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Relationship of presaccadic activity in frontal eye field and supplementary eye field to saccade initiation in macaque: Poisson spike train analysis

Abstract The purpose of this study was to investigate the temporal relationship between presaccadic neuronal discharges in the frontal eye fields (FEF) and supplementary eye fields (SEF) and the initiation of saccadic eye movements in macaque. We utilized an analytical technique that could reliably identify periods of neuronal modulation in individual spike trains. By comparing the observed activity of neurons with the random Poisson distribution generated from the mean discharge rate during the trial period, the period during which neural activity was significantly elevated with a predetermined confidence level was identified in each spike train. In certain neurons, bursts of action potentials were identified by determining the period in each spike train in which the activation deviated most from the expected Poisson distribution. Using this method, we related these defined periods of modulation to saccade initiation in specific cell types recorded in FEF and SEF. Cells were recorded in SEF while monkeys made saccades to targets presented alone. Cells were recorded in FEF while monkeys made saccades to targets presented alone or with surrounding distractors. There were no significant differences in the time-course of activity of the population of FEF presaccadic movement cells prior to saccades generated to singly presented or distractor-embedded targets. The discharge of presaccadic movement cells in FEF and SEF could be subdivided quantitatively into an early prelude followed by a high-rate burst of activity that occurred at a consistent interval before saccade initiation. The time of burst onset relative to saccade onset in SEF presaccadic movement cells was earlier and more variable than in FEF presaccadic movement cells. The termination of activity of another population of SEF neurons, known as preparatory set cells, was time-locked to saccade initiation. In addition, the cessation of SEF preparatory set cell activity coincided precisely with the beginning of the burst of SEF presaccadic movement cells. This finding raises the possibility that SEF preparatory set cells may be involved in saccade initiation by regulating the activation of SEF presaccadic movement cells. These results demonstrate the utility of the Poisson spike train analysis to relate periods of neuronal modulation to behavior.

Introduction

A central problem confronting neuroscientists is how to relate neuronal activity to behavior (Teller 1984). We are specifically interested in relating neuronal activity in the frontal eye fields (FEF) and supplementary eye fields (SEF) of macaque to saccade initiation. FEF and SEF are separate but interconnected areas (Huerta and Kaas 1990; Schall et al. 1993) that are known to be involved in saccade generation based on anatomical connections (Huerta et al. 1986; Segraves and Goldberg 1987; Stanton et al. 1988; Huerta and Kaas 1990; Segraves 1992), single-unit recordings (Bruce and Goldberg 1985; Schlag and Schlag-Rey 1987; Mann et al. 1988; Schall 1991a,b), and electrical microstimulation (Robinson and Fuchs 1969; Bruce et al. 1985; Gould et al. 1986; Schall 1991b; Russo and Bruce 1993; Tehovnik and Lee 1993).

Previously, changes in neuronal modulation have been identified by a number of methods, for instance, by detecting inflections in the cumulative sum of spikes across trials (Falzett et al. 1985; Schall 1991a,b) or by testing successive values of average peri-event time histogram bins or spike density functions against some baseline or threshold value (Levick and Zacks 1970; Richmond et al. 1987; Maunsell and Gibson 1992; Oram and Perrett 1992; Segraves and Park 1993). For our investigation these standard techniques had some limitations. First, defining a threshold that represents a significant change in discharge rate can be problematic because of the difficulty in determining the appropriate threshold value. Also, questions arise about what criteria to apply to define a

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threshold crossing. For example, what if the threshold is crossed more than once in succession? In this case, would the first threshold crossing be considered the beginning of the change in activation or would it be a later crossing, when perhaps the firing rate is above the threshold for a longer period of time? Typically, some period of integration is used. Furthermore, to investigate the variability of saccade latency as the performance of a monkey changes, it is useful to analyze neuronal data on a trial-by-trial basis. When neuronal discharge rates are high and regular, the criteria for defining thresholds that indicate changes in modulation on a trial-by-trial basis seem clear (e.g., Sparks and Mays 1980). Neurons in the cortex, however, are more variable in their activation, being subject to more contingencies (Bruce and Goldberg 1985; Schlag and Schlag-Rey 1987; Schall 1991a,b). Therefore, we wanted to develop a less arbitrary method for determining times of neuronal modulation on a trial-by-trial basis.

The current paper describes the application of a Poisson spike train analysis derived from Legéndy and Salcman (1985) to analyze the activity of presaccadic movement neurons recorded in FEF and SEF and preparatory set cells recorded in SEF of monkeys performing goal-directed, visually guided saccades. The Poisson spike train analysis compares the number of spikes that actually occurred within a given time interval with the number of spikes that would be predicted to occur in that time interval if the spikes had occurred in a random Poisson fashion given the mean discharge rate of the cell. If the spikes are distributed nonrandomly across the spike train being analyzed, then the Poisson spike train analysis determines the times of significant changes in neuronal activity. A Poisson distribution serves as a good null hypothesis, because interspike intervals have been shown to be distributed in an approximately Poisson fashion (Rodieck et al. 1962; Smith and Smith 1965).

A number of studies have shown that neurons can display different modes of activity, either tonic or bursting (McCormick and Feeser 1990; Esguerra and Sur 1993; Wang and McCormick 1993). These different modes of activation may correspond to the prelude and burst of activity previously described for neurons in oculomotor regions of the brain (Wurtz and Goldberg 1972; Sparks 1978; Schlag and Schlag-Rey 1987; Glimcher and Sparks 1992). In the past, the differentiation between these periods of modulation have been accomplished by somewhat arbitrary methods (e.g., Glimcher and Sparks 1992). Here we demonstrate the ability of the Poisson spike train analysis to distinguish the prelude of activity from the burst in presaccadic movement cells in FEF and SEF, and to determine the period of significant activity in SEF preparatory set cells. Using this new analytical method, we found a consistent and precise connection between presaccadic movement cell discharge in FEF and SEF and saccade initiation. In addition, the cessation of activity of SEF preparatory set cells coincided precisely with the beginning of the burst of SEF presaccadic movement cells. Some of these results have previously appeared in abstract form (Hanes and Schall 1993).

Materials and methods

Subjects and surgery

Data were collected from three rhesus monkeys (*Macaca mulatta*). The animals were cared for in accordance with the National Institute of Health's *Guide for the care and use of laboratory animals* and the guidelines of the Institutional Animal Care and Use Committee. All surgical procedures were carried out under aseptic conditions. Initially the monkeys were tranquilized with ketamine (15 mg/kg) for intubation, catheterization, and cleaning. During surgery the monkeys were anesthetized with a N_2O/O_2 mixture and isofluorane (2–3%). Electrocardiogram (ECG), rectal temperature, and respiration were monitored. Expired pCO_2 was maintained at approximately 4%. A scleral search coil (Judge et al. 1980) was implanted subconjuntively, and a stainless steel post was attached to the skull using metal plates (Synthese) to restrain the head during testing. Once the monkey mastered the tasks at a 90% criterion level, a recording chamber was implanted over a craniotomy that exposed either the frontal eye field or the supplementary eye field.
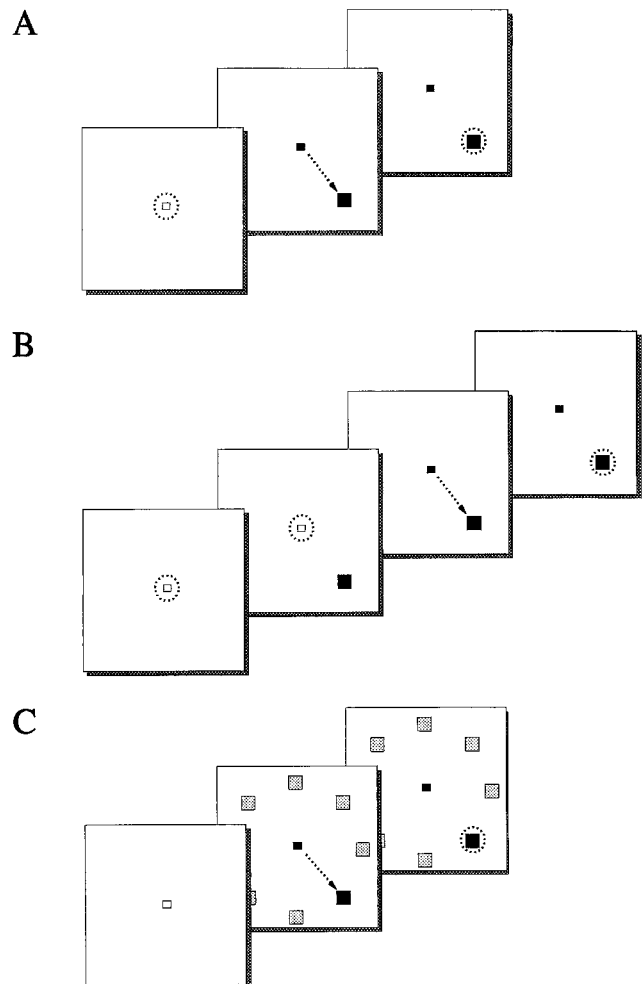
Fig. 1A–C Schematic representation of saccade tasks: **A** single target, **B** single target with instructed delay, and **C** single target with surrounding distractors. The *overlying panels* show the temporal sequence of displays for each of the three task conditions. The *dashed circle* represents the monkey's current point of fixation. The *arrow* represents the saccade to the target

Tasks

Using operant conditioning, all monkeys were trained to perform a go/no-go saccade task to a single target with and without an instructed delay (Fig. 1A,B). In addition, monkeys Q and B were trained to perform a saccade task to a single target with surrounding distractors (Fig. 1C). During the go/no-go saccade task to a single target without an instructed delay (Fig. 1A), the monkey fixated a central spot, after which a single target was presented at one of four or eight equally eccentric positions (4–12°) around the central spot. Simultaneously, the central spot changed color (trigger signal) signaling the monkey either to make a saccade to the target or to hold fixation on the central spot. In the instructed delay task (Fig. 1B), a single target was presented, but the monkey was required to maintain fixation on the central spot for a varying length of time (150–750 ms) before the color change of the central spot signaled the monkey to make a saccade to the target. During the saccade task in which the target was presented with surrounding distractors (Fig. 1C), the target was presented at one of four or eight positions around the central fixation spot and distractors at the other three or seven equally eccentric positions. The monkey was required to make a saccade to the stimulus that was different. Targets and distractors could be red and green squares or checkerboards of differing spatial frequencies and contrasts.

Each animal was tested for approximately three 3 h/day, 5 days/week. During testing, fruit juice was given as positive reinforcement. Monkeys' access to water in the home cage was controlled and monitored, fluids were supplemented as needed. Monkeys were seated in an enclosed chair within a magnetic field to monitor eye position using a scleral search coil. In all experiments using monkey M and the earlier experiments using monkey Q, stimuli were presented on a tangent screen in which five light-emitting diodes were embedded. In the later experiments using monkey Q and in all experiments using monkey B, the stimuli were presented on a video monitor (Conrac 7241, 60 Hz interlaced) using computer-controlled raster graphics (PDP 11/73, Peritek VCH-Q, 512×512 resolution). The fixation spot subtended 0.2° of visual angle and the target stimuli subtended from 0.3–1° of visual angle, depending on their eccentricity and were 30 cd/m² on a 1 cd/m² background.

Data collection

The experiments were under computer control (PDP 11/73), which presented the stimuli, recorded the eye movements, collected single-unit activity, and delivered the juice reward. Eye position was monitored with a scleral search coil (Robinson 1963) sampled at 200 Hz and stored with event times on disk for off-line analysis. Single units were recorded using glass-coated platinum-iridium or insulated tungsten microelectrodes that were under control of a hydraulic microdrive. The action potentials were amplified, filtered, and discriminated conventionally with a time-amplitude window discriminator and sampled at 1 kHz. Single units were admitted to the data base if the amplitude of the action potential was sufficiently above background to reliably trigger the time-amplitude window discriminator, the action potential waveshape was invariant, and the isolation could be sustained for a sufficient period for testing.

Data analysis

Saccades were detected using an algorithm that searched first for significantly elevated velocity (≥30 /s). Saccade initiation and termination then were defined as the beginning and end of the monotonic change in eye position, before and after the high-velocity gaze shift. Presaccadic movement cells and preparatory set cells were classified based upon their timecourse of activity according to the criteria of Schall (1991a,b). Only successful trials in which the saccade was generated for the cells' optimum direction and amplitude were analyzed.

Times of neuronal modulation were determined by employing the Poisson spike train analysis described below. The times of neu-

ronal modulation were determined to be stimulus- or movement-related with a linear regression analysis and by comparing the variances of the neuronal modulation times relative to the different events (i.e., trigger signal, saccade initiation; Seal et al. 1983; Commenges and Seal 1985). We compared these variances by using an F-test. The F-ratio was calculated by dividing the larger variance by the smaller variance (Kirk 1982). If this ratio was significant, then the neuronal modulation was related to the event, either the trigger signal or saccade initiation, that had the least variance. A Kolmogorov-Smirnov test was implemented to compare nonnormal distributions of the times of neuronal modulation of the different cell populations (Siegel and Castellan 1988).

Spike train analysis

Times of neuronal modulation were determined for each spike train using an adaptation of the Poisson spike train analysis originally described by Legéndy and Salcman (1985). It has been shown that a distribution of interspike intervals (ISIs) approximates a Poisson distribution (Rodieck et al. 1962; Smith and Smith 1965) and thus provides a good null hypothesis to detect changes in neuronal modulation (Legéndy and Salcman 1985). To verify that our data could also be fit with Poisson distributions, we determined the distributions of ISIs of cells recorded in both FEF and SEF. Previous work has shown that the saccade-related neurons in these areas exhibit at least two phases of activity, a resting level during fixation followed by an activated level prior to and during the saccade. Based on this common observation and for purposes of clarity, we tested the ISIs from these two periods separately. The best-fit Poisson distributions were determined using the method of least squares. The distributions of ISIs closely matched the best-fit Poisson distribution (Fig. 2). Figure 2A shows the ISIs for the period when the monkey began fixating the central fixation spot until the peripheral target was presented. Figure 2B shows the ISIs for the period from the presentation of the peripheral target until the reward was given. It is essential to understand that this separation of ISIs into two phases was shown only to demonstrate that the Poisson distribution provided an adequate null hypothesis for these cells. The actual method we applied to determine times of neuronal modulation does not compare two distributions of ISIs.

The Poisson spike train analysis determines how improbable it is that the number of action potentials within a specific time interval is a chance occurrence. This is achieved by comparing the actual number of spikes within a time interval to the number of spikes predicted by the Poisson distribution derived from the mean discharge rate during the entire time period in which deviations from randomness are sought. Legéndy and Salcman (1985) define this measure of improbability as the surprise index (SI), which generates higher values with increasing improbability and is computed with the following formula:

$$SI = -\log P$$

Thus, a large SI indicates a low probability that a specific elevation in activity was a chance occurrence. P is determined by Poisson's formula:

$$P = e^{-rT} \sum_{i=n}^{\infty} (rT)^i / i!$$

In our analysis, P is the probability that, given a mean discharge rate r, a spike train of a time interval T contains n or more spikes.

The determination of the mean discharge rate, r, is crucial. The goal of the Poisson spike train analysis is to determine whether or not the action potentials within a given interval of time are distributed randomly. In order to achieve this goal, we determined the Poisson distribution of action potentials for a spike train of the same time interval and containing the same number of spikes as the spike train being analyzed. Thus, r was defined as the mean discharge rate of the entire spike train being analyzed. We were interested in defining periods of neuronal modulation while the monkey was preparing and executing a goal-directed saccade; therefore, we analyzed the spike train recorded during each trial
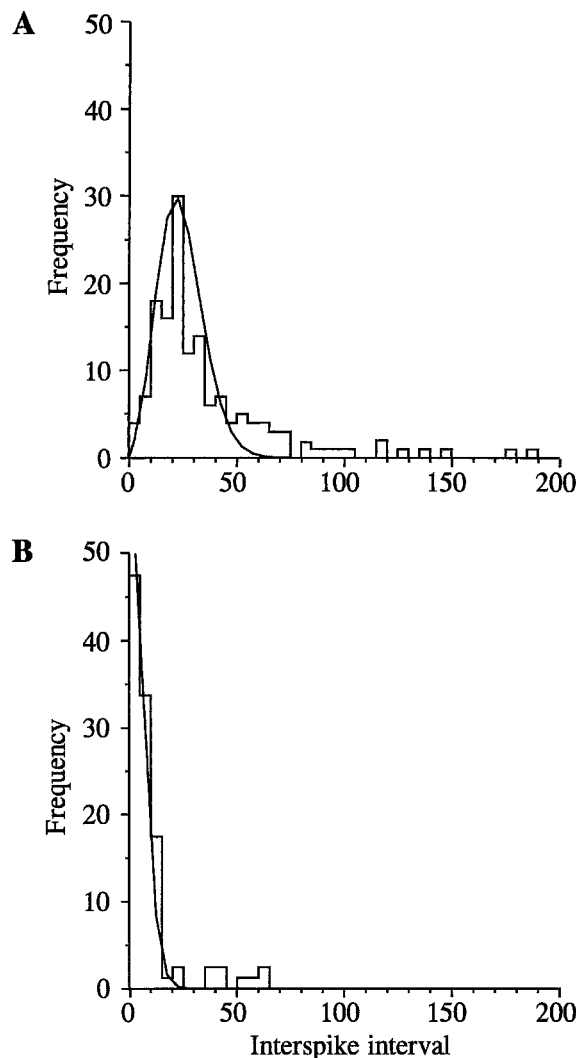
Fig. 2A,B Histograms of interspike intervals from a frontal eye field presaccadic movement neuron with superimposed best-fit Poisson distributions. **A** The interspike intervals for the period when the monkey began fixating the central fixation spot until the peripheral target was presented. **B** The interspike intervals for the period from the presentation of the peripheral target until the reward was given. The distributions of interspike intervals closely matched the best-fit Poisson distribution

greater than or equal to r. The time between these two spikes was the initial value of T. Then, the next spike was indexed and the ISI between this spike and the previous spike was added to T. The corresponding SI was then calculated. Successive spikes were then indexed and their ISIs were added to T until the end of the spike train. The SI was calculated after the addition of each ISI to T. The spike at the end of the interval T with the maximum SI value was defined as the end of the putative burst.

3. Next, the program indexed to the last spike in the spike train, and the SI was calculated for the time interval T from the last spike to the first spike after target presentation. The program then removed spikes from the beginning of the spike train until reaching the spike defining the end of the burst. The spike at which the SI was maximized was defined as the beginning of the putative burst. If the SI from the beginning of the burst to the end of the burst was not significant ($p<0.005$), the trial was defined as having no burst.

The method described to this point corresponds to that of Legéndy and Salcman (1985) who were interested in defining periods of bursts. For our purposes, it is important that many saccade-related neurons have a slow rise in activation before the burst (Wurtz and Goldberg 1972; Sparks 1978; Schlag and Schlag-Rey 1987; Glimcher and Sparks 1992), while other cells do not burst. Thus, it was necessary to determine when a nonbursting change in the neuron's activity became significantly different from the expected Poisson distribution. The significance level was usually set at $p<0.01$; however, some cells recorded in SEF did not exhibit bursting activity. For these cells the significance level was adjusted to $p<0.05$.

4. To determine the beginning of activation, the SI first was calculated from the end of the burst to the beginning of the burst, and then spikes before the beginning of the burst were added in succession until the SI fell below the desired significance level.

5. To determine the end of activation, the SI was first calculated from the beginning of the burst to the end of the burst, and then spikes after the end of the burst were added in succession until the SI fell below the desired significance level.

For nonbursting cells (SEF preparatory set cells), only the beginning of activation and the end of activation were used in subsequent analyses. For bursting cells (FEF and SEF presaccadic movement cells), if the beginning of activation and beginning of the burst occurred at the same point in time, the trial was defined as having no prelude of activity. Similarly, if the end of activation and the end of the burst occurred at the same point in time then the end of the burst was considered the end of significant activity. A copy of the Poisson spike train analysis algorithm written in C can be obtained via electronic mail from schalljd@ctrvax.vanderbilt.edu.

period (i.e., from the fixation of the central fixation spot until the juice reward was given). This length of time was sufficient to include the opportunity for a full cycle of activity for the cells being studied, but not too long to fail to account for changes in the overall level of excitability or arousal. If the spikes within the trial period occurred in a nonrandom fashion, then the Poisson spike train analysis determined the times at which the discharge rate changed from one level to another. It is important to note that the Poisson spike train analysis does not operate by finding deviations from some specified baseline level.

We applied the spike train analysis for each trial separately. The steps of the algorithm are as follows:
1. The mean discharge rate, r, during the trial being analyzed was determined as the number of spikes in the interval from the fixation of the central spot to the time the reward was given divided by the duration of that interval.
2. The program then indexed to the first spike after target presentation and advanced through the spike train until finding the first two consecutive spikes that had a mean discharge rate that was

Results

Thirty-one presaccadic movement cells in FEF and SEF, and preparatory set cells in SEF were analyzed in this study. Nine of these were FEF presaccadic movement cells (177 trials), 9 were SEF presaccadic movement cells (285 trials), and 13 were SEF preparatory set cells (275 trials). Thus, a total of 737 individual trials were analyzed.

FEF presaccadic movement cells

An example of an FEF presaccadic movement cell analyzed with the Poisson spike train analysis is shown in Fig. 3. Only trials in which the saccade was of the cell's optimum direction and amplitude were analyzed. Presaccadic movement cells were defined as those with no acti-

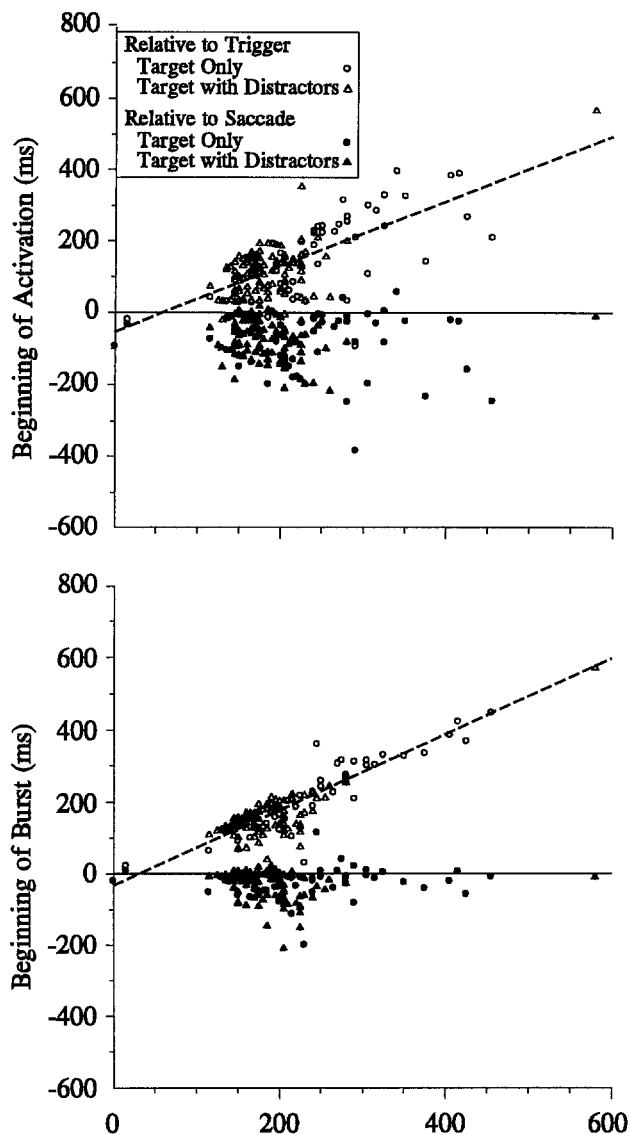Time from Saccade Initiation (ms)   *b171*
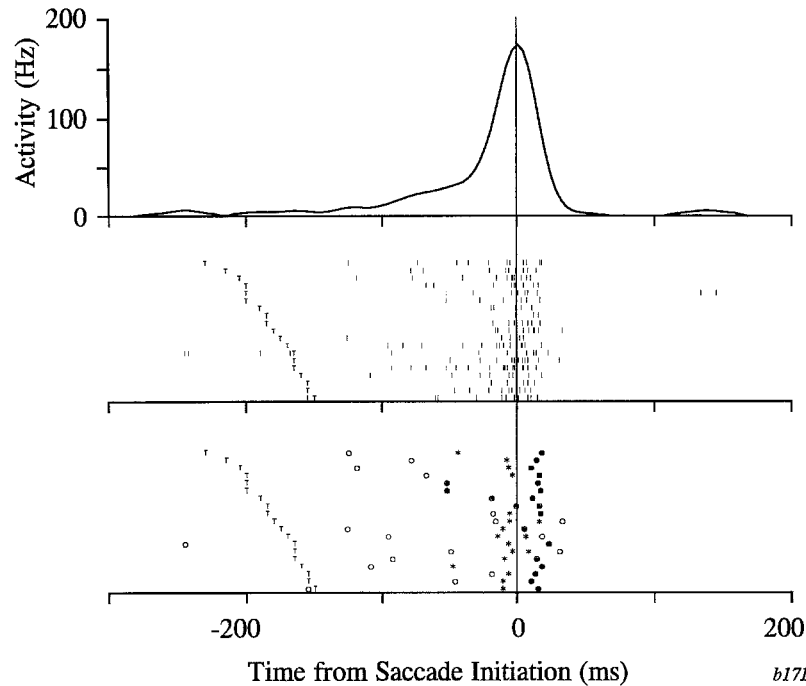
Fig. 3 A frontal eye field presaccadic movement cell recorded during the saccade task to a single target with surrounding distractors. Negative values on the *abscissa* indicate times before saccade initiation. The *top panel* shows the spike density function averaged across trials. The spike density function was generated by convolving the spike train with a Gaussian filter with σ=10 ms. In the *lower two panels* T represents the simultaneous presentation of the target and the trigger signal. The *middle panel* shows a raster display in which each *vertical tickmark* represents one action potential and each *row* represents a single trial. Trials are sorted by saccade latency. The *lower panel* shows the results of the Poisson spike train analysis from the corresponding trial in the *middle panel*. The *first open circle in each row* indicates the beginning of activation and the *second open circle* indicates the end of activation. The *first asterisk in each row* indicates the beginning of the burst and the *second asterisk* indicates the end of the burst. In some trials, the *asterisk* and *circle* are superimposed, indicating that the beginning or end of activation coincided with the beginning or end of burst

vation associated with target presentation and with a burst of activity occurring before and time-locked to saccade initiation. For all trials from the presaccadic movement cells analyzed, the beginning of activation, beginning of the burst, end of the burst, and end of activation were determined. Of the 177 trials analyzed, 85% exhibited a significant burst. In general, the beginning of the burst occurred a consistent interval before saccade initiation. As is evident from Fig. 3, the Poisson spike train analysis distinguishes between the prelude of activity and the burst on a trial-by-trial basis.
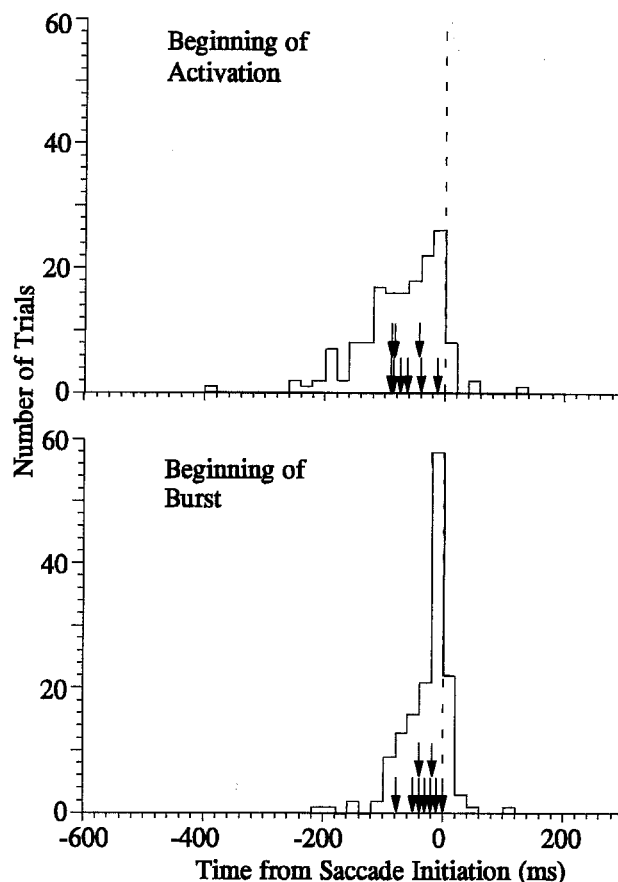
Data were collected from FEF presaccadic movement cells during all three task conditions. Of the 177 trials analyzed from FEF presaccadic movement cells, 52 trials were collected with the target alone and 125 trials were collected with a single target with surrounding distractors. We have recently reported that FEF visuomovement neurons, but not visual neurons, have a difference in modulation during these two task conditions (Schall and Hanes 1993; Thompson et al. 1993).

The ability to reliably identify periods of modulation within individual spike trains makes it possible to relate these periods to the behavioral events occurring during each trial. Figure 4 shows the relationship of the beginning of activation and beginning of the burst relative to both the trigger signal and saccade initiation as a function of saccade latency. The data when the target was presented alone or with surrounding distractors are shown separately in order to illustrate that these times of modulation were not significantly different during the two task conditions. For those trials in which the beginning of activation occurs earlier than the beginning of the burst, the beginning of activation serves as a measure of the beginning of the prelude of activity (e.g., Glimcher and Sparks 1992). The beginning of activation and beginning of the burst occurred at progressively longer intervals following trigger presentation for longer saccade latencies; for the longest latency saccade the beginning of activation and beginning of the burst occurred almost 600 ms after the trigger signal. A linear regression analysis showed that the time between the beginning of activation and the trigger signal increased significantly with saccade latency ($df=153$, $t=12.1$, $b=0.92$, $p<0.01$). Also, the time between the beginning of the burst and the trigger signal increased significantly with saccade latency ($df=148$, $t=21.2$, $b=1.00$, $p<0.01$). The beginning of activation generally occurred from 200 ms before to 10 ms after saccade initiation and the beginning of the burst from 100 ms before to 10 ms after saccade initiation. Although the beginning of activation was more variable than the beginning of the burst, both occurred at relatively consistent intervals before saccade initiation. The regression of the times of the beginning of activation and beginning of the burst relative to saccade initiation were not significant. In addition, the variance of the beginning of activation relative to the trigger signal was significantly greater than the variance of

Fig. 4 Scatterplots of the beginning of activation (*top*) and the beginning of the burst (*bottom*) as a function of saccade latency for all of the trials from nine frontal eye field presaccadic movement cells. A total of 177 trials are shown. For each trial the beginning of activation and the beginning of the burst is plotted twice, once relative to the trigger signal (*open symbols*) and once relative to saccade initiation (*solid symbols*). There were no significant differences between the trials collected during the saccade task to a single target (*circles*) and those collected during the saccade task to a single target with surrounding distractor (*triangles*). The time of neuronal modulation is negative if it occurred before the event relative to which it is measured. The *dashed diagonal lines* indicate a significant linear regression ($p<0.01$)


Fig. 5 Histograms of the beginning of activation times (*top*) and the beginning of burst times (*bottom*) relative to the time of saccade initiation for all rewarded trials from the frontal eye field presaccadic movement cells analyzed. Binwidths are 20 ms. Negative values on the *abscissa* indicate times before saccade initiation; *arrows* indicate individual cell means

the beginning of activation relative to saccade initiation ($F_{154,154}=1.91$, $p<0.01$). Likewise, the variance of the beginning of the burst relative to the trigger signal was significantly greater than the variance of the beginning of the burst relative to saccade initiation ($F_{149,149}=4.03$, $p<0.01$). The outcomes of these statistical tests indicate that both the beginning of activation and the beginning of the burst were more linked to saccade initiation than the presentation of the trigger signal (Commenges and Seal

1985). Although the results of these analyses are not surprising for this cell type, they illustrate that the Poisson spike train analysis reliably determines the temporal relationship between neural activity and saccade generation on a trial-by-trial basis.

The results of the Poisson spike train analysis indicate a functional linkage between the activity of FEF presaccadic movement neurons and saccade initiation. This relationship is evident in Fig. 5, which shows the distributions of the beginning of activation and the beginning of the burst for all trials from the FEF presaccadic movement cells relative to saccade initiation. The mean (±SEM) of the beginning of the burst for FEF presaccadic movement cells was 28±3.3 ms before saccade initiation, with a mode of 0–20 ms before saccade initiation. The times of neuronal modulation measured relative to saccade initiation are shown in Table 1. This mean latency corresponds to the latency of saccades evoked by suprathreshold microstimulation of FEF (Robinson and Fuchs 1969; Bruce et al. 1985). The beginning of activation occurred on average 73±5.5 ms before saccade initiation, with a mode of 0–20 ms before saccade initiation. The means of the beginning of activa-

Fig. 6 A supplementary eye field presaccadic movement cell. This cell was collected during the saccade task to a single target with an instructed delay. Conventions are as in Fig. 3, except that the *C* in the *middle panel* indicates the presentation of the trigger signal, and trials are sorted according to the interval between target presentation and saccade initiation
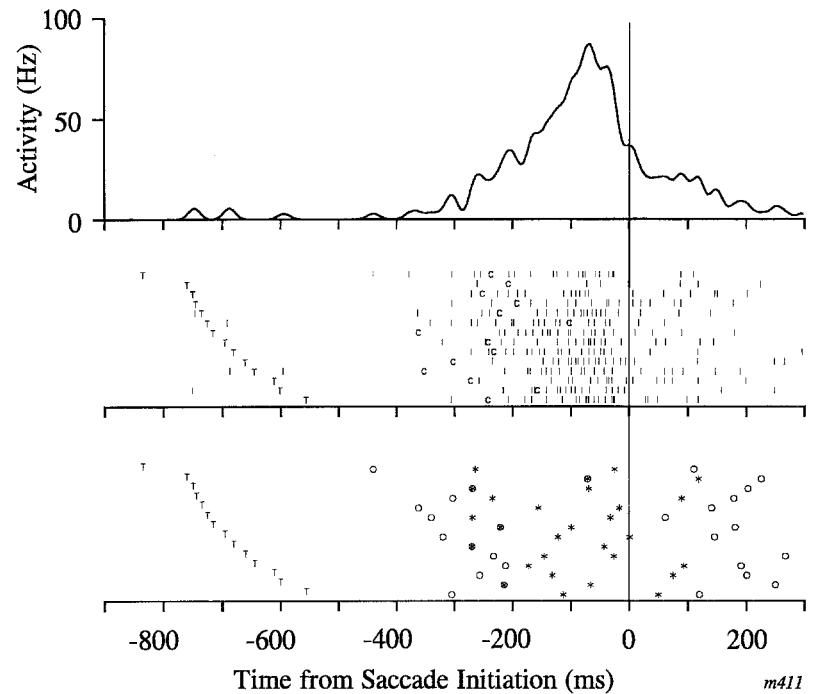
tion of each individual cell ranged from 13 to 90 ms before saccade initiation. The means of the beginning of the burst for each individual cell ranged from 13 to 78 ms before saccade initiation. In 55% of the trials that contained a burst, the beginning of significant activation occurred from 237 to 8 ms before burst onset (mean 83±7.3; mode 40–60 ms). In the remaining trials, the beginning of significant activation coincided with the beginning of the burst. In all FEF presaccadic movement cells analyzed, there were trials in which the beginning of activation occurred before the beginning of the burst, but some cells exhibited a prelude of activity more often than others.
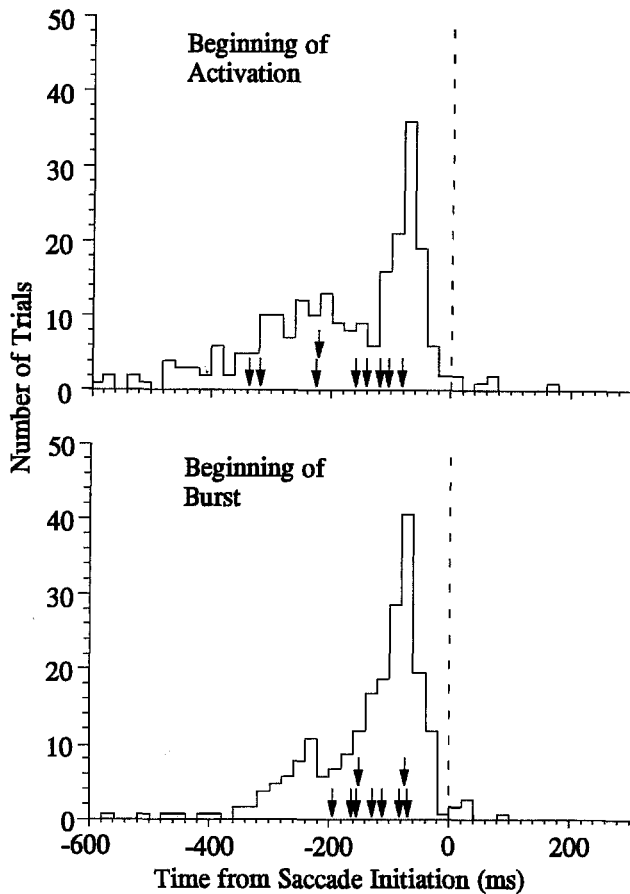
SEF presaccadic movement cells

Analyses identical to those performed on FEF presaccadic movement cells were performed on presaccadic movement cells recorded in SEF. An example of an SEF presaccadic movement cell analyzed with the Poisson spike train analysis is shown in Fig. 6. This cell was recorded while the monkey made saccades to the target presented alone, following an instructed delay ranging from 400 to 600 ms. Of the 285 trials analyzed, 78% exhibited a significant burst of activity. All SEF cells were tested using a single target with an instructed delay. SEF presaccadic movement cells differed from FEF presaccadic movement cells in that the times of modulation were more variable, the end of the burst occurred sometimes before and sometimes after saccade initiation, and the beginning of the burst generally occurred earlier in SEF than in FEF presaccadic movement cells.

In SEF presaccadic movement cells, the beginning of activation and beginning of the burst occurred at progressively longer interva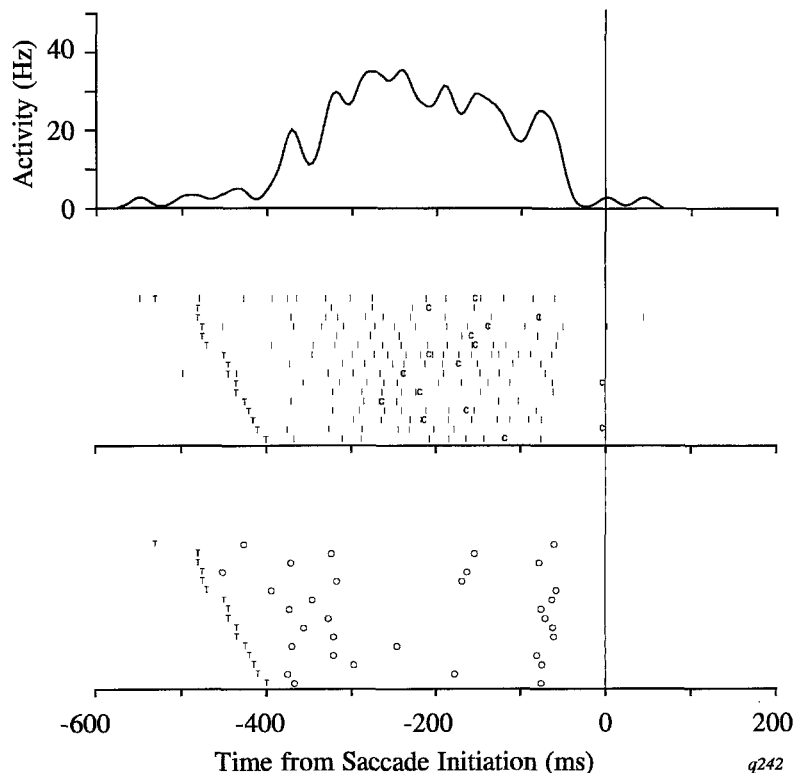ls following the trigger signal for longer saccade latencies. The scatterplots of these data were very similar to those from FEF presaccadic movement cells and thus are not illustrated. The beginning of activation generally occurred from 0 to 300 ms before saccade initiation. The beginning of the burst generally occurred from 20 to 250 ms before saccade initiation. A linear regression of the time between the beginning of activation and the trigger signal increased significantly with saccade latency (df=236, t=8.4, b=1.2, p<0.01), as did the time between the beginning of the burst and the trigger signal (df=221, t=12.0, b=1.2, p<0.01). The regression of the times of the beginning of activation and beginning of the burst relative to saccade initiation as a function of saccade latency were not significant. In addition, the variance of the beginning of activation relative to the trigger signal was significantly greater than the variance of the beginning of activation relative to saccade initiation ($F_{237,237}$=1.29, p<0.01). Also, the variance of the beginning of the burst relative to the trigger signal was significantly greater than the variance of the beginning of the burst relative to saccade initiation ($F_{222,222}$=1.64, p<0.01). The outcomes of these statistical tests indicate that both the beginning of activation and the beginning of the burst of SEF presaccadic movement cells were more linked to saccade initiation than the presentation of the trigger signal (Commenges and Seal 1985).

The distributions of the beginning of activation and beginning of the burst were more variable in SEF than in FEF presaccadic movement cells (Fig. 7). The overall mean of the beginning of the burst of SEF presaccadic movement cells was 135±6.6 ms before saccade initiation, with a mode of 60–80 ms before saccade initiation. The beginning of activation occurred on average 187±9.1 ms before saccade initiation, with a mode of 60–80 ms before saccade initiation (Table 1). The means

Fig. 7 Histograms of the beginning of activation times (*top*) and the beginning of burst times (*bottom*) relative to time of saccade initiation for all rewarded trials from the supplementary eye field presaccadic movement cells analyzed. Conventions are as in Fig. 5

for each individual cell range from 82 to 338 ms before saccade initiation for the beginning of activation and from 70 to 195 ms before saccade initiation for the beginning of the burst. In 43% of the trials that contained a burst, the beginning of significant activation occurred from 467 to 10 ms before burst onset (mean 148±11.2; mode 80–100 ms). In the remaining trials, the beginning of significant activation coincided with the beginning of the burst. In all SEF presaccadic movement cells analyzed, there were trials in which the beginning of activation occurred before the beginning of the burst, but some cells exhibited a prelude of activity more often than others.

SEF preparatory set cells

A particular population of cells in SEF has been identified that begin to discharge in relation to target presentation and cease firing before saccade initiation. This population has been referred to as preparatory set cells (Schall 1991a). An example of the activity of an SEF preparatory set cell is shown in Fig. 8. This type of cell does not exhibit burst-like activity, therefore the Poisson spike train analysis only generated reliable estimates of the beginning of activation and end of activation. Of the 275 trials analyzed, 88% exhibited significant levels of activation.

In SEF preparatory set cells, the end of activation occurred at progressively longer intervals following the trigger presentation for longer saccade latencies (Fig. 9). However, for shorter saccade latencies, less than 200 ms, the end of activation tended to occur before the trigger signal as indicated by the negative times. Despite the wide variation of the end of activation measured relative

Fig. 8 An SEF preparatory set cell. This cell was collected during the saccade task to a single target with an instructed delay task. Conventions are as in Fig. 6. Preparatory set cells did not exhibit bursting activity, therefore the Poisson spike train analysis only generated reliable estimates of the beginning of activation and end of activation

Time from Saccade Initiation (ms)

q242
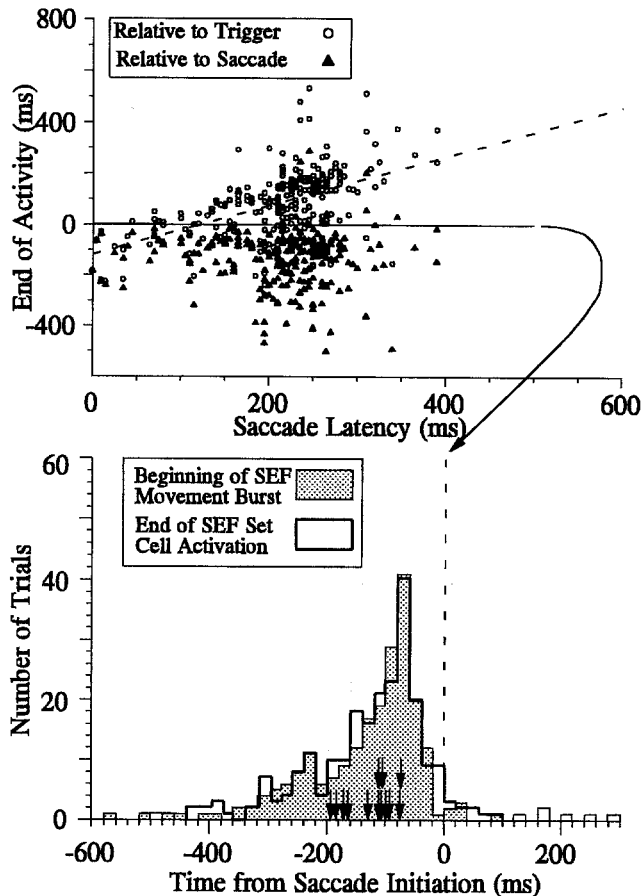
Fig. 9 Scatterplot (*top*) and histogram (*bottom*) of end of activation times relative to the time of saccade initiation for all rewarded trials from the SEF preparatory set cells analyzed. Conventions are the same as in Figs. 4 and 5, except that all SEF preparatory set cells were collected during the saccade task to a single target with an instructed delay. Overlayed on the histogram of the end of activation times for the SEF preparatory set cells is the histogram of the beginning of burst times for SEF presaccadic movement cells (*shaded area*). The distribution of the beginning of the burst of SEF presaccadic movement cells was not significantly different from the distribution of the end of activation of SEF preparatory set cells

to the trigger signal, this cessation of activity generally occurred from 0 to 250 ms before saccade initiation. A linear regression of the time between the end of activation and the trigger signal increased significantly with saccade latency (df=239, t=9.1, b=0.96, p<0.01). The regression of the time of the end of activation relative to saccade initiation as a function of saccade latency was

not significant. In addition, the variance of the end of activation relative to the trigger signal was significantly greater than the variance of the end of activation relative to saccade initiation ($F_{240,240}$=1.34, p<0.01). This demonstrates for the first time that the end of activation of preparatory set cells is significantly related to saccade initiation and not to the presentation of the trigger signal.

The distribution of the end of activation for all trials from SEF preparatory set cells relative to saccade initiation is shown in Fig. 9. The overall average end of activation for all trials from SEF preparatory set cells was 126±10.7 ms before saccade initiation, with a mode of 60–80 ms before saccade initiation (Table 1). The means of the end of activation for each individual cell ranged from 76 to 193 ms before saccade initiation.

The trial frequency distribution of the beginning of the burst of SEF presaccadic movement cells is replotted in Fig. 9, to provide a direct comparison between SEF presaccadic movement and preparatory set cells. The distribution of SEF preparatory set cell cessation of activity was not significantly different from the distribution of the beginning of the burst of SEF presaccadic movement cells, both occurring on average around 130 ms before saccade initiation (Kolmogorov-Smirnov; D=0.032, df=44,44).

Discussion

By implementing the Poisson spike train analysis on individual spike trains, times of modulation of neurons involved in saccade generation can be identified reliably on a trial-by-trial basis. We have used this approach to identify four separate times of change in neuronal activity: the beginning of activation, the beginning of the burst, the end of the burst, and the end of activation.

Three major findings emerged from the application of the Poisson spike train analysis to cells recorded in FEF and SEF. First, there are no significant differences in the timecourse of activity of the population of FEF presaccadic movement cells prior to saccades to singly presented or distractor-embedded targets. Second, the beginning of the burst of FEF presaccadic movement cells occurs at a time that corresponds to the latency of saccades generated by microstimulation of FEF (Robinson and Fuchs 1969; Bruce et al. 1985). Our inter-

Table 1 The mean, SEM, and mode of the times of the beginning of activation and the beginning of the burst measured relative to the time of saccade initiation are shown for frontal eye field (*FEF*) and supplementary eye field (*SEF*) presaccadic movement cells. The mean, SEM, and mode of the times of the end of activation measured relative to the time of saccade initiation are shown for SEF preparatory set cells. Times are measured in milliseconds

	FEF presaccadic movement cells		SEF presaccadic movement cells		SEF preparatory set cells
	Beginning of activation	Beginning of burst	Beginning of activation	Beginning of burst	End of activation
Mean	73	28	187	135	126
SEM	5.5	3.3	9.1	6.6	10.7
Mode	0–20	0–20	60–80	60–80	60–80

pretation of this result is that the beginning of the burst of FEF presaccadic movement cells may provide a signal to subcortical structures to generate a saccade. Third, the end of activation of SEF preparatory set cells coincides with the beginning of the burst of SEF presaccadic movement cells, suggesting that SEF preparatory set cells may influence movement cell activity and thus saccade initiation. The timecourse of neuronal modulation of cells recorded in FEF could not be reliably compared with neuronal populations recorded in SEF, because all cells recorded in SEF were collected during saccades to single targets with an instructed delay and very little of the data we analyzed from FEF was collected during the instructed delay task. It is noteworthy for further investigation, however, that we observed earlier activation in movement cells in SEF as compared to movement cells in FEF.

Poisson spike train analysis

In neurophysiological studies using awake behaving monkeys, many investigators are attempting to determine the relationship between a cell's discharge pattern and the concurrent behavior. The link between physiology and the behavior is a long-standing, fundamentally difficult problem (Teller 1984), hinging in part on how the discharges of neurons are described (Moore et al. 1967; Perkel et al. 1967). The relationship between neuronal modulation and visuomotor behavior has commonly been made by averaging some measure of cell activity across many trials (e.g., Bruce and Goldberg 1985; Waitzman et al. 1988; Schall 1991a,b; Segraves and Park 1993). A full understanding of saccade initiation, however, requires an account of the intrinsic variability of the spike train and saccade latency across trials. To do this, when one investigates the issue of saccade initiation, each trial should be analyzed individually. For cells that are well modulated, such as movement neurons in the superior colliculus, it has been effective to use prespecified criteria to determine times of neuronal modulation and relate these times to the gaze behavior on a trial-by-trial basis (e.g., Sparks and Mays 1980). However, for neurons with more variable activity, statistical criteria are necessary to define times of neuronal modulation on a trial-by-trial basis.

The analytical technique we applied identified segments of spike trains that are statistically unlikely to occur in comparison with the random Poisson distribution derived from the cell's mean firing rate. Legéndy and Salcman (1985) utilized the Poisson spike train analysis to determine the times of multiple bursts within spike trains of spontaneously active neurons in cat visual cortex. Another method has also been developed, by Cocatre-Zilgien and Delcomyn (1992), to determine the times of bursts within a single spike train, which is based on histograms of interspike intervals. For our analysis, we needed to determine the time of the burst within a relatively small period of time (a single trial from about 200 to 800 ms in duration). Often only ten or so spikes occurred during this interval, thus the method of Cocatre-Zilgien and Delcomyn (1992) would not be able to deter-

mine burst times because of an insufficient number of histogram bins. We based our analysis on the Poisson surprise index, because it did not require a large number of spikes within a spike train to identify periods of bursting activity.

Recently, there has been interest in differentiating between the low-frequency prelude of activity and the more vigorous burst of activity (e.g., Glimcher and Sparks 1992; see also Sparks 1978). We believe it is necessary to develop a method that can reliably and statistically differentiate the time of prelude onset from the onset of the burst on a trial-by-trial basis and also to identify periods of activation in nonbursting cells. Thus, we extended the method of Legéndy and Salcman (1985) to identify periods of activation at a specified significance level based on the intrinsic statistical properties of the individual spike train. It should also be stressed that the Poisson spike train analysis is capable of being applied to many different types of neuronal modulation. All that is required is a sufficient length of time that contains a full cycle of a cell's activity. Within that time interval either significant increases or decreases in activity can be detected.

Numerous studies have shown that ISI distributions can be fit with a Poisson distribution (Weiss 1964; Smith and Smith 1965; reviewed by Moore et al. 1967). Thus, a Poisson distribution serves as a good null hypothesis to detect changes in neuronal modulation. The analysis of our data utilizing the Poisson distribution revealed a precise and plausible temporal relationship between cell modulation and saccade generation. Of course, ISIs may be better fit by a nonPoisson distribution, for example, a Gamma distribution (Kuffler et al. 1957; Bishop et al. 1964). Further work is needed to determine whether the type of analysis we applied is improved by using another type of distribution as the null hypothesis (Commenges and Seal 1985), although the benchmark by which to judge improvement is not presently clear.

Implications for the neural regulation of saccade initiation

While much is understood about the final stages of saccade generation within the brainstem, little is known about mechanisms regulating when gaze shifts will occur. For example, there is insufficient experimental data to understand what accounts for the apparent "oculomotor procrastination" (Carpenter 1981). Nevertheless, circuits have been identified that are believed to initiate saccades. Saccade initiation and termination are gated by omnipause neurons in the nucleus raphe interpositus that cease firing about 15 ms before saccade initiation (Henn and Cohen 1976; Keller 1974). Control of these pause neurons arrives from the superior colliculus, the frontal eye fields, and the supplementary eye fields (Raybourn and Keller 1977; Huerta et al. 1986; Stanton et al. 1988; Huerta and Kaas 1990; Shook et al. 1990; Segraves 1992). Movement neurons in the superior colliculus exhibit a low frequency prelude of activation that can begin 100–200 ms before the saccade, followed by a high-fre-

quency burst immediately before and during the saccade (Wurtz and Goldberg 1972; Sparks 1978; Glimcher and Sparks 1992). Sparks (1978) showed that this burst of activity of movement neurons in superior colliculus precedes saccade initiation by approximately 20 ms.

Currently, the temporal relationship of FEF and SEF cell activity to saccade initiation is less clear. Previously, two studies have investigated the timecourse of activity of neurons in FEF (Schall 1991b; Segraves and Park 1993). Schall (1991b) identified the onset of activity of FEF presaccadic movement neurons by detecting changes in the slope of the cumulative sum of the spikes across all trials (Falzett et al. 1985). He determined the mean onset time of FEF presaccadic movement neurons to be approximately 130 ms before saccade initiation. Recently, Segraves and Park (1993) analyzed FEF presaccadic movement neurons identified by antidromic stimulation of the nucleus raphe interpositus and superior colliculus. Using Gaussian spike density functions averaged across trials, they defined the onset of activity as the first time the activity rose above the baseline firing rate. Based on this analysis, Segraves and Park found that the FEF corticopontine and corticotectal neurons begin to discharge approximately 150 ms before the saccade. The peak activity of corticopontine and corticotectal cells occurred around 13 ms before the saccade. They surmised that this mean time of peak discharge may signal the time to initiate a saccade. In the present study, we showed that the beginning of the burst of FEF presaccadic movement neurons occurred, on average, 28 ms before saccade initiation. Both Schall (1991b) and Segraves and Park (1993) determined the time of the onset of activity by measuring elevations above a baseline level. The times they determined are closer to the time we found for the beginning of significant activation (73 ms before saccade onset). We determined burst onset and the beginning of significant activation by implementing the Poisson spike train analysis. Thus, the different quantities obtained by Schall (1991b), Segraves and Park (1993), and ourselves are probably due to the use of different methods for determining times of neuronal modulation and because we were identifying two different states of neuronal activity (i.e., prelude and burst).

Schall (1991a) determined the timecourse of activity of neurons in SEF by detecting changes in the slope of the cumulative sum of the spikes across all trials. In the present study we analyzed a subset of the SEF data analyzed in Schall (1991a). The difference is that our analysis utilized the Poisson spike train analysis to determine times of neuronal modulation on a trial-by-trial basis. Schall (1991a) determined the mean offset time of SEF preparatory set neurons to be approximately 112 ms before saccade initiation. Schall (1991a) also determined the mean onset time of SEF presaccadic movement neurons to be approximately 144 ms before saccade initiation. Using the Poisson spike train analysis, we discovered that the distribution of SEF preparatory set cell cessation of activity was not significantly different from the distribution of the beginning of the burst of SEF presac-

cadic movement cells, both occurring on average around 130 ms before saccade initiation. Once again, the different quantities obtained by Schall (1991a) and ourselves is surely due to the use of different methods for determining times of neuronal modulation.

Conclusions

In the current study we show that the activity of FEF presaccadic movement cells, SEF presaccadic movement cells, and SEF preparatory set cells is related to saccade initiation. The time of the onset of bursting activity (on average 28 ms before saccade initiation) in FEF presaccadic movement cells corresponds to the latency of saccades generated by microstimulation of FEF (Robinson and Fuchs 1969; Bruce et al. 1985). Further, we were surprised to find that the distributions of the end of activation of SEF preparatory set cells and the beginning of the burst of SEF presaccadic movement cells were virtually identical. This result suggests that preparatory set cell activity in SEF may gate the activation of SEF presaccadic movement cells, thus leading to saccade generation. While we are cautious about attributing a specific causal sequence of events, we think these results indicate the potential power of the Poisson spike train analysis to address other questions about the neural mechanisms that guide saccade selection and regulate saccade initiation.

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Note added in proof. The Poisson spike train analysis reported in this paper identified one burst of activity. We have subsequently modified the algorithm to identify multiple bursts of activity within a single spike train.