Comparison of Saccade-Associated Neuronal Activity in the Primate Central Mesencephalic and Paramedian Pontine Reticular Formations

Jason A. Cromer and David M. Waitzman

1University of Connecticut Health Center, Department of Neurology and 2Program in Neuroscience, Farmington, Connecticut

Submitted 19 March 2007; accepted in final form 27 May 2007

Cromer JA, Waitzman DM. Comparison of saccade-associated neuronal activity in the primate central mesencephalic and paramedian pontine reticular formations. J Neurophysiol 98: 835–850, 2007. First published May 30, 2007; doi:10.1152/jn.00308.2007. The oculomotor system must convert signals representing the target of an intended eye movement into appropriate input to drive the individual extracocular muscles. Neural models propose that this transformation may involve either a decomposition of the intended eye displacement signal into horizontal and vertical components or an implicit process whereby component signals do not predominate until the level of the motor neurons. Thus decomposition models predict that premotor neurons should primarily encode component signals while implicit models predict encoding of off-cardinal optimal directions by premotor neurons. The central mesencephalic reticular formation (cMRF) and paramedian pontine reticular formation (PPRF) are two brain stem regions that likely participate in the development of motor activity since both are anatomically connected to nuclei that encode movement goal (superior colliculus) and generate horizontal eye movements (abducens nucleus). We compared cMRF and PPRF neurons and found they had similar relationships to saccade dynamics, latencies, and movement fields. Typically, the direction preference of these premotor neurons was horizontal, suggesting they were related to saccade components. To confirm this supposition, we studied the neurons during a series of oblique saccades that caused “component stretching” and thus allowed the vectorial (overall) saccade velocity to be dissociated from horizontal component velocity. The majority of cMRF and PPRF neurons encoded component velocity across all saccades, supporting decomposition models that suggest horizontal and vertical signals are generated before the level of the motoneurons. However, we also found novel vectorial eye velocity encoding neurons that may have important implications for saccade control.

INTRODUCTION

Separate groups of extracocular muscles are responsible for moving the eye horizontally and vertically. However, the location of the target of an eye movement is typically encoded in the cortex and tectum as a vector relative to the current position of the fovea. For instance, it is known that individual neurons in the motor layers of the superior colliculus (SC) are active before saccades of particular amplitude and direction, constituting a movement field (Sparks 1975; Wurtz and Goldberg 1972). As a population, the neurons of the SC form a topographical map of contralateral oculomotor space (Robinson 1972). Thus the vector displacement required to move the eye to a target is encoded by the activation of a specific group of neurons on the collicular map.

This topographic, population-based coding of intended saccadic eye displacement is drastically different from the activity of motor neurons that move the eyes. For example, in the abducens nucleus individual neurons encode the amplitude of the horizontal component of all ipsilateral eye movements via the number of spikes in their burst and the speed of the horizontal movement component by their rate and timing of discharge (King et al. 1986). A similar encoding for the vertical movement component is found in the neurons of the rostral interstitial nucleus of the MLF (riMLF) (Moschovakis et al. 1991a,b). Thus the two-dimensional, spatial code of activated SC neurons is eventually transformed into a single dimensional, temporal pattern of activity necessary to drive separate groups of horizontal and vertical eye muscles (Sparks and Mays 1990).

To address where this transition might occur, we recorded the discharge of neurons in two brain stem regions receiving input from the SC: the central mesencephalic reticular formation (cMRF) and the paramedian pontine reticular formation (PPRF). The cMRF has been hypothesized to extract the desired horizontal eye-movement component from the collicular output (Cohen et al. 1986; Sparks and Mays 1990), and the PPRF is believed to generate the command for horizontal saccades which is passed to the abducens (Keller 1974; Luschei and Fuchs 1972; Schiller 1970). If these ideas are true, then neurons in each of these structures should encode the horizontal component of oblique saccades. To test this hypothesis, we recorded cMRF and PPRF neurons during a series of oblique saccades for which the vertical component of movement was allowed to vary but for which horizontal component amplitude remained fixed. These saccades caused stretching of the horizontal component (Fig. 1). Thus we could distinguish whether neuronal discharge varied in accordance with vectorial saccade velocity or horizontal component velocity. A preliminary report of these findings has been presented previously (Cromer and Waitzman 2005).

METHODS

Animal preparation

Data were collected from four adult, male rhesus monkeys. All experiments were approved by the Animal Care and Use Committee of the University of Connecticut Health Center and complied with the principles enunciated in the “Guide for the Care and Use of Laboratory Animals”, (National Institutes of Health Publication No. 86-23, revised 2001). Each animal was fitted with a scleral search coil for monitoring eye position (Judge et al. 1980; Robinson 1963) and a stainless steel recording chamber for access to the selected brain region. Cylinders targeting the cMRF were positioned (angle: 0°) over...
midline trephine holes (A3.0–A4.0), which allowed access to both the left and right cMRF. Neurons were recorded in the cMRF of all four monkeys. In two of the monkeys, neurons from the PPRF were also recorded. In one case, these neurons were accessed using the same cylinder from which the cMRF was recorded. The other monkey was fitted with a second chamber (angled 22° from the coronal plane) positioned at P2.5 to record from the PPRF.

Behavioral training

Three of the monkeys were trained to perform an overlap saccade task with the head restrained. This task was chosen so that visual (sensory) activity could be easily dissociated from motor activity and because it allowed the collection of saccades of specific amplitude and direction. Each trial started with a ~1,000 ms period of fixation at primary position followed by the presentation of a peripheral visual target. Monkeys were cued to make a saccade to the target when the fixation point was extinguished 500-1,000 ms later. A fourth monkey was trained in another visually guided saccade paradigm, the “no gap” task, during which the fixation light is extinguished and simultaneously the target light is illuminated. This monkey was permitted to immediately saccade to the target.

For all experiments, the fixation spot was presented at primary position to avoid confounding our baseline signal in cases where neurons were modulated by eye position in the orbit. This was especially important in this study because eye position has been suggested to modulate the discharge of PPRF neurons (Henn and Cohen 1976). Each trial consisted of a single visually guided saccade to an eccentric target. We positioned targets (up to 30° around the primary position) to obtain a complete movement field within our range of recording. After collecting sufficient trials to establish a movement field for the cell, we recorded a series of saccades with a fixed horizontal component (e.g., 5°) but increasing vertical components (or a fixed vertical component and increasing horizontal components for those cells with vertical preferences). This technique generated a dataset for which the peak component eye velocity was dissociable from peak vectorial eye velocity and thus allowed us to distinguish cells encoding either property (see RESULTS). Note that cells in the cMRF and PPRF typically respond to all movements with components for those cells with vertical preferences). This technique generated a dataset for which the peak component eye velocity was dissociable from peak vectorial eye velocity and thus allowed us to distinguish cells encoding either property (see RESULTS). Note that cells in the cMRF and PPRF typically respond to all movements with a component of movement in a given cardinal direction (i.e., straight right, left, up, or down). Therefore the tested oblique saccades were within a neuron’s active range.

Data collection and analysis

Neurons in the cMRF were identified 2–4 mm lateral to the oculomotor nucleus (Cromer and Waitzman 2006). The PPRF was identified based on its relationship to the abducens nucleus, the raphe interpositus (RIP), and the nucleus reticularis tegmenti pontis (NRTP). Neurons in the PPRF were recognized by their characteristic burst discharge with saccades and were located in a 2-mm-wide region adjacent to the RIP that extended from the rostral pole of the abducens nucleus to the caudal portion of the NRTP (based on the presence of vector neurons and stimulation induced staircase saccades) (Crandall and Keller 1985; Kaneko 2006). The location of recording tracts in the cMRF and PPRF were confirmed by subsequent histology and were identified in all animals. Localization was facilitated by injection of fluorescent latex microspheres (Lumafuor) into the cMRF in two of the four animals.

Single neurons were recorded using tungsten microelectrodes (FHC) and isolated on-line via threshold and window discrimination (BAK). We selected for analysis those cells that had a robust motor burst that began before eye movement by comparing the mean activity in a motor epoch around the peak saccade velocity (100 ms before to 100 ms after peak saccade velocity) for the visually guided movements of all correct trials to the mean neuronal activity during a 500-ms epoch of the fixation period (while the monkey fixated the central target spot). Neurons were considered to have a presaccadic motor burst if the average activity in the motor epoch was significantly greater than that during the period of fixation (paired t-test, P < 0.01) and if the peak of the neuronal activity occurred before the peak saccade velocity.

Once motor cells were identified, analysis procedures were similar to that recently described (Cromer and Waitzman 2006) and are summarized here. Saccade onset and offset were marked when the vectorial eye velocity exceeded or fell below 15%/s, respectively. For each saccade, we counted the number of spikes in the interval from 100 ms prior to saccade onset to 10 ms after the end of the saccade (and adjusted for spontaneous background activity if necessary). The average spike count was plotted as a function of saccade amplitude and direction to generate movement fields for each neuron. Our analysis of movement fields included the activity associated with all visually guided and spontaneous saccades. For each neuron, a spike density function was created by convolving the unit activity with a Gaussian (σ = 5 ms) (MacPherson and Aldridge 1979; Richmond and Optican 1987; Silverman 1986). The peak firing rate of a cell was determined for each saccade based on the peak of the spike density function during the same interval used to count the number of spikes (100 ms before to 10 ms after the saccade). An optimal direction was calculated for each cell by fitting the number of spikes versus saccade direction curve for all saccades with a Gaussian curve. The direction at the peak of the Gaussian was selected as the “optimal direction” (Kaneko et al. 1981; Russo and Bruce 1996; Scudder et al. 1988; Waitzman et al. 1996).
Horizontal and vertical component velocities were calculated by differentiating the eye position signals. Vectorial eye velocity was then calculated as the square root of the sum of the squares of the component velocities on a point by point basis. To determine whether cMRF and PPRF cells were better related to the component or vectorial velocity of saccades, we compared the activity of each neuron associated with saccades where these features were dissociated from component velocity difference, we performed a dynamic analysis that tested whether predicted neuron associated with saccades where these features were dissociated (see RESULTS). Predicted neuron activity was dissociated from component or vectorial velocities on a point by point basis. To determine whether these features were differentiated in the population of cells in the given brain region. The basis for a second approach to the analysis of the data is a model that described in previous studies (Handel and Glimcher 1997; Richmond et al. 1987; Sylvestre et al. 2003). The models for neuronal firing rate based on either the component velocity ($E_x$) or vectorial velocity ($E_v$) accounted for more variance in the neuronal discharge (Cullen et al. 1996). The models compared were

$$FR(t) = a + b \times E_x(t + td)$$  (1)
$$FR(t) = a + b \times E_v(t + td)$$  (2)

Either horizontal or vertical component velocity was tested depending on the direction preference of the neuron. Predicted firing rates were generated using the recorded eye velocity (either vectorial or component), shifting it in time by $td$ (representing the time delay, or latency, from neuronal activity to movement), scaling it by parameter $b$ (gain), and adjusting it with a bias parameter ($a$). Note that both models have the same number of free parameters. For every neuron, these parameters were optimized for each model using a linear least squares method. The goodness of fit between the predicted firing rate and the actual neuronal firing rate was assessed by the variance accounted for (VAF). The model with the higher VAF suggested a neuron’s response was better related to that variable (Cullen et al. 2000). We used a bootstrapping technique to determine confidence intervals for the likelihood that one model consistently provided a better fit to the data (Manly 2007; Richmond et al. 1987; Sylvestre et al. 2003).

The basis for a second approach to the analysis of the data is a recent neural model that uses the linear vector sum of the amplitude and direction of each activated SC neuron to generate an ideal straight trajectory for a saccade and the cumulative spike count from the activated SC neurons to determine how far along that ideal trajectory the eye has moved (Goossens and Van Opstal 2006). If the cumulative spike count of the SC is actually used to drive the tecto-recipient burst neurons in the PPRF and cMRF in this way, then the cumulative spike count of these neurons should predict how far along either the vectorial or component direction the eye has moved. We tested this relationship between cumulative spikes ($cs$) and eye displacement along either the saccade or component vector. For the vectorial test, movements were expressed in terms of the saccade vector as described by Goossens and Van Opstal (2006). This resulted in a measure of the change in eye displacement (vectorial $\Delta E$) along the straight-line vector from the saccade start point to the endpoint. Similarly, we calculated the eye displacement along the component vector (component $\Delta E$), which corresponded to either the horizontal or vertical movement of eyes. The progress of the eye along these two vectors was then compared with the cumulative spikes the tested neuron elicited during saccades. For display purposes, the cumulative spike count started at the beginning time of the display. For regression analysis, we set the time delay for each neuron by optimizing the linear regression between $cs$ and $\Delta E$.

RESULTS

We recorded from 85 PPRF neurons and 143 cMRF neurons that appeared to be modulated during any portion of the behavioral trial. For this analysis, we selected presaccadic cells based on the presence of significantly increased activity prior to saccades (paired $t$-test, $P < 0.01$, see METHODS). This accounted for 62% of the PPRF neurons (53/85) and 38% of the recorded cMRF neurons (55/143). The latency from peak discharge to peak saccade velocity for each of these neurons is shown in Fig. 2 (A, PPRF; B, cMRF). We measured the peak-to-peak latency rather than the latency from burst onset to movement onset for several reasons. First, in temporally coded neurons, the peak-to-peak latency is a better measure of the relationship of the neuronal burst to the saccade because the main burst is temporally coded not the lead activity. Second, the presence of long-lead activity in many neurons can make identifying burst onset less precise. Thus the peak-to-peak latency could be more reliably measured. The mean peak-to-peak latencies in the PPRF ($-17.9 \pm 10.4$ (SD) ms) and cMRF ($-17.9 \pm 6.8$ ms) were not significantly different (paired $t$-test, $P = 0.92$).

We then assessed the directional tuning of the movement fields of both the PPRF and cMRF neurons. For the majority of neurons in both regions, the predominant activity was centered on a given cardinal direction (left, right, up, or down) and neurons fired before movements of all amplitudes into the hemifield in that direction (Fig. 3, A and B). This movement field pattern was present in 83% of PPRF (44/53) and 65% of cMRF (36/55) motor-associated neurons and was similar to that described in previous studies (Handel and Glimcher 1997; Moschovakis et al. 1988; Waitzman et al. 1996). Atypical motor cells were excluded form further analysis. These included 1) cells that were omnidirectional with movement fields that were less uniform and firing that was generally less robust (PPRF: 17%, 9/53; cMRF: 24%, 13/55); 2) neurons that showed activity limited to a specific vector (i.e., had closed movement fields) similar to typical neurons in the SC (PPRF: 0%; cMRF: 4%, 2/53); and 3) neurons that had movement fields...
with no discernable pattern of directional tuning (PPRF: 0; cMRF: 8%, 4/53). Although the latter four neurons met the statistical criteria for inclusion in the data set and did display a visible increase in activity around the time of the saccade, the activity was minimal (no robust burst) and likely accounted for the lack of a clear movement field.

From the population of cardinally tuned cells with motor-associated activity, 89% of PPRF neurons (39/44) and 83% of cMRF neurons (30/36) were horizontally tuned (Fig. 4, A and B). It is important to clarify here that we use “horizontally tuned” to specify that the center of the neuron’s active range was close to the horizontal. Almost all neurons in both the cMRF and PPRF were active for all movement to a particular hemifield (e.g., any movement in the leftward direction). In the PPRF, neuronal response occurred before ipsilateral saccades, whereas cMRF neurons responded predominantly before contralateral saccades. The remainder of the cardinal cells showed activity for vertical movements, either up or down (Fig. 4, C and D). In the cMRF, neurons with vertical movement fields were usually located in the more rostral portions of the MRF, as has been reported previously (Handel and Glimcher 1997; Waitzman et al. 1996). In the PPRF, neurons with the majority of activity in the vertical direction (and the omnidirectional cells) were typically located near the midline dorsal to the level of the RIP. Note that for simplicity, we will limit the discussion in the remainder of the paper to the horizontal cells, but an identical analysis was performed on the vertical cells, and they are included in all results.

A common approach to the analysis of saccade-associated neurons in the oculomotor literature has been to determine various parameter relationships along a cell’s optimal direction (for a review, see Moschovakis et al. 1996). For example, the two neurons displayed in Fig. 3 had optimal directions that were within 4° of the horizontal (PPRF: 178°; cMRF: 184°). For both of these neurons, the number of spikes in the motor burst was tightly correlated with horizontal saccade amplitude for all saccades with a leftward component, although the PPRF neuron had lower residual standard error than the cMRF neuron (C, SD = 4.72) compared with the PPRF neuron (C, SD = 3.15).
peak firing rate of the neurons (Fig. 5B: PPRF, bottom row; Fig. 5C: cMRF, bottom row) increased directly with peak saccade velocity (Fig. 5A: saccades, bottom row). Second, the duration of the neuronal bursts increased with the duration of the saccades. The tight coupling of the bursts of these neurons to saccade amplitude, peak velocity, and duration suggested that the temporal firing pattern of both of these neurons encoded instantaneous saccade velocity (Cromer and Waitzman 2006; Cullen and Guittion 1997; Van Gisbergen et al. 1981). In fact, the primary difference between the displayed cMRF and PPRF cardinal neurons was the “long-lead,” build-up of activity in the sample cMRF neuron that occurred before the robust presaccadic burst. With the exception of this long-lead activity, these neurons were nearly indistinguishable based on the analysis of trials along each cell’s optimal direction. Note that the robust eye-movement-associated burst of each cell occur at similar latencies despite the difference in lead activity. It should also be noted that whereas the depicted PPRF neuron had no lead activity, many neurons in both the cMRF and PPRF have long-lead activity.

Despite the similarities between the metrical properties of cMRF and PPRF neurons along their optimal directions and between their movement fields (e.g., both example neurons responded to all leftward movements), there was one salient difference between some movement fields that can be seen in the two example cells. The PPRF movement field displayed vertical stripes such that the number of spikes appeared to be identical for the series of movements for which horizontal component amplitude remained fixed (Fig. 3A). Therefore this neuron discharged for the horizontal component of oblique saccades. In contrast, the cMRF movement field displayed curved bands (Fig. 3B). Hence, the movement field of this cMRF neuron showed activation for the vectorial amplitude rather than the horizontal component of movement.

Because the optimal movement direction for nearly all PPRF and cMRF neurons was within ±20° of the horizontal (Fig. 4), an analytic technique that examined the metrical properties of the neurons along their optimal direction (e.g., Fig. 5) would have been unable to distinguish if the discharge of a neuron encoded vectorial or horizontal component velocity. However, there would have been a marked difference between the response of vectorial and component encoding neurons during oblique saccades the horizontal components of which were stretched. For example, consider the series of oblique saccades consisting of a small, fixed horizontal component (5°) and increasing amplitude of vertical components executed by one of our monkeys (Fig. 6B). Like saccades along the horizontal, the vectorial eye velocity of these oblique saccades (Fig. 6C, v) followed the saccadic main sequence (Fig. 6A: left). Thus saccade duration increased with amplitude (Fig. 6A: right, top) and peak velocity increased with saccade amplitude up to the point where it began to saturate for the largest-amplitude saccades (Fig. 6A: right, bottom). On the other hand, due to component stretching the velocity of the shorter, horizontal component of movement (Fig. 6C, c_h) was altered in the opposing pattern (Fig. 6D: left). Peak horizontal component velocity declined for saccades of increased vectorial amplitude even though the horizontal amplitude of the saccades was the
FIG. 5. Responses of PPRF and cMRF neurons (same cells as in Fig. 3) to saccades of increasing amplitude along their optimal direction of movement (leftward). These neurons display a number of metrical relationships to the eye movements that were characteristic of PPRF and cMRF cells. Each column shows ≥20 trials overlaid, all aligned on saccade onset (vertical lines). A, top: saccades to a set target were stereotypic. Bottom: peak velocity of the saccades increased as saccade amplitude increased up to the point where it saturated (≈20–25°). Saccade duration also increased linearly with increasing saccade amplitude. B, top: spike rasters (each dot represents 1 neuronal discharge and each row represents a single trial) for the PPRF neuron during saccades of the size displayed in A. Note the tight burst of action potentials that started just prior to eye movement initiation (vertical lines). The number of spikes in the burst increased monotonically with increasing saccade amplitude (cf. Fig. 3C). The duration of the burst also increased linearly with the increase in saccade duration. B, bottom: spike density functions for the spike rasters of the PPRF neuron shown in top. Note the clear temporal relationship of the neuron to instantaneous eye velocity (A, bottom), including an increase and saturation of peak discharge rate corresponding to peak saccade velocity. C: with the exception of the long-lead activity, this cMRF neuron was quite similar to the PPRF neuron shown in B. This neuron also showed metrical relationships between number of spikes (in the burst) and saccade amplitude, peak discharge and peak saccade velocity, and burst duration and saccade duration. Although the compact burst is harder to identify in the rasters (C, top) due to the long-lead activity of the cMRF neuron. It is clear when examining the spike density function (C, bottom) that like the PPRF neuron (B, bottom), this cMRF neuron had a strong temporal relationship to instantaneous eye velocity. Note that the actual saccades recorded with the cMRF neuron are not shown but were virtually identical to those depicted in A (both cells were recorded in the same monkey making saccades to the same targets).
Because the amplitude of the horizontal component vector remained constant, the area under the velocity curve must have remained constant. As a result, there was a corresponding increase in the duration of the component velocity with larger-amplitude oblique saccades (Fig. 6D: right, top). In sum, we could determine if cells with similar cardinal directional tuning were more closely associated with vectorial or component velocity by examining their response during a series of increasingly larger oblique saccades with similar horizontal components.

Neurons in the abducens have previously been shown to respond to saccade horizontal component velocity (King et al. 1986). Thus as proof of principle, we show the results of this test on an abducens motor neuron (Fig. 7A). Note how the burst of the motoneuron changed in conjunction with horizontal component velocity (cf., Fig. 7A, right and middle). Corresponding to the effect of component stretching on horizontal component velocity, the peak discharge rate of the neuron was reduced and the duration of the burst was stretched in time. Also note how the tonic discharge rate after saccade offset remained constant because horizontal saccade amplitude, and thus the final horizontal position of the eye, remained constant.

Using this approach, we then examined whether neurons in the cMRF and PPRF responded to component or vectorial velocity. Because PPRF neurons provide the burst of activity to abducens motor neurons, which results in movement of the eyes along the horizontal plane (Sasaki and Shimazu 1981; Strassman et al. 1986), we predicted that the discharge of PPRF neurons would encode horizontal component eye velocity. Our test of PPRF neurons confirmed the presence of cells with this type of encoding (Fig. 7B). Note that the temporal pattern of the discharge (Fig. 7B, right) was closely associated with the...
FIG. 7. Responses of individual neurons recorded during saccades with component stretching. Left: vectorial eye velocity for saccades of increasing vertical amplitude but fixed horizontal amplitude (See Fig. 6B). Middle: horizontal component eye velocity for the same saccades. Note how the peak horizontal velocity decreased due to component stretching even though the horizontal amplitude of all the saccades was the same. Right: neuronal response of each of the cells. A: abducens motoneuron the burst of which showed evidence of component stretching. There was a decrease in peak firing rate and an increase in burst duration that paralleled the decrease in peak component velocity and increase in component velocity duration. Thus neuronal activity followed the same pattern as component velocity. This indicated that the motoneuron encoded component, not vectorial velocity. Note that the tonic activity of the neuron ended at the same firing rate since the horizontal amplitude of all saccades was the same. B: temporal activity of a PPRF neuron clearly follows the instantaneous eye velocity associated with the horizontal component (middle) not the vectorial eye velocity (left). C: cMRF neuron the peak activity of which declined as the vertical amplitude of the oblique saccades increased. This demonstrated that the temporal activity of this neuron was best related to component eye velocity (middle) similar to the PPRF neuron shown in B. D: the discharge of the cMRF neuron which paralleled vectorial eye velocity (left). This neuron demonstrated an increase in peak discharge and spike number for oblique saccades whose vertical amplitude increased, whereas the horizontal component amplitude remained fixed. Note that the activity of this neuron increased despite the fact its “optimal” direction was for purely horizontal movements.
temporal waveform of the component saccade velocity (Fig. 7B, middle). In a similar vein, neurons in the cMRF have been hypothesized to extract the horizontal component of movement from the population of active SC neurons (Cohen et al. 1986; Sparks and Mays 1990). Therefore we predicted that cMRF neurons would also encode horizontal component velocity. Again we found cMRF neurons the peak discharge of which declined in conjunction with the decrement in peak horizontal component velocity (Fig. 7C, middle and right). However, we also found neurons in both the cMRF and PPRF (e.g., Fig. 7D, right) the discharge of which was best associated with vectorial saccade velocity (Fig. 7D, left) and not horizontal component velocity (Fig. 7D, middle). Thus the peak discharge of these neurons increased with increasing peak vectorial velocity. We performed a preliminary quantification of the number of cells in each category using this approach of comparing neuronal responses during oblique saccades the horizontal components of which were stretched. These data suggested that the majority of cells in the cMRF (12 of 16) and PPRF (12 of 19) encode saccade components, whereas approximately a quarter of cells in both regions were related to vectorial velocity (4 of 16 in cMRF, 7 of 19 in PPRF).

If cells in the cMRF and PPRF are separated into those encoding the vectorial eye movement versus those encoding only the horizontal component of eye movement, then this dissociation should also be evident on examination of the relationship between the cumulative number of spikes (cs) a neuron elicits during each saccade and the displacement of the eye along either the intended saccade vector or the horizontal component vector (see METHODS). Examination of these variables resulted in the same conclusions (Fig. 8). Those cells related to horizontal saccade components (Fig. 8, A–C) show a convergence in their cumulative spike profile (neuronal activity; Fig. 8, right). That is, the total number of spikes during each of the oblique saccades remains essentially constant, presumably because the horizontal component amplitude of the saccades was constant—fixed at 5° (component ΔE; Fig. 8, middle). In addition, slight temporal shifts in the horizontal velocity due to component stretching are reflected in the cs profile (cf. right to middle panels). Conversely, the number and timing of spikes for the neuron the discharge of which was most closely associated with vectorial velocity (in Fig. 7D) most closely tracked the increased vectorial amplitude of oblique saccades instead of their horizontal component (Fig. 8D, cf. neuronal activity: right panel to vectorial ΔE: left panel).

To more precisely quantify the influence of component and vectorial velocity on the population of cMRF and PPRF neurons in our sample, we tested whether dynamic models of component or vectorial velocity accounted for more of the variance in each neuron’s firing (see METHODS). We included all visually guided saccades made to targets with component amplitudes of ±10°. This restriction generated a pool of saccades whose directions ranged from cardinal (e.g., purely horizontal) to extremely off-axis (e.g., 5° horizontal component and 25° vertical component). For this subset of saccades, the peak velocity of the horizontal component of saccade amplitude ranged from equaling overall saccade velocity (during cardinal saccades) to displaying a distinct dissociation from overall saccade velocity (i.e., during saccades of increasing obliquity, horizontal peak component velocity decreases as vectorial peak velocity increases, as seen in Fig. 6). Restricting the analyzed pool of saccades to those with small component amplitude was critical to increase the number of saccades with “component stretching” (e.g., those saccades where the off-component amplitude was double or more the component amplitude) and thus increased the variance between component and vectorial velocity. We confirmed the existence of this dissociation because it was critical to our ability to distinguish between component and vector encoding neurons. The mean difference between component and vectorial velocity in the selected pool of saccades was 38 ± 13%.

We then optimized the parameters for the vectorial (see METHODS, Eq. 1) and component (see METHODS, Eq. 2) models and assessed how well each velocity model fit the actual neuronal firing rate by computing the VAF. A higher VAF for a given model suggested that a neuron was better related to either component or vectorial velocity. For example, the component model accounted for 91% of the variance in firing rate of the PPRF component cell in Fig. 7B, whereas the vectorial model accounted for only 55% of the variance. Likewise, for the component cMRF cell in Fig. 7C, the component model was better (39% vs. vectorial 32%). Thus for those cells that were related to component velocity, the component velocity model (Eq. 2) accounted for more of the variance in neuronal firing rate than the vectorial model (Eq. 1). Likewise, the vectorial model accounted for more variance in the vectorial cell of Fig. 7D (component 59%, vectorial 70%). For all cases where we performed both an explicit test (i.e., Fig. 6) and this dynamic analysis, the dynamic models accurately identified the response of the cell (n = 28). Thus we could confidently apply the dynamic analysis to the population data to distinguish component from vectorial encoding cells.

We had sufficient data in the restricted pool of saccades to compare the dynamic analysis for component and vectorial models on 19 of the 44 cardinal PPRF cells and 23 of the 36 cardinal cMRF cells. The excluded cells represent those for which there were insufficient visually guided saccades (n < 10) in the selected range for dynamic analysis (see above) or for which the pool of saccades did not show a large degree of separation between the vectorial and component velocity profiles (variance difference <10%, n = 4). A comparison of the VAF for the vectorial versus component model for the analyzed cells is displayed in Fig. 9, A and B. Although VAFs across models are directly comparable (the model with the higher VAF is a better fit), we took this analysis one step further by performing a bootstrap analysis of the data to obtain confidence intervals for the VAFs from each model. The points in black represent those cells for which the optimal model’s VAF was consistently higher (the difference between the component and vectorial VAFs did not overlap with 0) at the 95% confidence level. The majority of cells in both structures were better related to component velocity (PPRF, 67%; cMRF, 80%). However, about a quarter of cells in each population had higher VAFs with the vectorial model and appeared to encode vectorial velocity (PPRF, 33%; cMRF, 20%). Percentages were similar if the entire set (○ and ●) was considered or if just those cells that met the 95% bootstrap criteria were used (filled circles). Some cells did not have a large difference in the VAF between the two models (Fig. 9, A and B, cells falling near the line of unity). This finding suggests that there could be either a continuum of cells between those encoding vectorial velocity
FIG. 8. Change in eye position vs. cumulative spikes (cs). Vectorial change in eye position (ΔE; left) showed the position of the eye along the straight line vector from saccade start to end. Component change in eye position (ΔE) (middle) showed the position of the eye along the straight line vector representing the change in horizontal eye position during the saccades, which was fixed at 5°. Note how during the oblique saccades of increasing vertical amplitude, there was an increase in the vectorial ΔE due to the larger amplitude of the saccades, but the component (horizontal) ΔE had a constant start and endpoint and only differed in how quickly the eye moved along the vector. The cumulative spikes (right) shows a running count of the number of spikes generated by a neuron over time (counts start at the beginning of the time displayed in the figure). A: cumulative spikes of the abducens motor neuron remained constant across the different oblique saccades. Because the neuron had a tonic component related to eye position in the orbit, cumulative spikes continued to increase, but at a slower rate, after completion of the saccades. B: PPRF neuron the activity of which was related to component velocity (cf. Fig. 7B) showed a close relationship to component ΔE. Again cumulative spike numbers were the same across the set of saccades, with the temporal effect of component stretching evident in the cs profile. C: cMRF component neuron that also showed a close relationship between cumulative spike count and component ΔE. D: in contrast, this cMRF vectorial neuron showed increased spike number with increasing overall (vectorial) amplitude. In other words, the cumulative spike counts followed the pattern of the vectorial ΔE (left) instead of the component ΔE (middle).
and those encoding component velocity or could simply indicate that some cells are more influenced by other eye-movement metrics (Cromer and Waitzman 2006). As a secondary measure of the cell population, we also performed regression analysis on the cumulative spikes versus change in either vectorial or component eye displacement (as in Fig. 8). This analysis produced a similar breakdown of cells related to either component or vectorial velocity (data not shown). We reviewed the location of recorded PPRF and cMRF neurons that were best related to component versus vectorial velocity and found no obvious topography separating cells responding to either property.

Because the dynamic analysis provided a second measure of neuronal latency by optimizing the time delay parameter ($t_d$) from burst to saccade for each cell, we compared this measure of latency to the one we had previously calculated across the entire population (Fig. 2). The mean difference between the methods was 1.6 ± 1.5 ms, suggesting the two measures were nearly identical and confirming the validity of the methods (for 2 cells firing well before the saccade, there was more divergence between the methods). We then tested whether the populations of vectorial and component neurons showed a difference in latency using the $t_d$ values from the dynamic analysis (Fig. 9, C and D). There was a significantly longer latency ($P = 0.023$) for vectorial neurons ($-24.3 ± 10.7$ ms) versus component neurons ($-10.3 ± 5.3$ ms) in the PPRF (Fig. 9C). However, there was no significant difference in the cMRF population (vectorial: $-13.5 ± 2.1$ ms, component: $-14.0 ± 2.9$ ms; Fig. 9D). Again, results were similar if all tested cells (Fig. 9, A and B, ○ and ●) or just those meeting the 95% confidence interval (● in A and B) were analyzed.

**DISCUSSION**

A fundamental role of brain stem saccadic neurons is to generate signals used to drive the eye to a desired target location. Individual neurons in the cerebrum and SC encode intended movement displacement via a spatial code (Fig. 10A, SC cells). From the activated SC neuronal population, the temporal pattern of activity found in the motor neurons is eventually developed. We investigated two sites in the brain stem known to receive collicular output, the cMRF and PPRF. We demonstrate that the saccade-associated bursts of neurons in both structures typically had similar directional tuning, latencies, and temporal encoding of saccade metrics. The only obvious difference between the presaccadic responses of the
population of neurons in the PPRF and cMRF was the directional preference of each of the structures: ipsilateral for the PPRF versus contralateral for the cMRF.

To better understand the signals in these regions, we examined the response properties of neurons for evidence of a relationship (or lack thereof) to the horizontal component of saccades. One key characteristic of saccadic component stretching was critical to the current study and has been demonstrated previously (King et al. 1986). Specifically, during oblique saccades of increasing vertical amplitude and for which horizontal amplitude is fixed, peak horizontal component velocity declined, whereas overall vectorial velocity, overall saccade duration, and horizontal component duration increased. This characteristic permitted dissociation between
neurons related to horizontal component velocity from those associated with the overall vectorial velocity of the saccade. We found that the majority of neurons in the PPRF and cMRF were best correlated to component velocity. The phenomenon of component stretching was reflected in the discharge of these cells and indicated that a relationship to saccade components is likely developed before reaching the motor neurons (Fig. 10A, component). At the same time, the discharge of a substantial number of cMRF (20%) and PPRF (33%) neurons was most closely associated with vectorial velocity. Thus the activity of these cells increased directly with peak vectorial velocity and was not decomposed into horizontal and vertical components (Fig. 10A, vectorial).

Note that for both component- and vector-encoding cells, activity typically occurred for all saccades in a given cardinal direction (e.g., Fig. 10A shows vectorial and component cells that respond for any saccade to the left hemifield). Thus for similar neurons with large movement fields, analysis limited to an “optimal direction” (e.g., Fig. 5) would not be capable of distinguishing between cells encoding component and vectorial velocity. This result identifies the importance of examining oblique saccades in structures that are typically believed to be involved with the generation of the horizontal component of eye movement and may explain why neurons encoding vectorial velocity in the PPRF have not been previously reported. Alternatively, the relatively low percentage of these neurons in the population may account for their elusiveness. Although we have provided evidence for a population of cells encoding vectorial velocity by examining neuronal activity during component stretching, this was unexpected at the onset of the experiments. Thus further studies could more definitely determine whether cells truly encode a pure “vector” signal by also comparing saccades with fixed vector amplitudes but varying directions.

A number of stepwise models have been developed to explain the vector decomposition between the SC and the motor neurons. One point of contention among this type of model has been the order in which pulse generation (the development of a temporal velocity signal from a spatial signal) and vector decomposition occur. For instance, independent models decompose the two-dimensional signals originating in the SC into separate horizontal and vertical streams before pulse generation, whereas the common source model postulates that pulse generation occurs first, creating a signal specifying vectorial velocity (Van Gisbergen et al. 1985). In the common source model, the vectorial velocity signal is subsequently decomposed into temporal signals related to horizontal or vertical component velocity. Evidence that the discharge of some PPRF and cMRF neurons is most closely correlated with vectorial velocity supports the prediction of the common source model by demonstrating the physiological existence of a vectorial velocity signal. A major distinction between the vectorial velocity cells of the PPRF and cMRF reported here and SC cells associated with a two-dimensional “vector” signal for a fixed amplitude and direction that can also be influenced by saccade dynamics (Goossens and Van Opstal 2006; Munoz and Wurtz 1995; Waitzman et al. 1991), is that many SC neurons have closed, not open, movement fields (Fig. 10A, SC cells). Thus cMRF and PPRF vectorial velocity cells temporally encode vectorial velocity across saccades of all amplitudes to a given hemifield.

Based on the common source model, Fig. 10B illustrates a schematic identifying a potential pathway for the decomposi-
tion of the collicular gaze displacement signal into the temporal encoding of horizontal component velocity displayed by the abducens motor neurons (black arrows, SC→vectorial cells→component cells→motor neurons). In the two-dimen-
sional, topographic map of the SC, different regions are activated depending on the horizontal and vertical gaze displacement necessary to move the fovea onto a target (areas 1–3 from Fig. 10B elicit ideal saccade vectors corresponding to A–C from Fig. 10A, respectively). The SC may project to vectorial velocity neurons in the cMRF or PPRF, which fire a similar number of spikes for movements with similar vector amplitude (regardless of direction in the active hemifield; Fig. 10B, vectorial). These vectorial neurons could then project to component neurons that fire the same number of spikes before movements with the same horizontal component amplitude regardless of the vertical component (Fig. 10B, component). Note, however, for these component cells, while spike number remains constant (e.g., the parallel bands shown in the schematic movement field) the duration of the spike discharge is stretched based on the duration of the vectorial velocity of the saccade.

The idea that cMRF and PPRF neurons that temporally encode vectorial velocity are an intermediary step between closed movement field SC vector neurons and component velocity encoding neurons is attractive because the medium lead excitatory burst neurons (EBNs) in the PPRF, with latencies comparable to those included here, likely do not receive direct projections from the SC (Keller et al. 2000). This is important because the EBNs are believed to be the subpopulation of PPRF neurons that project directly to the abducens nucleus (Strassman et al. 1986). Instead it has been postulated that the SC projects to local, presumably long-lead, neurons in the PPRF, which in turn drive the EBNs. It is possible that the vectorial neurons represent this population. This idea is supported by the fact that the latency of vectorial neurons in the PPRF was longer than that of the PPRF component neurons, suggesting that the vectorial velocity encoding neurons may be activated first. A similar decomposition from vectorial to component signals could occur in the cMRF, however, the vector neurons in the cMRF had a similar latency to component cells making this less likely.

Despite the attractiveness of the vectorial velocity neurons being a distinct step in a serial decomposition process, there are a number of problems with this scheme. Saccades are not always as straight and fast as predicted by the common source model (Quaia and Optican 1997). In addition, if vectorial neurons were a necessary step in pulse generation, it would be expected that they would comprise a much larger percentage of the total population of PPRF and cMRF neurons than we found. There are several possible alternative explanations for our findings. One is that vectorial velocity neurons could play a role in accurately stopping saccades by providing the signal to latch the omnipause neurons (Fig. 10B, dashed lines) (Cro-
mer and Waitzman 2006; Rucker et al. 2005), which have been shown to respond to vectorial saccade velocity (Yoshida et al. 1999). Another possibility is that the vectorial velocity neurons in the cMRF and PPRF are used to generate the supplemental activity that occurs in the vertical eye muscles during hori-
zontal saccades (Cohen et al. 1964).
If any of these alternatives are true and the vectorial velocity neurons are not a requisite step in the decomposition process, then the activity observed in component velocity neurons must be generated directly from collicular output (Fig. 10B, solid gray lines). Many modeling studies have hypothesized that this direct transformation occurs via efferent mapping functions that assign particular weights to SC projections based on the location of neurons in the SC map (Moschovakis 1994; Scudder 1988; Van Gisbergen and Van Opstal 1989; Van Gisbergen et al. 1987). Furthermore, neural correlates of these weighting functions have been demonstrated in the form of differential neural density gradients (Grantyn et al. 2002) and locus-dependent weighting of the strength of efferent projections (Moschovakis et al. 1998). We suspect that the vectorial velocity neurons could also be generated through a similar, but altered, weighting function.

Our data also address the argument as to whether vector decomposition occurs prior to or not until the level of the motor neurons (Quaia and Optican 1997). The majority of PPRF and cMRF neurons in our sample had movement field activity that was clearly centered on a cardinal direction and had optimal directions that were close to cardinal. If vector decomposition did not occur until the level of the motor neurons as suggested by Quaia and Optican (1997), we would have expected to have found a more uniform distribution of neurons encoding vectors in all directions (i.e., Fig. 4 would be filled in, having all possible vectors, instead of showing vectors clustered around the cardinal directions). Although some past studies have indicated that the optimal direction of cells in the PPRF may be significantly tilted away from a cardinal direction (Hepp and Henn 1983; Strassman et al. 1986), most authors report that PPRF discharge is best associated with horizontal velocity (Cullen and Gutton 1997; Kaneko et al. 1981; Scudder et al. 1988; Sparks et al. 2002; Van Gisbergen et al. 1981). Furthermore, the existence of noncardinal PPRF neurons as suggested by Hepp and Henn (1983) has been questioned by a recent study that suggests that their “vector” long lead neurons were most likely recorded in the NRTP (Kaneko 2006). Finally, our results demonstrated that a majority of PPRF and cMRF neurons encoded horizontal component velocity during a behavioral paradigm that induced component stretching. Taken together, these data suggest that the development of signals encoding saccadic component velocity is completed before the level of the motor neurons. This conclusion is further supported by evidence that the interaction between voluntary and involuntary saccades occurs at a level of the system encoding saccade components (Van Beuzekom and Van Gisbergen 2002).

In addition to the finding that there are subpopulations of neurons in the brain stem encoding component or vectorial velocity, this study also provided a direct comparison of the presaccadic activity of cMRF and PPRF neurons recorded under identical experimental conditions. Although the similarities between neurons in these structures was not surprising given the results of past studies (Cromer and Waitzman 2006; Moschovakis et al. 1988; Pathmanathan et al. 2006), it raises the question as to the differential functions of these brain stem regions in saccadic control. One possibility is that cMRF neurons serve as intermediaries between the SC and PPRF (Pathmanathan et al. 2006). Our results show that although both cMRF and PPRF neurons have relationships to saccade dynamics, the discharges of PPRF neurons were in general more strongly correlated to saccade velocity (cf. cMRF vs. PPRF VAFs in Fig. 9). Thus the specificity of temporal encoding may gradually increase as information from the SC is funneled toward the motor nuclei (Cromer and Waitzman 2006; Grantyn et al. 2004; Hepp and Henn 1983). Although the physiological evidence fits nicely with this idea of an SC to cMRF to PPRF progression, anatomical evidence for the cMRF to PPRF connection is scant. This may imply that cMRF activity is used instead for another aspect of saccadic control such as efference copy, the feedback of the current motor command to the SC (see Cromer and Waitzman 2006 for a more detailed discussion of possible functions of the cMRF).

The current work may also answer a puzzling clinical question. Destruction of the PPRF in monkeys and man leads to a complete loss of rapid eye movement to the ipsilateral side (Goebel et al. 1971). However, a number of patients have been reported who have impaired conjugate movement but no evidence of a pontine lesion (Flannery et al. 2004). Moreover, patients with an infarction or a tumor confined to the midbrain may experience supranuclear vertical and horizontal gaze palsy (Bogousslavsky 1989) or a combined oculomotor paresis and a contralateral conjugate gaze palsy (Zackon and Sharpe 1984). Evidence of the close similarities between neurons in the cMRF and the PPRF suggest that damage to the cMRF in such patients could impair an alternate midbrain-dependent pathway that has direct access to the horizontal saccade generator, such as those connections from the region of the cMRF to the abducens described by Ugolini et al. (2006).

In summary, we have provided the first physiological documentation of the existence of a vectorial velocity signal within the saccadic control system. The discharge of vectorial velocity neurons could be a common source signal via which horizontal and vertical eye velocity is derived, or alternatively, vectorial velocity neurons could participate in a latch circuit to control saccade duration. Furthermore, we have shown that the majority of neurons in the cMRF and PPRF are tuned specifically to horizontal component velocity. This finding strongly supports the idea that component signals in the oculomotor system are developed prior to the level of the oculomotor neurons.

Acknowledgments

We thank Dr. Jay Pathmanathan for suggestions, assistance, and analysis routines; Dr. Paul May for providing expertise in anatomical reconstruction; Drs. Shig Kuwada, Kathleen Cullen, Lance Optican, and Rich Krauzlis for helpful discussions of the data and/or review of a previous version of this manuscript; Dr. Douglas Oliver for use of lab facilities and supplies; and J. Dearborn for assisting in experimental recordings and training.

Grants

This work was supported by the National Institutes of Health Grants EY-009481 to D. M. Waitzman and predoctoral National Research Service Award EY-015356 to J. A. Cromer and University of Connecticut Health Center Training Grant NS-041224 to J. A. Cromer.

References


Moschovakis AK, Scudder CA, Hightstein SM. The microscopic anatomy and physiology of the mammalian saccadic system. Prog Neurophysiol 50: 133–254, 1996.


