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Delay-Period Activity in Visual, Visuomovement, and Movement Neurons in the Frontal Eye Field

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Lawrence, B. M., R. L. White III, and L. H. Snyder. Delay-period activity in visual, visuomovement, and movement neurons in the frontal eye field. *J Neurophysiol* 94: 1498–1508, 2005. First published April 20, 2005; doi:10.1152/jn.00214.2005. In the present study, we examined the role of frontal eye field neurons in the maintenance of spatial information in a delayed-saccade paradigm. We found that visual, visuomovement, and movement neurons conveyed roughly equal amounts of spatial information during the delay period. Although there was significant delay-period activity in individual movement neurons, there was no significant delay-period activity in the averaged population of movement neurons. These contradictory results were reconciled by the finding that the population of movement neurons with memory activity consisted of two subclasses of neurons, the combination of which resulted in the cancellation of delay-period activity in the population of movement neurons. One subclass consisted of neurons with significantly greater delay activity in the preferred than in the null direction ("canonical"), whereas the other subclass consisted of neurons with significantly greater delay activity in the null direction than in the preferred direction ("paradoxical"). Preferred direction was defined by the saccade direction that evoked the greatest movement-related activity. Interestingly, the peak saccade-related activity of canonical neurons occurred before the onset of the saccade, whereas the peak saccade-related activity of paradoxical neurons occurred after the onset of the saccade. This suggests that the former, but not the latter, are directly involved in triggering saccades. We speculate that paradoxical neurons provide a mechanism by which spatial information can be maintained in a saccade-generating circuit without prematurely triggering a saccade.

INTRODUCTION

The frontal eye field (FEF), located on the anterior bank of the arcuate sulcus, is an important component of a cortical and subcortical network involved in the execution of visually guided and memory-guided saccades (see Munoz 2002; for a recent review of this network). This is evidenced by the fact that stimulation of the anterior bank of the arcuate sulcus at low electrical currents (<50 mA) generally results in fixed-vector saccadic eye movements, the amplitude of which decreases from medial to lateral locations (Bruce et al. 1985) and by the fact that either ablation or temporary inactivation results in a variety of deficits in both visually and memory-guided saccades (for a review, see Tehovnik et al. 2000).

FEF neurons are active around the time of onset of a visual stimulus ("visual" responses) and also immediately prior to a visually guided saccade ("movement" responses). This pattern is consistent with a role for the FEF in the sensory to motor transformation underlying visually guided eye movements (Bruce and Goldberg 1985). A subset of these neurons also responds in a delayed-saccade task in which the location of a transiently presented visual stimulus must be temporarily maintained for the execution of a memory-guided saccade (e.g., Funahashi et al. 1989). Such delay-period activity is often considered to be the neuronal substrate of spatial "short-term" or "working" memory (e.g., Fuster 1973; Fuster and Alexander 1971; Goldman-Rakic 1990).

Surprisingly, the role of visual, visuomovement, and movement neurons in memory-guided behavior has not been clearly defined. Funahashi and colleagues (1989) reported delay-period activity primarily in visual and visuomovement neurons, but this conclusion was based on a small sample of cells. More recently, Sommer and Wurtz (2000) found significant delay-period activity in 44% of the movement neurons with direct projections to the superior colliculus. This percentage approached the percentages found in visual and visuomovement neurons in the same study (47 and 57%, respectively). However, these responses were obtained late in a delay period and therefore may have been contaminated by anticipatory responses (e.g., Bruce and Goldberg 1985).

In the present study, we examined the role of FEF visual, visuomovement, and movement neurons in the maintenance of spatial information in a delayed-saccade paradigm. We quantified the response to the presentation of the visual target, the response during the delay period, and the response at the time of a memory-guided saccade. We found that the percentage of neurons with significant delay-period activity was similar across cell types. When neurons with significant delay-period activity were averaged together separately for each cell type, however, significant modulation was found in the population-averaged response of visual and visuomovement but not movement neurons. This seemingly contradictory finding was reconciled by the fact that the population of movement neurons was composed of two subclasses of neurons with complementary patterns of delay-period activity. Potential implications for the role of these subtypes in memory-guided behaviors are explored.

METHODS

Recording procedure

Animals were seated in a monkey chair (Crist Instrument, Hagerstown, MD). Stimuli were back projected by a CRT projector (Electrohome, Kitchener, Ontario, Canada) onto a touch panel located 25 cm in front of the animals. Unlike an LCD projector, a CRT projector

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casts no extraneous light, so that other than the visual stimuli, experiments took place in complete darkness.

Recordings were made from three adult male Rhesus macaques (*Macaca mulatta*). Recording chambers were placed flush with the skull at Horsley-Clarke coordinates of 25 mm anterior and 20 mm lateral. Structural MRI was used to confirm the placement of each chamber with respect to the arcuate sulcus and also to localize each recording site (Fig. 11; Caret and Surefit software packages; http://brainmap.wustl.edu/caret) (Van Essen et al. 2001).

Neurons recorded within 200 μm of sites at which electrical microstimulation of <50 μA (biphasic stimulation, 250 μs/phase, 350 Hz, 70-ms duration) (Bruce et al. 1985) evoked a consistent saccadic eye movement were defined as FEF neurons. To make this determination, the animal began by fixating a blue target for 400 ms. The fixation target was extinguished and 100 ms later was followed with equal probability by either a 70-ms interval of stimulation or no stimulation. The fixation point reappeared 230 ms later on stimulation and no-stimulation trials. The animal was rewarded on every stimulation trial, and also on each no-stimulation trial in which the eyes remained within 4.5° of the extinguished fixation point. The same electrode was used for both microstimulation and unit recording within a given session.

Spatially selective FEF neurons were identified using a non-delayed saccade-plus-reach task. On a given trial, this task required the animal to execute a combined movement to one of 24 targets presented in one of eight directions at 14, 20, or 28° of eccentricity. The response field (RF) of each spatially selective neuron was then carefully mapped using a delayed-response task (Dickinson et al. 2003) (target-delay-cue task). In two animals, memory responses were then tested during interleaved delayed-saccade and -reach trials (only the results of delayed-saccade trials are presented; see following text). As a control, memory responses were also obtained during delayed-saccade trials in a third animal that had never been trained on reach-related tasks. In this animal, spatially selective neurons were identified and mapped using saccade-only trials.

Delayed-saccade task

Each delayed-saccade trial began when the animal fixated and, in two of the three animals, touched a central blue fixation point for 500 ms. A red target was then presented for 300 ms in one of two symmetric locations, chosen to fall either inside of the RF or on the opposite side of the fovea, outside of the RF. Eccentricity was adjusted based on the size of the stimulation-evoked eye movement and the responses during the saccade-plus-reach and mapping tasks. Each monkey was required to maintain central fixation throughout the delay period (800 ms in the 1st 2 animals; 1.4–5.6 s in the control animal). The end of the delay period was signaled by the offset of the fixation point. Each monkey then had $\leq$700 ms to saccade to within 7° of the location at which the target had previously appeared. Saccade reaction time was 162 $\pm$ 33 (SD) ms in constant delay period trials (1st 2 animals) and 225.3 $\pm$ 14 ms in variable delay period trials where the timing of the movement could not be anticipated (3rd animal). Saccades terminated within 3.30 $\pm$ 1.34° (mean $\pm$ SD) of the target location.

Because the data reported in the present study were collected as a part of a larger study examining effector specificity in FEF, delayed-reach trials were interleaved with delayed-saccade trials in two monkeys. (Again, it is important to note that the 3rd animal performed *only* delayed-saccade trials.) Delayed-reach trials were similar to delayed-saccade trials but were signaled with a green rather than a red peripheral target and required a peripheral reach while maintaining central fixation. In the present study, however, we examine only the results from the delayed-saccade trials. In each animal, correct trials were rewarded and incorrect trials (e.g., trials on which the animal moved prematurely, moved too late, or landed outside the target window) were aborted ($<$20% of all trials). Aborted trials incurred a short ($\sim$1 s) time out. Animals performed 10 interleaved repetitions of each of two trial types (saccades into or out of the RF). Eye position was monitored using a scleral search coil (CNC Engineering, Seattle, WA). All tasks were conducted in a dark sound-attenuated room.

Data analyses

The classification of FEF neurons into visual, visuomovement, and movement cell-types was based on visual and motor responses in the delayed-saccade task. The visual response (the interval spanning 50–300 ms after target appearance) was measured relative to baseline activity (the interval spanning 200–400 ms before target appearance) and the peri-saccadic response (100-ms interval prior to saccade onset) was measured relative to late delay-period activity (the interval spanning 200–300 ms prior to saccade onset). Measurements of movement responses relative to late delay activity prevented prolonged delay-period responses from being misinterpreted as movement-related responses (see Sommer and Wurtz 2000 for a similar subtraction). Changes in the duration or alignment of the intervals used to measure the responses (e.g., 50 to 150 or 150 to 300 ms visual interval; 100 ms presaccadic interval aligned on the onset of the saccade or -100 to 100 ms interval surrounding the peak velocity of the saccade) had minimal effect on the classification of cell types and had no effect on the conclusions of the study.

A *visuomotor index* (Fig. 1*A*) was then constructed for each neuron by calculating the contrast ratio between visual and motor responses ([motor–visual]/[motor+visual]). For this purpose, visual responses that were less than baseline and peri-saccadic responses that were less than the late delay-period activity were rounded to zero. In addition to this continuous measurement, we also employed a discrete categorization. Neurons with indices from -1.0 to -0.4 were classified as visual neurons, those between -0.4 and 0.4 as visuomovement neurons, and those from 0.4 to 1.0 as movement neurons (Fig. 1*B*). Visual neurons were largely responsive to the presentation of the target and were largely nonresponsive immediately prior to the saccade, whereas visuomovement neurons were equally responsive during both intervals. Movement neurons, in contrast, were largely nonresponsive to the presentation of the target but were largely

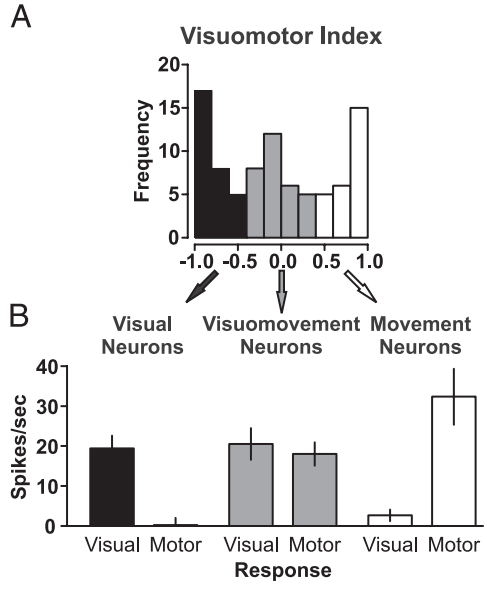

FIG. 1. Cell-type classification. *A*: the frequency distribution of cells across the visuomotor index. Neurons with indices less than -0.4 or >0.4 were classified as visual and movement neurons, respectively, whereas neurons with intermediate indices were classified as visuomovement neurons. *B*: mean visual and motor responses in the delayed-saccade task, with SEs, plotted for each cell type.

responsive immediately prior to the saccade. It is important to note that although we separated continuous data into discrete categories, we would not argue that these categories necessarily represent three distinct subtypes of FEF neurons. Indeed, even Bruce and Goldberg (1985) noted in their classic paper, which defined visual, visuomovement, and movement neurons, that the categorization of these subtypes is somewhat arbitrary because the underlying distribution is continuous. It is also important to note that while the criterion value of ± 0.4 was selected post hoc to approximate previously reported percentages of each cell type (Bruce and Goldberg 1985), large changes in the criterion values used for classification (e.g., 0.1 or 0.8 instead of 0.4) had no effect on our conclusions (see RESULTS).

Delay-period activity was measured as the difference in activity between preferred and null trials in the interval spanning 450 to 950 ms after the presentation of the target unless otherwise noted. This interval begins late to minimize the contribution of transient visual responses to the onset or offset of the target and ends early to minimize the contribution of anticipatory presaccadic responses at the end of the delay period. Significance of the delay-period activity was assessed by comparing the activity in preferred and null trials using 2-tailed *t*-test ($P < 0.05$). Correlation was used to examine delay-period activity as a continuous function of the visuomotor index. Because the populations of visual, visuomovement, and movement neurons of both monkeys exhibited similar patterns of activity, data were pooled across monkeys. For display purposes only, traces were smoothed using a 181-point digital low-pass filter with a transition band spanning 2–15 Hz (−3 dB point = 9 Hz); all statistics, however, were obtained from unsmoothed data.

RESULTS

Data in the delayed-saccade task were first collected from 87 FEF cells in two animals. Two interleaved trial types were examined: delayed saccades to a location inside (*preferred trials*) or outside (*null trials*) the RF of the neuron being recorded. In each case, animals fixated a central target while a peripheral target signaling a to-be-remembered location was presented for 300 ms, followed by an 800-ms delay period. At the end of the delay, the fixation target was extinguished, and the animal responded by executing a saccade to the remembered peripheral target location.

To examine the relationship between delay-period activity and FEF cell type, the activity on preferred trials was used to classify cells into visual, visuomovement, and movement categories, after Bruce and Goldberg (1985) (see METHODS). Visually related responses were quantified using the interval spanning 50 to 300 ms after the onset of the target. Motor-related responses were quantified using the 100-ms interval prior to the memory-guided saccade. Visuomotor index values, calculated as the contrast ratio between visual and motor responses, spanned the interval from −1 to +1, with peaks at +1, 0, and −1 (Fig. 1*A*). A random pairing of the FEF visual and movement responses would produce a bimodal distribution of indices with peaks at +1 and −1 (not shown). We tested the hypothesis that the seemingly tri-modal distribution that we observed reflected a nonrandom pairing of visual- and motor-related responses. Although the actual distribution was significantly different from uniform (Kolmogorov-Smirnov test, $P = 0.003$), it was not significantly different from the bimodal distribution that would be obtained by a random pairing (Kolmogorov-Smirnov, $P = 0.87$).

Neurons were further tested for the presence or absence of significant delay-period activity (assessed as the difference in activity between preferred and null trials in the 450–950 ms after target onset). Fifty-seven percent (17 of 30) of visual neurons, 32% (10 of 31) of visuomovement neurons, and 58% (15 of 26) of movement neurons had significant delay activity. The population-averaged time course of activity is presented for each cell type in Fig. 2. Responses from neurons with and without significant delay activity are separated (*left* and *right*, respectively). Solid traces represent activity on preferred trials and dashed traces represent activity on null trials.

As is evident in Fig. 2, visual and movement responses were larger in neuronal populations with significant delay activity (*left*) compared with those without significant activity (*right*). Visual responses were 14.7 ($P = 0.02$) and 23.0 sp/s ($P = 0.03$) larger in visual and visuomovement neurons, respectively, and movement responses were 12.1 ($P = 0.049$) and 28.7 sp/s ($P = 0.03$) larger in visuomovement and movement neurons, respectively, for neurons with significant delay activity compared with those without ($P < 0.05$ for all comparisons). These larger responses suggest that neurons with significant delay activity play a particularly prominent role in the FEF compared with those neurons without delay activity.

At the population level, delay-period activity was strongest in visual neurons and weakest in movement neurons (Fig. 2, *left*). Delay activity in visual neurons was maintained at a constant level (preferred minus null = 15.0 ± 6.1 spike/s, $P = 0.02$) and continued through the execution of the saccade (*top left*). Visuomovement neurons maintained slightly weaker delay activity (12.6 ± 2.2 spike/s; $P = 0.0003$) that increased markedly just prior to the execution of the saccade (*middle left*). Surprisingly, despite the fact that the population-averaged response of movement neurons consisted of only those movement neurons with significant delay-period activity, the population-averaged response of these neurons *was not* significant (0.4 ± 2.2 spike/s, $P = 0.87$; *bottom left*).

What could account for the presence of delay activity in individual movement neurons, and the absence of delay activity in the population-averaged response of these neurons? To examine the activity of individual neurons more closely, we plotted the delay-period activity of individual neurons as a function of the visuomotor index (Fig. 3*A*). Neurons with significant delay activity are represented by filled circles, whereas neurons without significant delay activity are represented by unfilled circles. There was a significant inverse correlation between delay-period activity and the visuomotor index among neurons with significant delay activity ($r = −0.4$; $P = 0.01$). Closer examination, however, reveals an interesting pattern. Overall, similar percentages of visual and movement neurons (~60%) showed significant delay activity (Fig. 3*B*, unfilled bars). But whereas most visual and all visuomovement neurons had greater delay activity in the preferred than in the null direction (black bars), this was true of less than half of movement neurons. Fully 60% of the movement neurons with significant delay activity (9 of 15) were more active during the delay preceding saccades in the *null* direction than during the delay preceding saccades in the preferred direction (gray bars). We will refer to those neurons with greater delay activity in the preferred direction than in the null direction as *"canonical"* neurons (solid black circles and black bars in Fig. 3) and those neurons with the reverse pattern as *"paradoxical"* neurons (gray circles and gray bars). When only canonical cells were considered, there was no longer a significant relationship between delay activity and visuomotor index.

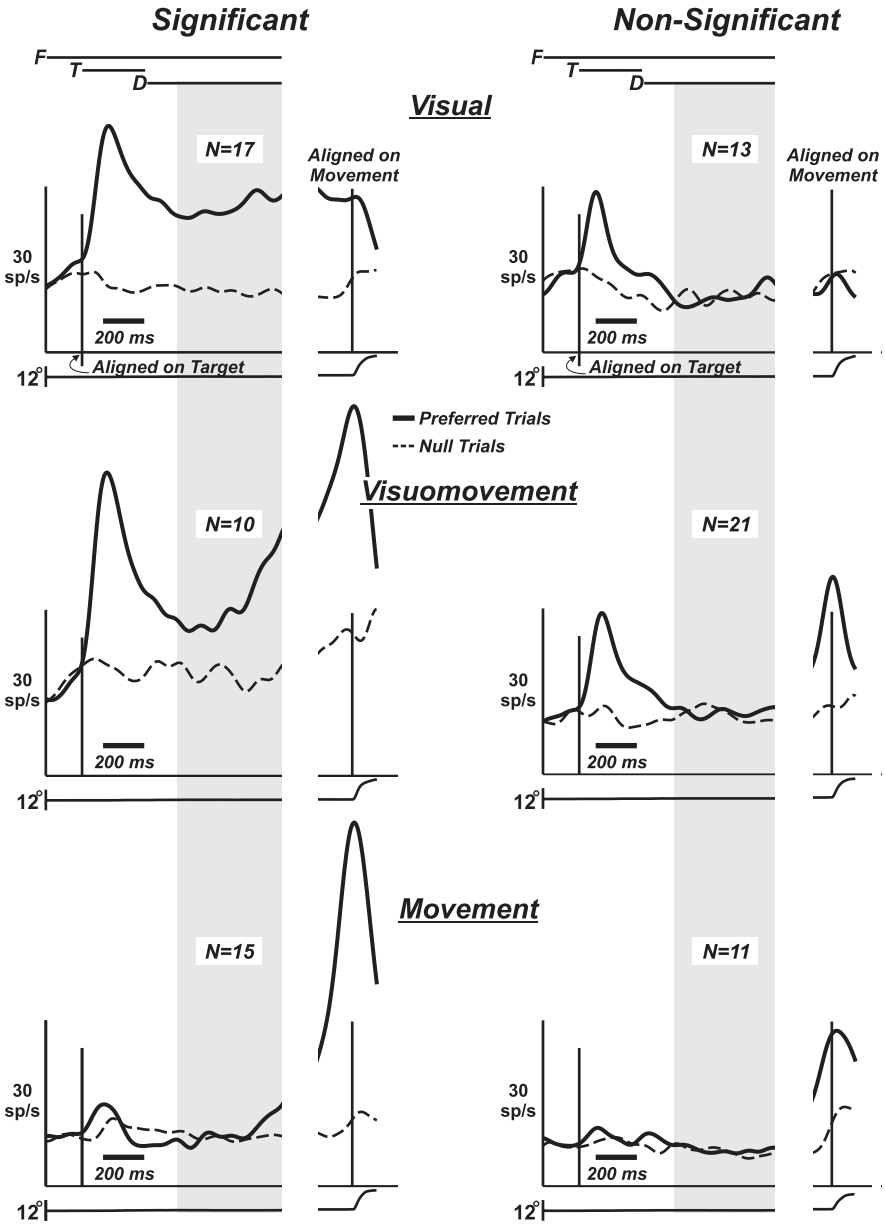

FIG. 2. Population-averaged responses. These population-averaged responses are composed of individual neurons that were classified as having visual, visuomovement, or movement responses and were further classified as having significant or nonsignificant delay-period activity (*left* and *right*, respectively). Activity on trials in which the target appeared in the RF (preferred trials) is represented by solid traces while activity on trials in which the target appeared out of the RF (null trials) is represented by dashed traces. Shaded regions represent the interval in which the delay-period activity was averaged (450 to 950 ms after the onset of the target). F, T, and D (*top*), fixation, target, and delay-period intervals.

An example of a movement neuron with canonical delay activity is presented in Fig. 4*A*. A saccade-related burst of activity establishes the preferred direction (leftward; *left*), whereas the absence of a saccade-related burst establishes the null direction (rightward; *right*). On preferred direction trials, there was an increase in activity during the delay period, hundreds of milliseconds prior to the saccade-related burst of activity. In contrast, on null direction trials, there was no change in delay activity. Delay activity for the preferred direction was significantly greater than delay activity for the null direction (difference of 12.8 spike/s; $P = 0.0008$). In contrast, an example of a movement neuron with paradoxical delay activity is presented in Fig. 4*B*. Once again, the presence of a saccade-related burst establishes the preferred direction (*left*), whereas the absence of a saccade-related burst establishes the null direction (*right*). In the preferred direction, activity did not increase until just prior to the saccade-related burst of activity. However, in the null direction, activity increased shortly after the presentation of the target and terminated around the onset of the saccade. The delay-period activity in the *null* direction was significantly greater than the delay-period activity in the preferred direction (15.1 spike/s; $P = 0.00001$).

While the activity of most visual and visuomovement neurons was canonical, the activity of a few visual neurons was paradoxical. An example of such a neuron is presented in Fig. 5. Notice that the presence of a visual burst of activity establishes the preferred direction (*left*), whereas the absence of a visual burst establishes the null direction (*right*). Although there was no delay activity during preferred direction trials, there was delay activity on null trials. This delay activity began well after target onset and ended just prior to the onset of the saccade. Similar to the paradoxical movement neuron, the delay-period activity in the *null* direction was significantly greater than the delay-period activity in the preferred direction (16.7 spike/s; $P = 0.00001$).

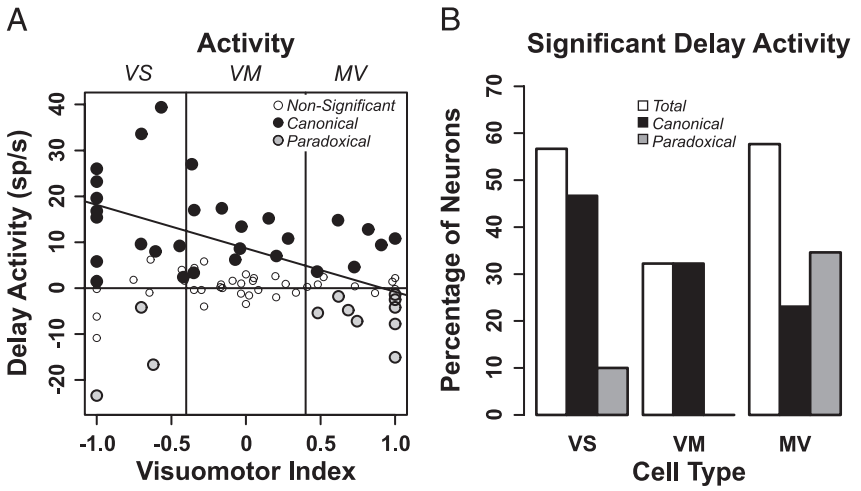

FIG. 3. *A*: a scatter plot of delay-period activity in single neurons plotted as a function of the visuomotor index. Black circles, neurons with canonical delay activity (preferred > null); unfilled gray circles, neurons with paradoxical delay activity (preferred < null). Neurons without a significant difference in delay-period activity on preferred and null trials are represented by unfilled circles (1 visual neuron with a firing rate of 90 spike/s falls outside the plotting area). Delay-period activity decreased from visual to movement neurons (*left* to *right,* respectively). This relationship was largely driven by the presence of movement neurons with paradoxical delay activity. *B*: this plot characterizes the percentage of neurons with significant delay-period activity (unfilled bars), broken down into canonical (black bars), and paradoxical (unfilled gray bars) delay-period activity for visual (VS), visuomovement (VM), and movement (MV) neurons. Although there are roughly equal percentages of visual and movement neurons with significant delay activity, movement neurons, unlike visual neurons, consisted of roughly equal percentages of neurons with paradoxical and canonical delay activity.

Because the information conveyed by neuronal activity depends not only on mean activity (e.g., Fig. 3*A*) but also on the reliability of that activity, we quantified the information conveyed by single neurons using receiver operating characteristic (ROC) analysis (Metz 1978). In our analysis, the area under a ROC curve reflects the accuracy of an "ideal observer," who uses the delay-period activity of individual neurons on individual trials to determine whether the target appeared inside or outside of the RF. This value is plotted for individual cells as a function of the visuomotor index in Fig. 6**.** As is evident in the figure, ROC analysis cleanly segregates neurons into canonical (black circles) and paradoxical (gray circles) subtypes. The mean ROC values for canonical visual, visuomovement, and movement neurons were 0.93, 0.86, and 0.86, respectively, whereas the mean ROC values for paradoxical visual and movement neurons were 0.91 and 0.81, respectively. (Because paradoxical neurons have greater activity in the null direction than in the preferred direction, the ROC value for paradoxical neurons is reported as 1 minus the ROC value. For graphical purposes, however, we did not perform this subtraction on paradoxical neurons in Fig. 6.) These results demonstrate that the amount of information conveyed by neurons with significant delay activity is relatively independent of cell type (visual, visuomovement, or movement) or subtype (canonical or paradoxical).

The averaged time course of activity for the subtypes of movement neurons with canonical and paradoxical delay activity are presented in Fig. 7. For canonical movement neurons (Fig. 7*A*), activity on preferred trials (*left*) increased during the delay period while the activity on null trials (*right*) decreased during the delay period. The delay activity on preferred trials was significantly greater (9.3 ± 1.8 spike/s; $P = 0.004$) than the delay activity on null trials. For paradoxical movement neurons (Fig. 7*B*), delay activity on null trials (*right*) was significantly greater (5.6 ± 1.4 spike/s; $P = 0.004$) than delay activity on preferred trials (*left*). The combined effects of canonical and paradoxical subpopulations resulted in the near cancellation of delay-period activity in the population-averaged response of movement neurons (Fig. 2, bottom left-hand panel).

It is possible, however, that the cancellation of delay-period activity across the population of movement neurons could be an artifact of the criterion value used for the classification of movement neurons. In Fig. 2, for example, neurons with a visuomotor index of ≥0.4 were classified as "movement neurons." However, as is evident in Fig. 8, shifting the criterion value anywhere from 0.0 to 0.9 neither changes the mean delay-period activity of canonical and paradoxical neurons nor changes the cancellation of delay-period activity in the population of movement neurons.

Timing of the saccade-related burst

Although initial inspection of Fig. 7 suggests that canonical and paradoxical subtypes were otherwise functionally equivalent, closer inspection reveals that the onset of the presaccadic activity occurred earlier in canonical neurons than in paradoxical neurons. Because the onset of the saccade-related activity can be gradual and occurs around the same time as fluctuations in late delay-period activity, we instead quantified the *peak* of the saccade-related burst, which was completely unambiguous in both individual neurons and in the population responses. The peak of the saccade-related burst was determined as the point of maximal activity in the 300-ms interval surrounding the onset of the saccade. The results of this analysis indicated that the peak of the burst occurred 20 ms *before* saccade onset in the population of canonical neurons, but 38 ms *after* saccade onset in paradoxical neurons. These values, obtained at the population level, are similar to the mean values obtained across all individual canonical neurons (−19.0 ± 5.6 ms) and across all individual paradoxical neurons (34.7 ± 12.0 ms).

Cumulative histograms showing the timing of the peak of the saccade-related burst, relative to saccade onset, in individual movement neurons with canonical, paradoxical, and for additional comparison, nonsignificant delay-period activity, are presented in Fig. 9. These histograms reveal that 50% of canonical neurons reached peak activation 27 ms prior to the onset of the saccade, whereas 50% of paradoxical neurons reached peak activation 53 ms after the onset of the saccade. The relationship between percentage and timing was strikingly similar in nonsignificant and canonical movement neurons (21 ms prior to the onset of the saccade in nonsignificant neurons; notice the overlap in histogram traces at ~50%), suggesting that nonsignificant and canonical movement neurons may be similarly involved in triggering saccades. Moreover, this similarity suggests that differences between canonical and para-

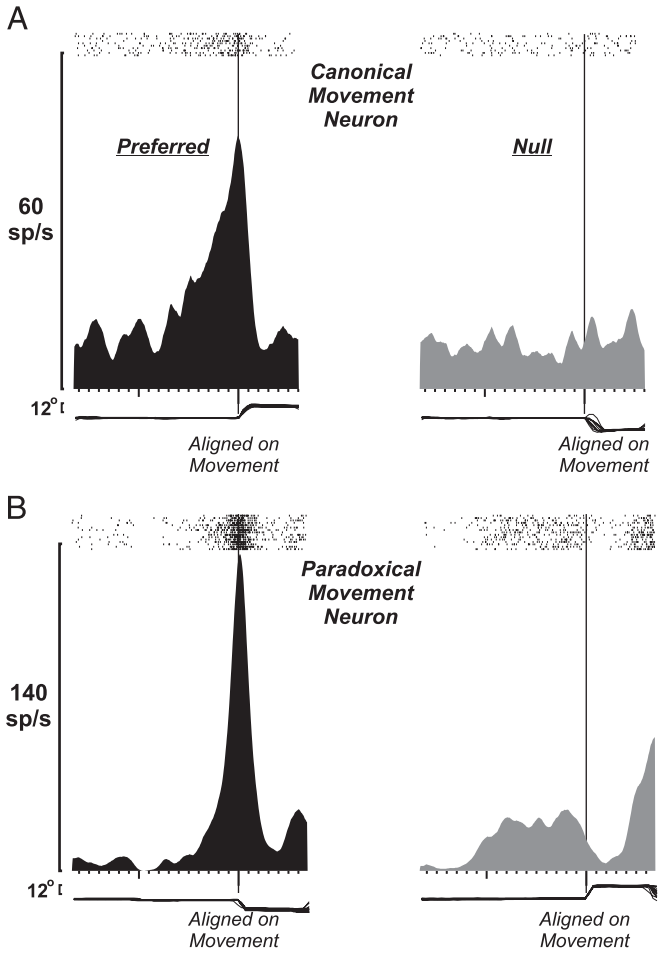

FIG. 4. *A*: an example of a movement neuron with canonical delay-period activity. In the preferred direction (*left*), there was an increase in activity during the delay period that culminated in a saccade-related burst of activity. In the null direction (*right*), there was neither an increase in activity during the delay period nor a saccade-related burst of activity. The delay-period activity on preferred trials was significantly greater than the delay-period activity on null trials. *B*: an example of a movement neuron with paradoxical delay activity. In the preferred direction (*left*), there was no increase in activity until just prior to the saccade related burst. In the null direction (*right*), there was an increase in activity that began shortly after the presentation of the target and terminated around the onset of the saccade. The delay-period activity on null trials was significantly greater than the delay-period activity on preferred trials. From top to bottom of each panel: rasters, smoothed single-unit activity, and horizontal eye position traces. Data are aligned on the onset of movement.

doxical neurons in peak activation times are not merely a byproduct of differences in delay-period activity. Taken together, these results provide additional support for the hypothesis that paradoxical and canonical neurons reflect two distinct subpopulations of movement neurons. Moreover, these results suggest that, unlike nonsignificant and canonical movement neurons, paradoxical movement neurons may not be *directly* involved in triggering saccades.

Additional control procedures

Finally, we examined the possibility that the delay-period activity found in movement neurons was an artifact of the experimental procedure used in the present study. We tested two possibilities. First, because the duration of the delay period was constant, it is possible that the delay-period activity in

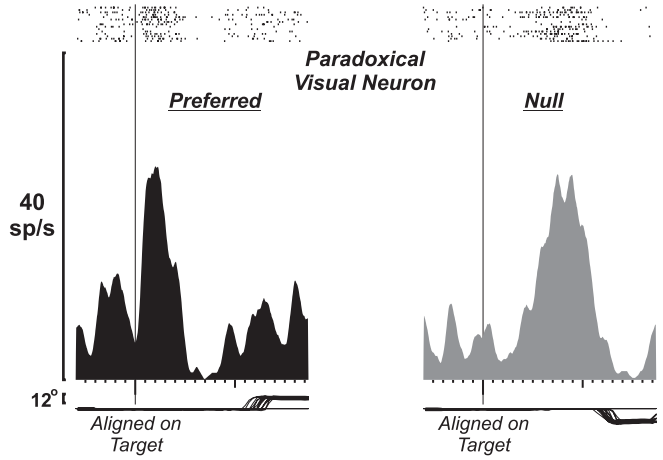

FIG. 5. An example of a visual neuron with paradoxical delay-period activity. Whereas there was a burst of activity in response to the onset of the target in the preferred direction (*left*), there was no increase in activity during the delay period. In contrast, whereas there was no burst of activity in response to the onset of the target in the null direction (*right*), there was a marked increase in activity during the delay period. The delay-period activity in the null direction was significantly greater than the delay-period activity in the preferred direction. Data are aligned on the presentation of the target.

movement neurons was due to the animals' anticipation of the upcoming movement. Second, the animals were also trained on a delayed-reach task, the trials of which were interleaved with delayed-saccade trials. Given the tight coupling between the saccade and reach systems (e.g., Abrams et al. 1990; Bekkering et al. 1994; Snyder et al. 2002), it is possible that either the training or the interleaving of delayed-reach trials may have influenced the pattern of neural activity during delayed-saccade trials. To rule out these two possibilities, we examined delay-period activity in a third animal in a delayed-saccade task with variable delay periods (1.4, 2.8, and 5.6 s). It is important to note that this control animal neither trained on, nor performed reach-related tasks of any kind. Otherwise, the procedure for

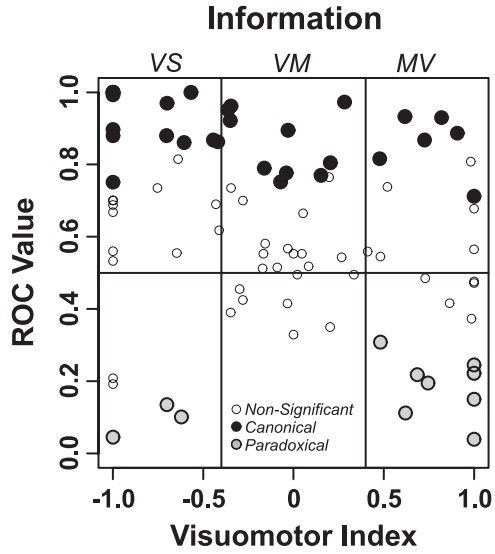

FIG. 6. A scatter plot of mean ROC values for nonsignificant (unfilled circles), canonical (black circles), and paradoxical (gray circles) neurons, plotted as a function of the visuomotor index. This plot reveals not only that canonical and paradoxical neurons convey roughly equal amounts of information during the delay period but also that the amount of information conveyed by these subtypes is similar across the visuomotor index.

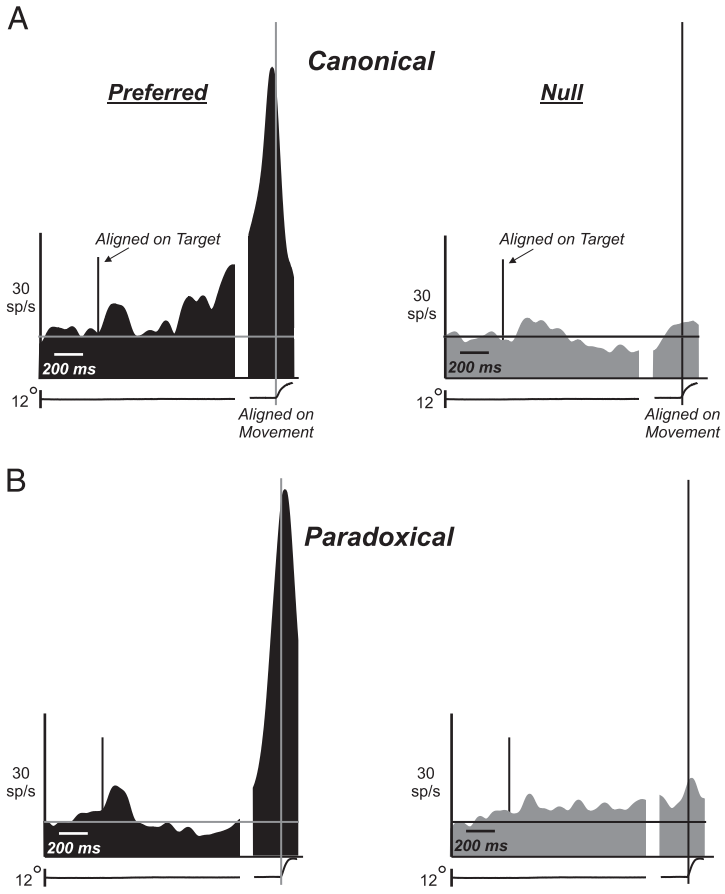

FIG. 7. The averaged time course of activity for the subpopulations of movement neurons with canonical and paradoxical delay activity. *A*: for canonical movement neurons, the delay-period activity on preferred trials (*left*) was significantly greater than the delay-period activity on null trials (*right*). *B*: in contrast, for paradoxical movement neurons, the delay-period activity on preferred trials (*left*) was significantly less than the delay activity on null trials (*right*).

the control animal was the same as the procedure used for the first two animals.

An example of a neuron with paradoxical activity from the control animal is presented in Fig. 10. Only data from trials with a 5.6-s delay period are shown. The presence of a pronounced presaccadic response for movements up and to the left, and the absence of a visual response for targets in any location, establishes this neuron as a movement neuron. During the delay, the cell was minimally active for movements in the preferred direction (up left) and maximally active for movements in the opposite, null direction (down right). The paradoxically opposed tuning of delay-period and saccade-related activity is evident in the tuning curve presented in the center of Fig. 10. Notice that those locations had the strongest saccade-related responses (thin line) also had the weakest delay-period responses (thick line), and vice versa. This pattern of tuning was independent of the delay interval (not shown). The presence of both canonical (not shown) and paradoxical neurons in the third monkey suggests that the results found in the first two monkeys were not due to fixed delay intervals or interleaved reach trials.

Anatomical localization of single units

Structural MRI was used to confirm the placement of each chamber with respect to the arcuate sulcus and also to localize each recording site (Caret and Surefit software packages, http://brainmap.wustl.edu/caret) (Van Essen et al. 2001). To view the anatomical locations of recording sites on a single surface, MRI data from both monkeys were first warped to a common atlas space. Recording sites from both monkeys were then projected onto the common atlas (Fig. 11). These sites are color coded by cell type (*left*; visual neurons, blue; visuomovement neurons, green; movement neurons, yellow) and delay-

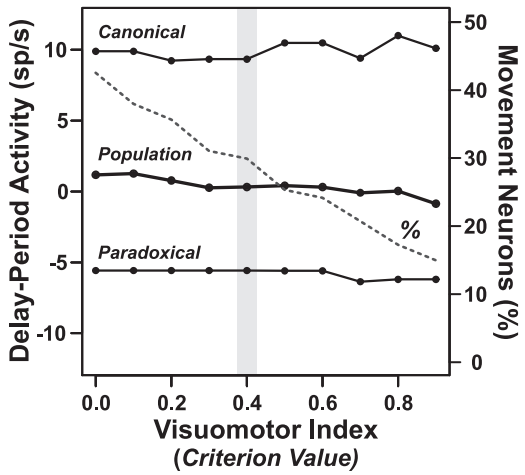

FIG. 8. The delay-period activity of canonical and paradoxical neurons, and of the population (left axis; solid lines), plotted as a function of the criterion value used to classify movement neurons. A criterion value of 0.4 on the visuomotor index was used in the present study (gray bar). As the criterion value shifts from 0.0 to 0.9, the percentage of neurons classified as movement neurons (right axis; dashed line) drops smoothly from ~45 to ~15%, yet the population mean of cells with delay-period activity (heavy solid line) remains within 1 or 2 spike/s of 0. This plot demonstrates that cancellation of delay-period activity in the population of movement neurons was not dependent on the criterion value.

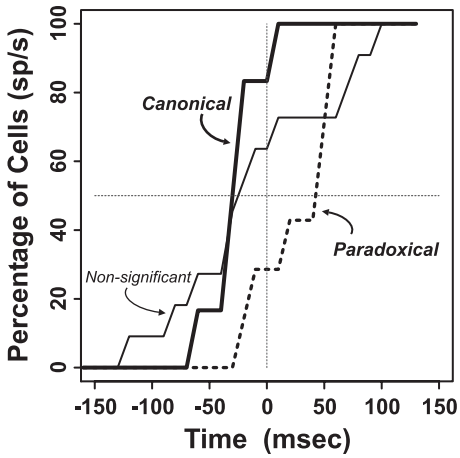

FIG. 9. Cumulative histograms showing the timing of the peak of the saccade-related burst, relative to saccade onset, for movement neurons with canonical (thick line), paradoxical (dashed line), and nonsignificant (thin line) delay-period activity. Half of all canonical neurons reached their peak activity 27 ms *before* saccade onset, while half of all paradoxical neurons reached their peak 56 ms *after* saccade onset.

period activity (*right*; canonical, red; paradoxical, white; nonsignificant delay activity, gray). To view recording sites in the sulci, a "flat map" was also constructed from the common atlas (*bottom*). With respect to medial-lateral topography, consistent with Sommer and Wurtz (2000), movement neurons tend to be clustered medially while visual neurons tend to be clustered laterally (Fig. 11, *left*). Because most paradoxical neurons *were* movement neurons, paradoxical neurons also tend to be clustered medially, whereas canonical neurons tend to be clustered laterally (Fig. 11, *right*). There was no obvious anterior-posterior topography by cell type or delay-period activity.

As is evident in Fig. 11, the majority of units were localized to the anterior bank of the arcuate sulcus (AS) and to the gyral surface just anterior to the AS. This localization of "low threshold" (i.e., <50 μA) recording sites is consistent with previous localizations of low-threshold FEF (e.g., Bruce et al. 1985). Although the continuation of low-threshold recording sites onto the gyral surface is consistent with previous accounts, the continuation of low-threshold recording sites to the posterior bank of the principle sulcus (PS) is not. It is likely,

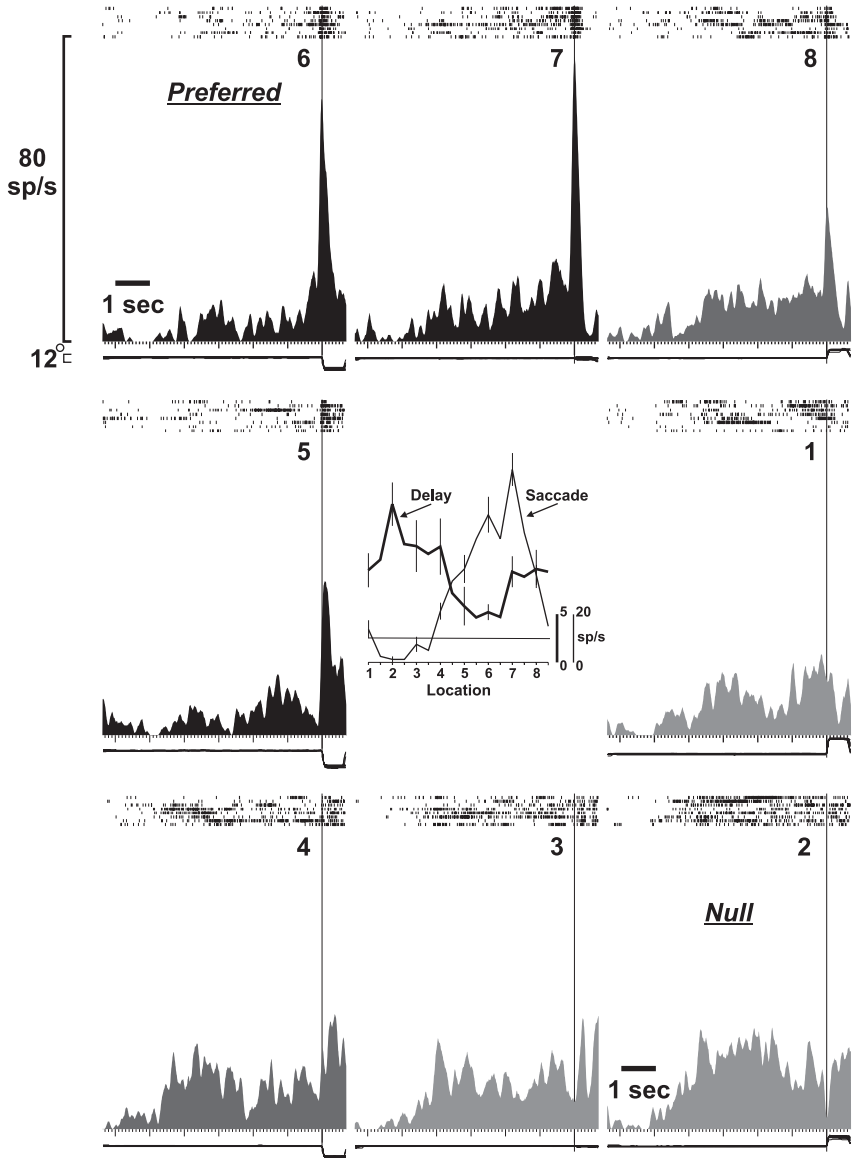

FIG. 10. An example of a paradoxical neuron collected from the frontal eye field of a control animal. Data were collected using 24 saccade directions; data from only 8 directions are shown. Multiple delay intervals were used (1.4, 2.8, and 5.6 s); data from only the longest delay are shown. Delay activity was most prominent for direction 2 (down left), whereas saccade-related activity was most prominent for directions 6 and 7 (up right and up). The tuning curve in the center panel shows mean firing rate during the delay period (thick line) and during the saccadic period (thin line) as a function of target location (mean $\pm$ 1 SE). Notice that different scales are used in the center panel for delay-period and saccade-related responses.

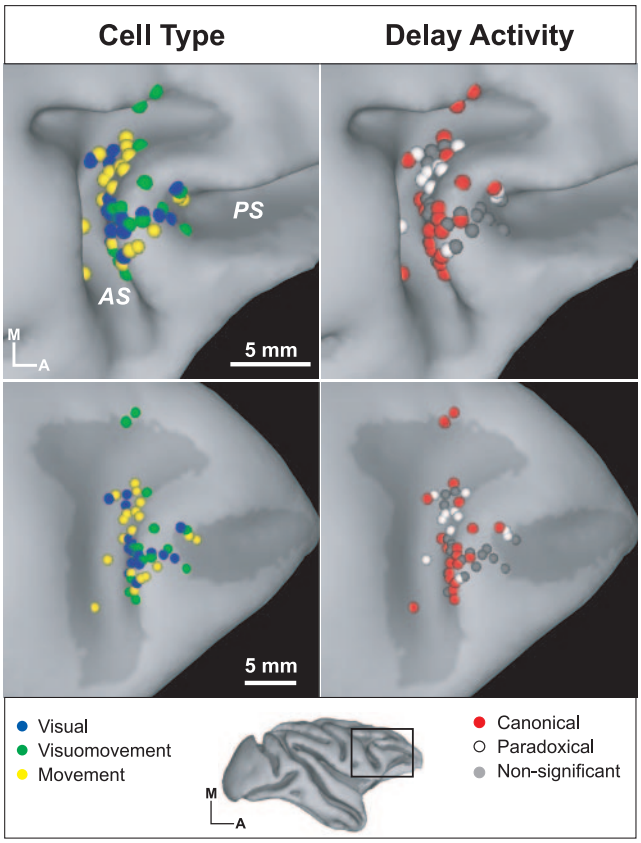

FIG. 11. Anatomical localization of recording sites, coded by cell-type (left) and by delay-period activity (right). Magnetic resonance images were obtained from both monkeys of the area in and around the arcuate sulcus (AS). These images were then warped to a common atlas using Caret and SureFit software packages (Van Essen et al. 2001). The recording sites of neurons were then projected onto 3-dimensional atlas space (*top*) and also onto the flattened representation (*bottom*). The fundi of the AS and the principle sulcus (PS) appear as dark gray lines. The recording sites are color coded by cell-type (*left*; visual neurons, blue; visuomovement neurons, green; movement neurons, yellow) and delay-period activity (*right*; canonical, red; paradoxical, white; nonsignificant memory, gray).

however, that the localization of recording sites proximal to the PS was the result of an artifact beneath the recording chambers of both animals in the structural MRI. This artifact effectively compressed the anterior extent of the gyral surface between the AS and the PS, resulting in the projection of anterior gyral recording sites onto the posterior-most aspect of the PS. Because this artifact was likely due to the absence of bone above the arcuate and principle sulci, we would suggest that for the most accurate reconstruction, the structural MRI be performed both before the craniotomy for accurate reconstruction and after the craniotomy for verification of the chamber location.

DISCUSSION

In the present study, we examined delay-period activity in FEF neurons in a delayed-saccade task. We found significant delay-period activity in individual visual, visuomovement, and movement neurons. Not only was the percentage of neurons with significant delay-period activity similar across cell types (Fig. 3*B*), but the amount of information conveyed by these neurons was also similar (Fig. 6). Interestingly, when neuronal responses were averaged separately for each cell type, there was significant delay-period activity in the population-averaged response of visual and visuomovement neurons but *not* in the population-averaged response of movement neurons (Fig. 2, *left*). This apparent contradiction was reconciled by the finding of two subclasses of movement neurons, the combination of which resulted in the near cancellation of delay activity.

In one subclass of movement neurons, the spatial tuning of delay-period activity was similar to that of presaccadic activity (Fig. 7*A*). More specifically, the spatial location that was associated with maximum delay-period activity evoked the maximum presaccadic response, whereas the spatial location that was associated with minimum delay-period activity evoked the minimum presaccadic response. The spatial alignment of excitatory responses is found not only in FEF (e.g., Chafee and Goldman-Rakic 1998; Funahashi et al. 1989), but also in many other areas of the brain. For example, visual, delay, and presaccadic activities in LIP neurons tend to be spatially aligned, both within and across tasks (Barash et al. 1991). Presaccadic activity in a delayed-saccade task and delay-period activity in a delayed-reach task are often aligned within PRR neurons (Snyder et al. 1998). The directions of greatest "build-up" and "burst" activity within single neurons in the superior colliculus are commonly aligned with one another (although dissociations are sometimes observed; see for example, Fig. 3, *C* and *D*, of Dorris et al. 1997); and all manner of visual, saccade-related, and reach-related responses are aligned within and across tasks in V6A neurons, a finding referred to as a "global tuning field" (Battaglia-Mayer et al. 2000). Many models of spatial memory capitalize on the idea that memory tuning is similar to that of visual and presaccadic tuning (for a recent review, see Constantinidis and Wang 2004). Because aligned tuning seems to be the rule and not the exception, we refer to neurons with such properties as "canonical" neurons.

In the other subclass of movement neurons, the spatial tuning of delay-period activity was *opposite* that of presaccadic activity (Fig. 7*B*). More specifically, the spatial location that was associated with maximum delay-period activity evoked little or no presaccadic response, whereas the spatial location that was associated with minimum delay-period activity evoked the maximum presaccadic response. In the latter case, minimal delay-period activity was often reduced to below baseline. Because it is difficult to reconcile this pattern with the simple idea that excitatory delay-period activity leads more or less directly to presaccadic activity, we refer to these neurons as "paradoxical." The term paradoxical has been used previously by Zhang and Barash (2000) to describe neurons that are responsive to the onset of a target that appears in the null direction, but signals a saccade in the preferred direction. Our use of the same term is not meant to imply that these are similar populations or effects.

The reduction of delay-period activity to a level below baseline is not, in itself, unusual. Inhibition of delay activity is a common finding in canonical FEF neurons, occurring on trials in which saccades are planned in the null direction (Funahashi et al. 1989). (Here and elsewhere we use "inhibition" to refer to a decrease in activity and not to the mechanism by which this decrease is accomplished.) In combination with excitatory delay activity on preferred direction trials, this inhibition creates a "push-pull" response pattern. Push-pull designs are familiar features of electronic circuits, where they

are used to improve linearity or to minimize the effects of extraneous factors such as temperature on circuit elements. Push-pull responses have been described in many parts of the brain, ranging from the vestibular periphery (Wilson and Melvill-Jones 1979) to the primary motor cortex (Georgopoulous et al. 1993). In the brain, push-pull designs may improve spatial tuning (Funahashi et al. 1989), help drive agonist-antagonist muscle pairs or extend the range or signal-to-noise ratio of sensory systems. Thus the inhibition found in paradoxical neurons is not in itself unusual. What is unusual is the fact that the delay-period activity and presaccadic responses of paradoxical neurons are tuned in opposite directions.

Neurons with inhibitory delay-period activity and excitatory saccade-related activity in the same direction were previously described by Funahashi and colleagues (1989). The saccade-related activity of these neurons, however, unlike the presaccadic activity of paradoxical neurons, is entirely *post*-saccadic, occurring hundreds of milliseconds following the onset of the saccade (see Fig. 10 in Funahashi et al. 1989). This pattern of activity is consistent with that of canonical neurons, which often show an excitatory postsaccadic response for saccades executed in the null direction (e.g., Barash et al. 1991; Funahashi et al. 1989).

The pattern of activity found in paradoxical neurons is also inconsistent with the pattern of activity found in "don't look" neurons in FEF (Hasegawa et al. 2004). "Don't look" neurons respond in a nonmatch-to-sample task and appear to encode the direction in which a saccade is *not* to be executed. Paradoxical neurons, in contrast, appear to prevent a saccade in the direction in which a saccade *will* be made, albeit not immediately. This is suggested by the fact that canonical and paradoxical delay-period activities so neatly cancel one another (compare the *2 left-hand panels* of Fig. 7 with the *bottom left-hand panel* of Fig. 2). By carrying a negative copy of the canonical delay-period signal, paradoxical neurons may represent an active mechanism to prevent the premature execution of a saccade. This notion assumes that a single read-out mechanism pools the responses of the two neuron types. This assumption could be tested by using anatomical or physiological methods to compare the projections of canonical and paradoxical neurons (e.g., Sommer and Wurtz 2000).

Canonical and paradoxical neurons differ not only in their spatial tuning characteristics, but also in the timing of their saccade-related activity (Figs. 7, *A* and *B,* and 9). Canonical neurons are likely involved in triggering memory-guided saccades. This view is supported by our finding that their peak saccade-related activity occurs before saccade onset and by the work of Schall and colleagues (Hanes and Schall 1996) showing that presaccadic FEF activity is tightly correlated with saccade onset. That peak activity occurs *after* saccade onset in paradoxical neurons, however, suggests that these neurons may not be directly involved in triggering memory-guided saccades. Because the paradoxical peak occurs, on average, 60 ms after the peak in canonical neurons, we speculate that the saccade-related activity of paradoxical neurons may reflect a corollary discharge. This signal could be driven directly by canonical neurons, or may reflect a canonical signal that has been passed through the superior colliculus back to FEF, perhaps via interneurons in the mediodorsal thalamus (Sommer and Wurtz 2004). The 60-ms timing difference, along with the fact that the early anticipatory build-up of activity that occurs late in the delay period of canonical neurons is nearly absent in paradoxical neurons, is consistent with a relatively indirect pathway.

It is important to note that paradoxical neurons are not simply neurons driven by inhibitory interneurons, which are in turn driven by canonical neurons; in this case, the very robust saccade-related responses of paradoxical neurons would be inhibitory rather than excitatory (Fig. 7, *bottom left-hand panel*). We suspect that there may be inhibitory interconnections between canonical and paradoxical neurons but that the strong excitatory movement response in paradoxical neurons is evidence of additional strong influences on these cells. Further experiments as well as computational modeling will be required to further explore these issues. Particular neural architectures could be simulated as well as tested to determine the most promising configurations. Finally, one might test the hypothesis that an imbalance in canonical and paradoxical delay-period activity could underlie the deficits in saccade inhibition seen in certain clinical populations (e.g., LeVasseur et al. 2001).

The present experiments demonstrate that visual, visuomovement, and movement neurons maintain roughly equal amounts of spatial information during the delay period of a memory-guided saccade task. This finding is inconsistent with previous suggestions that delay-period activity is primarily found in visual and visuomovement neurons (Funahashi et al. 1989). Delay-period activity may be problematic for movement neurons because activity above threshold may lead to the initiation of a saccade (Hanes and Schall 1996). We suggest that to prevent the premature initiation of a planned saccade, the activity of the population of paradoxical movement neurons effectively cancels out the activity of the population of canonical neurons during the delay period. This mechanism would allow spatial information to be maintained throughout the delay period without that activity reaching or even approaching the threshold for saccade initiation. During the delay, the spatial information may be used by canonical neurons to help prepare the upcoming saccade. The fact that paradoxical movement neurons are present in numbers just sufficient to balance the activity of canonical movement neurons, whereas paradoxical visual and visuomovement neurons are infrequent or absent, is further evidence for the special role of movement neurons in controlling the timing of the initiation of saccades.

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GRANTS

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