Abnormal Activity of Neurons in Abducens Nucleus of Strabismic Monkeys

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PURPOSE. Infantile strabismus is characterized by persistent misalignment of the eyes. Mounting evidence suggests that the disorder is associated with abnormalities at the neural level, but few details are known. This study investigated the signals carried by abducens neurons in monkeys with experimentally induced strabismus. We wanted to know whether the firing rates of individual neurons are exclusively related to the position and velocity of one eye and whether the overall level of activity of the abducens nucleus was in the normal range.

METHODS. We recorded 58 neurons in right and left abducens nuclei while strabismic monkeys (one esotrope and one exotrope) performed a saccade task. We analyzed the firing rates associated with static horizontal eye position and saccades by fitting the data with a dynamic equation that included position and velocity terms for each eye. Results were compared to previously published data in normal monkeys.

RESULTS. For both strabismic monkeys the overall tonic activity was 50 to 100 spikes/s lower, for every suprathreshold eye position, than what has previously been reported for normal monkeys. This was mostly the result of lower baseline activity; the slopes of rate-position curves were similar to those in previous reports in normal monkeys. The saccade velocity sensitivities were similar to those of normal monkeys, 0.35 for the esotrope and 0.40 for the exotrope. For most neurons the firing rate was more closely related to the position and velocity of the ipsilateral eye.

CONCLUSIONS. These data suggest that strabismus can be associated with reduced neural activity in the abducens nucleus.

Keywords: esotropia, strabismus, neurophysiology, exotropia, abducens nucleus

Infantile strabismus is a common disorder, characterized by a chronic misalignment of the eyes that often leads to significant impairment of oculomotor and visual function.^{1,2} The disorder can result from any of a large number of factors, including disturbance of binocular vision early in life,^{1,3–9} connective tissue abnormalities,^{10–14} and abnormalities of extraocular muscles and/or their innervation,¹⁵ but in many cases the cause is not understood. Given the above abnormalities, one might expect to also find differences in the signals carried by motoneurons. Indeed, this is the case for oculomotor nucleus,^{16,17} but the available data for abducens nucleus are limited to a recent preliminary report that abducens activity was reduced for an exotropic monkey performing a smooth pursuit task (Agaoglu MN, et al. *IOVS* 2014;55:ARVO E-Abstract 2572).

Since strabismus is associated with a loss of appropriately coordinated binocular visual responses, brainstem oculomotor structures might be abnormally monocular as well. For example, disordered premotor inputs might combine at the level of individual neurons to generate position and velocity commands exclusively related to one eye. Abducens motoneurons and internuclear neurons might carry different signals, with each monocularly encoding the position of the eye to

which they project. Alternatively, individual neurons might carry signals related to both eyes, perhaps with varying preferences for one eye or the other. Sylvestre and Cullen¹⁸ have tested these hypotheses in normal monkeys performing a saccade-vergence task. They found that many neurons encoded the motion of both eyes to varying degrees but that, overall, there was a preponderance of neurons encoding the position and velocity of the ipsilateral eye. However, given the well-documented loss of binocular visual responses and evidence for disordered development of oculomotor circuits in strabismus,^{16,17,19–21} one cannot assume, a priori, that the same is true of abducens neurons in strabismus. We repeated the dynamic analyses employed by Sylvestre and Cullen¹⁸ in two monkeys with experimentally induced strabismus, taking advantage of saccade disconjugacy and variation in horizontal strabismus angle to dissociate the velocity and position of the two eyes.²² Our findings were similar to those in the previous report in normal monkeys; the firing rates of most abducens neurons were more closely related to the position and velocity of the ipsilateral eye.

Several studies have provided evidence that strabismus can be associated with changes at the level of eye muscles. For example, magnetic resonance imaging studies in humans with

esotropia have demonstrated supranormal size of medial rectus muscles.²³ Scott²⁴ sutured the globe to the orbital wall in several monkeys and reported changes in sarcomere size associated with prolonged abduction. It has also been suggested that fiber type changes due to altered neuronal drive would also result in changes in muscle shortening velocity and overall muscle force generation capacity.^{25–27} Additionally, it has recently been reported that the superior and inferior halves of the lateral rectus muscle are innervated by different branches of the abducens nerve²⁸ that may have functional implications.²⁹ Furthermore, a variety of muscle fiber types have been described in extraocular muscles, falling into two broad categories: multiply innervated fibers (MIF) and singly innervated fibers (SIF).³⁰ Multiply innervated fibers and SIFs are innervated by different populations of motoneurons.^{31,32} It is possible that abnormalities of MIFs, or their innervation, may contribute to static eye misalignments in strabismus.

Taken together, these studies indicate previously unappreciated complexities at the level of eye muscles. A full understanding of the implications of these studies, however, will also require knowledge of the neural commands sent to these muscles. For example, several studies have convincingly shown that converged eye positions in normal primates are associated with less force in the horizontal rectus muscles than predicted by neuronal activity.^{29,33,34} Thus, contrary to what one might postulate, single-unit recording studies showed that motor neuron firing rates do not strictly correlate with measured muscle force.^{34,35} Given these complexities, one cannot assume, a priori, that horizontal strabismus angle is simplistically related to abnormal levels of activity in abducens neurons.

METHODS

Subjects and Surgical Procedures

Two female macaque monkeys (*Macaca mulatta*) served as subjects. The methods used to induce strabismus in these animals and the behavioral tasks are described in detail in our previously published work.^{20,22,36,37} Briefly, subject ET1 wore prism goggles for the first 3 months of life, resulting in incomitant esotropia (it was typically $\sim 15^\circ$ but could range from $\sim 25^\circ$ esotropia to 2° of exotropia). Subject XT1 underwent a bilateral medial rectus tenotomy during the first week of life, which resulted in a strong “A” pattern exotropia (25° when fixating with the right eye and 35° – 40° when fixating with the left eye). A small vertical nystagmus was occasionally observed when the animals were drowsy, but when they were alert and motivated, both monkeys were able to maintain fixation on the targets without detectable eye movements.

To prepare for neurophysiological experiments, the monkeys underwent placement of scleral search coils^{38,39} and recording chambers at 5 years of age (monkey ET1) and 7 years of age (monkey XT1). Detailed descriptions of our surgical procedures can be found in reports by Mustari et al.³⁶ and Ono and Mustari.³⁷ All procedures complied with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals. Experimental protocols were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Washington. For six recordings in monkey XT1, the position of the left eye was measured using an Eyelink 1000 tracking system (SR Research Ltd., Kanata, ON, Canada). For these recordings the camera was positioned to the left of the sagittal plane and

oriented at an angle, such that the left eye's typical range was aligned with that of the camera.

The behavioral tasks and visual display were identical to those described by Walton et al.²² Briefly, the animals were rewarded with a small amount of apple juice or applesauce, delivered at regular intervals, whenever they positioned at least one eye within an imaginary 5° circular window surrounding the target. Saccadic eye movements and periods of steady fixation were elicited by stepping the target to various locations on the tangent screen within a range of $\pm 25^\circ$ horizontally and $\pm 15^\circ$ vertically, in 2° to 5° increments.

Single-Unit Recording and Localization of the Abducens Nucleus

Extracellular recording was performed using glass-coated tungsten microelectrodes (Alpha-Omega, Alpharetta, GA, USA) with impedances ranging from 1 to 5 mega-ohms. For each animal, the right and left abducens nuclei were identified based on the characteristic “beehive” burst-tonic activity and the location of each track with respect to well-known neurophysiological landmarks (i.e., paramedian pontine reticular formation [PPRF], superior colliculus, and the trochlear nucleus). The locations of PPRF and the abducens nucleus were further confirmed by observing the eye movements evoked by microstimulation (biphasic current pulses, 0.25 ms, 30–50 μ A, 100–400 Hz, 100- to 200-ms train duration; see Walton et al.²⁰).

Data Analysis

Spike2 software (Cambridge Electronic Design, Cambridge, UK) was used for the initial verification of unit isolation. A custom spike sorting algorithm was used for offline spike detection. All data analyses were conducted in Matlab (MathWorks, Natick, MA, USA) using custom software. Saccades were identified using the same sliding window algorithm we have described in a previous study.²²

Steady fixation was defined as a period of at least 350 ms during which the vectorial velocity (of either eye) never exceeded a threshold of 10° /s. To avoid postsaccadic drifts and perisaccadic neural activity, data from the first and last 100 ms of each fixation period were excluded. This done, the mean firing rate and the mean horizontal positions of each eye were computed over the remaining portion of each fixation period. Rate-position curves were then obtained for each eye by performing linear fits to these data. To avoid the well-known nonlinearity associated with subthreshold eye positions,^{40,41} these fits were based only on fixations with mean firing rates of at least 20 spikes/s. The tonic firing rate was considered significantly related to horizontal static eye position if the 95% confidence interval for the slope did not include zero for each eye and associated neural recording.

The above analyses can demonstrate that the firing rate is better correlated with the position of one eye than the other but cannot determine the unique relationship between each eye and firing rate.¹⁸ In order to do this, the rate-position curve data, for static eye positions, were fit with the following planar equation (Equation 1):

$$FR = b_{ic} + k_i IE + k_c CE \quad (1)$$

where b_{ic} , k_i , and k_c represent the bias, ipsilateral eye position, and contralateral eye position sensitivities, respectively. Using this equation, we estimated the probability distribution of model parameters using a nonparametric bootstrap approach. Briefly, 2000 data sets were obtained for each neuron by randomly resampling with replacement, and model parameters

were computed for each. A given parameter was taken to be statistically significant if the associated 95% confidence interval did not include zero. Parameters were taken to be significantly different from each other if their confidence intervals did not overlap.

For dynamic analyses involving saccadic eye movements, the relations between instantaneous firing rate and horizontal position and velocity were assessed separately for the two eyes according to the following equation (Equation 2):

$$FR(t) = b_{ic} + k_i IE(t - t_d) + k_c CE(t - t_d) + r_i \bar{IE}(t - t_d) + r_c \bar{CE}(t - t_d) \quad (2)$$

where b_{ic} , k_i , k_c , r_i , and r_c represent the bias, ipsilateral eye position, contralateral eye position, ipsilateral eye velocity, and contralateral eye velocity sensitivities, respectively. This analysis was confined to the periods corresponding to saccadic eye movements. The expression t_d represents the neuron's dynamic lead time. It was determined individually for each neuron by trying values of d ranging from 0 to 50 ms. The dynamic lead time for each neuron was defined as the value that yielded the best fit.^{18,42-44}

To measure the number of spikes and the firing rate specifically related to the saccade, we used the regression coefficients from the rate-position curves to compute the instantaneous firing rate expected for the current eye position. This was done in two different ways: by estimating the saccade-related activity for each eye (1) based on its own rate-position curve and (2) based on the position of whichever eye yielded the best rate-position curve fit. However, the results were highly similar between the two approaches, so we report results obtained using only the latter approach. As previous studies have done for normal monkeys, the number of spikes specifically related to the saccade was taken to be equal to the difference between the observed number of spikes and the number predicted on the basis of eye position.⁴⁵ Similarly, the difference between the actual firing rate and predicted eye position-related firing rate represents the saccade velocity response.

For each neuron we also analyzed the relationship between firing rate and horizontal saccade velocity using Equation 2. We estimated the probability distribution of model parameters using the same nonparametric bootstrap approach employed by Sylvestre and Cullen¹⁸ for normal monkeys performing a saccade-vergence task (see also Carpenter and Bithell⁴⁶) and similar to that described above for Equation 1.

RESULTS

We recorded 58 neurons in the abducens nucleus (24 for monkey ET1, 34 for monkey XT1). Neurons were recorded from both sides of the brain in both animals. Figure 1 shows 3 seconds of raw data from an example neuron, recorded from the left abducens nucleus in monkey ET1. Note that the tonic firing rate is <50 spikes/s, even when the left eye is several degrees to the left of straight ahead, and the right eye is deviated even further into the neuron's on-direction. One can also see an example of postsaccadic drift: After the leftward saccade, both eyes briefly drift to the right. Note, however, that the eye position quickly stabilizes, and the animal is able to maintain fixation on the target.

Eye Position Sensitivity During Steady Fixation

For all neurons from both monkeys in our sample, the tonic firing rate was significantly positively correlated with horizontal eye position. Figure 2 shows rate-position curves from two

example neurons. In both cases, the rate-position curve for the contralateral eye shifted, depending on which eye was fixating the target. The rate-position curve for the ipsilateral eye was consistent, regardless of which eye was fixating and regardless of the horizontal strabismus angle. For both neurons, the linear fits were much better for the ipsilateral eye than for the contralateral eye. This effect can be appreciated most easily by looking at the rightmost column, which shows fits for all fixation periods regardless of which eye was fixating.

As stated in the introduction, the present study was designed to determine whether the abducens nucleus shows abnormal levels of activity in strabismus and whether individual neurons carry monocular signals. These two issues are not entirely independent, however, since, in a strabismic animal, the rate-position curves will differ for the two eyes. This raises the issue of which eye one should use when attempting to estimate the population activity. With this issue in mind, Figure 3 compares the slopes (Fig. 3A) and R^2 values (Fig. 3B) for rate-position curves for the ipsilateral and contralateral eyes for all neurons from both monkeys in our sample. For many neurons the slope was clearly higher for one eye than the other. Data points were present on either side of the unity line. In Figure 3B, however, we see that a strong majority of the data points fell above the unity line. This indicates that, for most neurons, the regression equation for the ipsilateral eye provided a better fit than the equation for the contralateral eye. Nonetheless, for 10 neurons, the firing rate was better correlated with contralateral eye position.

Plane Fits to Rate-Position Curve Data

Figure 4 compares the estimated eye position sensitivities for the contralateral and ipsilateral eyes, derived from the bootstrap analysis (Equation 1, see Methods). Note that these coefficients represent the unique contribution of each eye to the tonic firing rate. A perfectly monocular neuron encoding the position of the ipsilateral eye would fall on the solid vertical line in Figure 4 since the coefficient for the contralateral eye would be zero. Similarly, a perfectly monocular neuron encoding the position of the contralateral eye would fall on the solid horizontal line. A perfectly binocular neuron would fall on the dashed line.

If horizontal strabismus angle is explicitly encoded in abducens nucleus by separate populations of right eye and left eye neurons, one might expect to find numerous monocular neurons near both the horizontal and vertical lines in Figure 4. For a strong majority of the neurons in both strabismic monkeys, however, the slope was larger for the ipsilateral eye (data points above the dashed unity line), as is typical for abducens neurons in normal monkeys.¹⁸ Particularly noteworthy is the fact that 6 of 24 neurons in monkey ET1 had significantly negative slopes for the contralateral eye. This indicates that, for a given position of the ipsilateral eye, these neurons discharged at a higher rate if the contralateral eye was deviated further in the neurons' off-direction. For monkey ET1 this equated to a less pronounced esotropia. One neuron had a negative slope for the ipsilateral eye. For monkey XT1, 1 of 34 cells showed a significantly negative slope for the contralateral eye. Based on the resulting distributions of eye position coefficients, the neurons were classified as follows: conjugate (16/58 neurons, see Fig. 5A for bootstrap distributions for one example neuron in this category), monocular ipsilateral (18/58 neurons, Fig. 5B), monocular contralateral (2/58 neurons), binocular with ipsilateral preference (12/58 neurons), binocular with contralateral preference (2/58 neurons), and opposite where the slopes for the two eyes were in opposite directions (8/58 neurons, Fig. 5C). For monkey ET1, the mean slope was 0.58 for the contralateral eye and 3.96 for the

FIGURE 1. Three seconds of raw data from an example abducens neuron, recorded from monkey ET1. *Blue*: left eye position; *red*: right eye position. For the horizontal position traces, an upward deflection indicates rightward movement. The neuron displayed a burst-tonic pattern of activity. However, the tonic firing rate was quite low. For example, even when both eyes were abducted, the firing rate was below 50 spikes/s.

ipsilateral eye (Table, part A). The mean R^2 was 0.63. For monkey XT1 the mean slope was 1.70 for the contralateral eye and 3.75 for the ipsilateral eye. The mean R^2 was 0.75. It must be emphasized that while these correlational analyses and classifications cannot distinguish between abducens motoneurons and internuclear neurons,⁴⁷ they do indicate which eye's motion is coded by a given neuron.

Estimating the Population Drive

Based on Figure 3 and our subsequent bootstrap analysis (Figs. 4, 5), it is clear that neuronal activity is most tightly linked to the movement of the ipsilateral eye. Therefore, we used coefficients from the ipsilateral eye's rate-position curve to compute an estimated firing rate for eye positions ranging from 0° to 30° of abduction, in 5° increments. This was based on the linear rate-position curve fits, not the coefficients obtained from the bootstrap analysis. Any predicted firing rates that fell below zero were set to 0. For each monkey, the estimated population firing rate associated with each eye position was taken to be the mean firing rate across the entire sample for that eye position. In normal monkeys, nearly all abducens neurons are active when the eyes are directed straight ahead.^{34,41,42,47} That being the case, one can use the published mean regression coefficients to estimate the population firing rate over this range of eye positions. Figure 6 compares the estimated population firing rates for our two monkeys and for the normal monkeys in four previous studies. Note that the existing literature is highly consistent with respect to normal monkeys. By comparison, for both of our strabismic monkeys, the estimated population firing rate was notably reduced. Two of the above reports (Gamlin et

al.⁴⁷; Miller et al.³⁴) provided tables showing the regression coefficients for each neuron. For both of our strabismic monkeys the mean y -intercept was significantly lower than the means for either of these previous studies (two-tailed t -tests, Bonferroni correction; $P < 0.01$ for all four pairwise comparisons). The slight nonlinearity for the strabismic animals was due to recruitment of neurons that were quiescent when the eyes were centered in the orbits.

In Figure 6 one can see that the slopes are similar across studies employing normal monkeys (5.2 spikes/s/deg in Sylvestre and Cullen⁴³; 4.99 spikes/s/deg in Miller et al.³⁴; 4.6 spikes/s/deg in Mays and Porter⁴⁸; 5.3 spikes/s/deg for identified internuclear neurons and 6.0 spikes/s/deg for unidentified abducens neurons in Gamlin et al.⁴⁷). In the present study we obtained a value of 3.9 spikes/s/deg for monkey ET1. This was significantly smaller than the values obtained by Gamlin et al.⁴⁷ ($P < 0.01$) and Miller et al.³⁴ ($P = 0.03$). For monkey XT1 the mean slope was 4.99 spikes/s/deg, which is clearly within the range of what has been reported for normal animals. Indeed, this value was identical to that reported by Miller et al.³⁴

Saccade Velocity Sensitivity

We also computed the instantaneous firing rate specifically related to saccade velocity by subtracting the eye position component (see Methods). Because these dynamic analyses involved correlating firing rate with instantaneous eye position and comparing the results between the two eyes, it was necessary to use the same method (eye coils) to measure the positions of both eyes. Therefore, we excluded six neurons recorded in monkey XT1 because the position of the left eye

FIGURE 2. Rate-position curves for two example neurons. Data were plotted separately for periods in which the left eye was fixating (*left column*) and the right eye was fixating (*middle column*) and were pooled across all fixation periods (*right column*). Right eye: red; left eye: blue. The neuron shown in (A–C) was recorded from the right abducens nucleus in monkey ET1. Note that the rate-position curve for the right eye was consistent, regardless of fixation condition. In contrast, the rate-position curve for the left eye shifted, depending on which eye was fixating the target. The neuron shown in (D–F) was recorded from the left abducens nucleus in monkey XT1. Note that the rate-position curve was consistent for the left (ipsilateral) eye but shifted noticeably for the right eye.

was measured with the Eyelink system. This left 52 neurons for the dynamic analyses.

Figure 7 compares the slopes (Fig. 7A) and R^2 values (Fig. 7B) for the two eyes for each neuron. From these data it is clear that the firing rates of most neurons were more predictive of the horizontal velocity of the ipsilateral eye, as is typical for abducens neurons in normal monkeys.¹⁸ This bias was particularly strong for neurons in the left abducens nucleus in monkey XT1, presumably because leftward saccades were larger for the right eye for this animal,²² which translated to higher velocities for a given firing rate and therefore a smaller slope. For each neuron we performed an F -test to determine if the regression lines differed between the two eyes. For 42 of 52 neurons the regression lines were significantly different from each other (the slope and/or intercept). The slopes were significantly different for 20 of 52 neurons. The mean eye velocity sensitivities were 0.35 for monkey ET1 and 0.40 for monkey XT1, similar to the 0.42 reported by Sylvestre and Cullen⁴² for normal monkeys. A two-tailed t -test failed to show a significant difference between the data for our two strabismic monkeys ($P = 0.53$). Comparisons between left and right

abducens nuclei also failed to reach significance for either monkey (ET1: $P = 0.32$; XT1: $P = 0.06$).

For the bootstrap analysis (see Methods), the results were highly similar to those reported above for static eye position. For a purely monocular neuron encoding ipsilateral eye velocity, fitting the data with Equation 2 should yield a value of 0 for r_c and a value for r_i that is significantly different from 0. In contrast, for a neuron that perfectly encodes conjugate (cyclopean) eye velocity, r_i and r_c should be equal. Based on the eye velocity coefficients for each neuron, the neurons were classified as follows: monocular ipsilateral (8/52 neurons), monocular contralateral (4/52 neurons), binocular with ipsilateral preference (12/52 neurons), binocular with contralateral preference (5/52 neurons), conjugate (12/52 neurons), and opposite (9/52 neurons). Two neurons showed no significant velocity component. The Table, part B, shows mean position and velocity coefficients, dynamic lead times, and R^2 values.

Thus, across all of these analyses, the firing rates of most abducens neurons were more highly correlated with the position and velocity of the ipsilateral eye.

FIGURE 3. Comparison of rate-position curve slopes (A) and R^2 values (B) for the two eyes for all neurons in our sample. *Black*: monkey XT1; *green*: monkey ET1. *Filled circles* in (A) represent neurons for which the slopes were significantly different for the two eyes. For several neurons, the slopes appeared to be quite different but the F -test failed to reach significance. These were neurons with unusually high thresholds, which meant a reduced range of eye positions at which they were active. For a clear majority of neurons, the tonic firing rates were more predictive of the position of the ipsilateral eye than the contralateral eye (B). Nonetheless, some neurons appeared to encode contralateral eye position.

DISCUSSION

For both of our strabismic monkeys we found abnormally low tonic firing rates associated with a given static eye position, compared with data from previous studies that employed normal monkeys. This was mostly due to abnormally low y -intercepts for the rate-position curves, more than a reduced slope. In both strabismic monkeys, therefore, it appears that the main abnormality lies with the level of tonic activity needed to hold the eyes in a given position, more than the overall position sensitivities.

It is surprising that the tonic firing rates were abnormally low for both the esotrope and the exotrope. In monkey ET1, esotropia was induced by prism rearing. It is possible that

FIGURE 4. Comparison of eye position slopes, derived from a bootstrap analysis based on Equation 2 (see Methods). *Green*: monkey ET1; *black*: monkey XT1. *Filled circles* indicate neurons for which the slopes were significantly different from 0 for both eyes. *Open circles* above the *dashed unity line* represent neurons for which the slope was significantly different from 0 only for the ipsilateral eye. *Open circles* below the unity line represent neurons for which the slope was significantly different from 0 only for the contralateral eye.

muscle length adaptation might have occurred as a result of prolonged, sensory-induced esotropia,²⁴ but there is no reason to expect this to be associated with weaker activity in the abducens nucleus. If esotropia is associated with an excessively strong convergence drive to the medial rectus muscles, then one might have expected increased activity for the abducens nucleus, since this is what is observed for normal monkeys with converged eye positions.³⁴ Since we observed the opposite effect, it would seem that esotropia in this animal is more complex than simply an “excessive convergence.” Monkey XT1’s exotropia was the result of a bilateral medial rectus tenotomy in infancy. As a result, a weaker drive to the lateral rectus muscle may be sufficient to attain a given abducted eye position. If so, then the brain apparently responds to surgical tenotomy and the resulting decrease in muscle force-generating capacity by altering the neural drive to the antagonist muscle.

In any event, the finding of an abnormal constant term is consistent with a preliminary report involving one exotropic monkey (Agaoglu MN, et al. *IOVS* 2014;55:ARVO E-Abstract 2572). These authors suggested muscle length adaptation²⁴ as an explanation, which might explain the present results as well. A clearer picture might emerge by recording from both medial rectus motoneurons and abducens motoneurons in monkeys with muscle surgery-induced strabismus.

Another issue that deserves discussion is the question whether the abducens neurons in our monkeys carry a signal related to horizontal strabismus angle. Our Equation 2 is identical to the dynamic equation used by Sylvestre and Cullen¹⁸ to describe the firing rates of abducens neurons in normal monkeys performing a saccade-vergence task. As these authors pointed out, this equation is, in its nonreduced form, mathematically equivalent to their equation “EST-cv-all,” which has a term explicitly related to vergence angle. Since horizontal strabismus angle is mathematically equivalent to vergence

FIGURE 5. Distributions of static eye position coefficients for the right (red) and left (blue) eyes, obtained from a bootstrap analysis (see Methods) for three example neurons. For the “conjugate” neuron shown in (A), the distributions for the two eyes almost perfectly overlapped. For the “monocular, ipsilateral” neuron shown in (B), the distribution for the left eye was centered on 0. The distribution for the right eye was positive and did not overlap with 0 or with the distribution for the left eye. A different pattern was seen in (C). For this

angle, the coefficients from our Equation 2 can be used to determine whether there is a sensitivity to horizontal strabismus angle. For a purely binocular neuron the coefficients for the two eyes will, by definition, be statistically the same. This is equivalent to saying that there is no sensitivity to horizontal strabismus angle. For any other neuron, the firing rate will vary as a function of horizontal strabismus angle. Therefore, one interpretation of our results is that the majority of abducens neurons modulate their firing rate in association with the horizontal angle of strabismus. In fact, this description might be particularly appealing given the fact that the horizontal position and velocity coefficients sometimes had opposite signs for the two eyes.

For a majority of neurons, in both strabismic monkeys, the firing rates were better correlated with the position and velocity of the ipsilateral eye. Previous studies in normal animals have suggested that internuclear neurons comprise approximately 30% to 40% of the neurons in abducens nucleus.^{18,41} Thus, our sample must have included many internuclear neurons, yet relatively few carried signals that consistently predicted the horizontal position and velocity of the contralateral eye. Similar effects have been reported for normal monkeys performing a combined saccade-vergence task.¹⁸ It has also been reported that antidromically identified internuclear neurons, recorded during vergence eye movements, carry a signal appropriate for the ipsilateral eye but inappropriate for the medial rectus motoneurons to which they project.⁴⁷ These latter authors suggested that this inappropriate signal is simply overcome by a stronger convergence-related drive from the supraoculomotor area (SOA). If this is correct, then it follows that maintenance of normal eye alignment depends upon balancing the connection weights of the internuclear pathway and the vergence-related drive from SOA. How this is achieved is unknown, but it suggests the possibility that horizontal misalignments in strabismus may be partly due to a failure to balance these competing drives.

The shifting of the rate-position curves for one eye may be the result of signals added at the level of medial rectus motoneurons and/or oblique muscle motoneurons.^{16,47,49} The most obvious possibility is that input from a disordered vergence system to medial rectus motoneurons causes the actual position of the contralateral eye to differ from that requested by abducens internuclear neurons. Indeed, this is almost certainly the case, given that the tonic firing rates of neurons in SOA are correlated with the horizontal angle of strabismus.^{19,21} Recently, Van Horn et al.⁵⁰ have provided evidence that the rostral portion of superior colliculus contains neurons that encode vergence angle in normal monkeys. Repeating their study in strabismic animals might reveal relevant abnormalities that may affect horizontal eye alignment.

However, these explanations may not account for all of the available data in strabismic animals. For example, microstimulation of PPRF evoked conjugate movements in a normal monkey but disconjugate movements in the same two strabismic monkeys used in the present study.²⁰ Since PPRF has not been shown to project to any vergence-related areas, it is not clear how we could have obtained this result if the horizontal misalignment were determined solely by disordered

neuron, recorded from the right abducens nucleus in monkey ET1, the slopes for the two eyes were clearly in opposite directions (positive for the right eye and negative for the left eye). Thus, for a given position of the ipsilateral eye, the neuron discharged at a higher rate when the contralateral eye was farther to the left, which was the neuron's off-direction.

TABLE. Mean Slopes and R^2 Values for Static Rate-Position Curves for Each Eye, Derived From Planar Equation 1 (Part A), and Mean Sensitivities to Eye Position and Horizontal Velocity During Saccadic Eye Movements (Equation 2), Mean Dynamic Lead Times, and R^2 Values (Part B)

Subject	Contralateral Eye Position	Ipsilateral Eye Position	Contralateral Eye Velocity	Ipsilateral Eye Velocity	Dynamic Lead Time	R^2
A. Static eye position, Equation 1						
ET1	0.58 (1.72)	3.96 (2.56)				0.63
XT1	1.70 (2.08)	3.75 (2.89)				0.75
B. Dynamic analysis, Equation 2						
ET1	-0.44 (4.59)	3.15 (4.18)	0.14 (0.42)	0.19 (0.40)	10 ± 4.6	0.42
XT1	0.54 (4.66)	2.90 (4.95)	0.12 (0.22)	0.35 (0.31)	11.66 ± 3.9	0.45

input from SOA. Even if every premotor saccadic burst neuron projected to both abducens motoneurons and internuclear neurons, disconjugacy would still occur if the connection weights were unbalanced. As noted above, for example, horizontal saccade disconjugacy will occur if the activation of the lateral rectus by motoneurons is not balanced with the strength of the internuclear pathway. If this is the case, then stimulation of PPRF would evoke disconjugate movements even without activation of vergence pathways and even if every premotor burst neuron was binocular in terms of its anatomical connectivity in the abducens nucleus.

With the above considerations in mind, we suggest that saccade disconjugacy and horizontal strabismus angle reflect at least two influences. First, neurons that would normally encode disparity vergence develop abnormal tuning characteristics, inappropriately modulating their activity when monkeys perform oculomotor tasks on a tangent screen.^{19,21} Second, an imbalance develops between the strength of the PPRF-abducens motoneuron pathway and the PPRF-abducens

FIGURE 6. Estimated population activity. The mean firing rates were plotted for various eye positions from straight ahead to 40° of abduction for ET1 (green) and XT1 (black). The green and black lines were drawn to connect these points. We also plotted estimates of population activity (derived from mean slopes and intercepts) from four published studies employing normal monkeys.^{34,42,47,48} For both strabismic monkeys, the tonic eye position activity was substantially reduced, suggesting that a given amount of abduction could be attained with a weaker drive to the lateral rectus muscle.

FIGURE 7. Relationship between instantaneous firing rate (adjusted to compensate for the eye position sensitivity) and instantaneous horizontal saccade velocity. Linear fits were performed separately for the ipsilateral and contralateral eyes. The resulting slopes (A) and R^2 values (B) were plotted for all neurons in our sample. Black: monkey XT1; green: monkey ET1. Triangles: left abducens nucleus; circles: right abducens nucleus. Filled circles represent neurons for which the regression lines (slope and/or y -intercept) were significantly different between the two eyes. Some filled circles sit on or very near the unity line because, although the slopes were very similar, the y -intercepts were different. For most neurons the correlation was stronger for the ipsilateral eye. This ipsilateral bias was mild in monkey ET1 and stronger in monkey XT1, particularly for neurons recorded from left abducens nucleus.

internuclear neuron-medial rectus motoneuron pathway. This would account for the fact that PPRF stimulation evokes disconjugate eye movements.²⁰

In any case, the present data suggest that strabismus is associated with altered activity in abducens nucleus compared to that seen in normal monkeys.

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