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Threshold mechanism for saccade initiation in frontal eye field and superior colliculus

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Jantz JJ, Watanabe M, Everling S, Munoz DP. Threshold mechanism for saccade initiation in frontal eye field and superior colliculus. *J Neurophysiol* 109: 2767–2780, 2013. First published March 13, 2013; doi:10.1152/jn.00611.2012.—In an influential model of frontal eye field (FEF) and superior colliculus (SC) activity, saccade initiation occurs when the discharge rate of either single neurons or a population of neurons encoding a saccade motor plan reaches a threshold level of activity. Conflicting evidence exists for whether this threshold is fixed or can change under different conditions. We tested the fixed-threshold hypothesis at the single-neuron and population levels to help resolve the inconsistency between previous studies. Two rhesus monkeys performed a randomly interleaved pro- and antisaccade task in which they had to look either toward (pro) or 180° away (anti) from a peripheral visual stimulus. We isolated visuomotor (VM) and motor (M) neurons in the FEF and SC and tested three specific predictions of a fixed-threshold hypothesis. We found little support for fixed thresholds. First, correlations were never totally absent between presaccadic discharge rate and saccadic reaction time when examining a larger (plausible) temporal period. Second, presaccadic discharge rates varied markedly between saccade tasks. Third, visual responses exceeded presaccadic motor discharges for FEF and SC VM neurons. We calculated that only a remarkably strong bias for M neurons in downstream projections could render the fixed-threshold hypothesis plausible at the population level. Also, comparisons of gap vs. overlap conditions indicate that increased inhibitory tone may be associated with stability of thresholds. We propose that fixed thresholds are the exception rather than the rule in FEF and SC, and that stabilization of an otherwise variable threshold depends on task-related, inhibitory modulation.

visuomotor neuron; motoneuron; prosaccade; antisaccade; inhibition

THE FRONTAL EYE FIELD (FEF) and midbrain superior colliculus (SC) are brain regions that are critical for saccade initiation. Both structures contain neurons with retinotopically organized visual and motor response fields (RFs) (Bruce and Goldberg 1985; Mohler et al. 1973; Wurtz and Goldberg 1971), and electrical microstimulation in either the FEF or the SC can elicit saccades of a specified direction and amplitude (Bruce et al. 1985; Robinson 1972; Stryker and Schiller 1975). Furthermore, permanent lesion of the FEF (Schiller and Chou 1998) or the SC (Schiller et al. 1980, 1987) produces lasting deficits in saccade initiation, and reversible inactivation of the FEF (Dias et al. 1995; Dias and Segraves 1999; Sommer and Tehovnik 1997) or the SC (Hanes and Wurtz 2001; Hikosaka and Wurtz 1985) transiently impairs production of saccades, revealed as increases in saccadic reaction time (SRT). Lesion of both structures together abolishes saccades (Schiller et al. 1979, 1980). After reversible deactivation of the SC, electrical microstimulation of the FEF cannot elicit saccades (Hanes and Wurtz 2001), implying that FEF signals pass through the SC to the brain stem saccade-generating circuit to influence saccade initiation.

An influential model was proposed suggesting that saccade initiation occurs when the discharge rate of neurons encoding saccadic movement in the FEF and SC reaches a threshold level of activation (Brown et al. 2008; Hanes and Schall 1996; Paré and Hanes 2003; Schall et al. 2011; Sparks et al. 2000). According to this model, when FEF or SC neuronal activity reaches a threshold, saccade-generating burst neurons downstream in the brain stem reticular formation are disinhibited to activate an eye movement (for review, see Moschovakis et al. 1996; Scudder et al. 2002; Sparks 2002). However, conflicting evidence has been reported as to whether this threshold is fixed or variable (Munoz and Schall 2003; Stuphorn and Schall 2002). A fixed threshold implies that saccade initiation occurs when FEF or SC presaccadic motor activity reaches a predetermined level of activity and SRT depends on changes in the rate of increase of neural activity, a change in baseline activity, or both (Carpenter and Williams 1995; Hanes and Schall 1996; Ratcliff et al. 1999). Alternatively, a variable threshold implies that SRT relies on a threshold level that may change depending on task demands, in addition to changes in the rate of increase of neural activity and/or a shift in baseline (Grice et al. 1982; Lo and Wang 2006).

Strong evidence supporting a fixed threshold for FEF and SC movement-related neurons was found in the "countermanding" saccade task (a sudden "stop" cue during movement preparation, which can instruct saccade cancellation), in which lower presaccadic activity existed in canceled saccades compared with successful trials (Brown et al. 2008; Hanes and Schall 1996; Paré and Hanes 2003). These studies concluded that there is a fixed saccade threshold within FEF and SC single neurons, because there was presumably an invariant level of activity above which saccades could not be canceled. Pooled activity from FEF movement-related neurons also supports a fixed-threshold hypothesis across a greater population of neurons (Brown et al. 2008). However, only one saccade task was used to show physiological evidence for a fixed threshold in FEF and SC neurons, and only one 10-ms temporal epoch was examined in detail.

In addition to single neurons, it is possible that a fixed threshold for saccade initiation exists at a population level (i.e.,

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saccades are initiated when the population of FEF or SC neurons reaches a fixed level of activity). Many studies have assumed that saccade initiation relies on a fixed population threshold in light of the single-neuron findings in the FEF and SC described above (Boucher et al. 2007; Brodersen et al. 2008; Cutsuridis et al. 2007; Dean et al. 2011; Dorris et al. 1997; Dorris and Munoz 1998; Pouget et al. 2011; Schall et al. 2011; Trappenberg et al. 2001). However, it was found that evoking reflexive eyeblinks immediately prior to saccade onset decreased presaccadic motor activity in SC saccade-related burst neurons, while saccade initiation still occurred (Goossens and Van Opstal 2000a, 2000b). This may implicate compensatory mechanisms at the population level that facilitate saccade initiation despite decreased activity of SC saccade-related burst neurons during blinks. This may be inconsistent with a fixed population threshold in the SC, because saccade initiation is not dependent on presaccadic motor activity reaching a fixed point. However, the implications for a population threshold are not yet conclusive, because only burst neurons were analyzed.

Here, we test the fixed-threshold hypothesis at the single-neuron and population levels by recording from saccade neurons across different behavioral conditions, in order to help resolve the conflict between previous studies. We recorded neurons in both the FEF and SC, during an antisaccade task (look away from a visual stimulus) and a prosaccade task (look toward a visual stimulus). In the antisaccade task, a monkey must resolve a conflict between visual and motor signals. Because the antisaccade is made toward a blank screen, it is conceivable that the FEF and SC saccade motor burst is lower than that of a comparable saccade made toward a visual target (Edelman and Goldberg 2001). Therefore, the antisaccade task allows us to test threshold in a scenario most likely not to conform to a fixed-threshold prediction. We analyzed the discharge of visuomotor (VM) and motor (M) neurons to test three specific predictions of the fixed-threshold hypothesis: *1*) for single neurons, no correlation should exist between presaccadic discharge rate and SRT; *2*) no difference in presaccadic activity should exist between pro- and antisaccade tasks; and *3*) the visual discharge rate should be lower than the presaccadic motor discharge rate during correct antisaccade trials.

MATERIALS AND METHODS

Preparation of experimental animals. All experimental procedures were in accordance with the Canadian Council on Animal Care policy on the use and care of laboratory animals and were approved by the Queen's University Animal Care Committee. Surgical and electrophysiological procedures were described previously (Munoz and Istvan 1998). Briefly, two male monkeys (*Macaca mulatta*) were implanted with scleral search coils, a head restraining device, and two recording chambers: one centered above the arcuate sulcus (right hemisphere in *monkey A* and left hemisphere in *monkey B*) to access the FEF and the second centered on the midline and tilted 38° posterior of vertical to access the SC. The FEF region and intermediate layers of the SC (SCi) were identified by neuronal recordings and microstimulation. These were the same animals used previously for behavioral and single-neuron recordings in the SC and FEF with the same paradigm (Bell et al. 2000; Everling et al. 1998b, 1999; Everling and Munoz 2000). Data from these neurons were previously published with different analysis techniques (Everling et al. 1999; Everling and Munoz 2000).

Behavioral paradigms. Monkeys were trained to perform blocks of randomly interleaved pro- and antisaccade trials. Details of the animal training, experimental setup, and paradigms were described previously (Bell et al. 2000). Briefly, visual stimuli were back-projected onto a tangent screen by light-emitting diodes (red and green, 0.3 cd/m^2). The REX real-time data acquisition system (Hays et al. 1982) was used to control the behavioral paradigms and visual displays and to digitize data. Horizontal and vertical eye position from one eye was digitized at 500 Hz. Each trial began with the presentation of a central fixation point (FP) on the screen. The monkey was required to look at the FP and maintain fixation for 700–900 ms. A red FP signaled a prosaccade trial, and a green FP signaled an antisaccade trial. Note that in Figs. 1–4 prosaccade trials have been assigned the color blue and antisaccade trials have been assigned the color red. An eccentric red visual stimulus (S) was then presented pseudorandomly with equal probability either at the position that yielded the optimal saccade-related response of the neuron being recorded (RF) or at a location on the opposite side of the horizontal and vertical meridians. In the prosaccade task (Fig. 1*A*) the monkeys were required to look toward the eccentric red stimulus, and in the antisaccade task (Fig. 1*B*) the monkeys were required to look 180° away from the stimulus. On half of the trials, the FP remained illuminated throughout the trial (Fig. 1*C*; overlap condition). On the other half of the trials (Fig. 1*D*; gap condition), the FP disappeared 200 ms (gap period) before stimulus presentation. Because of the need to inhibit a reflexive saccade toward the visual stimulus, the antisaccade task spatially dissociates visual and motor signals across hemispheres in the brain (Fig. 1*F*; for review, see Munoz and Everling 2004). Temporal overlap of visual and motor signals may exist globally during the antisaccade task (such that visual activity and motor activity are present concurrently but in different hemispheres). To address whether a differential intrusion of spikes from the visual response to the presaccadic motor burst (because of trial-by-trial differences in SRT) was a confounding factor on motor activity, we verified that no significant difference in presaccadic motor burst exists between fast SRT and slow SRT saccade trials (data not shown). The trials in each neuron were separated according to a median split (2 bins, capturing discharge rate in all trials below and above median SRT in each neuron). Comparisons were performed with a paired Student's *t*-test.

The optimal vector RF was determined with prosaccade trials only, by systematically moving the stimulus across the contralateral hemifield to find the location that elicited the greatest saccade response, as determined from an online raster display. The monkeys received a liquid reward if they looked to the correct location within 500 ms and maintained fixation there for at least 200 ms. An online accuracy window of ±3° around the central FP and ±60% of the saccade vector length around the peripheral stimulus was used. This large window was necessary because the end points of antisaccades had great variability (Amador et al. 1998; Bell et al. 2000; Dafoe et al. 2007; Hallett 1978; Smit et al. 1987). However, for off-line quantification of saccade-related activity, the accuracy criteria were extremely stringent, which involved filtering for saccade metrics (see below). Trials with saccade latencies (defined as the delay between eccentric stimulus appearance and saccade onset) above 500 ms were excluded as no-response trials, and latencies below 80 ms were excluded because they had a 50% probability of being correct, reflecting anticipatory responses (Dorris and Munoz 1998). During the recording of each neuron eight conditions (pro/anti, gap/overlap, in/180° out of neuronal RF as defined below) were presented in a pseudorandom order, and 15–20 correct trials were collected in each condition.

Filtering for saccade metrics between tasks. Antisaccades are associated with greater variability in saccade metrics (amplitude and direction) compared with prosaccades (Amador et al. 1998; Bell et al. 2000; Dafoe et al. 2007; Hallett 1978; Smit et al. 1987), because the saccade goal in the antisaccade task is not explicitly visible and must be extrapolated (Fig. 1*B*). VM and M neurons in the SC and FEF have

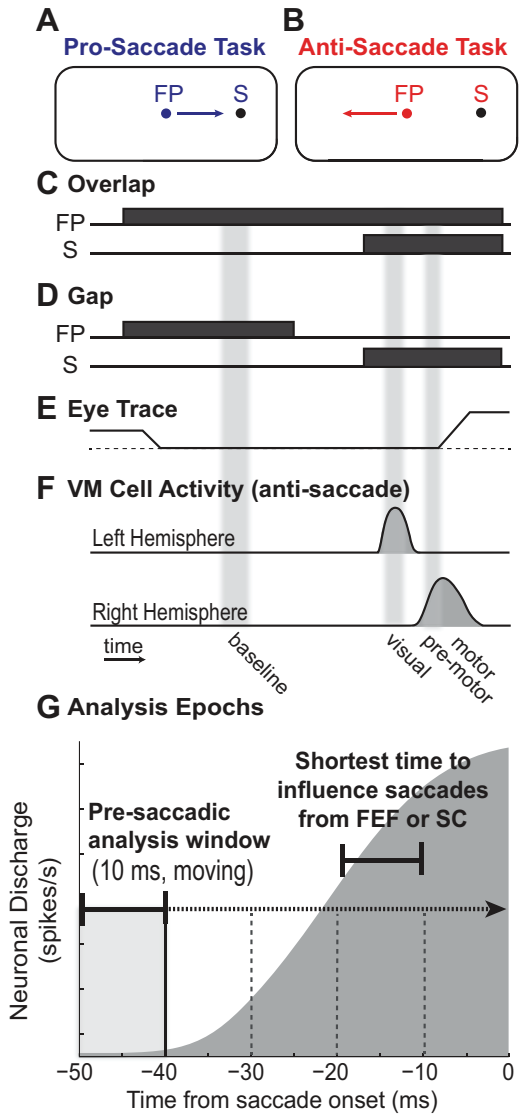

Fig. 1. Behavioral tasks employed. Monkeys performed interleaved pro- and antisaccade tasks, in which the monkey was required to either look toward (*A*) or 180° away from (*B*) an eccentric visual stimulus, based on a central color cue. On half of the trials, a central fixation point remained illuminated throughout the trial (*C*, overlap condition). On the other half of the trials (*D*, gap condition), the fixation disappeared 200 ms (gap period) before stimulus presentation. *E*: cartoon of eye position with respect to fixation point and stimulus appearance. *F*: in the antisaccade task, visual and motor activity is dissociated across hemispheres in the brain. This can be observed within individual visuomotor neurons. *G*: we used a moving 10-ms window to sequentially examine the entire presaccadic element of the frontal eye field (FEF) and superior colliculus (SC) saccade motor burst. FP, fixation point; S, eccentric visual stimulus.

spatially tuned RFs, and optimal discharge occurs in these neurons when a saccade is made into the center of an RF (Bruce and Goldberg 1985; Munoz and Wurtz 1995; Sparks et al. 1976; Sparks and Mays 1980). Variability in antisaccade metrics could result in saccade vectors that do not land in the center of the neuron's RF, resulting in lower saccadic discharge (Munoz and Wurtz 1995; Sparks and Mays 1980). In this case, the presaccadic motor discharge rate in the less variable prosaccade task would artificially appear higher compared with the more variable antisaccades in recorded neurons. To eliminate this confound, we excluded any correct antisaccade trials with >10% deviation of amplitude or direction from mean prosaccade amplitude and direction within data collected for each neuron. Any neuron with <25% antisaccade trials, or <5 correct trials in each condition remaining after exclusions, was removed from analysis. This method of off-line filtering of saccade metrics in the antisaccade task allowed us to make more meaningful conclusions about saccadic threshold.

Recording techniques. Extracellular single-neuron activity was recorded in both the SC and the FEF in two monkeys with commercially available tungsten microelectrodes (Frederick Haer) with impedances of 0.5–5 MΩ. Electrodes were driven by a hydraulic microdrive (MO-95, Narishige, Tokyo, Japan) through stainless steel guide tubes that were held firmly in position by a Delrin grid inside the recording chamber (Crist et al. 1988). Single-neuron activity was sampled at 1 kHz after passing through a window discriminator (Bak Electronics), which excluded action potentials that did not meet both amplitude and time constraints.

FEF and SC neuron classification and recording. To test hypotheses regarding a fixed threshold for saccade initiation in the FEF and SC, only neurons with an increase in activity corresponding to saccade onset were included for analysis. These neurons were grouped into M and VM classes on the basis of whether the neuron was also activated by the appearance of a visual stimulus in the RF. We focused on the analysis of these two neuron types because previous studies have shown that they project from the FEF to the SC (Everling and Munoz 2000; Segraves and Goldberg 1987; Sommer and Wurtz 2000) and from the SC to the paramedian pontine reticular formation (Rodgers et al. 2006). A subset of the neurons described here were antidromically confirmed as projecting from the FEF to the SC (Everling and Munoz 2000).

The dorsal surface of the SC was determined by the electrode depth where visual background activity was first noticed as the electrode was lowered into the midbrain. Visual and saccade-aligned discharge in the antisaccade gap condition was used to separate neurons in the SC into M and VM classes on the basis of visual and motor criteria described previously (Mohler and Wurtz 1976). To be classified as a motor-only neuron, a neuron had to be located 1–3 mm below the dorsal surface of the SC and possess the following discharge characteristics: *1*) statistically significant increase in mean discharge rate when the monkey made successful antisaccades into the RF during an epoch around saccade onset (−10 to +10 ms), compared with activity measured in the baseline epoch 500–700 ms before stimulus appearance, during fixation (paired *t*-test, $P < 0.05$) and *2*) when the stimulus was presented into the RF in the antisaccade task, no significant increase in peak firing rate measured 50–150 ms after stimulus appearance compared with activity measured in the baseline epoch defined above. Some M neurons also exhibited buildup or prelude activity before the saccade that was described previously (Glimcher and Sparks 1992; Munoz and Wurtz 1995). To be classified as a VM neuron, a neuron had to possess the following discharge characteristics: *1*) statistically significant saccade-related activity, as defined for M neurons (paired *t*-test, $P < 0.05$) and *2*) when the stimulus was presented into the RF in the antisaccade task, a statistically significant increase in peak firing rate measured 50–150 ms after stimulus appearance compared with activity measured in the baseline epoch defined above (paired *t*-test, $P < 0.05$).

The FEF was first localized as the low-threshold (<50 μA) region in the arcuate sulcus that could elicit saccades with microstimulation (Bruce et al. 1985). We used the same criteria described above to classify M and VM neurons in the FEF. Classification criteria did not require motor activity to reach an arbitrary discharge rate (Everling and Munoz 2000). This was designed to capture a range of VM neurons in which either visual or motor activity predominated, as described previously (Bruce and Goldberg 1985). Motor neurons with low (but significantly higher than baseline) saccade discharge rate were tolerated, because the antisaccade task removed contaminating signals from motor activity that otherwise would prevent a more sensitive analysis.

Data analysis. Data analyses were performed off-line with custom software (MATLAB, MathWorks) that marked the beginning and end of a saccade on the basis of radial eye velocity criteria (30°/s) described previously (Watanabe and Munoz 2010). Saccades marked on each trial were verified by an experimenter and corrected if necessary. For all analyses, only neurons with at least five correct trials for each condition (after filtering for saccade metrics) were included. A Gaussian activation function (Richmond and Optican 1987) with a σ of 4 ms was used to convolve the spike train and construct continuous spike density waveforms.

For comparing visual responses in the antisaccade task, when the stimulus was presented into the RF (i.e., saccade away from RF) we determined the peak of neuronal activation (visual response) in a time window from 50 to 200 ms after stimulus appearance (White et al. 2009). To be conservative, all analyses involving stimulus-related responses were also performed with mean neuronal activity in the interval $\pm$5 ms around peak neuronal activation in the above time window, and we found no qualitative difference in results (data not shown). Comparisons were performed with a paired Student's *t*-test.

It is unclear what temporal period relative to saccade onset might best represent a threshold for saccade initiation. It was previously assumed that threshold was dependent on presaccadic epochs spanning the shortest time at which a neural signal could influence saccade initiation, and some evidence supporting a fixed threshold for saccade initiation was found in this epoch (Hanes and Schall 1996; Paré and Hanes 2003). On the basis of previous physiological and anatomical studies, the latest 10-ms epoch with respect to saccade onset at which saccade initiation can be influenced by an FEF signal is 20 ms to 10 ms before saccade onset (Bruce et al. 1985; Büttner-Ennever et al. 1988; Hanes et al. 1995; Hanes and Schall 1996; Segraves 1992; Segraves and Goldberg 1987). Similarly, the latest 10-ms presaccadic epoch at which saccade initiation can be influenced by an SC signal is 18 ms to 8 ms before saccade onset (Miyashita and Hikosaka 1996; Munoz et al. 1996; Munoz and Wurtz 1993). To quantify changes in FEF and SC activity in a manner comparable to previous studies (Hanes and Schall 1996; Paré and Hanes 2003), we first analyzed our results in the FEF and the SC with the respective 20 ms to 10 ms and 18 ms to 8 ms presaccadic epochs above. In addition, we tested a range of epochs and repeated all analyses using mean discharge rate in a 10-ms moving window from 50 ms before saccade onset to saccade onset (Fig. 1*G*; 5 bins of 10 ms each), because this period encompassed the presaccadic element of the FEF and SC saccade motor burst (see Fig. 2*B*, Fig. 3*B*, *bottom*) and contained predominantly motor (and not visual) signals in FEF and SC VM neurons during the prosaccade task (see Fig. 2*B*, Fig. 3*B*, *top*). Comparisons were performed with a paired Student's *t*-test.

Spearman's rank correlation. We used a Spearman correlation to investigate the relationship between neuronal activity and SRT (*test 1*, RESULTS) because this is a nonparametric analysis designed for nonuniformly distributed populations. We did not have enough trials to bin data according to SRT as performed by Hanes and Schall (1996), while ensuring that more than one trial was included in each bin. This is because of the comparatively large number of interleaved conditions for our experiment (8 in total, prosaccade and antisaccade with gap and overlap fixation conditions, toward and away from the RF) and because our technique of filtering for saccade metrics between tasks dramatically decreased the number of trials available for analysis.

Bootstrap analyses. Neurons exhibiting visual and saccade motor activity can project from the FEF to the SC (Everling and Munoz 2000; Segraves and Goldberg 1987; Sommer and Wurtz 2000) and from the SC to the paramedian pontine reticular formation (Rodgers et al. 2006). We found differences in VM and M neuronal activity with respect to saccade threshold, and differences have also been reported in the FEF with the countermanding saccade task (with different cell classification criteria; Brown et al. 2008). Because the relative influence of VM and M neurons on downstream structures is currently unclear, we used bootstrap analyses to examine the aggregate activity of several modeled proportions of VM and M neuron types and compared these to previously reported anatomical distributions. We sequentially varied the sampled distribution of VM to M neurons, because previous studies have reported different VM and M neuron distributions in the FEF and SC (Rodgers et al. 2006; Segraves and Goldberg 1987; Sommer and Wurtz 2000), making the distribution of VM to M corticotectal and tectoreticular saccade projection neurons unclear. We began by calculating mean presaccadic discharge and visual peak response within a sample of VM and M neurons randomly sampled at a particular neuron distribution (such as 95% VM and 5% M neurons). We used a 20 ms to 10 ms presaccadic epoch in the FEF and an 18 ms to 8 ms epoch in the SC to probe presaccadic motor discharge rate because it is consistent with previous studies (Brown et al. 2008; Hanes and Schall 1996; Paré and Hanes 2003) and because there was no significant difference in trend from 50 ms to 0 ms before saccade onset when testing single M or VM neurons alone (see RESULTS). Using a bootstrap analysis, we performed a random sampling 500 times at the 95% VM to 5% M neuron distribution, to repeatedly determine mean presaccadic motor and visual peak discharge rate. This resulted in a normally distributed cluster of points centered on the mean presaccadic motor discharge rate, and the visual peak discharge rate, under the model that the actual downstream-projecting population consisted of 95% VM and 5% M neurons (clusters not shown; normal distribution was verified by Kolmogorov-Smirnov test). This analysis was repeated with multiple distributions of VM to M neurons at 5% intervals, so that neurons were sampled at 90% VM to 10% M in the next analysis, and so on.

For this analysis, we assumed that VM and M neuronal discharge are weighted equally in the FEF and SC to initiate saccadic eye movement. It is possible that differences exist in the synaptic strength of VM and M neuron populations, which would skew our bootstrap results. However, because we have no way of assessing the likelihood of this, and because individual differences in synaptic strength of VM and M neurons are also plausible, we have chosen an equal weighting between neuron types as the most conservative estimate.

RESULTS

We recorded 78 task-related neurons in the FEF while monkeys performed pro- and antisaccade tasks (see Table 1). Of these, 35 VM and 18 M neurons were classified. During some sessions the monkeys were implanted with stimulation electrodes inserted in the SCi for antidromic identification of corticotectal projection neurons, and 33 corticotectal task-related neurons were identified as described previously (Everling and Munoz 2000). Twelve recorded VM neurons and 6 recorded M neurons in the FEF were confirmed as corticotectal projection neurons. After filtering for saccade metrics, and

Table 1. *Numbers of neurons recorded*

	Frontal Eye Field	Superior Colliculus
Task related	78	87
VM neurons	35	48
M neurons	18	13
VM neurons, filtered for saccade metrics	29	34
M neurons, filtered for saccade metrics	12	10
Antidromically identified projection neurons	33	
Antidromically identified VM projection neurons, filtered for saccade metrics	9	
Antidromically identified M projection neurons, filtered for saccade metrics	6	

Values are numbers of neurons recorded in the frontal eye field and superior colliculus. VM, visuomotor; M, motor.

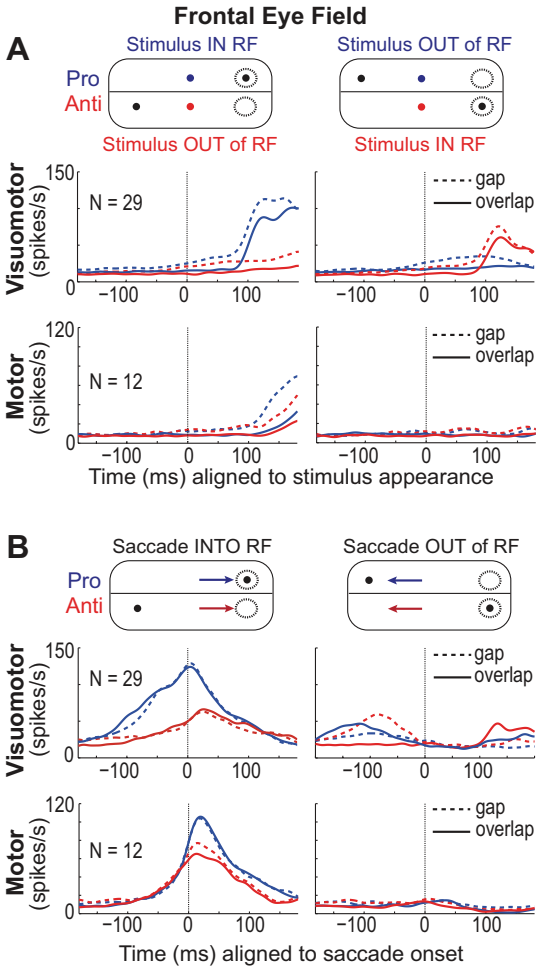

Fig. 2. Population spike density plot for visuomotor (VM; $n = 29$) and motor (M; $n = 12$) neurons in the FEF. *A*: spike density plots aligned to stimulus appearance can dissociate visual activity from motor activity in VM neurons during the antisaccade task, depending on whether the visual stimulus or the saccade occurs in the response field. M neurons demonstrate no significant visual response. *B*: spike density plots aligned to saccade onset show an increase of activity preceding saccade onset (presaccadic motor activity) in VM and M neurons in the FEF when a saccade is made into the response field. No significant difference in presaccadic motor activity exists between gap and overlap conditions in any cell type, from 50 ms to 0 ms before saccade initiation. RF, response field.

removing all neurons with less than five successful trials remaining per condition after the filter, 29 VM and 12 M neurons remained for analysis, which included 9 VM and 6 M antidromically confirmed corticotectal projection neurons. In the SC, we recorded 87 task-related neurons. Of these, 48 VM and 13 M neurons were classified. After filtering for saccade metrics, and removing neurons with less than five successful trials per condition remaining after the filter, 34 VM and 10 M neurons remained for analysis.

Averaged population spike density plots were calculated to qualitatively illustrate neuronal responses in the FEF (Fig. 2) and in the SC (Fig. 3) to visual stimuli and saccades either into or out of the RF. Aligning population spike density plots to stimulus appearance revealed the VM visual response, with peak activation occurring 100–150 ms after stimulus appearance in the RF (Fig. 2*A*, Fig. 3*A*). In the prosaccade task, VM stimulus-aligned visual activity was contaminated with presaccadic motor buildup activity. However, in the antisaccade task, visual activity and presaccadic motor activity were separated spatially across hemispheres, by placing the stimulus either out of or into the RF, respectively. We used the visual and motor discharges dissociated by the antisaccade task to classify recorded cells as M (discharge only during saccades) or VM (discharge for both visual stimuli and saccades). Aligning spike density plots to saccade onset illustrates the saccade motor activity in the FEF (Fig. 2*B*) and in the SC (Fig. 3*B*).

Test 1: Is there a correlation between presaccadic motor discharge rate and saccadic reaction time? Correlation analyses between SRT and presaccadic motor activity have been used previously as a key test for saccade threshold in FEF and SC M neurons (Hanes and Schall 1996; Paré and Hanes 2003). The prediction is of a negative result: a fixed threshold implies that motor activity is constant at saccade initiation regardless of when the initiation occurs. However, these studies examined only the countermanding task and relied on the assumption that the 20 ms to 10 ms presaccadic epoch in the FEF and the 18 ms

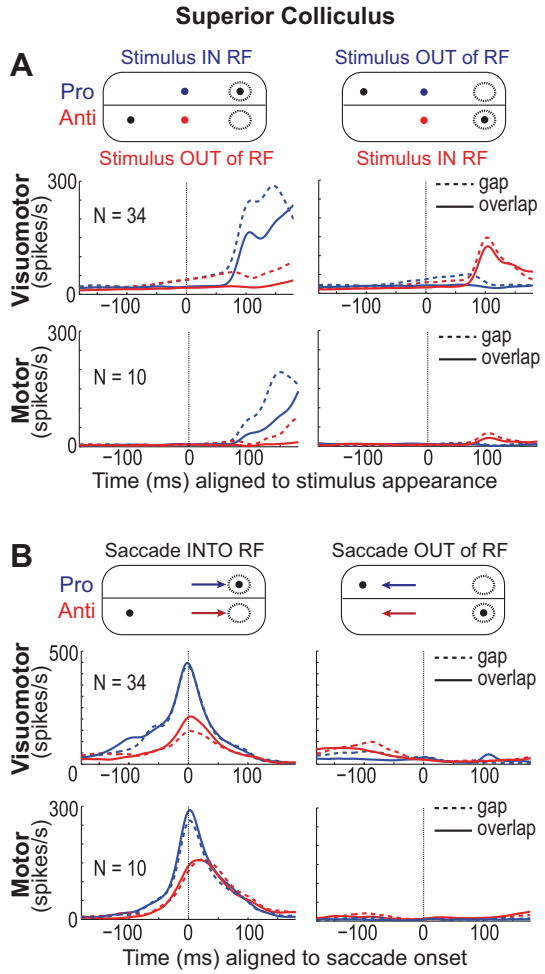

Fig. 3. Population spike density plot for VM ($n = 34$) and M ($n = 10$) neurons in the SC. *A*: spike density plots aligned to stimulus appearance can dissociate visual activity from motor activity in VM neurons during the antisaccade task, depending on whether the visual stimulus or the saccade occurs in the response field. *B*: spike density plots aligned to saccade onset show an increase of activity preceding saccade onset (presaccadic motor activity) in VM and M neurons in the SC when a saccade is made into the response field. No significant difference in presaccadic motor activity exists between gap and overlap conditions in any cell type, from 50 ms to 0 ms before saccade initiation.

to 8 ms presaccadic epoch in the SC accurately reflected saccade threshold. We tested fixed threshold within single VM and M neurons in the FEF and SC, by comparing SRT and presaccadic motor discharge rate during different saccade tasks, from 50 ms to 0 ms before saccade onset using a moving 10-ms window (Fig. 1*G*; 5 bins of 10 ms each). We determined the Spearman's rank correlation coefficient, which assesses how well the relationship between SRT and presaccadic neuronal discharge can be described with a monotonic function, for VM and M neurons in each task condition. A fixed threshold for saccade initiation predicts a nonsignificant Spearman correlation (no relationship between discharge rate and SRT). Alternatively, a significant positive or negative correlation would contradict a fixed-threshold model at that time point.

In temporal periods prior to 50 ms before saccade onset, we found a correlation between SRT and VM or M neuron discharge rate in both the FEF and the SC. This reflected changes in the neuronal rate of rise with respect to SRT (Hanes and Schall 1996); however, these correlations could occur irrespective of a fixed or variable threshold, and are therefore not shown. In addition, this positive correlation prior to 50 ms before saccade onset was not always consistent because of the inclusion of both buildup and burst motor neurons.

Similar to the findings of Hanes and Schall (1996), we found that the mean Spearman's rank correlation coefficient comparing presaccadic motor discharge rate and SRT in FEF M and VM neurons was not statistically different from 0 in the 20 ms to 10 ms epoch before saccade onset (Bonferroni correction for multiple comparisons; Fig. 4, *A* and *C*), indicating no relationship as predicted by a fixed threshold, in the epoch spanning the shortest time at which a neural signal can influence saccade initiation (see MATERIALS AND METHODS). Similarly, in SC M and VM neurons, mean Spearman's correlation coefficient was not statistically different from 0 in the 18 ms to 8 ms epoch before saccade onset (Fig. 4, *B* and *D*).

To address the possibility that presaccadic neural activity in the 20 ms to 10 ms epoch and the 18 ms to 8 ms epoch does not accurately reflect FEF and SC saccade threshold (respectively), we also tested the relationship between SRT and motor discharge in other presaccadic epochs. We found that FEF neurons and SC neurons demonstrate a statistically significant relationship between presaccadic motor discharge rate and SRT in some presaccadic window positions after a Bonferroni correction for five tests (i.e., 5 presaccadic epochs) on the same data sets (therefore $P < 0.05/5 = 0.01$ criterion; Fig. 4). However, this did not occur consistently across all task conditions. Because it is currently unclear when a fixed saccade threshold would take effect, and because results differ depending on the examined presaccadic epoch and condition, this test did not provide clear conclusions about a fixed threshold. Therefore we implemented additional tests of fixed threshold to search for more consistent results.

Test 2: Does presaccadic discharge vary between different behavioral conditions? A fixed threshold for saccade initiation predicts that signals contributing to saccade initiation will evoke a saccade when their activity reaches a fixed point, regardless of behavioral task. This would be revealed as no change in presaccadic motor discharge (sampled from equivalent time windows) between the pro- and antisaccade tasks.

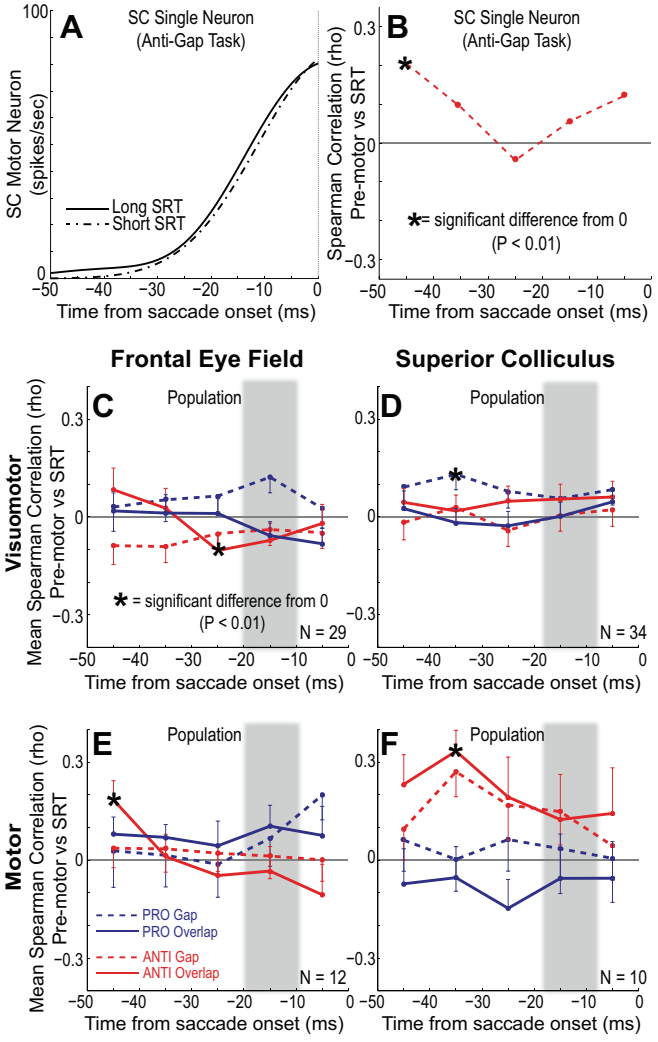

Fig. 4. A fixed threshold for saccade initiation predicts that no relationship exists between saccadic reaction time (SRT) and presaccadic motor discharge (Spearman correlation = 0). If a fixed-threshold mechanism exists, a saccade would be initiated when presaccadic activity reaches a fixed point, and SRT would rely only on changes in the rate of increase of neural activity, a change in baseline activity, or both. *A*: spike density functions for long-SRT and short-SRT trials (high and low SRT tertiles) are shown for an exemplar SC motor neuron ($n = 16$ trials, anti-gap task only). *B*: change in the Spearman correlation between SRT and the exemplar SC motor neuron firing rate in the anti-gap task is shown over time. *C–F*: change in mean population Spearman correlation over time, across all tasks. Any relationship between presaccadic motor discharge rate and SRT (Spearman correlation $\neq 0$) indicates that saccade latency is dependent on variations in presaccadic motor discharge rate and is inconsistent with a fixed-threshold model at that time point. However, it is not (currently) known when saccade threshold is reached, and a lack of correlation cannot on its own indicate the time at which saccade threshold is reached. *Significance after Bonferroni correction (paired *t*-test, $P < 0.01$). Gray shading illustrates previously examined epochs for threshold that span the shortest time at which a neural signal can influence saccade initiation: 20 ms to 10 ms before saccade onset in the FEF and 18 ms to 8 ms before saccade onset in the SC. Error bars indicate SD.

Alternatively, differences in FEF and SC presaccadic discharge rate between pro- and antisaccade tasks are inconsistent with a fixed-threshold prediction in single neurons in these structures. To facilitate a comparison between pro- and antisaccades, we matched saccade metrics stringently (see MATERIALS AND METHODS). Qualitatively, population spike density functions of VM

and M neurons in the FEF (Fig. 2*B*) and SC (Fig. 3*B*) revealed higher saccade-aligned motor activity in the prosaccade task compared with the antisaccade task. We first quantified differences in pro- and antisaccade activity in 20 ms to 10 ms and 18 ms to 8 ms presaccadic epochs in the FEF and SC, respectively. At these time points, presaccadic motor discharge rate was consistently higher in the prosaccade task than in the antisaccade task in SC VM, SC M, and FEF VM neurons (Fig. 5*A*; paired *t*-test, $P < 0.05$). For many individual FEF M neurons, we found significant differences in activity between the prosaccade task and the antisaccade task (Fig. 5*A*, *bottom left*; paired *t*-test, $P < 0.05$), but at the population level there was no significant difference or correlation (paired *t*-test, $P > 0.05$; Pearson linear correlation, $r = -0.30$, $P = 0.35$ gap task; $r = -0.23$, $P = 0.46$ overlap task).

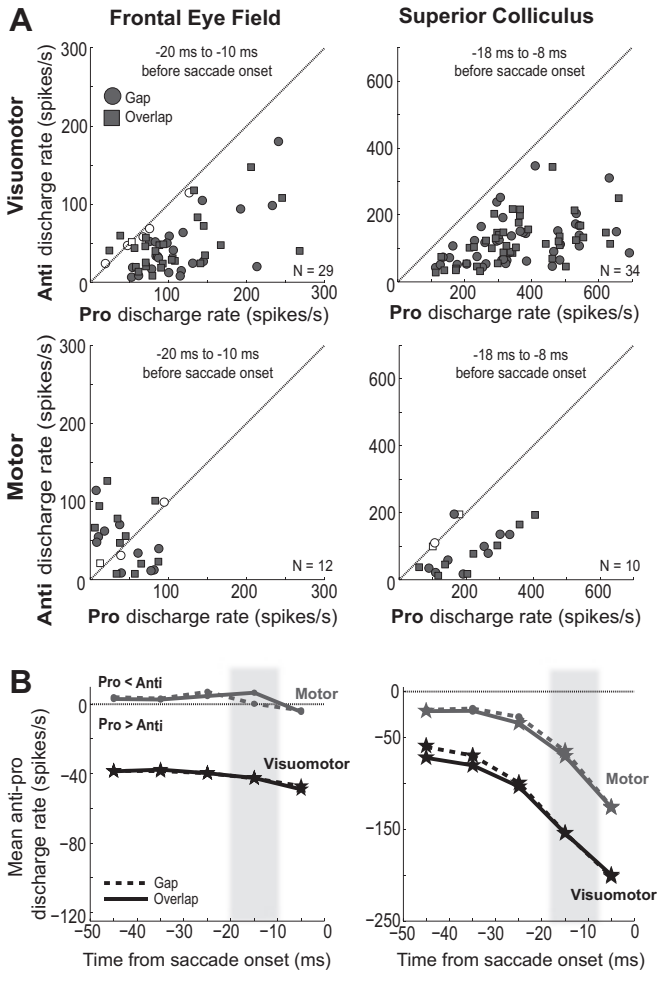

Fig. 5. Comparing presaccadic motor discharge rate between pro- and antisaccade tasks. A fixed-threshold model predicts that no variations exist in presaccadic motor discharge between saccade tasks. Alternatively, any variation in presaccadic motor discharge between saccade tasks is inconsistent with a fixed-threshold model. *A*: comparing mean motor discharge rates for neurons during previously examined presaccadic epochs for saccade threshold: 20 ms to 10 ms in the FEF and 18 ms to 8 ms in the SC. Deviation of the points from the line of unity (slope = 1) indicates a difference in presaccadic discharge rate between the saccade tasks during this epoch (filled symbols are significantly different, paired *t*-test, $P < 0.05$; open symbols are not significantly different). *B*: mean discharge rate in the prosaccade task was subtracted from the antisaccade task in a moving 50 ms to 0 ms presaccadic window. Values above 0 indicate a higher discharge rate in the antisaccade task, whereas values below zero indicate a higher discharge rate in the prosaccade task. ★, Significance after Bonferroni correction (paired *t*-test, $P < 0.01$).

We further quantified any differences in discharge rate from 50 ms to 0 ms before onset of pro- and antisaccades by subtracting mean presaccadic motor discharge rate of prosaccades from mean presaccadic motor discharge rate of antisaccades at each 10-ms window. Values above 0 indicate a higher discharge rate in the antisaccade task, whereas values below 0 indicate a higher discharge rate in the prosaccade task. After performing a Bonferroni correction for multiple comparisons, we found that the mean presaccadic motor discharge rate was consistently higher in the prosaccade task than in the antisaccade task in FEF VM neurons and SC VM neurons, regardless of presaccadic time point (Fig. 5*B*; paired *t*-test, $P < 0.01$). SC M neurons had qualitatively higher discharge rate in the prosaccade task across all presaccadic time points, although after Bonferroni correction this did not reach significance in the 40 ms to 30 ms window (gap and overlap conditions) and in the 30 ms to 20 ms window (gap condition only). Some FEF M neurons exhibited individual differences between tasks, which is inconsistent with a fixed threshold within individual FEF M neurons as proposed by Hanes and Schall (1996). However, average FEF M presaccadic discharge rate did not vary significantly between the pro- and antisaccade tasks, and therefore the population of FEF M activity in these tasks was largely consistent with the population fixed threshold proposed by Brown et al. (2008).

To account for the possibility that saccade initiation relies on a change in neuronal firing rate from baseline (rather than an absolute firing rate), and to account for task-dependent differences in baseline (e.g., higher baseline activity in the prosaccade task compared with the antisaccade task; reported by Everling et al. 1999; Everling and Munoz 2000), we repeated this test twice using presaccadic motor discharge after subtracting activity measured in the baseline epoch 500 to 700 ms before stimulus appearance (during fixation) as well as in the baseline epoch 400 to 300 ms before stimulus appearance (during fixation). We found no qualitative difference in results (not shown).

Test 2 also reveals time-dependent differences between pro- and antisaccade activity. Higher pro- compared with antisaccade activity exists as time progresses toward saccade initiation (Fig. 5*B*). We used a repeated-measures analysis of variance (ANOVA) to test this trend of increasing pro- compared with antisaccade activity and used sequential Bonferroni pairwise comparisons to determine the time points at which significant changes in activity occur.

ANOVA analysis revealed no significant upward or downward trend in FEF M cells in the gap condition [$F(4,44) = 1.08$, $P = 0.3762$] or in the overlap condition [$F(4,44) = 1.41$, $P = 0.2478$]. There was no significant trend in FEF VM cells in the gap condition [$F(4,108) = 1.46$, $P = 0.2196$]; however, there was a significant downward trend in the overlap condition [$F(4,108) = 4.05$, $P = 0.0042$]. Qualitatively for SC neurons, there was a downward trend in points when comparing pro- to antisaccade activity, in both gap and overlap conditions. In SC M cells, ANOVA analysis revealed a significant downward trend in the gap condition [$F(4,36) = 17.53$, $P = 4.55 \times 10^{-8}$] and in the overlap condition [$F(4,36) = 12.62$, $P = 0$]. Similarly, in SC VM cells, there was a significant downward trend in both the gap condition [$F(4,164) = 99.84$, $P = 0$] and

the overlap condition [$F(4,164) = 90$, $P = 0$]. Using a Bonferroni pairwise comparison (0.05/4 = 0.0125), we determined when a significant change of activity occurred by time point (Fig. 5*B*). In SC M cells, no significant difference existed between adjacent time points. However, a significant difference in activity existed between the −30 ms to −20 ms and −10 ms to 0 ms time points. In SC VM cells, a significant difference existed between the adjacent −30 ms to −20 ms and −20 ms to −10 ms time points. Predictably, there was also a significant difference between −30 ms to −20 ms and −10 ms to 0 ms time points in SC VM cells.

In sum, FEF M neurons showed the best evidence for a fixed-threshold mechanism according to *test 2* at the population level but not at the single-neuron level. FEF VM neurons and SC M and VM neurons failed *test 2* at both the population and single-neuron levels. For those neurons, the discrepancy from a fixed-threshold prediction grew as time proceeded toward saccade initiation.

Test 3A: Can visual response exceed presaccadic motor discharge in VM neurons? The possibility exists that saccade threshold may change based on the different demands between saccade tasks but remain fixed within a specific task condition (i.e., from task instruction to saccade initiation). To address this, we first compared visual and presaccadic motor activity of FEF and SC VM neurons for correct antisaccade trials. VM neurons can carry both visual and saccade activity from the FEF to the SC (Everling and Munoz 2000; Segraves and Goldberg 1987; Sommer and Wurtz 2000) as well as from the SC to the paramedian pontine reticular formation (Rodgers et al. 2006). In the antisaccade task, visual activity and presaccadic motor activity compete to initiate either an erroneous saccade (Fig. 6*A*; toward the visual stimulus) or a correct

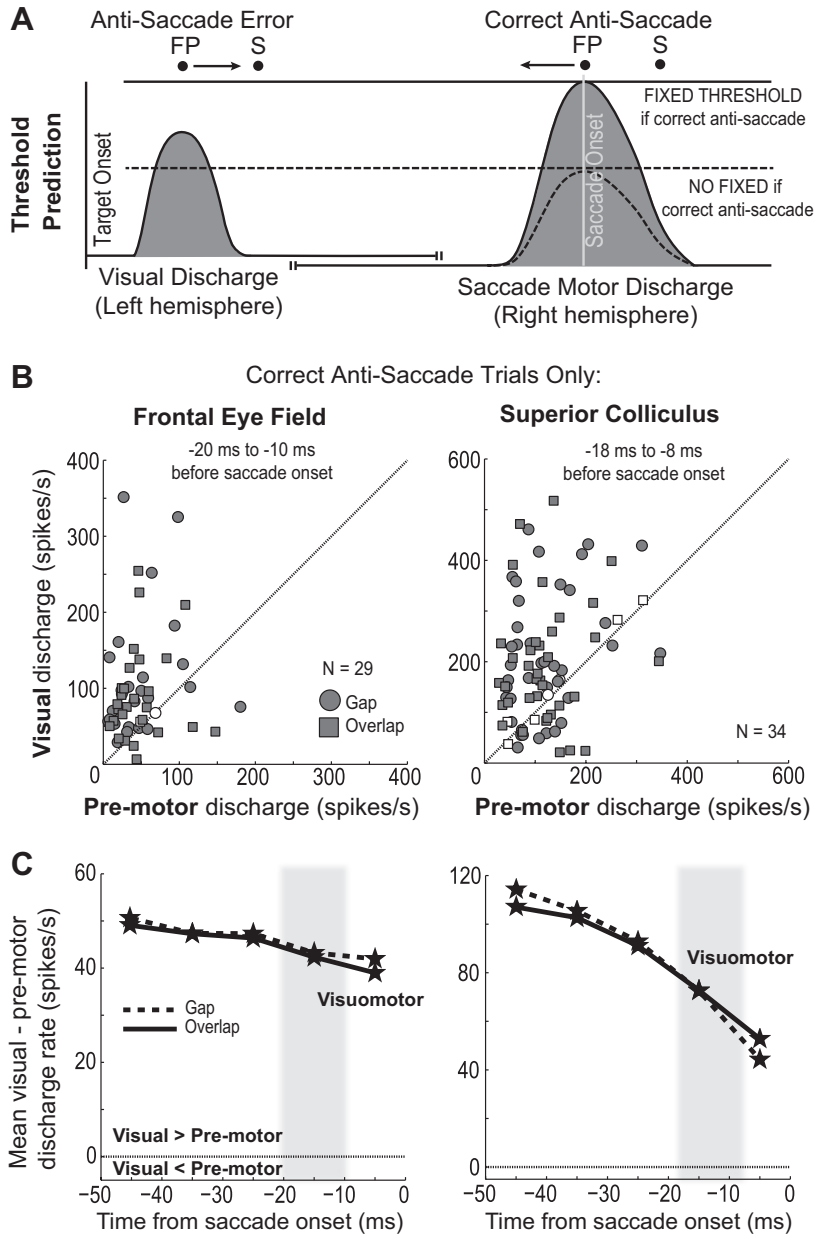

Fig. 6. Comparing visual and presaccadic motor discharges in visuomotor neurons. *A*: during correct antisaccade trials, visual and saccade motor discharges are spatially dissociated across hemispheres in the brain. Visual activity and presaccadic motor activity compete to initiate either an erroneous involuntary saccade or a correct antisaccade, respectively. Higher visual discharge rate than presaccadic motor discharge rate during correct antisaccade trials is inconsistent with a fixed threshold, as these signals should elicit an erroneous involuntary saccade toward the visual stimulus. *B*: clustering of points above the line of unity indicates higher visual discharge rate than presaccadic motor discharge during previously examined epochs for saccade threshold (filled symbols indicate significance, paired *t*-test, $P < 0.05$; open symbols indicate that visual and presaccadic motor discharges are not significantly different). *C*: mean presaccadic discharge rate was subtracted from visual discharge rate in a moving 50 ms to 0 ms presaccadic window. Values above 0 indicate higher mean visual discharge rate than presaccadic motor discharge rate (inconsistent with a fixed threshold). ★, Significance after Bonferroni correction (paired *t*-test, $P < 0.01$).

saccade (Fig. 6*A*; away from the visual stimulus), respectively. During a correct antisaccade, visual and presaccadic motor activity is spatially separated across hemispheres in the FEF and SC. A fixed threshold predicts that population visual activity in one hemisphere of the brain must be less than the presaccadic motor discharge in the other hemisphere during correct antisaccade trials, otherwise higher visual than presaccadic motor activity would initiate an erroneous involuntary saccade toward the stimulus (Everling and Munoz 2000; Munoz et al. 2007; Trappenberg et al. 2001). Alternatively, higher population visual activity relative to presaccadic motor discharge during correct antisaccade trials would be inconsistent with a fixed-threshold model (Fig. 6*A*).

Peak visual discharge rate (within 50–150 ms after stimulus appearance) was first compared to presaccadic motor discharge rate in the 20 ms to 10 ms and 18 ms to 8 ms presaccadic epochs in the FEF and SC, respectively. At these time points, visual discharge rate was significantly higher than presaccadic motor discharge rate (Fig. 6*B*; paired *t*-test, $P < 0.05$) in the majority of neurons ($n = 26$ of 29 FEF gap, $n = 24$ of 29 FEF overlap; $n = 24$ of 34 SC gap, $n = 23$ of 34 SC overlap).

To determine whether presaccadic motor activity in the 50 ms to 0 ms epoch before saccade onset is at any point higher than visual activity, we subtracted mean neuronal activity within a moving 10-ms window (Fig. 1*G*) from the visual response. Values below 0 indicate higher presaccadic motor discharge rate than visual peak discharge rate and are consistent with a fixed threshold. Alternatively, values above 0 indicate higher peak visual discharge rate than presaccadic motor discharge rate and are inconsistent with a fixed threshold. After performing a Bonferroni correction for multiple comparisons, we found that peak visual discharge rate was consistently higher than presaccadic motor discharge from 50 ms to 0 ms before saccade onset (Fig. 6*C*; paired *t*-test, $P < 0.01$). No difference in results was found between the gap and overlap conditions. Because the higher visual discharge rate did not elicit erroneous involuntary saccades toward the visual stimulus, this contradicts a fixed threshold within the antisaccade task among individual VM neurons. We repeated this test twice using presaccadic motor discharge after subtracting baseline activity measured during two different fixation periods (see MATERIALS AND METHODS) to account for the possibility that saccade initiation relies on a change in neuronal firing rate from baseline (rather than an absolute firing rate), and to account for task-dependent differences in baseline (e.g., higher pro- baseline activity compared with anti- baseline activity; Everling et al. 1999; Everling and Munoz 2000). We found no qualitative difference in results (not shown).

Test 3B: Can inhibitory state alter threshold in VM and M neuron populations within the antisaccade task? Because projections from the FEF to the SC (corticotectal) and from the SC to the brain stem saccade-generating circuit (tectoreticular) are critical for saccade initiation, any threshold for saccade initiation that involves the FEF and SC likely relies on these populations of projection neurons. Therefore it is possible that any findings in VM neurons alone cannot contradict a population fixed threshold, because they may not accurately represent all of the neural activity descending to the brain stem to trigger saccades. To address this possibility, we estimated visual activity and presaccadic motor activity within varied proportions of VM and M output neurons of the FEF and SC, which more accurately reflected physiological conditions. We chose the VM and M neuron types on the basis of antidromic identification of corticotectal projection neurons in our population (Everling and Munoz 2000) as well as previous studies reporting the percentages of saccade task-related neurons with projections from the FEF to SC (Segraves and Goldberg 1987; Sommer and Wurtz 2000) or from the SC to the paramedian pontine reticular formation (Rodgers et al. 2006).

We obtained mean presaccadic motor discharge rate and mean visual peak discharge rate using separate bootstrap analyses for varied distributions of VM and M neurons in the FEF and SC (see MATERIALS AND METHODS). This produced one data point for each distribution of VM and M neurons tested (Fig. 7). We constrained our analysis to the previously studied 20 ms to 10 ms and 18 ms to 8 ms presaccadic motor epochs in the FEF and SC, respectively, to capture the presaccadic motor discharge rate, because when testing VM neurons alone (*test 3A*) there was no change in results from 50 ms to 0 ms before saccade onset (Fig. 6*C*). Any data point showing higher visual than presaccadic motor discharge rate is inconsistent with a fixed threshold at that given distribution of VM and M neurons. Therefore, if any data point representing a VM to M distribution is below the line of unity (Fig. 7), then our estimate of population visual discharge rate is lower than the presaccadic motor discharge rate, which is consistent with a fixed threshold at that neuron distribution. Alternatively, if a data point representing a VM to M distribution is above the line of unity, then our estimate of population visual discharge rate is higher than the presaccadic motor discharge rate, which is inconsistent with a fixed threshold at that neuron distribution. Previously published neurophysiological estimations of corticotectal (Everling and Munoz 2000; Segraves and Goldberg 1987; Sommer and Wurtz 2000) and tectoreticular (Rodgers et al. 2006) VM to M projection neuron distributions are indicated in Fig. 7. This test investigates fixed threshold within an estimate of population VM and M neuron activity.

This approach is inconsistent with a fixed-threshold model for the majority of hypothetical FEF and SC neuron distributions in the gap task (Fig. 7, *A* and *B*) but not in the overlap task (Fig. 7, *C* and *D*). Increasing the percentage of sampled M neurons shifted the data points downward on the scatterplot (consistent with a fixed-threshold prediction), while increasing the percentage of sampled VM neurons shifted the points upward on the plot (contradicting a fixed-threshold prediction). In the gap condition, our data refute a fixed threshold for saccade initiation in both the FEF and the SC based on all previously published neuron distributions, with one exception (95% M, 5% VM neurons; Segraves and Goldberg 1987) that was previously suggested to underrepresent visual signals in the output of the FEF (Sommer and Wurtz 2000).

Overlap and gap conditions have been shown to increase or decrease SRT, respectively, implicating either an increase of inhibitory signals or decreased activation in structures critical for saccade initiation in the overlap condition compared with the gap condition (Dias and Bruce 1994; Dorris and Munoz 1995; Reingold and Stampe 2002). Because the pathway from the FEF to the SC is critical for saccade initiation, differences between SRT in the overlap and gap conditions must be reflected in FEF or SC neurons, or both. One model proposing a neural substrate and potential tuning mechanism of saccade threshold has suggested that elements of threshold may change

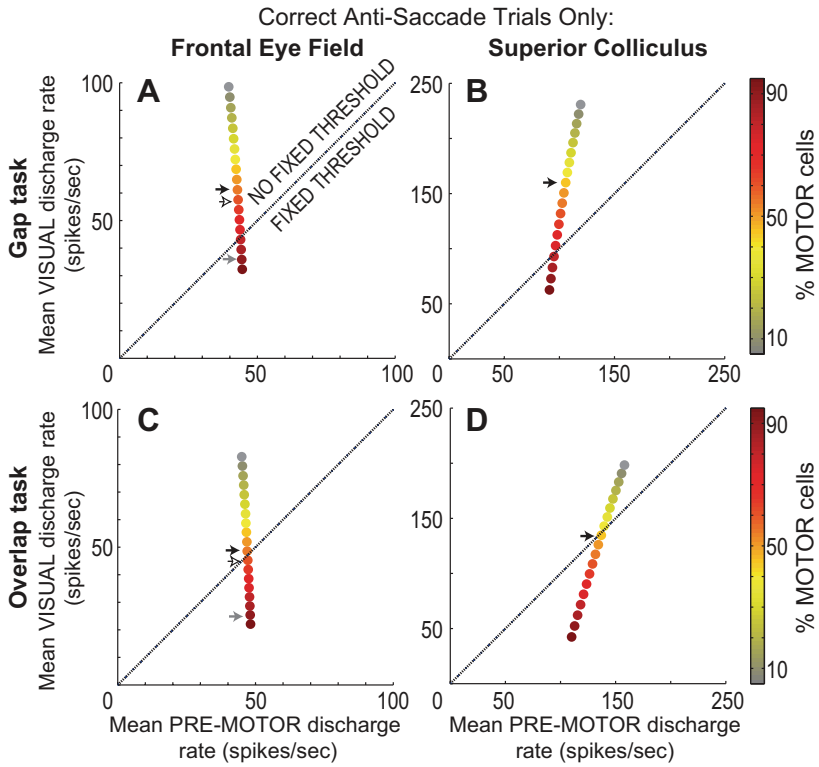

Fig. 7. Population analysis comparing aggregate visual and presaccadic motor discharges from varying distributions of VM and M neurons, to estimate the activity of corticotectal and tectoreticular projection neurons. Higher visual discharge rate than presaccadic motor discharge rate during correct antisaccade trials is inconsistent with a fixed threshold, as these signals should elicit an erroneous involuntary saccade toward the visual stimulus. Each data point represents the mean activity of an individual bootstrap analysis using a distinct ratio of VM and M neurons. Color bar indicates ratio of M to VM neurons. Increasing the percentage of M neurons shifts the data points below the line of unity (as predicted by a fixed-threshold model), while increasing the percentage of VM neurons shifts the points above the line of unity (inconsistent with a fixed-threshold model). Previously published anatomical estimations of corticotectal (*A* and *C*; gray arrow, Segraves and Goldberg 1987; open arrow, Everling and Munoz 2000; black arrow, Sommer and Wurtz 2000) and tectoreticular (*B* and *D*; black arrow, Rodgers et al. 2006) VM and M projection neuron distributions are indicated. Points are shifted downward in the overlap task (*C* and *D*; higher inhibition) compared with the gap task (*A* and *B*; lower inhibition).

according to inhibition of the SC from the basal ganglia (Lo and Wang 2006). Therefore, to address the possibility of changes in threshold due to inhibition, we manipulated the level of inhibition using overlap and gap fixation conditions while testing FEF and SC presaccadic motor activity. We found that there was an overall downward shift of data points in the overlap condition (Fig. 7, *C* and *D*) compared with the gap condition (Fig. 7, *A* and *B*) in both the FEF and the SC. Thus, in the overlap condition, our data were consistent with a fixed-threshold prediction using a 55% M and 45% VM neuron distribution (Fig. 7*D*). Therefore, when inhibitory signals are (presumably) increased in the overlap task a greater distribution of neurons show activity that agrees with a fixed-threshold prediction.

DISCUSSION

Here, we report tests of a within-neuron and a population fixed-threshold mechanism for saccade initiation in the FEF and the SC. Previous work has suggested that a fixed threshold for saccade initiation exists within FEF M and SC M neurons, and these results have been extrapolated to suggest that a population fixed threshold exists in these structures (Hanes and Schall 1996; Paré and Hanes 2003). However, these studies were limited to one saccade task and only examined one 10-ms presaccadic epoch. Our results contradict a within-neuron fixed threshold in FEF and SC M and VM neurons, and they suggest conditions where a population fixed threshold may not hold within only the FEF and SC. We found that in the FEF and SC presaccadic motor discharge rate and SRT were correlated in some presaccadic windows, presaccadic motor discharge rate varied across saccade tasks, and when a saccade was initiated visual signals were often higher than presaccadic motor discharge. These higher visual signals did not evoke a saccade (but the lower presaccadic motor discharge did evoke a saccade). We used a bootstrap analysis to incorporate a range of VM and M neuron proportions during our final analysis, to examine a population fixed threshold. We found that mean population visual activity was higher than presaccadic motor activity, but this depended on the distribution of sampled VM and M neurons and the inhibitory state as implied by gap and overlap conditions. Therefore, if a fixed threshold for saccade initiation exists, it may involve structures additional to only FEF and SC M and VM neurons, to balance the varying signals found in those neuronal populations.

If a saccade threshold mechanism exists, it is currently unknown when the threshold mechanism would take effect before saccade onset. However, because of our consistent results across examined temporal epochs, our conclusions remain valid regardless of saccade threshold timing in the FEF and SC (from 50 ms to 0 ms before saccade onset). Figures 5 and 6 were inconsistent with a fixed threshold in FEF VM and SC VM and M neurons, regardless of the presaccadic window position. In addition, our findings are inconsistent with a fixed threshold within individual FEF M neurons because of individual task-dependent differences (Fig. 5*A*, *bottom left*). However, averaged FEF M presaccadic activity is consistent with a fixed threshold across the population of FEF M neurons. In the gap task, all tests showed evidence against fixed saccade threshold for SC VM and M neurons, implying that a fixed threshold may not always exist at the SC population level. On the other hand, the FEF may or may not have a fixed threshold for saccade initiation based on the proportions of VM and M neurons and the relative influence of these neuron types on saccade initiation. In an estimate of FEF and SC population activity, only a (theoretical) strong bias for M neurons rendered the fixed-threshold hypothesis plausible. Therefore, identifying

the distribution of VM and M neurons in the circuit from FEF to SC to downstream targets is critical for supporting or rejecting a fixed-saccade threshold hypothesis for these structures.

Previous tests of threshold are dependent on what period a saccade threshold mechanism takes effect. Evidence supporting a within-neuron fixed threshold for saccade initiation in the FEF and SC (i.e., no significant correlation between presaccadic motor discharge rate and SRT) has been found previously in presaccadic epochs spanning the shortest time at which a neural signal can influence saccade initiation (Brown et al. 2008; Hanes and Schall 1996; Paré and Hanes 2003). In the FEF this is 20 ms to 10 ms before saccade onset (Bruce et al. 1985; Büttner-Ennever et al. 1988; Hanes et al. 1995; Hanes and Schall 1996; Segraves 1992; Segraves and Goldberg 1987), and in the SC this is 18 ms to 8 ms before saccade onset (Miyashita and Hikosaka 1996; Munoz et al. 1996; Munoz and Wurtz 1993). Similarly, during these epochs we found no correlation between presaccadic motor discharge rate and SRT in the FEF or the SC. However, a positive correlation was revealed in some FEF and SC neurons in a 50 ms to 40 ms and a 40 ms to 30 ms epoch before saccade initiation, respectively (Fig. 4). This emphasized the weakness of not knowing when a saccade threshold mechanism takes effect, in order to interpret the results of this test. Does saccade initiation occur directly after presaccadic motor activity crosses a threshold, as implied by previous studies that analyzed presaccadic epochs spanning the shortest time at which a neural signal can influence saccade initiation (Hanes and Schall 1996; Paré and Hanes 2003)? Or is it possible that a threshold could gate the saccade motor burst, which generally began 40 ms before saccade onset in the FEF (Fig. 2*B*; Hanes et al. 1995) and 30 ms before saccade onset in the SC (Fig. 3*B*; Munoz and Wurtz 1995; Sparks 1978)? These inconsistencies in results depending on epoch and task condition prompted us to use different tests of fixed threshold.

It is worth noting that, in some cases, there existed a low number of trials per neuron after our filtering of saccade metrics between tasks (minimum of 5 trials per condition after filter, see MATERIALS AND METHODS). In this case, it is possible that these fewer trials could be masking other weak correlations between firing rate and SRT, which may be statistically significant should more trials exist. However, this would provide more support for our conclusions below, not less.

Examining fixed threshold across saccade tasks. Our second test was an alternative examination of a within-neuron fixed threshold, which determined whether variations in the presaccadic motor discharge of VM and M neurons existed between saccade tasks. FEF VM neurons and SC M and VM neurons consistently showed higher presaccadic motor discharge rate in the prosaccade task compared with the antisaccade task. As mentioned above, FEF M neurons individually demonstrated task-dependent differences; however, averaged FEF M presaccadic activity was consistent with a fixed threshold across the population of FEF M neurons. Taken together, these data contradict a within-neuron fixed threshold in FEF and SC VM and M neurons. These data may also reveal characteristics of a population fixed threshold in the SC and FEF. A population fixed threshold in these structures would predict no variation in mean presaccadic motor discharge between saccade tasks, across all neurons contributing to saccade initiation. Because mean activity of sampled VM and M neurons in the SC shows higher presaccadic motor discharge rate in the prosaccade task, this may reflect neural characteristics inconsistent with a population fixed threshold in the SC. Because of the dissimilar results between FEF M and VM neurons in this test, there can be varying implications for a population fixed threshold in the FEF depending on the relative physiological contributions of VM and M neurons to saccade initiation. Dissimilarity between FEF M neurons and a subpopulation of FEF VM neurons has also been observed by Brown et al. (2008) in the countermanding saccade task while correlating behavior and neuronal activity, emphasizing the importance of controlling VM and M neuron distributions when examining saccade threshold.

Our third test examined a within-neuron fixed threshold in VM neurons and then a population fixed threshold in the FEF and the SC. We compared visual discharge rate and presaccadic motor discharge rate in the antisaccade task, which dissociates visual and motor signals spatially across hemispheres in the brain (for review, see Munoz and Everling 2004). In the antisaccade task, it was previously proposed that top-down inhibitory signals are required to suppress the visual grasp reflex (i.e., reflexive saccade) to look toward the visual stimulus in favor of a voluntary saccade away from the visual stimulus (Munoz and Everling 2004). If these top-down inhibitory signals are too weak, then the addition of a visual response to the disinhibited pretarget activity will be enough to cross the saccade threshold and trigger an erroneous reflexive saccade toward the visual stimulus (Everling et al. 1998a; Everling and Munoz 2000). The initial level of preparatory activity for a reflexive saccade toward the visual stimulus must be considered when determining whether a smaller visual response (compared with saccade motor activity in the opposing hemisphere encoding a voluntary saccade) can trigger an incorrect reflexive saccade. In addition, if a fixed threshold exists, a higher visual response (compared with saccade motor activity in the opposing hemisphere) should always trigger an incorrect reflexive saccade toward the visual stimulus (see Fig. 6*A*). In correct antisaccade trials, we found that the mean visual discharge rate was higher than the presaccadic motor discharge rate in FEF and SC VM neurons. This is inconsistent with a within-neuron fixed threshold in VM neurons, as the higher visual discharge rate did not elicit a saccade whereas the lower presaccadic motor activity did elicit a saccade. To more accurately represent output signals from the FEF and SC, we also used bootstrap analyses of varied distributions of VM and M neurons to estimate population activity. Because M neurons do not demonstrate a significant visual response, increasing the percentage of M neurons compared with VM neurons naturally reduces mean sampled visual activity compared with presaccadic motor activity. Utilizing ratios of VM and M output neurons previously estimated in the FEF (Everling and Munoz 2000; Sommer and Wurtz 2000) and in the SC (Rodgers et al. 2006), we found higher mean visual activity than presaccadic motor activity in the anti-gap task, but not in the anti-overlap task. Therefore, these results contradict a fixed threshold within the gap task but not within the overlap task. This phenomenon may help explain conflicting conclusions regarding fixed and variable saccade threshold mechanisms among previous studies (Goossens and Van Opstal 2000a, 2000b; Grice et al. 1982; Hanes and Schall 1996; Paré and Hanes 2003; Lo and Wang 2006).

Role of inhibitory signals in saccade threshold. Hanes and Schall (1996) found support for a fixed threshold for saccade initiation in the FEF with variable rate of rise that accounted for variability in SRT in a countermanding saccade task (for review, see Munoz and Schall 2003; Stuphorn and Schall 2002). In the countermanding task, the monkey must look toward a peripheral visual stimulus, except in random trials when a "stop" signal is presented before stimulus appearance, which indicates that saccadic eye movement must be inhibited. Decreasing the delay between stop signal onset and stimulus appearance in "stop signal" trials can reduce the ability to inhibit a saccadic eye movement to the visual stimulus. However, the need to quickly inhibit a visually guided saccade without warning (Hanes and Schall 1995, 1996) might require high global inhibition throughout the countermanding task, which could mask early changes in threshold associated with appearance of a visual stimulus.

A computational accumulator model by Lo and Wang (2006) proposed a variable threshold for saccade initiation in which corticotectal projections discharge to initiate a saccadic eye movement by crossing a threshold state that is set by local recurrent excitatory and inhibitory connections in the SC but can be tuned by basal ganglia loop inhibitory outputs. Previous neurophysiological evidence has also indicated that a fixed threshold may not hold in all cases (Everling and Munoz 2000; Everling et al. 1999; Goossens and Van Opstal 2000a, 2000b). A trend emerges when comparing these observations to our results in the overlap and gap tasks (Fig. 7). Overlap and gap conditions have been shown to increase or decrease SRT, respectively, implicating either an increase of inhibitory signals or decreased activation in structures critical for saccade initiation in the overlap condition compared with the gap condition (Dorris and Munoz 1995; Dorris et al. 1997; Reingold and Stampe 2002). Because the FEF to SC pathway is critical for saccade initiation (Dias et al. 1995; Dias and Segraves 1999; Hanes and Wurtz 2001; Schiller and Chou 1998; Schiller et al. 1979, 1980, 1987; Sommer and Tehovnik 1997), changes in SRT in the overlap and gap conditions should be reflected in the FEF and the SC. Indeed, increases in FEF saccade neuron presaccadic activity have been proposed as a physiological correlate of reduced inhibition in the gap task (Dias and Bruce 1994). If the observed difference between gap and overlap conditions in our study is due to changing inhibitory signals, it is possible that inhibition is a contributing influence to saccade threshold, such that higher inhibitory signals create conditions where a fixed threshold may hold. If this is the case, previous studies supporting a fixed threshold in the FEF and SC with the countermanding saccade task (Brown et al. 2008; Hanes and Schall 1996; Paré and Hanes 2003) may have been influenced by increased global inhibition in this task.

Alternate possibilities for saccade threshold. If a fixed threshold for saccade initiation does not exist within the SC, we can speculate that a variable threshold may therefore exist. It has been shown previously that GABAergic nigrotectal projection neurons from the substantia nigra pars reticulata (SNr; output structure of the basal ganglia) must be inhibited prior to initiation of some saccades (Hikosaka et al. 2000; Hikosaka and Wurtz 1981, 1983). Because of its inhibitory influence on SC activity, signals from the basal ganglia loop are likely critical for a saccade threshold mechanism. Therefore, the basal ganglia may modulate a variable threshold, as proposed by Lo and Wang (2006). Within the basal ganglia loop, an excitatory projection exists from the SC to the subthalamic nucleus (STN) carrying visual information (Coizet et al. 2009). Activating the STN increases inhibitory signals from the SNr basal ganglia output structure (Nambu et al. 2002). It is possible that this SC-STN-SNr-SC feedback loop could contribute to a variable thresholding mechanism dependent on visual input, and could underlie our results during the antisaccade task in the SC. In this case, a higher SC visual response may subsequently increase bilateral SNr inhibitory signals to the SC (through an SC-STN-SNr-SC feedback loop), which could inhibit the subsequent SC presaccadic motor discharge. However, other structures are implicated in addition to the basal ganglia loop to explain differences in the effect of visual stimulation across behavioral conditions, and to explain how a saccade is initiated by a smaller FEF and SC motor burst in the antisaccade task compared with the prosaccade task.

While our results contradict a population fixed threshold for saccade initiation in the SC, and in the FEF depending on VM to M neuron distribution, it is possible that a fixed threshold may exist at the scale of a larger network of oculomotor structures. For example, in addition to the FEF, the SC, and the basal ganglia, the oculomotor network includes the supplementary eye field (SEF; Schlag and Schlag-Rey 1987), which is associated with a higher presaccadic motor discharge during the antisaccade task compared with the prosaccade task (Schlag-Rey et al. 1997). While SEF neurons alone do not demonstrate activity consistent with a saccade threshold (So and Stuphorn 2010; Stuphorn et al. 2010), if considered as part of a larger oculomotor network, higher FEF and SC presaccadic motor discharge in the prosaccade task could be balanced by higher SEF presaccadic motor discharge in the antisaccade task, at the level of the brain stem saccade-generating circuit downstream of the SC. In the brain stem saccade-generating circuit, omnipause neurons (OPN) are located near the midline of the caudal pontine reticular formation within the nucleus raphe interpositus (Büttner-Ennever et al. 1988; Langer and Kaneko 1990) and are associated with tonic activity that inhibits saccade generation (Horn et al. 1994; Keller 1974; Scudder et al. 2002). OPN must therefore be inhibited to generate a saccade in any direction (Everling et al. 1998b; Keller et al. 1996). Neurons exhibiting visual and saccade motor activity project from the SC to the brain stem saccade-generating circuit (Rodgers et al. 2006), and a transient increase in OPN activity corresponding to visual stimulus appearance has also been observed (Everling et al. 1998b), but the consequences of this phenomenon are so far unclear. Because of the requirement for OPN to pause to generate a saccade of any vector, and because OPN are part of the brain stem saccade-generating circuit through which every signal must pass to produce an eye movement, these neurons may be good candidates controlling the threshold for saccade initiation downstream of the FEF and SC.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: J.J.J. and M.W. conception and design of research; J.J.J. analyzed data; J.J.J., M.W., and D.P.M. interpreted results of experiments; J.J.J. prepared figures; J.J.J. drafted manuscript; J.J.J., M.W., S.E., and D.P.M. edited and revised manuscript; J.J.J., M.W., S.E., and D.P.M. approved final version of manuscript; S.E. performed experiments.

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