Response Properties of Cells Within the Rostral Superior Colliculus of Strabismic Monkeys

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Purpose. The superior colliculus (SC) is an important oculomotor structure which, in addition to saccades and smooth-pursuit, has been implicated in vergence. Previously we showed that electrical stimulation of the SC changes strabismus angle in monkey models. The purpose of this study was to record from neurons in the rostral SC (rSC) of two exotropic (XT; divergent strabismus) monkeys (M1, M2) and characterize their response properties, including possible correlation with strabismus angle.

Methods. Binocular eye movements and neural data were acquired as the monkeys performed fixation and saccade tasks with either eye viewing.

Results. Forty-two cells with responses likely related to eye misalignment were recorded from the rSC of the strabismic monkeys of which 29 increased firing for smaller angles of exotropia and 13 increased firing for larger exotropia. Twenty-six of thirty-five cells showed a pause (decrease in firing rate) during large amplitude saccades. Blanking the target briefly during fixation did not reduce firing responses indicating a lack of visual sensitivity. A bursting response for nystagmus quick phases was identified in cells whose topographic location matched the direction and amplitude of quick phases.

Conclusions. Certain cells in the rSC show responses related to eye misalignment suggesting that the SC is part of a vergence circuit that plays a role in setting strabismus angle. An alternative interpretation is that these cells display ocular preference, also a novel finding, and could potentially act as a driver of downstream oculomotor structures that maintain the state of strabismus.

Keywords: Strabismus, nonhuman primate, fixation, saccades, vergence, superior colliculus, nystagmus

Approximately 5% of all infants in the world have some form of strabismus (ocular misalignment).[1,2] This developmental disorder is treated mostly at the level of muscles using surgical methods where the position of eye muscles are altered to correct for the eye misalignment.[3] Recent data from animal models of strabismus acquired using neurophysiological methods such as electrical stimulation, muscimol inactivation, and single cell recording within numerous brain areas including the motor nuclei, supraoculomotor area (SOA), fastigial and posterior interposed nuclei of the cerebellum, paramedian pontine reticular formation (PPRF), and the superior colliculus (SC), have shown that various structures within a vergence neural circuit contributes toward maintenance of the state of strabismus.[4–11]

The SC has been extensively studied for its involvement in saccadic eye movements,[12–14] and this structure also appears to have a role in vergence. Van Horn et al.,[15] in a study in normal monkeys, have shown that the rostral SC (rSC) contains vergence related neurons (convergence and divergence), which modulate with eye movements made to sinusoidal target motion in depth.[15] It has also been shown that stimulation of the rSC during an asymmetric vergence task affects vergence eye movement in normal monkeys.[16,17] Vergence related neurons have also been recorded in rSC of cat.[18] In humans, a case of bilateral SC lesion resulted in convergence and accommodation palsy.[19] Afferent and efferent anatomical connections from cerebellar areas and to the central mesencephalic reticular formation (cMRF) and SOA provide supporting evidence that the SC is part of a vergence and accommodation circuit.[20,21]

Motivated by the evidence in normal monkeys and humans, we recently investigated the SC in strabismic monkeys using electrical stimulation techniques and showed that low current electrical stimulation within mostly the rostral part of the SC of strabismic monkeys resulted in a change in strabismus angle.[7] Depending on the site of stimulation, we elicited either convergent or divergent change of strabismus angle. Other studies in strabismic monkeys using SC electrical stimulation have also shown that there were influences on strabismic angle during electrical stimulation.[10,22,23] Our analysis of the stimulation data indicated that the strabismus angle changes were a consequence of disconjugate saccades and also disconjugate postsaccadic drift.[7] We hypothesized that the disconjugate postsaccadic drift could be the consequence of stimulating a population of vergence (misalignment in the case of strabismic monkeys) cells within the SC. Therefore, the goal of the current study was to identify and examine the response properties of cells within the SC whose firing was potentially related to eye misalignment. Since the previous work by Van Horn et al.[15] and also our own electrical stimulation study was primarily focused in the rostral regions of the SC, we focused the neural recording also in the rSC. Some of these data have appeared before in

abstract form (Upadhyaya S, Das V. *IOVS* 2018;59:ARVO E-Abtract 1021).

Methods

Subjects, Rearing Paradigms, and Surgical Procedures

The subjects of this study were two adult exotropic (divergent strabismus; Monkey M1 and Monkey M2) monkeys whose strabismus was previously induced in infancy by disrupting binocular vision during the critical period of development using an optical prism-rearing method. In the optical prism rearing paradigm, the infant monkeys wore lightweight helmets fitted with either a base-in or base-out prism in front of one eye and a base-up or base-down prism in front of the other eye starting from day 1 after birth till they were 4 months of age after which they were allowed to grow under unrestricted viewing conditions.[24,25] This paradigm decorrelates binocular vision during the critical period for visual development thus resulting in development of strabismus.[26]

When the animals were ~4 years of age, they underwent a surgical procedure carried out under aseptic conditions with isoflurane anesthesia (1.25%–2.5%) to implant a head stabilization post.[27] Later in a second surgery, we stereotaxically implanted a 21-mm diameter titanium recording chamber in each animal. Chamber location in M1 was 3 mm anterior, 1 mm lateral, and 8 mm dorsal with respect to ear-bar-zero and a 20° tilt angle to the left with respect to the sagittal plane. Chamber in M2 was located on the mid-sagittal plane and 15 mm above ear-bar-zero and a 38° tilt angle to the coronal plane. In the same surgery, we also implanted a scleral search coil in one eye using the technique of Judge et al.[28] and in a third surgery, a scleral search coil was implanted in the fellow eye. All procedures were performed per National Institutes of Health guidelines and the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the protocols were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Houston. Monkey M1 was used in our previously published study (Monkey H) that examined effect of electrical stimulation within the SC of strabismic monkeys.[7]

Experimental Paradigms, Data Acquisition, and Analysis

Monkeys were trained on a variety of oculomotor tasks prior to data collection for this study. Eye movements were calibrated as the monkey monocularly viewed target stimuli at ±15° horizontally and vertically. A 2° sized white optotype target (luminance 470 cd/m^2) on a black background (luminance 0.5 cd/m^2) was used in the study. Targets were back-projected onto a tangent screen at a distance of 57 cm using a DepthQ LCD projector (Lightspeed Design, Inc., Bellevue, WA, USA) running at 120-Hz frame rate. Liquid crystal shutter goggles (Citizen Fine Devices, Nagano, Japan) under computer control were used to facilitate monocular viewing. Changing the viewing eye (by occluding the fellow eye) resulted in a change in strabismus angle that we were able to leverage to identify cells potentially related to eye misalignment, which were the target population for this study.

The SC was identified by visual responses from cells in the superficial layer followed by saccade related bursting as we descended into the intermediate and deep layers. Electrical stimulation, resulting in staircase saccades, was also used to map the area, and the area that evoked a radial saccadic amplitude of <5° was defined as our target zone (rSC) for neural recording. Once a cell in the rSC was isolated and then identified as being potentially related to eye misalignment, the following four tasks were performed:

- Monocular fixation with either eye for 4 to 7 seconds each. Data acquired in these trials were used to correlate firing rate with strabismus angle.
- Horizontal smooth-pursuit (0.2–0.3 Hz, ±10–15°) during monocular viewing to determine whether firing rate is correlated to eye position.
- 10° to 15° amplitude ipsilateral and contralateral saccades during monocular viewing to examine whether the misalignment-related cells paused during large saccades, similar to previously described fixation cells in the rSC.[29]
- A target blink paradigm in which the animal monocularly fixated a straight-ahead target for 1 to 3 seconds during which the target was randomly blanked for 300 to 400 ms. Data acquired in these trials were used to determine if the cell showed visual sensitivity.
- Fixation at vertical ±10° to determine whether changes in horizontal strabismus angle at different vertical gaze positions (A/V pattern strabismus) was accompanied by changes in firing rate of the misalignment related cells.

Eye movement data were processed with anti-aliasing filters at 400 Hz before sampling at 2.79 kHz with 12-bit precision (Alpha Lab SNR System; Alpha-Omega Engineering, Nazareth, Israel). All eye movement data were additionally calibrated offline and filtered using a software finite impulse response (FIR) low-pass filter with a pass-band of 0 to 80 Hz. Single cell recording was performed using epoxy coated tungsten electrode with ~1 Mohm resistance (Frederik Haer, Brunswick, ME, USA). Raw spike data were acquired at a sampling rate of 44 kHz. Spike sorting was performed offline using a template matching algorithm (Spike 2 Software; Cambridge Electronics Design, England). Unit response was represented as a spike density function that was generated by convolving action potential time stamps with a 15-ms Gaussian.[30] Data analysis was performed with custom software routines developed in MATLAB (MathWorks, Natick, MA, USA), and SigmaPlot 12.0 (Systat, Inc.; San Jose, CA, USA) was used for statistical analysis. The overall goal of the data analysis was to establish whether changes in neuronal firing rates observed in the rSC cells corresponded to the changes in angle of misalignment.

Results

Properties of Strabismus

Animal M1 presented with an exotropia of ~3° to 18° during right eye viewing and ~10° to 20° during left eye viewing, and monkey M2 had an exotropia of ~15° to 22° during right eye viewing and ~20° to 28° during left eye viewing. Monkey M1 did not show any prominent pattern deviation (i.e., no change in strabismus angle with up or down viewing), whereas monkey M2 showed A-pattern strabismus (i.e., reduction in angle of exotropia during up-gaze compared to down-gaze). Both animals showed small amplitude and low velocity downbeat and right-beat nystagmus. The strabismus angle range reported for monkey M1 in our earlier stimulation study was slightly different from the current values because the stimulation study was conducted several months prior to collecting these data, and the strabismus angle tends to vary slightly with time and was especially the case for this animal. When we were conducting the electrical stimulation study, the animal sometimes presented with a few degrees of esotropia, whereas for the duration of the current study, he was consistently exotropic. The reason for this variability is unclear

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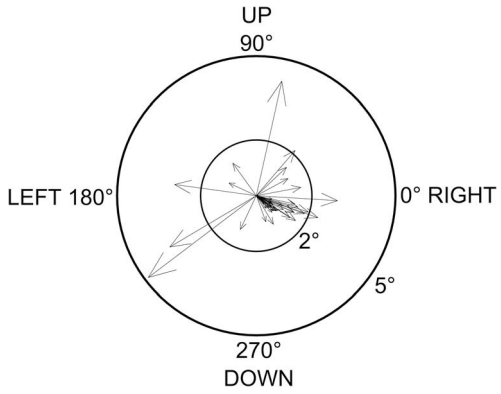

Figure 1. Polar plot showing amplitude and direction of the electrically evoked saccade vector in the viewing eye at sites where misalignment-related cells were recorded.

but could be a function of the tonic accommodative state of the animal. The refractive error of M1 was right eye: +4.50 diopters (D) and left eye: +0.75 D, and in M2 was right eye: −4.50 D and left eye: −1.50 D. All the recordings were performed under proper refractive correction. Note though that including refractive correction did not much alter their strabismus angle nor did it affect their ability to perform the task.

SC Recording Locations

Cells related to eye misalignment, which were the target of this study, were generally recorded ∼1.5 to 2 mm deeper than the initial visual background response that was characteristic of penetrating the superficial layers of the SC. Once an individual cell was isolated and neuronal data collected within the various paradigms described earlier, we delivered small amplitude electrical stimulation (10–40 microamp, 400 Hz, 500 ms) to evaluate the amplitude of the evoked staircase saccades and thereby get an estimate of our recording location within the SC. The mean radial amplitude of saccades evoked in the viewing eye at the recording sites were 2.2° ± 1.3° (M1) and 0.90° ± 0.50° (M2) with range of 0.6° to 4.9° (M1) and 0.2° to 2.1° (M2). Forty-two cells were recorded in total from the two animals of which six cells were recorded from the right SC and 36 cells were recorded from the left SC. The polar plot in Figure 1 shows amplitude and direction of the first electrically evoked saccade from the two monkeys. By design, all of the recording sites were within the rSC as shown by electrically evoked radial saccade amplitude being less than 5° (∼85% of recording sites evoked saccades less than 2°).

SC Cell Responses Associated With Change in Strabismus Angle

A total of 42 cells that were modulated by a change in eye misalignment were recorded in the two animals (M1: 25 cells; M2: 17 cells). Two different types of strabismus-related cells were encountered: cells that increased firing rate when exotropia became smaller and those that increased firing rate when exotropia became larger. Since exotropia is a divergent strabismus, an increase in exotropia is an increase in divergence (far-response) and a decrease in exotropia is an increase in convergence (near-response). The more commonly encountered cells (M1: 15/25 cells and M2: 14/17 cells) were near-response cells; far-response cells were fewer (M1: 10/25 cells and M2: 3/17 cells). Note however that our use of near- and far-response terminology is based only on the mathematical equivalency to a convergent or divergent response with respect to the state of misalignment and the cell response is not driven by a change in target distance.

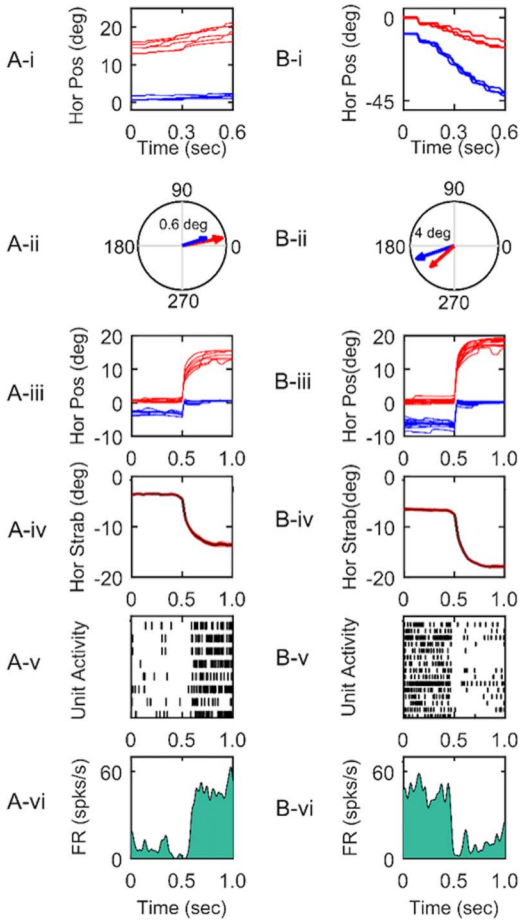

Figure 2. Raw eye and neural data from a far response cell (*left column*, **A**) and near response cell (*right column*, **B**) in monkey M1. **A-i** and **B-i** show horizontal component of staircase eye movement responses following electrical stimulation. Multiple trials are aligned on the start of stimulation. **A-ii** and **B-ii** are polar plots showing amplitude and direction of the first saccade vector elicited by electrical stimulation at this site. **A-iii** to **A-vi** and **B-iii** to **B-vi** are data associated with the neural response. **A-iii** and **B-iii** show right and left horizontal eye positions during multiple trials of an alternate cover test where the monkey was first viewing a straight-ahead target with his right eye and then his left eye. Positive numbers denote rightward eye positions. **A-iv** and **B-iv** show horizontal strabismus angle (left eye position minus right eye position as calculated from **A-iii** and **B-iii**) and illustrate the change in strabismus angle due to fixation switch with central *black line* and *red error bars* showing standard errors. **A-v** and **B-v** are raster plots showing timestamps of neural spiking during each trial shown in **A-iii** and **B-iii**. **A-vi** and **B-vi** are spike density functions showing average firing rate of the far response cell and near response cell. Data in **A-iii** to **A-vi** and **B-iii** to **B-vi** are aligned with change in fixation from right eye to left eye. In all plots, right eye is denoted in *red* and left eye is denoted in *blue*.

Figure 2 shows an example of a far-response cell (left column, A) and a near-response cell (right column, B) in the rSC of monkey M1. The far-response cell was recorded from the left rSC, and the near-response cell was from the right rSC. In this figure, panels A-i and B-i show the staircase saccades evoked due to electrical stimulation at these sites. Electrical stimulation not only evoked staircase saccades but also produced a divergent change in strabismus angle as we have

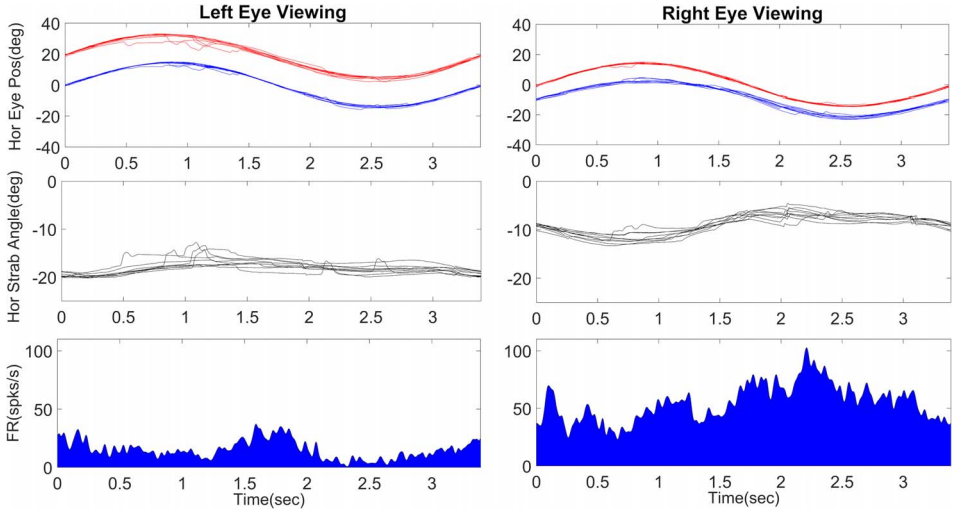

Figure 3. Eye and neural responses during horizontal smooth-pursuit (0.3 Hz, ±15°) with either the left or right eye viewing. The neuron is same as shown in the *right column* of Figure 2. *Red* denotes right eye; *blue* denotes left eye.

shown in our previously published study.[7] The small amplitude of the electrically evoked saccade at the two sites (panels A-ii [mean: 0.3° ± 0.1°] and B-ii [mean: 3.6° ± 0.3°]) indicate that these cells were located in the rostral part of the SC.[12]

In these data, the strabismus angle (left eye position – right eye position) is modulated depending on which of the two eyes is fixating (panels A-iii and B-iii), and this property was leveraged to investigate the correlation between strabismus angle and neural response rate within the subpopulation of rSC cells. Mean change in strabismus angle (calculated as difference between left and right eye position; shown in panels A-iv and B-iv) was ∼8° in M1 and ∼10.0° in M2. Raster plot of neural responses during several trials of monocular fixation (panels A-v and B-v) and the average neural responses shows that in the cell shown in the left column (panel A-vi), there was increased firing rate when angle of exotropia was greater (far-response cell) and in the example cell in the right column (panel B-vi), there was an increased firing rate when the angle of exotropia was small (near-response cell). There was also a significant pause or burst in firing that occurred at the time of the fixation switch (described later).

In order to verify that the tonic modulations in firing rate were related to eye misalignment and not just eye position, monkeys were tasked to perform horizontal smooth-pursuit (0.2–0.3 Hz, ±10°). Figure 3 shows smooth-pursuit data obtained for the near response cell in Figure 2 (right column). Although there is a change in baseline firing for right eye viewing versus left eye viewing, there is no significant modulation with eye position during smooth-pursuit.

Quantification of Eye Misalignment Sensitivities of rSC Cells

For each cell, data obtained during several trials of change in fixation with each eye viewing were averaged, and linear regresion was performed between mean firing rate and the corresponding strabismus angle to estimate sensitivity to eye misalignment. Data selection of eye and neural data to perform the linear regression proved to be challenging. A fundamental problem that we faced was that the data consisted of basically two values of strabismus angle (one for right eye viewing and one for left eye viewing). There was some moment to moment variation in strabismus angle during each eye viewing, but these variations were generally too small (∼1 deg) to be identifed within the neural response that was quite choppy to begin with. We attempted to improve the robustness of the fitting by including within the analysis, the data epoch signifying the slow change in strabismus angle that occurred immediately after switching fixation (see Fig. 2; A–iv and B–iv). Thus, the start and end of fixation-switch saccades were detected using a velocity threshold of 30°/s and an acceleration threshold of 3000°/s^2 and aligned to the start of the neural pause or burst that occurred due to change in fixation. After eye and neural data were aligned, we took three sections of data (300 ms each): one section before change in fixation (i.e., during either right or left eye viewing), a second section during the change in fixation that occurred immedately after the pause or burst ended, and a third section after change in fixation. Moment-to-moment neural firing rate during these three sections (900 ms in total) were plotted against the corresponding eye data, and the resultant slope of a linear fit was estimated to be the eye misalignment sensitivity. This method only improved matters slightly since the pause or burst were usually longer than the fixation-switch saccade itself and therefore included part of the duration of the postsaccadic drift. We also attempted to alter strabismus angle by either introducing lenses of power 1 to 2D or by changing viewing distance, but these methods did not induce significant changes in strabismus angle. Therefore, the estimates of neural sensitivity and threshold that we provide below must be interpreted with caution as they are limited by the limitations in the range and distribution of strabismus angle.

The slope of the regression line for each cell is a measure of the neuronal sensitivity (spikes/s/deg of strabismus angle) of the cell, and the threshold is a measure of the angle of misalignment at which the cell commenced firing. For the representative far-response cell shown in Figure 2A, mean sensitivity was –3.1 spikes/s/deg of strabismus angle and mean threshold was approximately 0.4°, and for the representative near-response cell shown in Figure 2B, mean sensitivity was 3.7 spikess/s/deg of strabismus angle and mean threshold was approximately –21.6°. Note that negative angles imply exotropia (XT) or divergence and positive angles imply esotropia (ET) or convergence.

A distribution of misalignment sensitivity and thresholds is shown in Figure 4. For the population, thresholds for near and far-response cells in the strabismic animals were substantially shifted toward exotropia in comparison to normal monkeys (near-response cells: M1 21.4° exotropic (XT), M2: 33.7° XT,

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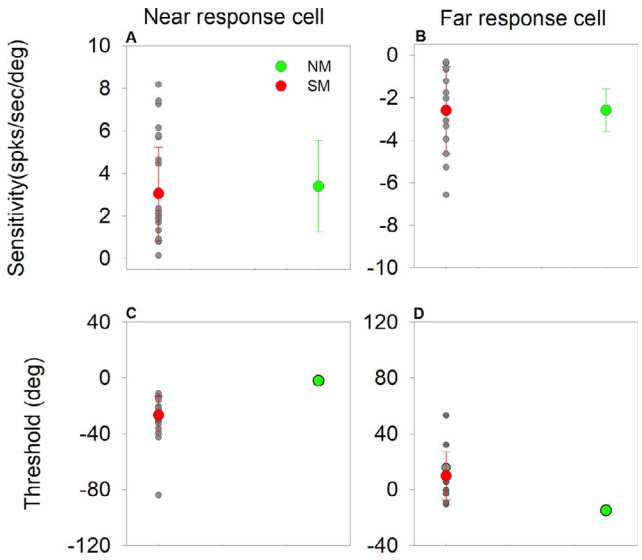

Figure 4. Distribution of sensitivity and threshold of near and far-response cells in the strabismic monkeys. *Gray symbols* are individual cell values, *red symbols* are population averages, and *green symbols* are population average values of normal monkeys derived from a previous publication by Van Horn et al.[15]

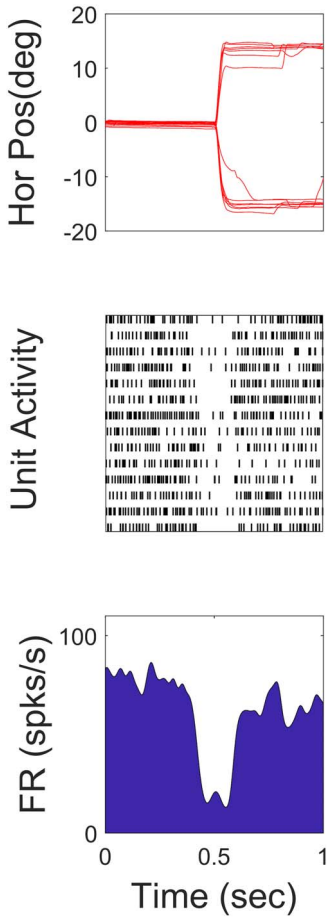

Figure 5. Firing properties of a misalignment cell during saccadic eye movements. *Top panel* shows raw traces of multiple saccades in rightward and leftward directions aligned on saccade onset. *Bottom* and *middle panels* show average firing rate of the cell and raster information.

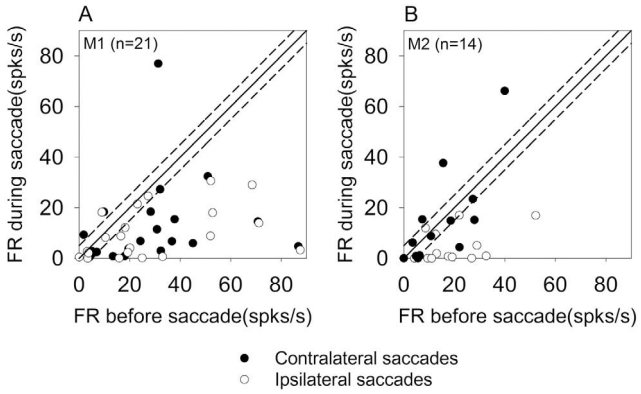

Figure 6. Scatter plot showing firing rate of misalignment-related cells during ipsilateral and contralateral saccades (*y*-axis) and over a 50-ms period that began 100 ms before the saccade onset (*x*-axis). *Solid line* is 1:1 line, and *dashed line* shows change of 5 spikes/s above or below the equality line. Panel **A** is from monkey M1, and panel **B** is from monkey M2. *Filled* and *unfilled black dots* represent firing rate during contralateral and ipsilateral saccades, respectively.

normal monkey (NM) ~2.0° divergence; far-response cells: M1 9.4° ET, M2 11.3° XT, NM ~15.0° convergence). Normative data (NM) are derived from the population data of Van Horn et al.[15] (Cullen KE, personal communications, 2017). The implication here is that, in the strabismic animals, unlike in the normal animals, these cells are active even under conditions of substantial divergence. The population sensitivity of near-response and far-response cells in rSC of strabismic monkeys (SM) and convergence and divergence cells found in rSC of normal animals were similar (near-response cells: SM 3.0 ± 2.2 spikes/s/deg [M1 3.3 ± 2.0 spikes/s/deg; M2 2.6 ± 2.5 spikes/s/deg]; NM 3.3 ± 2.3 spikes/s/deg; *t*-test df = 38, $t = -0.4$, $P = 1.00$ and far-response cells: SM −2.6 ± 2.0 spikes/s/deg [M1−3.1 ± 2.1 spikes/s/deg; M2 −1.0 ± 1.0 spikes/s/deg]; NM −2.6 ± 1.0 spikes/s/deg; *t*-test df = 19, $t = 0.0$ $P = 1.00$).

Response During Contralateral and Ipsilateral Saccades

Certain cells within the rSC pause during large saccades in any direction.[29] Previously called "fixation cells," these cells have now been shown to be sensitive to microsaccades.[31] We wondered if the misalignment-related cell sample in strabismic monkeys also show these pause properties. Figure 5 shows the saccade-related response of one of the misalignment-related cells (near response cell) from the left rSC of monkey M1 and illustrates the decreased discharge rate during both contralateral and ipsilateral saccades. Also note that the pause duration was longer than the saccade duration. We examined saccade related responses of 21 misalignment-related cells in M1 and 14 in M2 by calculating average firing rate during ipsilateral and contralateral saccades and comparing with average firing rate during a 50-ms period, 100 ms prior to saccade onset (Fig. 6). The solid black diagonal line in Figure 6 represents the equality line, and the dotted lines denote increase or decrease in firing rate of 5 spikes/s around the equality line.[29] The data in Figure 6 show that 26/35 cells reduced discharge rate during saccades, among which 20 reduced discharge for both ipsilateral and contralateral saccades, and six reduced discharged only for ipsilateral saccades. Among these six cells, four cells showed bursting activity and two cells remained unchanged for contralateral saccades. Additionally, 6/35 cells remained unchanged during both ipsilateral and contralateral

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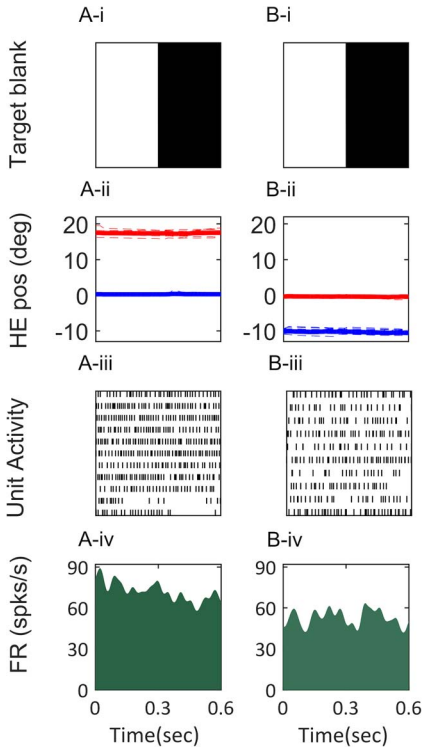

Figure 7. Data acquired during target-blank paradigm in which the visual target was turned off for 300 ms (*dark area* in panels **A-i** and **B-i**). *Column A* shows left eye viewing condition, and *column B* shows right eye viewing condition. Panels **A-ii** and **B-ii** show mean eye position (right eye, *red*; left eye, *blue*) during the paradigm. Panels **A-iii** and **B-iii** show raster plot of spiking during each trial, and panels **A-iv** and **B-iv** show the cell's average firing rate.

saccades, and 3/35 cells burst during both ipsilateral and contralateral saccades.

Visual Response of SC Misalignment Related Cells

Firing activity of the microsaccade cells in rSC persists during momentary blanking of the fixation target, that is, their responses are not visually driven.[29] We employed a similar fixation-blank paradigm to test visual responsiveness of 17 misalignment-related cells in M1 and 14 cells in M2. In this paradigm, the animal fixated the straight-ahead target for a period of 1000 to 1500 ms during which time the target was randomly blanked for a period of 300 to 400 ms. Figure 7 shows raw data during the blank paradigm from a far-response cell during left eye viewing conditions (column A; right eye is shifted to the right indicating exotropia ~18°) and right eye viewing conditions (column B; left eye is shifted to the left indicating exotropia ~10°). There was no visible difference in firing pattern due to blanking of the target. Figure 8 summarizes the results of the target blank paradigm for all 31 cells. Again, the solid black diagonal line represents the equality condition, and the dotted lines represent an increase or decrease of 5 spikes/s over the equality condition. The firing rate of the cells are close to equality suggesting that there was little effect of target blanking, that is, little effect of loss of visual information.

An Alternative Interpretation: Ocular Preference in SC Cell Responses

The primary observation of changes in firing rate in our cell sample was associated with the large changes in strabismus

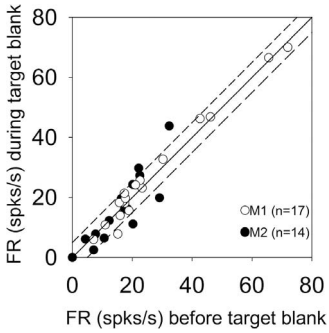

Figure 8. Comparison of firing rate of misalignment-related cells ($n = 31$) before target-blank and during the blank period. *Solid black line* is the equality line, and *dashed lines* indicate increase or decrease in discharge of 5 spikes/s during the blank period. *Filled black dots* are data from animal M2, and *unfilled black dots* are data from M1.

angle, leading to the label of "misalignment-related" cells. However, these large changes in strabismus angle were associated with a change in eye of fixation and therefore an alternative explanation of the firing rate changes is that the rSC cells recorded in this sample have an ocular preference. In other words, there are certain cells that prefer right eye fixation and other cells that prefer left eye fixation. Note that monocular preference has not been described before in the SC and so if it were to exist, it would be a novel finding. Further, since the cells show no visual sensitivity or eye position sensitivity (Figs. 3, 7, 8), the ocular preference would have to be driven by previous fixation or a nonvisual, possibly top-down, mechanism that determines eye of fixation.

We calculated an index for ocular preference (OPI) during fixation for each cell as a ratio of firing rate during left eye fixation to the sum of firing rate during left eye fixation and right eye fixation [$OPI = FR_{LE}/(FR_{LE} + FR_{RE})$]. A cell with strong firing during left eye fixation and weak to no firing during right eye fixation would result in an OPI of close to 1.0, and a cell with strong firing during right eye fixation and weak to no firing during left eye fixation would result in an OPI of close to 0.0. Figure 9 shows the distribution of OPI for all the cells in our sample and shows that there is a relatively uniform distribution of OPI within the rSC cell sample. Only 15/43 cells could be identified as being significantly monocular (by using an arbitrary criterion of OPI of <0.3 or OPI >0.7). Although all

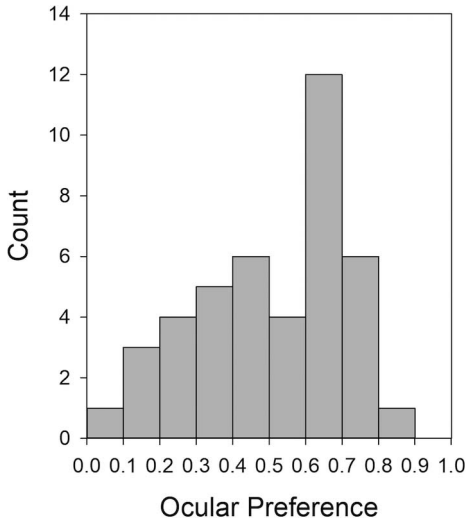

Figure 9. Distribution of ocular preference index for cell sample.

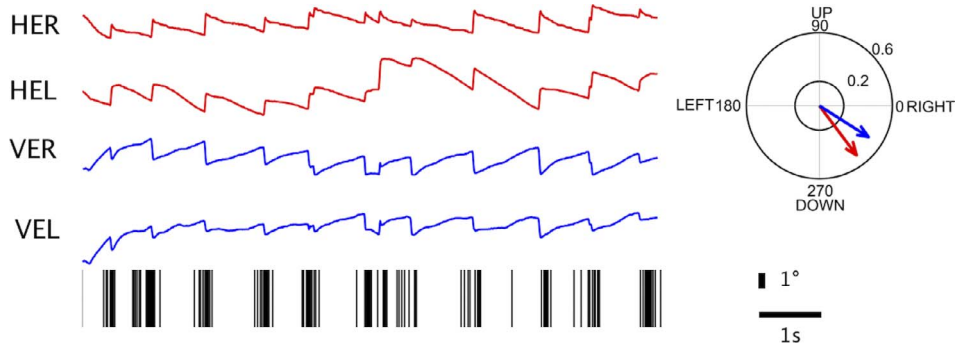

Figure 10. Example of cell that shows burst activity associated with nystagmus quick phases. *Top four rows* show horizontal and vertical eye positions of right and left eyes. The *bottom trace in black* shows time stamp of neural spikes. The *inset polar plot* on the right shows the amplitude and direction of the electrically evoked saccadic vector of the right (*red*) and left (*blue*) eyes. HER, horizontal eye position of right eye; HEL, horizontal eye position of left eye; VER, vertical eye position of right eye; VEL, vertical eye position of left eye.

the cells showed differences in responses between right and left eye fixation (basis for determination of eye misalignment related responses), a reason that only few cells appear strongly monocular is that all the cells showed some level of response during both right and left eye fixation conditions (e.g., numerator of the OPI ratio is never zero) and the overall firing rate of the cells is relatively low (i.e., denominator of OPI ratio is not a large number).

rSC Cells Show Correlated Activity With Quick Phases of Nystagmus

Previous studies have shown that collicular cells show responses correlated with quick phases of physiological nystagmus such as optokinetic or vestibular nystagmus.[32] To our knowledge, collicular responses during pathological nystagmus have not been reported. Since the strabismic monkeys showed significant nystagmus, we had an opportunity to investigate whether the SC cell responses correlated to the quick phase of nystagmus. Nystagmus quick-phases in both the animals were directed down and right. Since some of our penetrations were in the appropriate topographical location corresponding to the amplitude and direction of nystagmus in these strabismic monkeys (as determined by electrical stimulation), we were able to identify nystagmus related activity.

We found seven cells (M1 = 1 and M2 = 6) in the rSC of these strabismic monkeys that showed bursts correlated with the nystagmus quick phase. Figure 10 shows eye position data (top four traces in red and blue) along with corresponding time stamps of a sample cell that showed responses correlated with the rightward and downward quick phases of nystagmus. The same site in monkey M2 also yielded electrically evoked saccade directional responses that were in the same direction as the direction of nystagmus quick phases and of similar small amplitude. The mean amplitude and direction of right and left eye vector obtained by electrical stimulation at this site is shown in the inset.

Figure 11 shows the average neural response to multiple quick phases of nystagmus during both right eye and left eye viewing conditions. It is clear from the mean data that this cell shows bursts that are correlated with the quick phase of nystagmus. In addition, the baseline firing rate during right eye viewing (larger angle of exotropia) was less than during left eye viewing (smaller angle of exotropia). Another interesting observation was the apparent build-up in firing rate just before the burst, which possibly could be related to the slow phase. Unfortunately, we did not have enough of a sample of cells to make any further conclusions on properties of the nystagmus related response. This small sample of cells serve as a proof of existence of SC responses related to quick phases of pathological nystagmus.

Note that nystagmus related bursting is also likely influencing the misalignment-related responses that we have reported (Fig. 2 for example). However, these two types of activity are distinct because the quick-phase activity is "bursty" (Figs. 10, 11) while the misalignment-related activity is "tonic." Further, in our analysis for misalignment-related activity (Fig. 2), the responses are aligned on the switch in fixation from right eye to left eye and so the nystagmus quick-phases (if any) are not aligned across the multiple trials, partially muting their average contribution.

SC Cell Responses to A-Pattern Deviation

Monkey models of strabismus have reported presence of A-V pattern deviation,[8,33–35] a strabismus property also sometimes observed in human strabismic patients.[36,37] A-V pattern deviation is basically a characteristic change in horizontal

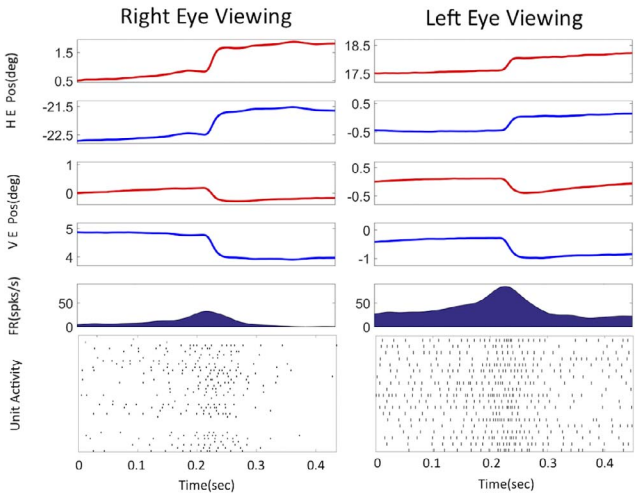

Figure 11. Eye position and neural data from a cell encoding quick-phases of nystagmus during right eye viewing and left eye viewing of a stationary straight-ahead target. *Red trace* denotes right eye positions, and *blue trace* denotes left eye positions. Data are aligned on quick-phase onset, and eye movement plots show the mean eye position over multiple quick-phases. Note the burst associated with the quick-phase in the spike density function and also in the raster plots. Also note that the baseline firing rate is less during right eye viewing (larger exotropia) than during left eye viewing (smaller exotropia) indicating that this cell is also encoding eye misalignment (near-response cell).

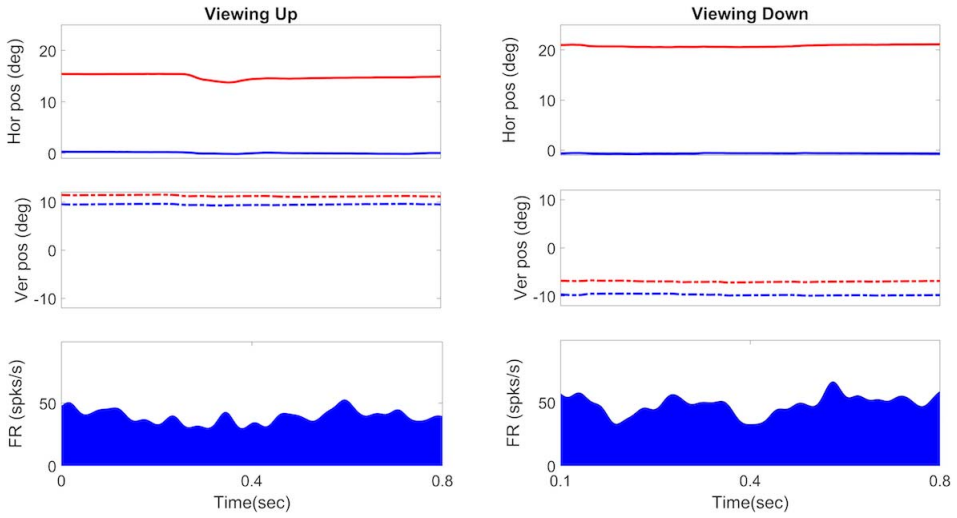

Figure 12. Firing rate of near-response cell at different vertical gaze positions. *Top row* shows horizontal eye position (*red traces*, right eye; *blue traces*, left eye), *middle row* shows vertical eye position, and *bottom row* shows corresponding firing rate of the near response cell from monkey M2 while monkey was fixating a 10° up target (*left column*) and 10° down target (*right column*). Although the horizontal strabismus angle varies at different vertical gaze positions (less in up-gaze compared with down gaze and therefore indicative of A-Pattern), there is no change in firing response of the cell.

strabismus angle with vertical gaze position. Studies in nonhuman primates have shown that activity at the motor neuron level[4] and within the PPRF[11] in strabismic monkeys is correlated to the cross-axis movements that leads to the appearance of A/V patterns. However, near response cells in the SOA of strabismic monkeys do not show the same correlation. One of our strabismic monkeys, animal M2, had an A-pattern deviation (reduction in exotropia in up-gaze compared with down-gaze), and so we were able to test whether changes in misalignment due to pattern deviation was reflected in SC activity. In order to determine whether firing rate of misalignment related cells change during up and down gaze, we collected data from 11 misalignment-related cells in monkey M2 while this animal fixated at targets 10° up and 10° down.

Figure 12 shows average strabismus angle and average neural response of a near response cell from the left rSC of monkey M2, while this animal fixated at different vertical gaze positions. Although strabismus angle was higher at down-gaze compared with up-gaze, the firing rate of the cell was not significantly different (up: 42.6 ± 5.1 spikes/s; down: 47.2 ± 8.1 spikes/s; *t*-test df = 10, $t = 1.2$, $P = 0.26$). A similar analysis was performed for nine cells in M2 where we acquired data during up and down gaze. Figure 13 plots summary data during both right eye viewing and left eye viewing conditions while the monkey viewed 10° up and 10° down targets. There was no significant difference (Wilcoxon signed-rank test df = 17, $t = 0.37$, $P = 0.72$) in firing rate during up gaze and down gaze, implying that these misalignment related cells do not encode changes in misalignment due to A-pattern deviation.

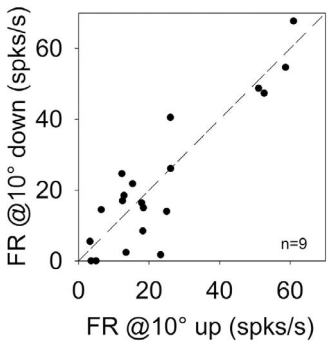

Figure 13. Plot comparing firing rate of misalignment-related cells ($N = 9$) in monkey M2 during 10° gaze up and 10° gaze down. *Diagonal dashed line* is the equality line.

Discussion

In this study, neurons that appear to carry a signal related to horizontal eye misalignment have been identified for the first time within the rSC of strabismic monkeys. An alternative interpretation is that these cells develop an ocular preference in strabismic monkeys, which has not been shown before in normal animals. These results, therefore, provide new insight into the role of the SC in the neural circuits that leads to the appearance of problems in binocular eye alignment and binocular coordination.

Role of rSC in Strabismus

The anatomical location and physiological properties of cells recorded in this study suggest that these cells are likely the same as those that have been reported to encode vergence angle in normal animals.[15] The anatomical locations of vergence cells in normal monkey SC overlaps the location of our cell sample in the strabismic monkeys since electrical stimulation within this area in our sample animals also produced contralateral staircase saccades of <5° radial amplitude. Our search area was limited to the rostral part because of the report of slow convergence and divergence cells in this area by Van Horn et al.[15] and so we cannot exclude the possibility of cells with similar response properties residing within caudal colliculus. Keeping in mind the caveats associated with estimates of population threshold and sensitivity, comparison of the population response properties of misalignment-related (slow vergence) cells in strabismic and normal animals yielded significant differences in threshold but not neuronal sensitivity. The threshold of both the near- and far-response cells were shifted toward exotropia in the strabismic monkey compared with the normal animals

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reported in the study by Van Horn et al.[15] The shift in threshold suggests that these cells are still active despite the significant exotropia and therefore this area and downstream areas receiving projections from the rSC cells still influence the state of misalignment on a moment-to-moment basis. Conversely, in the normal animal, these cells could help maintain the state of normal alignment. Neuronal sensitivity of near and far-response cells were not significantly different between normal and strabismic monkeys. So, it is unlikely that these cells alone are providing the reduced vergence tone responsible for the strabismus. Note that a difference in alignment with viewing condition that we observed in the animal model is not uncommon in human strabismus. There are numerous reports of dissociated deviations and other paralytic and nonparalytic incomitancy in strabismus.[3,36,38] It is possible, but rather unlikely in our opinion, that the identified neuronal responses are related to only the delta change in strabismus angle with viewing condition and that there exists an underlying comitant misalignment due to an altogether different mechanism.

Previously we have shown that the SOA that normally contains convergence and divergence cells shows responses related to strabismus angle.[39] Note that the SOA area is distinguished from the SC by the anatomical location[40] (SC is more caudal and dorsal), response to electrical stimulation (no staircase saccades in SOA), and also general firing characteristics (SOA cells showed higher baseline firing rates compared with the SC misalignment-related cells reported here). Analysis of the SOA cells showed that both threshold (same result as rSC cells) and sensitivity (different result from rSC cells) was reduced in animals with exotropia leading to the hypothesis that the SOA connection to medial rectus motoneurons was resulting in reduced vergence tone and therefore contributing to exotropia. Recently Pallus et al.[41] also recorded from SOA of strabismic monkeys and showed similar results in their exotropic monkeys. The SOA connects monosynaptically to the medial rectus motoneurons[42,43] and therefore changes in SOA sensitivity and threshold are likely to directly impact the state of strabismus via changes in medial rectus contractility. On the other hand, the SC projects to the cMRF, which in turn projects to the SOA, the oculomotor nucleus, and the abducens nucleus.[20] Our finding of SC misalignment-related cells places the SC within a vergence and accommodation circuit that is potentially disrupted in strabismus. Interestingly, Pallus et al.[41] found that the esotropic animals they tested also showed reduced SOA sensitivity but not altered thresholds compared with normal animals. In order to account for the reduced SOA sensitivity in esotropic animals (i.e., reduced vergence tone to medial rectus muscle), vergence input to the lateral rectus must be reduced even further. It is possible that the SC input to the abducens via the cMRF can account for the reduced vergence input to the lateral rectus in an esotrope. In this scenario, the misalignment-related cells in SOA play a stronger role in maintenance of exotropia, while the misalignment-related cells in SC play a stronger role in maintaining esotropia. Recording studies must be performed in esotropic animals to test this hypothesis. The SC also projects to the nucleus reticularis tegmenti pontis (NRTP), which subsequently projects to the cerebellum. Both the NRTP and the deep cerebellar nuclei have been shown to contain vergence related neurons.[44–46] We also showed, via muscimol inactivation studies of deep cerebellar nuclei in strabismic monkeys, that the caudal fastigial nucleus and the posterior interposed nucleus can influence the state of strabismus.[6] Since the deep cerebellar nuclei project to the SOA, this provides another pathway by which the SC could influence the state of strabismus.[47]

Analysis of the rSC responses could also be performed in terms of ocular preference (Fig. 9), that is, the preference of a cell to respond vigorously to fixation with a particular eye. We are unable to unequivocally determine whether the cell response is directly related to eye misalignment or ocular fixation preference because of the limitation of firing rate changes being driven largely by change in eye of fixation. Ocular preference or ocular selectivity has been described in other parts of the brain like the PPRF, neural integrator, and motoneurons but has not been studied in the SC. The lack of visual sensitivity or eye position sensitivity in our cell sample, the report of slow vergence neurons in the rSC of normal monkeys, and the absence of investigation into ocular preference in rSC of normal monkeys all suggest that describing the observed cell responses in terms of eye misalignment is a more parsimonious explanation than using an ocular preference framework. Nevertheless, we cannot distinguish between the two frameworks at this time. Note that the ocular preference framework also implicates the rSC in strabismus because normal monkey studies would suggest that cells are all binocular, whereas there are many cells with monocular weighting in the strabismic monkeys. Since there are direct and indirect connections between rSC and the SOA, eye misalignment sensitivity in SOA that we have described previously could be developed from the monocular weighting within the rSC cells.

Are the Vergence and Misalignment-Related Cells Distinct From Other Cell Types Found in rSC of Normal Monkeys?

Previous studies in cats and monkeys have described the so-called "fixation cells" in rSC that are tonically active when an animal fixates straight ahead and pause during saccades. These cells are not influenced by the presence or absence of a visual target.[29,48,49] In later studies, these cells have been shown to burst during microsaccades.[31] Other than the newly identified property of being sensitive to eye misalignment or ocular preference, the cells that we have described in this study appear to duplicate the properties of the previously described population. The anatomical location of these cells is within the rSC. Misalignment-related far response and near response cells found in strabismic monkeys show sustained discharge before and during blanking of a visual target. Most of the misalignment-related cells decrease their firing rate during large ipsilateral and contralateral saccades. They also show responses during quick-phases of nystagmus whose amplitudes overlap that of microsaccades.[50] We therefore suggest that misalignment-related cells are the same population of previously described fixation/microsaccade cells or perhaps constitute a subset that also carries information about strabismus angle.

Misalignment-Related Cells Also Encode Nystagmus Quick-Phases in Strabismic Monkeys

Nystagmus is observed in most strabismic humans and is replicated in monkey models of strabismus. Although strabismus in humans in often associated with fusion maldevelopment nystagmus (latent nystagmus) that is a predominantly horizontal nystagmus, nystagmus in the vertical plane is also common.[3,51,52] The predominant nystagmus observed in the two study animals was a downbeat (jerk) nystagmus coupled with a smaller amplitude horizontal component. Jerk nystagmus has both a quick phase during which the eye moves quickly with microsaccade-like amplitude and velocity and a slow phase drift. There is considerable evidence that microsaccades are generated within rSC in normal monkeys, and we

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have shown in our study (Fig. 8) that some neurons in the SC also encode the quick phases of nystagmus in strabismus. These neurons are located topographically in the same location of the motor map of SC as saccades of similar small amplitude and direction, as we found that electrical stimulation resulted in saccades of similar amplitude and direction as the nystagmus quick phases. Examination of SC misalignment-related cell activity during the slow phase period (immediately before and after the quick-phase) shows some indication that slow phases may also be encoded in these same cells. The low sample size of the nystagmus cells in our study ($n = 7$) precludes further conclusions on the role of SC in generating nystagmus. Note that we did not set out to obtain a sample of cells whose responses were related to nystagmus quick phases. Therefore, our electrode penetrations were not focused on that portion of the SC map, which matched the nystagmus quick-phase amplitude and direction, and which would likely have yielded more nystagmus cells. So, the small sample size in our study should not be interpreted as the relative proportion of SC cells with responses related to nystagmus.

Influence of Ocular Accommodation

SC has been shown to also be involved in accommodation in cats (Billitz MS, Mays LE. *IOVS* 1997;38: ARVO Abstract 984).[53] Further, an anatomical connection between the Edinger-Westphal nucleus and the SC through the cMRF has been found in monkeys.[21,54] Thus, it is possible that these misalignment-related cells also or perhaps only encode ocular accommodation. The relatively low firing rates of these cells also suggest that accommodation could be encoded within these cells, but the substantial and relatively rapid change in firing rate with strabismus angle suggests that it is unlikely that accommodation alone is encoded. In addition to refractive correction during recording, we tried to minimize accommodative cues as much as possible by using a white optotype shaped target against a dark background and a fixed target distance of 57 cm (1.75 D accommodative demand) making it further unlikely that changes in firing rate (due to change in fixation for example) are solely due to changes in accommodation. Future experiments that include measurement of accommodation as a variable could be useful in characterizing these cells more thoroughly.

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