Response Properties of Fixation Neurons and Their Location in the Frontal Eye Field in the Monkey

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Izawa Y, Suzuki H, Shinoda Y. Response properties of fixation neurons and their location in the frontal eye field in the monkey. J Neurophysiol 102: 2410–2422, 2009. First published August 12, 2009; doi:10.1152/jn.00234.2009. Electrical stimulation of the frontal eye field (FEF) has recently been reported to suppress the generation of saccades, which supports the idea that the FEF plays a role in maintaining attentive fixation. This study analyzed the activity of fixation neurons that discharged during fixation in the FEF in relation to visual fixation and saccades in trained monkeys. The neural activity of fixation neurons increased at the start of fixation and was maintained during fixation. When a fixation spot of light disappeared during steady fixation, different fixation neurons exhibited different categories of response, ranging from a decrease in activity to an increase in activity, indicating that there is a continuum of fixation neurons, from neurons with foveal visual-related activity to neurons with activity that is related to the motor act of fixating. Fixation neurons usually showed a decrease in their firing rate before the onset of visually guided saccades (Vsacs) and memory-guided saccades in any direction. The direction in activity of fixation neurons nearly coincided with, or occurred slightly before, the increase in the activity of saccade-related movement neurons in the FEF in the same monkey. Although fixation neurons were scattered in the FEF, about two thirds of fixation neurons were concentrated in a localized area in the FEF at which electrical stimulation induced strong suppression of the initiation of Vsacs bilaterally. These results suggest that fixation neurons in the FEF are part of a suppression mechanism that could control the maintenance of fixation and the initiation of saccades.

INTRODUCTION

The frontal eye field (FEF) has been implicated in saccade generation. It has been well established that electrical stimulation of the FEF produces saccades (Bruce et al. 1985; Robinson and Fuchs 1969). In addition, recent studies have shown that the FEF exerts suppressive control on saccades. Electrical stimulation studies in the monkey have shown that FEF stimulation suppressed the generation of saccades to visual targets (Azuma et al. 1986; Burman and Bruce 1997; Izawa et al. 2004a,b). Stimulation of a wide area of the FEF suppressed only ipsiversive visually guided (Vsacs) and memory-guided saccades (Msacs) at stimulus intensities lower than those that elicited electrically evoked saccades (Esacs) (Izawa et al. 2004a), whereas stimulation of a localized area of the FEF in the caudal part of the arcuate gyrus facing the inferior arculate sulcus suppressed both Vsacs and Msacs in any direction (Izawa et al. 2004b). The suppressive function of the FEF on the generation of saccades may be related to visual fixation. When an interesting object appears in a visual field, visual fixation is required to hold the image of the target on the fovea. Therefore the suppression of potential saccades to other objects in the visual field must be essential during fixation. In fact, the FEF contains fixation neurons that show continuous activity during fixation (Bizzi 1968; Suzuki and Azuma 1977), and fixation neurons have been found at suppression sites in the FEF (Izawa et al. 2004b).

Bizzi (1968) first noted that FEF neurons discharge during fixation. He classified such neurons with responses that depended on the direction of gaze as type II neurons. Suzuki and Azuma (1977) reported that many prefrontal neurons showed an increase in their firing rate during fixation. They stated that such “gaze neurons” showed a variety of discharge patterns during fixation with and without a target and suggested that their activity was not simply visual but was related to active fixation (Suzuki et al. 1979). Later, fixation neurons were shown to consist of corticofugal neurons in the FEF projecting to the superior colliculus (SC) (Segraves and Goldberg 1987; Sommer and Wurtz 2000) and the pons (Segraves 1992), consistent with anatomical projections from the FEF (Komatsu and Suzuki 1985; Künzle and Akert 1977; Stanton et al. 1988).

In the FEF, the activity of visual- or movement-related neurons has been extensively analyzed. On the other hand, to our knowledge, the activity of fixation neurons in the FEF must be studied further from the perspectives of both the maintenance of fixation and the initiation of saccades.

This study was performed to systematically analyze the activity of fixation neurons in the FEF in relation to the maintenance of fixation and the initiation of saccades in trained monkeys. In addition, we studied the distribution of fixation neurons in the FEF in relation to the ipsilateral (Izawa et al. 2004a) and bilateral suppression areas in the FEF (Izawa et al. 2004b). This report will describe the discharges of FEF fixation neurons and discuss their functional implication in relation to visual fixation and the initiation of saccades.

METHODS

Experiments were performed in two male Japanese monkeys (Macaca fuscata) weighing 7 and 9 kg, respectively. The surgical procedures have been described in previous reports on experiments in which the same monkeys were used (Izawa et al. 2004a,b). All animal experimentation was conducted in accordance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council, 1996), and the Guiding Principles for the Care and Use of Animals in the Field of Physiological Sciences (The Physiological Society of Japan, revised in 2001). All
surgical and experimental protocols were approved by the Animal Care Committee of Tokyo Medical and Dental University.

Behavioral training

During training and experimental sessions, the monkey was seated in a primate chair facing a tangent translucent screen 1.5 X 1.5-m square and 57 cm in front of it. Each monkey was first trained to fixate on a tiny spot of light (0.4° in visual angle, 2 cd/m²) that was back-projected at the center of the screen using a pair of mirrors attached to galvanometers (Suzuki and Azuma 1977; Wurtz 1969). The screen was evenly illuminated at 1 cd/m² to eliminate stray light around the spot. Monkeys were trained to perform a fixation task, fixation blink task, Vsac task, and M sac task:

FIXATION TASK. The monkey fixated on a center spot and pressed a bar with its right hand on the appearance of the spot, which occurred after an intertrial interval of 3–5 s. While the bar was held down, the spot remained illuminated for a variable duration of 1–4 s. The monkey was required to maintain its line of sight within an error window of ±2° around the fixation target. The target was then slightly brightened (0.3 log unit) for 0.5 s. If the monkey released the bar during this short brightening period, it received 0.2 ml of juice as a reward. Otherwise, the trial was terminated without a reward, and a new trial began. Fixation behavior was elicited, because the monkey had to look at the spot to notice its brightening for rapid bar-release.

FIXATION BLINK TASK. In the fixation blink task, the monkey first fixated on a center spot. After 1 s, the spot disappeared for 400 ms while the monkey had to maintain fixation. The spot then reappeared at the same place. The monkey was rewarded if it steadily fixated throughout the trial and if it released the bar only during the short brightening period, as in the fixation task. This paradigm enabled us to test the effects of visual stimulation of the fovea on neuronal activity.

VSAC TASK. When training of the fixation task was completed, the monkey was required to make Vsacs. For this task, after a fixation period of 1–1.5 s, the center spot was turned off, and another light spot was simultaneously turned on elsewhere on the screen as a target. The monkey learned to make a saccade to the target, because it had to observe its brightening. If the monkey released the bar during brightening of the target light, it received a reward.

MSAC TASK. In the M sac task, the monkey first fixated on a center spot. During this fixation, another instruction target spot was flashed at a location on the screen for 0.5 s. This time, the monkey was required to maintain its line of sight on the center spot, while the center spot remained on. At 0.5–1.5 s after the flashed target, the center spot was turned off as a cue to make a saccade. The monkey had to make a saccade to the previously instructed location within an error window of ±3° around the visual target. Otherwise, the trial was terminated without a reward, and a new trial began. At 0.6–1.5 s after the disappearance of the center spot, the target spot that had been flashed previously was turned on again for 0.5 s to confirm a correct Msac. If the monkey released the bar only during this short brightening period, it received a reward. This task enabled us to distinguish between visual- and movement-associated neuronal activity during fixation and the presence of tonic activity during fixation (fixing rate during steady fixation 0.5–2.5 s after bar-press onset was > 2 SD above the mean fixing rate during a 2-s period in the intertrial interval ≥1 s before bar-press onset in the fixation task) (Suzuki and Azuma 1977). Although we mainly searched for FEF fixation neurons during penetrations, we also identified other classes of FEF neurons using the criteria of Bruce and Goldberg (1985). Neurons with peripheral visual activity but no presaccadic burst activity were classified as visual neurons, neurons with presaccadic burst activity but no peripheral visual activity were classified as movement neurons, and neurons with both peripheral visual and presaccadic burst activity were classified as visuomovement neurons, and their activity was compared with that of fixation neurons. Discrimination of the action potentials of single neurons from the extracellular recording signal was achieved with a conventional amplitude window discriminator. Several representative recording sites in one monkey were marked with iron deposits by passing currents (electrode positive, 400 μCoulombs) through the elgiloy microelectrode (Suzuki and Azuma 1987). At the end of the experiment, the monkeys were deeply anesthetized with pentobarbital sodium and perfused with 6 l of saline followed by 6 l of a fixative solution containing 10% formalin. For one monkey with marked recording sites, the fixative solution also contained 2% ferrocyanide. Serial frozen sections of 80 μm were cut coronally from the frontal cortex and stained with thionine. The sections were reconstructed using a camera lucida system and three-dimensional graphics software (Rhinoeros, Robert McNeel and Associates, Seattle, WA). Recording sites were histologically verified to be located in the prearcuate gyrus.

Experimental control and data acquisition

The behavioral tasks, presentation of light spots, and data acquisition were controlled by IBM-compatible computers. Eye movements were recorded by a camera measurement system, using the corneal reflection image of infrared light (Azuma et al. 1996), with which we could measure horizontal and vertical eye positions with an accuracy of 0.3° and at a sampling rate of 4 ms. Eye-position signals were calibrated by having the monkey fixate on targets at known eccentricities (10, 20, and 30°) on the horizontal and vertical meridians and diagonal axes. Horizontal and vertical component signals of eye movements and neuronal activity with respect to behavioral event indicators were stored on computer hard disks and displayed on an oscilloscope. Eye position and neural activity were sampled every 4 and 1 ms, respectively. The onset of each saccade was identified in the eye-position traces by a mouse-controlled cursor. Subsequent off-line data analyses were performed using Matlab (MathWorks, Natick, MA) programs. To determine the onset of modulation in the firing rate, we applied the one-sample Kolmogorov-Smirnov test to spike histograms with 10- or 20-ms binwidths aligned with respect to behavioral events (Mano and Yamamoto 1979; Yamauti 1972). We calculated the largest difference between the cumulative distribution curves of sample spike frequencies and spike frequencies during the control period (300 ms for each measurement as described in RESULTS). The modulation in firing rate was considered to be signifi-
cant when the difference exceeded a 0.01 level of significance for a sample of four consecutive bins (1 sided), and the onset time was determined when the increase or decrease in spike frequencies reached a 0.05 level. The one-sample Kolmogorov-Smirnov test was also applied to interspike intervals of spike rasters to confirm measurements made from histograms. Statistical analysis was performed with a Kruskal-Wallis ANOVA. Correlations between data sets were assessed by measuring the Pearson correlation coefficient.

RESULTS

We studied single units in the FEF and its vicinity in the prearcuate gyrus. Before extracellular recording experiments, we systematically examined the effects of stimulation of the FEF and its vicinity in the prearcuate gyrus (Izawa et al. 2004a,b). In each track, we examined depth-thresholds for evoking Esacs and suppressing Vsacds. This large database of depth-threshold curves for Esacs and the suppression of sac- cades was used to delineate the FEF physiologically in these monkeys. We recorded the activity of 113 fixation neurons in this region. In addition, we studied other classes of FEF neurons during penetrations to identify differences in activity between them. We recorded the activity of 18 visual neurons, 13 visuomovement neurons, 17 movement neurons, 4 smooth pursuit neurons, and 18 other neurons, including neurons that discharged during the intertrial interval before the beginning of a trial. Therefore virtually all of the identified classes of FEF neurons were encountered during these penetrations (Bruce and Goldberg 1985).

Activity of FEF fixation neurons during attentive fixation

The activity of a fixation neuron during visual fixation is shown in Fig. 1A. During an intertrial interval of 3–5 s, the monkey was free to move its eyes. The activity of this neuron was relatively low and variable during this period. When a fixation spot appeared and the monkey foveated it, this neuron increased its firing rate. This increase in activity persisted during fixation. When the fixation spot disappeared, this neuron ceased discharging and the monkey started to make sac- cades spontaneously. In this way, this neuron increased its firing rate at the start of fixation and discharged during fixation, so that it was classified as a fixation neuron (Suzuki and Azuma 1977). To examine the effect of fixation duration on the activity of the fixation neuron, the fixation point was turned on for a random duration of 1–4 s in the fixation task (Fig. 1B). As the fixation duration increased, the activity of the fixation neuron persisted throughout the fixation period. As in this example, the activity of fixation neurons continued during

FIG. 1. Activity of single fixation neurons in the frontal eye field (FEF) during visual fixation. A: activity of a fixation neuron in fixation trials with a constant fixation duration. Neuronal discharges in successive fixation trials are represented by rasters. The histogram is the result of summing the dots on the raster in 50-ms bins. Horizontal and vertical components of eye position are shown above the raster. The rasters and histogram displays are aligned with respect to bar-press onset (vertical dotted line). The 1st to 4th triangles below each raster line mark the appearance of the central fixation point, the brightening of the fixation point (cue onset), bar-release (reward onset), and reward offset, respectively. The label in the top left of the histogram is the cell number. B: activity of a fixation neuron in fixation trials with a random fixation duration of 1–4 s. Same neuron as in A. The rasters are arranged according to fixation duration. The histogram shows the discharge rate of this neuron before and during the fixation period, so that the discharge rate after the fixation period is not included. Note that sustained activity of the fixation neuron persisted during fixation regardless of the fixation duration. The calibration in B applies to A. C: activity of a fixation neuron aligned with respect to fixation onset (a) and fixation offset (b). The histogram shows the sum of the discharge in 20-ms bins. D: latency histogram of the increase in activity of fixation neurons that was related to fixation onset (arrowhead).
fixation regardless of whether the target had a fixed or random duration.

We studied the relationship between the discharge onset of fixation neurons and behavioral events in more detail. Figure 1Ca shows the activity of a fixation neuron aligned with respect to the onset of fixation. The activity of the fixation neuron increased after the monkey foveated the target light. Usually, a fixation spot was turned on first, and the monkey made a saccade to foveate it. However, the monkey sometimes started fixation before the onset of the fixation point. This behavior was observed even when we used a random intertrial interval of 3–5 s. In this case, a fixation spot of light illuminated the fovea after the onset of fixation. However, the activity of the fixation neuron increased soon after spontaneous fixation onset and before the appearance of the fixation spot. Therefore this result indicated that this fixation neuron discharged in relation to the motor act of fixating rather than in response to visual stimulation of the retinal fovea. We also aligned raster and histogram displays with respect to the onset of a fixation spot or bar-press. As in this example, fixation neurons usually increased their firing rate at the start of fixation rather than the onset of a fixation spot or bar-press. We assessed the increase in the activity of fixation neurons at the start of fixation in the fixation task compared with the firing rate during the intertrial interval (600–900 ms before fixation onset; 1-sample Kolmogorov-Smirnov test, \( \alpha < 0.05 \)). The latencies of the increase in activity from fixation onset had a mean of 50.1 ± 69.3 (SD) ms (\( n = 78; \) Fig. 1D). The remaining 16 neurons increased their firing rate in relation to the appearance of a fixation spot, and 19 neurons showed a gradual increase in activity after bar-press or had a discharge onset that was not clearly related to behavioral events.

In a similar way, we studied the relationship between the discharge offset of fixation neurons and behavioral events. Figure 1Cb shows the activity of the same fixation neuron as in Fig. 1Ca, aligned with respect to the offset of fixation. The decrease in the activity of this fixation neuron occurred ~130 ms before the monkey moved its eyes after the disappearance of the fixation spot. We also aligned raster and histogram displays with respect to the onset of brightening of the fixation spot and the offset of the fixation spot (bar-release). As in this example, fixation neurons with a decreased firing rate at the end of fixation preceding the onset of eye movements were prevalent.

We observed some different patterns of activity of fixation neurons during the fixation task. Figure 2A shows the discharge pattern of a fixation neuron with tonic activity at a maintained firing rate throughout fixation. Figure 2B shows another discharge pattern of a fixation neuron for which the greatest activity occurred at the very beginning of fixation, and this was followed by tonic discharge during the rest of fixation. Figure 2C shows another discharge pattern of a fixation neuron with tonic activity that occurred after the monkey attained fixation, and this was followed by increased activity at the end of the trial when the monkey was close to getting a reward. The other neurons had increased activity at the beginning and end of fixation, with a lower tonic level in between. The whole population of fixation neurons is shown in Fig. 2D, where a late fixation activity at the end of the central fixation period is plotted against an early fixation activity at the beginning of the fixation period. This scatter plot indicates that these populations of fixation neurons were not discrete but rather formed a continuum.

In the fixation task, the fovea was illuminated with a fixation spot of light during fixation. To separate the visual and motor aspects of activity during fixation, the monkey was required to perform the fixation blink task. Figure 3A shows the response of a fixation neuron that showed a decrease in activity when the fixation spot was turned off during the trial and that resumed firing in response to reappearance of the fixation point. The intensity of the reduction in activity during the disappearance of the fixation spot varied among fixation neurons, from a total cessation of spontaneous activity to a slight decrease. We considered a neuron to be a foveal visual neuron if it ceased firing just after disappearance of the visual fixation spot. Figure 3B shows the response of a fixation neuron that maintained activity after disappearance and reappearance of the fixation spot. In contrast, Fig. 3C shows the response of a fixation neuron that showed a vigorous increase in activity after the fixation spot disappeared, and its usual activity returned after the fixation spot reappeared. The increase in activity persisted for hundreds of milliseconds after the fixation spot disappeared and was sometimes followed by a decrease.
When the light intensities of the fixation spot were gradually decreased from 0.3 to 1.5 log unit at an interval of 0.2 log unit, no change in activity was observed for two of these neurons. Eleven neurons showed an increase in activity with a phasic component just after disappearance of the fixation spot, which died away before reappearance of the spot. These fixation neurons may respond to both fixation and foveal visual-off-signal. Fixation neurons with activity during the period when the fixation spot was off tended to be active during spontaneous fixations on the blank screen during an intertrial interval or in the dark. To study the effects of blink manipulation, we compared the activity during the same epoch in the fixation and fixation blink tasks for 80 fixation neurons. In Fig. 3D, activity during fixation without a target (100–400 ms after the disappearance of the fixation point in the fixation blink task) is plotted against activity during fixation with a target (0–300 ms before disappearance of the fixation point) for all of the neurons tested. The distribution of fixation neurons in this scatter plot formed a continuum, from neurons with a decrease in firing to neurons with an increase in firing in response to the offset of visual stimulation of the fovea.

We assessed the change in activity of fixation neurons after disappearance of the fixation spot in the fixation blink task compared with the firing rate during fixation with a target (0–300 ms before disappearance of the fixation point) and measured the latency of the reduction (Fig. 4A) or increase in activity (Fig. 4B; 1-sample Kolmogorov-Smirnov test, α < 0.05). The latencies of the reduction in activity from fixation spot offset had a mean of 75.7 ± 26.3 ms (n = 21; Fig. 4C), whereas the latencies of the increase in activity from fixation spot offset had a mean of 84.7 ± 30.6 ms (n = 36; Fig. 4D). Compared with the latency of the decrease in activity, the latency of the increase in activity for a fixation neuron from fixation spot offset tended to be longer and more variable in the fixation blink task.

**FIG. 3.** Different patterns of activity of single fixation neurons in the FEF during the fixation blink task. A: an example of neuronal discharge with a decrease in activity during the disappearance of the central fixation point. B: an example of neuronal discharge where tonic activity was maintained during the disappearance of the fixation point. C: an example of neuronal discharge with an increase in activity during the disappearance of the fixation point. The histogram is aligned with respect to the disappearance of the fixation point (vertical dotted line). The 1st to 3rd triangles below each histogram mark press-onset, the reappearance of the fixation point, and cue onset, respectively. The small bar below each histogram marks the 300-ms period measured in D. D: summary of activity of fixation neurons during fixation with and without a target (n = 80). Firing rate during a 300-ms period beginning 100 ms after the disappearance of the fixation point in the fixation blink task plotted against the firing rate during the same 300-ms period in the fixation task. Dashed line has a slope of 1.0.

**FIG. 4.** The onset of the change in activity of single fixation neurons at the disappearance and reappearance of the fixation point in the fixation blink task. A: decrease in activity during the disappearance of the fixation point. Same neuron as in Fig. 3A but in expanded time scale to show the onset of the decrease in activity. B: increase in activity during the disappearance of the fixation point. Same neuron as in Fig. 3C but in expanded time scale to show the onset of the increase in activity. Vertical dotted line, disappearance of the fixation point; Triangle below each histogram, reappearance of the fixation point. C: latency histogram of the decrease in activity of fixation neurons from fixation spot offset (arrow). D: latency histogram of the increase in activity of fixation neurons from fixation spot offset (arrow).
Activity of FEF fixation neurons during saccades

To study the activity of fixation neurons during saccades, the monkey was required to perform the Vsac task. The example in Fig. 5A shows the activity of a fixation neuron during the Vsac task. This neuron was active while the monkey maintained fixation and stopped firing before the beginning of ipsiversive Vsacs (Fig. 5Aa). This reduction in activity was not caused by disappearance of the fixation point in the Vsac task but was related to making a Vsac, because this neuron showed an increase in activity during disappearance of the fixation point in the fixation blink task. After the Vsac, the monkey fixated on the target presented at the new location and the neuron resumed its firing rate. This neuron also stopped firing before the beginning of contraversive Vsacs and resumed its firing rate during steady fixation, as indicated by the firing rate during steady fixation (300–600 ms before Vsac onset; Fig. 5Ab). We examined activity during Vsacs in 74 of 113 fixation neurons, and most of them (62%, 46/74) decreased their firing rate before the onset of Vsacs in both directions, as in this example. The reduction in activity of the fixation neuron preceded the onset of ipsiversive Vsacs by ~110 ms compared with the firing rate during steady fixation (300–600 ms before Vsac onset). Overall, the latencies of the reduction in activity from the onset of ipsiversive Vsacs had a mean of −102.6 ± 40.8 ms (n = 46; 1-sample Kolmogorov-Smirnov test, α < 0.05; Fig. 5Ca). In a similar range, the reduction in activity of the fixation neuron preceded the onset of contraversive Vsacs compared with the firing rate during steady fixation (300–600 ms before Vsac onset), and the latency was ~90 ms (Fig. 5Bb). Overall, the latencies of the reduction in activity from the onset of contraversive Vsacs had a mean of −100.2 ± 35.8 ms (n = 46; 1-sample Kolmogorov-Smirnov test, α < 0.05; Fig. 5Cb). In the remaining fixation neurons, nine showed a reduction in activity only during or biased for ipsiversive Vsacs (Fig. 5D), whereas one showed a reduction in activity only during or biased for contraversive Vsacs, six showed no change in activity during Vsacs in both directions, five showed an increase in activity only during or biased for contraversive Vsacs, and seven showed an increase in activity during Vsacs in both directions.

In seven of the above fixation neurons with a bilateral reduction in activity during Vsacs, we examined the effects of the direction of Vsacs in more detail. The directions of Vsacs were varied among eight cardinal directions with the amplitude fixed at 10°. We found that the activity of a fixation neuron decreased during both ipsiversive and contraversive Vsacs in any direction (Fig. 6). This reduction in activity preceded the onset of Vsacs, and the latencies of the reduction in activity before the onset of Vsacs were not significantly different among the eight directions (Kruskal-Wallis ANOVA, P = 0.43). In addition, the reduced activity during saccades and the duration of the reduction in activity were not significantly different across the eight directions (Kruskal-Wallis ANOVA, P = 0.13 and P = 0.43, respectively), indicating that the fixation neuron was untuned for saccade direction. Usually, fixation neurons also showed a decrease in activity during saccades in any direction in the dark.

Next, we analyzed the change in activity of fixation neurons in relation to refixation after Vsacs. During the period when the monkey fixated on the target presented at the new location after a saccade, the firing rate of the fixation neuron shown in Fig. 5A did not differ from its firing rate during the central fixation period. In the example in Fig. 6, the firing rate of the fixation neuron during maintained period of refixation (600–900 ms after target onset for Vsac) did not differ among the eight directions (Kruskal-Wallis ANOVA, P = 0.11). Although four fixation neurons showed direction sensitivity, the activity of fixation neurons usually did not depend on the position of the eye in the orbit. At the beginning of refixation, 15 fixation neurons showed a transient increase in activity followed by tonic discharge at a constant rate. This transient increase in activity persisted for 100 ms with a mean latency of 46.3 ± 10.2 ms compared with the firing rate during steady fixation (300–600 ms before Vsac onset).

In contrast, the tonic activity of fixation neurons usually showed a transient increase in activity followed by tonic discharge at a constant rate. This transient increase in activity persisted for 100 ms with a mean latency of 46.3 ± 10.2 ms compared with the firing rate during steady fixation (300–600 ms before Vsac onset).
activity occurred ~80 ms after the onset and ~50 ms after the offset of both ipsiversive and contraversive Vsacs. This transient increase in activity that accompanied the beginning of refixation was usually observed in fixation neurons, with their greatest activity at the beginning of fixation in the fixation task. At the beginning of refixation without a target (i.e., after Msac, which is described in detail below), a transient increase in activity was not seen in 8 of these 15 neurons, indicating that this was a visual response. However, the remaining seven fixation neurons also showed a transient increase in activity even at the beginning of refixation after Msacs.

To examine the effects of the amplitude of Vsacs on the activity of a fixation neuron, the Vsac amplitude was changed from 5 to 20° at 5° intervals (Fig. 7A). The activity of the fixation neuron decreased before the onset of both ipsiversive and contraversive Vsacs with any amplitude. The ratio of the firing rate during the minimum reduction in activity associated with Vsacs to that during steady fixation was similar for Vsacs with any amplitude in the seven fixation neurons tested (Fig. 7B). In these neurons, the duration of the reduction in activity was longer during larger Vsacs with a longer duration. The correlation between the duration of the reduction in activity and the amplitude of saccades was significant for both ipsiversive ($r = 0.52, P < 0.05$) and contraversive Vsacs ($r = 0.77, P < 0.001$; Fig. 7C). The firing rate of fixation neurons during refixation after saccades of any amplitude did not differ from their firing rate during the central fixation period. Therefore as shown in the direction-variation series in Fig. 6, the activity of fixation neurons usually did not depend on the eye position.

To study the activity of fixation neurons during saccades without visual targets, we used the Msac task. In this task, the memory period was varied randomly from 0.5 to 1.5 s, so that the monkey could not predict the onset of the cue to make an Msac. The example in Fig. 8A shows the activity of a fixation neuron during the Msac task to a remembered target location. The activity of this neuron increased while the monkey maintained fixation and firing stopped before the beginning of Vsacs (see Fig. 6). In the Msac task, this neuron stopped firing before the beginning of ipsiversive Msacs (Fig. 8Aa). After Msacs, the monkey had to maintain fixation on the blank screen, and the fixation neuron resumed its firing rate. This neuron also stopped firing before the beginning of contraversive Msacs and resumed its firing rate after Msacs (Fig. 8Ab). We examined the activity during Msacs in 87 of 113 fixation neurons, and many of them (74%, 64/87) showed a decreased firing rate before Msacs. The reduction in activity of the fixation neuron preceded the onset of Msacs in both directions, as in this example. We measured the preceding time for the reduction in activity of neurons before the onset of Msacs. The reduction in activity of the fixation neuron preceded the onset of ipsiversive Msacs by ~120 ms compared with the firing rate during steady fixation (300–600 ms before Msac onset; Fig. 8Ba). Overall, the latencies of the reduction in activity from the onset of ipsiversive Msacs had a mean of $-98.4 \pm 38.2$ ms ($n = 64$; 1-sample Kolmogorov-Smirnov test, $\alpha < 0.05$). There was no significant difference in the latencies of the reduction in activity between ipsiversive Msacs and Vsacs (Wilcoxon signed-rank test, $P = 0.66; n = 28$; Fig. 8Ca). In a similar range, the reduction of activity of the fixation neuron preceded the onset of contraversive Msacs compared with the firing rate during steady fixation (300–600 ms before Msac onset), and the latency was ~140 ms (Fig. 8Bb). Overall, the latencies of the reduction in activity from the onset of contraversive Msacs had a mean of $-102.7 \pm 40.2$ ms ($n = 64$; 1-sample Kolmogorov-Smirnov test, $\alpha < 0.05$). Similar to the results for ipsiversive saccades, there was no significant difference in the latencies of the reduction in activity between contraversive Msacs and Vsacs (Wilcoxon signed-rank test, $P = 0.49; n = 28$; Fig. 8Cb). In the remaining fixation neurons, 12 showed no change.
in activity during Msacs in both directions, 5 showed a reduction in activity only during ipsiversive Msacs, 2 showed a reduction in activity only during contraversive Msacs, and 4 showed an increase in activity during Msacs. Some fixation neurons showed changes in their firing rate during the memory period (i.e., the delay period) in the Msac task. An increase in activity during the memory period was observed in 6 fixation neurons, and a decrease in activity during the memory period was observed in 15 fixation neurons. It is possible that the reduction in activity during the memory period was related to the preparation of Msacs. However, the latencies of Msacs in such cases were in the range of regular saccades but not express saccades. After Msacs, many fixation neurons resumed their firing rate during refixation on a remembered target location without a visual target, as shown in the example in Fig. 8. However, the latency of the resumption of activity after Msacs tended to fluctuate between fixation neurons. This tendency was observed even in fixation neurons that maintained activity after disappearance of the fixation spot in the fixation blink task and that did not exhibit sensitivity to eye position in the Vsac task.

In the same monkey, we recorded the activity of movement neurons and visuomovement neurons as well as fixation neurons. Movement neurons and visuomovement neurons showed increased activity before saccades, whereas fixation neurons showed decreased activity before saccades. Next, we compared the timing of the increase in activity for movement and visuomovement neurons to the timing of the reduction in activity for fixation neurons in relation to the onset of Vsacs and Msacs.

When we aligned the activity of a fixation neuron (Fig. 9Aa) and a movement neuron (Fig. 9Ab) with respect to the onset of Vsacs, the reduction in activity of the fixation neuron almost coincided with, or occurred slightly before, the increase in activity of the movement neuron. In addition, the duration of the reduction in activity of the fixation neuron almost corresponded to the duration of the increase in activity of the movement neuron. As in the case of Vsacs, we also aligned the activity of the fixation neuron (Fig. 9Ba) and the movement neuron (Fig. 9Bb) with respect to the onset of Msacs. The responses of the fixation neuron and the movement neuron over time for Msac were similar to those for Vsac. To further compare the timings of these signal changes leading up to Msac, we computed population spike density waveforms for the population of fixation neurons (n = 47; Fig. 9Ca) and movement and visuomovement neurons in this monkey (n = 18; Fig. 9Cb). The results indicated that, before the onset of saccades, the reduction in activity of fixation neurons occurred with a time course similar to, or slightly before, the increase in activity of movement and visuomovement neurons.

Distributions of fixation neurons and suppression sites in the FEF

As previously mentioned, before these extracellular recording experiments, we had systematically examined the effects of stimulation of the FEF and its vicinity in the prearcuate gyrus and found two types of suppression for saccades: ipsilateral and bilateral suppression of saccades (Izawa et al. 2004a,b). In
the present study, we performed the histological reconstruction of stimulation sites and compared them with the distribution of fixation neurons in the FEF. The stimulation tracks and the location of low-threshold sites for bilateral suppression of Vsacs were plotted on representative frontal sections of the FEF (Fig. 10A). The three-dimensional histological reconstruction of stimulation tracks showed that the bilateral suppression sites were predominantly confined to an area ∼2 mm in diameter in the caudal part of the arcuate gyrus facing the inferior arcuate sulcus (Fig. 10B). Although fixation neurons were scattered in the FEF, we encountered many fixation neurons at the same depths in the same tracks as the bilateral suppression sites, indicating that fixation neurons were concentrated in the bilateral suppression area. We observed that 72 of the 113 fixation neurons recorded (64%) were located in the bilateral suppression area, and many of them decreased their firing rate before the onset of Vsacs in both directions (69%, 33/48). On the other hand, six fixation neurons with a reduction in activity only during ipsiversive saccades were found in the ipsilateral suppression area.

**DISCUSSION**

We analyzed the activity of fixation neurons in the FEF in relation to the maintenance of visual fixation and the initiation of saccades. The activity of fixation neurons increased at the start of fixation with a mean latency of ∼50 ms and was maintained during fixation. When a fixation spot of light disappeared during steady fixation, different fixation neurons exhibited different responses, ranging from a reduction in activity to an increase in activity, indicating that there is a continuum of fixation neurons, from neurons with foveal visual-related activity to neurons with activity related to the motor act of fixating. Fixation neurons usually showed a decrease in their firing rate before the onset of Vsacs, Msacs, and spontaneous saccades in any direction. Some fixation neurons showed a reduction in activity only during ipsiversive saccades. Before these extracellular recording experiments, we systematically examined the effects of stimulation of the FEF and its vicinity in the prearcuate gyrus (Izawa et al. 2004a,b). In the present study, we performed the histological reconstruction of stimulation tracks and identified whether recorded fixation neurons were located in either the ipsilateral or bilateral suppression area for saccades in the FEF. We found that about two thirds of fixation neurons were concentrated in the bilateral suppression area of the FEF localized in the caudal part of the arcuate gyrus facing the inferior arcuate sulcus (Fig. 10). Therefore this area may correspond to the foveal representation of the FEF.

The activity of fixation neurons in the FEF was first reported by Bizzi (1968), and Suzuki and Azuma (1977) described how many prefrontal neurons showed an increase in their firing rate...
during fixation with a variety of discharge patterns. Later, fixation neurons in the FEF were found to project to the SC (Segraves and Goldberg 1987; Sommer and Wurtz 2000) and the pons (Segraves 1992) and to influence the activity of neurons in these structures. Functionally, the generation of saccades to other objects in the visual field must be suppressed to maintain attentive fixation. In this study, we systematically correlated the activity of fixation neurons while maintaining fixation and the suppressive effects of their location on saccades. These results suggest that fixation neurons in the FEF are part of a suppression mechanism that could control the maintenance of visual fixation and the initiation of saccades.

Fixation neurons in the FEF showed some different patterns of activity during fixation. In addition to tonic activity during fixation, some fixation neurons showed a transient increase in activity at the beginning of fixation that might include activity reflecting the internal status of the monkey related to attention. We also observed tonic activity followed by an increase in activity at the end of the trial that might include activity reflecting anticipation of a reward. In the fixation blink task, fixation neurons in the FEF also showed some different patterns of activity. Although we showed three typical examples of activity during fixation without a visual target in Fig. 3, the patterns of activity during the fixation blink task were not discontinuous, and there were fixation neurons with activity lay between them. Therefore foveal visual responsiveness appeared to vary continuously between fixation neurons. This result was similar to the result reported by Suzuki et al. (1979), who evaluated visual stimulus factors that contributed to the activity of fixation neurons. In their report, most fixation neurons showed a decrease in activity during fixation without a visual target. However, our results differ from those of Suzuki et al. (1979) in this respect. In addition to fixation neurons that showed a decrease in activity, we observed that many fixation neurons maintained or increased their activity during fixation without a visual target. FEF neurons suppressed by foveal light stimuli were reported to be quiet during fixation with a target (Segraves 1992; Segraves and Goldberg 1987). The present fixation neurons discharged during fixation with a target, indicating that the increase in activity during fixation without a target could not be attributed to passive disinhibition in response to the offset of foveal visual stimulation. The activity of these fixation neurons may be related more to the motor act of fixating than to foveal visual stimulation. Therefore these results indicated that there is a continuum of fixation neurons in the FEF from visual to motor extremes. This continuum from visual to motor extremes may be equivalent to that of visuomovement neurons reported by Bruce and Goldberg (1985).

Fixation neurons usually showed a decrease in their firing rate before the onset of Vsacs and Msacs in both ipsilateral and contralateral directions, and we found many fixation neurons in the bilateral suppression area in the FEF. Therefore it seems highly likely that these fixation neurons with bilateral activity reduction during saccades may be related to the bilateral suppression of saccades induced by electrical stimulation of the FEF (Izawa et al. 2004b). On the other hand, we also observed fixation neurons with a reduction in activity only during ipsiversive saccades. These fixation neurons with a reduction in activity only during ipsiversive saccades were most likely to be
related to the suppression of ipsiversive saccades induced by electrical stimulation of the FEF (Izawa et al. 2004a). Some fixation neurons showed an increase in activity during saccades. Most of these neurons showed an increase in activity during fixation without a visual target in the fixation blink task. Therefore an increase in activity during saccades may be a response to the disappearance of the fixation spot rather than movement-related activity. This interpretation may be supported by the fact that the increase in activity usually occurred before saccades in both ipsiversive and contraversive saccades without a directional preference. Although we observed few fixation neurons with an increase in activity only during contraversive saccades, they may resist simple classification with regard to their functional meaning.

In addition to the FEF, the posterior parietal cortex has been shown to contain many fixation neurons (Mountcastle et al. 1975). Fixation neurons in the posterior parietal cortex continued to discharge during fixation and ceased firing during saccades. They varied in activity during fixation without a target, from a decrease to an increase in activity relative to that during fixation with a target (Ben Hamed and Duhamel 2002; Sakata et al. 1980). Therefore fixation neurons in the posterior parietal cortex resemble the present fixation neurons in the FEF, suggesting that they might play a role in the common fixation system. In fact, the FEF has connections to the posterior parietal cortex (Pandya et al. 1971). However, there are some differences between them. Most fixation neurons in the posterior parietal cortex have been reported to have activity that is modulated by eye position (Mountcastle et al. 1975). On the other hand, as shown in this study, the activity of fixation neurons in the FEF during refixation after saccades usually did not differ from that during the central fixation period. This was true when we changed the direction and amplitude of saccades, indicating that the activity of fixation neurons usually did not depend on the position of the eye in the orbit. Similar results were reported by Suzuki and Azuma (1977), in that the activity of fixation neurons in the FEF was little influenced by the position of the fixation spot. Although we also observed activity that was sensitive to position, the number of fixation neurons with such activity was small. This may correspond to the fact that Segraves (1992) found few corticopontine neurons that were sensitive to eye position.

Fixation neurons have also been described in the rostral SC (Munoz and Guittion 1991; Munoz and Wurtz 1993). These neurons are active during steady fixation and show a decrease in activity during most saccades. Therefore fixation neurons in the rostral SC resemble fixation neurons in the FEF with regard to their discharge pattern. In fact, fixation neurons in the FEF were found to project to the ipsilateral SC (Segraves and Goldberg 1987; Sommer and Wurtz 2000). This projection of fixation neurons in the FEF to the SC may contribute to the activity of rostral SC fixation neurons, although the projection was not preferential to the rostral SC. On the other hand, there were some differences between fixation neurons in the FEF and those in the SC. Fixation neurons in the SC were reported to be localized to the rostral pole of the SC (Munoz and Wurtz 1993), whereas FEF fixation neurons were found in a relatively wide area in the FEF. However, we also found FEF fixation neurons that were concentrated in a localized area corresponding to the bilateral suppression area in the FEF. Therefore fixation neurons in the FEF may be topographically organized in the FEF. Another difference between fixation neurons in the FEF and those in the rostral SC was their activity during small saccades. Many fixation neurons in the rostral SC have been
reported to show an increase in activity during small contraversive saccades (Hafed et al. 2009; Munoz and Wurtz 1993). Therefore the activity of fixation neurons in the rostral SC should not always be assumed to preserve fixation (Gandhi and Keller 1999) but instead seems to provide a position-error signal for both small saccades and smooth pursuit (Krauzlis et al. 2000). In this study, fixation neurons in the FEF usually showed a decrease in activity during saccades with any of the amplitudes tested, suggesting that they contribute to an inhibitory process for saccades to maintain fixation. However, we also observed some, albeit not many, fixation neurons in the FEF with increased activity during saccades. These fixation neurons in the FEF may correspond to those in the SC that possess movement fields.

The other neurons that show continuous activity during fixation are omnipause neurons (OPNs) in the brain stem. They discharge tonically during fixation and stop firing during all saccades, regardless of their amplitude or direction (Cohen and Henn 1972; Keller 1974; Luschei and Fuchs 1972; Ohgaki et al. 1987; Strassman et al. 1987). In addition, Missal and Keller (2002) recently reported that the activity of OPNs decreased during smooth pursuit, suggesting that OPNs are involved in a common inhibitory mechanism for saccades and pursuit. Usually, the discharge rate of OPNs during fixation is not correlated with eye position (Keller 1974). Therefore the activity of OPNs is very similar to that of fixation neurons in the FEF, except for the higher firing rate in OPNs. Fixation neurons in the FEF may suppress the initiation of saccades during visual fixation by projecting to OPNs directly or indirectly via the SC. Segraves (1992) reported that fixation neurons in the FEF directly projected to the OPN region, although OPNs were not excited by FEF stimulation. In the indirect pathway, fixation neurons in the FEF project to the SC (Segraves and Goldberg 1987; Sommer and Wurtz 2000) and the SC projects to the OPN region predominantly, but not exclusively, from the rostral pole where fixation neurons were found (Büttner-Ennever et al. 1999; Gandhi and Keller 1997; Paré and Guitton 1994). Therefore it seems likely that inputs from fixation neurons in the FEF to OPNs form at least part of a fixation system, although the functional contribution of collicular projection to OPNs for fixation is controversial (Gandhi and Keller 1999; Kaneko 1996).

Fixation neurons in the FEF that showed an increase in activity during the memory period in the Msac task may have a delay signal that may reflect cognitive processes (Funahashi et al. 1989; Kubota and Niki 1971). The decrease in activity of some fixation neurons during the memory period may be related to the preparation of regular Msacs. Therefore the FEF seemed to contain some fixation neurons that also carried a delay signal similar to that carried by neurons in the adjacent prefrontal cortex.

The activity of fixation neurons in the FEF usually decreased before the onset of Vsacs and Msacs. This decrease in the activity of fixation neurons almost coincided with, or occurred slightly before, the increase in activity of movement neurons or visuomovement neurons in the same monkey. Therefore both the disinhibition produced by the reduction in activity of fixation neurons and the excitation produced by the increase in activity of movement and visuomovement neurons in the FEF may occur at the level of the brain stem saccade generator and contribute to the initiation of saccades (Izawa et al. 1999, 2007; Sugiuichi et al. 2005; Takahashi et al. 2005). A reversed pattern of activity, i.e., an increase in the activity of fixation neurons and a decrease in the activity of movement and visuomovement neurons in the FEF, may contribute to cancel a planned saccade (Hanes et al. 1998). During fixation including spontaneous fixation, the present fixation neurons in the FEF maintained their tonic discharge. These fixation neurons were found in the FEF and were concentrated in the bilateral suppression area for saccades. To further understand the functional role of fixation neurons in the FEF in relation to the control mechanism between visual fixation and other eye movement systems, further studies are needed to understand the exact pathways that are responsible for fixation and the suppression of saccades.

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REFERENCES


