Abnormal tuning of saccade-related cells in pontine reticular formation of strabismic monkeys

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Walton MM, Mustari MJ. Abnormal tuning of saccade-related cells in pontine reticular formation of strabismic monkeys. J Neurophysiol 114: 857–868, 2015. First published June 10, 2015; doi:10.1152/jn.00238.2015.—Strabismus is a common disorder, characterized by a chronic misalignment of the eyes and numerous visual and oculomotor abnormalities. For example, saccades are often highly disconjugate. For humans with pattern strabismus, the horizontal and vertical disconjugacies vary with eye position. In monkeys, manipulations that disturb binocular vision during the first several weeks of life result in a chronic strabismus with characteristics that closely match those in human patients. Early onset strabismus is associated with altered binocular sensitivity of neurons in visual cortex. Here we test the hypothesis that brain stem circuits specific to saccadic eye movements are abnormal. We targeted the pontine paramedian reticular formation, a structure that directly projects to the ipsilateral abducens nucleus. In normal animals, neurons in this structure are characterized by a high-frequency burst of spikes associated with ipsiversive saccades. We recorded single-unit activity from 84 neurons from four monkeys (two normal, one exotrope, and one esotrope), while they made saccades to a visual target on a tangent screen. All 24 neurons recorded from the normal animals had preferred directions within 30° of pure horizontal. For the strabismic animals, the distribution of preferred directions was normal on one side of the brain, but highly variable on the other. In fact, 12/60 neurons recorded from the strabismic animals preferred vertical saccades. Many also had unusually weak or strong bursts. These data suggest that the loss of corresponding binocular vision during infancy impairs the development of normal tuning characteristics for saccade-related neurons in brain stem.

strabismus; PPRF; esotropia; exotropia; monkey

Infantile strabismus is a disorder of eye alignment affecting ~3% of children in the United States. The condition is associated with numerous visual and oculomotor abnormalities, including amblyopia, impaired motion perception, asymmetrical smooth pursuit gain (Mustari and Ono 2011; Tychsen et al. 1985), latent nystagmus (Mustari et al. 2008; Richards et al. 2008), and an absence of disparity vergence (Kenyon et al. 1981). A number of studies have also shown that saccades are disconjugate in humans with infantile strabismus (Bucci et al. 2002; Kapoula et al. 1997; Maxwell et al. 1995) and in monkeys with experimentally induced strabismus (Fu et al. 2007; Walton et al. 2014b). In at least some cases, saccades in the two eyes can differ in both amplitude and direction (Walton et al. 2014b). In monkeys (Walton et al. 2014b) with pattern strabismus, saccade disconjugacy shows a cross-axis pattern similar to that observed for static eye position. In that study, we suggested the possibility that this may be associated with abnormal cross talk between horizontal and vertical saccadic circuitry in brain stem.

A good target to investigate this issue is the pontine paramedian reticular formation (PPRF). Single neurons in this structure exhibit a high-frequency burst associated with any saccade with an ipsiversive component (Hepp and Henn 1983). In normal monkeys, PPRF neurons are characterized by strong correlations between the number of spikes and horizontal amplitude, and between instantaneous firing rate and saccade velocity (Hepp and Henn 1983; Ling et al. 1999; Strassman et al. 1986; Walton and Freedman 2011). In strabismus, impaired binocular vision early in life might impede the development of normal tuning of neurons related to eye movements. If so, then one should find abnormalities in the normally machine-like relationships between saccade kinematics and measures of neural activity in PPRF. One goal of the present study, therefore, was to compare the basic tuning properties of PPRF neurons in normal and strabismic monkeys.

PPRF sends strong projections to abducens nucleus and to nucleus prepositus hypoglossi (NPH). This latter structure is believed to generate a horizontal eye position command by mathematically integrating eye velocity signals from PPRF (Chu and Kaneko 1995; Kaneko 1997). It has been argued that many individual neurons in PPRF carry signals specifically related to movement of one eye in normal primates (Van Horn et al. 2008; Zhou and King 1998). Thus abnormalities in PPRF have the potential to impact static eye misalignments in strabismus. If the directional component of saccade disconjugacy is due to abnormal cross talk between horizontal and vertical burst generators, then recordings of single neurons in this structure might reveal an abnormal distribution of preferred directions. Another goal of the present study, therefore, was to compare the distributions of preferred directions of saccade-related neurons in PPRF between normal and strabismic monkeys.

When normal monkeys perform saccades between targets that differ in both direction and distance, one eye must move more than the other. If one records from saccadic premotor burst neurons while normal monkeys perform this task, the activity of many neurons is more predictive of the motion of one eye than the cyclopean eye, or of the other eye (Van Horn and Cullen 2008; Zhou and King 1998). This remains a controversial issue with respect to normal monkeys, as some authors have argued that apparently disconjugate saccades result from a nonlinear interaction between saccadic and vergence systems (Bussetini and Mays 2005; Mays 1998), but...
there might be additional reasons to hypothesize some degree of monocular control of saccades in strabismus. Previous studies have demonstrated evidence of impaired binocular visual responses in cortical areas of strabismic monkeys (Crawford and von Noorden 1979; Kumagami et al. 2000; Mori et al. 2002). It is possible that impaired binocularity in the visual system might influence neurons in the saccadic system to develop in abnormal ways. Given these reports, one might expect PPRF neurons in strabismic monkeys to be strongly monocular. Another goal of the present study, therefore, was to determine whether the firing rates of PPRF neurons of strabismic monkeys specifically encode the velocity of one eye.

MATERIALS AND METHODS

Subjects and surgical procedures. Four adult female rhesus macaque monkeys (Macaca mulatta) served as subjects. Two (monkeys N1 and N2) had normal eye alignment and the other two (monkeys ET1 and XT1) had experimentally induced strabismus. The behavioral characteristics of strabismus in the latter two animals is described more thoroughly in our previously published work (Walton et al. 2014b). In brief, monkey XT1 had incongruent exotropia (25° when fixating with the right eye, and 35° to 40° when fixating with the left eye), resulting from a bilateral medial rectus tenotomy performed during the first week of life. Monkey ET1 wore prism goggles for the first 3 mo of life (left eye: 20 prism diopter, base-down; right eye: 20 prism diopter, base-in), resulting in incomitant esotropia (15°). After the procedures described above, strabismic monkeys were allowed to develop, without further intervention, until they reached maturity (7 yr of age for monkey XT1, and 5 yr for monkey ET1).

To prepare for neurophysiological experiments, the animals had head stabilization posts, recording chambers and scleral search coils surgically implanted. All surgical procedures were in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. All experimental protocols were approved by the Institutional Animal Care and Use Committee at the University of Washington. More detailed descriptions of our procedures are available in previously published work (Mustari et al. 2001; Ono and Mustari 2007). To allow the head to be restrained during experiments, a titanium post (Crist Instruments, Hagerstown, MD) was affixed to the skull with titanium bolts. To permit access to the brain stem, a titanium recording chamber (Crist Instruments) was positioned over a 16-mm craniotomy. Eye movements were measured with an electromagnetic system (CNC Engineering) employing scleral search coils, which were implanted underneath the conjunctiva of each eye (Fuchs and Robinson 1966; Judge et al. 1980). Most of our studies were conducted with binocular coils. Eye coil signals were calibrated independently for each eye under monocular viewing conditions. For some recordings, however, (17 from subject XT1; 8 from subject N1), the position of one eye was measured using an Eyelink 1000 tracking system (SR Research, Kanata, Ontario, Canada) (Walton et al. 2014a). We have verified that this system provides high temporal (1-kHz sampling) and spatial resolution (<0.5°) by directly comparing the Eyelink system signal with the search coil signal from the same eye. Calibration of Eyelink signals was performed during monocular viewing. Marking lesions were placed on some representative tracks in the vicinity of oculomotor nucleus and the supraoculomotor area. We used those landmarks, electrode track trajectories confirmed in histology and functional criteria, to identify the PPRF. We did not place marking lesions in the PPRF per se.

Behavioral tasks and visual display. During experimental sessions, the animals sat in a specially designed primate chair, at the center of a 1.5-m magnetic coil frame (CNC Engineering). Their heads were restrained during each recording session.

Visual targets consisted of a 0.25° red laser spot, back-projected onto a tangent screen. The viewing distance was 57 cm. When the animals fixated the target (within an invisible 5° window) with at least one eye; a small amount of applesauce or apple juice was delivered every 300 ms. Every 1.5–5 s, the target stepped to a new location, chosen at random (possible target locations were 0°, 2°, 4°, 6°, 8°, 10°, 12°, 15°, 18°, and 20° right or left, and 0°, 2°, 4°, 6°, 8°, 10°, 12°, and 15° up or down). Since the target could step directly between any of these positions, these locations could elicit saccades of >25° in any direction. In previous studies, this range of amplitudes has been sufficient to characterize horizontal premotor burst neurons in PPRF (Hepp and Henn 1983) and vertically tuned neurons in the rostral interstitial nucleus of the medial longitudinal fasciculus (MLF) (Moschovakis et al. 1991a, 1991b).

For each neuron recorded, we attempted to collect data with each eye fixating. For monkey ET1, monocular viewing was accomplished through the use of liquid crystal shutter goggles (Micron Technology, Boise, ID). When using the goggles, monkey XT1 had difficulty seeing targets presented far into the contralateral hemifield. Without the goggles, however, monkey XT1 consistently used the right eye to fixate targets of 15° or more to the right, and the left eye to fixate any target presented to the left of straight ahead. Between these two points, monkey XT1 alternated frequently. Due to this animal’s exotropia, one eye was always directed to a point in darkness, 25° to 40° away from the target. Thus we were able to elicit saccades of 20° or more in various directions, from both strabismic animals, with either eye fixating.

Unit recording and localization of PPRF. Single-unit activity was recorded using glass-coated tungsten microelectrodes (Alpha-Omega, Alpharetta, GA). The unit recording channel was sampled at 50 kHz. The timing of each detected spike (specifically, the time of minimum voltage) was coded, with high precision, as an event mark in our data acquisition system (CED Power 1401, Cambridge Electronic Design, Cambridge, UK). The isolation of each neuron was verified offline using Spike 2 (Cambridge Electronic Design, Cambridge, UK) and a custom spike sorting algorithm written in Matlab (Mathworks, Natick, MA).

PPRF is a physiologically defined structure that lies adjacent to other oculomotor areas. Immediately dorsal to the classic excitatory burst neurons, one often encounters fibers with burst-tonic discharge properties in the MLF. For this reason, we took the following steps to ensure that the recording locations were as similar as possible for strabismic and control animals. First, we found neurons that burst for all ipsiversive saccades and that were quiescent during steady fixation. Next, we systematically shifted the recording sites caudally, in steps of 0.25 mm, until abducens nucleus was found, identified by the characteristic “beehive” sounding burst-tonic activity heard on the audio monitor associated with the microelectrode signal. In all animals, we targeted the region of PPRF located 0.5–2 mm rostral to the previously identified border of abducens nucleus. Additional confirmation of our recording locations was based on MRI images and localization of other well-known neurophysiologically identified landmarks (omnipause neurons, superior colliculus, and the trochlear nerve). After saccade-related burst neurons were isolated and data collected, microstimulation was often used to verify that characteristic “ramp” eye movements could be evoked with parameters similar to those used in previously published PPRF stimulation studies [30–50 μA, 200 ms, 200–400 Hz (Gandhi et al. 2008; Walton et al. 2013)]. A given burst neuron was only included in our sample if it was well isolated; 2) background bursting was heard in association with ipsiversive saccades but no eye position related tonic activity was heard; and 3) the neuron burst for saccades and was quiescent during steady fixation.

Data analysis. Spike2 software was used for data acquisition and offline verification of unit isolation. All data were then imported into Matlab and analyzed using custom software. Eye and target position signals were passed through an anti-aliasing, 6-pole Bessel filter
Hz) and digitized at 1 kHz with 16-bit precision. Eye velocity was computed using five-point differentiation. Saccade onset was defined as the first point in time at which eye velocity exceeded 50°/s and acceleration exceeded 10,000°/s². Offset was defined as the first point in time at which either of two criteria were met: 1) eye velocity fell below 50°/s; or 2) eye velocity fell below 100°/s and the absolute value of acceleration fell below 10,000°/s². In previous studies, we have found that this algorithm successfully excluded the occasional large postsaccadic drift that resulted in a late reacceleration of the eye (Walton et al. 2013, 2014b). Saccade amplitude was computed separately for each eye and was defined as the change in eye position between movement onset and offset.

Long-lead burst neurons (LLBNs) and medium-lead burst neurons (MLBs) were distinguished based on the lead time between the onset of the burst and saccade onset, with a cutoff of 12 ms to separate the two cell classes (Hepp and Henn 1983). The onset of the saccade-related burst was considered to be the first time (within a 50-ms presaccadic window) at which the firing rate exceeded 100 spikes/s. The offset of the burst was defined as the time at which the firing rate fell below 100 spikes/s, or saccade offset, whichever came first. This frequency threshold is lower than that used by Hepp and Henn (1983). We used this approach to accommodate neurons with unusually weak bursts (see RESULTS). For each saccade, the number of spikes was counted between these threshold-defined points. For each neuron, linear regression analysis was used to investigate the relationship between the number of spikes in the burst and horizontal saccade amplitude.

The preferred direction for each LLBN or MLB was estimated by selecting amplitude-matched saccades (vectorial amplitudes of 8–12°) and plotting the number of spikes in the burst as a function of the polar direction of the saccade (Cullen and Guitton 1997). If this procedure yielded fewer than 25 trials, the Gaussian fits were based on all trials (Kaneko 2006). The location of the peak was taken to be the neuron’s preferred direction. The height of the function provides a direction-independent measure of the strength of the neuron’s bursts.

The relationships between instantaneous firing rate (FR) and the horizontal and vertical velocities of each eye were assessed using the following equation (Eq. 1):

\[
\text{FR}(t) = b_h + r_h E_{\text{hr}}(t - t_d) + r_{vv} E_{\text{vv}}(t - t_d) + r_v E_{\text{vl}}(t - t_d) + r_{vl} E_{\text{vvl}}(t - t_d)
\]

where \(E_{\text{hr}}, E_{\text{vv}}, E_{\text{vl}},\) and \(E_{\text{vvl}}\) are the horizontal left, horizontal right, vertical left, and vertical right eye velocities, respectively; \(b_h\) is the left right bias; and \(r_{vv}, r_{vvl}, r_{vl},\) and \(r_v\) are the horizontal left, horizontal right, vertical left, and vertical right eye velocity sensitivities, respectively. The expression \(t_d\) is designed to ensure temporal alignment of unit recordings with eye movements (i.e., by compensating for delays associated with neural processing). The dynamic lead time, \(t_d\), was calculated separately for each neuron by trying values of \(d\) ranging from 0 to 50 ms. The value that yielded the best fit was defined as the dynamic lead time. The results of this analysis were compared with reduced versions of Eq. 1 that only included the terms for one eye.

When fitting complex data with equations such as these, there is a well-known danger of using unnecessarily complex models, since adding additional terms will often result in slight increases in the variance accounted for. To address this, we computed the Bayesian Information Criterion (BIC), which imposes a compensatory penalty for adding terms to the model. To do this, we used the same equations and procedures described in Cullen et al. (1996). The use of the full Eq. 1 (i.e., with terms for both eyes) was considered justified if it resulted in a reduction in the BIC, compared with either of the monocular versions.

It is possible that saccade disconjugacy in strabismus might reflect, at least in part, contextually inappropriate commands from a disordered vergence system. To investigate this, we also tested a conjugate model (Eq. 2) that included terms for the first derivatives of the horizontal and vertical angles of strabismus:

\[
\text{FR}(t) = b_h + r_h E_{\text{hr}}(t - t_d) + r_{vv} E_{\text{vv}}(t - t_d) + r_v E_{\text{vl}}(t - t_d) + r_{vvl} E_{\text{vvl}}(t - t_d)
\]

where \(b_h\) is the bias; \(r_{vv}, r_{vvl},\) and \(r_v\) are the sensitivities to cyclopean horizontal, vergence, and cyclopean vertical velocities, respectively; \(r_{vvl}\) is the sensitivity to the first derivative of vertical strabismus angle; and \(E_{\text{hr}}, E_{\text{vv}}, E_{\text{vl}},\) and \(E_{\text{vvl}}\) are the cyclopean horizontal, vergence velocity, cyclopean vertical velocity, and first derivative of vertical strabismus angle eye velocities, respectively.

Data for the normal monkeys were analyzed using a simpler model with terms for conjugate horizontal and vertical velocity (Eq. 3):

\[
\text{FR}(t) = b_h + r_h E_{\text{hr}}(t - t_d) + r_v E_{\text{vl}}(t - t_d)
\]

For all dynamic analyses, we used a nonparametric bootstrap approach to estimate the probability distributions of model parameters (Carpenter and Bithell 2000; Sylvèstre et al. 2003; Walton et al. 2014a). By randomly resampling, with replacement, we obtained 2,000 data sets for each neuron. A given coefficient was considered significantly different from zero, if the 95% confidence interval did not include zero. Similarly, if the confidence intervals for two parameters did not overlap, then they were considered significantly different from each other.

RESULTS

The behavioral characteristics of monkeys ET1 and XT1 (and the results of visual acuity testing in monkey ET1) have been described in detail in a previous study (Walton et al. 2014b). Both animals were able to perform our saccade task with >90% accuracy, with either eye fixating, in several previously published studies (Walton et al. 2013, 2014a, 2014b). This is consistent with previous work showing that monkeys with surgically-induced exotropia are able to perform oculomotor tasks with either eye fixating (Economides et al. 2007). Monkey ET1 frequently altered her fixating eye, but showed a clear preference for the right eye. Monkey XT1 always used the left eye to fixate targets to the left of straight ahead (0°), and the right eye to fixate targets to more than 10° to the right. Between these two points, the animal frequently alternated, with a preference for the left eye.

Both strabismic monkeys displayed the characteristics of pattern strabismus (see Fig. 1 of Walton et al. 2014b). Monkey ET1 showed a modest increase in the angle of esotropia for upward eye positions. The vertical strabismus angle increased for leftward eye positions. Monkey XT1 showed a strong “A” pattern: the angle of exotropia increased for downward eye positions; the vertical strabismus angle increased for leftward eye positions.

Relationship between the number of spikes and horizontal amplitude

We recorded from a total of 84 saccade-related neurons in PPRF, while monkeys performed the target step task. Of these, 32 were classified as LLBNs and 52 as MLBs. Data were pooled for the two normal animals (12 LLBNs and 12 MLBs). For the strabismic animals, there were 20 LLBNs (6 from monkey ET1 and 14 from monkey XT1) and 40 MLBs (19 from monkey ET1 and 21 from monkey XT1).

As a first step, we assessed the relationship between the number of spikes in the burst and horizontal amplitude for normal and strabismic monkeys. Figure 1 plots the regression lines for all neurons in our sample. For all subjects,
there were many neurons for which the number of spikes increased linearly with horizontal saccade amplitude. In both strabismic animals, however, we also isolated many neurons that burst for saccades, and were quiescent during steady fixation, but that showed little or no correlation between the number of spikes and horizontal amplitude. Note that, for monkey XT1, there were eight neurons that discharged more than 40 spikes for 30° saccades; this was the case for only one neuron recorded from the normal monkeys. Figure 2 compares the slopes for the linear regression relating number of spikes to horizontal amplitude for the two eyes for all neurons in our sample. For the normal animals, the absolute values of the slopes ranged from 0.12 to 1.01 (mean 0.63 ± 0.27); all but two had slopes between 0.3 and 1.0 (circles). For the strabismic animals, the absolute values of the slopes ranged from 0.01 to 1.66. For more than one-third (23/60), the absolute value of the slope was <0.25. The absolute value of the slope exceeded 1.00 for 13/60 neurons. For subject XT1, the mean slopes for the left and right eyes were 0.69 ± 0.57 and 0.53 ± 0.54, respectively. Interestingly, there appeared to be a bimodal distribution, one group with unusually large slopes and a second with unusually small slopes. For subject ET1, the mean slopes for the left and right eyes were 0.33 ± 0.34 and 0.55 ± 0.38, respectively. Welch’s test was used to compare the slopes for normal vs. strabismic monkeys. No significant differences were found for LLBNs (P = 0.21), MLBs (P = 0.52), or when the comparison was done with the two cell types pooled (P = 0.28).

Fig. 1. Linear regression fits relating the number of spikes in each burst to horizontal saccade amplitude. Each line represents data from a single neuron. A, C, and E: left eye. B, D, and F: right eye. A and B: for almost every long-lead burst neuron (LLBN; red) and medium-lead burst neuron (MLB; blue) recorded from the normal animals, the number of spikes increased monotonically with horizontal amplitude. For many neurons recorded from subjects ET1 (C and D) and XT1 (E and F), however, the slopes were near 0. On the other hand, there were eight neurons recorded from monkey XT1 for which the number of spikes exceeded 40 for 30° saccades (horizontal dashed lines). This was the case for only one neuron recorded from the normal animals.
As noted above, some of the neurons recorded from strabismic animals had unusually large slopes, while others had unusually small slopes. With this in mind, we sought to compare the variability of the slopes for normal and strabismic animals. Indeed, the variability was significantly higher for the strabismic monkeys than for the normal monkeys (χ² test for equal variances, P < 0.01 for both eyes).

**Untuned cells.** In recordings from the strabismic animals, 7/60 (12%) cells consistently burst for ipsiversive saccades, but showed little or no modulation with any kinematic variables we tested. A cell was considered “untuned” if the fits (see MATERIALS AND METHODS) relating the number of spikes to horizontal amplitude, vertical amplitude, and polar direction all yielded R² values < 0.10. This did not include four LLBNs (three from monkey ET1 and one from monkey XT1) that preferred saccades of a particular amplitude and direction (all preferred saccades of less than 10°). Since horizontal and vertical target steps could be up to 30° vertically, or 40° horizontally, linear fits such as those depicted in Fig. 1 often showed low correlations for neurons with vector-specific movement fields. For the Gaussian fits to polar direction, however, all four neurons had R² values > 0.30. In contrast, the untuned cells did not appear to have movement fields, at least over the range of saccade vectors we were able to elicit. No untuned cells were found in the normal monkeys.

**Distribution of preferred directions.** Some neurons recorded from strabismic animals clearly modulated their activity in association with vertical amplitude (either the amplitude of an upward or downward saccade or the vertical component of an oblique saccade). Figure 3 shows four groups of trials from one such neuron, recorded from the right PPRF of monkey XT1. The cell consistently burst for upward saccades (A), but was quiescent for downward (B), rightward (C) and leftward (D) saccades. Figure 4 shows the relationships between the number of spikes in the burst and amplitude for another example neuron. The number of spikes was strongly related to the amplitude of the vertical component, but the neuron was almost completely insensitive to horizontal amplitude.

We sought to quantitatively determine the preferred direction for each neuron. To do this, we plotted the number of spikes in the burst as a function of polar direction, then fit these data with Gaussian functions (see MATERIALS AND METHODS). Untuned cells were excluded from this analysis, as were three other neurons for which the Gaussian fits yielded R² values less than 0.10. For the strabismic animals, this left a sample of 36 MLBs and 14 LLBNs. Figure 5 shows an example fit for one neuron in monkey ET1. This neuron discharged at least a small burst for saccades in all directions, but showed a clear preference for upward saccades (left eye Gaussian peak = 77.8 ± 4°, 95% confidence interval; right eye Gaussian peak = 88.4 ± 5.3°, 95% confidence interval).

Figure 6 plots the preferred directions of all neurons for which the Gaussian fits were performed. For subjects N1 and N2, all neurons had preferred directions within 30° of horizontal. For both strabismic animals, the preferred directions were far more variable on one side of the brain than the other. For right PPRF in subject ET1, all neurons had preferred directions within 30° of horizontal for at least one of the two eyes (one cell had a predominantly vertical preferred direction only for the right eye). In contrast, the preferred directions for left PPRF varied widely within a range that spanned more than 180°. A χ² test for equal variances showed that the variance of preferred direction was significantly greater for left PPRF (P < 0.001). A similar result was found for subject XT1, except that it was left PPRF that was less variable. This difference was also highly significant (P < 0.001). For subject ET1, the variance for left PPRF was significantly greater than for the pooled PPRF data from the control animals (P < 0.001). No significant difference was found between the variance for right PPRF of monkey ET1 vs. the normal controls (P = 0.64). For subject XT1, a significant difference was found between the variance for right PPRF and pooled data from the control animals (P < 0.001). No significant difference was found between left PPRF of subject XT1 and data from the control animals (P = 0.33).

One might wonder if the larger variability of preferred directions on one side of the brain might be a consequence of poor Gaussian fits for the strabismic animals. However, there was no significant difference between the mean R² for predominantly vertical neurons (0.51) and predominantly horizontal ones (0.49) (two tailed t-test, P > 0.05).

For the strabismic animals, some neurons (MLBs: 11/36 for the left eye, 12/36 for the right eye; LLBNs: 1/14 for the left eye, 0/14 for the right eye) had preferred directions that deviated more than 45° from the horizontal. For the more abnormal side of the brain, over half of MLBs (11/21 for the left eye; 11/21 for the right eye) had predominantly vertical preferred directions. Vertically tuned neurons were found throughout PPRF, often after encountering numerous horizontally tuned burst neurons.

It is possible that a given neuron’s preferred direction might vary, depending on which eye views the target. This could happen, for example, if saccade direction is influenced by visually dependent signals along a parallel pathway. To address this issue, the above analyses were repeated, separating the data according to which eye was directed at the target. This was done only for neurons recorded from the strabismic ani-
mals, and only if a minimum of 25 saccades existed for each eye. These criteria were met for 24 neurons, including 6 predominantly vertical neurons. The locations of the Gaussian peaks were compared for the two fixation conditions; if the 95% confidence intervals did not overlap, the shift was taken to be statistically significant. For the right eye, 4/24 neurons (all from monkey XT1) showed a significant shift, 3 of which had predominantly vertical preferred directions. Three of those four neurons also showed a significant shift for the left eye, including two with predominantly vertical preferred directions. However, only one neuron had a predominantly vertical preferred direction when one eye was fixating and a predominantly horizontal preferred direction when the other eye was fixating (left eye fixating: 50.9°; right eye fixating: 13.6°).

As noted above, PPRF is defined in terms of neuronal response properties. Since other oculomotor related structures may be found nearby, one must consider the possibility that the vertically-tuned neurons in our sample might reside outside of PPRF. For example, in normal monkeys, vertically tuned burst activity has been reported from recordings of fibers in the adjacent MLF (Pola and Robinson 1978). It is very unlikely that our vertically-tuned neurons were in MLF, however, for several reasons. First, since MLF is a fiber track, the spikes have a very different waveform from those recorded from soma (see Fig. 1D in King et al. 1976). We examined the spike waveforms for all neurons in our sample, and none exhibited a waveform characteristic of MLF fibers (i.e., a positive phase that precedes the negative phase).

Second, microstimulation of MLF causes adduction of the ipsilateral eye at latencies of ~7 ms (Pola and Robinson 1978). Importantly, the eye quickly moves back toward the starting position after stimulation offset, since the artificially imposed phasic signal does not pass through the neural integrator. In contrast, PPRF stimulation evokes “ramp-like” eye movements with a velocity waveform that approximates a low-pass filtered square-wave (Cohen and Komatsuzaki 1972; Gandhi et al. 2008). We collected microstimulation data from 26 PPRF sites, including 6/12 sites at which vertically-tuned neurons were recorded. Some of these data have been reported previously (Walton et al. 2013). Figure 7 shows the effects of microstimulation of a site where one vertically-tuned neuron was recorded in monkey XT1. With the exception of small deviations at the beginning and end, the horizontal velocity was constant for the duration of the stimulation. This pattern, which is very typical of PPRF stimulation (Cohen and Komatsuzaki 1972; Gandhi et al. 2008; Walton et al. 2013), was observed for all sites.
Movements typical of MLF stimulation were not observed for any of the 23 sites.

**Dynamic analysis.** The above data suggest that pattern strabismus might be associated with abnormally strong cross talk between horizontal and vertical saccadic commands in brain stem. We wondered whether the predominantly vertical neurons described above carry signals related to vertical saccade velocity. We tested this possibility by performing dynamic analyses, using Eqs. 1–3, all of which contain terms related to vertical velocity. Since these analyses often involved comparing the correlations between instantaneous firing rate and eye velocity separately for the two eyes, it was important to ensure that the positions of both eyes were measured using the same technology (eye coils). We, therefore, excluded any neuron recorded while the Eyelink was used for one eye. This left a sample of 43 neurons from the strabismic animals (25 from monkey ET1, 18 from monkey XT1) and 16 from normal subject N2.

We first fitted the data with dynamic Eq. 1, which contains separate terms for horizontal and vertical saccade commands. Figure 8 shows the horizontal and vertical eye velocity coefficients, derived from the bootstrap analysis. Note that each coefficient represents the slope of the unique relationship between firing rate and the associated eye velocity variable, corrected for each of the other terms in Eq. 1. Interestingly, for 20/43 neurons for the strabismic animals, the horizontal velocity coefficients were significantly different from zero for both eyes, yet were opposite in sign (Fig. 8, compare A and B).

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**Fig. 4.** Relationship between the number of spikes in the burst and the horizontal (A and B) and vertical (C and D) amplitude of the left (A and C) and right (B and D) eyes for a neuron recorded from left PPRF of monkey ET1. The neuron clearly has a downward preferred direction and quite strong bursts.

**Fig. 5.** Example Gaussian fits, relating the number of spikes in the burst to polar saccade direction for the left eye (A) and right eye (B). This example neuron was recorded from monkey ET1. The inset in A illustrates the convention: 0° = right, 180° = left, 90° = up, 270° = down.
Mathematically, this is equivalent to saying that these neurons modulated their firing rate in association with the first derivative of strabismus angle, which would be vergence velocity in a normal monkey. We tested this more explicitly by fitting the data with a dynamic, conjugate model that included a term for vergence velocity ($\text{Eq. 2}$). For 13/43 neurons, the addition of vergence velocity to the model reduced the BIC by at least 0.1 and increased the mean $R^2$ from 0.15 to 0.33. For 6 of these 13 neurons, there was virtually no correlation between firing rate and cyclopean horizontal velocity ($R^2 < 0.10$). For four of those six, the further addition of vertical velocity to the model reduced the BIC.

Based on a reduction of at least 0.1 in the BIC for $\text{Eq. 1}$, the firing rates of 12/43 neurons were related to the vertical velocity of at least one of the two eyes. Of the 12 neurons classified as vertically tuned by the Gaussian fits, 9 were included in the dynamic analysis. Adding vertical velocity (of at least one of the two eyes) to the model reduced the BIC by at least 0.1 for 7/9 of these neurons. Similar results were found when the data were fit with the conjugate $\text{Eq. 3}$. By comparing...
son, none of the neurons from monkey N2 had a vertical velocity sensitivity larger than 0.28, and the addition of vertical velocity to the model never reduced the BIC.

As noted above (see MATERIALS AND METHODS), we also sought to determine whether the full Eq. 1, which included terms for both eyes, would provide a better fit than either of the reduced, monocular versions. For 8/43 neurons, the BIC criteria was lower (by at least 0.1) for the full version of Eq. 1, compared with either of the reduced, monocular versions. For a further 8/43 neurons, the full model reduced the BIC by >0.05 for one eye but <0.1 for the other eye. For these neurons, the firing rate could be predicted using terms for only reduced, monocular versions. For 8/43 neurons, the BIC criteria was lower (by at least 0.1) for the full version of Eq. 1, compared with either of the reduced, monocular versions. For a further 8/43 neurons, the full model reduced the BIC by >0.05 for one eye but <0.1 for the other eye. For these neurons, the firing rate could be predicted using terms for only

Fig. 7. Microstimulation of an example site at which a vertically-tuned burst neuron was recorded from monkey XT1. The gray shaded area indicates the period of stimulation (200-ms train, 400 Hz, 30 μA). Three traces are plotted, aligned with respect to the first pulse in the stimulation train. A and B: horizontal eye position and velocity traces, respectively. C and D: vertical position and velocity traces, respectively. Note that stimulation evoked constant velocity ramp eye movements that persisted for the duration of each train. These characteristics are typical of PPRF stimulation. Insets compare superimposed example spike waveforms from all vertically-tuned neurons to that of a burst-tonic neuron recorded more dorsally.

Fig. 8. Eye velocity sensitivities, derived from a dynamic analysis that predicts the instantaneous firing rate based on horizontal and vertical velocity terms for each eye: A, left eye; B, right eye. Red, subject XT1; blue, subject ET1. For the sake of comparison, A also shows data from subject N2 (black), derived from a simplified version of the model that included terms for horizontal and vertical cyclopean velocity. A: the horizontal gray bar indicates the range of vertical sensitivities found in this normal subject. Triangles represent neurons recorded from left PPRF; circles represent right PPRF. For neurons recorded on both sides of the brain, the horizontal velocity coefficients were usually positive for the left eye (A) and negative for the right (B). Note that this does not mean that cells in left PPRF discharged for rightward saccades. Rather, it means that, for a given (leftward) velocity of the right eye, these neurons discharged at a higher rate if the left eye’s (leftward) velocity was lower. Most neurons in right PPRF, however, discharged at a higher rate if the (rightward) velocity of the left eye was higher (independent of the velocity of the right eye). Mathematically, this is equivalent to saying that most PPRF neurons in the strabismic animals were sensitive to dynamic changes in strabismus angle, independent of cyclopean horizontal velocity. Note also that many neurons had vertical velocity sensitivities outside the range obtained from the normal subject.

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one eye, but adding terms for the other eye did little or nothing to improve the fit. For a further five neurons, the BIC was the same, or lower, for one eye’s reduced monocular model compared with the full version of Eq. 1, but the BIC for the other eye was reduced by $>0.05$ and $<0.1$. For the remaining neurons, the full model improved the fit more for one of the monocular models than the other, but that difference was small. Thus similar to what has been reported for premotor burst neurons in normal monkeys (Van Horn et al. 2008), there appears to be a continuum between fully binocular neurons and fully monocular ones, with a majority of neurons falling somewhere in between. Interestingly, however, for the strabismic monkeys in our sample, there were far more neurons with a bias for the left eye than for the right. This can be seen in Fig. 9, which compares the $R^2$ values obtained from the monocular right eye and left eye models (Eq. 1). Overall, for both strabismic monkeys, there was a clear bias in favor of the left eye. This was the case for PPRF neurons on both sides of the brain. This is surprising for monkey ET1, which preferred to use the right eye.

The presence of the “untuned” and vertically-tuned neurons suggests the possibility that the neural signals carried by these cells might be noisier in the strabismic animals. If so, the $R^2$ values for the dynamic analysis should be lower for these subjects. The mean $R^2$ value for Eq. 3 appeared to be higher for the normal animals (normal: 0.36; strabismus: 0.27), but this difference did not reach statistical significance (two-tailed t-test, $P = 0.07$).

**DISCUSSION**

The tuning characteristics of PPRF neurons differed in several respects between the strabismic animals and the normal controls. First, the relationship between the number of spikes in the burst and horizontal amplitude was more variable for the strabismic animals. Second, for both strabismic animals, we found an unexpectedly broad distribution of preferred directions on one side of the brain. Third, for seven neurons in the strabismic monkeys, the number of spikes in the burst and the firing rate were not correlated with any of the variables we tested. We found no such neurons in the normal monkeys.

The vertically-tuned neurons recorded from the strabismic animals deserve further discussion. Comparisons with previous literature are complicated by the fact that PPRF is a physiologically-defined structure that lies adjacent to other oculomotor areas, such as the MLF. For this reason, we sought to directly compare data from strabismic and normal monkeys, using the same approach for identification of PPRF, the same inclusion criteria, and the same statistical procedures. We adopted fairly strict criteria that excluded several vertically-tuned burst-tonic neurons that were likely to be in MLF. The vertically tuned cells we included in our sample were quiescent during steady fixation. Indeed, approximately one-third (31% for the left eye, 33% for the right eye) of the MLBs in the strabismic monkeys had preferred directions tuned more than 45° away from horizontal, but only one of the LLBNs did (left eye only). We used identical procedures in our recordings from normal animals, but found no neurons in PPRF with predominantly vertical preferred directions. Furthermore, for our strabismic animals, the distributions of preferred directions were strikingly different between the two sides of the brain. The “normal” side was different for subjects ET1 and XT1, even though the chambers were on the same side of the head in both animals. Thus, given our angled approach, it is hard to see how the asymmetry could be attributed to faulty localization of PPRF. Finally, microstimulation was performed at 6 of the 12 sites at which vertically-tuned neurons were recorded. For all six sites, the evoked movements displayed characteristics typical of PPRF stimulation, including relatively constant velocity ramp eye movements (of both eyes) that persisted for the duration of each train (Cohen and Komatsuzaki 1972; Gandhi et al. 2008). Taken together, these observations strongly suggest that the tuning of many PPRF neurons is abnormal in some forms of strabismus.

As noted in the MATERIALS AND METHODS section, subject ET1 preferred to use the right eye, while subject XT1 preferred to use the left eye. Thus, for both strabismic monkeys, the more normal side of the brain was the one that corresponded to oculomotor control of the preferred eye.

The present results should be discussed in the context of our previous study (Walton et al. 2013) of microstimulation in PPRF in the same two strabismic animals used for the present report. One of the more surprising aspects of the stimulation data was the high degree of variability with respect to the conjugacy and direction of evoked movements. At some sites, the evoked movements were nearly conjugate; at others, the directional disconjugacy exceeded 45°. In contrast, evoked movements are consistently conjugate in normal monkeys (Cohen and Komatsuzaki 1972; Walton et al. 2013). The variability of the stimulation data implies that the abnormalities in PPRF may be somewhat “patchy”. If so, then the large differences between right and left PPRF in the present study should be interpreted with a degree of caution. A significant number of vertically-tuned neurons might be present on the more normal side of the brain, in areas we failed to sample.
Conversely, on the more variable side of the brain, we might have failed to sample areas of PPRF with no vertically tuned neurons. However, we did attempt to provide the same sampling across the PPRF in electrical stimulation and single unit studies. One cannot determine, on the basis of the present data, whether or not the vertically tuned neurons actively contribute to directional saccade disconjugacy, or to vertical duction at all. If they project to abducens nucleus, then their primary contribution would be to horizontal movement, despite their preference for vertical saccades. That is, they might be passing inappropriate vertical signals to horizontal rectus motoneurons. Indeed, this type of abnormal horizontal-vertical cross talk has been suggested as an explanation for cross-axis patterns of saccade disconjugacy (Walton et al. 2014b). If this is the case, then the neural basis for the vertical component of movements evoked by PPRF stimulation may lie elsewhere. The integration of such disordered signals in NPH would, presumably, cause the static horizontal eye position to be influenced by the vertical component of the saccade. In this way, a disordered saccadic system might contribute to the cross-axis positional deviations that characterize pattern strabismus.

There is another possibility. Recent studies have provided anatomical evidence that extraocular muscles have distinct compartments (Demer and Clark 2015, 2014). These authors have suggested that differential contraction of dorsal and ventral compartments of horizontal rectus muscles may contribute to vertical duction. If the vertically tuned neurons in the present study preferentially drive abducens neurons innervating one compartment, then they might have an influence on vertical duction. Since the anatomical targets of vertically-tuned PPRF neurons are unknown, however, the present data cannot resolve this issue.

We found seven “untuned” cells in the strabismic monkeys, but none in the normal animals. As far as we are aware, no previous studies have reported such neurons in PPRF. Cramer and Waitzman (2007) reported finding four cells fitting that description (out of a sample of 53 recorded from normal monkeys) in the central mesencephalic reticular formation, but they found none in their sample of 44 PPRF neurons. Thus our untuned cells might be another abnormality. In any case, between the predominantly vertical neurons and the untuned neurons, we found that, for 23/60 (38%) saccade-related neurons in PPRF in the strabismic animals, the number of spikes in the burst was unrelated, or only weakly related, to the horizontal saccade amplitude (i.e., $R^2 < 0.25$). This was the case for only 1/24 neurons (4%) in the normal monkeys. Note that this cannot be attributed to the use of a video eye tracker for one eye in some of the recordings, since the percentage of neurons recorded using two eye coils was similar between strabismic (71%) and normal (67%) monkeys.

When we performed a dynamic analysis, the slopes relating firing rate to horizontal velocity were often in opposite directions for the two eyes. This could happen if a strong additional drive, added downstream, influences the horizontal velocity of the two eyes in opposite directions. For example, consider a neuron in right PPRF with a positive slope for the left eye and a negative slope for the right eye. This means that, if one holds the instantaneous horizontal velocity constant for the left eye, the neuron fires at a higher rate if the right eye’s rightward velocity is lower. This is exactly what would happen if a contextually inappropriate convergence signal was added downstream during the saccade. However, comparable results have not been reported for premotor saccadic burst neurons recorded from normal monkeys performing a combined saccade-vergence task (Van Horn et al. 2008). It is also worth noting that a similar dynamic analysis of the saccade-related bursts of abducens neurons yielded different results from the same strabismic subjects, with only 9/52 neurons classified as “opposite” (Walton et al. 2014a).

An alternative interpretation is that the opposite direction horizontal sensitivities are related to cross-axis brain stem saccadic signals that may underlie pattern strabismus. For these animals, saccades that were purely vertical for the cyclopean eye were usually associated with opposite-direction horizontal movement. For example, monkey XT1 exhibited a strong “A” pattern (Walton et al. 2014b), which means that downward saccades were nearly always associated with increased exotropia, while upward saccades were almost always associated with decreased exotropia. This means that a given neuron’s vertical sensitivity could also be described in terms of opposite-direction horizontal velocity sensitivities. Thus the apparent vergence sensitivities of some neurons should be interpreted with some caution. It may reflect a contribution of contextually inappropriate slow vergence to saccade disconjugacy, but it may also be the case that the dynamic model was unable to distinguish between vergence sensitivity and vertical velocity sensitivity for our subjects with pattern strabismus.

It is possible that this association between apparent vergence sensitivity and vertical velocity sensitivity may be more than a statistical oddity. Perhaps, at birth, there is greater cross talk between horizontal and vertical burst generators in normal primates, with many of the “inappropriate” synaptic connections being lost in an experience-dependent fashion. In normal monkeys, PPRF neurons discharge, while monkeys make vertical saccades between near and far targets, even when there is no horizontal component for the cyclopean eye (Van Horn and Cullen 2008). We propose that, in pattern strabismus, the consistent association between upward or downward saccades and a particular vergence change might result in greater retention of connections between PPRF neurons and the vertical burst generator. Mathematical integration of these cross-axis signals in NPH might lead to some of the clinical symptoms associated with pattern strabismus.

Due to the complexity of these effects, it was not possible to determine with certainty how many individual neurons exclusively encoded the velocity of one eye. Given that little is known about oculomotor neurophysiology in strabismus, it is difficult to say whether the observed movement of one eye is being influenced by events downstream, such as a muscle weakness and/or a contextually inappropriate (and possibly asymmetrical) slow vergence command. What we can say is that there appears to be considerable diversity with respect to how well the firing rates of individual PPRF neurons reflect the velocity of one eye, or the cyclopean eye. The fact that microstimulation of PPRF in these same subjects (including many of the recording sites for the present report) evokes disconjugate movements (Walton et al. 2013) suggests that this diversity may be sufficient to partially account for the observed disconjugacy of visually guided saccades (Walton et al. 2014b). Taken together, these data suggest that PPRF neurons in strabismic monkeys carry a mixture of signals not typically observed in normal primates.
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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: M.M.G.W. conception and design of research; M.M.G.W. performed experiments; M.M.G.W. analyzed data; M.M.G.W. and M.J.M. interpreted results of experiments; M.M.G.W. prepared figures; M.M.G.W. and M.J.M. edited and revised manuscript; M.M.G.W. and M.J.M. approved final version of manuscript.

REFERENCES


