Correlation of Cross-Axis Eye Movements and Motoneuron Activity in Non-Human Primates with "A" Pattern Strabismus

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Purpose. The authors showed earlier that animals reared with certain types of visual sensory deprivation during their first few months of life develop large horizontal strabismus, A/V patterns, and dissociated vertical deviation (DVD). Cross-axis eye movements were observed in the nonfixating eye that reflected pattern strabismus and DVD. The purpose of this study was to investigate whether neuronal activity within the oculomotor nucleus could be driving the abnormal cross-axis eye movements observed in the nonfixating eye.

Methods. Burst-tonic activity was recorded from oculomotor nucleus neurons in three animals with A-pattern exotropia as they performed horizontal or vertical smooth pursuit during monocular viewing. Two animals were reared by alternate monocular occlusion for 4 months, and one animal was reared by binocular deprivation for 3 weeks.

Results. In this study, efforts were focused on neurons modulated for vertical eye movements. Vertical burst-tonic motoneurons were strongly correlated with vertical eye movements regardless of whether the movement was purposeful, as in vertical smooth pursuit, or whether it was inappropriate, as in a vertical component observed in the nonfixating eye during horizontal smooth pursuit. Quantitative analysis of position and velocity sensitivities of the cells measured during the different tracking conditions suggested that motoneuron activity was sufficient to account for most of the inappropriate vertical cross-axis component.

Conclusions. Results suggest that, in animals with sensory-induced strabismus, innervation to extraocular muscles from motor nuclei produce the inappropriate cross-axis eye movements, resulting in change in ocular misalignment with gaze position associated with pattern strabismus and DVD. (*Invest Ophthalmol Vis Sci.* 2007;48:665–674) DOI:10.1167/iovs.06-0249

Binocular alignment and binocular coordination of eye movements are important in primates, who have frontal vision and foveae to direct gaze at a particular object.[1,2] Loss of sensory or motor fusion early in postnatal development leads to binocular misalignment (strabismus). Various studies conclude that infantile forms of strabismus occur in as many as 5% of all children.[3] Incomitant strabismus is one in which ocular misalignment varies with gaze position. A relatively common form of incomitant strabismus is A/V pattern strabismus.[4,5] An increase in esotropia or a decrease in exotropia in supraduction and an increase in exotropia or a decrease in esotropia in infraduction is called an "A" pattern. Similarly, an increase in exotropia or a decrease in esotropia in supraduction and an increase in esotropia or a decrease in exotropia in infraduction is called a "V" pattern.[6,7] One study suggests that more than 50% of patients with horizontal misalignment also show A/V pattern incomitance.[5] Though the nomenclature primarily refers to variation of horizontal misalignment with vertical gaze position, often a vertical misalignment that changes with horizontal gaze position is present as well. Earlier we showed that we are able to reproduce these properties of strabismus in monkeys reared using visual sensory deprivation paradigms.[8] Our animals also displayed dissociated vertical deviation (DVD), another common disorder observed in humans with strabismus, by which the nonfixating eye is elevated compared with the fixating eye. In our published study, we measured binocular eye movements in these animals and showed that static alignment patterns were reflected in their eye movements. Thus, during monocular viewing, the animal was able to track a horizontally or vertically moving pursuit or saccadic target with purely horizontal or vertical eye movements of the viewing eye. However, the nonviewing eye displayed significant cross-axis components (i.e., vertical components during horizontal tracking and horizontal components during vertical tracking; see Figs. 1 and 2).[8]

There are at least two possible sources for the abnormal cross-axis eye movements associated with A/V pattern strabismus and DVD. One possibility involves only the periphery and is what we refer to as the *mechanical hypothesis*. Thus, in the mechanical hypothesis, either static malpositioning of extraocular rectus muscle pulleys or sideslip of extraocular muscle because of dynamic instability of muscle pulleys could result in A/V patterns and associated eye movements. This hypothesis has support from human MRI studies that examined patients with incomitant strabismus and showed pulley location problems and problems with muscle stability.[9–12] Another hypothesis is that disruptive changes in neural circuits result from visual sensory deprivation rearing, leading to an inappropriate neural drive to extraocular muscles that leads to cross-axis eye movements and the A/V patterns and DVD observed in the strabismic animals. We refer to this as the *neural hypothesis*. Though the neural hypothesis may be attractive for animals with sensory induced strabismus that were part of this study (given that the rearing paradigm putatively does not interfere with extraocular muscle), a few studies have provided genetic/molecular evidence that suggests visual sensory deprivation may alter the development of extraocular muscle structure.[13,14]

In this study we report results from experiments aimed at testing the neural hypothesis for generating cross-axis eye movements leading to A/V patterns and DVD in our animals. We recorded from extraocular motoneurons in the oculomotor nucleus and analyzed neuronal activity when the animals at-

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tempted a sinusoidal smooth pursuit task with either eye viewing. We focused this study on vertical motoneurons. The question we asked was whether neuronal responses of the oculomotor neurons could account for the abnormal cross-axis movements observed in our animals. Our working hypothesis was that if there were a neural source for the inappropriate cross-axis movements, the relationship between motoneuron unit activity and eye motion should remain consistent during purposeful tracking and during cross-axis eye movements. On the other hand, if the A/V patterns and associated cross-axis eye movements were caused by nonneural sources (for example, mechanical problems at the periphery), the lawful relationship between the neuronal responses and eye movements should break down during the inappropriate cross-axis eye movements. It is important to note that we were not investigating the source of the horizontal or vertical misalignment. Rather, our goal was to determine whether the change in ocular misalignment with eye position was caused by a neuronal drive. Some of the results have appeared before in abstract form (Das VE, et al. *IOVS* 2004;45:ARVO E-Abstract 2545).[15]

METHODS

Subjects and Rearing Paradigms

Behavioral and neurophysiological data were collected from three strabismic (S1, S2, S3) juvenile rhesus monkeys (*Macaca mulatta*) weighing 3 to 7 kg each. Monkeys with strabismus were reared at the Yerkes National Primate Research Center using visual sensory deprivation methods for the first few months of life designed to induce ocular misalignment but not to affect visual acuity.[8,16] S2 and S3 were reared using an alternating monocular occlusion (AMO) method. In the AMO-rearing procedure, soon after birth (within the first 24 hours), an occluding patch (either opaque goggles or dark contact lenses) is placed in front of one eye for a period of 24 hours and switched to the fellow eye for the next 24 hours. The patch is alternated daily for a period of 4 months. In this method, binocular vision is severely disrupted during the first few months of life, the critical period during which the monkeys normally develop proper eye alignment, stereovision, and binocular sensitivity in the brain.[17–19] S1 was reared using a binocular deprivation (tarsal plates intact; BDTP) method. With this method, the animals' eyelids were kept closed by tarsoraphoplasty for the first 3 weeks of life. Tarsal plates contained inside the lids were left intact. Earlier we reported that BDTP results in large strabismus and small latent nystagmus (LN).[16] Even though visual function was not directly tested in these animals, they were all able to perform the oculomotor tasks used in this study. Further, all these animals also showed evidence for alternating fixation during binocular viewing, suggesting that there was not a significant bias toward any one eye. Additional details on rearing and visual function of similarly reared animals have been described.[8]

Surgical Procedures and Eye Movement Measurements

After special rearing, the animals were allowed to grow normally, until they were approximately 2 to 3 years of age, before behavioral and neurophysiological experiments were begun. Sterile surgical procedures performed under aseptic conditions using isoflurane anesthesia (1.25%–2.5%) were used to stereotaxically implant a head stabilization post and a recording chamber. The recording chamber was a 21-mm–diameter stainless steel cylinder implanted at a stereotaxic location (3-mm anterior, 1-mm lateral, and 20° angle to the sagittal plane). This chamber placement allowed full access to both oculomotor nuclei. During the same surgical procedure, a scleral search coil was implanted in one eye according to the Judge et al.[20] technique. Later, in a second surgery, a second scleral search coil was implanted in the other eye. All procedures were performed in strict compliance with National Institutes of Health and the Association for Research in Vision and Ophthalmology guidelines, and the protocols were reviewed and approved by the Institutional Animal Care and Use Committee at Emory University.

Binocular eye position was measured using the magnetic search coil method (CNC Engineering, Seattle, WA [S1 and S2]; Primelec Industries, Regensdorf, Switzerland [S3]).[21,22] Calibration of the eye coil signal was achieved by giving the monkey a small amount of juice or another reward when the animal looked within a small region (±2° window) surrounding a 0.25° target spot that was rear projected on a tangent screen 57 cm away from the animal. All stimuli were under computer control. Animals were trained for approximately 2 to 3 months before data collection. Calibration of each eye was performed independently during monocular viewing.

Single Unit Recording and Experimental Paradigms

The oculomotor nucleus was clearly identified by its stereotaxic location, characteristic "beehive" sound, and burst-tonic (BT) activity of the cells during eye movements made in the on-direction of the cells. During initial electrode penetrations, we mapped the rostrocaudal extent of the oculomotor nucleus and established the midline. Based on the change in tonic activity with eye position, the cells could be classified into horizontal (left/right) BT cells that project to the medial rectus or vertical (up/down) BT cells that project to the superior/inferior recti or the inferior oblique muscle. We did not attempt to identify the particular muscle to which the cell was projecting, but, based on the recording location in the chamber, we were able to estimate with reasonable certainty in which oculomotor nucleus (i.e., right OMN or left OMN) we were recording. Based on this, the down-BT neurons could be localized to the ipsilateral inferior rectus while the up-BT neurons could be projecting to either the ipsilateral inferior oblique or the contralateral superior rectus. For the purposes of this study, we decided to simply refer to the cells as down-BT or up-BT neurons.

The goal of the experiments was to compare neuronal responses during purposeful tracking eye movements and during inappropriate cross-axis eye movements. To achieve this goal, we acquired neuronal activity as the animals performed each of four tracking tasks: sinusoidal horizontal smooth pursuit, 0.2 to 0.3 Hz, ±10° to 15°, left eye viewing; sinusoidal vertical smooth pursuit, 0.2 to 0.3 Hz, ±10° to 15°, left eye viewing; sinusoidal horizontal smooth pursuit, 0.2 to 0.3 Hz, ±10° to 15°, right eye viewing; sinusoidal vertical smooth pursuit, 0.2 to 0.3 Hz, ±10° to 15°, right eye viewing. Thus, when the left eye is viewing, cross-axis movements are observed in the right eye and vice versa.

Data Acquisition and Analysis

For S1 and S2, binocular eye and target position feedback signals were processed with anti-aliasing filters at 200 Hz using six-pole Butterworth filters before digitization at 1 kHz with 16-bit precision (Labview Software and DAQ board [National Instruments, Austin, TX]). Unit activity was recorded with epoxy-coated tungsten electrodes (1–5 megaohm; Frederick Haer, Brunswick, ME). Action potentials were identified using a windowing method (Bak Instruments, Rockville, MD), and time stamps were stored. In addition, raw unit data were acquired at 25 kHz (CED 1401 and Spike2 software [Cambridge Electronic Design, England]). Spike sorting was also performed offline, and spike times were calculated with the use of an offline template-matching algorithm (Spike2 software; CED). Because of a change in data acquisition systems in the laboratory, eye, target, and unit data for animal S3 were acquired differently (AlphaLab system; Alpha Omega Inc., Nazareth, Israel). Binocular eye and target data were acquired at 781.25 Hz, and raw unit data were acquired at 25 Khz. A time stamp representing isolated unit activity was generated by an online hardware spike sorter (AlphaLab Spike Detector; Alpha Omega Inc.). In addition, the raw unit data were saved, and an offline template-matching algorithm was used for spike sorting (Spike2 software; CED) as in

animals S1 and S2. Generally, the online and offline sorting methods were in close agreement.

Data analysis was performed with custom software built in Matlab (Mathworks, Natick, MA). Velocity arrays were generated by digital differentiation of the position arrays using a central difference algorithm. Unit response was represented as a spike density function that was generated by convolving the spike times with a 10-ms Gaussian.[23] We used a model estimation procedure to calculate position and velocity sensitivities of the motoneurons. Similar procedures have been used with success by us and other investigators in various parts of the ocular motor system, including the motor nuclei.[24–28]

Eye position and velocity data were filtered using an 80-point finite impulse response (FIR) digital filter with a passband of 0 to 50 Hz. Saccades were identified using a 50°/s velocity criterion and were removed from the sinusoidal tracking eye data. Corresponding spikes were also removed after adjusting for an average motoneuron lead time of approximately 10 ms.[24] Desaccading the data was important because it has been shown that motoneuron position and velocity sensitivities may be different during saccades and smooth pursuit.[24] Averaged data from multiple trials in which the animal was judged to be tracking the sinusoidal target were then used to identify coefficients in the following model:

$$FR(t) = KE(t) + RE'(t) + C$$

where $E(t)$ denotes the eye position at time t, $E'(t)$ denotes the eye velocity at time t, and $FR(t)$ is the estimated value of the unit spike density function at time t. Coefficients K and R are the position and velocity sensitivities of the cell, and C is a constant that represents unit firing rate when the animal is fixating straight ahead. We did not include latency because the data used for the model estimation was low-frequency sinusoidal tracking, and adjusting the neuronal response by 10 ms would have made little difference in the model fits. We estimated the parameters K, R, and C in each of the four tracking conditions for every cell. The regstats function available through the statistics toolbox in Matlab was used for this purpose. We also calculated goodness-of-fit based on the coefficient of multiple determination (CD). This is equivalent to an R^2 measure for linear regression. Repeated-measures ANOVA on ranks and multiple comparison tests were used to compare the estimated parameters (K, R, C) in conditions of purposeful tracking and during conditions that elicited cross-axis movements.

Results

Cross-Axis Eye Movements in Monkeys with Strabismus

All strabismic animals tested in this study showed evidence of A pattern exotropia, as shown in the Hess screen chart representation in Figure 1. We have earlier described in detail the eye alignment and the behavioral eye movements in two of the animals (S1 and S2) in this study.[8] Figure 1 repeats data from these animals and includes data from a new animal, S3. Alignment data were collected from periods of postsaccadic fixation of at least 5 seconds each. The main points to be noted in this figure are that all animals were exotropic, horizontal misalignment varied with vertical gaze position, and vertical misalignment varied with horizontal gaze position.

Figure 2 shows the eye movements in animal S2 collected under monocular viewing during smooth pursuit tracking. It also shows that tracking eye movements reflect the static alignment patterns depicted in Figure 1. Figure 2A plots horizontal position of the viewing (right, black trace) and nonviewing (left, gray trace) eyes, and Figure 2B plots the vertical positions. Thus, the viewing right eye tracks the smooth pursuit target with a purely horizontal or vertical eye movement, whereas the eye movement in the nonviewing left eye includes an inappropriate cross-axis component (i.e., an inappropriate vertical eye movement during horizontal tracking (Fig. 2A) and an inappropriate horizontal eye movement during vertical tracking (Fig. 2B), resulting in oblique trajectories). The question we asked in this study was whether motoneuron activity could account for the inappropriate cross-axis eye movements observed in these animals.

Horizontal Motor Neuron Activity

We encountered many medial rectus motoneurons (related to horizontal eye movements) in the three strabismic animals in this study. However, we collected limited data from medial rectus motoneurons and performed limited analysis on these data, primarily because two of the three animals in the study had large exotropia. In this form of strabismus, the medial rectus of the nonfixating eye is relaxed because of its abducted state, which in turn makes it likely that neurons projecting to the relaxed medial rectus are mostly inactive. Figure 3 shows an example of such a neuron. This particular cell showed robust modulation for rightward movements (therefore projecting to the medial rectus of the left eye) when the left eye was viewing (Fig. 3A) but showed no activity when the right eye was viewing (Fig. 3B) because the left eye was now exotropic (i.e., in an abducted position). During our experiments, most isolated medial rectus cells were completely shut off when the eye to which the neuron projects was nonfixating, making estimation of parameters during the cross-axis tracking conditions difficult. One strategy could have been to offset the target so that the neuron remained above threshold even when the eye to which it projected was not viewing (i.e., abducted). However, because the exotropia of S1 and S2 was so large, we were unable to sufficiently offset the target and to obtain consistent tracking behavior from the animal. Therefore, we decided to focus the rest of this study on motoneurons that are modulated for vertical eye movements.

Vertical Motor Neuron Activity

We recorded from 20 motoneurons that were modulated for vertical eye movements in the three strabismic animals. The goal with these data was to compare neuronal response during a) vertical tracking when the eye to which the neuron projected was viewing (VerSP), b) horizontal tracking when the eye to which the neuron projected was viewing (HorSP), c) horizontal tracking when the eye to which the neuron projected was the nonfixating eye (paradigm during which an inappropriate vertical cross-axis component was observed in the eye to which the neuron projected; cross-axis HorSP), and d) vertical tracking when the eye to which the neuron projected was nonviewing (cross-axis VerSP).

Figure 4 shows data from a sample neuron in animal S3 that was modulated for upward eye movement and projecting to the left eye extraocular muscle (EOM). The figure shows nondesaccaded data, but saccades were removed before regression analysis to estimate coefficients. The middle and top rows show averaged horizontal and vertical positions of the right and left eyes during the four sinusoidal smooth pursuit tasks listed in Methods. The bottom row shows the neuronal response in each condition. Data show that a cross-axis component is present in the nonfixating eye during vertical (cross-axis horizontal component) and horizontal (cross-axis vertical component) tracking. Neuronal responses in the bottom row show that the cell is well modulated during vertical tracking eye movements with either the right or the left eye viewing (Figs. 4A, 4B). Neuronal modulation was not observed when the animal tracked a horizontal target with the left eye (i.e., no vertical component in left eye; Fig. 4C), but a clear modulation was observed when the animal tracked the horizontal target with the right eye (inappropriate cross-axis vertical component

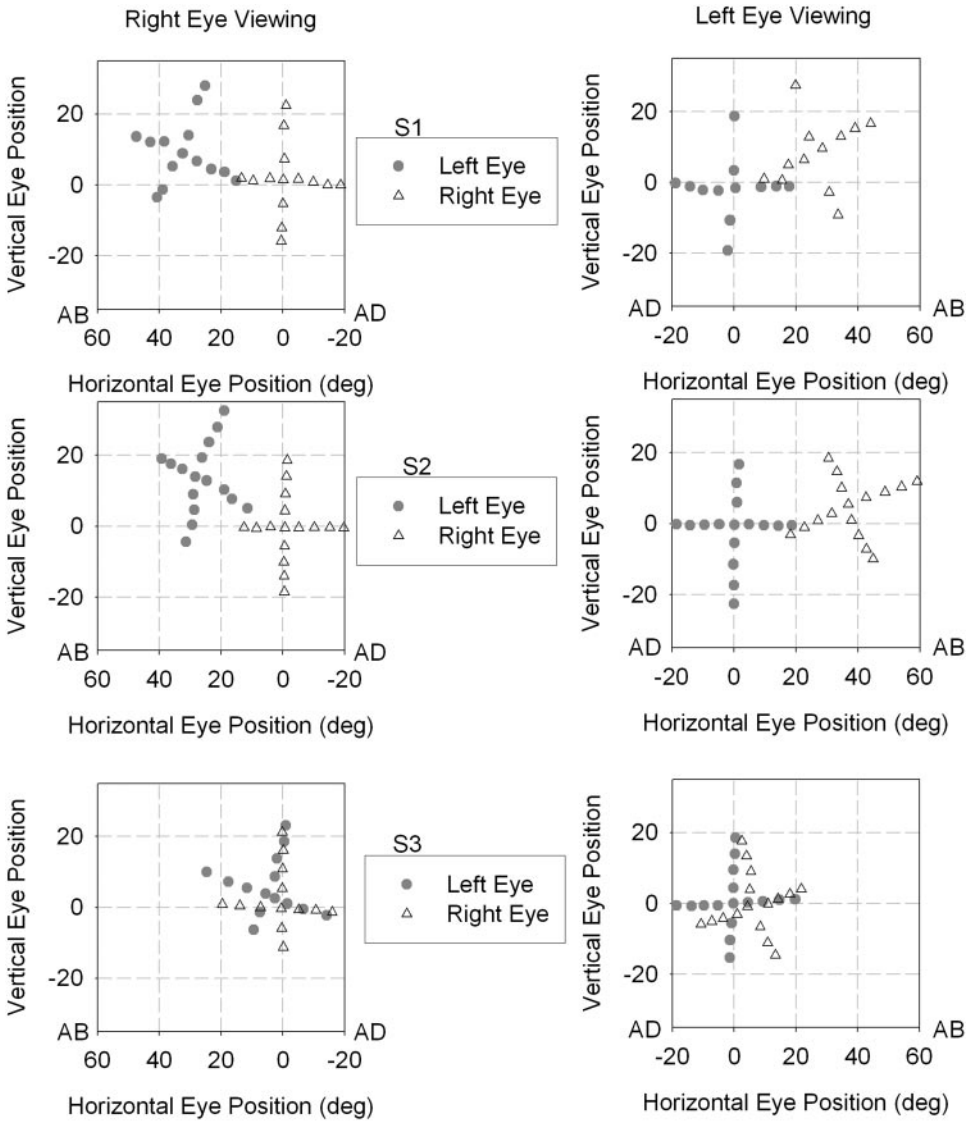

FIGURE 1. Hess screen chart showing alignment patterns during monocular viewing in strabismic monkeys S1, S2, and S3. *Left*: alignment data collected during right eye viewing; *right*: alignment data collected during left eye viewing. Abduction is positive, and adduction is negative. Upward eye positions are positive, and downward eye positions are negative. All the strabismic animals had significant horizontal (exotropia) and vertical misalignment during viewing with either eye. The following eye was generally higher (except in S3, left eye viewing) than the fixing eye, suggesting the presence of DVD. In each case, a change occurred in horizontal misalignment, with vertical gaze position consistent with an A pattern. In addition, all the strabismic animals also showed a change of vertical misalignment with horizontal gaze position.

observed in left eye; Fig. 4D). Thus, this particular BT cell showed activity correlated with upward movements of the left eye whether they were associated with a vertical tracking task (Figs. 4A, 4B; VerSP and cross-axis VerSP) or an inappropriate vertical component observed in the left eye during horizontal tracking with the right eye viewing (Fig. 4D, cross-axis HorSP).

Figure 5 shows another sample neuron from animal S3 modulated for downward movement of the left eye. A result

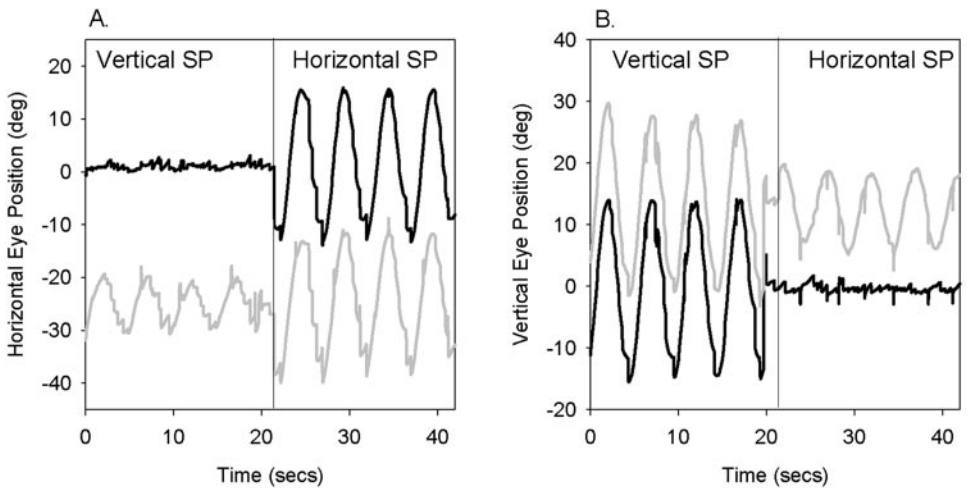

FIGURE 2. Raw data plot of eye movements in animal S2 during horizontal and vertical smooth pursuit. Target amplitude was ±15°. The viewing eye (right eye, *black trace*) makes purely horizontal or vertical tracking eye movements. However, the nonviewing eye (left eye, *gray trace*) shows an inappropriate cross-axis component (i.e., inappropriate horizontal component during vertical tracking and inappropriate vertical component during horizontal tracking). In this plot and other data plots, positive values indicate rightward or upward eye positions.

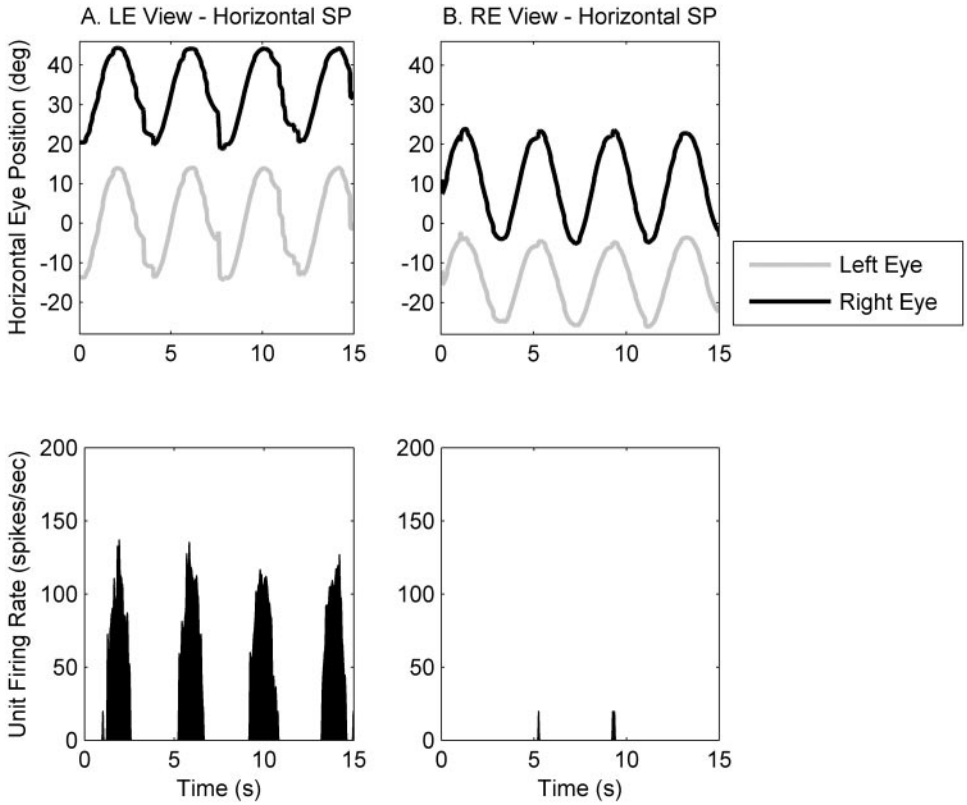

FIGURE 3. Single-unit activity in an example horizontal motoneuron. (**A**, **B**) Smooth pursuit eye movements with either the right eye or the left eye viewing. (**C**, **D**) Associated neuronal activity. This sample neuron was sensitive to rightward eye movements (left medial rectus motoneuron). Thus, during left eye viewing (**A**, **C**), the neuron is well modulated for rightward eye movements. When the eye of fixation is switched and the animal is tracking the target with the right eye viewing (**B**, **D**), the left eye is deviated to the left (exotropic). The neuron is completely shut off during this task, as would be expected when the medial rectus of the left eye is relaxed. A fixation offset of 10° was added during the right eye viewing (RE View) tracking condition (**B**) to bring the eye position closer to the neuron's threshold, but this offset was insufficient.

similar to that in the previous example is demonstrated. This particular BT cell showed activity correlated with downward movement of the left eye, whether associated with a vertical tracking task (Figs. 5A, 5B) or an inappropriate vertical component observed in the left eye during horizontal tracking with the right eye viewing (Fig. 5D). These data are consistent with the neural hypothesis, which states that the inappropriate cross-axis vertical component observed in the nonfixating eye during horizontal tracking is driven by neuronal activity in motoneurons projecting to vertical muscles of the nonfixating eye.

Quantification of Position and Velocity Sensitivities

The data illustrated in Figures 4 and 5 show that vertical components of cross-axis movements are possibly driven by activity in vertical motoneurons. We examined the relationship quantitatively by comparing position and velocity sensitivities of these neurons across the different tracking conditions. To achieve this, we used multiple linear regression to fit the unit response to the vertical component of the eye movement in the four tracking conditions according to the equation described in Methods. For the sample neuron in Figure 5, the equations describing the fit for the four tracking tasks are provided in the legend to Figure 5.

In each of the equations, fits are made using vertical movement data of the left eye. The first two fit equations represent fits to unit responses during conditions of vertical tracking, when the neuron is strongly modulated. The third fit equation represents the condition in which no vertical movement occurred in the left eye, and, accordingly, the neuron was not modulated and the goodness-of-fit was very low ($CD = 0.1$). The last fit equation represents the cross-axis condition in which a vertical component is observed in the left eye during horizontal tracking with the right eye viewing. The neuron is modulated, and a good fit was obtained with a high *CD* similar to the vertical tracking conditions. Parameters from the third fit equation (horizontal SP, left eye view) were meaningless because the *CD* was very low.

Figures 6 and 7 are comparative plots of position and velocity sensitivities under the three high *CD* conditions for all the cells in the sample. The *x*-axis in each plot of Figure 6 shows the coefficient estimate for the vertical tracking condition, where the eye to which the neuron is projecting is the fixating eye (VerSP). The *y*-axis shows the coefficient estimate for the vertical tracking condition, where the eye to which the neuron is projecting is the nonfixating eye (cross-axis VerSP). For example, coefficients obtained from data in Figure 5A and equation A are on the *x*-axis, and data obtained from Figure 5B and equation B are on the *y*-axis. As would be expected, the data points are close to the unity line.

Figure 7 plots parameters estimated during vertical smooth pursuit (Fig. 5A, equation A [VerSP]) and during the vertical cross-axis component during horizontal tracking (Fig. 5D, equation D [cross-axis HorSP]). These plots illustrate two points. The first is that, like the sample cells illustrated in Figures 4 and 5, all the cells in our sample showed significant sensitivity to vertical eye position and velocity associated with the inappropriate vertical component observed in the nonfixating eye during horizontal tracking. The distribution of sensitivities showed a strong correlation between the estimated values in the two conditions plotted. The second point is that for all neurons we collected, the phase of modulation observed during cross-axis vertical movements was always consistent with the phase observed during vertical tracking. Thus, neurons classified as sensitive to upward or downward eye movements (up-BT or down-BT) during vertical smooth pursuit were similarly sensitive to either upward or downward eye movements during the vertical components of cross-axis eye move-

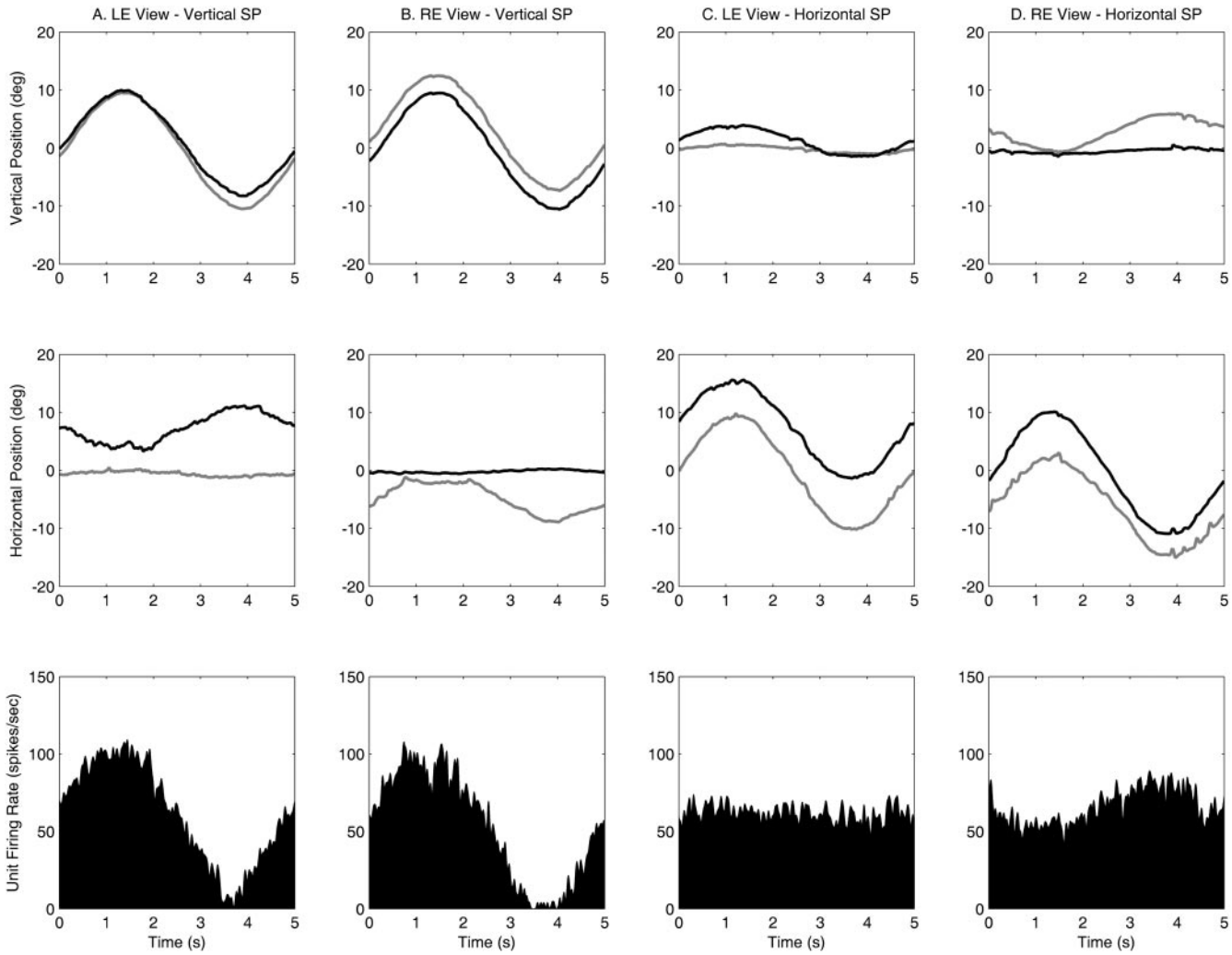

FIGURE 4. Single-unit activity in an sample motoneuron that was sensitive to upward eye movements and projected to the left eye. Plots show the pattern of eye movements (*black*, right eye; *gray*, left eye) and the associated neuronal activity during the four tracking conditions (horizontal or vertical smooth pursuit with either the left or the right eye viewing). Each column shows eye data averaged over multiple trials from a single tracking paradigm. *Top*: vertical position; *middle*: horizontal position; *bottom*: neuronal activity. Data plotted are not desaccaded, though saccades were removed before regression analysis. Note the inappropriate cross-axis component in the left eye traces when the animal is performing horizontal or vertical smooth pursuit with the right eye viewing (**B**, *middle*; **D**, *top*). The neuron is well modulated during vertical tracking with either the left eye (**A**) or the right eye (**B**) viewing. The neuron shows an increase in activity associated with upward eye movements. When the animal is performing horizontal smooth pursuit with the left eye viewing (**C**), no vertical component of movement in the left eye and no modulation in activity of the neuron developed. (**D**) Data from trials when the animal is performing a horizontal smooth pursuit task with the right eye viewing. The left eye shows an inappropriate vertical component of motion (*top*) and the neuron (*bottom*) shows a modulation in activity associated with the vertical component of the movement. The neuron's phase is similar to that observed during vertical smooth pursuit tasks.

ments observed during horizontal tracking. Together these points suggest that the modulation of the neuron was actually driving the inappropriate vertical cross-axis eye movement during horizontal tracking. Although not plotted to prevent redundancy, it is immediately apparent that coefficients estimated for cross-axis VerSP (Fig. 6, *y*-axis) and cross-axis HorSP (Fig. 7, *y*-axis) were also closely correlated.

Statistical Comparison of Coefficients

We performed a series of statistical tests to compare fit coefficients in the different tracking conditions. We first compared the coefficients of the entire population. Average coefficients (SD in parentheses) of the entire cell sample for the four tracking conditions are shown in Table 1.

Note that the fits in the third column of Table 1 have low *CD*; therefore, position and velocity coefficients were meaningless because they represented the condition when no vertical cross-axis component in the eye was observed to which the neuron projected and no modulation was observed in the cell. Therefore, we only performed statistical comparisons of coefficients obtained using the other three high *CD* tracking conditions. Comparison of the population means with one-way ANOVA showed no significant differences in position ($P = 0.45$), velocity ($P = 0.13$), or constant ($P = 0.86$) across the tracking tasks.

Because each neuron was studied during all four tracking tasks, we were able to use pairwise tests to study their behavior further. We used repeated-measures ANOVA on ranks (similar to Wilcoxon paired *t* test, but for three or more treatments) to compare each coefficient during the three tracking conditions in which the neuron was modulated. We found no significant difference in the constant ($P = 0.21$) in the three

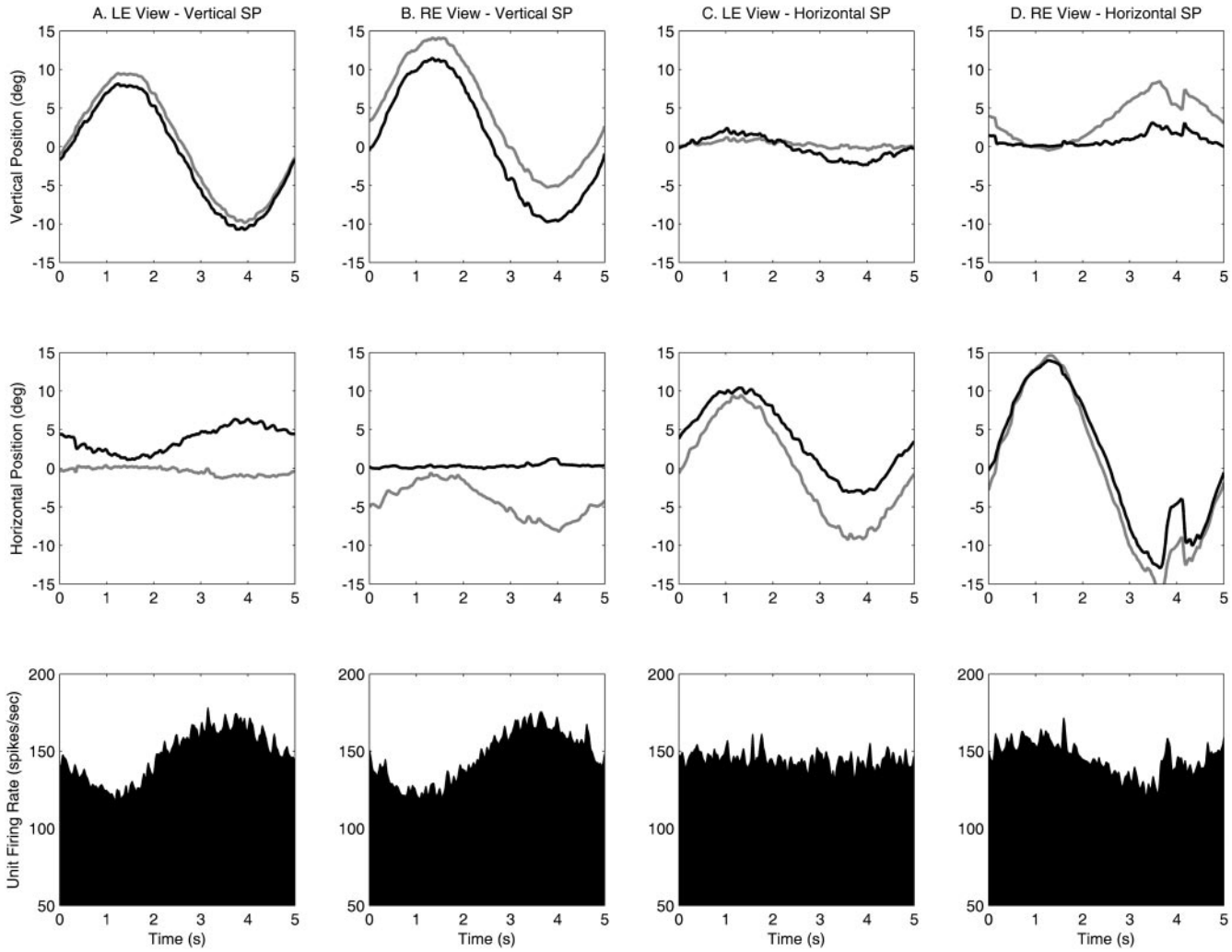

FIGURE 5. Single-unit activity in an sample motoneuron that was sensitive to downward eye movements and projected to the left eye. Similar to the previous example, this neuron showed modulation of activity during vertical smooth pursuit with either the left eye (**A**) or the right eye viewing (**B**). When the animal performed a horizontal smooth pursuit task with the left eye viewing, there was no vertical component of left eye movement (**C**, *top*), and there was no modulation of activity in the neuron (**C**, *bottom*). When the animal performed a horizontal smooth pursuit task with the right eye viewing, an inappropriate vertical component of eye movement was observed in the left eye trace (**D**, *top*). The amplitude of tracking was ±15° because the ±10° tracking data did not yield enough analyzable trials. Neuronal activity was modulated (**D**, *bottom*) for downward eye movements, similar to the vertical smooth pursuit tasks. Fit equations describing motoneuron activity in each tracking condition are as follows: (**A**) vertical SP, left eye view – VerSP: $FR(t) = 2.15^*E(t) + 0.7^*E'(t) + 147$; $CD = 0.95$; (**B**) vertical SP, right eye view – cross-axis VerSP: $FR(t) = 2.25^*E(t) + 0.61^*E'(t) + 156$; $CD = 0.96$; (**C**) horizontal SP, left eye view – HorSP: $FR(t) = -4.2^*E(t) + 0.26^*E'(t) + 143$; $CD = 0.10$; (**D**) horizontal SP, right eye view – cross-axis HorSP: $FR(t) = 3.13^*E(t) + 0.97^*E'(t) + 156$; $CD = 0.91$.

tracking conditions. However, significant differences ($P = 0.003$) were noted in the position coefficients across the three tracking conditions (VerSP, cross-axis VerSP, and cross-axis HorSP) and in the velocity coefficients ($P = 0.006$) across the three tracking conditions. A pairwise multiple comparison procedure (Dunn test; Sigma Stat 3.0) was used to identify the source of these differences. Given that there were three sets of comparisons (VerSP vs. cross-axis VerSP; VerSP vs. cross-axis HorSP; cross-axis VerSP vs. cross-axis HorSP), we applied a Bonferroni correction and chose a significance level of 0.01 (approximately 0.05/3). Using the pairwise multiple comparison procedure, we found for position and velocity coefficients no significant difference between VerSP and cross-axis VerSP conditions (Fig. 6) or cross-axis VerSP and cross-axis HorSP conditions (data not plotted). However, we observed a significant difference between VerSP and cross-axis HorSP conditions (Fig. 7).

Finally, given that we used two types of rearing paradigms, we grouped the neurons according to the rearing paradigm (AMO or BDTP) and performed repeated-measures ANOVA comparison separately for each group. The AMO group (S2, 3 up-BT neurons; S3, 7 down-BT and 2 up-BT neurons) followed the previous result, and we found no significant differences in the constant terms ($P = 0.51$) but did find significant differences in the position ($P = 0.004$) and velocity ($P = 0.003$) coefficients. Once again, as in the previous result, the pairwise multiple comparison procedure yielded no significant difference between the VerSP and the cross-axis VerSP conditions or the cross-axis VerSP and the cross-axis HorSP conditions. However, there was a significant difference between the VerSP and the cross-axis HorSP conditions. Conversely, the BDTP group (S1, 6 up-BT and 2 down-BT neurons) did not show any significant differences in the constant ($P = 0.23$), position coefficient ($P = 0.24$), or velocity coefficient ($P = 0.53$). These

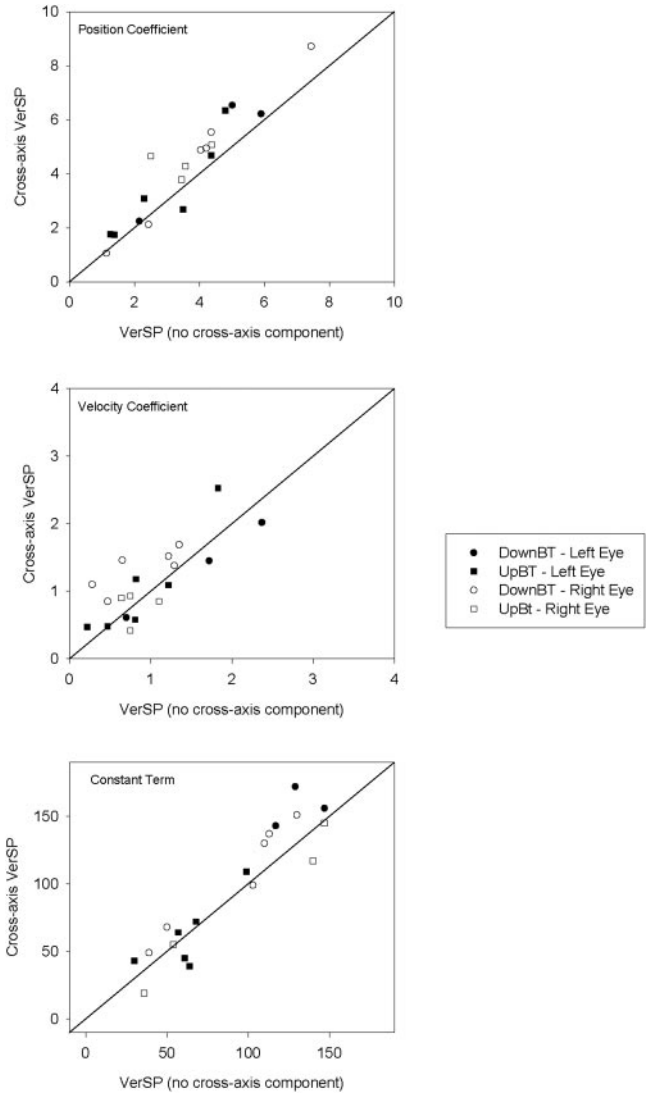

FIGURE 6. Comparison of position (*K*), velocity (*R*), and constant (*C*) coefficients during the two vertical tracking conditions. On the *x*-axis is the estimated value during vertical smooth pursuit when the eye to which the neuron projects is viewing the target (VerSP), and on the *y*-axis is the estimated value during vertical smooth pursuit when the eye to which the neuron projects is the nonfixating eye (cross-axis VerSP). For example, parameters estimated from Figures 4A and 5A would be on the *x*-axis, and parameters estimated from Figures 4B and 5B would be plotted on the *y*-axis. Note that for sake of simplicity of illustration, the parameter estimates are plotted without signs; otherwise down-BT motoneurons would have negative position and velocity coefficients.

results suggest that BDTP pathophysiology may be different from AMO pathophysiology.

DISCUSSION

A/V patterns and DVD are common phenomena observed in humans with strabismus. However, the extent of our understanding of these phenomena is limited. We have developed a monkey model for these problems and thus are in a position to conduct invasive studies that will increase our understanding of the actual mechanisms involved. To our knowledge, these are the first studies of A/V patterns and DVD in an awake and behaving animal model. Our studies provide new insight into strabismus properties that were thus far inadequately explained.

In this study, we show evidence that the inappropriate vertical cross-axis movements observed in the nonfixating eye during horizontal tasks are driven by motoneuronal activity. Therefore, our results point to an innervational source to inappropriate cross-axis movements leading to A/V patterns and DVD. Although our results may appear to be in contradiction to the results of Oh et al.,[12] who showed strong evidence from MRI data in patients that inappropriately placed extraocular muscle pulleys or unstable EOM might result in various forms of incomitant strabismus, we believe that the apparent differences can be resolved by considering the etiology of the

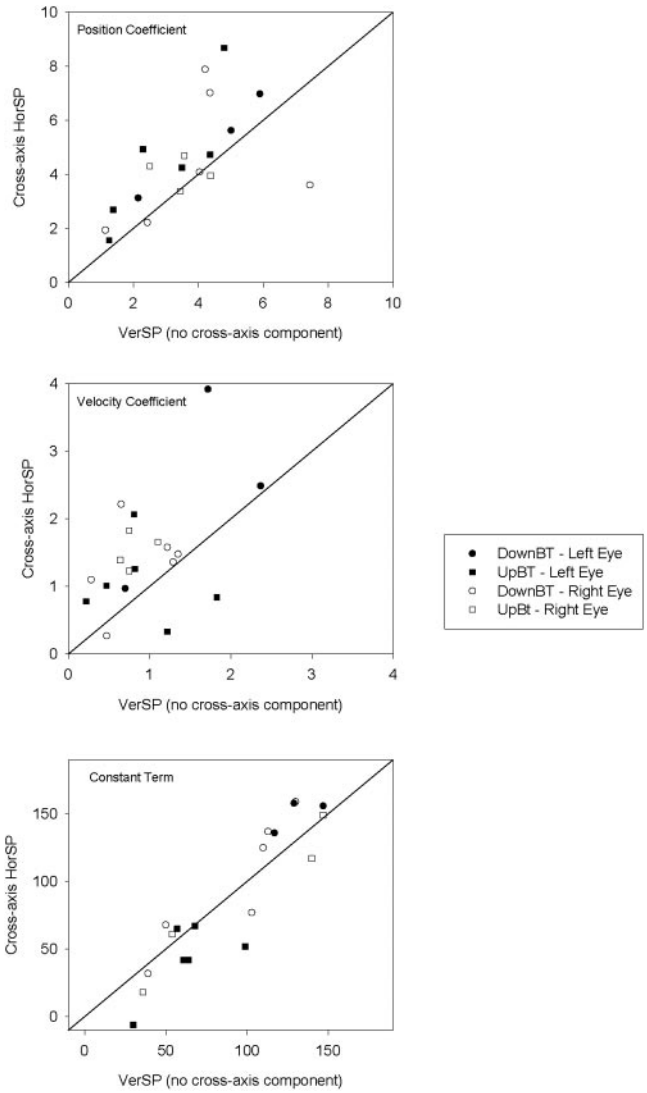

FIGURE 7. Comparison of position (*K*), velocity (*R*), and constant (*C*) coefficients during vertical smooth pursuit (VerSP) and inappropriate vertical component observed in nonfixating eye during horizontal smooth pursuit (cross-axis HorSP). Vertical eye movement data and associated motoneuron responses were used for the curve-fit. On the *y*-axis is the estimated value associated with the vertical cross-axis component during horizontal tracking. For example, parameters estimated from Figures 4D and 5D would be plotted on the *y*-axis. Comparison of the parameter estimates in these two tracking conditions show that all neurons in the sample were modulated during vertical cross-axis movements. The distribution of sensitivities shows a strong correlation in neuronal activity during the vertical tracking condition and during the vertical cross-axis condition.

TABLE 1. Population Characteristics of Vertical Motoneuron Sample

	VerSP	Cross-Axis VerSP	HorSP	Cross-Axis HorSP
Position coefficient, *K*	3.59 (±1.64)	4.10 (±2.04)	0.46 (±5.22)	4.36 (±2.04)
Velocity coefficient, *R*	0.98 (±0.56)	1.10 (±0.57)	0.02 (±2.45)	1.41 (±0.85)
Constant, *C*	89.00 (±40.00)	94.00 (±47.00)	92.00 (±48.00)	86.00 (±51.00)
Coefficient of determination, *CD*	0.92 (±0.06)	0.92 (±0.07)	0.21 (±0.19)	0.79 (±0.13)

Values are coefficients (±SD).

strabismus. The patients in the Oh et al.[12] study all had a predilection to muscle and orbit problems, such as Brown syndrome and Marfan syndrome. In contrast, our animals were reared under sensory deprivation without any manipulation of the peripheral apparatus. Therefore, we suggest that if the strabismus is exclusively caused by sensory factors, problems are likely in innervational drive to EOM. For example, congenital cataracts could lead to sensory-induced strabismus in humans. In support of our hypothesis, Narasimhan et al. found, using histologic techniques and MRI on naturally strabismic and prism-induced strabismic monkey cadavers, no apparent problem with pulley location or pulley structure (Narasimhan A, et al. *IOVS* 2006;47:ARVO E-abstract 5068). Even though we have not directly examined EOM in our animals, we would expect no gross muscle abnormalities based on our hypothesis and on the motoneuronal data.

In performing quantitative comparisons of the vertical position and velocity sensitivities during the different tracking conditions, we found no statistical differences for the entire cell sample. However, closer examination using pairwise comparisons indicated that there were statistically significant differences in position and velocity coefficient estimates between the vertical tracking condition (verSP) and the vertical component during horizontal tracking condition (cross-axis HorSP), shown in Figures 7A and 7B. However, no significant difference was found when coefficients were compared in the vertical tracking condition, when the eye that the neurons projects to is not viewing (i.e., cross-axis VerSP), and the cross-axis HorSP condition. One possible reason for the difference in coefficient estimates in the first comparison is the large difference between horizontal and vertical positions of the eye in the orbit in the two tracking conditions. It is possible (especially in strabismic animals with large angles of strabismus) that, because of the different horizontal and vertical eye positions, different sets of motor units were recruited in the two tracking conditions. Given the complex interactions between muscle fibers and recruitment properties, the force generated at the tendon and, therefore, the relationship between the eye movement and the neuronal response could vary slightly in the two tracking conditions we compared.[29] There may be other reasons for the statistical differences in coefficients we observed. The differences could indicate the presence of a missing variable in our equations used for fitting the data, and this variable could be torsion, as suggested by Guyton.[7,30] Alternatively, a secondary contribution of EOM pulleys, as indicated by the mechanical hypothesis, cannot be ruled out. In any case it appears that the contribution of any of the factors is small and that the primary driver for vertical cross-axis movements is motoneuron activity.

Because the rearing paradigm used for S1 (BDTP) was different from that used for S2 and S3 (AMO), we separated the neurons according to these two groups and performed statistical comparisons. We thought such a grouping would be useful considering that S3 did not appear to show a DVD. AMO animals had results similar to those of the group results described in the previous paragraph. However, we found no statistically significant differences in estimated coefficients of BDTP animals (S1) in the three tracking conditions. These results could suggest that the BDTP pathophysiology was different from the AMO pathophysiology and that the missing variable we alluded to earlier was specific to AMO pathophysiology. However, such an interpretation must be treated with caution because the sample size of neurons within each group was small.

The classical notion of A/V pattern strabismus is the idea that individual oblique muscles might be overacting or underacting (for a review, see von Noorden[1]). Overaction of the inferior oblique is most often associated with V patterns, whereas overaction of the superior oblique is associated with A patterns. Why a single muscle should be mysteriously overactive or underactive is unclear. In our analysis of vertical motoneurons, we found that all the cells we recorded from were active during inappropriate vertical cross-axis movements. Although we did not identify the particular muscle to which the cell was projecting, it is likely that we sampled from neurons projecting to all the cyclovertical muscles—that is, the superior rectus, inferior rectus, and inferior oblique. Hence, it appears that in sensory-induced strabismus, altered innervation occurs to all cyclovertical muscles, leading to A/V patterns and DVD. Therefore, our results support the assessment of Demer[10] that the classical notion of overacting or underacting oblique muscles is erroneous and refers to a functional description rather than a representation of muscle state.

As discussed in Results, our analysis of horizontal motoneurons was limited because two animals had large angle exotropia. A second issue with analyzing medial rectus motoneurons in animals with strabismus is the potential role of eye accommodation.[31,32] Thus, Zhang et al.[32] showed that near response cells that projected monosynaptically to the medial rectus motoneurons carried a signal related to eye accommodation. The accommodation signal tends to cancel out when the population of near response cells projecting to oculomotor nucleus is considered, so the net signal reaching the medial rectus motoneurons appears to be exclusively related to vergence. Because eye accommodation is typically accompanied by a change in vergence, accounting for eye accommodation may not be critical when investigating motoneuron activity in animals with normal vision. However, monitoring eye accommodation could be critical in animals with strabismus because AC/A ratios (ratio of accommodation-related convergence to accommodation) and control of eye accommodation are most likely abnormal and could potentially vary from trial to trial with the eye of fixation and with gaze eccentricity.

Because we did not analyze motoneurons associated with horizontal eye movements, one question that arises is whether the mechanisms that drove the vertical cross-axis movements were the same as those that drove horizontal cross-axis movements. It is possible that our results with vertical motoneurons were exclusively related to mechanisms that mediated the change in *vertical misalignment with horizontal position* and therefore did not translate to the source for change in *horizontal misalignment with vertical eye position*. In the animals with exotropia, single-unit recording from neurons in the oculomotor nucleus was not efficient; therefore, single-unit stud-

ies targeted at the abducens nucleus during horizontal and vertical smooth pursuit can be used to test whether change in horizontal misalignment with vertical eye position (i.e., horizontal cross-axis movements during vertical tracking) results from an innervational source. The prediction would be that abducens neurons will be well modulated during horizontal tracking and horizontal cross-axis movements during vertical tracking.

Correlated vertical motoneuron activity with the vertical component of cross-axis movements does not imply that pattern strabismus and cross-axis movements are generated in the oculomotor nuclei. It is likely that premotor structures are the real source for these cross-axis movements. Single-unit studies focusing on premotor structures and using similar behavioral paradigms and conceptual framework will help determine the actual source of the inappropriate cross-axis eye movements.

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