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Role of Supplementary Eye Field in Saccade Initiation: Executive, Not Direct, Control

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Stuphorn V, Brown JW, Schall JD. Role of supplementary eye field in saccade initiation: executive, not direct, control. *J Neurophysiol* 103: 801–816, 2010. First published November 25, 2009; doi:10.1152/jn.00221.2009. The goal of this study was to determine whether the activity of neurons in the supplementary eye field (SEF) is sufficient to control saccade initiation in macaque monkeys performing a saccade countermanding (stop signal) task. As previously observed, many neurons in the SEF increase the discharge rate before saccade initiation. However, when saccades are canceled in response to a stop signal, effectively no neurons with presaccadic activity display discharge rate modulation early enough to contribute to saccade cancellation. Moreover, SEF neurons do not exhibit a specific threshold discharge rate that could trigger saccade initiation. Yet, we observed more subtle relations between SEF activation and saccade production. The activity of numerous SEF neurons was correlated with response time and varied with sequential adjustments in response latency. Trials in which monkeys canceled or produced a saccade in a stop signal trial were distinguished by a modest difference in discharge rate of these SEF neurons before stop signal or target presentation. These findings indicate that neurons in the SEF, in contrast to counterparts in the frontal eye field and superior colliculus, do not contribute directly and immediately to the initiation of visually guided saccades. However the SEF may proactively regulate saccade production by biasing the balance between gaze-holding and gaze-shifting based on prior performance and anticipated task requirements.

INTRODUCTION

The frontal cortex of primates includes two areas identified with the control of movements of the eyes (Schall 1997; Tehovnik et al. 2000). The function of the frontal eye field (FEF), located in the rostral bank of the arcuate sulcus, is reasonably well understood. FEF is involved in selecting targets for orienting (Schall 2002, 2004) and in controlling whether and when a saccade to such a target is made (Stuphorn and Schall 2002). The function of the supplementary eye field (SEF) is less well understood. It contributes to saccade production somehow because microstimulation with low currents evokes saccades. SEF neurons respond to visual and acoustic stimuli and are active before and during pursuit and saccadic eye movements (Berdyyeva and Olson 2009; Bon and Lucchetti 1991, 1992; Fujii et al. 1995, 2002; Hanes et al. 1995; Heinen 1995; Lee and Tehovnik 1995; Moorman and Olson 2007a,b; Nakamura et al. 2005; Ohmae et al. 2008; Pouget et al. 2005; Russo and Bruce 1993, 2000; Schall 1991; Schlag and Schlag-Rey 1987; Schlag et al. 1992; Uchida et al. 2007). SEF coincides with area F7 (Matelli et al. 1991), which projects to the FEF, superior colliculus (SC), the oculomotor region of caudate nucleus, and elements of the brain stem saccade generator (Huerta and Kaas 1990; Parthasarathy et al. 1992; Schall et al. 1993; Shook et al. 1990).

Anatomical and physiological similarities between SEF and FEF have led to the hypothesis that SEF functions in parallel with FEF and SC (Amador et al. 2004; Russo and Bruce 2000; Schlag 2002; Tehovnik et al. 2000). However, many characteristics distinguish SEF from FEF and SC. Whereas microstimulation of FEF or SC evokes fixed vector saccades, stimulation of SEF commonly produces convergent saccades (Martinez-Trujillo et al. 2004; Park et al. 2006; Russo and Bruce 1993; Schall 1991; Schlag and Schlag-Rey 1987; Tehovnik and Lee 1993). The connections between the SEF and the caudate, thalamus, and SC are more diffuse than those of the FEF (Huerta and Kaas 1990; Parthasarathy et al. 1992; Shook et al. 1990). The activity of many SEF neurons appears much more context dependent than that of FEF neurons (Amador et al. 2004; Chen and Wise 1995a,b, 1996, 1997; Coe et al. 2002; Isoda and Tanji 2002, 2003; Lu et al. 2002; Mushiake et al. 1996; Nakamura et al. 2005; Ohmae et al. 2008; Olson and Gettner 1995, 1999; Schlag-Rey et al. 1997; Tremblay et al. 2002; Uchida et al. 2007). Last, the SEF exhibits patterns of activity that have no counterparts in FEF; notably, SEF neurons signal error- and conflict-related activity (Nakamura et al. 2005; Stuphorn et al. 2000) as well as the anticipation and delivery of reinforcement (Amador et al. 2000; Roesch and Olson 2003; Stuphorn et al. 2000).

In this study we asked whether SEF neurons, like those in the FEF and SC, generate signals sufficient to directly and immediately control the initiation of saccades or whether they play a more subtle and indirect role in the control of oculomotor behavior. The main result is that SEF neurons are markedly different from presaccadic movement-related neurons recorded in FEF or SC because they do not control the initiation of saccades. However, we found that neural activity in SEF is correlated with saccade response time, varies with sequential adjustments in response latency, and allows a partial prediction of the cancelation likelihood in stop signal trials. This result supports the hypothesis that SEF contributes to the proactive adjustment of saccade production.

METHODS

General procedures used for data collection and analysis were previously detailed (Hanes and Schall 1995; Hanes et al. 1998). Only details specific to the data reported in this study are included here.

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Subjects and surgery

Data were collected from four macaque monkeys (*Macaca mulatta*, *Macaca radiata*). The care and use of the animals were in accordance with the National Institute of Health's *Guide for the Care and Use of Laboratory Animals* and the guidelines of the Vanderbilt Animal Care Committee.

Data collection

In the first monkey used in this study (monkey A), action potentials were discriminated with a time–amplitude window discriminator (BAK Electronics) and were sampled at 1-kHz resolution. Single units were admitted to the database if: *1*) the amplitude of the action potential was sufficiently above background to reliably trigger the time–amplitude window discriminator, *2*) the action potential wave shape was invariant throughout recording, and *3*) the isolation could be sustained for a sufficient period. For the other three monkeys (F, H, N) all waveforms that passed a threshold were recorded digitally (Multichannel Acquisition Processor, Plexon, Dallas, TX). Action potentials from one to four neurons were discriminated from the electrode on-line using two-dimensional principal component analysis (PCA) and template matching (real-time acquisition system programs for unit timing in neuroscience [RASPUTIN], Plexon). The identification and isolation of individual spikes was reevaluated and corrected off-line using three-dimensional PCA and visual inspection of selected waveforms (Off-line Sort Program, Plexon).

Intracortical microstimulation was used to locate sites from which saccades could be evoked with low current thresholds. The stimulation parameters were conventional [70-ms trains of 330-Hz biphasic pulses (23 pulses per train) with duration of 0.2 ms per pulse]. Stimulation was applied 10–20 ms after the monkey received reinforcement for a saccade made from the central fixation point to one of four targets. All targets were 12° eccentric at the equidistant polar directions of 45, 135, 225, and 315°. SEF was defined as the region where we could evoke saccades by applying current <50 μA (Russo and Bruce 1993, 2000; Schlag and Schlag-Rey 1987).

Localization of recording sites

The location of sampled neurons was verified histologically in three of the monkeys (monkeys A, H, and N). They were deeply anesthetized with pentobarbital and perfused with saline, followed by 10% paraformaldehyde in 0.1 M phosphate buffer followed by buffered sucrose solution. The brains were photographed in situ and after removal. The frontal lobes were sectioned in either the coronal (A) or sagittal (H, N) plane at 50 μm and stained in alternating series for Nissl, SMI-32, and AChE. Area F7 was identified according to cytoarchitectonic criteria (Luppino et al. 1991; Schall et al. 1993). Fiduciary guide pins in the recording chamber were located relative to anatomical landmarks such as the hemisphere midline and the arcuate and principal sulci. The location of neurons with presaccadic activity and the sites from which saccades could be elicited by microstimulation were projected onto a dorsal surface view of the frontal lobe based on the grid coordinates.

Figure 1 shows a reconstruction of the recording sites in two of the monkeys. Research with monkey F is still in progress. In all monkeys we determined the location of the SEF by intracortical electrical microstimulation. Figure 1 shows an example of evoked saccades and the correspondence between the density of neurons with presaccadic activity and the low-threshold SEF. In all monkeys data were collected from the contiguous region from which saccades were evoked with low current thresholds (<50 μA) and which corresponded with area F7 in monkeys A, H, and N.

Visuomovement index

The activity of many of the neurons was different for memory-guided saccades and visually guided saccades that were produced to the same target location used for the countermanding task. To quantify these differences, we measured the mean activity of neurons during the 50 ms before saccade initiation for memory-guided and visually guided saccades, respectively. We subtracted the activity measured during visually guided saccades from that during memory-guided saccades and divided this difference by the sum of the two measurements. This visuomovement value indexed the balance of activity in the two conditions with values approaching $+1$, indicating activity associated more with memory-guided saccades, and values approaching -1, indicating activity associated more with visually guided saccades. If a neuron discharged equally in the two conditions, the index was close to 0.

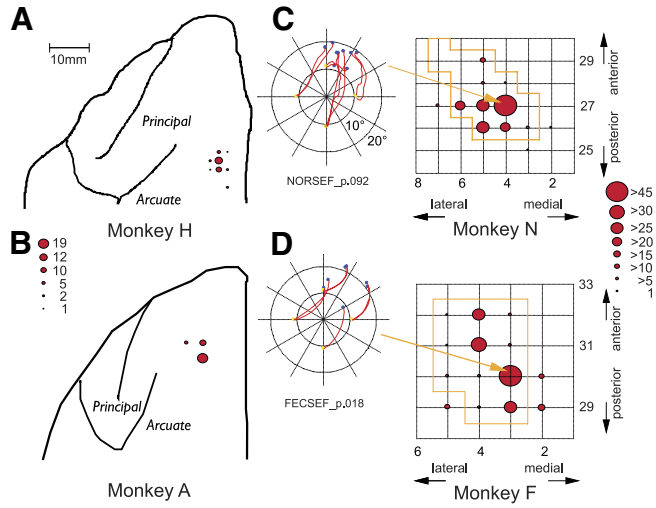


FIG. 1. Localization of supplementary eye field (SEF). Location of recording sites in monkeys H (*A*) and A (*B*) relative to sulcal landmarks based on histology. Location of recording sites in monkeys N (*C*) and F (*D*) relative to stereotaxic coordinates, with area indicated from which intracortical microstimulation-evoked saccades with low current thresholds. Patterns of saccades evoked by stimulation at representative sites are shown. Circle size indicates number of neurons with presaccadic activity according to respective scales.

Stop signal task

The stop signal task requires monkeys to prepare a saccade, which is occasionally withheld in response to a stop signal (Hanes and Schall 1995). Performance of the stop signal task is measured by the probability of not canceling a saccade as a function of stop signal delay (SSD) (referred to as the inhibition function) and the distributions of latencies of correct saccades in no-stop signal trials and of noncanceled saccades in stop signal trials. Performance of the stop signal task can be described as the outcome of a race between a GO and a STOP process (Logan and Cowan 1984). As previously described in detail, the race model permits calculation of the stop signal reaction time (SSRT) (Logan and Cowan 1984). A new, interactive race model of stop signal task performance demonstrates that SSRT measures the latency of the inhibitory process that interrupts movement preparation (Boucher et al. 2007; see also Camalier et al. 2007; Lo et al. 2009).

Identification of neuronal activity sufficient to control saccade initiation or inhibition

For a neuron to control saccade initiation during this task it must fulfill two criteria. First, the neuron must discharge differently when a saccade is initiated versus when the saccade is withheld. Second, this difference must occur within the SSRT; otherwise, it is too late to influence saccade initiation. Both criteria are fulfilled by movement-related neurons in the FEF and SC; these neurons exhibit activation on

noncanceled trials, equivalent to that on no-stop signal trials and a pronounced decrease of activation on canceled trials (Brown et al. 2008; Hanes et al. 1998; Paré and Hanes 2003). The activity of neurons with strong visual responses does not satisfy these criteria and recent work has shown that visuomovement neurons in FEF exhibit another pattern of modulation consistent with a function different from saccade control (Ray et al. 2009). The race model of the countermanding tasks specifies how to compare activation across trials. First, the activity on noncanceled trials must be compared with the activity on no-stop signal trials, with saccade latencies less than the combined duration of the SSD plus SSRT. This is because on these trials the GO process was so fast that if the stop signal had occurred, then the GO process would have finished before the STOP process. Second, the activity on canceled trials at a given SSD must be compared with the activity on no-stop signal trials with saccade latencies greater than the SSD plus SSRT. This is because on these trials the GO process was slow enough that if the stop signal had occurred, then the GO process would have been interrupted by the faster STOP process. To designate this comparison, we refer to *latency-matched* trials.

Relationship among neural activity, response time, and probability to cancel

Two approaches were used to determine whether the activity of SEF neurons influences the probability of saccade initiation. First, a linear regression analysis was used to determine the relationship between the magnitude of neural activity and saccade initiation times

$$SRT(t) = (b0 + b1)A(t)$$

where SRT(t) is the saccade response time and A(t) is the spike rate in a given time interval on trial t. We tested three intervals: *1*) the 100 ms preceding target onset (referred to as "baseline" interval), *2*) 100–200 ms following target onset (referred to as "target onset" interval), and *3*) the 100 ms preceding saccade onset (referred to as "movement generation" interval). Because inclusion of the noncanceled stop signal trials would have introduced a bias for shorter responses times, we included only no-stop signal trials in this analysis. The null hypothesis that SEF activity does not affect the average response time (H_0: b1 = 0) was evaluated using an F test for nested models (Taplin 1999).

Second, we compared the activity of neurons in different types of trials using a neuron–antineuron approach as described previously (Britten et al. 1996; Thompson et al. 1996). In short, the activity in canceled and noncanceled trials was compared by calculating the area under the receiver operating characteristic (ROC) curve derived from the respective distributions of activity as a function of time in 15-ms intervals.[1] Because this quantity describes the degree to which the discharge rate of the neuron predicts whether the monkey will cancel the saccade, it will be referred to as *outcome probability*. A bootstrap procedure provided 95% confidence intervals (Efron and Tibshirani 1993) that were used to identify neurons with outcome probability that was significantly different from 0.5. To be counted as significant, the area under the ROC curve had to be significantly different from 0.5 for a period continuing until the SSRT and lasting ≥50 ms. To have an influence on the monkey's decision, a neuron must at least carry a significant outcome probability signal before the choice is made between saccade initiation and suppression. Therefore we truncated each stop signal trial after either saccade initiation (noncanceled trials) or after SSD + SSRT (canceled trials). We performed the same analysis separately for three time periods: *1*) 400 ms before the target appeared (referred to as "pretarget"), *2*) from target onset to the earliest SSD + SSRT (referred to as "early"), and *3*) between the earliest and the latest SSD + SSRT (referred to as "late"). These three intervals assessed how neuronal activity was affected first by the anticipation and then by the increasing demand to suppress or to initiate the saccade.

RESULTS

Behavior

Figure 2 summarizes the performance for the 65 sessions in which neurons with presaccadic activity were recorded. Consistent with other stop signal task studies, saccade latencies on no-stop signal trials (mean value = 334 ms, n = 17,372) were longer than the saccade latencies on noncanceled trials (mean value = 310 ms, n = 2,661). Because it is well known that the

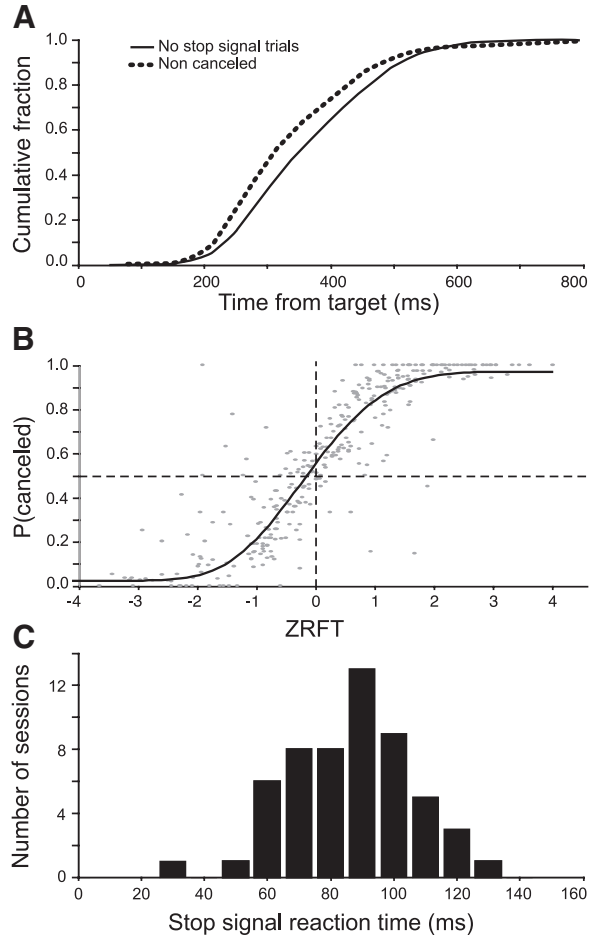


FIG. 2. Saccade countermanding performance. Data are combined across sessions with the 4 monkeys in which the 65 neurons were recorded whose activity in the countermanding task was analyzed for this study. *A*: cumulative distributions of saccade latencies in the no-stop signal (solid) and noncanceled trials (thick dotted). *B*: normalized inhibition function from all sessions. Abscissa plots the relative finishing time Z-score (ZRFT) = (mean saccade latency − SSD − SSRT)/SD of saccade latency, where SSD is stop signal delay and SSRT is stop signal reaction time. This quantity is the time relative to the finish times of the GO and STOP processes normalized by the SD of the saccade latencies in trials with a no-stop signal. Each point plots the probability of not canceling the saccade as a function of ZRFT for each session. A Weibull function is fit to the points to highlight the monotonic trend. *C*: distribution of SSRTs across all sessions for the 4 monkeys.

[1] Note that the previous publications of saccade stop signal neurophysiology have compared activity in canceled or noncanceled trials with activity in latency-matched no-stop signal trials. This approach respects the difference in response time that results in canceled or noncanceled movements according to the race model. A different approach is taken in the current analysis to explore the origin of this difference in response time.

inhibition function changes with response time (Logan and Cowan 1984), we compared performance across sessions by applying a Z transform to data from each session. According to this transform the probability of responding is plotted as a function of the mean saccade latency on trials with the no-stop signal in a session minus the particular SSD minus the SSRT, all divided by the SD of the saccade latencies on trials with the no-stop signal (Band et al. 2003; Logan and Cowan 1984). The monotonic character of this plot for all sessions demonstrates the sensitivity of the monkeys to the stop signal, a prerequisite for the application of the race model to the data. The distribution of SSRT for all monkeys was unimodal with a mode of 90 ms. The average SSRT for monkey A was 108 ms, for monkey F was 62 ms, for monkey H was 106 ms, and for monkey N was 82 ms.

Neuronal data set

We recorded 478 neurons from the dorsomedial convexity of the four monkeys long enough that they could be tested with the countermanding task (Table 1). They were classified using a memory-guided saccade task (Bruce and Goldberg 1985; Hikosaka and Wurtz 1983) in conjunction with the criteria applied in an earlier description of SEF neuron properties (Schall 1991). In agreement with earlier findings, many neurons showed visual- or saccade-related activity (Schall 1991; Schlag and Schlag-Rey 1987). Here, the term "saccade-related" is applied to neurons with an increased firing rate before saccade initiation. Many neurons, including some visually responsive and saccade-related neurons, also showed activity carrying evaluative signals related to the detection of errors, conflict, or the anticipation and delivery of reward (Stuphorn et al. 2000).

This study analyzed the activity of presaccadic neurons in SEF. This included 103 neurons with only visual responses and 176 with elevated discharge rate before the saccade with or without visual responses. The other types of neurons identified in SEF were cells with reward-related and error-related signals. Finding these cells replicated the results of Stuphorn et al. (2000) in two more monkeys. We summarized these neurons as "evaluative" in Table 1, but we did not analyze them for this study.

Comparison of memory-guided and visually guided saccades

Figure 3*A* shows the visuomovement index values for 111 SEF neurons with sufficient data to compare the two conditions. The distribution of the indices peaks at 0, but spans the range with values significantly different from 0 (bootstrap test, $P < 0.05$) highlighted. Some neurons were more active before visually guided saccades (Fig. 3*B*); others were more active before memory-guided saccades (Fig. 3*D*), but most neurons were active to the same extent (although with different time courses of activity in some cases) in both conditions (Fig. 3*C*). Still, the mean of the distribution (0.14) was significantly >0 (permutation-test; $P \ll 0.01$), indicating a slight population preference for memory-guided saccades.

Activation during the countermanding task

We tested whether the activity of SEF neurons was sufficient to control saccade initiation in the countermanding task. We included data from 65 saccade-related neurons for which we collected sufficient data (at least five canceled and five noncanceled trials for at least one SSD).

Figure 4 shows the activity of a SEF neuron with strong modulation immediately before and during the saccade; this would be classified as a "movement neuron" in previous studies of sensorimotor cortex. The pattern of activity of this neuron is compared across stop signal and trials with shorter and longer SSD and latency-matched no-stop signal trials. The time course and magnitude of activation on noncanceled trials was identical to that observed in latency-matched no-stop signal trials. In canceled stop signal trials, though, the neurons exhibited a pronounced decrease in discharge rate following the stop signal. Qualitatively this pattern of activation is very similar to that observed for movement-related neurons in FEF and SC. However, the modulation exhibited by this SEF neuron occurred after SSRT. The SSRT determined directly from the trials during which this neuron was recorded was 100 ms. In both SSDs this neuron started to change its activity 9 and 17 ms after SSRT. The fact that the onset of the modulation, which we refer to as cancellation time, followed SSRT means that the modulation could not contribute to the inhibitory process measured by SSRT. Therefore the activity of this neuron is not sufficient to control whether a saccade is initiated. A few neurons in SEF did exhibit a cancellation time before SSRT (Fig. 5). However, these neurons had other properties different from those of FEF and SC such as modulation of activity in noncanceled trials that was different from that observed in no-stop signal trials.

Figure 6*A* plots the distribution of cancellation times for all 65 saccade-related SEF neurons for each SSD providing at least five trials. The majority of these neurons changed their activity either after the SSRT (41/65; 63%) or showed no significant difference in activity (16/65; 25%). Only 11% of the neurons (7/65) consistently exhibited modulation before the

TABLE 1. *Numbers of different types of neurons recorded in SEF*

	Modulation Type								
Monkey	V	S	E	VS	VE	SE	VSE	O	Sum
A	16	0	2	8	17	3	6	1	53
F	6	10	3	43	6	1	16	14	99
H	50	7	19	11	20	5	4	72	188
N	31	14	10	37	13	5	6	22	138
Sum	103	31	34	99	56	14	32	109	478

Three basic types of modulation were distinguished: visually responsive (V); presaccadic (S), and evaluative (E). Neurons showed either one of these types of activity, a combination of activity types, or did not exhibit modulation that could be categorized, identified as other (O).

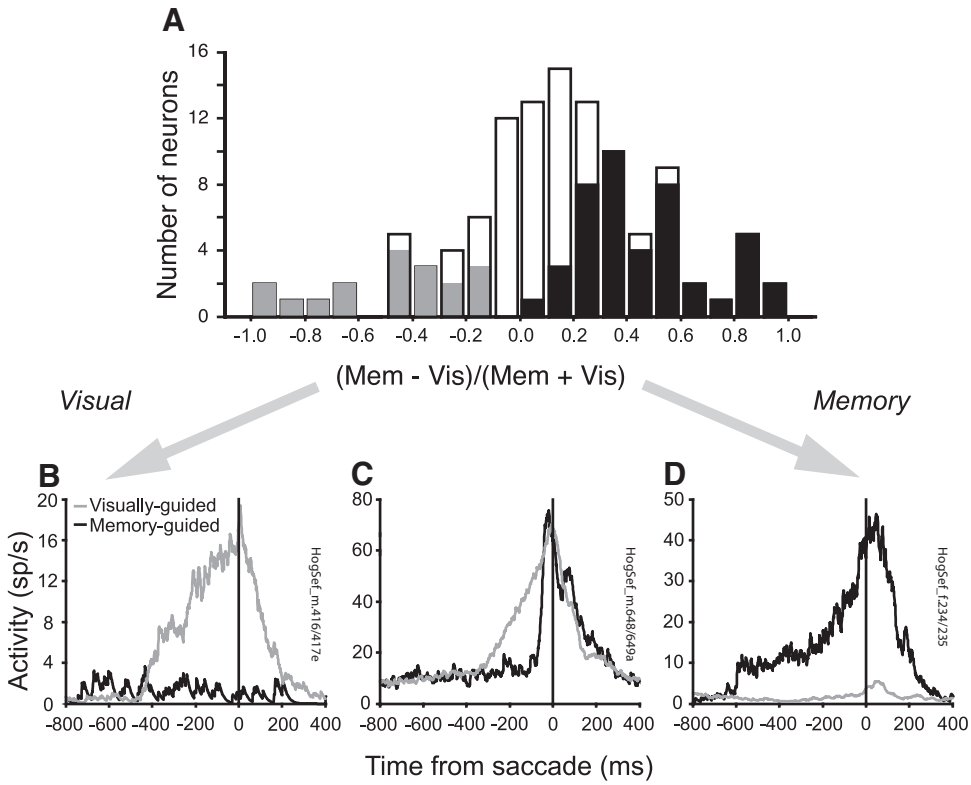


FIG. 3. Activity associated with visually guided and memory-guided saccades. *A*: distribution of the contrast between activity in the 50 ms before memory-guided saccades and that before visually guided saccades. Positive values indicate greater activity before memory-guided saccades; negative values indicate greater activation before visually guided saccades. Individually significant differences are filled. Representative neurons that were more active before visually guided (gray) compared with memory-guided (black) saccades (*B*), equivalently active before memory-guided and visually guided saccades (*C*), and more active before memory-guided saccades (*D*).

SSRT. For comparison, distributions of cancellation times for FEF and SC are shown (Hanes et al. 1998; Paré and Hanes 2003). Whereas the cancellation time of the minority of SEF neurons precedes SSRT, the modulation of nearly all SC saccade-related neurons (98%) and the majority of FEF saccade-related neurons (59%) precede the SSRT. The distributions were significantly different from each other (Kruskal–Wallis; P2 = 101.9; $P < 0.001$), with the modulation in SEF occurring significantly later than that in FEF (permutation test; $P \ll 0.001$) or SC (permutation test; $P \ll 0.001$).

The version of the countermanding task that we use in this study requires the control of saccades to visual targets. As noted earlier, some SEF neurons were significantly more active for memory-guided than for visually guided saccades and therefore were not optimally driven by this task. To determine whether these neurons behaved differently from the other saccade-related neurons, we separated the 16 neurons of this type from the rest and compared the cancellation time distribution of these two subpopulations (Fig. 6*B*). There is no significant difference between the two groups (Kolmogorov–Smirnov test; $P > 0.17$). Thus there seems to be no functional difference between saccade-related neurons that prefer memory-guided saccades, those that prefer visually guided, and those that are active during both types of saccades. None of them carries signals sufficient to control saccade initiation.

Threshold analysis

Previous work has shown that it is possible to define a threshold level of activity for movement-related neurons in the FEF and SC that specifies when a saccade will be initiated (Brown et al. 2008; Hanes et al. 1998; Paré and Hanes 2003). In contrast, the activity of visual neurons in FEF did not exhibit a unique relationship to saccade latency and so cannot contribute directly to controlling saccade initiation. The analysis described by Brown et al. (2008) was applied to the SEF neurons and confirmed that neurons in SEF do not generate a fixed level of activity before saccade initiation that could be described as a trigger threshold (data not shown). For a neuron to contribute to controlling saccade initiation, the activity must be different between noncanceled and canceled trials. Such a difference can be quantified by constructing ROC curves derived from the distributions of activity in the two types of trials (Fig. 7). This measure provides a robust test of whether the activity differs between trials in which saccades are initiated or withheld. According to the convention we used, if the area under the ROC curve is >0.5, then the distribution of activity in noncanceled trials exceeds the distribution of activity in canceled trials. If the area under the ROC curve is <0.5, then the distribution of activity on canceled trials exceeds that on noncanceled trials.

First, consider the typical SEF neuron that was modulated in canceled trials after SSRT (Fig. 4). Distributions of the maximum activity in the interval between target presentation and saccade initiation or SSRT, in canceled stop signal and noncanceled stop signal trials for this neuron, are shown in Fig. 7*A*. There was a great deal of overlap between the two distributions. Therefore the distribution of presaccadic activity measured in canceled trials when no saccade was produced was often of the same magnitude as the activity measured in trials when a saccade was produced. Consequently, the area under the ROC curve has a very low value of 0.59. Next, consider the neuron that modulated consistently within the SSRT (Fig. 5). The distributions of activity for canceled and noncanceled trials are more clearly separated, but the ranges of activity values still show extensive overlap (Fig. 7*C*). In this case the area under the ROC curve was 0.71.

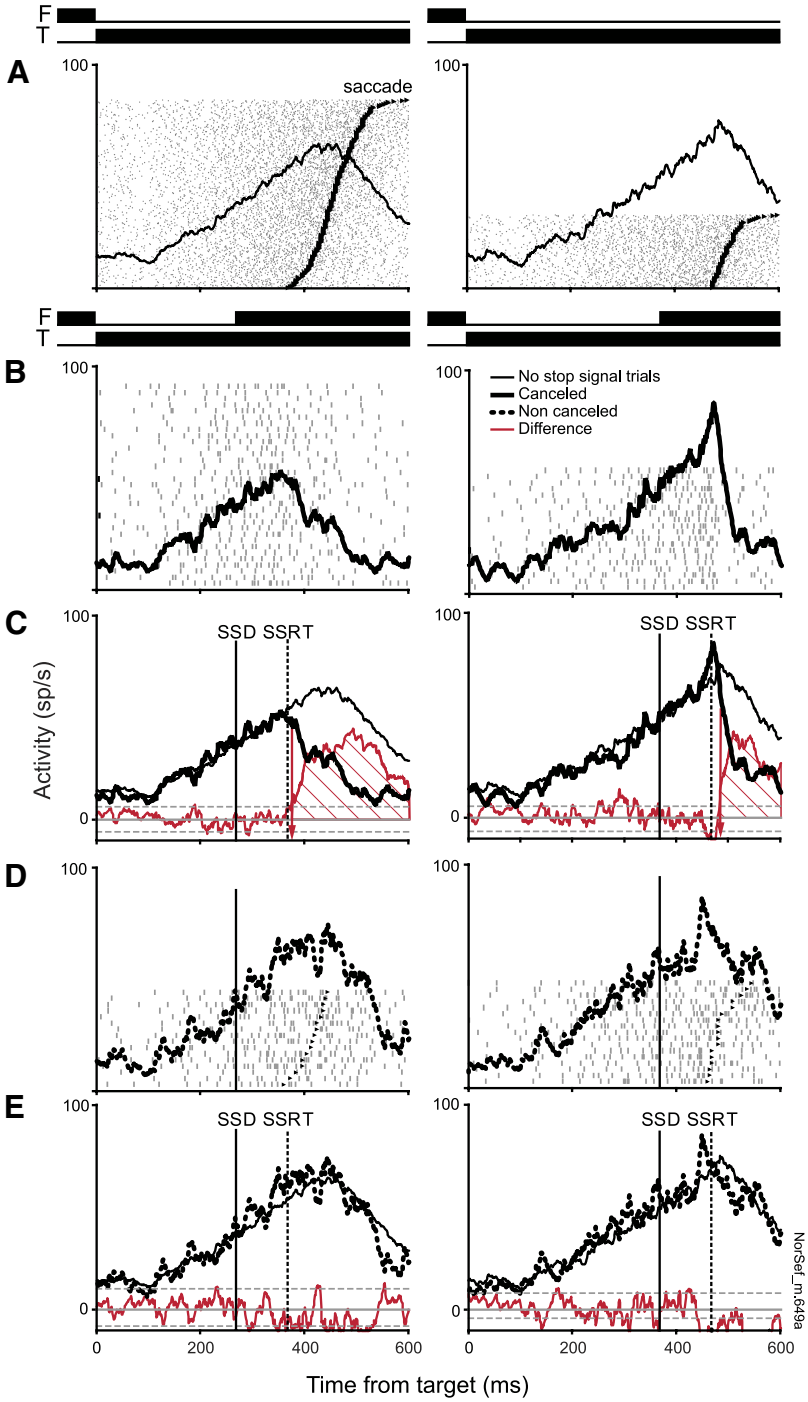
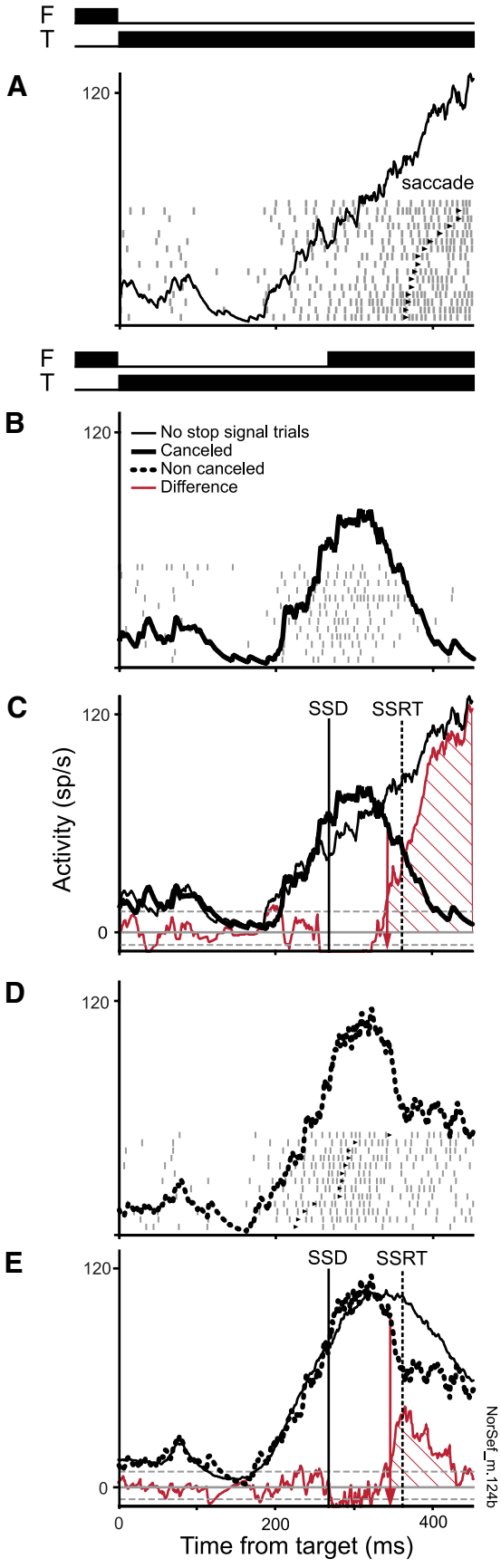


FIG. 4. Activity of representative SEF neuron with presaccadic activity in the countermanding task. Activity in canceled and noncanceled stop signal trials is compared with activity in latency-matched no-stop signal trials. The rasters and spike density functions are aligned on target onset. The state of the fixation spot (F) and target (T) are indicated above the panels. *A*: activity during subset of no-stop signal trials with latencies exceeding SSD + SSRT, which are long enough that they would have been canceled if a stop signal had been presented. *B*: activity during canceled trials with SSDs of 269 ms (*left*) and 369 ms (*right*). *C*: spike density functions of canceled (thick) and latency-matched no signal trials (thin) with their difference (red). The SSD is indicated by solid vertical line; the SSRT is indicated by dotted vertical line. Solid horizontal line indicates the mean difference between the spike density functions in the 600-ms time interval preceding the target onset; dashed horizontal lines mark 2SDs above and below this average. Red arrow marks the first time at which the difference in activity exceeds the criterion difference of 2SDs. Note that the difference in discharge rate arises after SSRT. *D*: activity during noncanceled trials with SSDs of 269 ms (*left*) and 369 ms (*right*). *E*: spike density functions of noncanceled (thick dotted) and latency-matched no signal trials (thin) with their difference (red). Note the lack of any difference in discharge rate.

The distribution of the ROC area values from the population of SEF neurons that were modulated in stop signal trials is shown in Fig. 8*A*. These values are compared with the distribution of values from movement-related neurons in FEF reported in Hanes et al. (1998) and Brown et al. (2008). The population analysis allows two conclusions. First, there is a significant difference between the ROC values of SEF and FEF populations (Wilcoxon rank-sum test, $P < 0.001$). The median value of the SEF neurons is 0.38, whereas the median value of the FEF neurons is 0.67. Second, the neurons illustrated in Figs. 4 and 5 are unusual within the sample of SEF neurons in exhibiting ROC area values >0.5. In fact, most SEF neurons exhibit ROC area values <0.5. In other words, most neurons in SEF produce more activity on canceled than on noncanceled trials. An example of such a neuron is shown in Fig. 8*B*. The neuron has a ROC area value (0.14) <0.5. However, the average cancellation time of this neuron across all SSD values is 182 ms following the SSRT. Thus the activity of this neuron is not sufficient to control saccade inhibition. This was true for almost all other neurons with low ROC area values as well. Some SEF neurons increase their activity following a successful cancellation, but this activity occurs after the SSRT when it could represent a putative conflict signal (Stuphorn et al. 2000).



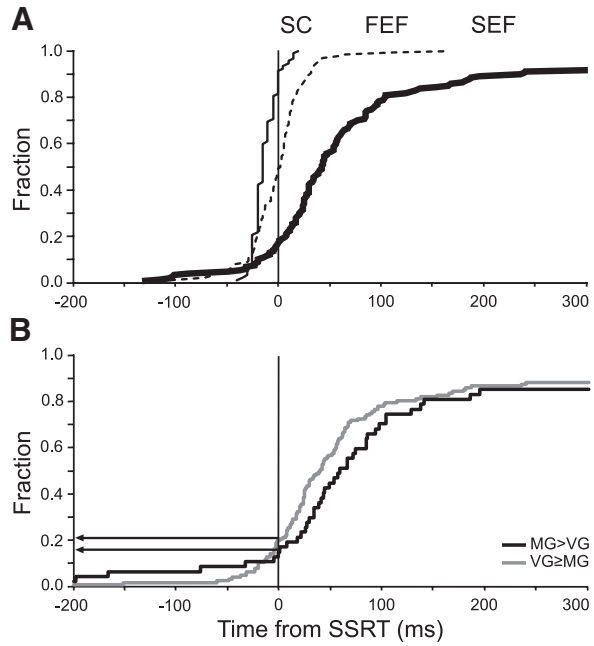


FIG. 6. *A*: distribution of times of modulation when saccades were canceled relative to stop signal response time for SEF neurons (thick) compared with movement-related neurons sampled in the superior colliculus (SC; thin solid) and frontal eye field (FEF, thin dashed). Unlike movement-related neurons in the SC or the FEF, most SEF neurons modulate well after SSRT and therefore cannot contribute to controlling directly or immediately saccade initiation. A few SEF neurons were modulated very early before SSRT in a proactive manner. SC data from Paré and Hanes (2003); FEF data from Hanes et al. (1998). *B*: distribution of times of modulation of SEF neurons that were significantly more active before visually guided saccades (black) and those that were significantly less active before visually guided saccades (gray). No difference was observed in the proportion of neurons modulating before SSRT (horizontal arrows).

Relationship to saccadic response time

The previous analysis has shown that SEF neurons do not modulate their activity in a manner sufficient to determine the monkeys' response to the stop signal. However, it has also shown that a more subtle relationship seems to exist between SEF neuron activity and the monkeys' responses in stop signal trials. In this case, we would expect to find SEF neurons with discharge rates correlating either negatively or positively with the saccade response time. In addition, since the speed of saccade generation strongly influences the odds of success during stop signal trials, we can expect to find some relationship between SEF neuronal activity and the likelihood of canceling a saccade in stop signal trials. Having no specific hypothesis about which neuronal population should carry such signals, we tested all 305 SEF neurons with visual and saccade-related activity with ≥15 canceled and noncanceled trials.

During performance of the saccade countermanding task, response time is influenced by variations in the fraction of stop signal trials on a short timescale (Emeric et al. 2007; Mirabella et al. 2006; van den Wildenberg et al. 2002). To test the hypothesis that the SEF might be the origin of these sequential effects, we performed a linear regression between the saccade response time and the activity of SEF neurons. We concen-

FIG. 5. SEF neuron that could contribute to controlling saccade initiation. Note, though, the significant modulation in stop signal trials with noncanceled responses. Conventions as in Fig. 4.

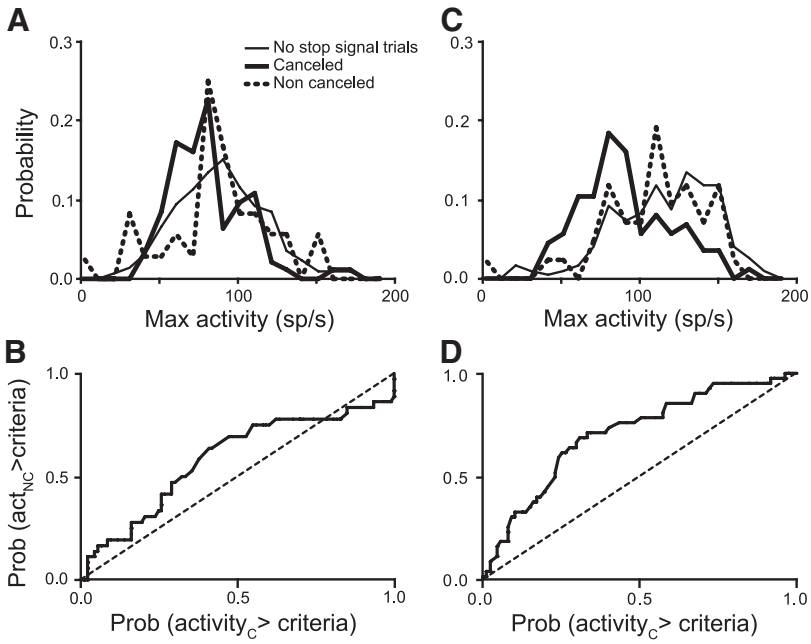


FIG. 7. Receiver operating characteristic (ROC) curve analysis of activity in canceled and noncanceled trials for the neuron shown in Fig. 4 (*A*, *B*) and for the neuron shown in Fig. 5 (*C*, *D*). *A* and *C*: frequency distributions of peak presaccadic discharge rates during canceled (thick solid) and noncanceled (thick dashed) trials. The values for trials with no stop signal (thin) are plotted for comparison. *B* and *D*: ROC derived from the distribution of activity measured during canceled and noncanceled trials (thick line). Chance is indicated by the thin dashed line. The activity of the neuron illustrated in Fig. 4 resulted in ROC area value of 0.59. The activity of the neuron illustrated in Fig. 5 resulted in ROC area of 0.71.

trated this regression analysis on no-stop signal trials to capture only the effect of stop signal expectation. We investigated three different trial periods before the saccade initiation: a baseline time period (100 ms before target onset), a period following target presentation that contained the strongest visual response (100–200 ms following target onset), and a period immediately before saccadic initiation (100 ms before the saccade). We found neurons with both a visual response and saccade-related activity that showed a significant regression of saccade response time on discharge rate. Examples of both types of cells are shown in Fig. 9. The spike density functions show the firing rate of each neuron for three subsets of trials sorted by response time (RT): short (thin line), middle (medium line), and long (thick line). The time periods in which activity was measured against which to regress RT are highlighted. For some of the neurons, response time showed either a positive or a negative regression with activity during the target onset (Fig. 9, *A* and *C*) or movement-generation period (Fig. 9, *B* and *D*). Some neurons also showed a significant regression between response time and baseline period activity. In the example shown here, this regression is negative (Fig. 9*C*).

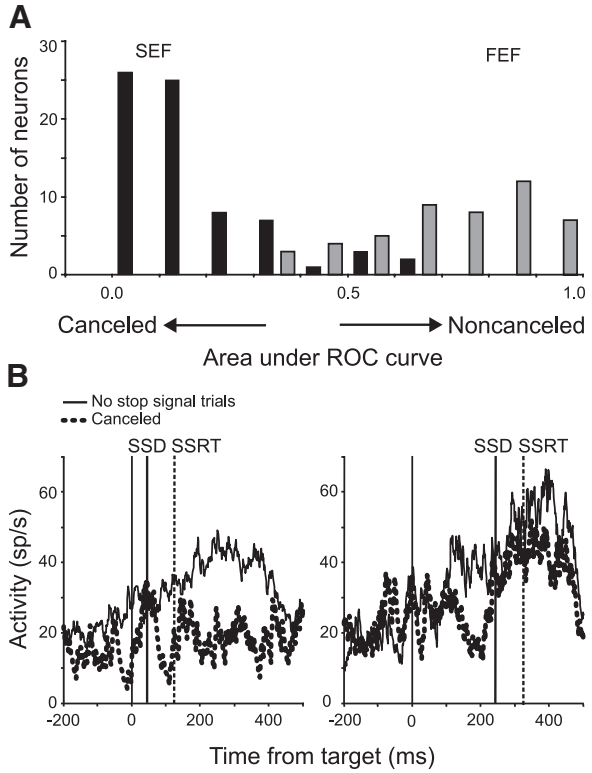


FIG. 8. *A*: comparison of the distribution of areas under the ROC curves for samples of SEF (black) and FEF (gray) neurons. Values approaching 1.0 indicate greater activity on noncanceled trials when saccades are produced. Values approaching 0.0 indicate greater activity on canceled trials when saccades are inhibited. The values of the SEF were significantly less than the FEF values. *B*: activity of representative neuron with greater activity on canceled compared with noncanceled trials during trials with shorter (*left*) and longer (*right*) SSDs.

Response time regressed significantly on the activity of numerous neurons in SEF. Figure 10 shows the distribution of regression slope coefficient values for the 305 neurons in the three time periods. The number of neurons with significant relationships of activity with response time increased with the time interval sampled: baseline activity (39/305; 13%), target-onset activity (71/305; 23%), and movement-generation activity (110/305; 35%).

Next, we analyzed how often neurons with one of the three functional types of modulation (visual, visual–saccadic, and saccadic) showed a particular regression for activity measured in the three time periods (Table 2). During the baseline and target-onset time periods, neurons of all three functional classes show with equal probability a significant negative or positive regression (χ^2 between 1.05 and 3.55). However, during movement generation, neurons with saccade-related activity often showed a disproportionately positive regression ($\chi^2 = 7.53$; $P < 0.006$) and rarely showed a disproportionately negative regression with response time ($\chi^2 = 6.73$; $P < 0.01$). Neurons with visual activity showed the opposite pattern during this time period.

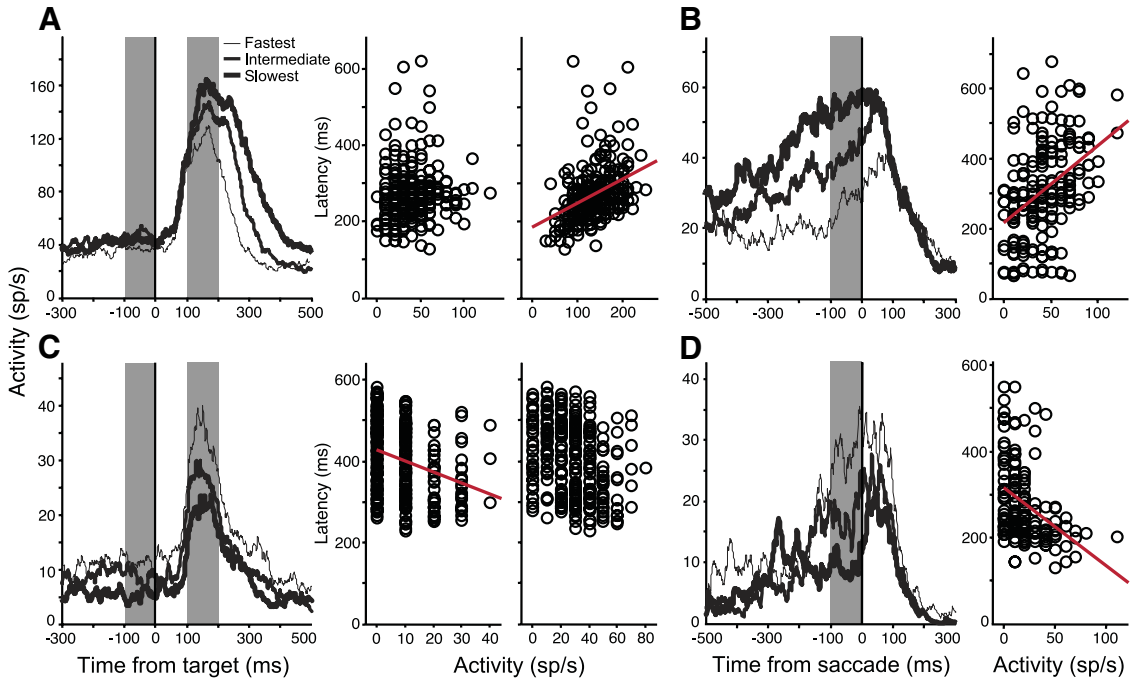


FIG. 9. Relationship of saccade response time to SEF activity. The activity of 4 representative neurons is illustrated aligned on target presentation (*A*, *C*) and on saccade initiation (*B*, *D*). All trials with no stop signal in which the target was presented in the neuron's receptive field were divided into 3 groups according to saccade response time: fastest (thin line), intermediate (middle line), and slowest (thick line). Discharge rate was measured in 3 intervals (indicated by gray background):100 ms before target onset (baseline), 100–200 ms following target onset (target onset), and 100 ms before saccade initiation (movement generation). The saccade response time is plotted against activity on that trial with the linear regressions indicated by the red line if it was significant ($P < 0.05$). The regression between baseline and target onset activity and saccade response time is shown in *A* and *C*, whereas the regression between movement generation activity and saccade response time is shown in *B* and *D*. The neuron shown in *A* and *B* was more active during saccades with longer response times. The neurons shown in *C* and *D* were more active during saccades with shorter response times.

Relationship to probability of cancelation

Thus saccadic response time is correlated with the neural activity of a substantial minority of visual and presaccadic SEF neurons. Since a slower saccade-generation process increases the chances of responding appropriately to the stop signal, the activity of these neurons might be related to the success rate during the countermanding task. To investigate this hypothesis, we compared the activity of the same 305 SEF neurons across both canceled and noncanceled trials. We measured the area under the ROC curve derived from the distributions of activity in the two sets of trials, to describe the likelihood of a specific response given the momentary firing rate of the neuron. In parallel with the term *choice probability*, this relationship will be referred to as *outcome probability*. Figure 11 shows the activity of two neurons exhibiting significant outcome probabilities. During memory-guided saccades, both neurons showed a strong visual response and sustained activity until saccade initiation with no saccade-related modulation. This pattern of activity resembles the set neurons described by Schall (1991). In comparing the average spike density functions for canceled and noncanceled stop signal trials aligned on target presentation, it is clear that the discharge rate covaried with the behavior, but the sign of the modulation differed for the two neurons. One neuron was more active during noncanceled stop signal trials and this activity difference became pronounced before target presentation. The other neuron was more active on canceled stop signal trials with the activity difference developing after target presentation (Fig. 11, *B*, *D*, and *F*). The differential activity of both neurons lasted until the SSRT elapsed or the saccade was initiated.

We analyzed the neural activity in three consecutive time periods. The first time period was the 400 ms before the target onset ("Pretarget"). Since no target was present during the pretarget period, any neural modulation must represent some proactive process. The second time period spans from target onset through the earliest SSD + SSRT ("Early"). The third time period spans the end of the earliest SSD + SSRT through the latest SSD + SSRT ("Late"). We used a bootstrap procedure to compute confidence intervals to test whether the outcome probabilities were significantly different from 0.5 ($P < 0.05$). The distributions of outcome probability values in the three time periods are shown in Fig. 12. The SEF population as a whole forms a unimodal distribution that is centered on 0.5, corresponding to no bias toward gaze-holding or gaze-shifting. Neurons at the low and high ends of the distributions showed significantly different activity for canceled versus noncanceled stop signal trials. The number of neurons whose activity was significantly correlated with outcome increased from the pretarget period (57/305; 18%), to the early period (64/305; 20%), and finally to the late period (103/305; 33%). However, the signal strength did not increase over the three time periods.

Relationship to trial sequence

We do know that a higher fraction of stop signal trials results in a delayed response time on subsequent trials with the no-stop signal (Emeric et al. 2007). Thus if the influence of SEF neurons is related to saccade response times, then

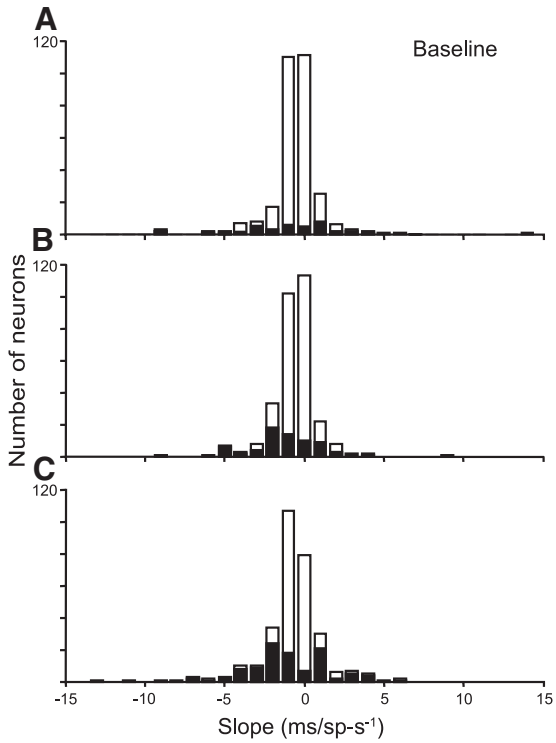


FIG. 10. Distributions of slopes of regressions of saccade latency as a function of discharge rate in the baseline (*A*), target onset (*B*), and movement generation (*C*) intervals. Significant values are filled. The number of significant values and their strength increases the later the time period.

these neurons should change their activity following stop signal trials. To investigate this hypothesis, we compared the activity of the same 305 SEF neurons across trials with the no-stop signal that either followed another no-stop signal trial or a stop signal trial (Fig. 13). We measured discharge rate in three intervals: *1*) the 100 ms preceding target onset ("baseline"), *2*) 100–200 ms following target onset ("target onset"), and *3*) the 100 ms preceding saccade onset ("movement generation"). This analysis shows that a substantial number of SEF neurons show significant differences of activity depending on the immediate trial history: baseline activity (40/305; 13%), target-onset activity (43/305; 14%), movement-generation activity (42/305; 14%). We found both neurons with visual activity (Fig. 13, *A* and *B*) and neurons with presaccadic activity (Fig. 13, *C* and *D*) that were modulated by trial history. The modulation could be an increase as well as a decrease in discharge rate. Examples of all four combinations are shown in Fig. 13. The significance of the difference in discharge rate was tested using a permutation test.

The neuron with visual activity shown in Fig. 13*A* discharged significantly more during no-stop signal trials following stop signal trials (S-N) than in trials following no-stop signal trials (N-N), both during the baseline period (N-N: 5.2 Hz; S-N: 11.8 Hz, $P < 0.0001$) and the target-onset period (N-N: 53.7 Hz; S-N: 59.8 Hz, $P < 0.03$). In contrast, the neuron with visual activity shown in Fig. 13*B* discharged significantly more during N-N trials than during S-N trials, but only during the baseline period (N-N: 16.8 Hz; S-N: 10 Hz, $P < 0.008$). The activity difference during the target-onset period, although showing the same trend, was not significantly different (N-N: 63.4 Hz; S-N: 57.6 Hz, $P > 0.14$). During the movement-generation period, the neuron with presaccadic activity shown in Fig. 13*C* discharged significantly more during S-N trials than during N-N trials (N-N: 38.2 Hz; S-N: 51.1 Hz, $P < 0.001$), whereas the neuron shown in Fig. 13*D* showed the opposite effect (N-N: 11 Hz; S-N: 7.2 Hz, $P < 0.02$).

The patterns of modulation were as expected from the results of the regression analysis. For example, the neuron shown in Fig. 13*C* increased its activity for longer response times, as shown in Fig. 9*B*. Following stop signal trials, when the monkey responded more carefully, the neuron also increased its activity, corresponding to a longer response time. It is of further note that many neurons showed significant changes of activity in the baseline period before the target for the next movement was presented (Fig. 13, *A* and *B*).

We found a substantial number of neurons that showed a relationship between their activity rate and either the saccadic response time, the probability of successful saccade cancellation, or the presence of a stop signal in the last trial. Accordingly, we tested whether the three different effects were independent among the SEF neurons in the three time periods. In the case of the outcome probability, we compared activity during the "early" with the "target-onset" time period and activity during the "late" with the "movement-generation" time period. For each pairwise comparison, we counted the number of neurons that showed either both effects, only one of them, or none. Using a χ^2 test, we compared the frequency occurrence of these four types against the one expected, based on the frequency of the two effects in the population independent of each other. The results of the three comparisons for the baseline, target-onset, and movement-generation time periods are listed in Table 3. In three cases, the number of neurons that showed both effects significantly exceeded what would be expected if the two effects were independent. Two of these cases

TABLE 2. *Distribution of SEF neuron types providing sufficient data with significant positive or negative relationship to saccade response time in three time periods relative to the entire test sample*

		Neuron Types/Cells Tested (%)		
Time Period	Sign of Slope Coefficient	Visual/140 (46)	Visual–Saccadic/122 (40)	Saccadic/43 (14)
Baseline	positive	10 (53)	4 (20)	5 (26)
	negative	6 (30)	10 (50)	4 (20)
Target onset	positive	11 (44)	11 (44)	3 (12)
	negative	23 (50)	14 (30)	9 (20)
Movement generation	positive	12 (29)	17 (40)	12 (30)
	negative	37 (54)	30 (44)	2 (2)

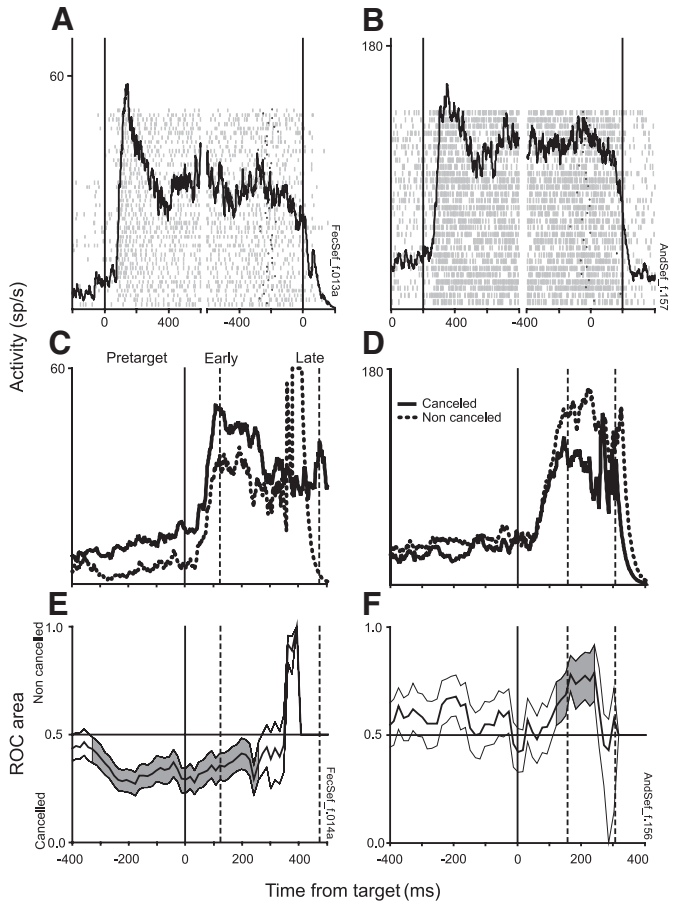


FIG. 11. Outcome probability analysis. Two SEF neurons biasing for (*left*) and against (*right*) stopping saccade responses. *A* and *B*: activity during memory-guided saccade trials. Note the pronounced visual response and delay period activity with no presaccadic modulation. *C* and *D*: superimposed average spike density functions for canceled (solid) and noncanceled (dashed) stop signal trials aligned on target presentation. Activity on canceled trials was truncated after the SSRT and activity on noncanceled trials was truncated after saccade initiation. Dotted vertical lines indicate the earliest (Early) and latest (Late) SSRT based on the variability of SSD. *E* and *F*: plot of average (thick) and confidence intervals (thin) of the area under the ROC curve constructed from the distributions of activity on canceled and noncanceled trials as a function of time. Gray background highlights periods during which the area under the ROC was significantly different from a chance value of 0.5. The neuron illustrated on the *left* exhibited significantly higher discharge rate beginning 300 ms before the target was presented and persisting until about 200 ms after target presentation on noncanceled trials. The neuron illustrated on the *right* was slightly more active before target presentation and significantly more active before SSRT on canceled trials.

involve activity during the movement-generation time period, during which each expression of proactive bias of trial outcome was most prominent. During this time period, neurons that show a significant regression with response time during no-stop signal trials were also more likely to significantly predict the outcome during stop signal trials ($P < 0.05$) and to reflect the presence or absence of a stop signal in the preceding trial ($P < 0.04$). On the other hand, outcome probability and sequential effects, by themselves, were not more likely to be present in the same cells ($P > 0.8$). This supports the hypothesis that the relationship between SEF activity and the latency with which a saccade is initiated is more closely associated with the basic function of these neurons, whereas their relationship with cancellation probability and the sequential effect are derived from the relationship with motor readiness as measured by response time.

We tested whether the few SEF neurons with activity modulating before SSRT on canceled trials (7/65; 11%) differed from the majority that did not (59/65; 91%), in the frequency with which they exhibited the three kinds of proactive motor readiness. We did not find any significant difference in the frequency of these effects in the two populations ($\chi^2 = 0.4$).

DISCUSSION

In this study we used the countermanding paradigm to test the role of SEF neurons in controlling saccade initiation. Previous work has demonstrated that saccades are initiated when the discharge rate of movement-related neurons in the FEF and SC reaches a threshold (Brown et al. 2008; Fecteau and Munoz 2007; Hanes and Schall 1996; Hanes et al. 1998; Paré and Hanes 2003; Sparks 1978). The first new observation from this study was that despite the concomitance of the activity exhibited by presaccadic neurons in SEF, FEF, and SC, we found very little evidence that SEF neurons contribute to the immediate, direct control of saccade initiation. Our second new observation was that the activity of SEF neurons was related to the level of responsiveness of the oculomotor system. These findings—together with the discovery of neurons in SEF that signal error, response conflict, and reinforcement (Stuphorn et al. 2000) and the discovery that weak electrical

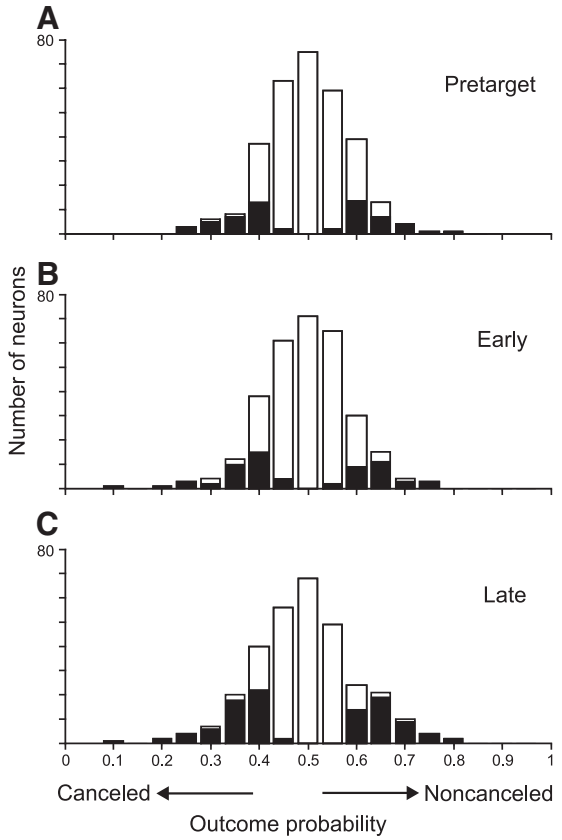


FIG. 12. Distributions of outcome probability values in stop signal trials during the pretarget (*A*) and the early (*B*) and the late (*C*) intervals. Values significantly different from 0.5 are filled. The number of significant values increases in the later time periods.

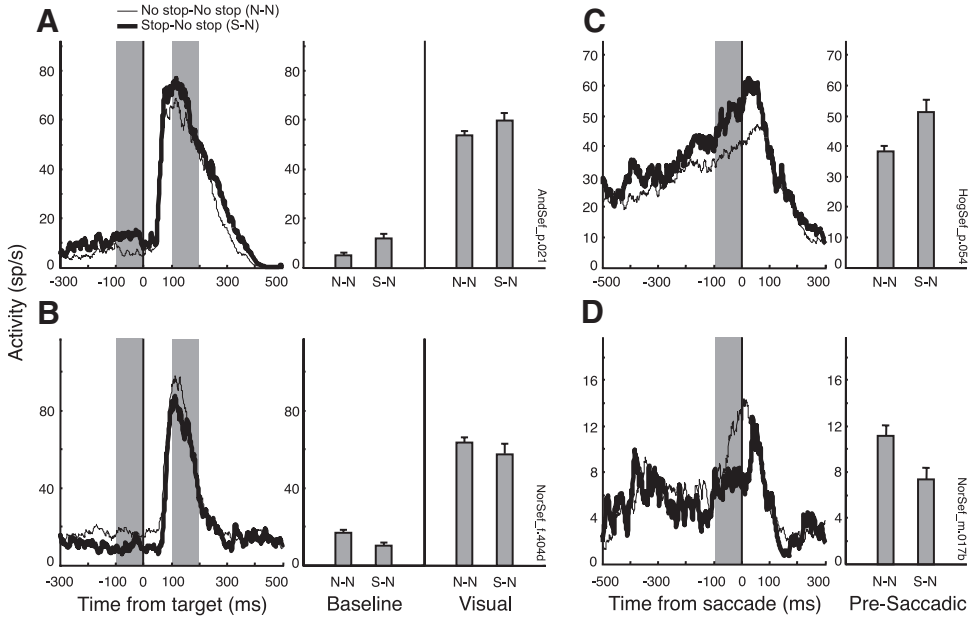


FIG. 13. Relationship of SEF activity to trial sequence. The activity of 4? (4 cell labels?) representative neurons is illustrated aligned on target presentation (*A*, *C*) and on saccade initiation (*B*, *D*). All trials with no stop signal in which the target was presented in the neuron's receptive field were divided into 2 groups: those that followed another no-stop signal trial (N-N; thin line) and those that followed a stop signal trial (S-N; thick line). Discharge rate was measured in 3 intervals (indicated by gray background): 100 ms before target onset (baseline), 100–200 ms following target onset (target onset), and 100 ms before saccade initiation (movement generation). Average discharge rate is plotted for N-N and S-N trial sequences; the error bars plot SD and asterisks highlight significant differences.

stimulation of SEF can bias saccade countermanding performance (Stuphorn and Schall 2006)—suggest a reevaluation of the role of the SEF in gaze control. Thus we suggest that the most comprehensive, plausible theoretical framework identifies SEF neurons with indirect, proactive control of gaze.

SEF neurons do not control whether and when saccades are initiated

The stop signal paradigm affords a rigorous examination of the role of neurons in motor control. To be sufficient to control the initiation of movements, neuronal signals must fulfill two criteria. First, the neuron must produce different discharge rates if a movement is initiated or canceled. Notably, some saccade-related neurons in the SEF do not fulfill even this minimal condition. The second criterion is that the difference in activity must occur within the SSRT because that is the interval taken to cancel the partially prepared movement. Those SEF neurons that satisfied the first criterion almost never fulfilled the second, since they changed their activity well after the SSRT. Accordingly, these saccade-related SEF neurons cannot contribute to canceling the saccade. It is of course possible that the SSRT was not estimated accurately in every case (Band et al. 2003; Hanes et al. 1998), but the procedures for SSRT estimation were identical for the studies of the SC, FEF, and SEF (Hanes et al. 1998; Paré and Hanes 2003); therefore we do not believe this can explain the large difference between the modulation times observed in this study of the SEF and the modulation times observed in previous studies of the FEF and SC.

These physiological results lead to the conclusion that saccade-related neurons in SEF do not contribute directly to the control of saccade initiation. However, intracortical microstimulation of the SEF with low currents evokes saccades (Martinez-Trujillo et al. 2004; Park et al. 2006; Russo and Bruce 1993; Schall 1991; Schlag and Schlag-Rey 1987; Tehovnik and Lee 1993). This efficacy of microstimulation would seem to contradict the claim that the SEF does not contribute to saccade initiation. This apparent paradox can be resolved by

TABLE 3. *Comparison of the correlation of significant effects on activity of SEF neurons during three time periods*

	Effects		Pattern of Significant Effects: Actual Number of Neurons (Expected)					
Time Period	First	Second	Both	First	Second	None	χ^2	*P*
Baseline	S	O	9 (7)	31 (33)	42 (44)	221 (219)	1.057	0.304
	S	R	5 (5)	35 (35)	32 (32)	231 (231)	0.003	0.951
	O	R	**11 (6)**	**40 (45)**	**26 (31)**	**226 (221)**	**5.008**	**0.024**
Target onset	S	O	12 (8)	31 (35)	43 (47)	217 (213)	3.209	0.072
	S	R	12 (10)	31 (33)	55 (57)	205 (203)	0.977	0.321
	O	R	17 (12)	38 (43)	50 (55)	198 (193)	3.019	0.081
Movement generation	S	O	13 (12)	29 (30)	76 (77)	185 (184)	0.058	0.809
	S	R	**21 (15)**	**21 (27)**	**86 (92)**	**175 (169)**	4.604	**0.032**
	O	R	**39 (31)**	**50 (58)**	**68 (76)**	**146 (138)**	3.992	**0.046**

Three different effects were compared: correlation of activity with response time (R), outcome probability (O), and sequential adjustments (S). For each pairwise comparison, neurons showed either both effects, only one of them, or none. The frequency of these four types was tested for independence of the two effects, using a a χ^2 test. The table compares the number of neurons that we actually observed in our sample with the number we expected if the effects were independent of each other (in parentheses). In three cases (bold type), the test showed that the number of neurons, which showed both effects, was significantly larger ($P < 0.05$) than would be expected if they were not correlated.

recognizing that electrical stimulation can evoke saccades from parts of the brain that indirectly influence more primary ocular motor structures. For example, we know that saccades can be evoked by electrical stimulation of the primary visual cortex (V1) (Keating et al. 1983; Schaeffer 1888; Schiller 1977; Wagman et al. 1958; Walker and Weaver 1940), under certain conditions with very small currents (Tehovnik et al. 2003). This effect is likely mediated through the projection from V1 to SC (Keating et al. 1983; Schiller 1977). Thus the fact that microstimulation of a part of the brain evokes saccades does not establish that the part in question produces signals necessary and sufficient to initiate saccades.

A possible objection to the conclusion that SEF is not involved in saccade initiation is related to the suggestion that the SEF predominantly plays a role in the initiation of internally guided, self-generated eye movements, as opposed to saccades triggered by external events (Amador et al. 2004; Coe et al. 2002; Schlag 2002; Schlag and Schlag-Rey 1987). Thus the fact that SEF neurons do not control saccade initiation in the countermanding task might be explained by assuming that these saccades are guided by external cues on the computer screen. However, lesion studies indicate that the SEF by itself is not sufficiently able to activate the brain stem saccade generator to initiate saccades. Saccade initiation is completely abolished after a combined bilateral lesion of FEF and SC, even though SEF remained intact (Schiller et al. 1980, 1987). On the other hand, ablation of the SEF alone does not affect the production of visually guided saccades at all (Schiller and Chou 1998, 2000a,b). Furthermore, one human patient with a highly selective lesion of the SEF showed no abnormality in producing antisaccades, which are internally guided (Husain et al. 2003; Sumner et al. 2007). This represents a serious problem for the hypothesis that SEF is responsible for producing internally guided saccades.

SEF neurons may contribute to proactive control

An alternative hypothesis is that the SEF is part of an executive control system indirectly influencing the production of saccades. Executive control can take place on at least two different levels (Braver et al. 2007). First, *reactive control* cancels or modifies the ongoing response preparation. Reactive control is a transient process that is triggered by an unexpected cue in the environment that indicates a change in the task requirements. Second, *proactive control* adjusts the response selection and preparation process in anticipation of the task demands. Proactive control does not rely on an external trigger. Instead, it is guided by endogenous signals such as prior knowledge about the task and the environment and is constantly present throughout the response selection and preparation process. It can reflect a variety of factors such as the motivation and likely outcome of different responses and the probability of the occurrence of task-relevant events.

The countermanding task evokes both forms of control. The STOP process, triggered by onset of the stop signal, is a form of reactive control and has been the focus of most physiological studies in the oculomotor system (Hanes et al. 1998; Ito et al. 2003; Paré and Hanes 2003; Stuphorn et al. 2000). However, independent of the presence of a stop signal, stop signal task performance can be influenced by proactive control (Verbruggen and Logan 2009). Behavioral studies in monkeys show that the mean saccadic response time during no-stop signal trials is delayed by about 100 ms relative to a situation where the monkey does not expect any stop signal at all (Stuphorn and Schall 2006). Similar effects have been found in humans (Verbruggen et al. 2004, 2006). Stop signal trials occur randomly in the stream of no-stop signal trials so that, on a shorter timescale, the frequency of stop signal trials changes, which can lead to behavioral adjustments as well (Emeric et al. 2007).

We believe the current results are consistent with the hypothesis that SEF can contribute to proactive control of saccade production. The signals produced by the SEF neurons with trial-to-trial variability in activity correlated with the response time and changing with trial history can influence the overall level of excitability in the saccade production system through projections to FEF, SC, basal ganglia, and brain stem nuclei, such as the nucleus raphe interpositus in which the omnipause neurons reside. We suggest that the weak correlation between SEF neuron activity and response time is the basis of the other relationships we observed between SEF activity and performance of the stop signal task. Two earlier studies failed to observe this relationship between SEF activity and response time (Genovesio et al. 2006; Ohmae et al. 2008). This is most likely explained by the different task design. In the delay tasks used in the earlier studies, there was never any reason to cancel a prepared saccade. Therefore we would expect that the saccadic responsiveness level was set to an optimal level that depended on the difficulty of the target selection and that was uniform across trials. Thus the remaining variance in saccadic reaction time was not related to fluctuations in SEF activity and no relationship between SEF activity and reaction time was detectable.

The mechanisms by which SEF can influence saccade production are not known and we do not have enough information to meaningfully constrain hypotheses. Proactive control is typically assumed to be inhibitory (Boulinguez et al. 2008; Frank 2006; Jaffard et al. 2008; Lo et al. 2009), although results from SEF indicate that both inhibitory and excitatory effects can occur. First, we found SEF neurons with both negative and positive correlations with response time. Second, an early study investigating the effects of electrical microstimulation of SEF on performance of the saccade stop signal task found sites with opposite influences (Stuphorn and Schall 2006). Recent anatomical studies are consistent with this possibility and suggest that medial prefrontal cortex projects primarily to inhibitory interneurons in the lateral prefrontal cortex (Medalla and Barbas 2009). Third, impaired executive control is demonstrated in the case of a human patient with a lesion restricted unilaterally to the left SEF (Husain et al. 2003). The patient was tested in a task in which he had to switch saccade targets infrequently and unpredictably. The patient could still switch from one saccade to another, but only when the task demands were easy. At higher difficulty levels, performance was very much impaired. Further behavioral testing of this patient revealed that he was also significantly impaired when required to switch between anti- and prosaccades, when there were conflicting rules governing stimulus–response mappings for saccades, and when required to select the appropriate saccade from conflicting eye movement responses during an arbitrary stimulus–response associative learning task (Parton et al. 2007).

The human lesion studies suggest that we might extend our findings in the countermanding task by hypothesizing that SEF cells represent the incentive value of a wide variety of response strategies. These incentive signals compete with each other for access to the oculomotor system (i.e., FEF and SC) and, as a whole, bias the motor system toward responding in a particular fashion. In situations without any salient external stimuli that favor one saccade target over another or when a salient stimulus has to be ignored, the balance of signals from SEF can tip the competition within the oculomotor system toward the actual chosen action. Examples of such situations are free-choice tasks (Coe et al. 2002) or the antisaccade task (Schlag-Rey et al. 1997). However, even in this case the final commitment takes place in FEF and SC (Hanes and Schall 1996; Sparks 1978), but not in SEF.

Anatomical pathways for executive control by SEF

SEF can set the excitability level in the saccade system through multiple neural pathways. First, the SEF has direct projections to the FEF (Huerta and Kaas 1990; Schall et al. 1993), the SC (Huerta and Kaas 1990), and to the saccade brain stem generator (Huerta and Kaas 1990; Shook et al. 1990). Second, the SEF provides input into the basal ganglia that could affect the oculomotor system indirectly via the excitatory direct and inhibitory indirect and hyperdirect pathways (Nambu 2004; Parthasarathy et al. 1992). In particular, a recent human neuroimaging study suggests a role of the subthalamic nucleus (STN) in countermanding (Aron and Poldrack 2006). Single-unit physiology experiments are necessary to clarify the role of the STN and other basal ganglia structures during the countermanding task.

We do not think SEF is the only cortical area involved in the executive control of gaze. We showed in earlier work that neurons in anterior cingulate cortex signal the consequences of saccades in the stop signal task (Ito et al. 2003). Also, in other saccade tasks neurons in the dorsolateral prefrontal cortex carry task set and task-switch–related information (Johnston et al. 2007). Presupplementary motor area neurons were recruited rapidly when an automatic response had to be suppressed to allow the generation of an unexpected saccade (Isoda and Hikosaka 2007) and therefore might work in a complementary fashion to SEF by providing reactive control. Understanding the specific role of each of these areas requires systematic experimental study. We believe the saccade stop signal task can provide necessary information through the deadline imposed by SSRT and the particular pattern expression of executive control.

Conclusion

We found that apparent saccade-related neurons in the SEF are not involved immediately or directly in controlling the initiation of visually guided saccades during the countermanding task. However, subtle modulation of SEF activity that related to the balance between gaze-shifting and gaze-holding coupled with signals of error, conflict, and reinforcement (Stuphorn et al. 2000) as well as the contextual effects of SEF microstimulation (Stuphorn and Schall 2006) support the hypothesis that SEF contributes to proactive control of gaze.

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