initially positive following the switch but became reliably positive by the sixth post-switch trial. The fact that the shift did not occur instantly indicates, surprisingly, that it depended on the monkey’s registering the information conveyed by the cue and did not depend just on the experienced duration of the delay. We conclude that it took the monkeys around five trials to register in their performance an awareness of the switched cue-delay contingencies.

We also assessed how quickly delay-related activity during the pre-delay epoch (beginning with delay-cue onset and ending with directional-cue offset) was re-established after the switch. To do so, we combined data from all neurons with a significant tendency to fire more strongly during short-delay trials during the pre-delay epoch, collapsing the data across areas and
monkeys. We restricted consideration to trials that the monkey completed successfully. The results are shown in Fig. 12B. This plots, as a function of trial number, the mean firing rate on trials involving the pre-switch short-delay cue minus the mean firing rate on trials involving the pre-switch long-delay cue. The values were positive before the switch because the neurons fired more strongly on short-delay trials and negative after the switch for the same reason. The shift to reliable negativity was established by the fifth trial after the switch.

It is conceivable that neuronal activity following the switch might have adjusted at different rates in different areas. To assess this possibility, we analyzed data separately from each area with a marked pre-delay firing rate enhancement on short-delay trials. To compensate for the smaller sample sizes, we carried out a coarser analysis, analyzing firing rates on blocks of four consecutive correct trials, with blocks demarcated so that the time of the switch fell at a between-block boundary. The results are shown in Fig. 12C-E. These plots indicate that neuronal activity adjusted quickly to the new rule regardless of area. We conclude that the rule-switching design did not lead to a major attenuation of neuronal activity dependent on expected delay length.

**Selectivity for Anticipated Delay: Location of Selective Neurons**

To analyze the fine distribution of neurons sensitive to anticipated delay, we projected recording sites onto the cortical surface. For three lateral chambers (in monkeys P, N and F), we collected MR images parallel to the cortical surface. Using a set of slices that contained both the cortex and fiducial markers at known locations relative to the recording grid, we determined the locations of recording sites relative to the arcuate (AS) and principal (PS) sulci. The results are shown in Fig. 13A-C. In this figure, the size of each symbol indicates the proportion of neurons at the corresponding site in which there was a significant enhancement of firing rate on short-delay trials during the pre-delay epoch (delay-cue onset to directional-cue offset). The general
tendency for enhancement under short-delay conditions to occur at posterior sites, and, in particular, at sites behind the arcuate sulcus, is clear. In the vicinity of the FEF and FEF/PM, neurons represented by a single symbol occupied depths ranging from 0 to 4 mm (mean = 2.60 mm; median = 2.00 mm). However, there was no consistent trend with respect to depth. Neurons showing a significant effect of delay length were observed throughout the range of recording depths (mean = 2.34 mm; median = 2.25 mm). For three midline chambers (in monkeys A, N and F), we collected frontoparallel MR images showing the cortex and fiducial markers at known locations relative to the recording grid. These images were used to determine the locations of recording sites relative to the interhemispheric midline and the frontal plane containing the genu of the arcuate sulcus (AS genu). The results are shown in Fig. 13D-F. The general tendency for enhancement under conditions of anticipated short delay to occur at relatively posterior sites - in monkey F - as contrasted to relatively anterior sites - in monkeys A and N - is clear.

Selectivity for Anticipated Delay: Relation to Selectivity for Anticipated Reward

Like anticipation of a big reward, anticipation of a short delay increased the monkey’s level of task engagement as demonstrated by a reduced fixation break rate (Fig. 2C). Were the two effects related? To answer this question, we recorded from a large number of neurons in the context of both the variable-reward and the variable-delay task: 186, 113, 34, 66, 136 and 84 neurons in PFC, FEF, FEF/PM, PM, SEF and SMAr, respectively. To determine whether neurons that fired more strongly (or more weakly) under big-reward conditions also fired more strongly (or more weakly) under short-delay conditions we computed a reward index and a delay index on firing rates during the pre-delay epoch for each neuron averaged across direction. The
reward index was \((BR - SR)/(BR + SR)\) where \(BR\) and \(SR\) were the firing rates on big and small reward trials during the pre-delay epoch (reward-cue onset to directional-cue offset). The delay index was \((SD - LD)/(SD + LD)\) where \(SD\) and \(LD\) were the firing rates on short- and long-delay trials during the pre-delay epoch (delay-cue onset to directional-cue offset). On plotting the reward index against the delay index for all neurons in each area, we found that there was a significant positive correlation in each area (Fig. 14). The correlation coefficient, like the incidence of big-reward and short-delay enhancement, increased with posteriorward movement across the cortical surface both in lateral frontal cortex (PFC < FEF < FEF/PM < PM) and in medial frontal cortex (SEF < SMAr). We conclude that neurons that fired more strongly when a big-reward was anticipated also tended to fire more strongly when a short delay was anticipated.

To assess the impact of this effect at the level of population activity, we divided neurons into two groups on the basis of their activity during the variable-reward task: those that did and those that did not show significant big-reward enhancement during an epoch extending from onset of the reward-cue to initiation of the saccade. We then constructed population histograms representing mean firing rate as a function of time for neurons exhibiting big-reward enhancement (Fig. 15) and those not exhibiting it (Fig. 16). Neurons classified as exhibiting big-reward enhancement did indeed fire more strongly when a big reward was anticipated (each blue curve is higher than the corresponding red curve in Fig. 15A-F) whereas the other neurons did not (blue and red curves are overlapping in Fig. 16A-F). If we now ask, for the same groups of neurons, what was the pattern of activity on short-delay and long-delay trials, we find that neurons exhibiting big-reward enhancement also exhibited short-delay enhancement except in the SEF (each blue curve is higher than the corresponding red curve in Fig. 15G-J and L) whereas the other neurons did not (blue and red curves roughly overlap in Fig. 16G-L). These
observations indicate that the correlation between reward-related and delay-related activity not only was statistically significant but also was robust.

**Selectivity for Elapsed Delay: Comparison among Areas**

To determine whether the impact of elapsed delay length varied systematically across areas, we carried out area-to-area comparisons based on counts of neurons in which firing depended significantly on delay length, direction and their interaction (Table 3; Fig. 17). Several systematic inter-areal differences were clearly evident upon comparison.

**Sign of delay sensitivity.** Among delay-sensitive neurons, those firing more strongly after a long delay (red bars in Fig. 17A) outnumbered those firing more strongly after a short delay (blue bars in Fig. 17A) in three relatively anterior areas (PFC, FEF and SEF) whereas the opposite pattern was present in three relatively posterior areas (FEF/PM, PM and SMAr). Statistical analysis bore out this picture (Table 4). The ratio of long-delay-preferring to short-delay-preferring neurons was significantly different in every case where an area from the anterior triad was compared to an area from the posterior triad whereas it fell short of significance in every case where two areas from the same triad were compared, with the sole exception of SMAr and FEF/PM.

**Direction selectivity.** Neurons exhibiting a statistically significant dependence of firing rate on response direction (blue and red bars in Fig. 17B) were more numerous in the eye fields (FEF and SEF) than in other areas. The frequency of such neurons was significantly higher in the FEF than in the PFC (p < 0.05) and PM (p < 0.05) and was significantly higher in the SEF than in the PFC (p < .001), PM (p < 0.001) and SMAr (p < 0.05) (chi-squared test). As an incidental point, we may note that the direction selectivity in both FEF and SEF appeared to reverse after
completion of the saccade (Fig. 6A2 and Fig. 9A2). The apparent reversal may have reflected the preparation and initiation of a return saccade - in the neuron's preferred direction after an initial saccade in its non-preferred direction and vice versa.

Impact of elapsed delay on directional signals. In data pooled across all areas, neurons exhibiting a statistically significant dependence of firing rate on the interaction between delay length and response direction were observed with a frequency (0.11) significantly in excess of the frequency (0.05) expected by chance (chi-squared test, p < 0.0001). Nevertheless, the counts in individual areas were so low as to render all inter-areal differences insignificant.

Selectivity for Elapsed Delay: Time Course of Long-Delay Enhancement in Rostral Areas

The tendency for neurons in PFC, FEF, and SEF to exhibit enhanced activation at the end of a long delay (Fig. 17A, red bars; Table 3) could have arisen from either of two mechanisms. 1) The activity of these neurons could have depended solely on elapsed time. In other words, their firing rate could have increased gradually over the course of the delay, attaining a higher level after a long delay because the increase was more prolonged. 2) Their activity could have depended on some process set in motion at the time of presentation of the delay cue. In other words, they could have exhibited enhanced firing even early in a long-delay trial.

To distinguish between these possibilities, we constructed population histograms representing firing rate as a function of time during the trial for all neurons in PFC, FEF and SEF that fired significantly more strongly at the end of a long delay than at the end of a short delay (Fig. 18A-C). As a basis for comparison, we also constructed population histograms for the remaining neurons, which did not show significant long-delay enhancement (Fig. 18G-I). In each display, blue and red lines represent population activity on preferred-direction short-delay
and long-delay trials respectively, with alignment on directional-cue onset. For short-delay conditions, activity starting 250 ms before the imperative cue (offset of the fixation spot) is replotted (in light blue) with alignment on the imperative cue to allow for comparison of pre-movement activity between short-delay and long-delay conditions.

Among neurons exhibiting long-delay enhancement, firing increased steadily during the long delay period (each red curve rises steadily over the course of the trial in Fig. 18A-C), as expected if enhancement depended on elapsed time (hypothesis 1 above). However, enhancement was present even very early in the trial (each red curve is above the corresponding blue curve during the “analysis epoch” in Fig. 18A-C), as expected if enhancement depended on a process set in motion by presentation of the delay cue early in the trial (hypothesis 2 above). In SEF, the enhancement developed immediately after the delay-predicting cue, whereas in PFC and SEF, it developed only at a visual response latency after the appearance of the targets. The dependence of enhancement in PFC and FEF on the presence of the targets suggests a mechanism based on attentional accentuation of visually elicited activity. Neither effect was present among neurons lacking pre-movement enhancement (Fig. 18G-I). To quantify the degree to which firing was enhanced early in the trial when a long delay was expected, we computed a delay index for each neuron based on firing during an epoch (“analysis epoch” in Fig. 18) beginning with onset of the delay cue and ending at the instant when, on short-delay trials, the imperative cue (offset of the fixation spot) occurred. The delay index was (SD – LD)/(SD + LD) where SD and LD were the firing rates on short- and long-delay trials). The early delay indices were significantly (t-test, p < 0.05) skewed to negative values among neurons showing late long-delay enhancement (Fig. 18D-F) and not among other neurons (Fig. 18J-L). We conclude that neurons exhibiting enhanced firing on long-delay trials were not simply responding passively to the passage of time
but rather were participating in an active process (possibly attentional) called into play by the cue predicting a long delay.

**Selectivity for Elapsed Delay: Relation to Saccadic Reaction Time**

Behavioral reaction times were significantly shorter on short-delay than on long-delay trials in all monkeys. This raises the question: Was delay-related neuronal activity directly correlated with the length of the delay or, alternatively, was it directly correlated with the behavioral reaction time and correlated with delay only through this dependence? To resolve this issue, for each neuron, we used a multiple least-squares regression approach to optimize the parameters of three models representing firing rate as a linear function of reaction time and delay length: (1) a reduced model incorporating reaction time only, (2) a reduced model incorporating delay length only and (3) a full model incorporating both. This analysis was based on a period (immediately before the behavioral response) extending from 250 ms before to 100 ms after the imperative cue (offset of the fixation spot). It was carried out independently for trials involving each neuron's preferred and antipreferred directions because the relation between reaction time and neuronal activity might vary according to whether the saccade was toward or away from the neuron’s response field. We compared each of the reduced models to the full model using a nested F-test (see Methods). Then we calculated the percentage of neurons in each area that showed a significant improvement of fit only when the variable of delay length was added to the model (Fig. 19; black bars), only when reaction time was added (Fig. 19; white bars) or when either was added (Fig. 19; hatched bars).

**Relation of firing rate to delay length.** The relation between firing rate and delay length, as revealed by this analysis, was the same as the relation revealed in analyses described above. In
each anterior area (PFC, FEF and SEF), neurons firing more strongly after a long delay (Fig. 19: upward pointing black and darkly hatched bars) outnumbered neurons firing more strongly after a short delay (Fig. 19: downward pointing black and darkly hatched bars), whereas, in each posterior area (FEF/PM, PM and SMAr), the opposite pattern was observed. This was true both on trials requiring a saccade in the neuron’s preferred direction (Fig. 19A) and on trials requiring a saccade in the anti-preferred direction (Fig. 19B). On analyzing data from all three anterior areas combined and likewise from all three posterior areas combined, we found (a) that in each group the difference in frequency between long-delay-preferring neurons and short-delay-preferring neurons was significant (chi-squared test, $p < 0.05$) and (b) that the difference between groups in the relative frequency of long-delay-preferring and short-delay-preferring neurons was significant (chi-squared test, $p < 0.05$). This was true both for trials requiring a response in the neuron’s preferred direction and for trials requiring a response in the anti-preferred direction.

**Relation of firing rate to reaction time.** In all areas, neurons firing significantly more strongly on trials with short reaction times were preponderant (Fig. 19: downward pointing white and lightly hatched bars). The preponderance achieved significance in all cases except the case of neurons in the posterior group of areas (FEF/PM, PM, SMAr) before saccades in the anti-preferred direction (chi-squared test, $p < 0.05$).

**Relation of delay-related to reaction-time-related effects.** Delay-related effects frequently occurred in the absence of reaction-time-related effects (black portions vs. hatched portions of dark bars in Fig. 19). Moreover, in anterior areas (PFC, FEF and SEF), the preponderant form of delay-related activity (stronger firing after a long delay) was opposite to the preponderant form of reaction-time-related activity (stronger firing before a fast reaction). Thus we can rule out the interpretation that delay-related firing was simply a secondary consequence of the relation
between neuronal firing rate and reaction time. Nevertheless, in the posterior group of areas (FEF/PM, PM and SMAr), the tendency for a neuron to exhibit one kind of effect might have been correlated with its tendency to exhibit the other. To investigate this possibility, we computed, for each neuron in the posterior group, (1) the mean firing rate on short-delay trials minus the mean firing rate on long-delay trials and (2) the slope of a line fitted to points representing firing rate vs. reaction time across all trials requiring a response in a given direction. We then analyzed the correlation across neurons between the two measures. On preferred-direction trials, the correlation was weak ($r^2 = 0.196$) but significant ($p < 0.0001$). On antipreferred-direction trials, it was very weak ($r^2 = 0.010$) but still significant ($p < 0.0001$). Thus, in posterior areas, there was a weak but significant tendency for neurons that fired more strongly after a short delay (with reaction time factored out) to fire more strongly before a faster behavioral reaction (with the length of the antecedent delay factored out).

**EMG Measures**

Activity related to the length of the delay might have been present during the delay period in PM, FEF/PM and SMAr because monkeys were engaging in skeletomotor behavior or were preparing to make skeletomotor responses at the trial's end. The monkeys in this study did not engage in consistent overt licking during the delay period, such as has been observed in tasks involving multi-second delays (Hassani et al. 2001; Watanabe 2001). Nevertheless, they may have contracted the muscles of the jaw and neck during the delay period or may have maintained a state of preparation for contraction of these muscles. If they did so at all, then they might have done so to different degrees when anticipating a long or a short delay. Thus firing dependent on the length of the anticipated delay might have been related primarily to contracting or preparing
to contract the neck and jaw muscles and only secondarily to delay length. To assess this possibility, we recorded EMG activity in the three monkeys (P, N and F) still available for study after the collection and analysis of single-neuron data, focusing on the splenius capitus, responsible for ipsilateral version and torsion of the head, and the masseter, responsible for jaw closure and a commonly used indicator of oral activity (Apicella et al., 1991; Huang et al. 1989; Murray et al. 1991). We collected data from the splenius capitus during 8, 12, and 10 400-trial sessions and from the masseter during 8, 8 and 9 400-trial sessions in monkeys N, P and F respectively. Combining the data for each muscle in each monkey across all trials in all sessions, we analyzed the dependence of the EMG on experimental condition by carrying out an ANOVA with delay length and direction as factors for each of the epochs (I-VII in Fig. 20) on which neuronal data analysis had been based.

**EMG in splenius capitus.** The pattern of EMG activation and its dependence on condition varied across monkeys. In monkey P the level of activation was approximately the same for short- and long-delay conditions until very late in the trial (Fig. 20A). EMG activation was significantly greater following a short delay only around the time of reward (epoch VII, p < 0.001). In monkey N, EMG activation was enhanced after a short delay from shortly before the saccade onward (Fig. 20B). This trend reached significance in epochs IV - VII (p < 0.0001). In monkey F, activation was stronger under short-delay than under long-delay conditions throughout much of the trial, with the difference attaining significance in epochs I-III and VI-VII. We note incidentally that EMG activation was stronger when the impending saccade was in the muscle's 'preferred' (ipsiversive) direction than when it was in the muscle's 'antiprefered' (contraversive) direction (thick vs. thin lines) in monkey P (epochs V-VII), monkey N (epochs VI-VII) and monkey F (epochs IV-V). These EMG results indicate that short delay periods
resulted in greater tonus of the neck muscles and stronger torque to the head. However, the
effect was present for anticipated delay (rather than after the delay had elapsed) only in monkey F.

**EMG in masseter.** Effects of predicted length of delay on masseter EMG for the most part paralleled those on splenius capitus EMG (Fig. 20D-F). In monkey P (Fig. 20D), EMG activity was enhanced under short-delay conditions, with the enhancement attaining significance in epochs I and III (p < 0.05). EMG activity was stronger under short-delay conditions in multiple epochs in monkey N (I and IV-VII) and in monkey F (I-VII). Thus expectation of a short delay induced stronger contraction of the jaw muscles in all three monkeys.

**EMG in relation to neuronal activity.** The fact that motor output before the onset of the delay reflected the delay’s anticipated length underscores the complexity of the processes set in motion by the delay-cue. These were not restricted to central processes concerned with attention, arousal and motor readiness but included overt motor output. Thus delay-dependent neuronal activity could have been related either to covert or to overt processes.

**Delay-related EMG in comparison to reward-related EMG.** There is a striking similarity between EMG activity dependent on predicted delay, as described here, and EMG activity dependent on predicted reward, as described in a previous study (Roesch and Olson 2003, Fig. 16). Under each manipulation, two monkeys (P and N) showed relatively weak anticipatory EMG effects, while the third monkey (F) showed intense anticipatory activation of both the splenius capitus and the masseter muscles from the instant of the predictive cue onward. This parallel argues for the view that predicting a large reward and predicting a short delay called into play equivalent state changes in each monkey.
DISCUSSION

Overview

We have recorded single-neuron activity in a large set of frontal cortical areas in monkeys performing an ocular delayed response task in which a cue presented early in the trial predicted whether a short or long delay would elapse before the signal to respond. The areas studied were dorsolateral prefrontal cortex in and around the principal sulcus (PFC), the frontal eye field (FEF), a rostral zone of premotor cortex in which electrical stimulation elicited both upper-body and eye movements (FEF/PM), premotor cortex proper (PM), the supplementary eye field (SEF) and a rostral region of the supplementary motor area (SMAr). During the early period of the trial, when events and their timing were identical but the anticipated delay was either short or long, neuronal activity in comparatively posterior areas (FEF/PM, PM and SMAr) was markedly higher when a short delay was anticipated. During the interval preceding and accompanying saccade initiation, when the immediately preceding elapsed delay had been either short or long, neuronal activity in comparatively anterior areas (PFC, FEF and SEF) was markedly higher when a long delay had occurred.

Neuronal Activity Dependent on Anticipated Delay: Does It Reflect Motivation?

Delay-related neuronal activity might have arisen from motivational modulation of neuronal activity related to motor preparation, attention or arousal. Monkeys perform with greater speed and accuracy when a more valued reward is expected (Hassani et al. 2001; Hollerman et al. 1998; Kawagoe et al. 1998, 2004; Kobayashi et al. 2002; Lauwereyns et al. 2002a, b, Leon and Shadlen 1999; Musallam et al. 2004; Roesch and Olson 2003, 2004; Takikawa et al. 2002; Tremblay et al. 1998; Watanabe 1990; Watanabe et al. 2001) just as rats run faster for a higher
concentration of sucrose (Stellar 1982). This indicates that neural processes underlying response preparation and execution are enhanced when motivation is high (Stellar and Stellar, 1985). In the present experiment, speed and accuracy measures were not appropriate indicators of motivational level because they were influenced by elapsed delay as distinct from anticipated delay. However, the fact that fixation breaks were less frequent on short- than on long-delay trials (Fig. 3C) indicates that the monkeys were more motivated.

If one grants the proposition that the prominent delay-related activity of neurons in FEF/PM, PM and SMAr could have been due to motivational modulation of preparatory processes, then the question arises: What was the nature of the modulated processes? There are at least four plausible scenarios that involve motivational modulation of motor signals in delay tasks such as the one used here. 1) *Response preparation*. Neurons representing the plan for the instructed response might fire more strongly when the monkey is more motivated. This scenario can account for the relatively infrequent effect whereby directional signals were enhanced on short-delay trials (Fig. 11C: blue bars). However, it cannot explain the much more frequent cases in which enhancement occurred independently of response direction (Fig. 11A: blue bars). Nor can it account for the fact that areas in which directionally selective neurons were most prominent (Fig. 11B: FEF and SEF) do not coincide areas in which delay effects were most prominent (Fig. 11A: FEF/PM, PM and SMAr). 2) *Arousal*. Neuronal activity sensitive to arousal or responsible for generalized motor readiness might be enhanced when the monkey is more motivated. It is not clear why a state of behavioral arousal should be accompanied by an increase in population activity in premotor cortex or how such an increase could give rise to generalized motor readiness. However, with such issues resolved, this scenario could explain the fact that neuronal activity tends to increase on short-delay trials. 3) *Synergistic muscle activity*. Neuronal activity
governing overt behaviors that automatically accompany response planning, such as increases of axial tonus, might be enhanced when the monkey is more motivated. This scenario is compatible with our finding that delay-period neck EMG activity was enhanced on short-delay trials in one monkey. The absence of delay-induced modulations of neck EMG in the other two monkeys seems to argue against it. However, the other monkeys may have engaged in overt behaviors involving other muscles in which we did not monitor EMG. 4) *Ingestive preparation.* Neurons involved in preparing ingestive movements might be more active when the monkey is more motivated. None of these scenarios can be absolutely ruled out. Each may contribute to delay-related activity. To assess the contribution of each will require further experiments.

**Neuronal Activity Dependent on Anticipated Delay: Does It Represent Value?**

Neurons that fired more strongly when the monkey anticipated receiving reward at a shorter delay might have represented the time-discounted value of the reward. It is reasonable to assume that monkeys placed greater value on the reward if they expected it after a shorter delay. It has been observed across a wide range of species, extending from pigeons to humans, that animals prefer a smaller, immediate reward over a larger, delayed one (Cardinal et al. 2001; Evenden and Ryan 1996; Herrnstein 1961; Ho et al. 1999; Lowenstein 1992; Mobini et al. 2002; Montague and Berns 2002; Thaler 1981). The view that monkeys placed greater value on a reward anticipated at short delay is supported both by the fact that they were more motivated (as discussed in the preceding section) and by the fact that neurons in orbitofrontal cortex fired more strongly in response to the cue predicting a reward at short delay (unpublished observations from ongoing experiments). Neuronal activity in orbitofrontal cortex is related to the magnitude of reward value and not to the intensity of movement preparation (Roesch and Olson 2004).
These considerations support the view that the monkeys placed greater value on the reward when the anticipated delay was short but they do not establish that delay-related neuronal activity represented value in an economically meaningful sense – as a factor in the decision how hard to work. There is a fundamental difference between (a) neuronal activity representing reward value in the service of an economic decision process and (b) neuronal activity reflecting motivational modulation of preparatory processes. However, in experimental paradigms involving manipulation of the value of the anticipated reward, value and motivation are correlated, so that it is impossible to determine whether neuronal activity is related to one or the other (Maunsell 2004; Roesch and Olson 2004). Under these circumstances, neuronal firing even in parietal area LIP is correlated with value (Dorris and Glimcher, 2004; Glimcher 2002; Platt and Glimcher 1999; Sugrue et al. 2004). However, it would be wrong to conclude from the presence of a correlation that this activity represents value.

A simple example will suffice to make this point. In some monkeys, EMG activation of the neck muscles is greater when they are working for a more valued reward (Roesch and Olson 2004). It does not follow that neck muscle activation represents value in an economically meaningful sense (in other words, that the monkey decides to work harder because the neck muscles are more tense). Causality works in the other direction: the neck muscles are more tense because the monkey has decided to work harder. The same cautionary note applies to neuroeconomic interpretations of reward-related activity anywhere in the brain. Neuronal activity may be correlated with “experienced value” (Sugrue et al., 2004), or “action value” (Barraclough et al., 2004), or “subjective desirability of action” (Dorris and Glimcher, 2004) and yet not represent value in any sense more meaningful than the sense in which the neck muscles represent it.
It might be argued that neurons active in conjunction with planning a particular behavior (for example, making a saccade to the left) represent value in at least a narrow sense – that they represent the value to the monkey of executing the action with which their firing is associated (Barraclough et al., 2004; Dorris and Glimcher, 2004; Glimcher 2002, 2003; Platt and Glimcher 1999; Sugrue et al. 2004). This argument is questionable for two reasons. First, if we consider the case of value-dependent neck-muscle activation, then it is clear that activity correlated with value need not represent value in an economically meaningful sense. Second, it seems unlikely that decision processes are based on the values associated with actions (as encoded by neurons representing those actions) rather than on the values associated with goals (as encoded by neurons representing those goals). Animals decide between possible goals on the basis of their value (an affective process). They select actions on the basis of their efficacy in contributing to the attainment of the chosen goal (a strategic process). In Lashley’s classic study, rats trained to run to the baited arm of a maze, if prevented by a cerebellar lesion from running, would roll (Lashley and McCarthy 1926). They chose the goal on the basis of its value and utilized whatever means they could to get to it. For these reasons, we reject the conclusion that neuronal activity in any of the areas that we have studied represents the time-discounted value even in the limited sense of representing the value attached to the action with which their firing is associated.

Neuronal Activity Dependent on Anticipated Delay: Does It Reflect Temporal Preparation?

The tendency of neurons in FEF/PM, PM and SMAr to fire more strongly following a cue predicting a short delay than following a cue predicting a long delay might be related to the timing of the monkey’s preparation to respond – early under expectation of a short delay but late – and therefore outside the immediate post-cue period – under expectation of a long delay. In
tasks where a warning cue precedes an imperative cue at a predictable interval, preparation is maximal (as indicated by the fact that reaction times are minimal) at the predicted time (Nobre, 2001). Neuronal signals apparently related to temporal preparation have been observed in several cortical areas. In the supplementary eye field of monkeys performing a smooth pursuit task, neuronal activity peaks around the time of an expected change in direction of the target (Heinen and Liu, 1997). In area V4 of monkeys performing a detection task, attentional modulation of neuronal visual responses peaks around the time when the target event is most likely to occur (Ghose and Maunsell, 2002). In the lateral intraparietal area of monkeys performing an interval estimation task, neuronal activity shifts from representing the saccade by which a short interval will be reported to representing the saccade by which a long interval will be reported at the point in time demarcating a short from a long interval (Leon and Shadlen, 2003). Might it be true, by analogy to the findings described above, that stronger firing immediately following the cue on short-delay trials reflected early preparation to respond? We believe that this cannot be the entire explanation of the effect. If the neuronal firing rate were related simply to timed preparation, then one would expect that the firing should be as strong on long-delay as on short-delay trials immediately before the behavioral response. Contrary to this prediction, firing was consistently weaker on long-delay trials even during the period immediately preceding the response. This was true in all three areas showing robust short-delay enhancement: FEF/PM (Fig. 7A2), PM (Fig. 8A2) and SMAr (Fig. 10A2).

**Comparison to Results Obtained by Manipulating Reward Size**

In a previous paper (Roesch and Olson 2003), we reported that the incidence of reward-related neuronal activity increased steadily along the anterior-posterior axis in both the lateral
frontal lobe (PFC < FEF < FEF/PM < PM) and the medial frontal lobe (SEF < SMAr). This finding was surprising because the comparatively posterior areas in which reward-related activity was strongest (FEF/PM, PM and SMAr) were farthest removed connectionally from limbic cortex and thus might have been supposed to be least likely to process information related to reward value. As a means for resolving this paradox, we suggested that the robust reward-related activity of neurons in FEF/PM, PM and SMAr reflected the monkey's motivationally modulated level of motor readiness, arousal or attention and did not represent the value of the reward in an economically meaningful sense. The results of the previous study (concerned with anticipated reward size) and the results of the present study (concerned with anticipated delay to reward) are mutually illuminating. 1) The results of the current study replicate, under a new set of experimental conditions, the results of the old. The tendency for neurons to fire strongly in anticipation of the more valuable outcome (a short delay) was far more robust in posterior areas, FEF/PM, PM and SMAr, than in anterior areas, PF, FEF and SEF (cf. Fig. 11A in this report and Fig. 11A in Roesch and Olson 2003). 2) The results of the previous study constrain the interpretation of results from the current one. Inasmuch as areas showing a strong dependence on reward size also showed a strong dependence on delay length, and inasmuch as individual neurons sensitive to one factor tend also to be sensitive to the other (Fig. 14), we conclude (a) that one mechanism underlies both forms of sensitivity and (b) that short-delay enhancement therefore cannot be due exclusively to timed readiness.

Neuronal Activity Dependent on Elapsed Delay

Anterior areas: enhanced activity after long delays. In around a fifth of neurons in PFC, FEF and SEF, the firing rate was significantly enhanced, immediately before the behavioral response,
at the end of a long delay as compared to a short delay (Fig. 17A). These neurons significantly outnumbered neurons showing the opposite effect in all three areas. To understand better the nature of long-delay enhancement, we analyzed firing rate as a function of time for all neurons that exhibited the effect in each area. In all three areas, we found that neurons with long-delay enhancement exhibited a steady build-up of firing rate during the delay period as if their activity were indeed dependent on elapsed time (Fig. 18A-C). However, we also found that their firing rate was somewhat enhanced, even very early in the trial, when the monkey merely anticipated a long rather than a short delay (Fig. 18A-C). In the SEF, the enhancement appeared at a visual-response latency after onset of the delay cue (Fig. 18C). In PFC and FEF, it appeared only in response to onset of the target array, as if dependent on gating of neuronal visual responsiveness by the monkey’s anticipatory state. One possible explanation for these effects is that PFC, FEF and SEF are recruited into a higher rate of activity under more challenging circumstances. This would fit with other findings on the SEF indicating that net neuronal activity is higher when monkeys (1) must select targets according to a pattern-location-association rule vs. a spatial rule (Olson et al. 2000), (2) are being guided by a novel vs. familiar set of pattern-location associations (Chen and Wise 1995a, b), (3) are preparing to look away from vs. toward a cue (Olson and Gettner 2002), or (4) are preparing to follow an antisaccade vs. a prosaccade rule (Amador et al. 2004; Schlag-Rey et al. 1997). The nature of the contribution to task performance arising from difficulty-related enhancement is not known. One important insight arising from the present study is that the enhancement of firing rate occurring on long-delay trials is not ubiquitous but rather is confined to comparatively anterior frontal areas.
ACKNOWLEDGMENTS

We thank Karen McCracken for excellent technical assistance. Support was provided by the Center for the Neuroscience of Mental Disorders (DBCNR/NIMH MH45156) and NIH RO1 EY11831. Technical support was provided by an NIH core grant (EY08098). Collection of MR images was supported by an NIH center grant (P41RR03631).
BIBLIOGRAPHY


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**Table 1. Anticipated Delay: Incidence of significant effects in the long anticipatory epoch**

Counts of neurons exhibiting significant effects in an ANOVA taking firing rate as the dependent variable and employing as factors the length of the delay (short or long) and the direction of the response (ipsiversive or contraversive). Main effects of delay were assessed during an epoch extending from onset of the delay cue to a point in time 250 ms after offset of the directional cue. Interaction effects involving delay and direction were assessed during an epoch extending from onset of the directional cue to a point in time 250 ms after its offset. S>L or L>S: firing rate significantly greater for short than for long delay conditions or vice versa. C>I or I>C: firing rate significantly greater for contraversive than for ipsiversive response or vice versa. Ds>Dl or Dl>Ds: Directional signal (difference in firing rate between the two directions) significantly greater for short than for long delay conditions or vice versa. Percentages are expressed relative to all neurons in recorded in that area in that monkey.
### Table 2. Comparison between areas: anticipated-short delay > anticipated-long delay

Significance of pairwise differences between areas in the frequency with which neurons showed enhanced activity in anticipation of a short delay period (p value obtained with a chi-squared test). For each area, the measure of frequency was the number of neurons firing significantly more strongly under the short- than under the long-delay condition during the pre-delay epoch (category S>L in Table 1) over the total number of neurons recorded in the area. Ns: not significant.

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<td>&lt;&lt; 0.0001</td>
<td>ns</td>
<td>&lt; 0.001</td>
<td>&lt; 0.01</td>
<td>ns</td>
</tr>
<tr>
<td>FEF</td>
<td>&lt; 0.001</td>
<td>ns</td>
<td>&lt; 0.01</td>
<td>&lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>FEF/PM</td>
<td>ns</td>
<td>&lt; 0.001</td>
<td>ns</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PM</td>
<td>ns</td>
<td>&lt;&lt; 0.0001</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SEF</td>
<td>&lt;&lt; 0.0001</td>
<td></td>
<td></td>
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</table>
### Table 3. Elapsed Delay: Incidence of significant effects in the long pre-movement epoch

Counts of neurons exhibiting significant effects in an ANOVA taking as the dependent variable the mean firing rate during the pre-movement period (starting 250 ms before the imperative cue and ending at the time of saccade initiation) and employing as factors the length of the delay (short or long) and the direction of the response (ipsiversive or contraversive). Same format and conventions as in Table 1.

<table>
<thead>
<tr>
<th>Area</th>
<th>PFC</th>
<th>FEF</th>
<th>FEF/PM</th>
<th>PM</th>
<th>SEF</th>
<th>SMAr</th>
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<tr>
<td>Monkey</td>
<td>N</td>
<td>P</td>
<td>N</td>
<td>P</td>
<td>F</td>
<td>N</td>
</tr>
<tr>
<td>Total Neurons</td>
<td>102</td>
<td>102</td>
<td>42</td>
<td>38</td>
<td>44</td>
<td>20</td>
</tr>
<tr>
<td>Delay Main Effect</td>
<td>S&gt;L</td>
<td>5 (5%)</td>
<td>11(11%)</td>
<td>6 (14%)</td>
<td>3 (8%)</td>
<td>7 (16%)</td>
</tr>
<tr>
<td></td>
<td>L&gt;S</td>
<td>19 (19%)</td>
<td>13(13%)</td>
<td>17 (40%)</td>
<td>8 (21%)</td>
<td>12 (25%)</td>
</tr>
<tr>
<td>Direction Main Effect</td>
<td>C&gt;I</td>
<td>31 (31%)</td>
<td>9 (9%)</td>
<td>22 (52%)</td>
<td>8 (21%)</td>
<td>17 (39%)</td>
</tr>
<tr>
<td></td>
<td>D&gt;C</td>
<td>2 (2%)</td>
<td>8 (8%)</td>
<td>2 (5%)</td>
<td>5 (13%)</td>
<td>6 (14%)</td>
</tr>
<tr>
<td>Reward x Direction Interaction</td>
<td>Ds&gt;Dl</td>
<td>5 (5%)</td>
<td>2 (2%)</td>
<td>5 (12%)</td>
<td>1 (3%)</td>
<td>3 (7%)</td>
</tr>
<tr>
<td></td>
<td>Dl&gt;Ds</td>
<td>13(13%)</td>
<td>5(5%)</td>
<td>5 (12%)</td>
<td>2 (5%)</td>
<td>0 (0%)</td>
</tr>
<tr>
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<td>SMAr</td>
<td>SEF</td>
<td>PM</td>
<td>FEF/PM</td>
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<td></td>
</tr>
<tr>
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</tr>
<tr>
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<tr>
<td>FEF/PM</td>
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<td>&lt; 0.005</td>
<td></td>
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<tr>
<td>PM</td>
<td>ns</td>
<td></td>
<td>&lt;&lt; 0.0001</td>
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<tr>
<td>SEF</td>
<td>&lt; 0.05</td>
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<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

**Table 4. Comparison between areas: proportion of neurons exhibiting enhancement after long vs. short delay**

Results of a pairwise comparisons between areas (p value obtained with a chi-squared test). The null hypothesis was that the relative frequency of neurons firing significantly more strongly after a long delay (L>S in Table 3) and those firing significantly more strongly after a short delay (S>L in Table 3) was not different between the pair of areas. Ns: not significant.
FIGURE LEGENDS

Figure 1. Variable-delay task and variable-reward task. A. All potential targets were at 10° eccentricity. One pair of diametrically opposed targets was used during each recording session (1 and 1’, 2 and 2’, or 3 and 3’). The pair was selected to include the target at the neuron's preferred location. B-I. The panels represent the screen in front of the monkey during successive epochs of a single representative trial. The center of the dashed circle indicates the monkey’s direction of gaze during the corresponding trial epoch, the arrow indicates the direction of the eye movement. All other items represent images visible to the monkey. B. A white fixation spot appeared at the center of the screen and monkey achieved foveal fixation. C. After 50 ms, the fixation spot was replaced by a cue the shape and color of which signified the length of the upcoming delay period (C1) in the variable-delay task and the size of the upcoming reward (C2) in the variable-reward task. D. After 400 ms two targets appeared at diametrically opposed locations. E. A cue was then presented for 250 ms in superimposition on one of the targets. F1. A delay period of 500 ms (short) or 2500 ms (long) ensued in the variable-delay task. F2. A fixed delay of 1500 ms ensued in the variable-reward task. G. The fixation spot was extinguished. H. The monkey was required to make a saccade directly to the previously cued target. I. After maintaining fixation on the target for 300-450 ms, the monkey received a juice reward.

Figure 2. Recording sites in all monkeys were localized relative to gross morphological landmarks visible in structural MR images and relative to regions from which motor responses could be elicited by low-threshold electrical microstimulation (200 ms 300 Hz trains of 1.65 ms...
biphasic pulses at currents ≤40 µA). A. MR slice tangential to the surface of the cortex underlying the lateral chamber of monkey N. Black dots indicate MR-visible fiducial markers at known locations relative to the recording grid. Black rectangles indicate, for comparison, the approximate locations of fiducial markers in the midline chamber. This is for illustrative purposes only. Localization of midline recording sites was accomplished by use of a separate set of frontoparallel slices. AS: arcuate sulcus. PS: principal sulcus. B. Results of microstimulation mapping in the lateral chamber of monkey N are projected onto an enlarged view of the cortex surrounding the arcuate (AS) and principal (PS) sulci. The symbols indicate particular patterns of motor response as defined in the legend at the bottom of the figure. Dashed perimeters enclose regions (PFC, FEF, FEF/PM and PM) defined by stimulation-based criteria described in the text. C. Results of microstimulation mapping in the midline chamber are projected onto an enlarged dorsal view of the underlying cortex. Anterior is to the right. Dashed perimeters enclose the SEF as defined by stimulation-based criteria described in the text. Black rectangles represent MR-visible fiducial markers at known locations relative to the recording grid in the midline chamber. The dashed vertical line indicates the frontal level of the genu of the arcuate sulcus.

Figure 3. Impact of the duration of the delay on behavior. A. Error rate. The height of each bar indicates the mean across all recording sessions in all monkeys of the error rates on short-delay (black) and long-delay (gray) trials. Each value was obtained by first computing the mean for each session and then taking the average of the session means. Error bars indicate SEM for the latter step. *: p < 0.0001. B. The mean behavioral reaction times on short-delay (black) and long-delay (gray) trials were computed similarly. *: p < 0.0001. C. Distribution of fixation
breaks across the 4000 ms period extending from initiation of fixation (panel B in Fig. 1) to offset of the fixation spot (panel G in Fig. 1). For each 500 ms epoch in short-delay (black square) and long-delay (gray circle) trials, the height of the symbol indicates the percentage of all fixation breaks that occurred during that epoch. Each value was obtained by first computing the mean for each session and then taking the average of the session means. Error bars indicate SEM for the latter step. D. Means across all recording sessions for individual monkeys. Error rate and reaction time were computed as above. Fixation-break rate, computed independently for short- and long-delay conditions, was the percentage of trials on which the monkey broke fixation at any point during the first 1000 ms period extending from fixation attainment to offset of the fixation spot. An asterisk next to any value in the short-delay column indicates that this value differed significantly from the juxtaposed value in the long-delay column (t-test comparing the distributions of session means, p < 0.05).

Figure 4. Data from two neurons exhibiting significant effects of delay length. Yellow: a period in which trials differed with respect to the length of the anticipated delay (data aligned on delay-cue onset). Green: a period around the time of the response when trials differed with respect to the duration of the antecedent elapsed delay (data aligned on saccade initiation). A. Data from a neuron in PM that fired more strongly in anticipation of a short delay (yellow panels). Other functional attributes included firing more strongly after a short delay had elapsed (green panels) and exhibiting direction selectivity during the postsaccadic period. B. Data from a neuron in the FEF that fired more strongly, on trials requiring a leftward response, at the end of a long delay (green panels). Other functional attributes included firing more strongly in anticipation of a
short delay (yellow panels) and firing much more strongly throughout the delay period when the impending saccade was in a leftward direction.

Figure 5. Impact of delay length on 204 PFC neurons. A. Curves representing mean population firing rate as a function of time under the four task conditions defined by two lengths of delay (short = blue, long = red) and two directions (preferred = thick, antipreferred = thin). Data to the left are aligned on the onset of the directional cue (Fig. 1E). Data to the right are aligned on saccade initiation (Fig. 1H). B. Difference in population firing rate between short-delay and long-delay conditions as a function of time during the trial. Positive (blue) values indicate that the firing rate was higher on short-delay trials. C. Frequency of cases in which there was a significant main effect of delay length on neuronal firing rate (blue = stronger firing on short-delay trials, red = stronger firing on long-delay trials) during seven trial epochs (I-VII) indicated at the bottom of the figure. D. Difference in population directional signal between short-delay and long-delay conditions as a function of time during the trial. Positive (blue) values indicate that the directional signal was stronger on short-delay trials. The directional signal was taken as the firing rate on preferred-direction trials minus the firing rate on antipreferred-direction trials. E. Frequency of cases in which firing rate depended significantly on the interaction between delay length and response direction (blue = stronger directional signal on short-delay trials, red = stronger directional signal on long-delay trials) during seven trial epochs (I-VII) indicated at the bottom of the figure.

Figure 6. Impact of variable delay on 124 FEF neurons. Same conventions as in Fig. 5.
Figure 7. Impact of variable delay on 34 FEF/PM neurons. Same conventions as in Fig. 5.

Figure 8. Impact of variable delay on 76 PM neurons. Same conventions as in Fig. 5.

Figure 9. Impact of variable delay on 147 SEF neurons. Same conventions as in Fig. 5.

Figure 10. Impact of variable delay on 84 SMAr neurons. Same conventions as in Fig. 5.

Figure 11. Frequency with which neuronal activity, as measured across the long anticipatory epoch (delay-cue onset to 250 ms after directional-cue offset), depended on delay length (A), direction (B) or their interaction (C). A. Blue (or red) bars indicate the percentage of neurons in which the firing rate was significantly higher (or lower) under the short-delay condition. B. Blue (or red) bars indicate the percentage of neurons in which the firing rate was significantly higher (or lower) on trials requiring a contraversive saccade. C. Blue (or red) bars indicate the percentage of neurons in which there was a significant interaction such that the directional signal was stronger (or weaker) under the short-delay condition.

Figure 12. Impact of the reversal of cue-delay associations that occurred after each block of 40 successful trials. A. Behavioral reaction time effect averaged across all data collection sessions in all monkeys. The vertical axis represents the reaction time on trials involving the cue initially associated with a short delay (but associated with a long delay after the reversal) minus the reaction time on trials involving the cue initially associated with a long delay (but associated with a short delay after the reversal). B-E. Impact of anticipated delay on firing rate averaged
across all neurons that showed significantly enhanced pre-delay activity on short-delay trials (represented by blue bars in Fig. 11A). B. Data from all areas combined. C-E. Data from posterior areas where neurons exhibiting short-delay enhancement were most common. The vertical axis represents the firing rate after presentation of the cue initially associated with a short delay (but associated with a long delay after the reversal) minus the firing rate after presentation of the cue initially associated with a long delay (but associated with a short delay after the reversal). Data are shown as a function of trial number for 20 successful trials (-20 to -1) preceding the reversal and 20 trials (+1 to +20) following the reversal.

Figure 13. Frequency with which neuronal firing rate was significantly increased on short-delay trials as a function of recording-site location. A-C: For three lateral chambers (in monkeys P, N and F), recording sites are shown in relation to the arcuate (AS) and principal (PS) sulci. The size of each filled dot indicates the proportion of neurons at the corresponding site that exhibited a significant increase in firing rate on short-delay trials during the pre-delay epoch (delay-cue onset to directional-cue offset). Unfilled dots indicate recording sites at which no significant effect was obtained. D-F: for three midline chambers (in monkey A, N, and F), recording sites are shown in relation to the interhemispheric midline and the frontal level of the genu of the arcuate sulcus. Other conventions as in B.

Figure 14. Relation of firing dependent on anticipated reward size to firing dependent on anticipated delay length for all neurons studied in the context of both the variable-reward task and the variable-delay task. Reward index: delay-period (onset of the reward-cue to saccade initiation) firing rate on big-reward trials minus delay-period firing rate on small-reward trials
divided by their sum. Delay index: pre-delay (onset of delay cue to 250 ms after offset of

directional cue) firing rate on short-delay trials minus pre-delay firing rate on long-delay trials

divided by their sum.

Figure 15. Population curves representing mean population firing rate as a function of time for

all neurons (a) studied in both the variable-reward task and the variable-delay task and (b)

meeting the criterion that they fired significantly more strongly (in the variable-reward task)

when the monkey expected a large reward (measurement epoch beginning with onset of the

reward-cue and ending with saccade initiation). Activity is averaged across direction and is

defined by two levels of reward (big = blue, small = red) in A-F and two lengths of delay (short

= blue, long = red) in G-L. Data to the left in each column is aligned on the onset of the

directional cue. Data to the right in each column are aligned on saccade initiation. A-F.

Population histograms for neurons that fired significantly more strongly for big reward during

performance of the variable-reward task. G-F. Population histograms for the same neurons

under conditions of short- and long-delay in the variable-delay task. The disparity between blue

and red curves in the lefthand column was imposed by the selection procedure whereas the

disparity in the righthand column was not.

Figure 16. Population curves representing mean population firing rate as a function of time for

all neurons (a) studied in both the variable-reward task and the variable-delay task and (b) not

meeting the criterion that they fired significantly more strongly (in the variable-reward task)

when the monkey expected a large reward. Conventions as in Fig. 15. Neurons contributing to

Fig’s 15 and 16 together represent all neurons studied in the context of both tasks.
Figure 17. Frequency with which neuronal activity at the end of the elapsed delay (epoch beginning 250 ms prior to fixation-spot offset and ending with saccade initiation) depended on delay length (A), response direction (B) or their interaction (C). Conventions as in Fig. 11.

Figure 18. A-C. Population curves representing mean population firing rate as a function of time for all neurons in PFC, FEF and SEF meeting the criterion that they fired significantly more strongly at the end of a long delay. Red and blue curves represent activity on long- and short-delay trials averaged across direction. A segment of the curve representing activity on short-delay trials is replotted after rightward transposition so as to bring activity at the time of the imperative cue on short-delay trials (blue) into alignment with activity at the time of the imperative cue on long-delay trials (red). The elevation of the red curve relative the blue curve at the end of the delay period (selection epoch) was imposed by the selection procedure. The elevation of the red curve early in the trial (analysis epoch) was not imposed by the selection procedure. D-E. For the same neurons, the distribution of indices reflecting sensitivity to anticipated delay during the early (analysis) epoch. The significant leftward shift in each distribution indicates that neurons firing more strongly in anticipation of a long delay were preponderant. The number of observations, the mean of the distribution and the level of significance at which it differed from zero (t-test) are given by n, µ and p respectively. G-L. Identical analysis for all neurons not meeting the criterion that they fired significantly more strongly at the end of a long delay. Neurons in A-F and G-L together represent all neurons studied in these areas in the context of the variable-delay task.
Figure 19. Dependence of firing rate (during the epoch beginning 250 ms before the imperative cue and ending 100 ms later) on delay length and behavioral reaction time as revealed by multiple regression analysis. *Dark bars* placed to the left in each side-by-side set represent neurons in which firing was significantly correlated with *delay length*. Upward (or downward) pointing bars represent cases in which the correlation between firing rate and delay length was positive (or negative). Hatched regions represent neurons exhibiting a significant dependence both on delay length and on reaction time (but sorted by delay length. *Light bars* placed to the right in each side-by-side set represent neurons in which firing was significantly correlated with *reaction time*. Upward (or downward) pointing bars represent cases in which the correlation between firing rate and reaction time was positive (or negative). Hatched regions represent neurons exhibiting a significant dependence both on reaction time and on delay length (but sorted by reaction time).

Figure 20. Impact of delay length on activation of neck and jaw muscles. EMG activity of right splenius capitus (A-C) and right masseter (D-F) muscles is shown as a function of time under the four task conditions for monkeys P, N and F. Blue and red lines represent conditions in which the monkeys expected short- and long-delay, respectively. Thick and thin lines represent conditions in which the saccade was in the muscle's 'preferred' direction (toward the ipsilateral side of the body) and its 'antipreferred' direction, respectively. Vertical axis: mean instantaneous firing rate (sec\(^{-1}\)). Horizontal axis: time during trial. The left segment of each histogram is aligned on directional-cue onset and the right segment on saccade onset. Boxes I-VII indicate epochs on which statistical analysis was based.
Variable-Delay Task

Variable-Reward Task

A. Potential Targets

\[ \text{\(\Box\)} = \text{Short Delay Condition (500 ms)} \]
\[ \text{\(\bigcirc\)} = \text{Long Delay Condition (2500 ms)} \]

\(=\) Big Reward Condition (0.3 cc)
\(\oplus\) = Small Reward Condition (0.1 cc)

Fig. 1
Fig. 2

A. MRI Scan

B. Lateral Microstimulation Sites

C. Medial Microstimulation Sites

- Arm/Face
- Arm/Face & Eye
- Eye
- No Effect
**Fig. 3**

### Individual Monkey Data

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Error Rate (%)</th>
<th>Reaction Time (ms)</th>
<th>Fixation Breaks(%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Short</td>
<td>Long</td>
<td>Short</td>
</tr>
<tr>
<td>N</td>
<td>0.3*</td>
<td>0.9</td>
<td>264</td>
</tr>
<tr>
<td>P</td>
<td>1.2*</td>
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<tr>
<td>A</td>
<td>0.9*</td>
<td>3.3</td>
<td>229*</td>
</tr>
</tbody>
</table>
Fig. 4
Fig. 6
Fig. 7
PM

Fig. 8
Fig. 9
Fig. 10
Pre-Delay Trial Epoch

A. Main Effects of Delay
- Higher firing rate for short delay
- Higher firing rate for long delay

B. Main Effects of Direction
- Higher firing rate for contraversive saccade
- Higher firing rate for ipsiversive saccade

C. Delay-Direction Interactions
- Stronger directional signal for short delay
- Stronger directional signal for long delay

Fig. 11
Fig. 12
A. Monkey P (L. hem.)

B. Monkey N (R. hem.)

C. Monkey F (R. hem.)

D. Monkey A

E. Monkey N

F. Monkey F

Fig. 13
Fig. 14

- **PFC**
  - Delay Index: -.3
  - Reward Index: .3
  - $n=186$
  - $p<0.0001$
  - $r^2=0.031$

- **FEF**
  - Delay Index: -.3
  - Reward Index: .3
  - $n=113$
  - $p=0.0005$
  - $r^2=0.099$

- **FEF/PM**
  - Delay Index: -.3
  - Reward Index: .3
  - $n=34$
  - $p=0.0006$
  - $r^2=0.294$

- **PM**
  - Delay Index: -.3
  - Reward Index: .3
  - $n=66$
  - $p=0.0001$
  - $r^2=0.384$

- **SEF**
  - Delay Index: -.3
  - Reward Index: .3
  - $n=136$
  - $p=0.0009$
  - $r^2=0.088$

- **SMA**
  - Delay Index: -.3
  - Reward Index: .3
  - $n=84$
  - $p=0.0001$
  - $r^2=0.233$
Fig. 15
Variable Reward Task

Variable Delay Task

Fig. 16
Pre-Movement Trial Epoch

A. Main Effects of Delay

- Higher firing rate for short delay
- Higher firing rate for long delay

B. Main Effects of Direction

- Higher firing rate for contraversive saccade
- Higher firing rate for ipsiversive saccade

C. Delay-Direction Interactions

- Stronger directional signal for short delay
- Stronger directional signal for long delay

Fig. 17
**Fig. 18**

Neurons Selected for \( L > S \) in Pre-Movement Epoch

- **A** and **B** for PFC and FEF, respectively.
- **C** for SEF.

**Delay Index During Analysis Epoch**

- **D** for PFC.
- **E** for FEF.
- **F** for SEF.

**Number of Neurons**

- **A** and **B** for PFC and FEF, respectively.
- **C** for SEF.

**Analysis Epoch**

- **PA** and **PB** for PFC and FEF, respectively.
- **PC** for SEF.

**Selection Epoch**

- **PA** and **PB** for PFC and FEF, respectively.
- **PC** for SEF.

**Delay Index**

- **PA** and **PB** for PFC and FEF, respectively.
- **PC** for SEF.

**Neurons Selected for \( L \leq S \) in Pre-Movement Epoch**

- **G** and **H** for PFC and FEF, respectively.
- **I** for SEF.

**Delay Index During Analysis Epoch**

- **J** for PFC.
- **K** for FEF.
- **L** for SEF.

**Number of Neurons**

- **G** and **H** for PFC and FEF, respectively.
- **I** for SEF.

**Analysis Epoch**

- **GA** and **GB** for PFC and FEF, respectively.
- **GC** for SEF.

**Selection Epoch**

- **GA** and **GB** for PFC and FEF, respectively.
- **GC** for SEF.

**Delay Index**

- **GA** and **GB** for PFC and FEF, respectively.
- **GC** for SEF.
Fig. 19

A

preferred direction

B

antipreferred direction

Percent of Neurons in Area

reaction time

both

delay length

- correlation +

- correlation +

PFC  FEF  FEF/PM  PM  SEF  SMAr

reaction time

delay length

both

- correlation +

- correlation +
Fig. 20