Exp Brain Res (2000) 130:227–237
Digital Object Identifier (DOI) 10.1007/s002219900239

RESEARCH ARTICLE

Edward L. Keller · Neeraj J. Gandhi
S. Vijay Sekaran

Activity in deep intermediate layer collicular neurons during interrupted saccades

Received: 24 November 1998 / Accepted: 18 August 1999 / Published online: 10 November 1999

Abstract The activity of neurons located in the deep intermediate and adjacent deep layers (hereafter called just deep intermediate layer neurons) of the superior colliculus (SC) in monkeys was recorded during saccades interrupted by electrical stimulation of the brainstem omnipause neuron (OPN) region. The goal of the experiment was to determine if these neurons maintained their discharge during the saccadic interruption, and, thus, could potentially provide a memory trace for the intended movement which ends accurately on target in spite of the perturbation. The collicular neurons recorded in the present study were located in the rostral three-fifths of the colliculus. Most of these cells tended to show considerable presaccadic activity during a delayed saccade paradigm, and, therefore, probably overlap with the population of SC cells called buildup neurons or prelude bursters in previous studies. The effect of electrical stimulation in the OPN region (which interrupted ongoing saccades) on the discharge of these neurons was measured by computing the percentage reduction in a cell's activity compared to that present during non-interrupted saccades. During saccade interruption about 70% of deep intermediate layer neurons experienced a major reduction (30% or greater) in their activity, but discharge recovered quickly after the termination of the stimulation as the eyes resumed their movement to finish the saccade on the target. Therefore, the pattern of activity recorded in most of the deep intermediate layer neurons during interrupted saccades qualitatively resembled that previously reported for the saccade-related burst neurons which tend to be located more dorsally in the intermediate layer. In contrast, some of our cells (30%) showed little or no perturbation in their activity caused by the saccade interrupting stimulation. Because all the more dorsally located burst neurons and the majority of our deep intermediate layer neurons show a total or major suppression in their discharge during interrupted saccades, it seems unlikely that the colliculus by itself could maintain an accurate memory of the desired saccadic goal or the remaining dynamic motor error required to account for the accuracy of the resumed movement which occurs following the interruption. However, it remains possible that the smaller proportion of our neurons whose activity was not perturbed during interrupted movements could play a role in the mechanisms underlying saccade accuracy in the interrupted saccade paradigm. Interrupted saccades have longer durations than normal saccades to the same target. Therefore, we investigated whether the discharge of our deeper collicular cells was also necessarily prolonged during interrupted saccades, and, if so, how the prolongation compared to the prolongation of the saccade. Sixty percent of our sample neurons showed a prolongation in discharge that was approximately the same as the prolongation in saccade duration (difference <15 ms in magnitude). The observation that temporal discharge in our neurons was perturbed to roughly match saccadic temporal perturbation suggests that dynamic feedback about ongoing saccadic motion is provided to the colliculus, but does not necessarily imply that this structure is the site responsible for the computation of dynamic motor error.

Key words Superior colliculus · Interrupted saccades · Intermediate layer · Feedback · Omnipause neurons

E.L. Keller (✉) · N.J. Gandhi · S. Vijay Sekaran
Smith-Kettlewell Eye Research Institute, 2318 Fillmore Street, San Francisco, CA 94115, USA
e-mail: elk@ski.org
Tel: +1 415 345 2102, Fax: +1 415 345 8455

E.L. Keller · N.J. Gandhi
Graduate Group in Bioengineering, University of California, San Francisco, CA 94143, USA

E.L. Keller · N.J. Gandhi
Graduate Group in Bioengineering, University of California, Berkeley, CA 94720, USA

Present address:
N.J. Gandhi, Division of Neuroscience, Baylor College of Medicine, One Baylor Plaza, Room S515, Houston, TX 77033, USA

Introduction

The mammalian superior colliculus (SC) is divided into *two main* functional layers, with each layer consisting of two or more sublayers (Wurtz and Albano 1980). The superficial (more dorsal) layer contains cells that respond to visual stimuli. The deep (more ventral) layer contains cells that may show visual or other sensory responses and, in addition, motor responses related to gaze shifts. Past descriptions of the discharge of individual neurons in this sensorimotor layer, which is often further divided into the intermediate layers and the deep layers, have emphasized their spatial coding properties for saccadic eye movements (Sparks and Hartwich-Young 1989). For instance, neurons in the rostral SC code for small saccades while neurons in the caudal regions discharge prior to large movements. However, more recent accounts have demonstrated that the discharge of these cells is also correlated with various dynamic eye motion variables (Berthoz et al. 1986; Munoz et al. 1991; Waitzman et al. 1991; Munoz and Wurtz 1995b; Anderson et al. 1998b). The invariant relationship between saccade size and duration and velocity makes it difficult to differentiate if the discharge correlation is the result of efferent feedback to the colliculus or merely reflects the feedforward signals relaying the main sequence properties of saccades.

The interrupted saccade paradigm is able to perturb the invariant relations present in normal saccades (Keller 1977; King and Fuchs 1977). In this task electrical stimulation in the region of the saccadic omnipause neurons (OPNs) – a group of midline cells in the brainstem that strongly inhibits the saccade-generating machinery – during an ongoing saccade stops the movement in midflight. When the stimulation is turned off, the movement resumes, and finally the eyes stop near the location of the flashed visual target with almost normal accuracy (Keller et al. 1996). As a result of the perturbation, saccade duration is prolonged compared to the non-stimulated saccades of the same size and the dynamics of the movement are grossly altered. Keller and Edelman (1994) used this paradigm to study the behavior of what they classified as saccade-related burst neurons (SRBNs), a group of SC neurons located dorsally within the intermediate layers. (Details on cell classification are discussed below.) They reported that the discharge of all active SRBNs was rapidly and completely inhibited by stimulation in the OPN region during the interruption period when the saccade was stopped. Following the termination of the stimulation the same previously active SRBNs then showed a second burst of discharge during the resumed movement, though this movement was smaller than the minimum-sized normal saccade that would have been necessary to produce significant discharge in these particular cells. Munoz et al. (1996) reported a similar pattern of activity in SC cells they called BNs during saccades perturbed by electrical stimulation of the rostral region of the SC at saccade onset. These neurons were probably located, on average, dorsally in the intermediate layers based on the Munoz and Wurtz (1995a) report.

These results raised the important question of how collicular discharge was re-kindled, and at the same previously active spatial location on the SC, to complete the resumed movement. It seems impossible that the resumption could have been produced by the collicular SRBNs themselves, because they were completely turned off during the stimulation and only resumed activity at some time after the termination of the stimulus. It is possible that other upstream brain structures involved in saccadic control maintain a memory of the desired saccadic goal, in contrast to the SRBNs in the SC, where it seems to be erased during the stimulation period, and reimpose this spatial goal on the SC motor map just before the resumption of the interrupted saccade. Another possibility is that other types of saccade-related burst cells located on average at deeper sites in the SC are not suppressed by the interrupting stimulation. These cells could then be responsible for regenerating a focus of activity in SRBNs at the originally active SC site. In any case, it should be emphasized that the resumed movement ends close to the location of the initial target despite the fact that the reactivated collicular site would not normally code the resumed movement.

The other type of collicular saccade-related neuron, the buildup neurons (BUNs), according to the nomenclature of Munoz and Wurtz (1995a), was not included in the population of SC cells studied in the previous work in which saccades were interrupted by stimulation in the OPN region (Keller and Edelman 1994), and only a few BUNs were included in the study of Munoz et al. (1996). Therefore, the goal of the current study was to record from cells located more ventrally in the deep intermediate layers during saccades interrupted by stimulation in the omnipause region. The intent of the primary analysis was to determine if the discharge of BUNs, like SRBNs, was totally suppressed by the stimulation. If so, we could conclude that the SC, by itself, is unlikely to be able to code the metrics or dynamics of interrupted, but still spatially accurate, saccades. On the other hand, if the discharge of BUNs remained constant during the interruption period, then it might be possible that they were responsible for the resumption of SRBN discharge and the resumed movement.

A second question about the behavior of collicular neurons during interrupted saccades is whether their discharge is prolonged to reflect the prolonged duration of the total sequence of movements present in the interrupted saccade. Both Keller and Edelman (1994) and Munoz et al. (1996) found that the discharge of SRBNs for perturbed movements was prolonged in comparison to control saccades of the same size and argued that this was evidence for feedback to the SC about the eye motions actually present during saccades. We also wanted to conduct similar analyses on the present group of deeper SC neurons to see if they showed similar prolongations of discharge.

Classification of neurons in the intermediate and deep layers of SC

Various anatomical and physiological criteria have yielded different schemes of nomenclature for the categorization of intermediate and deeper layer collicular neurons (for a review of earlier work, see Guitton 1991). In a particularly influential paper, Munoz and Wurtz (1995a) divided saccade-related cells in these layers into two classes, burst neurons (BNs) and buildup neurons (BUNs). They based their separation on a quantitative measure of the presaccadic pattern of activity in SC cells and suggested that BUNs showed a prolonged buildup of activity before optimal eye movements in a delayed-saccade paradigm, where optimal is defined as that saccade vector for which the cell shows the most intense discharge. Burst neurons in contrast only discharged in close temporal association with the saccade. They also reported that most BUNs continued to discharge (usually at lower intensities) for all saccades larger than the optimal amplitude movement, As a result of this trait, BUNs were also called "open movement field" cells. Finally Munoz and Wurtz (1995a) reported that BUNs tended to be located more ventrally within the intermediate and deeper layers of the SC than BNs.

The first two criteria to separate saccade-related cells in the deep layers of the colliculus into the BN and BUN classes have subsequently been criticized. The specific level of prelude activity was found to be a poor parameter to make the distinction (Anderson et al. 1998b). Freedman and Sparks (1997) have argued that SC neurons which appear to have open movement fields during saccades in the head-restrained preparation in fact exhibit "closed movement fields" because they stop firing for large enough gaze shifts in the head-free condition. Additionally, Dorris et al. (1997) have recently reported that discharge during the interval between fixation point offset and target onset in a gap paradigm may serve as a more definitive discriminate between cell classes in the SC. Thus, the relative depths of cells within the deeper layers of the SC may be the best of the three criteria to distinguish between these overlapping populations of collicular cells (Wurtz 1996; Anderson et al. 1998b).

Given the current controversy over the names and proper criteria that are used to distinguish between classes of saccade-related collicular cells, we have chosen in the present investigation to use the depth of the cell from the dorsal surface of the SC as a way to classify the neurons. We believe that recorded depth of the cells is a more objective measure less subject to variation in paradigm, but do not mean to imply that the choice of a particular depth divides the cells into two distinct classes. Quite the contrary is true. Just as is the case for amount of prelude discharge or size of the movement field, the properties of intermediate and deep layer SC cells seem to also change along a continuum as a function of depth (Mohler and Wurtz 1976; Munoz and Wurtz 1995a; Anderson et al. 1998b). We have chosen depth as the means to select the cells studied in the present investigation primarily to emphasize that as a group they are partially distinct from a more dorsally recorded group whose properties we reported in an earlier study (Keller and Edelman 1994).

A brief report of some of these findings has appeared previously in abstract form (Keller 1999).

Materials and methods

Three juvenile male *Macaca mulatta* monkeys were used for this study. The experiments involved measuring eye movements while recording from collicular neurons during control and interrupted saccades. All experimental protocols were approved by the Institutional Animal Care and Use Committee at the California Pacific Medical Center and complied with the guidelines of the "Principles of Laboratory Animal Care" (NIH publication No. 86–23, revised 1985).

Surgical preparation

Surgery was performed with the animals under isoflurane anesthesia and aseptic conditions. Heart rate, respiratory rate, body temperature, blood pressure and oxygenation were monitored for the duration of the surgery. Four devices were implanted in each monkey: (1) a stainless steel chamber was placed stereotactically on the skull, slanted posteriorly at an angle of 38° in the sagittal plane and aligned approximately perpendicular to the surface of the SC. (2) Another stainless steel chamber was mounted stereotactically on the skull, slanted laterally in the frontal plane at an angle of 25° and aligned on the OPN region. (3) A head restraint device consisting of two light, stainless steel tubes was positioned transversely. The chamber and the head bars were fixed to the skull with dental acrylic and small titanium bone screws. (4) A coil of Teflon-coated stainless steel wire was placed under the conjunctiva of one eye using the method developed by Fuchs and Robinson (1966) and modified by Judge et al. (1980). After surgery, the monkeys were returned to their cage and were allowed to fully recover from surgery. When the proper level of recovery was reached as determined by consultation with the attending veterinarian, daily recording sessions were begun. Antibiotics (Ancef) and analgesics (Buprenex) were administered under the direction of the veterinarian during the postoperative period.

Behavioral conditioning

Each monkey was trained to climb out of its cage into a primate chair and sit in it during the experiment. Training and subsequent experimental sessions were conducted 4–5 times a week. The monkey was given water or juice rewards for correctly executing behavioral paradigms and was allowed to work until satiation. Daily records were kept of the animal's weight and health status. Supplemental water was given as necessary, and unlimited access to it was provided on days when training or experimental sessions were not performed.

Behavioral paradigms, visual displays, and data storage were under the control of a real-time program running on a laboratory PC system. The targets were presented via a computer-controlled, analog oscilloscope which back-projected light spots on a 90°×90° translucent screen placed 40 cm in front of the monkey (Crandall and Keller 1985). The targets were 15 minarc in diameter and 2 cd/m^2 in intensity against a diffusely illuminated dim homogeneous background illumination (0.05 cd/m^2).

The eye movement signals were obtained by placing the head-restrained animal with an implanted scleral coil in a pair of orthogonally aligned 20-kHz magnetic fields maintained electronically in temporal quadrature. The voltage induced in the coil was passed through a phase detector which separated the eye position

signal into horizontal and vertical components with a sensitivity of 0.25°, zero drift and a bandwidth of 1 kHz (Robinson 1963). Horizontal and vertical eye velocity were obtained by analog differentiation (with a cutoff frequency of 170 Hz) of the position signals yielding an rms velocity noise of about 1°/s.

Experimental paradigms

Before each experimental session, both chambers were opened and cleaned under aseptic conditions. Double eccentric positioning devices were loaded in the chambers which allowed microelectrode penetrations at any location within the 12-mm diameters of the chambers. Sharpened guide tubes with tungsten microelectrodes inside were pushed through the dura in each chamber, and independent hydraulic drive systems were used to advance the microelectrodes to the desired locations in the brain.

The brainstem electrode was advanced into the region of the omnipause neurons (OPNs), which were identified by their unique discharge patterns in relationship to saccadic eye movements (Keller 1974). Once the OPN region had been located based on the recorded characteristics of the neurons, the electrode was fixed in place and the threshold current for interrupting saccades in midflight by electrical stimulation in the region was determined. Short trains of high-frequency, biphasic current pulses (0.25-ms pulse width for each phase in the pair) were delivered by a constant current stimulator. Stimulation onset was triggered by a computer program from the computed radial eye velocity. The trigger point was found by subsequent offline analysis to occur at 5 ms (±2 ms) from movement onset. Stimulus frequency was set at 400 pps and train duration was fixed at a duration that reliably interrupted ongoing saccades (nominally 18–20 ms). Current strength was increased until interrupted saccadic eye movements (Keller and Edelman 1994) were obtained on ~90% of the experimental trials. The range of current strengths used was 10–25 μA. At some sites train duration had to be shortened to prevent saccade truncation. In truncated trials the ongoing saccade was stopped in place and then did not resume when the stimulation was ended.

Once the microelectrode in the OPN region was fixed, the electrode in the SC chamber was advanced into the superficial layers of the SC (identified by neural "swishes" on the audio amplifier as the monkey scanned the visual field). It was then lowered further into the deeper layers as individual collicular neurons were isolated. Each isolated single neuron was tested qualitatively using online analyses. Pure visual neurons in the superficial layers and visuomotor neurons in the dorsal portion of the intermediate layers with spatially restricted movement fields and well-defined temporal bursts were discarded without further study. At depths around 1.5 mm from the dorsal surface single collicular neurons with very large movement fields began to be recorded. Neurons with these physiological characteristics were subjected to more detailed analysis and their behavior was recorded for offline analysis.

The behavior of each deeper (depth 1.5 mm or greater) SC neuron was studied using a delayed saccade paradigm. The monkey first visually acquired a fixation point that remained on for a quasi-random interval of time (500–900 ms). Next, a target appeared in the periphery for 300 ms. The fixation point remained illuminated for the duration of the target presentation, plus an additional randomly selected 0–300 ms. The offset of the fixation point was the cue that a saccade to the target was now to be made. The visual target was turned off before saccade onset to guarantee that all saccades were made to the remembered location of the target. The approximate center of the cell's movement field (the spatial region associated with the saccadic discharge) was located by preliminary online analysis. A grid of either 9 or 16 target locations centered on the movement field was then set up. The monkey was required to make a set of randomly chosen saccades to this grid of target locations (usually five to each location). The grid generally extended ±60° in direction from the center of the cell's movement field and from 2° to 40° in amplitude. For neurons with movement field centers near the fixation point, the lower limit of the grid was decreased to 1°. If the cellular recording remained stable, another block of trials was presented in which the target was presented at random locations in the contralateral visual field. Once the data necessary to plot the cell's movement field offline had been obtained with the two sets of target locations, the cell was next studied (using the same delayed-memory task) during a block of trials in which the target appeared at a 30° eccentric position in the direction of the center of the cell's movement field. For a few cells, data were collected for target eccentricities of 25° instead. Within this block of trials stimulation of the OPN region, which produced interrupted movements, was delivered on randomly selected trials with an average frequency of about 50%.

Data collection

Horizontal and vertical eye position, velocity and target position were sampled at 1 kHz and were stored on computer disk. The occurrence of unit discharge was determined with the use of a standard electronic window detector. The output of the window detector was stored to disk in temporal register with the analog data. During blocks of trials containing stimulation, raw neural activity was also sampled at 20 kHz and stored to disk in temporal register with the eye movement data. This was done to permit offline confirmation and correction of the unit spike occurrence from the windowed data which may be contaminated by shock artifacts in the raw unit potential recording during stimulated trials (Keller and Edelman 1994). A human operator scrolled through the trials with stimulations to correct falsely windowed spikes in the spike train data.

Data analysis

All offline analyses were performed in Matlab (The Mathworks Inc.). Radial eye position and velocity were computed by the Pythagorean theorem. The eye velocity signal was further digitally filtered with a five-pole Butterworth filter in the forward and reverse direction to produce zero phase distortion. This signal was then differentiated, and a threshold criterion on the resulting acceleration signal was used to detect saccade onset. As this method could erroneously mark the end of saccades, particularly for large eye movements, which were often slow when made to remembered target locations (White et al. 1994), a velocity threshold criterion (typically 90°/s) was used to mark the end point of saccades. In addition, all saccade onset and offset marks were checked by one of the investigators and manually changed if necessary.

Raw neural discharge was converted to spike density signals by convolving the spike trains with a Gaussian waveform (σ=4 or 10 ms; Richmond et al. 1990) to produce smooth continuous estimates of unit activity.

Histology

At the completion of the recording sessions in each animal an electrolytic lesion (20 μA for 30 s) was made at a depth below 1.5 mm from the dorsal surface of the SC at a site where deep SC neurons similar to those included in the present study were recorded. After a 4-day period the animals were deeply anesthetized and perfused through the left ventricle with isotonic saline followed by 10% formalin. Frozen 60-μm sections were cut in the plane of the electrode penetrations. Sections were stained with cresyl violet and the site of the lesion marker was recovered by microscopic examination. The distance from the dorsal surface of the colliculus to the center of the lesion was measured and compared with the depth recorded on the day the lesion was made.

Results

Cell classification

We recorded from 30 collicular neurons located in the intermediate and deep layers in the three monkeys. Their depth from the dorsal surface of the SC (determined physiologically on each penetration from the depth recorded at the first appearance of visual activity on the audio monitor) ranged from 1.5 to 2.8 mm. The lesion markers recovered from the histological material verified that depth in the SC could be estimated within ±0.2 mm using the depth of the sharp transition to background visual activity as a gauge for the dorsal surface of the SC. As a group, these cells were located more ventrally in the SC than the previously studied group of saccade-related burst cells (SRBNs), which were located from 1.0 to 1.6 mm from the dorsal surface (Keller and Edelman 1994). Nevertheless, there was overlap in the depth of the supposed two cell types. The group of cells included in the present study also tended to show more presaccadic discharge during the 150-ms period prior to saccade onset and more postsaccadic discharge after the end of the movement than the SRBNs previously studied (Keller and Edelman 1994). The deeper group of cells studied here had an average prelude discharge of 45 spikes/s (averaged over a 50-ms window centered at 100 ms before saccade onset of near optimal saccades in a delayed saccade paradigm). However, two cells had no discharge by this measure of prelude activity. The distribution of presaccadic discharge defined in this fashion for the 30 cells was rather uniform from zero to 100 spikes/s. Each of the cells included in the present sample continued to show significant discharge for the largest recorded saccades (usually for target displacements of 40°) in the cell's preferred direction. Thus, the discharge pattern of our group of cells resembles the class of deeper SC cells called buildup neurons (Munoz and Wurtz 1995a). They most likely also correspond to the prelude burst neurons studied by Glimcher and Sparks (1992), although no depths within the SC were reported by these authors for the latter class of neurons.

The 30 cells were recorded at various locations throughout the more rostral three-fifths of the SC, as illustrated in Fig. 1. Spatial movement fields were estimated for each of these cells at 2-ms time intervals from 20 ms before saccade onset to saccade end using a recently developed radial basis function method of smoothing spatiotemporal unit discharge (Anderson et al. 1998a). The movement field center was then defined as the location of the peak discharge on the smoothed fit of the cell's activity at 8 ms before saccade onset.

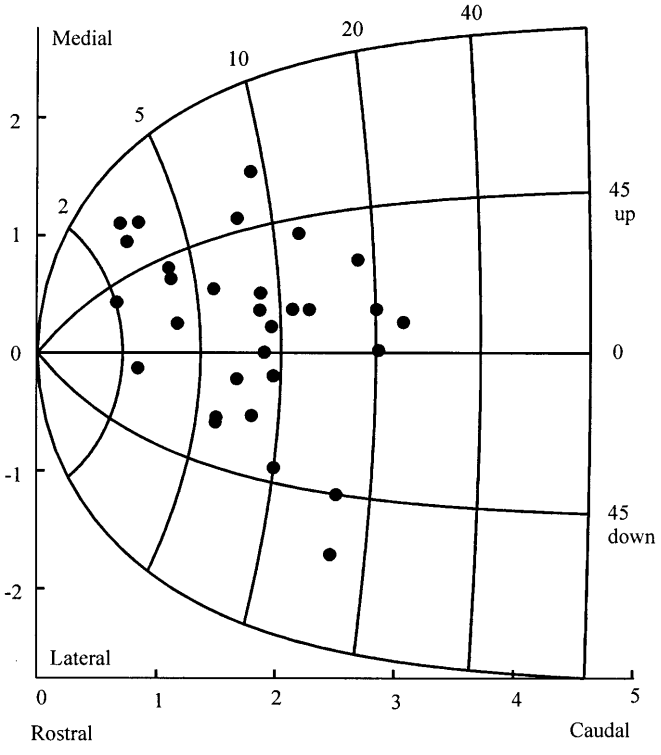

Fig. 1 Estimated location on the superior colliculus motor map of the sample (*n*=30) of intermediate and deep layer, saccade-related neurons characterized in the present study. Rostral/caudal and medial/lateral extent of one idealized colliculus is shown in millimeters. Cells were located on the basis of the saccade vector for which they displayed the maximum discharge. Polar coordinates of the saccade vectors were then transformed to collicular locations using the geometric formulae of Ottes et al. (1986)

Activity of deep intermediate layer neurons during interrupted saccades

Figure 2 shows the saccadic discharge patterns for two typical cells from our sample. Each row of panels illustrates the behavior of one cell. The left column is obtained for movements near the cells' optimal vectors and the right for much larger movements in the optimal direction. Each subsection in the figure shows the averaged spike density of the cell and the accompanying averaged radial eye positions. The cell shown in the upper row discharged at its highest peak rate for saccades ~7° in amplitude, but showed very little early presaccadic activity for this optimal amplitude. It had a broad movement field over which it reached almost the same level of peak discharge, including movements as large as 38°, as illustrated in the upper right panel. The cell also showed a shift in the peak of its temporal activity as a function of saccade size. For the optimal saccades shown in the left panel the peak led saccade onset by 4 ms, while for the larger movements shown on the right, the peak lagged saccade onset by 18 ms.

The cell illustrated in the lower row discharged best for saccades ~11° in amplitude as shown in the plot on the left. For these optimal movements the cell showed a considerable amount of presaccadic buildup activity and reached its peak response well after saccade onset. Its declining activity then outlasted the end of the saccade by ~100 ms. For the larger movements shown on the right the cell continued to discharge but at a lower rate.

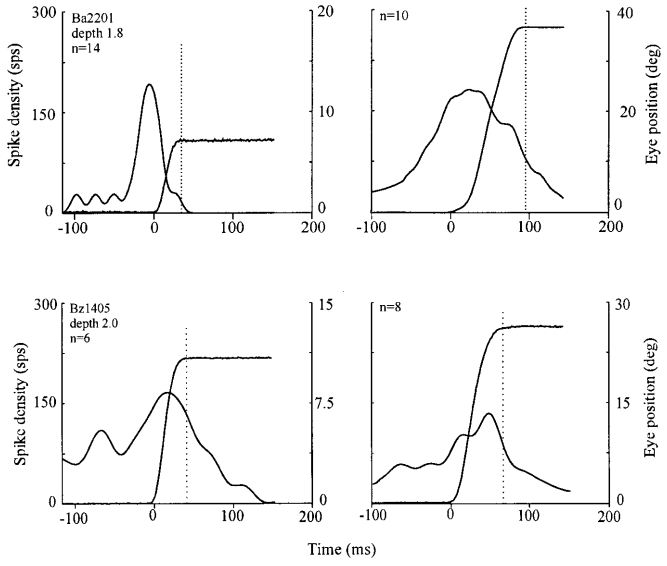

Fig. 2 Temporal discharge patterns for two neurons included in the present study. *Each panel* shows the averaged eye position record and the averaged spike density record for the discharge that accompanied the eye movements included in its average. Spike density records were aligned on saccade end (*vertical dotted lines in all plots*) so that the relationship of unit activity to saccade end can be seen clearly. Shifts in the location of the peak discharge toward the time of saccade end for larger saccades is a feature associated with many deep intermediate layer collicular neurons (Munoz and Wurtz 1995b). *The upper left panel* shows data for one cell for saccades to a location near its optimal saccade vector of 7° in amplitude and 46° in an up, oblique direction. *The upper right panel* shows data for much larger saccades in the optimal direction. The value of *n* shown in each section is the number of movements averaged to obtain the data shown in that plot. This cell's discharge was about the same for all saccades larger than 7° in the optimal direction. The peak of this discharge slightly led saccade onset for the smaller amplitude movements, but was shifted to produce a small lag following saccade onset for the larger movements recorded as shown *on the right*. *The lower set of panels* shows the behavior of another cell. *The panel on the left* shows average activity for saccades near the cell's optimal vector, which was ~11° and nearly horizontal. This cell remained active for the larger saccades shown *on the right* and its peak activity maintained a rather constant relationship to saccade end for both small and large movements. The smoothing time for all spike density traces shown in this figure was σ=10 ms

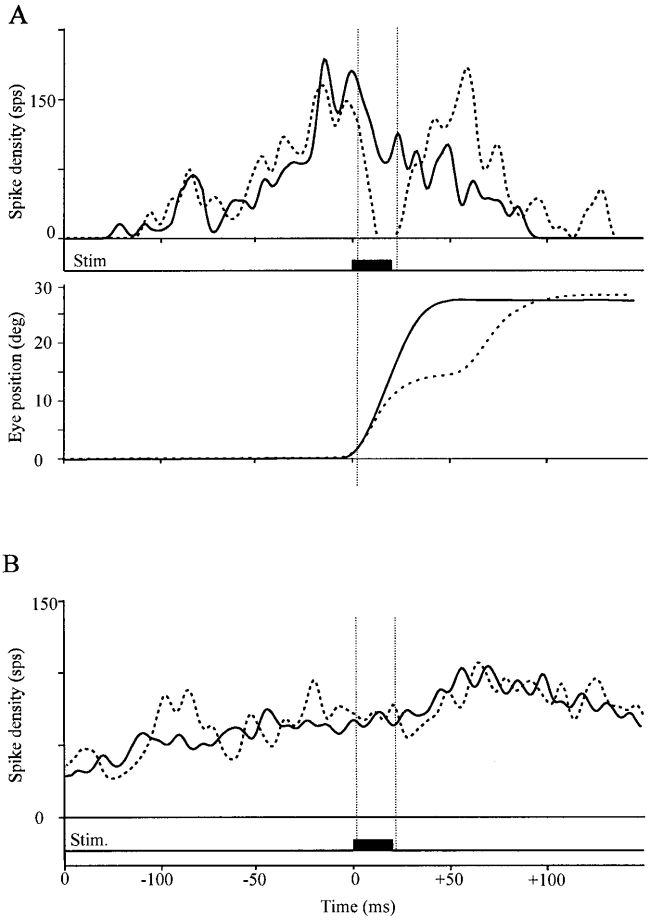

Fig. 3 **A** Comparison of the cellular discharge and eye movements for the same cell as that shown in Fig. 2 (*upper panels*) during a block of mixed trials (non-stimulated saccades, *n*=9, interrupted saccades, *n*=8). *Upper pair of traces* show averaged spike density data for unstimulated trials (*solid curve*) and stimulated trials (*dotted curve*). Stimulated trials were aligned on electrical stimulus onset (stimulus envelope shown by *filled rectangle in Stim*). Spike densities for the control trials were aligned on an equivalent time with respect to saccade onset. *The vertical dotted lines* indicate the time period that was used to quantify the amount of discharge reduction produced by the stimulus as described in the text. *The lower pair of traces* show the averaged eye movements that accompanied the spike densities shown above. *The solid curve* shows control saccades and *the dotted curve* interrupted trials. **B** Comparison of the discharge for another cell during a block of mixed trials (non-stimulated saccades, *n*=9, interrupted saccades, *n*=10). Conventions the same as in **A**. The smoothing time for all spike density traces shown in this figure was σ=4 ms

Time of peak activity with respect to saccade end remained about the same as for saccades of optimal size. There was never a clearly defined burst of saccade-related activity for any size of movement; rather the cell showed a phase of increasing activity followed by a decreasing phase that outlasted saccade end.

Figure 3 illustrates the method we used to quantify the possible reduction in discharge produced in deep intermediate SC cells as a result of the electrical stimulation in the OPNs. The data come from blocks of trials containing randomly interleaved stimulated and unstimulated saccades. The figure compares the averaged spike densities for the stimulated trials (dotted curves) with those without stimulation (solid curves). In this figure and for all analyses of discharge reduction a filter sigma of 4 ms was used. For stimulated trials the individual spike densities are aligned on stimulation onset (time zero in the figure) and then averaged. For unstimulated trials the individual spike densities are shifted to the left by 5 ms from a zero time at saccade onset and then averaged. The shift of 5 ms corresponded to the average time after saccade onset that the stimulator was triggered by the real time program when the shift was measured in subsequent offline analyses. This alignment procedure results in a close temporal match of the averaged data for stimulated and unstimulated trials as can be seen in Fig. 3A (lower section). These curves show the averaged eye

movement records for both types of trials. The individual eye movements that went into the averages were the movements that accompanied the spike density records shown above.

The two cells picked for inclusion in Fig. 3 exhibited OPN stimulation-induced reductions in discharge that lie near the opposite ends of a broad spectrum that we observed with respect to this behavior. The upper plot in Fig. 3A shows the averaged spike density records for a cell that was completely silenced for a short period of time following the onset of the stimulation. This can be seen even on the smoothed spike density trace for the stimulated trials, which drops to zero for a short period of time, but this time is smaller than the duration of the stimulation. Because the filter used in computing the spike density traces smooth rapid changes (either up or down) in activity, we needed to devise a measurement algorithm that would capture the essence of different levels of the evoked reduction.

The percentage reduction in discharge caused by the stimulation was computed in each cell by first shifting the averaged spike density trace for stimulated trials up or down so that it was at the same level as that for the control trials at the onset of stimulation. This shift is not shown in Fig. 3A for clarity. Then the percentage reduction in discharge was computed as the average spike density for non-stimulated trials during the time of the stimulus plus 4 ms, minus the average spike density for stimulated trials over the same interval of time divided by the first term (average non-stimulated spike density). The delay of 4 ms with respect to stimulus onset and end was chosen to compensate for the lag produced by the Gaussian filter. The reduction in discharge computed by this method for the cell illustrated in Fig. 3A was 65%. For other cells that experienced a complete inhibition of discharge that was longer in duration than the stimulus duration, it was possible to get reduction values of 100% with this method.

Figure 3B displays the discharge records for a cell that was virtually unaffected by OPN stimulation (the percentage reduction computed for this cell was only 0.8%). The target was located at a radial amplitude of 25° during the collection of the data shown in Fig. 3B. This cell showed a more vigorous peak of activity (165 spikes/s) for optimal saccade amplitudes of ~8°, and this peak slightly led saccade onset in contrast to the situation for the larger 25° movements analyzed in Fig. 3B, where the peak slightly lagged saccade end.

Inspection of the discharge data in Fig. 3A suggests that the saccade-related, higher frequency activity of some deep intermediate layer cells in the SC is prolonged during interrupted saccades in comparison to its duration during unstimulated movements. Since the time required to complete the saccade is also prolonged in interrupted trials, we wanted to compare the durations of the prolongation in eye movement behavior and unit discharge. The method we used to do so is shown in Fig. 4 using the activity of another neuron from our sample as an example. The upper set of traces again show the averaged spike densities with the dotted curve for interrupted trials and the solid curve for unstimulated trials. The data for both are aligned on saccade onset. The averaged saccadic responses for the two types of trials are shown by the lower two traces. On unstimulated trials the cell's discharge increased during the saccadic movement and reached a peak just before saccade end. The discharge then declined rapidly but did not approach background zero levels until well after the end of the movement. On interrupted trials it showed a reduction in discharge ~8 ms after the onset of the stimulation – the effect we have quantified in the previous section. The cell then produced a second peak of discharge that led the onset of the resumed movement. The second burst was prolonged and did not fall to levels that compared to those on control trials until much later after the end of the resumed movement. We quantified the apparent prolongation of activity on interrupted trials for each cell by measuring the difference in time it took for the activity to fall to a discharge level of 50 spikes/s in comparison to the time it took to reach this level on control trials (the time difference indicated by the label SD). We also measured the prolongation in the set of interrupted saccades that accompanied the spike density records directly from the eye movement records (the time difference EM). We then computed the difference in SD and EM for each cell. For the cell shown in Fig. 4 the spike density prolongation was 69 ms and the saccadic eye movement prolongation was 61 ms. The difference, SD–EM, was +8 ms. The filter sigma used in all the analyses of discharge prolongation was 10 ms in order to smooth the

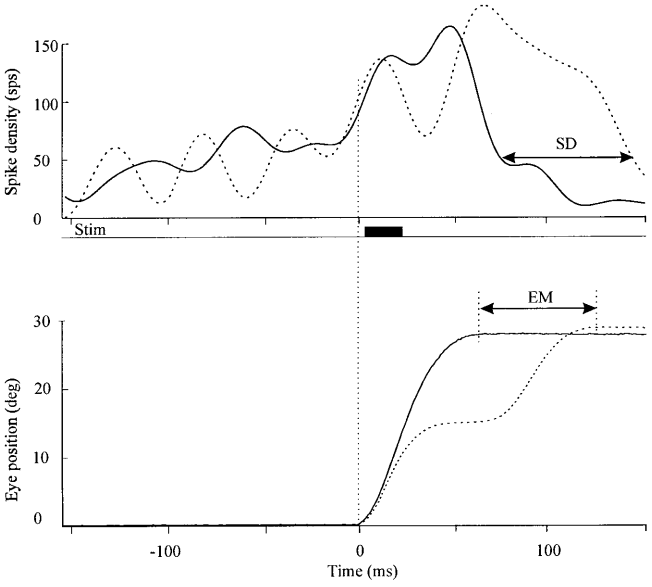

Fig. 4 Illustration of the method used to measure and compare the prolongation of spike density (*SD*) and the eye movement (*EM*) for unstimulated (*solid curves*) and interrupted (*dotted curves*) trials. Data obtained from a cell during a block of mixed trials (non-stimulated saccades, n=12, interrupted saccades, n=10). All data were aligned on saccade onset. The smoothing time for all spike density traces shown in this figure was σ=10 ms

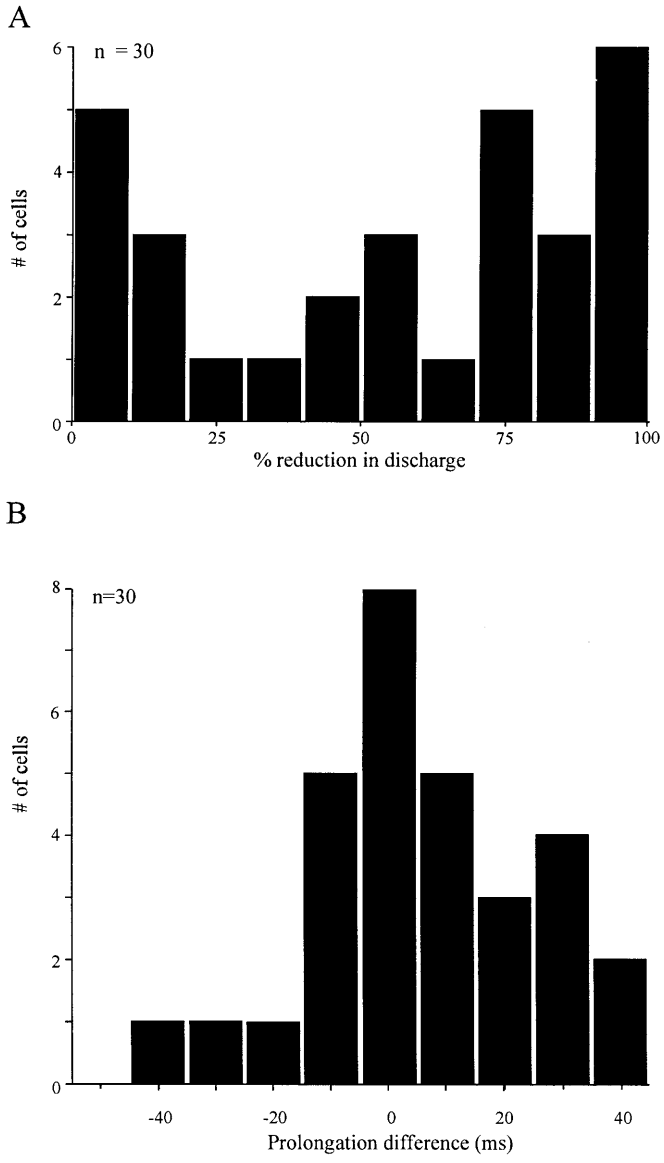

Fig. 5A,B Summary of percentage reduction in discharge and spike density prolongation measurements presented as frequency histograms. **A** Distribution of measured values of percentage reduction in discharge produced by OPN stimulation measured for all cells included in the study. **B** Temporal difference in milliseconds between the prolongation of the cell's activity as measured on the spike density records and the prolongation in saccade duration produced during interrupted saccades

uneven declining tail of unit discharge that occurs with a sigma of 4 ms (see the spike density traces in Fig. 3A).

The reason we chose a level of 50 spikes/s for level of activity at which to measure the end of discharge was that the discharge of our cells frequently showed a rapid decline near the end of the saccadic movement on control trials, but then the decline reached a shoulder at about this level before dropping further to zero or background levels of activity. We also used an alternative measure of discharge prolongation. The peak discharge was measured on the averaged spike density traces and the time required for the discharge to decline to a value of 25% of the cell's peak value was measured. This measurement was made for both interrupted and unstimulated trials, and the difference was considered the amount of discharge prolongation. We found no noticeable difference in the average value obtained with the two ways of measuring discharge prolongation.

Figure 5 gives a summary of the results obtained with the two measurements that we made on the spike density and eye movement data for the cells in our sample. The frequency histogram in Fig. 5A shows the distribution of the percentage reduction in discharge caused by the interrupting stimulus for the 30 cells. Stimulation produced a major reduction in discharge of 30% or greater in a majority of the cells (21 of 30 cells). The activity in several was completely suppressed by the stimulation. However, there were some cells (8 of 30) that exhibited less than 30% reduction of their discharge during the stimulation even though eye movements were always interrupted.

We attempted to find a relationship between the percentage reduction in a cell's discharge and its rostral/caudal location on the SC, but found no significant correlation (r=0.22) when all 30 measured cells were included. Likewise there was no correlation between percentage reduction in discharge and the additional depth below the 1.5-mm limit for our sample of cells.

The histogram in Fig. 5B shows the distribution of the measurements of the difference between the prolongation in a cell's discharge and the prolongation of saccade duration caused by the stimulation. If the perturbation in a cell's discharge compensated for the prolongation in saccade duration caused by interrupting the saccade, this difference would be close to zero. There were 8 cells out of the 30 tested that fell into this category as illustrated by those in the modal bin of the histogram. However, all the cells in the central three bins of the histogram (18 of 30) showed approximate compensation (the prolongation of discharge was within ±15 ms of the prolongation in saccade duration). The discharge in the cells in the right bins was prolonged for a longer period of time than the accompanying saccadic duration perturbations. The three cells in the left bins of the histogram show less prolongation in their discharge than the accompanying saccadic prolongation. Overall the mean value of the difference was 5.7 ms (±19.4 SD) for the group of 30 cells included in the present study. The mean value of saccadic prolongation was 47 ms (±14.8 SD) in comparison.

Discussion

Stimulation produced perturbation in neural discharge in the colliculus

The major finding in the present study was that the discharge of most deep intermediate layer saccade-related neurons the superior colliculus undergoes a major reduction during stimulation of the omnipause neuron (OPN)

region in the brainstem, a procedure that also interrupts saccades. This behavior during interrupted saccades resembles that previously reported for a group of collicular cells called SRBNs and which tended to be located more dorsally in the intermediate and optic layers (Keller and Edelman 1994). However, unlike the SRBNs, the inhibition in the deeper cells studied here was not total in most cells and some cells showed no effect of the stimulation during the period that it was being applied. It has been reported that SRBNs also undergo a complete suppression of their discharge during stimulation in the rostral region of the SC, a process that also interrupts saccades (Munoz et al. 1996). In the latter study the discharge pattern of nine cells called buildup neurons (BUNs) was also observed during saccades interrupted by rostral collicular stimulation. The locations of these BUNS in depth in the SC was not given, and no quantitative measures were made on their discharge during or after the electrical stimulation of the rostral SC. At least one, based on the data given in single-trial figures, appeared to be totally turned off during the stimulation and the discharge of another was less suppressed. We have extended these results in the present study by making quantitative measurements on the amount of suppression produced in a larger group of cells that most likely correspond to BUNs based on an early report from the same laboratory (Munoz and Wurtz 1995a) that showed a deeper location on average for BUNs in contrast to SRBNs. The cells included in the present study were also located over a wider spatial extent of the SC.

In comparison to SRBNs, the deeper cells included in the present study are on average less affected by interrupting stimulations. This observation provides one more criterion with which to separate collicular cells in addition to the level of their presaccadic discharge, the size of their movement fields and their location below the visual layers of the SC (Munoz and Wurtz 1995a). Unfortunately, the two populations overlap in behavior for all of these distinguishing dimensions (Munoz and Wurtz 1995a; Anderson et al. 1998b).

The most straightforward conclusion based on all three studies (Keller and Edelman 1994; Munoz et al. 1996; and the present study) is that the entire active population of saccade-related collicular neurons, both dorsal and ventral groups, undergoes a major perturbation in discharge during stimulations that interrupt saccades. Since these saccades resume and end on the target with almost equal accuracy to non-stimulated saccades (Keller et al. 1996; Munoz et al. 1996; Gandhi and Keller 1998), it seems unlikely that the SC by itself could store the appropriate spatiotemporal pattern of activity during the perturbation necessary to code for the accuracy of the resumed saccade. However, this conclusion must be made with the caveat that a few cells in our present sample showed little or no perturbation of their discharge during interrupted saccades. Before one could be certain that these few cells could not retain a memory of the desired saccade goal or the dynamic motor error, it would be necessary to compute the complete spatiotemporal population activity in cells of this type during and after the interruption period. We have recently carried out a study of the population discharge of these deeper intermediate layer cells during non-stimulated saccades (Anderson et al. 1998b), but the present sample is too small and spatially restricted in location on the collicular motor map to carry out similar estimations during interrupted saccades.

It would also be helpful to know the effect of similar stimulation-induced perturbations of saccades on the population discharge of neurons in the cortical frontal eye fields (FEF). Neurons with discharge patterns on delayed saccade tasks qualitatively similar to the cells included in the present study have been found in the FEF (Segraves and Park 1993; Hanes et al. 1998), but no studies have assessed the ability of these cells to maintain a memory of the movement goal or dynamic motor error during perturbed saccades.

As an additional caveat, it should be noted that we only studied the reduction in discharge in our cells for relatively large saccades (25° or 30° in amplitude). We did this because saccades directed at targets of less than about 20° in eccentricity are difficult to interrupt reliably (Keller et al. 1996). Thus, we are not certain if the reduction or lack of reduction we measured in a cell's discharge for large ongoing saccades would have remained the same for saccades of near optimal vector for the cell. For example, the discharge for the cell shown in Fig. 3A suffered a major reduction in activity when 30° saccades were interrupted, but this cell's optimal saccade was only about 7° in amplitude.

Dynamic adjustment of temporal discharge pattern for perturbed saccades

The other goal of the present study was to examine the temporal discharge records of deeper intermediate layer cells during the resumed movements following interruptions to determine if the prolongation produced in saccade duration during interrupted saccades was also reflected in a similar prolongation in cellular discharge in the deeper group of cells included in the present study. Neural discharge in the colliculus has been reported to be correlated in time (Waitzman et al. 1991) or space (Munoz and Wurtz 1995b) with saccade dynamics during unperturbed saccades, but it is difficult to establish causality from these correlations for saccades which normally have a rather fixed amplitude/duration relationship.

It has already been shown that SRBN discharge, although silenced during interrupting stimulations, returns to the control trajectory during the resumed movement on phase plots of discharge versus dynamic motor error (Keller and Edelman 1994; Munoz et al. 1996). This dynamic relationship was interpreted as evidence for feedback about ongoing eye motion during saccades to this class of SC neuron. Similar analyses have not been applied to the deeper group of intermediate layer cells in-

cluded in the present study, and we were unable to conduct the phase analysis on our sample of cells in the present study. The reason this type of analysis can be applied incisively for SRBNs is that the peak discharge of these cells is reached slightly before saccade onset (~7 ms on average) and then tends to decline to zero levels without inflection points on the descent. When the spike density records for this type cell are shifted backward in time by about 7 ms (to align the decline of discharge with the start of declining motor error) and plotted against dynamic motor error, single-valued, relatively straight phase curves are obtained on control trials (Waitzman et al. 1991). During interrupted saccades the spike density of SRBNs is suppressed to zero, but then rapidly reestablishes itself on the phase curve for control saccades as the movement resumes and motor error again begins to decline (Keller and Edelman 1994; Munoz et al. 1996). In contrast, most cells in the deeper intermediate layer reach their peak discharge only after saccade onset or near saccade end (e.g., the cell shown in Fig. 4) for the large saccades necessarily used here to study interrupted movements. Many also continue to discharge after saccade end. When we attempted to plot the dynamic relationship between these cells' discharge and motor error, the phase plots, even for control trials, were highly curved and some were not single valued. When we further attempted to add the phase trajectories for interrupted movements to these plots, we were unable to produce interpretable results. Therefore, we chose instead to make a single measurement that estimated the prolongation in discharge for a fixed level of declining discharge for all cells.

The quantitative measurements made in this way in the present study suggest that, on average, the discharge of deeper collicular cells is prolonged during interrupted saccades. For the fixed level of discharge picked to quantify this prolongation, the effect was nearly the same as the amount of movement prolongation produced in the saccades that accompanied the discharge for many cells. These results, and similar results obtained in the previous studies of collicular SRBNs (Keller and Edelman 1994; Munoz et al. 1996), show that the discharge of some collicular neurons remains correlated with movement dynamics even during perturbed saccades. The combined results suggest that both types of cells in the SC receive feedback about the ongoing saccade, but do not prove that the neural computation that determines dynamic motor error to guide saccade accuracy occurs only in the colliculus.

Acknowledgements This work was supported by National Institutes of Health grants R01 EY06860 (E.L.K.) and 5 T32 GM08155 (UCSF/Berkeley Bioengineering; N.J.G.).

References

Anderson RW, Das S, Keller EL (1998a) Estimation of spatiotemporal neural activity using radial basis function networks. J Comput Neurosci 5:1–21

Anderson RW, Keller EL, Gandhi NJ, Das S (1998b) Two-dimensional saccade-related population activity in superior colliculus in monkey. J Neurophysiol 80:798–817

Berthoz A, Grantyn A, Droulez J (1986) Some collicular efferent neurons code saccadic eye velocity. Neurosci Lett 72:289–294

Crandall WF, Keller EL (1985) Visual and oculomotor signals in nucleus reticularis tegmenti pontis in alert monkey. J Neurophysiol 54:1326–1345

Dorris MC, Pare M, Munoz DP (1997) Neuronal activity in monkey superior colliculus related to the initiation of saccadic eye movements. J Neurosci 17:8566–8579

Freedman EG, Sparks DL (1997) Activity of cells in the deeper layers of the superior colliculus of the rhesus monkey: evidence for a gaze displacement command. J Neurophysiol 78: 1669–1690

Fuchs AF, Robinson DA (1966) A method for measuring horizontal and vertical eye movement chronically in the monkey. J Appl Physiol 21:1068–1070

Gandhi NJ, Keller EL (1998) Comparison of saccades perturbed by stimulation of the superior colliculus and the omnipause region. Soc Neurosci Abstr 24:1498–1498 (abstract)

Glimcher PW, Sparks DL (1992) Movement selection in advance of action in the superior colliculus. Nature 355:542–545

Guitton D (1991) Control of saccadic eye and gaze movements by the superior colliculus and basal ganglia. In: Carpenter RHS (ed) Eye movements. CRC Press, Boca Raton, pp 244–276

Hanes DP, Patterson WF II, Schall JD (1998) Role of frontal eye fields in countermanding saccades: visual, movement and fixation activity. J Neurophysiol 79:817–834

Judge SJ, Richmond BJ, Chu FC (1980) Implantation of magnetic search coils for measurement of eye position: an improved method. Vision Res 20:535–538

Keller EL (1974) Participation of the medial pontine reticular formation in eye movement generation in monkey. J Neurophysiol 37:316–332

Keller EL (1977) Control of saccadic eye movements by midline brain stem neurons. In: Baker R, Berthoz A (eds) Control of gaze by brain stem neurons. Elsevier, Amsterdam, pp 327–336

Keller EL (1999) Activity in collicular buildup neurons during interrupted saccades. Arch Ital Biol 137 (Suppl):22

Keller EL, Edelman JA (1994) Use of interrupted saccade paradigm to study spatial and temporal dynamics of saccadic burst cells in superior colliculus in monkey. J Neurophysiol 72: 2754–2770

Keller EL, Gandhi NJ, Shieh JM (1996) Endpoint accuracy in saccades interrupted by stimulation in the omnipause region in monkey. Vis Neurosci 13:1059–1067

King WM, Fuchs AF (1977) Neuronal activity in the mesencephalon related to vertical eye movements. In: Baker R, Berthoz A (eds) Control of gaze by brain stem neurons. Elsevier, Amsterdam, pp 319–326

Ma TP, Cheng HW, Czech JA, Rafols JA (1990) Intermediate and deep layers of the macaque superior colliculus: A Golgi study. J Comput Neurosci 295:92–110

Mohler CW, Wurtz RH (1976) Organization of monkey superior colliculus: intermediate layer cells discharging before eye movements. J Neurophysiol 39:722–744

Munoz DP, Wurtz RH (1995a) Saccade-related activity in monkey superior colliculus. I. Characteristics of burst and buildup cells. J Neurophysiol 73:2313–2333

Munoz DP, Wurtz RH (1995b) Saccade-related activity in monkey superior colliculus. II. Spread of activity during saccades. J Neurophysiol 73:2334–2348

Munoz DP, Guitton D, Pélisson D (1991) Control of orienting gaze shifts by tecto-reticulo-spinal system in the head-free cat III. Spatiotemporal characteristics of phasic motor discharges. J Neurophysiol 66:1642–1666

Munoz DP, Waitzman DM, Wurtz RH (1996) Activity of neurons in monkey superior colliculus during interrupted saccades. J Neurophysiol 75:2562–2580

Ottes FP, Van Gisbergen JAM, Eggermont JJ (1986) Visuomotor fields of the superior colliculus: a quantitative model. Vision Res 26:857–873

Richmond BJ, Optican LM, Spitzer H (1990) Temporal encoding of two-dimensional patterns by single units in primate primary visual cortex I. Stimulus-response relations. J Neurophysiol 64:351–369

Robinson DA (1963) A method of measuring eye movement using a scleral search coil in a magnetic field. IEEE Trans Biomed Eng BME-10:137–145

Segraves MA, Park K (1993) The relationship of monkey frontal eye field activity to saccade dynamics. J Neurophysiol 69: 1880–1889

Sparks DL, Hartwich-Young R (1989) The deep layers of the superior colliculus. In: Wurtz RH, Goldberg ME (eds) The neurobiology of saccadic eye movements. Reviews of oculomotor research, vol III. Elsevier, Amsterdam, pp 213–256

Waitzman DM, Ma TP, Optican LM, Wurtz RH (1991) Superior colliculus neurons mediate the dynamic characteristics of saccades. J Neurophysiol 66:1716–1737

White JM, Sparks DL, Stanford TR (1994) Saccades to remembered target locations: an analysis of systematic and variable errors. Vision Res 34:79–92

Wurtz RH (1996) Vision for the control of movement. The Friedenwald Lecture. Invest Ophthalmol Vis Sci 37:2130–2145

Wurtz RH, Albano JE (1980) Visual-motor function of the primate superior colliculus. Annu Rev Neurosci 3:189–226