Neurons in Monkey Prefrontal Cortex Whose Activity Tracks the Progress of a Three-Step Self-Ordered Task

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INTRODUCTION

The prefrontal cortex is critical for the ordering of behavior that transcends simple stimulus-response reflexes. Neuropsychological tasks such as the self-ordered task probe these more complicated behaviors, and are particularly difficult for subjects with prefrontal lesions (Petrides and Milner 1982). In the self-ordered task, the subject must choose different objects, one by one, on successive steps of the task. Because the positions of the objects are shuffled spatially in each step, the subject cannot use a simple spatial strategy. Instead, the subjects must remember the objects that they have chosen and make behavioral choices based on their actions in previous steps of the trial. Although the experimenters dictate the outline of the task, they do not dictate the individual steps. The subjects must order their own behavior and remember what they have done. Monkeys with lesions of the mid-dorsal prefrontal cortex cannot perform a three-step self-ordered task in which they must show their stimulus choice by reaching (Petrides 1995).

To investigate this possibility, we trained monkeys on an oculomotor version of the self-ordered task (Hasegawa et al. 2000a) and studied the activity of neurons in this area while the monkey performed it. In agreement with previous studies, we found few neurons selective for the memory of object pattern, even across steps of the trial. Instead, the largest number of classifiable neurons belonged to a new class of neurons whose activity increased as the step—and hence task difficulty—increased. Activity of these neurons correlated with the monkeys’ success or failure on the task. The overwhelming majority of these neurons were not tuned for object pattern, although we also found step modulated neurons that were tuned for the direction of the saccade that the monkey chose to make. Some of these results were presented in abstract form (Hasegawa et al. 1999).
METHODS

Subjects

We prepared two adult male rhesus monkeys (Macaque mulatta, weighing 7–11 kg), using standard sterile surgical techniques and isoflurane/ketamine anesthesia, for head restraint and eye position recording with a magnetic search coil (Colby et al. 1996). After the monkeys had learned the tasks, we placed recording cylinders over the prefrontal cortex of each hemisphere, under sterile surgical conditions, using isoflurane/ketamine anesthesia. The National Eye Institute Animal Care and Use Committee approved all animal protocols, which were in compliance with the National Institutes of Health Guide on the Care and Use of Laboratory Animals.

Behavioral tasks

Monkeys were trained on an oculomotor self-ordered task and its control task (Fig. 1A), using the REX system (Hays et al. 1982) running on a Dell Pentium II computer for behavioral control and eye position monitoring. A second PC, controlled by the REX machine via an Ethernet, generated visual stimuli and back-projected them on a tandem screen 57 cm in front of the monkey, using the VEX software. We prepared two adult male rhesus monkeys (Macaque mulatta, weighing 7–11 kg), using standard sterile surgical techniques and isoflurane/ketamine anesthesia. The National Eye Institute Animal Care and Use Committee approved all animal protocols, which were in compliance with the National Institutes of Health Guide on the Care and Use of Laboratory Animals.

Each recording cylinder was placed at a location, the centers of which were anterior to the arcuate and medial to the principle sulcus, determined by prior MRI using a 1.5-T GE Sigma 2 imager. We recorded the activity of single neurons using standard tungsten microelectrodes (FHC) and used a neural network spike sorter (Chandra and Optican 1997) running on a Dell Pentium II to do on-line spike discrimination and generate single neuron pulses, which were collected by the REX system. We analyzed data off-line using programs written in C and Matlab. We examined neuronal activity in six distinct epochs during each trial. The “fixation” period was 400 ms before the appearance of the cues. The “early cue” period was 50–250 ms after the cue appearance. This period included any stimulus-related tran-
sient responses. The “late cue” period was 250–450 after the appearance of the cue. The “presaccade” period was 200–0 ms before the onset of the saccade. The “postsaccade” period was 50–250 ms after the end of the saccade. The “reward waiting” period was 430–30 ms before the reward delivery.

Data analysis

We determined the statistical significance of data using the ANOVA functions in Matlab, applying the Tukey HSD correction for multiple comparisons, and using corrections for repeated measures (Winer et al. 1991). To verify this method, we checked our results with the Geisser-Greenhouse and Huyn-Heldt corrections using the general linear model in SPSS. Since these corrections yielded nearly identical results, we adopted the results produced by our custom-made Matlab analysis program. For display purpose, we convolved the impulse function of the spike train with a Gaussian with a \( \sigma \) of 10 ms (Richmond et al. 1987) and averaged this function throughout a given epoch.

We reconstructed the surface location of the electrode penetrations from the MR localization of the recording grid and the adjacent principal and arcuate sulci (Fig. 1C).

RESULTS

Behavior

The monkeys performed this difficult task well, but not perfectly. The chance levels of succeeding on each step of the task, given that the monkey reaches it, are 100% (3/3) for the first step, 67% (2/3) for the second step, and 33% (1/3) for the third step. The chance level of reaching the third step and succeeding on it is 22% (2/3 \( \times \) 1/3), and the monkeys performed much higher than chance: the averages of both monkeys (64% for monkey 1 and 53% for monkey 2) were significantly (\( z \) test, \( P < 0.001 \)) better than the chance level (22%), data for which were sampled over the recording sessions (99 sessions for monkey 1 and 45 sessions for monkey 2). The majority of the errors were on the third step—the monkeys did the first step perfectly and were roughly 95% correct on the second step. We presented objects pseudorandomly so that the monkeys saw many different combinations of objects. This made it difficult for the monkeys to form a spatial or pattern habit, which could reduce the memory load. In fact, they did not develop a bias for a given combination of objects (Fig. 2). If they had, they would have chosen a given target 100% of the time it appeared. Instead, their pattern choices were never >40% in average, indicating that the monkeys did not habitually choose a specific pattern. The error trials were related to the monkeys’ repeated choice of an object that had been chosen in the previous step. Although the monkeys had different levels of accuracy, there was no evidence that they used different strategies.

For both self-ordered and control tasks, we measured reaction times (saccade latency; Fig. 3). Reaction time has been viewed as an index of the import of the reward schedule on an animal’s behavior (Bowman et al. 1996), and monkey 2 showed

**FIG. 2.** Lack of pattern bias. **A:** 3 different examples for each monkey, randomly chosen from different months of experimentation, of the percentage that a given pattern was chosen in step 1 of the task. **B:** choices of each monkey for each pattern in steps 1, 2, and 3 during a single session. All plots are distributed around chance level (33.3%).
the expected reduction of reaction time for both tasks as the reward increased. Monkey 1 did not show a consistent relationship for either task, although the latency values slightly but significantly reduced from the first step to second and third steps. The monkey showed this pattern in both tasks. Although reaction time can be affected by task difficulty, the monkeys were able to decide which saccade to make during the lengthy delay period, rendering it unlikely that task difficulty was a factor in determining the saccadic reaction time.

Neuronal data set

We recorded neuronal activity from the dorsal part of the prefrontal cortex, medial to the principal sulcus and anterior to the arcuate sulcus (Fig. 1C). We usually recorded from surface of the cortex and did not search for neurons in the deeper bank of the principal sulcus. We did not prescreen neurons, but instead recorded the activity of each neuron we encountered through a number of sets of trials. We recorded from 156 neurons (79 from monkey 1 and 77 from monkey 2). Of them, 66 (42%) neurons were selective for the target location during any one of six task epochs, while only a few neurons (6%, 9/156) were selective for pattern, and this selectivity was very weak. Seventy-four neurons showed a new and unexpected kind of activity: step modulation, in which activity increased or decreased monotonically through the three steps of the self-ordered task (Fig. 4). Forty-three of these neurons also showed selectivity for saccade target location.

Step modulation during the self-ordered task

The neuron in Fig. 4 exhibited a visual response to cue onset and the response increased with increasing step (Fig. 4A, top). This increase was significant in the late cue period (250–450 ms after the appearance of the stimulus); there was a significant interaction between step and epoch by a two-way ANOVA for these factors \([F(5,355) = 9.09, P < 0.001]\). Because reward size also increased with step, we used the control task to see if the increase in activity were simply related to the expectation of increasing reward (Fig. 4A, middle). In this task, with a reward structure and motor response identical to the self-ordered task but with no increase in task difficulty, the visual response and the degree of increase were far less than that in the self-ordered task (Fig. 4A, bottom). The average activity of the most active period (late cue period) significantly increased by steps only in the self-ordered task (Fig. 4B); two-way ANOVA for task and step revealed that there were significant \((P < 0.001)\) main effects of task \([F(1,121) = 86.14]\) and step \([F(1,121) = 33.43]\), as well as an interaction between them \([F(1,121) = 12.71]\). The neuron was not significantly selective for pattern (2-way ANOVA for step and pattern; 1-way ANOVA for pattern; Fig. 4C, left). The neuron was also unselective for saccade direction (2-way ANOVA for saccade direction and step; 1-way ANOVA for saccade direction; Fig. 4C, right). On any given step of a trial, three patterns appeared, and it is possible that one of those patterns, or one of those locations, was driving the neuron, and that the step modulation appeared because of an unequal distribution of a given stimulus or pattern through the trials. To rule this out, we conducted “minus-one” analysis, where we eliminated one pattern or one saccade direction from the data set and reanalyzed it. We did this 12 times to have a complete set of minus-one data sets (6 single directions and 6 single patterns). No minus-one data set lost significant step modulation.

The step effect was not limited to the cue period, but could occur in the fixation, presaccade, postsaccade, or reward waiting periods as well (Fig. 5). For example, the neuron in Fig. 5A showed modulation in the period immediately around the saccade in the self-ordered task but not in the control task. The neuron in Fig. 5B showed step modulation maximally throughout the post-saccade period, from saccade to reward delivery. For this neuron, activity in the control task was also modulated for step, but its activity in the control task was significantly lower than that in the self-ordered task. Even though these neurons had their most dramatic modulation in the periods around the saccade, neither was selective for saccade target location by one-way ANOVA \([F(5,113) = 0.199, P = 0.41]\) for...
presaccade activity of the neuron in Fig. 5A; \( F(5,100) = 1.54, P = 0.3 \) for postsaccade activity of the neuron in Fig. 5B]. Most of the neurons with step modulation changed their activity in monotonically increasing fashion, although we also found a few cells with decreasing step modulation (Fig. 5C). The neuron shown has the greatest decrease in activity in the fixation period (400-ms interval preceding the cue onset) of the self-ordered task but not in the control task.

For all 156 recorded neurons, we used a two-way ANOVA (\( \alpha = 0.05 \)) to analyze the effects of step and epoch (see METHODS). Seventy-four (47%, 74/156) neurons were related to the step effect, 16 had a significant (\( P < 0.05 \)) main effect of step, and 58 had a significant (\( P < 0.05 \)) interaction between step and epoch. For the neurons with such an interaction, step modulation could have occurred independently during the various periods of the task. In any given period, step modula-
tion occurred in 16–24% of the sample of 156 neurons (Table 1). Of all 74 step neurons, 49 neurons showed increasing modulation in at least one epoch, and 29 showed decreasing modulation in at least one epoch. Although most cells showed only increasing step modulation or decreasing modulation, four neurons showed increasing modulation in one or more epochs and decreasing modulation in one or more epochs. All of our significant neurons had either monotonic increases or decreases or a pattern in which the first two or last two steps had similar activity. No neuron had an egregious response to the second step which was higher or lower than the response to a successful third step. The population average, using the epoch for each

<table>
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<th>Late Cue</th>
<th>Presaccade</th>
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<th>Reward-Waiting</th>
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<td>38 (24%)</td>
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FIG. 5. Examples of step modulations in a variety of task epochs in the self-ordered task. Spike probability density histograms for 3 prefrontal neurons were shown around the epoch of interest. A: a neuron with increasing step modulation during presaccade period (200-ms interval just before saccade onset) in the self-ordered task. Vertical solid line indicates onset of saccade. B: a neuron with increasing step modulation during postsaccade period (50–250 ms after saccade offset). Vertical solid and dashed lines indicate offset of saccade and onset of reward delivery, respectively. C: a neuron with decreasing step modulation during the fixation period (400-ms interval just before cue onset). Vertical solid line indicates onset of visual stimuli. Modulation of these neurons in the self-ordered task was detected by a 2-way ANOVA for step and epoch (see METHODS). First 2 neurons (A and B) had a significant ($P < 0.001$) interaction of step and epoch [$F(5,180) = 6.12$ and $F(5,335) = 48.73$, respectively], in which there were significant ($P < 0.001$) increases in activity with step during the presaccade epoch of neuron A and postsaccade epoch of neuron B. The 3rd neuron (C) also had a significant interaction of step and epoch [$F(5,330) = 2.59$, $P < 0.05$], but it derived from a significant ($P < 0.001$) decrease in activity with step during the fixation epoch. Effect of step was also compared between self-ordered (solid line) and control (dashed line) tasks. Step modulation was generally stronger in the self-ordered than control task; for the neurons in A and B, a 2-way ANOVA for task and step detected a significant ($P < 0.001$) interaction between task and step [A: $F(1,123) = 14.84$; B: $F(1,112) = 15.52$]. For the neuron in C, only step modulation was significant [$F(1,99) = 4.82$, $P < 0.05$].
cell with the greatest modulation, showed striking, linear step modulation (Fig. 6), with the mean first-step response for both step increasing and step decreasing neurons being indistinguishable, and the modulation clear in the subsequent steps.

We studied the control task in 31 neurons with step modulation (19 step-increasing, 12 step-decreasing) in at least one epoch of the self-ordered task. Of them, 17 neurons (55%) also showed step modulation in at least one epoch of the control task. However, step modulation in the control task was usually much weaker than that in the self-ordered task. To examine the effect of task, we compared the activity of the 19 increasing step neurons that had been tested in both tasks. The population means for both the initial response and the step increment were much less in the control task (Fig. 7A). On a cell-by-cell basis, the step 3 activity was significantly greater in the self-ordered task than in the control (Wilcoxon sign-rank test, \( P < 0.01 \); Fig. 7B). This was not merely a gain effect. The ratio of step 1 activity to step 3 activity for each cell was also significantly greater in the self-ordered task (Fig. 7C, Wilcoxon sign-rank test, \( P < 0.01 \)) than in the control task. Therefore, although some neurons did show reward-related modulation, a far larger proportion showed modulation related to some aspect of behavior unique to the self-ordered task.

It is possible that, for the neurons that showed significant modulation in the control task, the effect of the self-ordered task was merely to amplify reward modulation, which, although real, was not statistically significant given the relatively small numbers of our population. However, for seven neurons with step modulation, activity in the third step of the control task was less (although not statistically significant) than the activity in the first step; therefore, at least for these neurons, a trivial amplification effect could not have been responsible for the step modulation.

Another possibility is that the step-related neural activity could have correlated with some motoric aspect of the task. Certainly, both monkeys had shorter reaction times on the third step than on the first, so there was a weak inverse correlation of reaction time with step. However, there was no significant correlation of neuronal activity with saccadic latency or peak velocity. We evaluated the contributions of step, saccade latency, and saccade velocity to the activity of step-increasing cells with a multivariate analysis, using a linear model

\[
y = w_1 \text{[step]} + w_2 \text{[latency]} + w_3 \text{[velocity]} + c
\]

where, \( y \) is neuronal activity on each trial, [step] is any of steps 1–3, [latency] is saccade latency, [velocity] is peak saccade velocity, \( w_1, w_2, w_3 \) are partial regression coefficients, and \( c \) is a constant term. We found that the effect of step was much greater than that of the eye movement parameters, which had a negligible effect (Fig. 8A). We used the same model to compare the control and self-ordered cases. The step coefficients (slopes) were significantly higher in the self-ordered task than in the control (Fig. 8B, left), but the constant terms (intercepts) were identical (Fig. 8B, right). We also applied another model that had interaction terms ([step \( \times \) latency], [step \( \times \) velocity],
and \([\text{latency} \times \text{velocity}]\) as well, but none of these terms had an effect nearly as strong as the single step effect.

Although step modulation did not correlate with the monkeys’ saccadic behavior, it did correlate with their performance in the task. The great bulk of the errors were on step 3. Across the population, there was a significant \((P < 0.001\) by \(t\)-test) increase in the average on correct trials, but no significant difference between the average response on step 2 and step 3 trials in error trials \((P > 0.05;\) Fig. 9A). Step 3 activity on error trials of increasing step neurons \((Fig. 9B)\) was significantly \((\text{Wilcoxon sign–rank test}; P < 0.01)\) smaller than that on correct trials. The ratio of activity on step 1 to step 3 was also significantly \((\text{Wilcoxon sign–rank test}; P < 0.001)\) smaller on error trials \((\text{Fig. 9C})\).

**Location-selective activity during the self-ordered task**

Forty-two percent \((66/156)\) of neurons were selective for the spatial location of a target when the monkey actually chose to make a saccade to that target \((2\text{-way ANOVA for location and epoch}, \alpha < 0.05)\). The neurons responded when the target appeared at the goal of the saccade that the monkey would ultimately make \((\text{Fig. 10A})\). The neuron responded to a stimulus appearing within its response field during the early cue period \((50–200\) ms after cue onset) when the monkey was going to choose it \((\text{Fig. 10A, left})\). However, the activity was suppressed when the monkey chose a target outside the response field even though there was a stimulus in its response field \((\text{Fig. 10A, right})\). Neurons selective for target location in the self-ordered task were generally selective for target location in the control saccade task \((evaluated by 2\text{-way ANOVA for location and epoch}, \alpha < 0.05)\); the effect was often observed after the late cue period \((\text{late cue, 22%}; \text{presaccade, 22%}; \text{postsaccade, 29%}; \text{reward waiting, 26%})\) compared with fixation \((6\%\) and early cue \((13\%)\) periods.

For the neuron in Fig. 10A, the activity was selective for target location, but the neuron had no step modulation. However, \(31/66\) \((47\%)\) of the target selective neurons were also modulated by step \((\text{Fig. 10B})\). This neuron discharged maximally during the late cue period when one of three objects was presented in the response field \((\text{right position})\), and the monkey actually made a saccade toward it. The neuron did not respond even when there was an object presented in the response field, but the monkey chose another object for the saccade target \((\text{bottom left position})\). When the monkey chose the stimulus in its response field, this neuron exhibited step modulation, but not when the monkey chose a target out of its response field. The neuron had no pattern selectivity.

Of 74 step modulated neurons, \(43\) \((58\%)\) were also selective for location in any epoch. The epochs of step modulation did not necessarily correlate with epochs of directional selectivity.

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**FIG. 8.** Multiple regression analysis of step modulation. A: comparison of standardized partial regression coefficients (step, saccade latency, and peak velocity) in the self-ordered task for increasing step neurons \((n = 49)\). B: comparisons of (unstandardized) partial regression coefficient (slope) for step effect and constant term (intercept) between self-ordered and control tasks. Error bar indicates SE.

**FIG. 9.** Analysis of error trials in 49 neurons with increasing step activity. A: Average activity on correct (solid line) and incorrect (dashed line) sets, as a function of step. Two-way ANOVA for performance (correct or incorrect) and step showed a significant interaction of performance and step \([F(2,96) = 14.01, P < 0.001]\), as well as a significant main effect of step \([F(2,96) = 67.35, P < 0.001]\). B: Effect of performance on step 3 activity. C: Effect of performance on ratio of step 1 to step 3 activity. Diagonal line \(x = y\) in both panels.
Thus 13 neurons (18%) showed nondirectional step modulation at different epochs from those in which they were directionally selective, and they were not step modulated. Fifteen neurons (20%) showed directional step modulation only. Fifteen neurons (20%) had directional step modulation in at least one epoch and nondirectional step modulation in a different epoch. To detect the change in activity quantitatively, we calculated step- and location-selectivity indices for 30 neurons with “directional step” activity (Fig. 10C). There was a positive correlation between the size of the location effect and the size of the step effect \( (r = 0.57, P < 0.01) \). Across the population, the location-selective index was significantly (Wilcoxon sign-rank test, \( P < 0.001 \)) greater than the step-selective index, indicating that the effect of target location or saccade direction was stronger than the step modulation.

**Discussion**

In this study, we recorded the activity of neurons in the dorsal prefrontal cortex while monkeys performed a three step oculomotor version of the self-ordered task, a task in which subjects had to remember their choices on previous steps of the task. We chose to study dorsal prefrontal cortex because the step effect \( (\text{signifcant interaction between step and period by 2-way ANOVA, } F(5,146) = 2.85, P < 0.05) \) for late cue period activity \( (250–450 \text{ ms after cue onset}) \), while no significant step modulation was detected in this period. This was visual response of a neuron that showed both step and location-selective activity inside (yellow) and outside (gray) the response field. In addition to the step effect \( \text{signifcant interaction between step and period by 2-way ANOVA, } F(5,540) = 4.76, P < 0.001 \), the neuron was selective for the target location during the late cue period \( [\text{1-way ANOVA, } F(5,180) = 4.22, P < 0.01] \). The overall effect of target location (or saccade direction) vs. step across 30 neurons with “directional step” activity. Location-selectivity index \( = \frac{\text{max location} – \text{min location}}{\text{max location} + \text{min location}} \). Step-selectivity index \( = \frac{\text{step 3} – \text{step 1}}{\text{step 3} + \text{step 1}} \). For 12 neurons with “directional step” activity at >1 period, we used an average for each index in each neuron.

**Figure 10.** A: visual response of a location-selective neuron to target (left) and distractor (right) within the response field. There was a significant difference among different locations \( [\text{1-way ANOVA, } F(5,146) = 2.85, P < 0.05] \) for late cue period activity \( (250–450 \text{ ms after cue onset}) \), while no significant step modulation was detected in this period. B: visual response of a neuron that showed both step and location-selective activity inside (yellow) and outside (gray) the response field. In addition to the step effect \( \text{signifcant interaction between step and period by 2-way ANOVA, } F(5,540) = 4.76, P < 0.001 \), the neuron was selective for the target location during the late cue period \( [\text{1-way ANOVA, } F(5,180) = 4.22, P < 0.01] \). C: overall effect of target location (or saccade direction) vs. step across 30 neurons with “directional step” activity. Location-selectivity index \( = \frac{\text{max location} – \text{min location}}{\text{max location} + \text{min location}} \). Step-selectivity index \( = \frac{\text{step 3} – \text{step 1}}{\text{step 3} + \text{step 1}} \). For 12 neurons with “directional step” activity at >1 period, we used an average for each index in each neuron.
saccade direction. Another 18% showed directionally nonselective step modulation during some epochs, and selectivity for saccade direction in other epochs. Despite this lack of mnemonic specificity, the activity of these neurons correlated with the monkey’s actual performance: when the monkeys failed to perform the third step, the population as a whole, and many cells individually, failed to show the expected increase in activity between the second and third steps of the task. Other neurons were selective for saccade target location and modulated by step. We will discuss these results in terms of the sensory and motor functions of prefrontal cortex and the problems of reward and task difficulty.

Sensory and motor functions of prefrontal cortex

The prefrontal cortex has been suggested to be involved in working memory for spatial locations or for objects that had to be remembered throughout a given trial (Funahashi et al. 1989; Fuster 1973; Kubota and Niki 1971). Although we found many neurons that were selective for target location when the monkey was going to use that target for a saccade, we found a statistically insignificant number of neurons specific for object pattern (ANOVA at α = 0.05) and those that were selective were only weakly selective. This is consistent with the results of a number of other investigators who found little object-related activity medial to the principal sulcus (Hoshi et al. 2000; Wilson et al. 1993). Therefore, as far as one can trust negative results, it is unlikely that dorsal prefrontal cortex is important in the mnemonic processes underlying the performance of single steps of the self-ordered task. In fact, prefrontal patients and monkeys with prefrontal lesions were able to hold objects in memory in the early steps (Petrides and Milner 1982) and therefore had some mnemonic capability. Inferior temporal cortex is clearly involved in these mnemonic processes: monkeys with inferior temporal cortex lesions cannot remember an object that they have chosen if they have to wait 90 s before making the choice (Petrides 2000). In contrast, monkeys with limited dorsolateral prefrontal lesions can perform a three-step, but not a five-step, self-ordered task (Levy and Goldman-Rakic 1999) even with long delays. Taken together, our results suggest that the contribution of dorsolateral prefrontal cortex to the accurate performance of the self-ordered task occurs after the identity of the target patterns has been established.

Another important component of the self-ordered task is the motor plan: the decision to make a specific movement—a saccade in our experiments, a reaching movement in Petrides’—out of a number of plausible potential movements. We did find a number of neurons that were selective for the location of the saccade target location that the monkey was going to choose. This is also consistent with previous studies: behaviorally relevant patterns and spatial locations evoke more activity in prefrontal cortex than irrelevant patterns (Boch and Goldberg 1989; Hasegawa et al. 1998, 2000b). It is possible that the prefrontal cortex is important in developing a movement plan for ordering behavior in a complicated context, like the later steps of the self-ordered task. Step-modulated neurons with directional selectivity may well provide this function, and the information carried by these neurons, the spatial location of the next movement target, could well be a major output of this area of cortex. However, we never saw neurons with activity selective for a specific step in the task, as reported, for example, in the supplementary motor area (Tanjii and Shima 1994) and prefrontal cortex (Barone and Joseph 1989; Funahashi et al. 1993). Because we only used saccadic eye movements as an index of the monkey’s behavior, we cannot tell if the activity was specific for the saccade that the monkey was going to make or the spatial location of the movement target regardless of the actual motion used to demonstrate the choice process.

The step-modulated neurons that had no selectivity for target location appear to be far removed from motor processes. Although there was a correlation between step and latency, within a given step, there was no correlation between neuronal activity and latency. There was also no correlation between neuronal activity and saccade velocity. It is therefore difficult to ascribe step modulation to variations in motor performance.

Reward expectation

Because we used an increasing reward schedule, it could be that the step modulation resulted from the expectation of increasing reward or from the motivation to earn an increasing reward. Certainly, studies in many part of the brain have shown activity increasing with increasing reward expectation: prefrontal cortex (Leon and Shadlen 1999), ventral striatum (Bowman et al. 1996), dorsal striatum (Schultz et al. 2000), and even the lateral intraparietal area (Platt and Glimcher 1999). We devised a three-step control experiment in which the monkey saw only one member of the stimulus set and had to make a saccade to it on step of the trial. Thus there was no difference in the task requirements for each step, and the monkeys performed the steps at equal rates. At the same time, there was a progression of reward value, using the same reward schedule as the self-ordered task. Thus there was a progression in reward value without a concomitant progression of task complexity. We chose this task, rather than one in which the monkey was rewarded for making a saccade to any of three simultaneously presented stimuli, because we could not prevent these overtrained monkeys from using a self-ordered strategy, despite the fact the task might not demand it. Classically, reaction time decreases with increasing reward expectation (Bowman et al. 1996; Brown and Bowman 1995). With one exception (step 2 for one monkey), the reaction times did not differ between the control and self-ordered tasks. One monkey adjusted the latency of its saccade according to the step of both tasks, suggesting that the reward schedule affected the monkey’s performance. The other monkey did not, but had almost identical latencies for each step of the task. Nonetheless, both monkeys had neurons that were pattern- and location-nonspecific, which showed step modulation in the self-order task and not in the control task. The increase in the self-ordered task was manifest in both the absolute number of spikes and the gain of response. This in turn suggests that the step modulation is not simply a result of the reward schedule, although we cannot rule out some complicated interaction between reward schedule and other aspects of the task. A smaller percentage of neurons responded equally to the reward schedule in both the control and self-ordered tasks. We suggest that these neurons are involved in the analysis of reward expectation.
Task difficulty

One measurable aspect of the self-ordered task is difficulty. The monkeys performed the first step perfectly, the second nearly perfectly (95%), and the third step much less well, at rates between 45% and 85% depending on monkey and day. Although it is not perfect, this performance is far above chance, which was 67% for the second step and 33% for the third step. The monkeys tended, as do humans (Tversky and Gilovich 1989), to perform in chance-engendered streaks. We have reported elsewhere that the background activity of dorsolateral prefrontal neurons during the first step of the self-ordered task varies with the monkey’s performance, with a temporal offset (Hasegawa et al. 2000a). Difficulty in the self-ordered task is related to set size. The performance of both normal subjects and humans with prefrontal lesions falls with increasing set size, and the error rate is skewed toward later steps in the task (Petrides and Milner 1982). This was true of our monkeys as well. It is clear therefore that, in our version of the self-ordered task, the difficulty of the task increased in the later steps.

A number of human studies have correlated increased blood flow in prefrontal cortex with task difficulty (Baker et al. 1996; Braver et al. 1997; Duncan et al. 2000; Owen et al. 1996). In the monkey, Lecas found that background activity of prefrontal neurons was modulated according to task difficulty in a simple visual discrimination task (Lecas 1995). Unlike in our study, the majority of neurons in his small sample decreased their activity under the more difficult condition. Task difficulty enhances responses in parts of the monkey visual system (Spitzer and Richmond 1991; Spitzer et al. 1988). In these studies, as in ours, there was no attempt to separate task difficulty from other factors or to use several different paradigms of equal difficulty to dissociate the abstract concept of difficulty from its determinants.

A number of factors can correlate with difficulty in our task: one is memory load. In the self-ordered task, as set size increases, the number of stimuli that must be held in working memory increases. Several human imaging studies used increasing memory load to render their task more difficult and found positive correlations between increasing memory load and prefrontal activation (Braver et al. 1997; Klingberg et al. 1997; Manoach et al. 1997). If, in fact, our results are due to the increased memory load required as the task progresses, then these results suggest that memory load, or capacity, is determined by a neuronal mechanism independent of the objects to be remembered.

Another factor that increases with step is the number of distractor stimuli. In step 1, all of three objects can be a target, but in steps 2 and 3, the monkey needs to avoid one and two objects as distractors. Hasegawa et al. (2000b) reported that neurons recorded in the same area of dorsal prefrontal cortex responded more to a visual search array that contained five distractors than they did to a single target. This phenomenon could be related to our finding that the single target in the control task evoked a lesser response than the three targets, even in the first step of the self-ordered task. Hence, the level of task difficulty in the self-ordered task and response modulation might be related not only to memory load but also to the need to ignore distractors. However, the performance in the two distractor self-ordered task was far worse than the performance of monkeys in a seven distractor stable-target match-to-sample task using stimuli of similar size and eccentricity (Gottlieb et al. 1998), suggesting that discounting distractors may not be a major component of task difficulty in this task.

A third category of factors is that of the internal psychological factors that change with task difficulty. Doing difficult tasks requires a host of yet poorly defined processes such as more motivation, more attention, more concentration, less ability to multitask, and more sophisticated executive control. We cannot even hypothesize what specific function mid-dorsal prefrontal cortex plays in the performance of the self-ordered task. We know that it does not provide a specific mnemonic function, and we know that certain neurons describe the spatial target of the movement that shows the monkey’s choice.

The most intriguing result of our studies is that there is a large population of neurons with no selectivity for spatial target or pattern that nonetheless track the progression of the task by monotonically increasing or decreasing their activity from step to step. Failure of the neurons to increase their activity between the second and third step correlates with the monkeys’ failure to perform the third step accurately. We suggest that these neurons are critical for the maintenance of performance as a cognitive task becomes more complex and difficult. Determining the exact contribution of these neurons will require much further work, but we can still suggest that the loss of step-modulated dorsal prefrontal neurons in human patients and lesioned monkeys may explain why those subjects have difficulty with the self-ordered task, even though they can hold objects in memory in the early steps.

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