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Similarity of superior colliculus involvement in microsaccade and saccade generation

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Hafed ZM, Krauzlis RJ. Similarity of superior colliculus involvement in microsaccade and saccade generation. *J Neurophysiol* 107: 1904–1916, 2012. First published January 11, 2012; doi:10.1152/jn.01125.2011.—The characteristics of microsaccades, or small fixational saccades, and their influence on visual function have been studied extensively. However, the detailed mechanisms for generating these movements are less understood. We recently found that the superior colliculus (SC), a midbrain structure involved in saccade generation, also plays a role in microsaccade generation. Here we compared the dynamics of neuronal activity in the SC associated with microsaccades to those observed in this structure in association with larger voluntary saccades. We found that microsaccade-related activity in the SC is characterized by a gradual increase in firing rate starting ~100 ms prior to microsaccade onset, a peak of neuronal discharge just after movement onset, and a subsequent gradual decrease in firing rate until ~100 ms after movement onset. These properties were shared with saccade-related SC neurons, recorded from the same monkeys but preferring larger eye movements, suggesting that at the level of the SC the neuronal control of microsaccades is similar to that for larger voluntary saccades. We also found that neurons exhibiting microsaccade-related activity often also exhibited saccade-related activity for slightly larger movements of similar direction, suggesting a continuity of the spatial representation in the SC, in both amplitude and direction, down to the smallest movements. Our results indicate that the mechanisms controlling microsaccades may be fundamentally the same as those for larger saccades, and thus shed new light on the functional role of these eye movements and their possible influence on sensory and sensory-motor processes.

voluntary eye movement; fixational eye movement; fixation

MICROSACCADES ARE TINY SACCADES that occur during gaze fixation (Barlow 1952; Steinman et al. 1973). Unlike larger saccades, which allow foveating new targets, microsaccades shift targets from one foveal position to another (see, for example, Ko et al. 2010). In other properties, microsaccades are similar to larger saccades. For example, microsaccades follow the “main sequence” relationship between peak eye velocity and movement amplitude that governs the metrics of larger saccades (Zuber et al. 1965). Also, like larger saccades during scene exploration, microsaccades during fixation occur at a rate of ~1–3 movements/s (Barlow 1952; Steinman et al. 1967, 1973).

The similarity between the characteristics of microsaccades and saccades suggests an underlying similarity in their control mechanisms. Even though neurophysiological stud-

ies of microsaccade generation (Guerrasio et al. 2010; Hafed 2011; Hafed et al. 2009; Van Gisbergen et al. 1981) are scarce relative to studies of these movements’ sensory consequences (Bair and O’Keefe 1998; Bosman et al. 2009; Hafed and Krauzlis 2010; Herrington et al. 2009; Kagan et al. 2008; Leopold and Logothetis 1998; Martinez-Conde et al. 2000; Snodderly et al. 2001), the existing evidence supports the hypothesis of a common mechanism. For example, preliminary results show that brainstem omnipause neurons (OPNs) pause during microsaccades just as they do during larger saccades (Brien et al. 2009; Van Gisbergen et al. 1981). In addition, premotor neurons in the brainstem reticular formation, which are involved in saccade generation, also likely drive microsaccades (Van Gisbergen et al. 1981). Finally, we recently identified neurons within the intermediate layers of the midbrain superior colliculus (SC), a structure well known for its involvement in saccade generation, that are active during microsaccades, and we showed that inactivation of the SC can impair microsaccade generation (Hafed et al. 2009).

In this study, we compared the temporal and spatial discharge properties of SC neurons that exhibit microsaccade-related activity to those of neurons that exhibit saccade-related activity for larger movements. We found that the temporal patterns of microsaccade-related SC activity are quantitatively similar to those of saccade-related activity. Moreover, we found that neurons with microsaccade-related activity lie predominantly in the foveal part of the SC map, consistent with this structure’s retinotopic representation (Robinson 1972), although activity during microsaccades involves a population of neurons that often extends to preferred eccentricities larger than the amplitudes of the movements themselves.

Our results support a population coding view of SC activity for representing eye movement goals (Lee et al. 1988), and they are consistent with such coding being present even in the foveal portions of the SC (Hafed et al. 2008, 2009; Hafed and Krauzlis 2008). Moreover, by virtue of the similarity between microsaccade and saccade generation, our results also suggest that microsaccadic influences on sensory processing (Bair and O’Keefe 1998; Bosman et al. 2009; Herrington et al. 2009; Kagan et al. 2008; Leopold and Logothetis 1998; Martinez-Conde et al. 2000; Snodderly et al. 2001) extend beyond retinal image refreshing and may include additional functions such as saccadic suppression (Hafed and Krauzlis 2010) and perisaccadic spatial updating.

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MATERIALS AND METHODS

General Laboratory Setup

The experimental setup was the same as that described previously (Hafed et al. 2008, 2009; Hafed and Krauzlis 2008, 2010).

Animal Preparation

We collected data from two (*J* and *W*) adult male rhesus monkeys (*Macaca mulatta*) that were 10–11 yr of age and weighed 15–16 kg. These monkeys were prepared with standard surgical techniques that have been described in detail previously (Krauzlis 2003), and all experimental protocols for the monkeys were approved by the Institutional Animal Care and Use Committee and complied with United States Public Health Service policy on the humane care and use of laboratory animals.

Behavioral Tasks

Fixation task. To investigate microsaccades, we employed a task involving prolonged periods of gaze fixation. Our monkeys fixated a small white spot presented at the center of the display. The fixation spot consisted of a single pixel of background luminance (18 cd/m² luminance) surrounded by a 1-pixel-thick white square (65 cd/m² luminance). With our display geometry and distance, this 3 × 3-pixel stimulus corresponded to ~9 × 9 min arc of visual angle. Each fixation trial lasted for 3,500 ms, and we typically collected ~60–100 trials per session. We enforced spatial accuracy of fixation by aborting a trial whenever we detected an eye position that was >1° away from the central spot.

Saccade task. To investigate the activity of SC neurons during movements larger than those occurring in the fixation task, we also employed a second task in which the monkeys generated delayed, visually guided saccades (similar to Krauzlis 2001, 2003, 2004; Munoz and Wurtz 1995a, 1995b). In each trial, a fixation spot appeared at the center of the display, as in the fixation task. Once the monkeys fixated this spot, a saccade target (another spot identical to the fixation spot) appeared in the periphery and remained on until trial end. After 500–1,000 ms from peripheral target onset, the fixation spot disappeared, instructing the monkeys to initiate a saccade. We collected 50–140 trials in this task, manually changing the saccade target location from trial to trial in order to map the neurons' response fields (RFs). Since such RFs were predominantly contralateral to the recorded neurons, the great majority of saccades collected in this task were directed to contralateral locations. We enforced saccade end point accuracy by only rewarding the monkeys for landing within 1–3° from the actual target. Given the range of target eccentricities we tested (<20°), this constraint was met by our monkeys with relative ease.

Fixation blink and ipsilateral memory-guided saccade tasks. For some neurons (see RESULTS), we presented the monkeys with a fixation task in which the fixated spot disappeared transiently (for 500 ms) 500 ms after trial onset. The spot then reappeared for another 500 ms, and the monkeys were rewarded for maintaining fixation for the entire 1,500 ms interval (with spatial accuracy similar to that in the fixation task above). We collected 35–45 trials from this task.

We also instructed our monkeys to perform a memory-guided saccade task to 10° ipsilateral locations along the horizontal axis. This task was similar to the saccade task described above, except that the saccade target remained visible for only 500–900 ms, after which it disappeared for 500–800 ms before the fixation spot was removed. The monkeys were trained to initiate a saccade after fixation spot offset toward the remembered location of the saccade target. This target only reappeared after the monkeys successfully made a saccade to within 2–3° of its location (as in Krauzlis 2001, 2003, 2004; Krauzlis et al. 2000; Munoz and Wurtz 1993a, 1993b). We collected ~35 trials from this task.

Single-Neuron Recordings

We used tungsten microelectrodes with impedances of 900 kΩ–1.7 MΩ to record extracellular action potentials of individual neurons in the intermediate layers of the SC (~1–2.8 mm below surface). Our criteria for inclusion of neurons were those described by Hafed et al. (2009) and required that neurons exhibited saccade-related increases or decreases in activity during the saccade tasks described above.

Data Analysis

Eye movement analysis and visualization. Saccades (and microsaccades) were detected with velocity and acceleration thresholds (Krauzlis and Miles 1996), also described in detail for microsaccades (Hafed et al. 2009; Hafed and Krauzlis 2010). We used a velocity threshold of 30°/s for the movements collected from our saccade tasks and a threshold of 8°/s for the significantly smaller (and therefore slower) movements collected in our fixation tasks; similarly, we used an acceleration threshold of 800°·s⁻¹·s⁻¹ for the movements of our saccade tasks and 550°·s⁻¹·s⁻¹ for the movements of our fixation tasks. For all movements, we manually inspected the results of the saccade detection algorithm and made adjustments for false positives or misses when they occurred.

For the purpose of classifying neurons as exhibiting microsaccade-related activity, we defined as a microsaccade any movement whose radial amplitude was <15 min arc (0.25°) (Collewijn and Kowler 2008). This definition, consistent with the classic literature on microsaccades (Collewijn and Kowler 2008), encompassed a majority of the movements that typically occurred in our fixation task (see, for example, Fig. 2), and it was deliberately chosen to be more stringent than several more recent studies of microsaccades (including Brien et al. 2009; Martinez-Conde et al. 2000), in order to allow us to characterize the activity of SC neurons associated with the smallest set of movements. To avoid contamination of the SC firing rates associated with the movements of interest by previous or later saccades occurring in the same trial, we only analyzed movements for which there were no other saccades or microsaccades from an interval starting 300 ms prior to their onset and ending 200 ms after their end.

Neuronal data preparation. All of our neuronal analyses were performed on firing rates, or neuronal spike densities. We obtained a spike density for each trial in every task by convolving spike times with a Gaussian of 10 ms SD. We then obtained an average spike density per condition (for example, contralateral movements of a certain amplitude and direction) for each neuron by averaging the individual spike densities. Whenever we computed average normalized spike densities across multiple neurons, we first normalized the spike density of each neuron before averaging across neurons. The normalization factor was the peak firing rate observed for a given neuron during the neuron's preferred saccades or microsaccades.

For several of our analyses of neuronal activity during the fixation task, we compared perimovement activity to the baseline activity of the neuron during saccade-free fixation. We calculated this baseline activity by measuring the activity of the neuron during every trial but after first excising all periods starting from 100 ms prior to the onset of any fixational saccade or microsaccade until 50 ms after the end of such movements (which was equivalent to ~75–85 ms after the beginning of these movements). We then averaged the measurements across trials to obtain a given neuron's average baseline. We also confirmed that this procedure produced reasonable estimates by comparing the baseline values obtained with this procedure to those obtained with an alternative procedure. In this alternative procedure, we measured the neuron's activity during a 50-ms interval centered around trial onset in the saccade task. Across neurons, both procedures gave similar baseline values ($P = 0.7$, rank sum, $N = 100$ neurons).

For the analysis of neuronal activity in the saccade task (as well as in the ipsilateral, memory-guided version), we also compared the

strength of perimovement activity to a saccade-free baseline. This baseline was measured as the activity during a 50-ms interval centered on a time point 250 ms prior to movement onset. This interval was well before movement onset, making it suitable as a non-movement-related reference, but it was late enough within trials to represent activity associated with steady-state fixation (as opposed to stimulus-induced transients). This interval also allowed us to assess saccade-related modulations that occurred in addition to any low-frequency modulations in firing rate associated with saccade preparation or the prior knowledge of the location of the upcoming saccade (Basso and Wurtz 1997; Dorris and Munoz 1998).

Response field maps. We plotted the horizontal (*x*-axis) and vertical (*y*-axis) amplitude of each detected microsaccade/saccade and the corresponding neuronal discharge during that particular movement (*z*-axis; color coded from low to high firing rate). We obtained RF maps for data from the fixation task alone (e.g., Fig. 1C), the saccade task alone (e.g., Fig. 1E), or both tasks combined (see, e.g., Figs. 5 and 6). For the combined case, we treated the small fixational saccades obtained from the fixation task and the larger voluntary saccades obtained from the saccade task as a single data set and plotted the neurons' firing rates for all of the movements combined. This allowed us to assess a single neuron's spatial RF characteristic for all possible saccade and microsaccade sizes that we collected, but it did mean that the range of movement amplitudes included for visualization was

fairly large and could span several orders of magnitudes. For example, for neurons exhibiting activity for 0.1° (6 min arc) microsaccades in the fixation task, we often tested amplitudes of 10° or 15° (>2 orders of magnitude larger) in the saccade task. To accommodate this range of values, we employed a "log-polar" coordinate system instead of a linear cartesian one. Specifically, we converted the movement horizontal and vertical amplitudes into polar format (radial amplitudes and angular directions) and then represented the movement end points such that the radial amplitudes were plotted on a logarithmic scale (see, e.g., Fig. 5). This format of displaying RF maps allowed us to easily assess the continuity of the RFs of SC neurons across a wide range of saccade amplitudes (microsaccades and larger saccades) and is consistent with the logarithmic compression of saccade amplitudes present in the SC topographic representation (Robinson 1972). We preferred using such a data-driven log-polar mapping over a model-based coordinate transformation into anatomical SC coordinates (Ottes et al. 1986) because it did not entail making assumptions about the anatomical structure of the portions of the SC representing small saccades (Marino et al. 2008).

On the basis of the RF maps of our neurons during the fixation and saccade tasks, we identified the axis of eye movements along which a neuron preferentially increased its activity during saccades or microsaccades. We defined this axis as the neuron's preferred direction, and we used it to select the neuron's preferred movements when analyzing the temporal dynamics of microsaccade- and saccade-related activity in the SC. These preferred movements were defined as the movements within 6 min arc (for neurons with microsaccade-related activity) or 3° (for neurons with saccade-related activity) from the movement causing maximum firing rate in a given neuron, with an additional constraint being that these saccades/microsaccades were associated with a firing rate higher than 2 SD above the movement-free fixation baseline.

Time course of microsaccade- and saccade-related activity. We estimated the time at which neuronal activity in the SC associated with microsaccades deviated away from the movement-free fixation baseline. For each neuron, we selected the movements along the neuron's preferred axis (based on the spatial RF profile; see above) that were associated with firing rates during a movement higher than

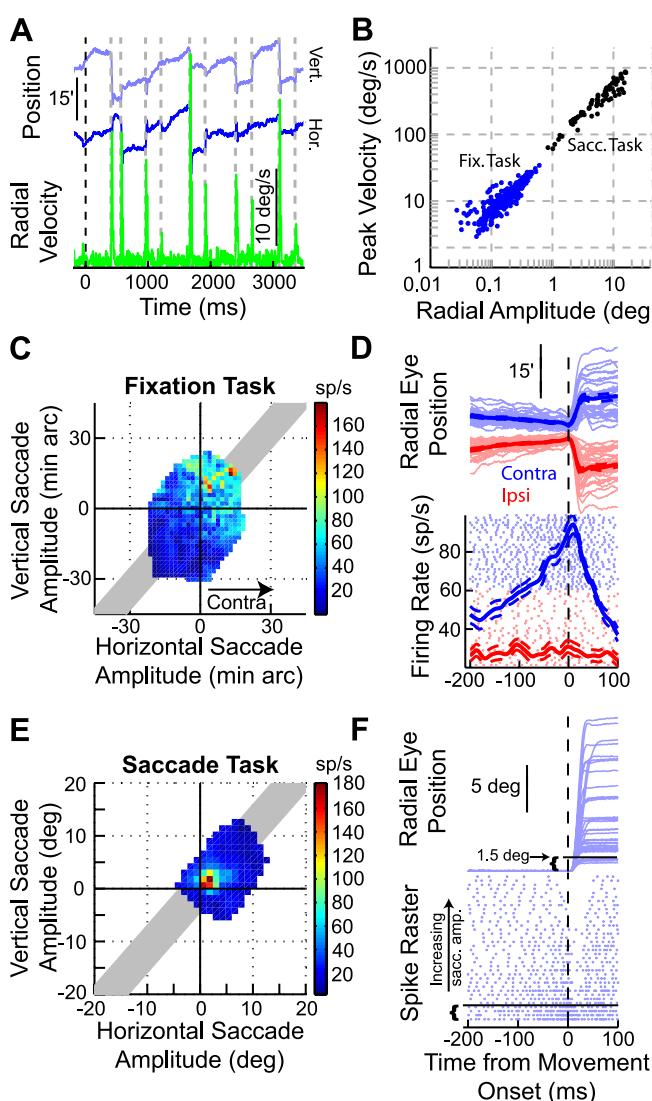


Fig. 1. Behavioral and neuronal results from a sample recording experiment investigating microsaccade-related activity in the superior colliculus (SC). *A*: eye position and radial eye velocity from a sample trial of the fixation task containing 10 fixational saccades (marked with gray vertical dashed lines). *B*: peak velocity vs. movement amplitude for all fixational saccades collected during the fixation task (blue) as well as all larger movements collected during the saccade task (black) (same session). All movements fell on the same "main sequence" curve (Zuber et al. 1965). *C*: movement-related activity of the neuron for all movements occurring during the fixation task in the session. Movement horizontal and vertical amplitude as well as the firing rate of the neuron observed during the movement (*z*-axis) are plotted. The neuron exhibited preferential activation during small movements directed to the upper right quadrant. The gray band indicates the preferred direction of the neuron and was used for the analysis of *D*. *D*: time course of microsaccade-related activity of the neuron. We plotted the perimicrosaccadic activity of the neuron for contralateral movements along the preferred direction of the neuron (blue) as well as for ipsilateral movements along the same axis (red). The neuron exhibited buildup activity for the preferred movements and no modulation for the nonpreferred ipsilateral movements. To obtain this figure, we only considered microsaccades for which there were no other movements within the shown analysis interval (to avoid contamination of firing rates by these movements). We also aligned/plotted movements/firing rates as described by Hafed et al. (2009). *E*: analysis similar to *C* for the same neuron but during the saccade task. The neuron exhibited elevated activity for small saccades along the same preferred direction as in *C*. *F*: perisaccadic activity of the same neuron during the saccade task for movements along the preferred direction (gray band in *E*). The spike rasters at bottom are sorted according to saccade size. The neuron was active during saccades smaller than $\sim 1.25^\circ$. For larger saccades, the neuron paused during the movements and the pause duration increased with increased saccade duration. Note that for this panel we did not plot an estimate of firing rate for the neuron because of the varying type of response (increase vs. pause) as a function of different movement amplitudes.

2 SD above the saccade-free baseline. We then normalized each neuron's firing rate by the maximum response observed during these microsaccades. On the basis of the distribution of population-averaged, normalized activity across neurons, we then estimated when this activity was significantly different from the population-averaged, normalized baseline activity by measuring when the 95% confidence intervals for the two distribution means (microsaccade vs. baseline) ceased to overlap (see, for example, Fig. 3A). We also estimated the time course of peri-microsaccadic SC activity with an alternative procedure, based on an individual neuron basis. For each neuron, we estimated the onset and end of microsaccade-related activity by identifying the earliest time point prior to microsaccade onset (or the latest time point after microsaccade onset) at which activity was still significantly higher than that during the saccade-free fixation baseline of the same neuron, according to a two-tailed *t*-test. We then plotted the fraction of neurons exhibiting significantly higher microsaccade-related activity than baseline activity at different times relative to movement onset. Both measures gave similar estimates of the time course of microsaccade-related activity in the SC.

To understand the potential relationship between microsaccade-related SC activity and movement kinematics, we also estimated the time of peak discharge observed during a microsaccade to the time of movement onset, movement peak velocity, or movement end. For each neuron, we obtained a mean and SD measure of the time of peak firing rate (across all of the neuron's preferred microsaccades), and we then obtained a population average across the neurons.

For comparing microsaccade-related activity to saccade-related activity observed for larger eye movements, we analyzed the firing rates of neurons recorded from the same animals but preferring larger saccades than those measured in the fixation task. We thus repeated the same analyses above but for the preferred movements of the neurons as assessed from the saccade task. As with microsaccade-related activity, the preferred axis was defined based on the spatial RF profile of the neurons, as described above.

Finally, depending on saccade size relative to a given neuron's RF profile, we sometimes observed decreases in firing rates during a movement, as opposed to increases (see, for example, Fig. 7). In these cases, we estimated the times of onset and end of activity reductions for these neurons in a manner that was identical to the description above, except that a significant movement-related change in firing rate relative to baseline was defined as a decrease rather than an increase.

RESULTS

Sample SC Neuron Exhibiting Microsaccade-Related Activity

Figure 1, *A* and *B*, show behavioral data collected from the fixation and saccade tasks during one sample session in our experiments. Trials in the fixation task required the monkey to maintain steady fixation of a small spot for 3,500 ms and resulted in frequent saccades (Fig. 1A), a majority of which were smaller than 15 min arc (0.25°) in amplitude (Fig. 1, *A–D*; also see Fig. 2 for data from all sessions). Apart from their small size, these movements looked almost indistinguishable from larger saccades and followed the same main sequence relationship of peak velocity versus movement amplitude as the $\sim 1\text{--}16^\circ$ movements that we collected in the saccade task during the same session (Fig. 1B) (Zuber et al. 1965).

We collected 122 trials of the fixation task during this sample session while we simultaneously recorded the activity of a single neuron located in the rostral region of the left SC. We detected a total of 1,323 fixational saccades during these trials, and when we plotted the neuron's activity during each of these movements as a function of the movements' horizontal and vertical amplitudes, we found that this neuron preferen-

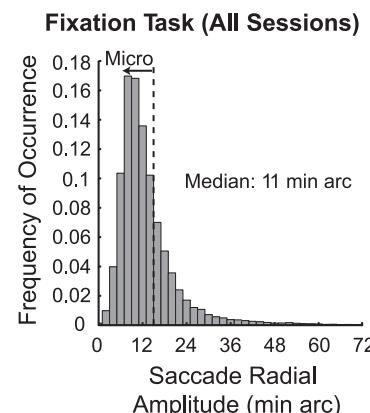


Fig. 2. Amplitude distribution of all saccades detected during the fixation task in our experiments. The median amplitude was 11 min arc (0.18°). To investigate the characteristics of SC activity associated with the smallest possible eye movements, we concentrated in this study on movements <15 min arc (0.25°) in amplitude (vertical dashed line). This included the majority of fixational saccades that occurred in the experiments.

tially increased its activity for contraversive movements directed toward the upper right quadrant (Fig. 1C). We analyzed the microsaccade-related elevation of the neuron's activity evident in Fig. 1C by plotting the time course of this activity aligned on movement onset for both preferred (toward the upper right quadrant) and nonpreferred (toward the lower left quadrant) movements that were <15 min arc (0.25°) in amplitude (Fig. 1D; similar to procedure in Hafed et al. 2009) (i.e., for microsaccades; Collewijn and Kowler 2008). For movements along the preferred axis of the neuron (Fig. 1C), the neuron preferentially responded for contraversive microsaccades (Fig. 1D) and exhibited a gradual increase in firing rate that started well before movement onset and reached a peak during movement execution. The neuron's activity then gradually decreased, only reaching premovement levels well after the microsaccades ended (Fig. 1D). When we repeated the same analysis but for similar-sized microsaccades in the opposite ipsiversive direction (i.e., movements to the lower left quadrant; Fig. 1D), we found that the neuron maintained its activity throughout the analysis interval and did not exhibit either a clear increase or a clear decrease in its firing rate. These results indicate that this neuron possessed a contralateral RF, within which it preferentially exhibited saccade-related increases in firing rate, and that this RF encompassed microsaccades directed to the upper right quadrant. Moreover, the time course of activity of this neuron for its preferred microsaccades was reminiscent of known saccade-related increases in activity in more caudal portions of the SC for larger, voluntary saccades (Gandhi and Katnani 2011; Munoz and Wurtz 1995a; Waitzman et al. 1991), a point summarized later.

To explore the RF characteristics of this neuron further, we also analyzed its activity during the saccade task. In this task, the monkey generated delayed, visually guided saccades to targets with locations that we manually selected for the purpose of additional RF mapping. Figure 1E shows an analysis similar to that of Fig. 1C but for the range of saccades tested during this task ($1\text{--}16^\circ$, predominantly to the upper right quadrant, which we assessed during the experiment as the preferred quadrant for the neuron). The neuron exhibited preferential increases in activity for small saccades of $\sim 1\text{--}1.25^\circ$ amplitudes in the same direction as that preferred for microsaccades

during the fixation task (Fig. 1E). For larger saccades and saccades of directions different from those preferred by the neuron, the firing rates during saccades were lower than those during the preferred movements. This latter point was more obvious when we plotted the perisaccadic time course of neuronal activity in this task in a format similar to that we used for the fixation task data. Specifically, in Fig. 1F, we plotted neuronal activity as a function of time from saccade onset for all the contralateral saccades along the preferred axis of the neuron (within the shaded region of Fig. 1E). We found that saccades larger than $\sim 1.25^\circ$ in amplitude were generally associated with a pause in the neuron's activity during movement execution, not an increase (Fig. 1F); for saccades of $\sim 1\text{--}1.25^\circ$ amplitude, the neuron exhibited perisaccadic increases (evidenced by the high density of spikes) that were similar to those observed for microsaccades in Fig. 1D.

Thus the results from both the fixation and saccade tasks combined indicate that the sample neuron of Fig. 1 exhibited saccade-related increases in its activity for movements inside its RF, which encompassed both microsaccades and small visually guided saccades, and that the RF of the neuron when estimated from microsaccades had a preferred direction similar to that when measured from the saccade task. Moreover, the neuron exhibited suppression of activity, or a saccade-related pause, for movements well outside its RF. In the following sections, we explore these observations further across our population of recorded neurons.

Time Course of SC Activity Leading Up To and Following Microsaccades

The fixation task allowed us to study neuronal activity within the SC associated with the smallest possible saccades. We found that the great majority of fixational saccades that the monkeys generated while performing this task were smaller than 30 min arc (0.5°), with a median amplitude of 11 min arc (0.18° ; Fig. 2). To assess the characteristics of SC activity associated with microsaccades, we concentrated our analyses in this report on neurons exhibiting significant movement-related activity for saccades < 15 min arc (0.25°). Even though this threshold of 15 min arc for defining microsaccades was more stringent than recent studies of these movements, such as those of Brien et al. (2009) and Martinez-Conde et al. (2000), it was similar to that used in classic studies of microsaccades (see Collewijn and Kowler 2008 for review), and it was large enough to include most of the movements in our data set of Fig. 2. More importantly, this threshold allowed us to compare the activity of neurons that exhibited movement-related activity for the smallest group of eye movements that occurred during our fixation task to the activity of neurons that only exhibited movement-related activity for significantly larger, voluntary saccades (collected during the saccade task, as we describe in a later section).

SC activity associated with microsaccades was characterized by a gradual elevation of firing rate leading up to microsaccade onset and then a gradual return to baseline after movement end. We analyzed the activity of 100 neurons from the intermediate layers of the SC during the fixation task, 45 of which exhibited microsaccade-related increases in activity similar to those of Fig. 1D (and for movements < 15 min arc in amplitude). For each of these 45 neurons we could identify a set of microsac-

cades, falling within a particular amplitude and direction range, that was associated with higher firing rates than those observed during saccade-free fixation epochs ($P < 0.05$; rank sum). We analyzed the time course of this activity around microsaccade onset in these neurons as shown in Fig. 3A. In this figure, we plotted the average normalized firing rate of all 45 neurons aligned on microsaccade onset, and we compared it to the average normalized firing rate of these same neurons during saccade-free fixation epochs. For each neuron's firing rate curve, we selected only the microsaccades along the neuron's preferred axis (e.g., the shaded gray region of Fig. 1 for that sample neuron) that caused a firing rate during the movement that was larger than 2 SD above the saccade-free fixation baseline and we normalized the neuron's firing rate curve by the maximum response during these selected microsaccades. Across the 45 neurons, we found that elevated SC activity associated with microsaccades started on average ~ 98 ms prior to microsaccade onset, reached a peak during the movement, and only returned back to baseline levels at ~ 108 ms after microsaccade onset (Fig. 3A). Given the relatively short duration of the microsaccades causing this activity ($32 \text{ ms} \pm 11 \text{ ms SD}$), these results therefore suggest that microsaccades were associated with elevated firing rates within the SC that started well before movement onset and that lasted well after movement end. We also found that the range of microsaccade amplitudes contributing to such a temporal pattern of movement-related activity in the SC was similar to the range of microsaccade amplitudes that we observed in general (compare Fig. 3A, *inset*, to the microsaccade range in the aggregate data in Fig. 2), suggesting that all microsaccade amplitudes appear to be represented within the SC (Hafed et al. 2009).

One characteristic of saccade-related activity in the SC is that the time of peak neuronal discharge associated with a given movement occurs early during the movement, contributing to at least the initial portions of the oculomotor drive signal (Lefevre et al. 1998; Munoz and Wurtz 1995a; Waitzman et al. 1991). Our results suggest that a similar property holds for microsaccade-related activity as well. We measured the time of peak neuronal discharge for microsaccade-related activity relative to the time of movement onset, movement peak velocity, or movement end (Fig. 3B). We found that peak neuronal activity associated with microsaccades occurred shortly after movement onset, with an average latency of ~ 5 ms (Fig. 3B, *left*), but before the time of peak eye velocity, with an average lead time of ~ 11 ms, and also preceding the time of movement end, with an average lead time of ~ 28 ms (all values significantly different from zero, $P < 0.001$, *t*-test). In addition, the time of peak discharge was always closest to the time of movement onset, and not to the time of peak velocity or movement end. Specifically, the mean time difference between peak activity and movement onset (~ 5 ms) was significantly smaller than the mean time difference between peak activity and movement peak velocity (~ 11 ms) or peak activity and movement end (~ 28 ms) ($P < 0.05$, 2-tailed, paired *t*-test for each comparison). These observations are consistent with previous findings of an early peak discharge for saccade-related activity in the SC (Waitzman et al. 1991); they are also consistent with saccade-related activity that we recorded from the same animals, as we describe next.

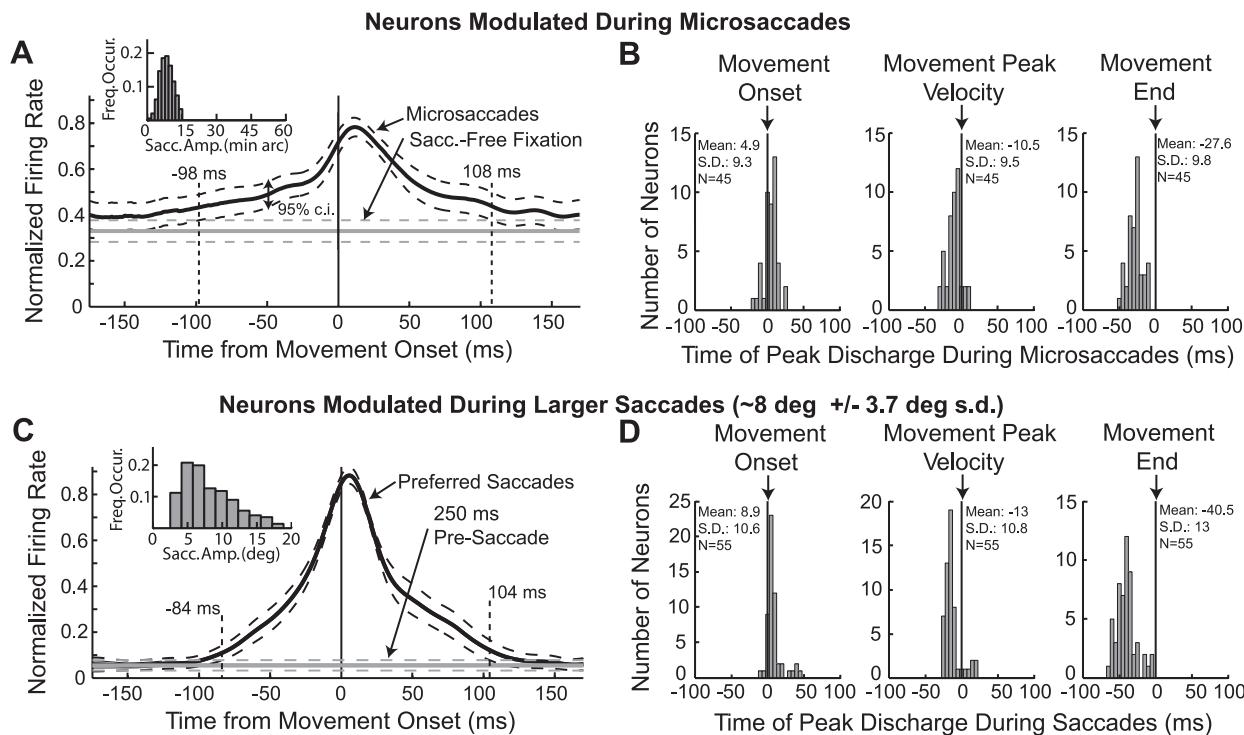


Fig. 3. Time course of movement-related activity in the SC for microsaccades and saccades. *A*: for each neuron exhibiting microsaccade-related activity, we identified the preferred movement amplitudes and directions and used those to obtain perimovement firing rates. We normalized each neuron's firing rate by the maximum observed for the best microsaccades and then combined data across neurons. Dashed lines indicate 95% confidence intervals (c.i.). We estimated the onset and offset times of microsaccade-related activity elevation by also measuring the activity of the same neurons, using the same normalization factors, during saccade-free fixation epochs (gray). Across the population, microsaccade-related activity started to increase ~98 ms prior to movement onset and returned to baseline ~108 ms after movement onset. *Inset*: amplitude distribution of microsaccades contributing to the firing rate profile shown, indicating that all microsaccade amplitudes that normally occur (compare to Fig. 2) appear to be represented. *B*: peak microsaccade-related activity in the SC generally occurred after movement onset (*left*) but before movement peak velocity (*center*) and movement end (*right*). For each neuron exhibiting microsaccade-related activity, we measured the time of peak movement-related discharge observed during the neuron's preferred microsaccades. Histograms summarize the data across neurons. These results indicate that peak neuronal firing associated with microsaccades occurred early in the movements, consistent with general saccade-related activity in this structure (see *D*). *C*: analysis similar to that of *A* but for the preferred saccades of each caudal neuron, collected during the saccade task. *Inset*: distribution of preferred saccades in this population of neurons, demonstrating that we compared microsaccade-related activity to the activity associated with neurons preferring significantly larger saccades. For this data set, we defined the saccade-free baseline as the firing rate observed 250 ms prior to visually guided saccade onset (see MATERIALS AND METHODS). The temporal profile of SC activity was similar to that observed for microsaccades, except that the baseline activity was lower for large saccades (compare baseline to that in *A*), indicating that more caudal SC neurons possessed less tonic activity (also see Figs. 4 and 7). *D*: time of peak activity around movement onset, movement peak velocity, and movement end in our saccade-related neurons. Similar to microsaccade-related activity, saccade-related activity exhibited a peak shortly after movement onset but before movement peak velocity and before movement end. Moreover, comparison of the time of peak discharge relative to movement end in these neurons to the value in microsaccade-related activity in the neurons of *B* reveals that the quantitative difference was related to the difference in duration between saccades and microsaccades (see text). Thus both saccade- and microsaccade-related peak discharge occurred early during the movements.

Comparison of Microsaccade-Related SC Activity to Activity of SC Neurons Preferring Larger Saccades

In addition to the above 45 neurons exhibiting activity for movements smaller than 15 min arc (0.25°), we also analyzed the activity of 55 neurons that did not exhibit microsaccade-related activity but exhibited firing rate increases during larger movements collected from the saccade task. These neurons were recorded from more caudal electrode locations than the first group, but they had similar depths relative to our estimate of SC surface ($1.53 \text{ mm} \pm 0.41 \text{ mm SD}$ vs. $1.63 \text{ mm} \pm 0.47 \text{ mm SD}$ for the first group; $P = 0.323$, 2-tailed t -test). We defined these additional neurons as our “saccade-related neurons,” and we compared their activity for their preferred saccades to the activity of our neurons modulated during microsaccades. Specifically, we repeated the same analyses presented above in Fig. 3, *A* and *B*, but for these additional saccade-related neurons and for larger movements. Our aim was to test whether there were any fundamental differences

between saccade-related and microsaccade-related SC activity; in a later section, we discuss differences in tonic activity during saccade-free fixation between the two groups of neurons.

Microsaccade-related activity that we observed in the SC shared many features with saccade-related activity in the more caudal neurons in our data set. Figure 3C plots the average normalized perisaccadic firing rate curve of our 55 saccade-related neurons (for saccades within 3° from each neuron's preferred movement). Figure 3C, *inset*, indicates that the neuronal activity shown was associated with saccades ranging in amplitude from $\sim 3^\circ$ to $\sim 20^\circ$, all of which were significantly larger than the microsaccades contributing to the activity shown in Fig. 3A. As in the case of the neurons of Fig. 3A, we normalized each neuron's activity in Fig. 3C by its maximum activity during its most preferred saccade. Across the population, we found that these saccade-related neurons exhibited elevated activity, relative to saccade-free fixation, starting ~ 84 ms prior to movement onset. In addition, the activity of these

neurons reached peak discharge shortly after movement onset, and it gradually declined to reach presaccadic baseline levels at ~ 104 ms after movement onset. These properties were similar to those of the microsaccade-related activity in Fig. 3A, except that the onset time of elevated saccade-related activity was shorter than that observed for microsaccades by ~ 14 ms. Inspecting individual neuron firing rates revealed that this difference was mainly due to a subset of neurons in Fig. 3A that had a particularly long lead of activity before some microsaccades. Thus perimicrosaccadic activity in the SC falls in the same general class of perisaccadic activity in this structure with regard to its time course around movement onset.

The similarity of microsaccade- and saccade-related SC activity was also evident in the timing of peak movement-related firing rates. For our saccade-related neurons, we analyzed the time of peak discharge relative to movement onset, movement peak velocity, and movement end, just as we did for the microsaccade-related neurons. The results of these analyses are shown in Fig. 3D. As in the case of microsaccade-related activity, peak saccade-related activity occurred after movement onset, evidenced by the positive average latency of ~ 9 ms relative to the beginning of saccades ($P < 0.05$, *t*-test). This value was only marginally significantly higher than that obtained in Fig. 3B, *left*, for microsaccade-related activity ($P = 0.051$, 2-tailed *t*-test; $N = 45$ for the neurons exhibiting microsaccade-related activity and $N = 55$ for the saccade-related neurons of Fig. 3D). On the other hand, peak saccade-related discharge was reached prior to peak eye velocity in the saccade-related neurons, consistent with previous reports (Waitzman et al. 1991) (Fig. 3D, *center*). This observation was again similar to that made for microsaccade-related activity (Fig. 3B, *center*). In fact, the lead time of peak discharge relative to peak eye velocity was statistically indistinguishable between the neurons exhibiting microsaccade-related activity and those exhibiting saccade related activity (~ 11 ms vs. ~ 13 ms; $P = 0.23$, 2-tailed *t*-test). Finally, for the saccade-related neurons, peak neuronal discharge led saccade end (Fig. 3D, *right*), as it did for microsaccade-related neurons. However, in this case, saccade-related activity peaked much earlier than microsaccade-related activity (~ 41 ms before movement end vs. ~ 28 ms; $P < 0.001$, 2-tailed *t*-test). Given the difference in duration between the saccades of Fig. 3D and the microsaccades of Fig. 3B (49 ms ± 12 ms SD vs. 32 ms ± 11 ms SD; $P < 0.001$, 2-tailed *t*-test), this difference between microsaccade-related activity and saccade-related activity can be explained by the difference in movement durations. Thus comparison of the timing of movement-related activity between microsaccades and saccades suggests that peak neuronal discharge in the SC, in general, is not synchronized with movement end. Instead, we found that peak movement-related activity was best synchronized with movement peak velocity, because it is this measure that depended the least on the different movement duration ranges in our data.

Continuity of Distributed Spatial Representation of SC for Microsaccades and Saccades

The similarity between perimovement neuronal discharge in the SC for microsaccades and for larger saccades suggests that neurons exhibiting microsaccade-related activity belong to the same functional class as saccade-related SC neurons, and

simply possess more foveal RFs. To test this possibility, we inspected the activity of microsaccade-related SC neurons in our population, but during the saccade task, and vice versa for the saccade-related SC neurons (i.e., we inspected these neurons' activity during the fixation task). We found a continuum of spatial representations within the SC for all movement amplitudes, such that some SC neurons were specifically tuned for microsaccades, showing no apparent preference for larger movements; other neurons had RFs that encompassed microsaccades and slightly larger saccades, in a continuous manner; and yet other neurons were only responsive during large saccades.

For neurons specifically tuned for microsaccades, we often observed a reduction in activity during larger saccades. The activity of one such neuron is shown in Fig. 4A. Even though the neuron exhibited significant movement-related elevation of activity for microsaccades (Fig. 4A, *left*), it paused for all tested visually guided saccades, including ones as small as $\sim 1.5^\circ$ (Fig. 4A, *right*). In addition, the duration of the pause appeared to increase for larger saccades, which also had longer durations (also see Fig. 1F and Fig. 4B, *right*, for similar examples). It thus appears that the neuron of Fig. 4A possessed a foveal, spatially restricted RF; this neuron exhibited two types of movement-related activity depending on whether the movements were inside or outside its RF: elevation for preferred movements and suppression for larger, nonpreferred ones.

Other SC neurons had similar patterns of activity, but their RFs encompassed both microsaccades and small saccades, in a spatially continuous manner. This can be seen from the example neuron of Fig. 1 and a second sample neuron shown in Fig. 4B. Both of these neurons increased their firing rates before and during microsaccades and small saccades but paused for larger saccades. Specifically, for the neuron of Fig. 4B, plotting the activity of the neuron for movements collected during the fixation task revealed that this neuron was not only active for microsaccades toward the neuron's preferred direction but also exhibited movement-related increases in activity for larger fixational saccades that occurred during the session (up to ~ 30 min arc in amplitude). During the saccade task, movements of a similar direction but with amplitudes that were as large as 3.5° (Fig. 4B, *right*) continued to be associated with saccade-related increases in this neuron's activity. It was only for larger saccades that the neuron exhibited saccade-related decreases in firing rate. Thus the RF of the neuron of Fig. 4B (as well as that of the neuron of Fig. 1) was also spatially restricted as in the case of the neuron of Fig. 4A, but its preferred saccade amplitudes were slightly larger (thus encompassing movements larger than microsaccades).

Finally, many of our saccade-related neurons were tuned for larger eye movements and did not discharge for microsaccades. An example of one such neuron is illustrated in Fig. 4C. This neuron was not active for microsaccades (in the fixation task; Fig. 4C, *left*) and visually guided saccades smaller than $\sim 6^\circ$ (in the saccade task; Fig. 4C, *right*). However, the same neuron did exhibit movement-related increases for larger saccades collected during the saccade task (Fig. 4C, *right*). Thus the neuron's RF had an organization similar to that of the neurons of Fig. 4, A and B, which exhibited microsaccade-related activity but represented larger eye displacements.

Taken together, these results (Fig. 4) suggest that a large part of the difference between SC neurons exhibiting microsaccade-

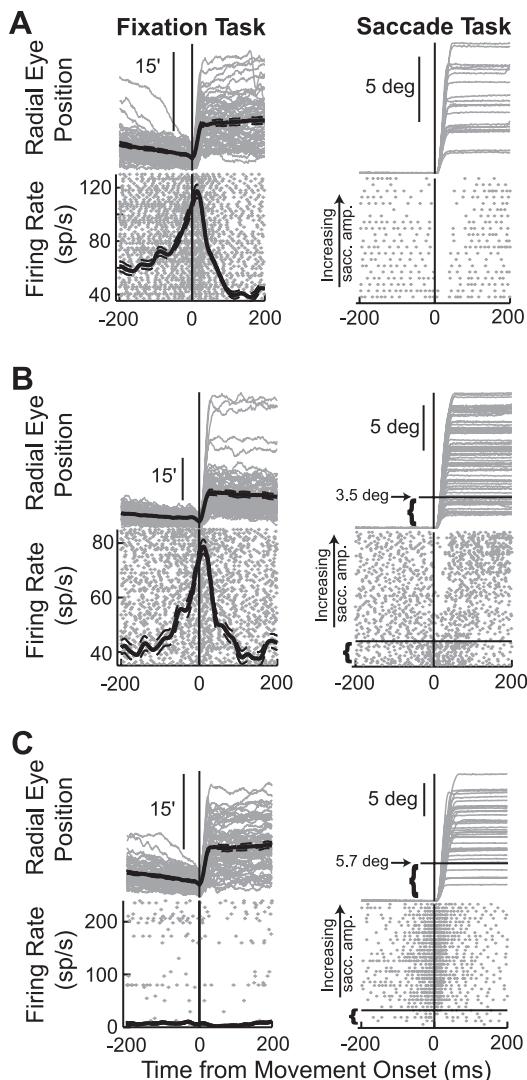


Fig. 4. Continuity of the representation of microsaccades and saccades in the same SC neurons. We plotted the activity of 3 sample neurons (*A*, *B*, *C*) for rapid eye movements during the fixation task (left) as well as during the saccade task (right). For the neuron in *A*, microsaccade-related elevation in firing rate was observed, but the neuron paused during all measured visually guided saccades (larger than $\sim 1^\circ$), suggesting a small, foveal response field specific to the smallest eye movements. For the neuron in *B*, a similar observation was made, but the response field of the neuron appeared to include some microsaccades as well as slightly larger movements (up to $\sim 3.5^\circ$). For even larger saccades, the neuron paused. For the neuron in *C*, no microsaccade-related elevation of activity occurred, and the neuron exhibited saccade-related activity only for movements larger than $\sim 6^\circ$. Thus the response field of this neuron was more eccentric than that of the neurons in *A* and *B*. The data in this figure are plotted as in Fig. 1, *D* and *F*, as well as in Hafed et al. (2009). For the saccade task data, we did not include a firing rate curve because of the variable types of responses across movement amplitudes (e.g., increase vs. decrease in firing rate).

related activity and those exhibiting only saccade-related activity can be explained by the different preferred movement amplitudes of the neurons: the neurons of Fig. 4, *A* and *B* (and Fig. 1) exhibited increases for small movements, and the neuron of Fig. 4*C* exhibited increases for large movements. Because the neurons preferring smaller movements also generally had higher tonic rates (Fig. 3*A*, saccade-free baseline), nonpreferred movements caused a reduction in these rates as these movements were associated with target locations not

represented by the neurons (Hafed and Krauzlis 2008) (also see next section and DISCUSSION).

The continuity of the spatial representation for movement amplitude, encompassing both microsaccades and larger saccades, also applied to movement direction. To demonstrate this, we plotted the RF maps of our neurons, but now taking into account both microsaccades and larger saccades in one grouping and taking into account both movement amplitude and movement direction. For this analysis (Fig. 5), we employed a “log-polar” coordinate transformation, that is, we displayed the firing rate of a neuron during a movement (*z*-axis) as a function of the radial amplitude and angular direction of the movement, and we used a logarithmic scale to display the radial amplitude data in order to account for the large range of amplitudes observed when both microsaccade and saccade data are combined (see MATERIALS AND METHODS). Figure 5 displays the RF maps of the three sample neurons of Fig. 4 in this format, and it demonstrates several aspects of the spatial representation within the SC that we observed across our population: 1) different neurons possessed different preferred directions and eccentricities (compare the hotspots in Fig. 5, *A*, *B*, and *C*), 2) neurons exhibiting microsaccade-related activity (Fig. 5, *A* and *B*) possessed RFs that were more central than neurons not exhibiting microsaccade-related activity (Fig. 5*C*), and 3) when a neuron was modulated for both microsaccades and slightly larger saccades (as in the case of Fig. 5*B*), this modulation was associated with a contiguous RF in which there was no distinction between microsaccades and larger saccades as far as the neuron’s modulation was concerned.

The observation that neurons exhibiting microsaccade-related activity in the SC possessed more central RFs than neurons not exhibiting microsaccade-related activity, while still preferring a range of directions and eccentricities, was consistent across our population. In Fig. 6*A*, we used the RF maps of individual neurons that were obtained according to the procedure of Fig. 5 in order to find the best movement end point (whether microsaccadic or not) preferred by a given neuron (i.e., the amplitude and direction that were associated with the highest firing rate during saccades or microsaccades). We then grouped neurons according to whether they exhibited microsaccade-related activity during the fixation task (i.e., the neurons of Figs. 1–3*B*) or not (i.e., the neurons of Fig. 3, *C* and *D*). As can be seen, neurons not exhibiting microsaccade-related activity generally had preferred eccentricities larger than $\sim 4^\circ$. On the other hand, neurons exhibiting microsaccade-related activity generally had preferred eccentricities smaller than $\sim 4^\circ$. Moreover, neurons exhibiting microsaccade-related activity possessed a wide range of preferred directions, consistent with the idea that the spatial representation in the SC extends down to represent the smallest movements. Finally, as described by Hafed et al. (2009), some of the neurons exhibiting microsaccade-related activity preferred small ipsilateral eccentricities in their responses, apparently bridging the representation of the foveal retinotopic space across the two colliculi.

Relation of Neurons Exhibiting Microsaccade-Related Activity to Previous SC Neuronal Classification

Finally, the saccade-related pauses in activity that some of our neurons exhibited for saccades outside their RFs (see, for example, the sample neurons of Fig. 1*F* and Fig. 4, *A* and *B*)

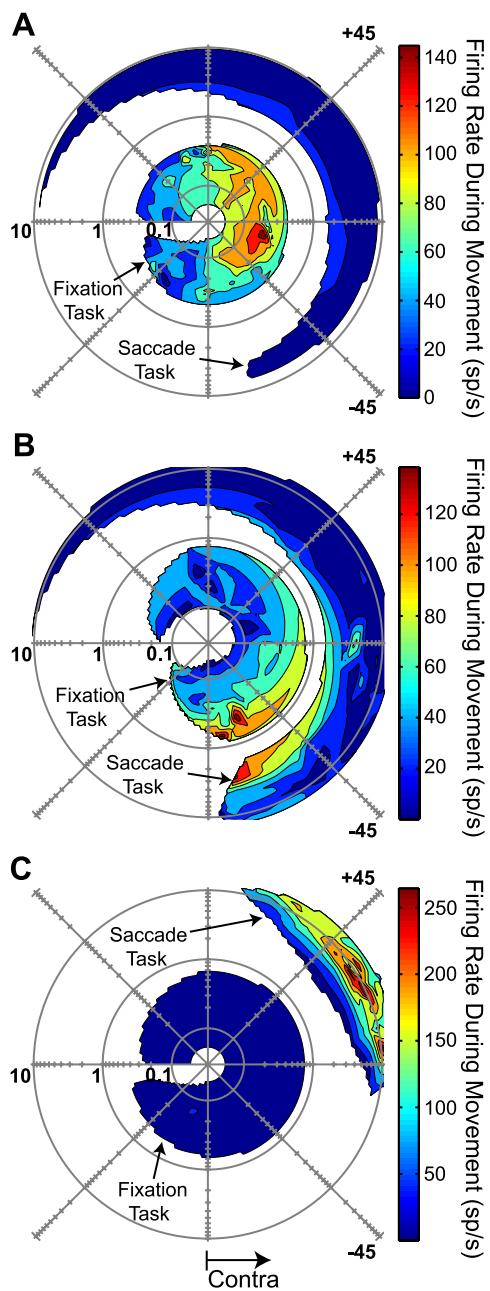


Fig. 5. Spatial contiguity of SC movement fields for microsaccades and saccades. For the same neurons of Fig. 4, we plotted the full 2-dimensional response fields of the neurons by combining data from the fixation and saccade tasks. We plotted saccade/microsaccade radial amplitude (on a logarithmic scale) and direction and then indicated the mean firing rate during a given movement on the z -axis (color coded). Data from the fixation task typically involved movements $<1^\circ$ in amplitude, contributing to the inner surface of each panel (labeled fixation task); data from the saccade task typically involved contralateral movements $>1^\circ$ in amplitude, contributing to the outer surface of each panel (labeled saccade task). White spaces are regions not sampled by either microsaccades or saccades, and all response fields are aligned such that the right half of each panel is the contralateral side relative to the recording location. When saccades and microsaccades are combined, the response fields of the neurons appear to form contiguous shapes with differing spatial preferences, the neuron of *A* preferring small eccentricities along the horizontal axis, the neuron of *B* preferring slightly larger eccentricities (encompassing both microsaccades and small saccades) to the lower visual field, and the neuron of *C* preferring even larger eccentricities (not encompassing movements less than $\sim 6^\circ$, whether saccadic or microsaccadic) to the upper visual field. The displayed origin on the logarithmic radial amplitude in this figure refers to 0.03° .

are reminiscent of pauses in activity that have been used previously to classify rostral SC neurons as fixation related (Munoz and Guitton 1989, 1991; Munoz and Wurtz 1993a). We thus investigated whether or not the neurons in our population that exhibited microsaccade-related activity were likely to be the same neurons as those that were previously classified in the literature to be “fixation neurons” (Munoz and Guitton 1989, 1991; Munoz and Wurtz 1993a, 1993b). To test this, monkeys performed a fixation-blink task, in which the fixation spot disappeared for 500 ms while the monkeys maintained fixation. We measured the average firing rate of the 45 neurons of Figs. 1–3*B*, Fig. 5, *A* and *B*, and Fig. 6 during the final 100 ms of the blink period, and we found that 80% (36/45) of these neurons (i.e., those that exhibited microsaccade-related activity) maintained >10 sp/s during this interval. Thus these

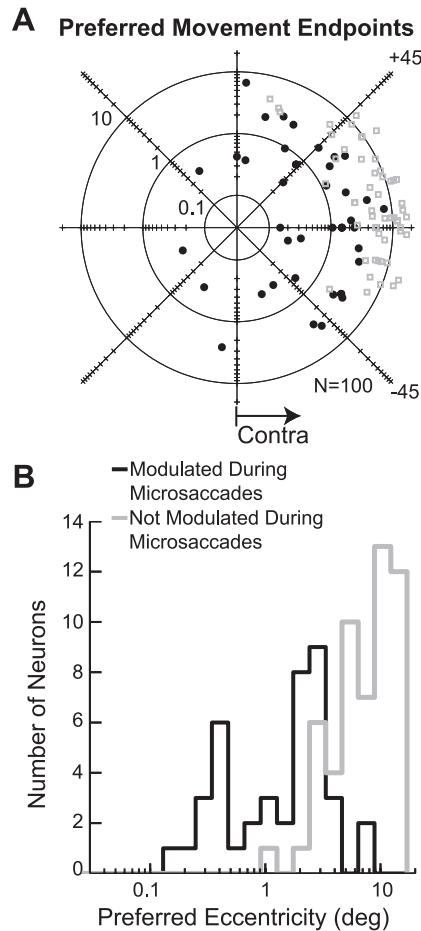


Fig. 6. Summary of response field centers for neurons exhibiting microsaccade-related activity (black) and neurons not exhibiting microsaccade-related activity (gray). *A*: each dot displays the estimated response field center of a neuron after combining the fixation and saccade task data as in Fig. 5. SC neurons exhibiting microsaccade-related activity tended to have more foveal response field centers than neurons not exhibiting microsaccade-related activity, consistent with a continuous spatial representation within the SC for all movement amplitudes. *B*: summary histograms of the radial amplitudes of the response field centers for the neurons exhibiting microsaccade-related activity (black) and those only exhibiting saccade-related activity (gray). Again, neurons with microsaccade-related activity had more foveal response field centers than neurons exhibiting only saccade-related activity. Moreover, microsaccade-related activity was observed even for neurons with parafoveal response field centers, suggesting that these neurons possessed response fields that extended significantly into the fovea. Note that these data are plotted on a logarithmic radial amplitude scale, as in *A* and Fig. 5.

neurons exhibited tonic activity during fixation (e.g., Fig. 3A, saccade-free fixation) that was not strictly visual in nature (i.e., dependent on the presence of the fixation spot), much like the previously identified putative fixation-related neurons (Munoz and Wurtz 1993a). The remaining 9 of 45 neurons exhibiting microsaccade-related activity were not tonically active and exhibited <10 sp/s rates during the blink interval, suggesting that they were more similar to neurons in more eccentric portions of the SC topographic map exhibiting saccade-related activity but without much baseline firing. We did not test the 55 saccade-related neurons in our population (those described in Figs. 3, C and D, 4C, 5C, 6) with the fixation-blink task because they did not exhibit high tonic activity during fixation. Thus, according to the fixation-blink test, most neurons with microsaccade-related activity appear to be the same neurons previously identified as “fixation neurons.”

We used a second test for classifying fixation-related activity in the SC, namely, the presence of saccade-related pauses for large ipsilateral memory-guided saccades (Munoz and Wurtz 1993a). Fixation-related neurons reliably pause for such saccades even when they do not pause for all contralateral movements (Munoz and Wurtz 1993a). For 31 of the 45 neurons exhibiting microsaccade-related activity, we also instructed our monkeys to generate 10° horizontal saccades to remembered targets in the ipsilateral visual field relative to the recorded neuron. All of these 31 neurons had exhibited tonic activity during the fixation-blink task as per the above description. We found that the majority of these neurons 74% (23/31) had significantly lower activity during ipsilateral saccades than during a baseline interval centered on 250 ms prior to movement onset ($P < 0.05$, *t*-test; see MATERIALS AND METHODS). Figure 7 shows this effect for the three sample neurons of Fig. 1 and Fig. 4, A and B, as well as for the entire population. This figure also indicates that the dynamics of activity reduction across the population of neurons (analyses similar in principle to those in Fig. 3, A and C) have characteristics similar to those observed earlier for putative fixation-related neurons (Munoz and Wurtz 1993a). For example, the onset of activity reduction for the 10° ipsilateral memory-guided saccades appears to happen closer to movement onset than the onset of activity elevation we observed in Fig. 3, A and C, for microsaccade-related and saccade-related activity. Taken together, these results indicate that SC neurons exhibiting microsaccade-related activity belong to the same group of neurons previously studied at similar locations in the SC and classified as “fixation neurons.”

DISCUSSION

We investigated the characteristics of neuronal discharge in the SC in relationship to microsaccades. We found that microsaccades are associated with a temporal pattern of SC activity that is nearly the same as that for larger saccades. Spatially, microsaccade-related activity is exhibited by neurons located in rostral regions of the SC, which represent the central visual field. These results, and the observation that individual RFs in the rostral SC can encompass movements of microsaccade amplitude as well as slightly larger saccades of similar direction, support the conclusion that the SC controls microsaccades in a fashion similar to larger saccades. These findings have implications for our understanding of the neural control of

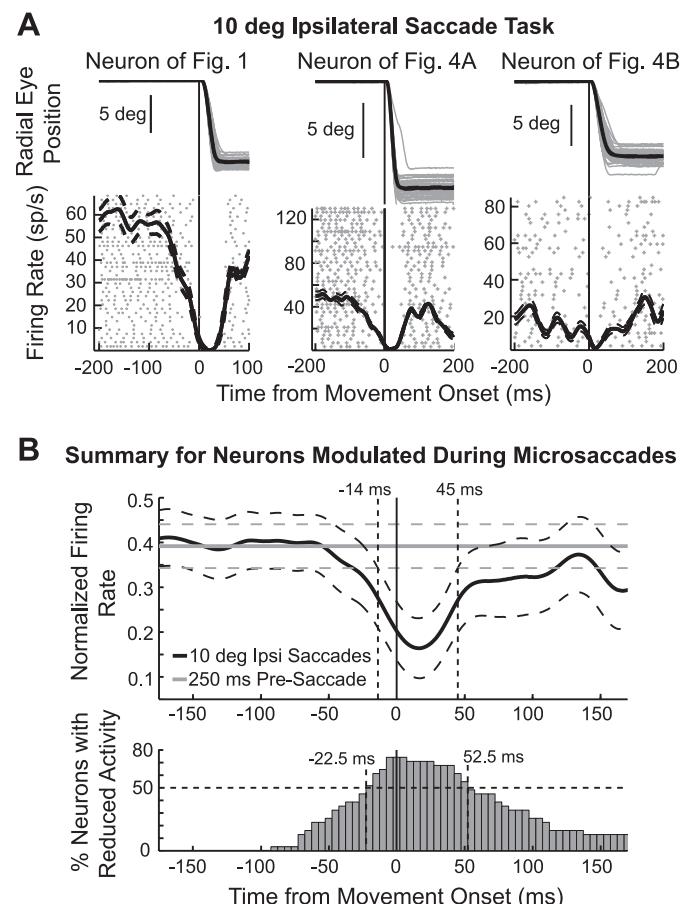


Fig. 7. Classification of neurons exhibiting microsaccade-related activity according to previous criteria in the literature for rostral SC neurons. *A*: activity of the neurons of Fig. 1, Fig. 4A, and Fig. 4B during 10° ipsilateral memory-guided saccades. All neurons exhibited tonic activity during fixation and reduced activity during ipsilateral saccades. *B*: for neurons exhibiting microsaccade-related activity, ipsilateral memory-guided saccades were almost always associated with reduction in firing rate, consistent with the conclusion that neurons exhibiting microsaccade-related activity are not distinct from neurons previously described in the rostral SC (e.g., Krauzlis et al. 1997; Munoz and Wurtz 1993a). The data in *A* were plotted by procedures similar to Fig. 4, and those in *B* were obtained as described in Fig. 3 and MATERIALS AND METHODS.

microsaccades, the nature of the functional representation within the SC, and the influence of microsaccades and saccades on visual function.

Neural Control of Microsaccades

Our results indicate that SC neurons contribute part of the drive signal for generating microsaccades. First, SC neurons exhibit elevated firing rates well before microsaccade onset, unlike sensory neurons (such as in V1) whose activity seems to reflect either the retinal consequences of microsaccades (Martinez-Conde et al. 2000) or an extraretinal saccadic suppression (Kagan et al. 2008; Snodderly et al. 2001). Specifically, we found that microsaccade-related activity in the SC begins to increase ~98 ms prior to movement onset, peaks before peak eye velocity, and returns to baseline ~108 ms after movement onset. Second, the time course of elevated microsaccade-related activity is similar to that observed in saccade-related neurons elsewhere in the SC, whose role in controlling saccades is well established (Gandhi and Katnani 2011). Third,

peak neuronal activity associated with microsaccades occurs in close temporal register with movement onset, consistent with the view that SC activity contributes to saccade target selection and saccade initiation, but not necessarily saccade termination or in-flight control of saccade trajectory (Lefevre et al. 1998).

The temporal pattern of microsaccade-related activity in the SC is also consistent with the frequency of microsaccades. We found that each microsaccade is associated with differential SC activity (relative to saccade-free fixation) that lasts ~200 ms or more (including pre- and postmovement activity). This observation places an upper bound on normal microsaccade frequency of <5 per second, consistent with numerous studies (Bosman et al. 2009; Hafed et al. 2009; Otero-Millan et al. 2008). It would be interesting to test whether such a pattern of SC activity has further implications on the recently discovered interactions between stimulus transients and microsaccade frequency, in which the probability of observing a microsaccade appears to be modulated by the onset of visual stimuli (Engbert and Kliegl 2003; Hafed and Clark 2002; Laubrock et al. 2005).

It is unclear whether the gradual buildup of activity prior to microsaccades arises locally within the SC or is a result of interactions with other structures. One possibility is that variability in the activity of individual SC neurons representing the location of the fixated target (Hafed et al. 2009) biases some neurons to be slightly more active than others. Through local excitation, this differential activation could get amplified and gradually lead to elevated firing rates until a movement is triggered. Alternately, modulation of inhibitory input (e.g., from substantia nigra; Hikosaka and Wurtz 1985; Isa and Hall 2009; Liu and Basso 2008) or excitatory input (e.g., from the prefrontal cortex; Sommer and Wurtz 2000; Wurtz et al. 2001) could shape SC population activity and contribute to microsaccade-related buildup. These mechanisms are sources of saccade-related buildup of activity elsewhere in the SC, highlighting the similarity between microsaccades and saccades at the mechanistic level.

Our results leave open the question of whether other brain structures known to contribute to saccade generation also contribute to microsaccade generation. For example, the frontal eye fields (FEF) contribute to saccade generation by sending sensory, motor, and cognitive signals to the SC (Johnston and Everling 2006, 2009; Koval et al. 2011; Sommer and Wurtz 2000; Wurtz et al. 2001). Does the similarity between microsaccades and saccades, at both the level of behavior and brainstem involvement, also include the FEF? If so, this would provide one candidate pathway for explaining evidence of voluntary control over microsaccades, which has been demonstrated in humans despite the fact that these movements are generally assumed to be involuntary (see, e.g., Ko et al. 2010; Steinman et al. 1967; also see Hafed et al. 2011a for evidence in monkeys).

Nature of Representation Within the SC

The preferred retinotopic locations of neurons exhibiting microsaccade-related activity in our population were restricted to the central visual field (extending to preferred eccentricities of up to ~4°), whereas the preferred locations of neurons not exhibiting microsaccade-related activity were more peripheral. This suggests that the well-known spatial topography within

the SC spans all possible retinotopic locations targeted by saccades and microsaccades. While parsimonious, this description contrasts with some gaze-error models of the SC (Bergeron and Guitton 2001, 2002) in which the rostral region of this structure issues fixation commands. According to this view, neurons in the rostral region of the SC, corresponding to the fovea, are classified as “fixation neurons” because they are tonically active during fixation and pause during some saccades.

Our results support the view that neurons described as fixation related have a broader functional role than fixation maintenance. We found that most neurons excited during microsaccades meet the previously established criteria for “fixation neurons”: they exhibit higher tonic activity during fixation than neurons preferring larger saccades (compare baseline rates in Fig. 3, A and C); they maintain >10 sp/s during fixation-blink trials; and they reduce their activity for large saccades (Fig. 7). The microsaccade-related (and potentially saccade-related; Figs. 1, 4, 5, 6 and Munoz and Wurtz 1993a) activity of these same neurons shows that they are involved in functions beyond fixation maintenance. This conclusion is further supported by the fact that these neurons are modulated during smooth pursuit (Krauzlis et al. 1997, 2000), as well as during cognitive tasks in which fixation can still be maintained despite modulations in the neurons’ activity (see, for example, Dorris and Munoz 1995; Munoz and Everling 2004). A unifying explanation for these seemingly disparate findings is that these neurons support multiple forms of orienting behavior through the representation of behaviorally relevant goal locations (Hafed et al. 2008; Hafed and Krauzlis 2008) and using a distributed population code (Lee et al. 1988).

A distributed representation including the foveal SC is consistent with an alternative model of how gaze fixation is achieved at the level of the SC, namely, through balance of bilateral activity corresponding to the goal location (Hafed 2011; Hafed et al. 2008, 2009). According to this model, fixation is maintained while the entire population remains balanced bilaterally across the two SCs. If individual neurons with small preferred eccentricities increase their activity, the overall population becomes biased and such imbalance may be sufficient to trigger microsaccades. Of course, in such a model, the most active neurons in the population become relatively more important than the rest of the SC map by virtue of their activity; thus, since foveal SC neurons are most active in representing the foveal goal locations associated with gaze fixation, these neurons play an important role for this aspect of gaze orienting.

Besides clarifying the role of the SC in multiple eye movement outputs (including fixation, microsaccades, larger saccades, and smooth pursuit) (Hafed et al. 2008, 2009; Hafed and Krauzlis 2008; Krauzlis et al. 1997), the above hypothesized mechanism for the role of the SC in supporting gaze fixation may also explain an additional property of microsaccades, that they reflect the allocation of covert visual attention (Engbert and Kliegl 2003; Hafed and Clark 2002; Hafed et al. 2011a). Because covert attention is associated with modulations in SC activity at the peripheral sites corresponding to attended locations (Ignashchenkova et al. 2004), it is possible that such modulations may directly or indirectly influence activity in the central SC representation and bias microsaccade directions

(Hafed et al. 2009) (also see Hafed et al. 2011b for a direct test of this hypothesis).

Microsaccades, Saccades, and Their Role in Vision

Finally, our results impose constraints on how to interpret the functional role of microsaccades in visual perception. The similarity between microsaccade and saccade generation at the level of the SC, and at downstream brainstem levels (Hafed 2011; Hafed et al. 2009; Van Gisbergen et al. 1981), suggests that microsaccadic influences on vision should be interpreted in a similar manner to larger saccades. For example, there is a rich literature on how saccades modulate neuronal activity in sensory (Bremmer et al. 2009; Ibbotson et al. 2008; Kagan et al. 2008; Rajkai et al. 2008; Reppas et al. 2002) and sensory-motor (Bremmer et al. 2009; Duhamel et al. 1992; Ibbotson and Krekelberg 2011; Phongphanphanee et al. 2011; Sommer and Wurtz 2006) areas and an equally rich literature on the effects of saccades on behavioral and perceptual performance (e.g., Cai et al. 1997; Diamond et al. 2000; Ross et al. 1997; Zuber and Stark 1966). The major themes of this literature are that saccades ultimately help vision by allocating the high-resolution fovea to interesting scene locations, which facilitates information acquisition, but that the preparation for and execution of these eye movements are associated with various forms of momentary suppression, or distortion, that can manifest themselves at both the neuronal and behavioral levels under the right circumstances. Similar influences of microsaccades seem to exist, an example being the phenomenon of saccadic suppression (Beeler 1967; Hafed and Krauzlis 2010; Zuber and Stark 1966), and it would be interesting to test for other perimicrosaccadic changes in perception, such as spatial mislocalization.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: Z.M.H. and R.J.K. conception and design of research; Z.M.H. and R.J.K. performed experiments; Z.M.H. and R.J.K. analyzed data; Z.M.H. and R.J.K. interpreted results of experiments; Z.M.H. and R.J.K. prepared figures; Z.M.H. and R.J.K. drafted manuscript; Z.M.H. and R.J.K. edited and revised manuscript; Z.M.H. and R.J.K. approved final version of manuscript.

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