

Abnormal Tuning in Nucleus Prepositus Hypoglossi of Monkeys With “A” Pattern Exotropia

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PURPOSE. In many individuals with pattern strabismus, the vertical misalignment varies with horizontal eye position. It has been proposed that these cross-axis effects result from abnormal cross-talk between brainstem structures that would normally encode horizontal and vertical eye position and velocity. The nucleus prepositus hypoglossi (NPH) is an ideal structure to test this overarching hypothesis. Neurons in the NPH are believed to mathematically integrate eye velocity signals to generate a tonic signal related to horizontal eye position. We hypothesized that, in monkeys with A-pattern exotropia and vertical incomitance, these neurons would show an abnormally large sensitivity to vertical eye position.

METHODS. Three rhesus monkeys (1 normal and 2 with A-pattern exotropia) were trained to maintain fixation on a visual target as it stepped to various locations on a tangent screen. Extracellular neural activity was recorded from neurons in the NPH. Each neuron’s sensitivity to horizontal and vertical eye position was estimated using multiple linear regression and preferred directions computed for each eye.

RESULTS. Unexpectedly, the mean preferred directions for the left eye were normal in the monkeys with A-pattern exotropia. For the right eye, there was a clear upward deviation for the right NPH and a downward deviation for the left NPH. In addition, the R^2 values were significantly lower for model fits for neurons recorded from the exotropic monkeys.

CONCLUSIONS. We suggest that vertical incomitance results from inappropriate vertical-to-horizontal cross-talk that affects the two eyes differently.

Keywords: exotropia, nucleus prepositus hypoglossi, pattern strabismus, monkey, electrophysiology

The infantile strabismus syndrome is a common disorder that is associated with numerous visual and oculomotor abnormalities.¹⁻⁷ In some patients, the vertical and horizontal misalignments of the eyes are variable and correlated with eye position along the orthogonal axis, a condition referred to as pattern strabismus.^{1,6} In many patients, there are associated torsional abnormalities that are suggestive of oblique muscle over- or underaction. However, recent evidence from nonhuman primate models⁸⁻¹¹ and from human patients⁷ have offered compelling evidence that the cross-axis pattern of eye misalignments that characterize pattern strabismus is associated with abnormalities within the midbrain and brainstem. Specifically, it has been suggested that there is abnormal cross-talk between pathways that, in normal primates, carry signals specific to either the horizontal or vertical components of eye movements.^{7,10-12}

We have recently proposed two computational models that place this hypothetical cross-talk at the level of the neural integrators in the midbrain and brainstem.¹³ One, the Integrator Crosstalk Model, assumes that the crosstalk occurs exclusively at the level of the horizontal and vertical neural integrators in the nucleus prepositus hypoglossi

(NPH) and the interstitial nucleus of Cajal (INC), respectively. This assumption was based on neuroanatomic studies that have shown projections from the NPH to the INC¹⁴ and from the INC to the abducens nucleus^{15,16} in normal primates. Another model, the Distributed Crosstalk Model, postulates a more general breakdown of directional tuning across many areas of the brainstem. Consistent with this latter idea, we have shown that saccade-related neurons in the pontine paramedian reticular formation (PPRF) that encode the amplitude and kinematics of the horizontal component of saccades in normal monkeys¹⁷⁻¹⁹ have an abnormally broad distribution of preferred directions in monkeys with pattern strabismus.¹⁰ Moreover, microstimulation of the PPRF in monkeys with pattern strabismus evokes directionally disconjugate eye movements from some sites and not others, suggesting the possibility that abnormalities may exist in the projections from the PPRF to downstream structures. Because the PPRF sends a strong projection to the NPH,^{19,20} we hypothesized that pattern strabismus is associated with aberrant directional tuning in the latter structure. Specifically, we predicted that the horizontal eye position sensitivity would be highly variable between neurons, and would be abnormally large for a subset of neurons.

METHODS

Subjects and Surgical Procedures

Three macaque monkeys participated in this study, including one normal animal (hereafter referred to as monkey N1) and two with “A” pattern exotropia (monkeys XT1 and XT2). Both of the strabismic animals underwent a bilateral medial rectus tenotomy, performed during the first week of life.²¹ Following this procedure, the animals were permitted to reach maturity (>3 years) without further intervention, until they underwent surgeries to prepare for neurophysiological experiments. The horizontal strabismus angle for monkey XT1 was typically close to 25° when fixating a visual target with the right eye and 35° to 40° when fixating with the left eye. For monkey XT2, the horizontal strabismus angle ranged from approximately 15° (right eye fixating a target in the left visual hemifield) to approximately 35° (left eye fixating a target near straight ahead). None of the three monkeys used in this study displayed a detectable nystagmus during the performance of our behavioral tasks.

Monkey XT1 was the same “XT1” animal used in several of our previous studies that targeted the PPRF, abducens nucleus, and superior colliculus.^{10,11,22–24} Monkey XT2 was used in our recent study of the preferred directions of vertical neural integrator neurons in the INC.²⁵

All surgical and experimental procedures were in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research and the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The protocol was approved by the Institutional Animal Care and Use Committee at University of Washington before any procedures were performed. Detailed descriptions of our surgical procedures are available in previously published studies.^{26,27} Briefly, t-bolts were used to attach a titanium head post (Crist Instruments Co., Inc., Hagerstown, MD, USA) to the skull, which allowed the head to be restrained during experiments. A 16 mm craniotomy was performed and a titanium recording chamber installed over it, positioned so that electrode tracks near the center of the chamber would be likely to reach abducens nucleus or the NPH. Eye coils were chronically implanted beneath the conjunctiva of both eyes so that eye position could be measured using the magnetic search coil technique.^{28,29}

Behavioral Tasks and Visual Display

The visual target was a red, 0.25° laser spot, displayed on a tangent screen at a distance of 57 cm from the monkey’s face. The target remained at a given location for intervals ranging from 1.5 to 5 seconds, after which it stepped to a new location, determined by randomly selecting horizontal and vertical Cartesian coordinates from a user selectable list (0°, 2°, 4°, 6°, 8°, 10°, 12°, 15°, 18°, and 22° left, right, up, or down). Thus, targets could appear along the horizontal or vertical meridians, or at tertiary positions. Monkeys were rewarded with a small amount of apple juice or applesauce every 300 ms whenever at least one eye was directed within 5° of the target.

Unit Recording and Localization of the NPH

Extracellular single unit recordings were made using tungsten and glass microelectrodes (Frederick Haer, Brunswick,

ME, USA). Before searching for the NPH, we first localized the abducens nucleus (based on the characteristic “beehive” sound), with burst-tonic activity that modulated in association with horizontal eye position during performance of the saccade task described above. Across different recording days, the medio-lateral recording site was systematically varied until the midline was located. Next, remaining within 3 mm of the midline, the electrode location was systematically shifted in the caudal direction until we began to isolate tonic (without a saccade-related burst) and burst-tonic neurons that were sensitive to the horizontal eye position (without the “beehive” sound characteristic of motor nuclei). Based on previous studies showing that the NPH functions as a horizontal neural integrator,^{14,30–34} microstimulation (100 ms, 300–400 Hz, 20µA) was applied to one site in monkey XT1 and 12 sites in monkey XT2, to verify that the eyes would remain at, or near, their new locations following the offset of the artificially imposed signal. Finally, a marking lesion was placed at the location of the stimulation site in monkey XT1, by passing 10 µA direct current for 10 seconds through the recording electrode. After the animal was euthanized, the brain was cut into 50 µm sections in the stereotaxic plane, and stained with Cresyl violet.

Data Analysis

The commercially available Spike 2 software package (Cambridge Electronic Design, Cambridge, UK) was used for both data acquisition and preliminary offline assessment of unit isolation. For all other analyses, custom functions were written in MatLab (Mathworks, Natick, MA, USA). The instantaneous eye velocity and acceleration were estimated by computing the first and second derivatives, respectively, of eye position using 7-point parabolic differentiation. The instantaneous horizontal and vertical strabismus angle (mathematically equivalent to horizontal and vertical vergence angle in a normal monkey) were computed using Equation 1:

$$S = P_{left} - P_{right} \quad (1)$$

where S is the horizontal or vertical strabismus angle, P_{right} is the horizontal or vertical position of the right eye, and P_{left} is the horizontal or vertical position of the left eye.

In strabismus saccades are often followed by an abnormally large postsaccadic drift^{35–37} that can, occasionally, lead to inaccurate estimates of saccade offset time when the detection algorithm is based entirely on a velocity threshold. To address this potential problem, saccade offsets were detected based on a combination of velocity and acceleration criteria.²²

In order to evaluate each neuron’s sensitivity to static vertical and horizontal eye position, periods of fixation were identified based on a set of three criteria, all of which had to be satisfied: (1) no saccades were detected during the preceding 100 ms, (2) vergence velocity remained below 10°/s, (3) version velocity remained below 25°/s, and (4) the duration of the fixation was at least 500 ms. Additionally, to ensure that tonic firing rates would not be influenced by perisaccadic activity, all fixation periods ended at least 50 ms before the next saccade.

Offline spike detection was performed using the same custom algorithm that we have used in several previous studies.^{10,23} The mean firing rate was then computed for

each period of fixation that was detected using the above procedure. In order to estimate the sensitivities to horizontal and vertical eye position, the data were then fit with Equation 2:

$$FR(t - t_d) = a + k_{hor}H + k_{vert}V \quad (2)$$

where V and H are the vertical and horizontal eye positions, respectively. K_{hor} and K_{vert} are the estimated sensitivities to horizontal and vertical eye position, respectively. In order to compensate for neural processing delays, the neural recording data were time-shifted by 20 ms ($t - t_d$). Note, however, that this time shift had very little effect on the data, because most fixation periods lasted for several hundred milliseconds. The resulting parameter estimates for horizontal and vertical eye position sensitivity were then converted to polar coordinates using the MatLab function “cart2pol.” Because of directional saccade disconjugacy,^{7,22} and the pattern strabismus, it is inevitable that the horizontal and vertical sensitivities will differ for the two eyes in the animals with exotropia. For this reason, the fits were performed separately for the two eyes, resulting in separate estimates of each neuron's preferred direction for the right and left eye.

Finally, the absolute deviation of each preferred direction from “pure” horizontal (either 0° or 180°, whichever was closer) was computed for each eye for each neuron, using Equation 3:

$$AbsDev_H = |D_{ideal} - D_{Actual}| \quad (3)$$

where $AbsDev_H$ is the absolute value of the deviation of the preferred direction from horizontal, D_{Actual} is the neuron's estimated preferred direction, and D_{ideal} is the nearest “pure” horizontal direction. When the estimated preferred direction was between 271° and 359°, we subtracted 360° before computing $AbsDev_H$.

RESULTS

Hess plots for both exotropic monkeys can be seen in Figure 1. These plots were obtained using horizontal and vertical smooth pursuit. Both of the monkeys with exotropia tended to use the left eye to fixate targets to the left of straight ahead and the right eye to fixate targets to the right. When the target was straight ahead both of these animals used either eye, but monkey XT1 preferred the left eye and monkey XT2 preferred to use the right eye. Both of these animals demonstrated the capability of fixating targets at least 15° into the contralateral hemifield (i.e. using the left eye to fixate a target 15° right of straight ahead), up to 40° into the ipsilateral hemifield, and $\pm 30^\circ$ vertically. Note, in all four panels, the vertical movement in the nonviewing eye during smooth pursuit of a target moving along the horizontal meridian.

We recorded a total of 57 neurons that showed statistically significant sensitivity to eye position, including 17 from monkey N1, 15 from monkey XT1, and 25 from monkey XT2. For monkey N1, four were classified as tonic neurons and 13 as burst-tonic. For the 2 monkeys with exotropia, there were 9 tonic neurons and 31 burst-tonic neurons.

Figure 2A shows a representative section that includes the NPH region of monkey XT1. The inset box, and associated photograph in panel B, indicates the location of a marking

lesion (red arrow) in the NPH placed at the site of recorded neurons and microstimulation.

Eye Position Model Fits

Panel A of Figure 3 shows histograms of the R^2 values resulting from fits of Equation 2 to the data for all neurons in our sample. Panels B through F show the fits for two example neurons. The neuron recorded from the normal monkey has little or no sensitivity to vertical eye position; the R^2 value of 0.52 is very close to the mean for that animal, across our data set. The neuron recorded from monkey XT2 (panels C–F) was quite typical for neurons recorded from the monkeys with exotropia (the R^2 value is lower than the mean, but the small vertical sensitivity for the right eye is quite typical). Panels G and H show three superimposed example microstimulation trials, at the site from which the neuron shown in panels C to F was recorded. The evoked movements are disconjugate but, after stimulation offset, the eyes remained near the new locations. This indicates that the artificially imposed signal was mathematically integrated, which is what we should expect from activation of the NPH. Figure 4 shows another less typical example neuron recorded from monkey XT2. It is clear that this neuron was far more sensitive to the vertical eye position than the horizontal. No such neurons were recorded from the normal monkey.

The mean R^2 value for monkey N1 was 0.50 for both eyes; this was significantly higher than the mean R^2 value for the monkeys with exotropia for either eye (two-tailed t -tests; left eye: mean = 0.34, $P = 0.04$; right eye: mean = 0.33, $P = 0.02$). This difference may have been driven largely by 11 neurons, recorded from the monkeys with exotropia, that showed R^2 values below 0.1, despite having a statistically significant sensitivity to eye position. No such neurons were found in the normal monkey.

In pattern strabismus, the inappropriate cross-axis movement occurs almost exclusively in the eye that is not viewing the target. With this in mind, one might wonder whether the low R^2 values for the model fits in the subjects with strabismus might be the result of pooling data from right-eye-on-target and left-eye-on-target conditions. With this concern in mind we also performed the model fits separately for right-eye-on-target and left-eye-on-target conditions. For the normal monkey, of course, both eyes always pointed in the same direction. Nonetheless, as a test of the reliability of our estimates of direction preference, the data from each neuron recorded from monkey N1 were divided into two groups, based on which eye was closer to the center of the target.

The mean absolute value of the horizontal position sensitivity was 1.93 for monkey N1 and 2.15 for the monkeys with exotropia. The SD for horizontal sensitivity was 2.0 for monkey N1 and 3.1 for the monkeys with exotropia. For monkey N1, 8 of 17 neurons had a statistically significant sensitivity to vertical eye position. The mean absolute value of the vertical eye position sensitivity was 0.40. For the monkeys with A-pattern exotropia, 20 of 40 neurons showed a significant sensitivity to the vertical eye position. The mean absolute value of the vertical eye position sensitivity was 0.66 for the left eye and 0.99 for the right eye. For the right eye, the mean absolute value of the vertical eye position sensitivity was significantly higher in the monkeys with exotropia (two-tailed t -test, $P = 0.008$) but there was no significant difference for the left eye ($P = 0.197$).

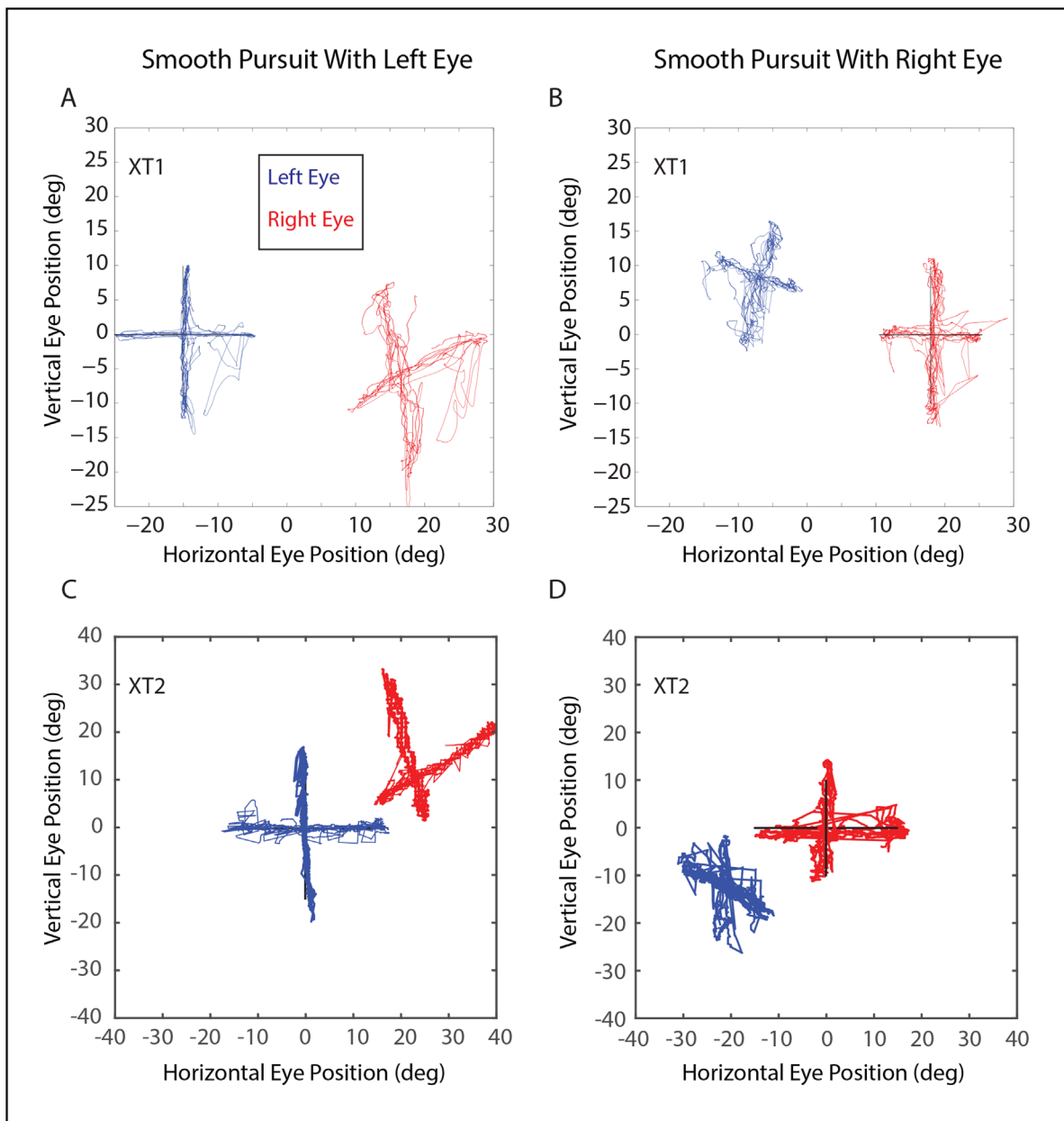


Figure 1

FIGURE 1. Hess plots for monkey XT1 (**A, B**) and XT2 (**C, D**). Data were collected during horizontal and vertical smooth pursuit. In the left column **A, C**, the monkey is using the left eye (blue) to pursue the target. In the right column **B, D**, the monkey is using the right eye (red) to pursue the target. In all four panels, note that the viewing eye follows the target as it moves along the horizontal or vertical meridian, but the nonviewing eye always moves at an oblique angle.

Figure 5 compares the vertical and horizontal eye position sensitivities for monkey N1 and the monkeys with exotropia.

Preferred Direction Analysis

As noted above, fitting Equation 2 to the data yielded very poor fits for some recordings. Including such neurons in the analysis of preferred directions would likely yield misleading results. With this in mind, we only included neurons for which the model yielded an R^2 value of at least 0.1. This criterion resulted in the exclusion of 11 neurons

(0 for monkey N1, 5 for monkey XT1, and 6 for monkey XT2). **Figure 6** shows the preferred directions for all of the remaining neurons. For the monkeys with A-pattern exotropia, there were several neurons (5 for the left eye and 6 for the right eye) with predominantly vertical preferred directions. No such neurons were found in the normal monkey. A 2-way ANOVA (strabismus status and eye used to predict the absolute deviation from horizontal) found a significant effect of strabismus ($P = 0.0044$) but not eye ($P = 0.37$). The interaction term was also not significant ($P = 0.3638$). For the left eye, the mean absolute deviation

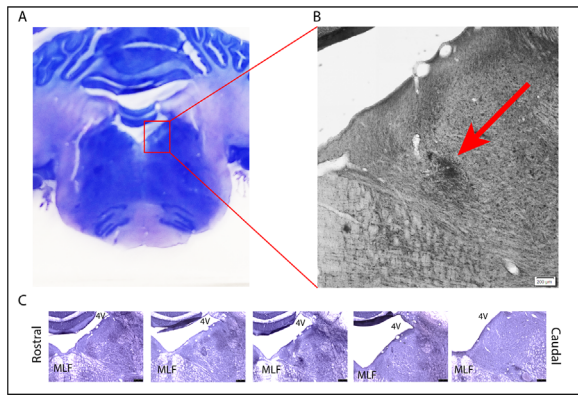


Figure 2

FIGURE 2. Marking lesions in monkey XT1. (A) Representative section, stained with cresyl violet, showing the brainstem region containing the NPH. The red box indicates the area shown in panel B. (B) Area of the NPH where one of the neurons was recorded, microstimulation applied, and a marking lesion placed (red arrow). (C) Series of five tissue slices, including two that were rostral to the one shown in panels A and B and two that were more caudal.

from horizontal was not significantly different between monkey N1 and the monkeys with exotropia (monkey N1: 11.9; exotropia: 22.1; two-tailed *t*-test, $P = 0.16$). For the right eye, the mean absolute deviation from horizontal was significantly larger for the monkeys with exotropia (monkey N1: 11.9; exotropia: 31.2; two-tailed *t*-test, $P = 0.01$).

In the normal monkey, the mean preferred direction was 176° for the left NPH and 353° for the right NPH (based on the right eye; mean preferred directions for the two eyes differed by $<1^\circ$ in monkey N1). For the left eye, in the monkeys with exotropia, the mean preferred directions were almost purely horizontal, for both the right and left NPH (right NPH: 1° ; and left NPH: 181°). It is clear that, for the right eye in the monkeys with exotropia, the preferred directions of the NPH neurons typically deviate from the horizontal; the preferred directions of most of the neurons recorded from the right NPH are clustered in the range of 0° to 30° (mean: 16.5°), whereas the majority of neurons recorded from the left NPH had preferred directions that had a downward component (mean: 201°).

Consideration of Alternative Explanations

It is possible that the lower R^2 values observed for the monkeys with exotropia might be a consequence of abnormalities downstream from the NPH, or in parallel pathways. From Figure 1, we can see that, for both of these animals, the vertical strabismus angle was correlated with horizontal eye position. In addition, it is known that, in strabismus, near and far response cells in the supraoculomotor area change their tonic firing rates in association with switches of the fixating eye.^{12,38} Thus, even if the NPH neurons were normal in monkeys XT1 and XT2, our Equation 2 might yield lower R^2 values if switches of the fixating eye cause changes in vertical eye position that were not “requested” by the neurons in our sample. If the relatively poor model fits are due solely to these effects, then Equation 2 should yield larger R^2 values if the fixating eye is held constant.

With these considerations in mind, we separated the data into left-eye-on-target and right-eye-on-target conditions. Left eye and right eye data in both groups were fitted

with Equation 2, yielding four conditions for this analysis. For the left-eye-on-target condition, the mean R^2 values were 0.28 (left eye) and 0.28 (right eye). For the right-eye-on-target condition, the mean R^2 value was 0.23 for both the left and right eyes. By comparison, when the data were pooled across all trials, the mean R^2 values were 0.33 (left eye) and 0.34 (right eye). Thus, the mean R^2 values were even lower when the viewing eye was held constant, although this difference might simply be the result of a decrease in the range of eye positions.

For each neuron, the preferred directions were estimated for each eye for the left-eye-on-target and right-eye-on-target conditions. When the monkeys with exotropia fixated the target with the left eye the mean absolute deviations from horizontal were 26° for both eyes. For the right-eye-on-target condition, the mean absolute deviations from horizontal were 27° (left eye) and 32° (right eye). ANOVA revealed a significant effect of strabismus status ($P < 0.001$) but not the viewing eye ($P = 0.88$). For all four of the eye + viewing eye conditions (left eye with left eye on target, right eye with left eye on target, left eye with right eye on target, and right eye with right eye on target), pairwise comparisons revealed that the mean absolute deviation from horizontal was significantly greater for the monkeys with exotropia, compared to monkey N1 (two-tailed *t*-tests, $P < 0.001$ for all four comparisons). Thus, the effects described in the present report were not due solely to the pooling of data from the right-eye-on-target and the left-eye-on-target conditions.

Monkeys XT1 and XT2 underwent bilateral medial rectus tenotomy in infancy. Eye muscle surgery has been used to experimentally induce strabismus in monkeys for many years.^{10,11,21–23,38–44} Nonetheless, one might wonder whether the pattern strabismus in these animals might be due to an abnormal pulling direction for the medial rectus muscles. To further test this possibility, we performed two additional analyses: First, we recorded 15 medial rectus motoneurons from monkey XT1 and plotted the preferred directions using the same procedure described above for the NPH neurons. Looking at Figure 1, the eye that is not being used to pursue a horizontally moving target consistently displays a downshoot on adduction. Because the viewing eye moves horizontally (following the target) this results in a relationship between horizontal eye position and vertical strabismus angle (Fig. 1). If this abnormality is due to an abnormal pulling direction for the medial rectus muscles, then the mean preferred direction for MR motoneurons should be significantly downward.

For neurons in the left oculomotor nucleus (OMN), the mean preferred direction was 7° . Note that this is slightly upward, which is the opposite of the vertical bias that would be required to explain the downshoot of the left eye associated with rightward eye positions (Fig. 1B). Note that there was also no consistent downshoot for the preferred directions of the right NPH neurons for the left eye (Fig. 6C).

This result indicates that the pattern strabismus in monkey XT1 cannot be fully accounted for by an abnormal pulling direction of the medial rectus muscles. This is consistent with a previously published study that showed that changes in the vertical strabismus angle with horizontal eye position are encoded by the firing rates of motoneurons serving vertically-acting eye muscles.⁸ In this earlier study, the pattern strabismus was induced using sensory deprivation methods, such as alternating monocular occlusion. To verify that this result also holds for our exotropic monkeys, who underwent medial rectus tenotomy

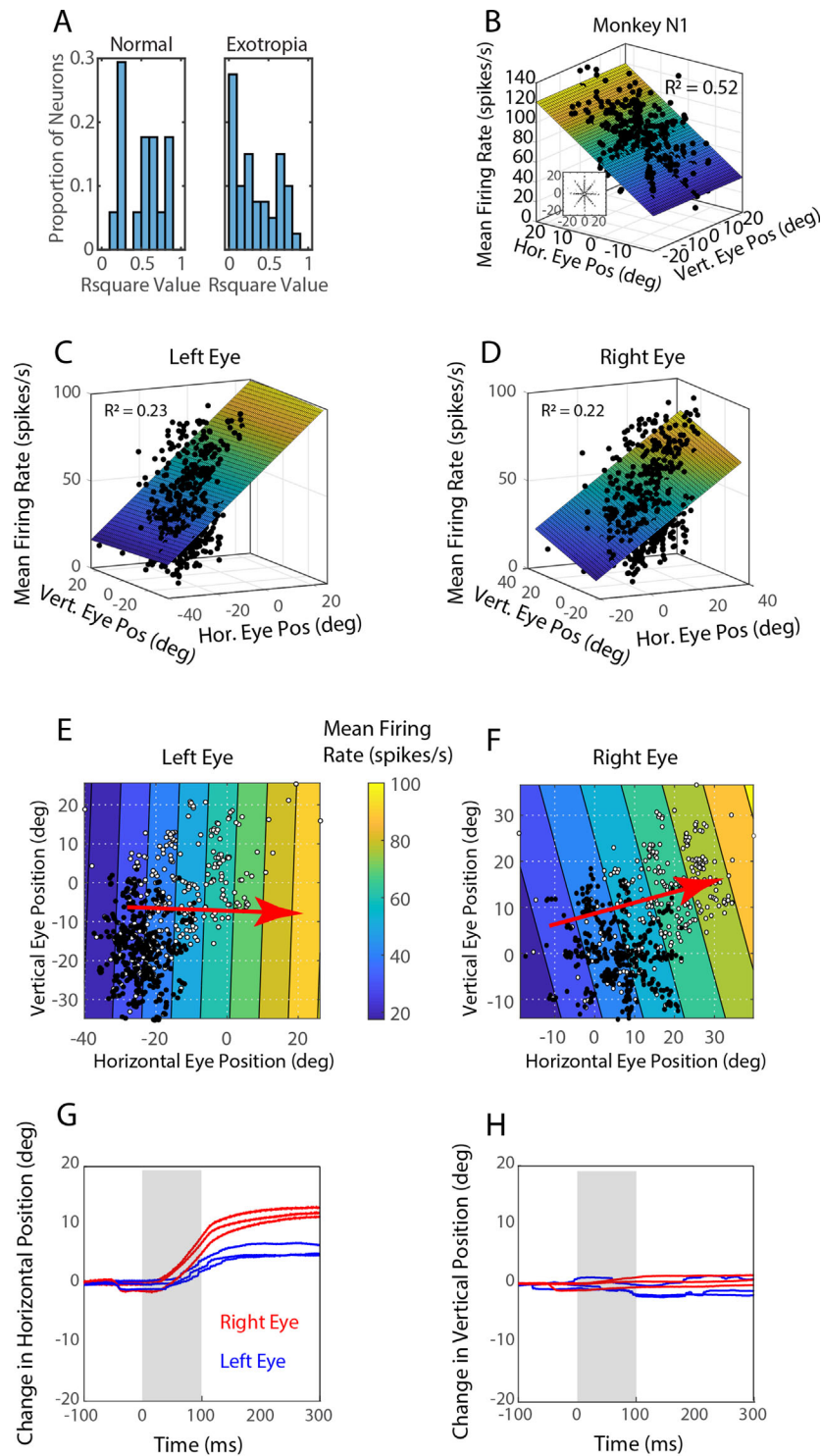


Figure 3

FIGURE 3. Results of model fits. **(A)** Distribution of R^2 values, across all recordings from normal and exotropic monkeys. For the normal monkey, a majority of neurons had R^2 values > 0.5 (mean = 0.5, range = 0.12–0.82); for the monkeys with strabismus, a majority had R^2 values < 0.5 (left eye mean = 0.34, range = 0–0.72; right eye mean = 0.33, range = 0–0.73). **(B)** Example plane fit for a typical neuron recorded from monkey N1. During periods of steady fixation, the tonic firing rate is strongly correlated with horizontal eye position but there is very little sensitivity to vertical eye position. The inset shows the horizontal and vertical eye positions. **(C, D)** Example plane fits for the left and right eyes for a typical neuron recorded from monkey XT2. Note the lower R^2 values and the small sensitivity to vertical eye position for the right eye. **(E, F)** The same data, and same model fit, shown in panels **C** and **D**, plotted using the SurfaceFit option in MatLab. Data points within the same color band have approximately the same firing rate. For the left eye, the color bands are nearly vertical, indicating that the neuron is almost exclusively sensitive to horizontal eye position. For the right eye, however, the color bands are clearly angled. As a crude demonstration of the neuron’s preferred direction, the red arrows are drawn perpendicular to the color bands. White

dots indicate that the left eye was fixating the target; black dots indicate that the right eye was fixating the target. (G, H) Microstimulation of the site where the example neuron in panels C–F was recorded. The gray shaded area represents the period of stimulation (100 ms, 300 Hz, and 20 μ A). Red = right eye; Blue = left eye.

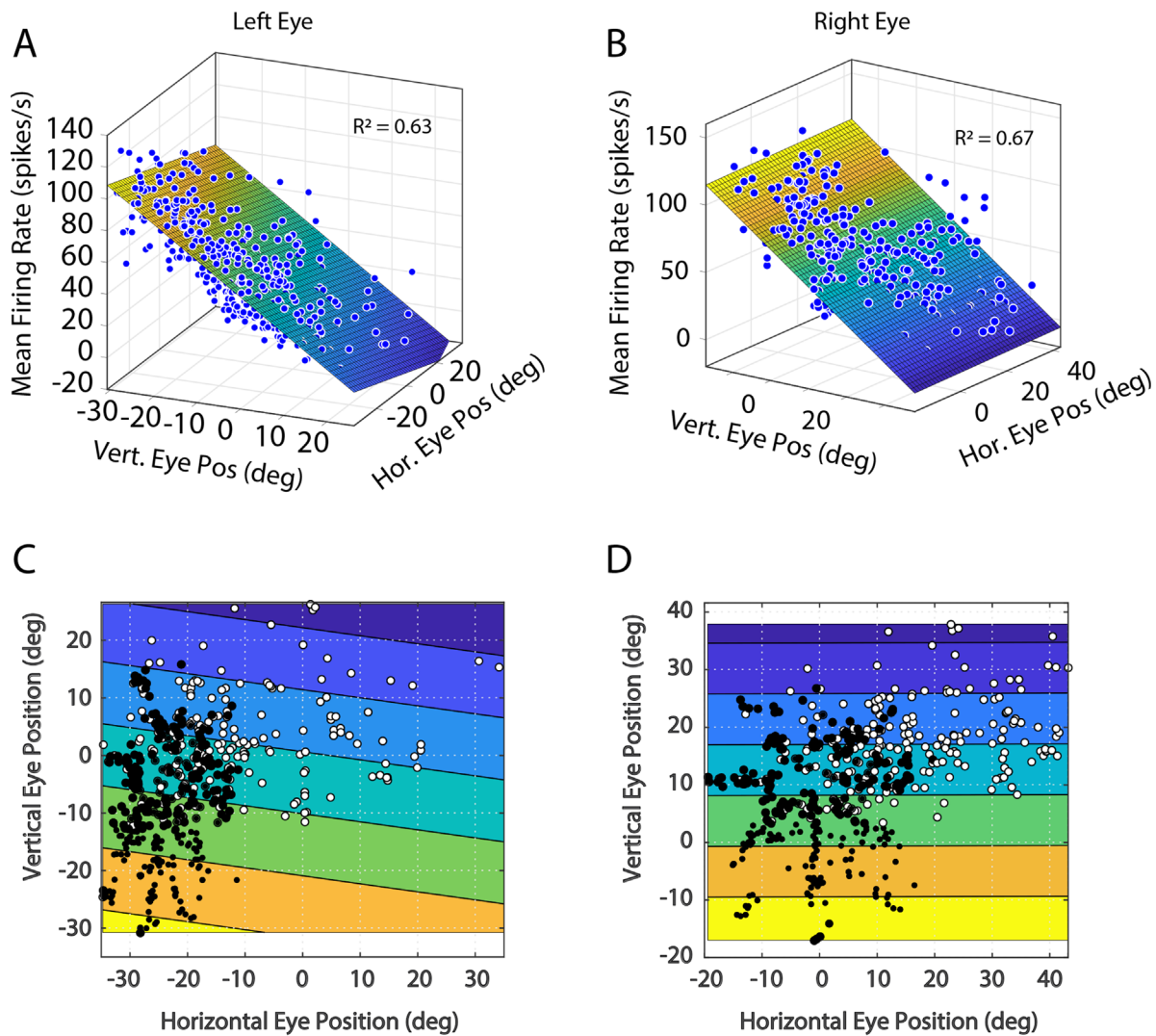


Figure 4

FIGURE 4. Another example neuron from monkey XT2. All conventions are the same as in Figure 2, C, D. (A, B) This neuron is clearly more sensitive to vertical eye position than horizontal eye position. (C, D) Surface fit plot of the same data shown in panels A and B. Horizontal and vertical eye positions. White dots indicate that the left eye was fixating the target and black dots indicate that the right eye was fixating the target.

in infancy, we recorded 43 vertically acting motoneurons. We then searched for mostly horizontal saccades in which only one eye showed a vertical component, or the vertical component was in opposite directions for the two eyes (i.e. one eye up and the other eye down). Example raw data from monkey XT1, from a left eye inferior rectus motoneuron, are shown in Figure 7. When the saccade has a downward component for both eyes, the neuron shows a saccade-related burst (arrows). For the left-eye-down/right-eye-up saccade (first gray shaded area) there is a burst and the tonic firing rate increases; for the left-

eye-up/right-eye-down saccade (second gray shaded area) there is a brief pause, after which the tonic firing rate decreases.

If the pattern strabismus is solely a consequence of an abnormal pulling direction for the medial rectus muscles, then for vertically disconjugate saccades like those described above, these cross-axis vertical components should not be encoded by vertically acting motoneurons. On the other hand, if the cross-axis vertical components are the result of abnormal cross-talk between horizontal and vertical pathways in the brainstem, then the tonic firing rates should

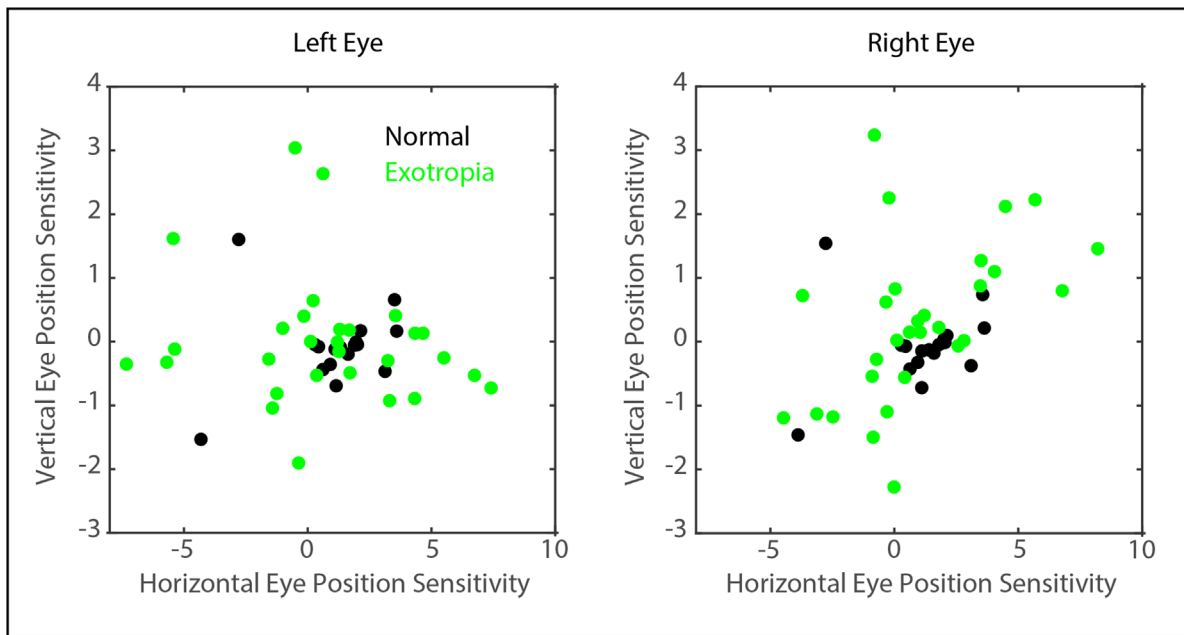


Figure 5

FIGURE 5. Relationship between the vertical and horizontal eye position sensitivity for all neurons for which the fit yielded an R^2 of at least 0.1. Black = Normal; Green = Exotropia.

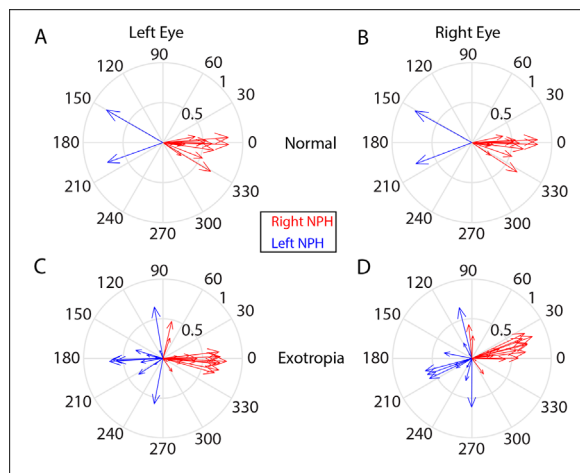


Figure 6

FIGURE 6. Preferred directions for all neurons for which the model fits yielded R^2 values ≥ 0.1 . Red = right NPH; Blue = Left NPH. The length of each arrow represents the R^2 value. For the normal monkey (A, B) nearly all neurons had preferred directions within 30° of horizontal. For the monkeys with A pattern exotropia, there were 5 to 6 neurons (out of 29 that were included in this analysis) with predominantly vertical preferred directions. In addition, for the right eye, there was a small, but fairly consistent, upward bias for neurons recorded from the NPH on the right side of the brain. The majority of the neurons recorded from the left NPH, however, had preferred directions with a downward component for the right eye.

increase when the ipsilateral eye moves in the on-direction and decrease when that eye moves in the off-direction. This analysis was only performed on a given neuron if at least six such trials were found ($n = 18$ neurons).

For each saccade that met the above inclusion criteria, the tonic firing rate was measured in two epochs,

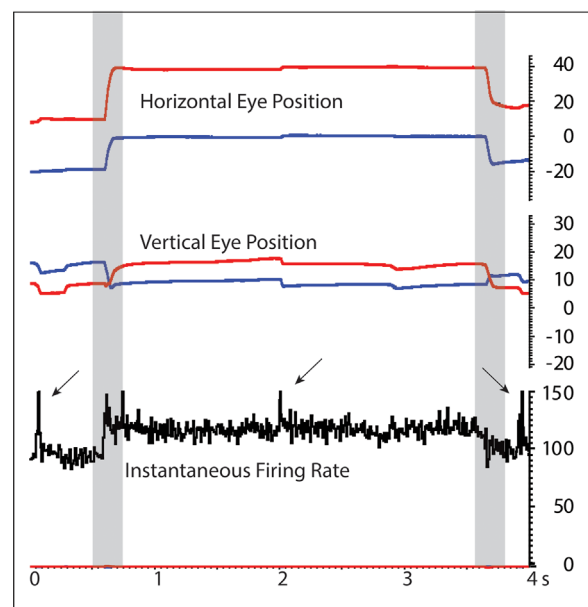


Figure 7

FIGURE 7. Raw data from an inferior rectus motoneuron, recorded from monkey XT1. Left eye position is shown in blue; right eye position is shown in red. When the saccade involves a downward component for both eyes the neuron shows a burst of spikes (arrows). When the vertical component is in opposite directions for the two eyes, the neuron shows a burst of spikes and an increase in the tonic firing rate when the left (ipsilateral) eye moves in the neuron's on-direction (i.e. first gray shaded area). In contrast, for left-eye-up/right-eye-down saccades (second gray shaded area) there is a brief pause followed by a decrease in the tonic firing rate. Thus, the neuron's firing rate appropriately reflects the movement of the ipsilateral eye, regardless of whether the contralateral eye moves in the on or off direction.

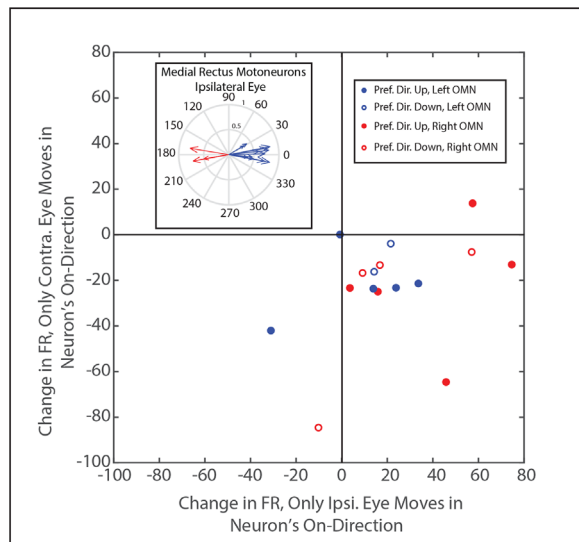


Figure 8

FIGURE 8. Change in the tonic firing rates of vertically-acting motoneurons, for saccades with opposite direction vertical components. The y-axis shows the change in firing rate when the contralateral eye moves in the on-direction and the ipsilateral eye moves in the off direction. The x-axis shows the change in firing rate when the ipsilateral eye moves in the on direction and the contralateral eye moves in the off-direction. Neurons recorded from the oculomotor nucleus (OMN) on the left side of the brain are shown in blue; neurons recorded from right OMN are shown in red. Filled circles indicate neurons with upward preferred directions (superior rectus motoneurons and inferior oblique motoneurons). Open circles represent inferior rectus motoneurons. For 16 of 18 neurons, the change in firing rate was consistent with the hypothesis that the discharge rate is higher for ipsi-on/contra-off movements than ipsi-off/contra-on movements. Because these saccades have large horizontal components, and smaller opposite direction vertical components, these data demonstrate that the dependence of vertical strabismus angle on horizontal eye position is the result of disjunctive eye position signals being sent to vertically acting motoneurons. Thus, the pattern strabismus in these animals cannot be fully accounted for by peripheral abnormalities, such as abnormal pulling directions of the eye muscles. Inset: Preferred directions for 15 medial rectus motoneurons recorded from monkey XT1.

presaccadic (300–100 ms before saccade onset) and post-saccadic (100–300 ms after saccade offset). The change in tonic firing rate (ΔFR) was measured as $TonicFR_{post} - TonicFR_{pre}$. Next, ΔFR was compared for trials in which only the ipsilateral eye moved in the on-direction (Ipsi-On) and those in which only the contralateral eye moved in the on-direction (Contra-On). Figure 8 plots the mean ΔFR for Contra-On versus Ipsi-On saccades for each of the 18 vertically acting motoneurons included in this analysis. If the pattern strabismus in monkeys XT1 and XT2 was the result of neural abnormalities, as is the case for AMO-reared monkeys,⁸ then most of the data points should fall into the bottom-right quadrant (ΔFR is positive for Ipsi-On and negative for Contra-On). Indeed, this was the case for 12 of 18 neurons. For one neuron, the ΔFR was positive for both Ipsi-On and Contra-On trials but was clearly higher for the former. For another neuron, ΔFR was negative for both, but was much lower for Contra-On trials. Thus, for 14 of 18 vertically acting motoneurons, the ΔFR was consistent with the hypothesis that vertically acting motoneurons fire at a higher rate for Ipsi-On trials than Contra-On trials, strongly suggesting that the vertical cross-axis disconjugacy

in monkeys XT1 and XT2 was the result of neural abnormalities, and not merely an abnormal pulling direction for the medial rectus muscles. The inset of this figure shows the estimated preferred directions for the 15 medial rectus motoneurons; note that there is no evidence of an overall downward bias, as there should have been if the medial rectus muscles had an abnormal pulling direction.

DISCUSSION

This study tested the hypothesis that neurons in the NPH show an abnormally strong sensitivity to vertical eye position in monkeys with surgically induced A-pattern exotropia. In both of our monkeys with exotropia, the vertical misalignment of the eyes varied with horizontal eye position (Fig. 1). Saccade direction often differs for the two eyes in both humans⁷ and monkeys²² with pattern strabismus. For example, when the left eye's saccade is purely horizontal, say to the right, the saccade for the right eye may have an upward component. For this reason, the horizontal and vertical sensitivities of the NPH neurons, and the estimates of their preferred directions, will inevitably differ for the two eyes. Interestingly, the overwhelming majority of the NPH neurons had normal preferred directions for the left eye in our subjects with A-pattern exotropia. Similarly, the left eye medial rectus motoneurons in monkey XT1 also had normal preferred directions. This would not be the case if the left medial rectus muscles in these two animals had abnormal pulling directions (compare red arrows in Figs. 6A and 6C). Additionally, when the saccade involved vertical components that were in opposite directions for the two eyes, vertically acting motoneurons on both sides of the brain modulated appropriately for the ipsilateral eye's vertical component. This would not happen if the pattern strabismus was merely the result of abnormal pulling directions for the eye muscles.^{8,9}

It is well known that many human patients with pattern strabismus display abnormal torsion.^{1,6} This being the case, one must consider whether some of the effects reported in the present paper might be due to torsion. However, as we have discussed in a recent study of the preferred directions of the INC neurons in pattern strabismus,²⁵ and in a recent review article,⁴⁵ it is very unlikely that the cross-axis effects we are interested in are primarily attributable to torsion. First, torsional abnormalities are not always present in human patients with pattern strabismus.⁴⁶ Second, the magnitude of torsional abnormalities, when they are present, is not correlated with the severity of the cross-coupling in human patients with pattern strabismus.⁷ Third, when monkeys with pattern strabismus perform horizontal smooth pursuit, the inappropriate cross-axis (i.e. vertical) movement of the nonviewing eye is associated with changes in the firing rates of motoneurons serving vertical rectus muscles.⁸ Similarly, during vertical smooth pursuit, the abnormal horizontal component of the movement in the nonviewing eye is associated with changes in the firing rates of medial rectus motoneurons.⁹ Fourth, the pattern of directional saccade disconjugacy can be complex, and inconsistent with overall torsional rotation.^{22,45} Fifth, microstimulation of some sites in the PPRF evoked conjugate, horizontal eye movements in monkey XT1, but microstimulation of other sites in the same animal evoked oblique movements with highly disconjugate vertical components.¹¹

Both of our exotropic monkeys underwent eye muscle surgery in infancy, which may have effectively weakened the

medial rectus muscles. If so, then the thresholds for rate-position curves of medial rectus motoneurons, abducens neurons, and the NPH neurons would be affected. It is also possible that early eye muscle surgery may trigger an adaptive response in oculomotor structures. Finally, there is the possibility that extraocular muscle (EOM) proprioception may be affected. These issues are discussed in detail in a recent review article.⁴⁷

Another potential limitation of the present study is that we had a relatively small number of recordings from the normal animal. However, McFarland and Fuchs⁴⁸ recorded 100 neurons from the NPH and the nearby medial vestibular nucleus (MVN) and reported estimates of horizontal sensitivity of 3.2 ± 1.3 (range = 1.2 to 6.4) for burst-tonic neurons and 2.4 ± 1.6 for tonic neurons. These horizontal sensitivity values were somewhat higher than the 1.93 we found for monkey N1, but this might be because we were careful to record, and analyze, every neuron that we isolated in the NPH. For our exotropic monkeys, the mean horizontal sensitivity was 2.15 when the neurons with R^2 values < 0.1 were included. When we excluded them, we obtained a mean sensitivity of 2.82; thus, the present data provide no convincing evidence that the horizontal sensitivity differs between normal and strabismic monkeys. However, the std of horizontal sensitivity from our monkeys with exotropia was 3.1 (2.37 if we excluded neurons with $R^2 < 0.1$), which is much larger than what McFarland and Fuchs⁴⁸ reported, and was also larger than the 2.0 we found for monkey N1.

It was common for neurons in all monkeys to have at least a small sensitivity to vertical eye position. In this respect, the only clear directional abnormality we found for the monkeys with exotropia was that the absolute value of the vertical sensitivity was significantly larger for the right eye in those animals, compared to monkey N1. We found several neurons in the monkeys with exotropia that were more sensitive to vertical eye position than horizontal eye position. No such neurons were found in the normal animal in the present study but there is a brief mention of such neurons in McFarland and Fuchs.⁴⁸ However, those recordings were excluded from analysis. In addition, from an examination of their Figure 2, it appears that the small number of vertically tuned neurons they found were not in the NPH.

In both of the monkeys with "A"-pattern exotropia, most of the neurons had normal preferred directions for the left eye. For the right eye, however, the preferred directions deviated from the horizontal in a manner consistent with the changes in vertical strabismus angle observed in the Hess plots for these animals (i.e. elevation of the abducting eye, relative to the adducting eye). A degree of caution is warranted when interpreting this result, however, because it might be that the directional deviations for the right eye are the result of events in parallel pathways or downstream from the NPH. For this reason, the present data cannot unequivocally place the key directional abnormality within the NPH.

When monkeys with pattern strabismus pursue a horizontally moving visual target, vertical rectus motoneurons serving the fellow eye modulate in association with the inappropriate vertical movement of that eye.⁸ It is likely that the same thing happens in association with horizontal saccades for the two monkeys with A-pattern exotropia in the present study. If so, then when the left eye makes a horizontal rightward movement, the upward movement in the right eye occurs because superior rectus motoneurons serving that eye increase their firing rates and/or inferior rectus motoneurons decrease their firing rates. The exact mecha-

nism by which this might occur is, at present, unknown, but we speculate that disconjugate input from the horizontal pathway causes the push-pull interactions that normally determine the tonic firing rates of motoneurons serving agonist and antagonist muscles to be asymmetrical for the two eyes.

The most robust effect we found was the relatively poor model fits for neurons recorded from the monkeys with exotropia. It is not unusual, of course, to find neurons with weak position sensitivity in the NPH (see the histogram for the normal animal in Fig. 3A) but, in the monkey with exotropia, there were very few model fits that yielded R^2 values > 0.7. Overall, the R^2 values were significantly lower in the monkeys with exotropia.

In previous studies, we have found similar directional abnormalities and poor model fits in the PPRF¹⁰ and the interstitial nucleus of Cajal²⁵ in monkeys with experimentally induced pattern strabismus. This is consistent with a view that is emerging from recent literature, that a chronic misalignment of the eyes in infancy, and the resulting disturbance of binocular vision, interferes with the development of normal tuning for neurons in the visual and oculomotor areas of the brain.^{45,47} At birth, neurons in visual and oculomotor pathways display coarse, immature tuning.^{45,49-52} For example, in monkeys with strabismus induced in infancy, the number of binocularly responsive neurons and disparity sensitivity is reduced in V1,^{39,53,54} MT⁴¹ and MST.⁵⁵ In the PPRF in monkeys with pattern strabismus, the number of spikes in saccade-related bursts is often poorly correlated with horizontal amplitude.¹⁰

It should be noted that monkey XT1 was also used in our single unit recording¹⁰ and microstimulation¹¹ studies in PPRF. As we have previously noted,^{11,45} eye movements evoked by the PPRF stimulation in this animal were conjugate and horizontal for some sites but, for other sites, the evoked movements were highly disconjugate with strong vertical components. Furthermore, stimulation of several sites in this animal caused the right eye to move up-left (see blue dots in Fig. 4D of this earlier study¹¹). In the present study, the preferred directions of neurons recorded from left NPH in the *same animal* showed a down-left bias (Fig. 6D).

The high degree of variability in the direction, and conjugacy/disconjugacy, of evoked movements is incompatible with the hypothesis that the pattern strabismus in this animal is solely a consequence of peripheral abnormalities (such as an abnormal pulling direction of the medial rectus muscle). Similarly, the high variability of the preferred directions in the right PPRF in this animal (see red arrows in Figs. 6E, 6F of Ref. 10) cannot be accounted for by peripheral abnormalities. In the present study, the preferred directions for the right NPH neurons were less variable (Fig. 6, red arrows), which might indicate convergent input, with some of the variability at the level of the PPRF being averaged out in the NPH. Nonetheless, there were still several neurons in the present study with predominantly vertical preferred directions. Similarly, in recordings of the INC of monkeys with pattern strabismus, some neurons had normal preferred directions, whereas a minority had mostly horizontal preferred directions.²⁵ This pattern of results is most consistent with the Distributed Crosstalk Model,¹³ which proposes that cross-axis disconjugacy in pattern strabismus is the result of an accumulation of (perhaps mild) directional abnormalities across numerous brainstem areas.

A similar failure of maturation has been proposed to account for the nasalward bias of smooth pursuit gain in

strabismus.⁵⁶ Normal infant primates show a clear nasalward bias of smooth pursuit and optokinetic nystagmus⁵⁷ and it has been proposed that the asymmetrical smooth pursuit gain in strabismus is the result of persistence of neural tuning in this immature state.^{56,58} Similarly, we suggest that the relatively poor R² values for the model fits and the abnormal directional tuning for the right eye in the present study reflect a failure of neurophysiological maturation in monkeys with exotropia induced in infancy.

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