Eye Movements, Strabismus, Amblyopia and Neuro-ophthalmology

Longitudinal Development of Ocular Misalignment in Nonhuman Primate Models for Strabismus

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Purpose. To investigate the longitudinal change in horizontal and vertical ocular alignment in normal and prism-reared infant monkeys during the critical developmental period.

Methods. Ocular alignment was measured using Hirschberg photographic methods in 6 infant monkeys reared under prism-viewing from day 1 after birth to 4 months, and 2 monkeys reared with normal visual experience. Photographs were acquired twice a week for the first 6 months of life and analyzed to identify pupil center and the first Purkinje image from which eye positions and strabismus angle were calculated.

Results. At 3 weeks after birth, prism monkeys presented with significant horizontal ocular misalignment. A gradual change in alignment was seen in all prism-reared monkeys stabilizing at approximately 11 weeks, at which time 5 monkeys were exotropic (mean, 16° XT; range, 13°–24°) and 1 monkey was esotropic (5° ET). A reduction in ocular misalignment was observed after exposure to normal visual environment at 16 weeks, but at 34 weeks of age, that is, 18 weeks after removal of prisms, prism-reared monkeys displayed a mean horizontal strabismus of 7° XT (range, 2° ET to 20° XT), which was still significantly different from normal monkeys.

Conclusions. Prism-rearing disrupts binocular fusion mechanisms, and horizontal and vertical strabismus is seen to develop as early as 3 weeks of age in monkey models, equivalent to approximately 3 months in humans. The time course of change in alignment overlaps with disruption in various visual sensory functions, suggesting a causal temporal link between sensory and motor mechanisms for alignment.

Keywords: strabismus, infant development, prism-viewing, binocular decorrelation, nonhuman primate

Binocular alignment and binocular coordination, like many other visual functions, develop over the postnatal period, and disruption of binocular vision during this critical period leads to strabismus in approximately 2% to 5% of children in the world.[1–4] Strabismus is associated with a plethora of visual and oculomotor dysfunctions that include reduced visual acuity, impaired stereoacuity, interocular suppression, disconjugate saccades, alternate fixation, fixation instability, and vergence dysfunction.[5–8] With the advent of animal studies,[6,9–13] we now have insight into the neural mechanisms involved in the development and maintenance of abnormal eye alignment and abnormal eye movements associated with strabismus.

Nonhuman primates have the closest genetic, physiological, and behavioral similarity to humans, and this similarity is observed in visual-oculomotor mechanisms and the associated neural pathway. The incidence of naturally occurring strabismus has been reported to be 4% in the *Macaca nemestrina* monkey,[14] but its incidence in other macaque species is not known. Therefore artificial induction of strabismus in nonhuman primates (monkey models) has been most commonly used to investigate the anatomic and neurophysiological basis for strabismus. Monkeys reared with monocular occlusion or monocular defocus, for a period of 3 months after birth, developed deprivation-induced strabismus.[15] To avoid amblyopia, strabismus was modeled in infant monkeys by alternating occlusion between the two eyes.[7,16–18] Visual sensory deprivation in the form of bilateral lid suture induced strabismus in infant monkeys,[19–21] which mimicked the sensory and motor deficits observed in children with strabismus and amblyopia. Infant monkeys with medial rectus tenotomy in each eye developed large angle exotropia with alternate fixation.[8] Other recession, resection, and extirpation surgeries of extraocular muscles have also been successful in inducing ocular misalignment.[22–26] Furthermore, temporary esotropia can be induced by injecting *Clostridium botulinum A* in the lateral rectus of infant monkeys.[27] Each of these strabismus induction methods come with advantages and disadvantages that are reviewed elsewhere.[6,28]

An optical prism-rearing paradigm that disrupts sensory fusion during the critical period of development is a popular method to induce strabismus and is the preferred method in our laboratory.[23,29–33] This established model for inducing sensory strabismus in monkeys replicates the clinical signs and symptoms seen in humans with sensory strabismus, and many insights into neural mechanisms have been gained through the use of this model.[6,9–13] This model is thought to most closely mimic human sensory strabismus because it involves decorrelation of visual information between the two eyes. Although it is evident that decorrelating binocular vision during development via prism-rearing induces misalignment, the evolution and progression of ocular misalignment in the primate model is still unknown. In this study, we assessed the development of ocular misalignment both during and after completion of a 16-week prism-rearing paradigm. To our knowledge, this study is the first to describe the longitudinal development of horizontal and vertical ocular alignment in prism-reared infant monkeys during the developmental critical period. Examining ocular alignment during this malleable period of development provides insight into the different mechanisms involved in maintaining ocular alignment.

Methods

All experimental procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Houston and conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Subjects and Rearing Paradigms

The subjects included in this study were eight infant monkeys (*Macaca mulatta*), born and bred at the Michale E. Keeling Center for Comparative Medicine and Research at the MD Anderson Cancer Center, Bastrop, Texas, for approximately the first 2 weeks of life. After this period, they were transferred to the primate facilities at the University of Houston. Two monkeys (NM) were reared under normal visual experience, whereas the other six animals (PM) were reared using an optical prism-rearing paradigm. Specifically, prism-reared monkeys were fit with a light-weight helmet that housed Fresnel prisms (right eye: 20Δ base-down; left eye: 20Δ base-in), which were worn for the first 4 months of life starting from day 1 of birth. After the prism-rearing period, these animals were reared in a normal visual environment. The magnitude and direction of the optical prism has been found to be effective in decorrelating binocular vision during the critical period of development, preventing binocular fusion and consequently leading to strabismus.[33,34]

Data Acquisition and Experimental Procedures

Ocular alignment was measured longitudinally using Hirschberg photographic methods during the 16 weeks of prism-rearing (first photos at ~11 day of age after transfer to the University of Houston facility), and for 18 weeks after the prism-rearing period.[35–37] Digital photographs of the infant animals were acquired twice a week in a dimly illuminated room using a D300 camera (Nikon, Tokyo, Japan) with an attached ring light placed at a distance of 60 cm from the animal in primary gaze. To facilitate photography, the infant animals were wrapped in a blanket and placed in a primate chair (Crist Instrument Co, Inc., Hagerstown, MD), while one of the investigators lightly held the animal's head to prevent excessive movement. Prism helmets were removed for the duration of the photographic procedure, and multiple photographs were taken during each session for each monkey. Considering a previous report suggesting

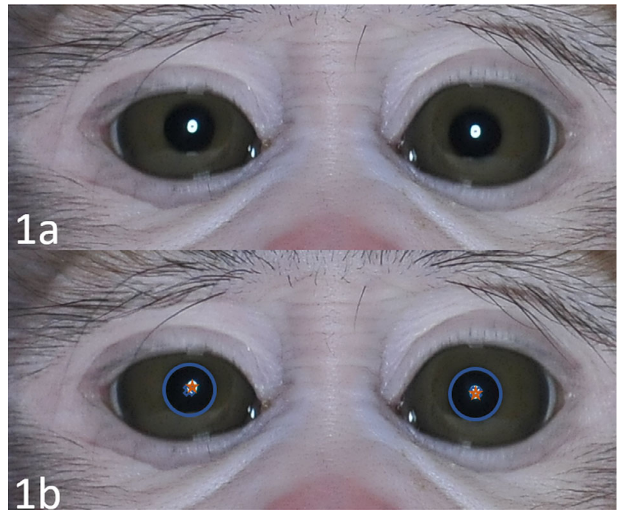

Figure 1. (a) Photograph of normal monkey (NM2) at 11 weeks of age displaying normal ocular alignment. (b) ImageJ analysis to calculate eye alignment. The *blue circle* marks the pupil and the *white regions* denote the first PI. The PC is represented by the *blue cross*, and the center of first PI by the *orange star*. Note that in the left eye, the PC and center of first PI match, whereas in the right eye, the PI is shifted nasally with respect to the PC. This small apparent exotropia is a consequence of angle κ.

that even 60 minutes of normal binocular vision each day might influence the final state of strabismus,[38,39] we were careful to minimize the overall photography time. The time spent under binocular vision, owing to the experimental procedure, was also recorded.

Ocular Misalignment Measurement

Photographs in which monkeys fixated centrally with one or both eyes were selected for further analysis, that is, photographs with off-axis fixation and out of focus photographs were rejected. Angular position of each eye (and consequently ocular misalignment) was calculated using the Hirschberg method, which uses estimates of the difference between the pupil center (PC) location and the location of the center of the first Purkinje image (PI).[35–37] ImageJ software (National Institutes of Health, Bethesda, MD) was used to analyze the data using the following steps. First, the contrast and brightness of each photograph was manually adjusted to visually delineate the borders of the pupil and first PI (Fig. 1). Using ImageJ tools, a circle was manually drawn over the pupil and the first PI, and the 'x' and 'y' coordinates of the PC and PI were automatically calculated. Horizontal and vertical angular position of each eye was calculated by taking the difference between the PI and PC in millimeters and converting it to angular position in degrees using a published Hirschberg ratio of 14°/mm for monkeys.[35] In our convention, a leftward or downward shift in PI with respect to PC is denoted as a positive difference and occurs when the eye is deviated to the right or up with respect to the camera, which is located straight-ahead of the monkey; a rightward or upward shift of PI with respect to PC yields a negative difference and occurs when the eye is deviated to the left or down. Horizontal and vertical strabismus angle were calculated as the difference between the position of the left and right eyes in horizontal and vertical planes, respectively. Because in our convention rightward eye positions are positive and leftward eye positions are negative, the difference between left and right eye positions (strabismus angle) yields positive values for esotropia and negative values for exotropia. Generally, horizontal locations of PI and PC are close to each other for one of the eyes (eye position close to 0°; labeled as the viewing eye) and are laterally shifted relative to each other for the other eye (labeled as the deviated eye). A temporally shifted location of PI with respect to PC describes esotropia (positive value for difference between left and right eye positions), and a nasally shifted location of PI with respect to PC represents exotropia (negative value for difference between left and right eye positions). A downward shift of PI with respect to PC of the deviated eye results in a positive value for vertical ocular misalignment. Ocular misalignment was averaged over the multiple measurements that were acquired each day and analyzed separately for right eye and left eye viewing when possible. The influence of age, viewing eye, and rearing paradigm on ocular misalignment was assessed (SigmaPlot V12.5; Systat Software, Inc., San Jose, CA) within and across groups by performing parametric and nonparametric tests.

Angle Kappa (κ) is the angle between the pupillary axis and the visual axis, manifesting typically as a nasal ward displacement of the corneal light reflex even in normal humans and monkeys.[15] Therefore normally aligned animals appear to display a small exotropia when Hirschberg photographic data are analyzed. Figure 1 displays the ocular alignment of infant monkey (NM2) reared under normal visual environment and illustrates both the Hirschberg methodology and the potential influence of angle κ. For each monkey reared with normal visual experience, the mean horizontal alignment during the 11-week measurement period was estimated to be the angle κ (NM1: 4.2° XT, NM2: 3.1° XT). This empirically calculated value is close to the reported average value for angle κ in nonhuman primates of 5°.[15] Because angle κ potentially influences the measurement of ocular misalignment in prism-reared monkeys, the angle κ of NM1 and NM2 was averaged and a convergent correction of 3.7° was applied when calculating the ocular misalignment in all prism-reared animals.

Results

Ocular alignment was measured twice a week in the eight infant monkeys (six males, two females; no significant birth history) starting from approximately 2 weeks of age after transfer to the University of Houston from the MD Anderson facility in Bastrop, Texas. Of these monkeys, two were reared under normal visual environment (NM), and six were reared with prism-helmets for the first 16 weeks (PM). The refractive error measured at 23 weeks of age and sex distribution are presented in the Table. In comparison to normal monkeys, prism-reared monkeys displayed significantly greater magnitude of hyperopia (PM: 4.69 ± 0.70 diopter [D], NM: 1.25 ± 0.14 D; $P \leq 0.005$, *t*-test). On average, 7 ± 3 photographs were analyzed per week per monkey to calculate ocular alignment. This number does not include the photographs that were rejected due to poor fixation, head turn and poor quality. On objective assessment of the selected photographs, the mean eye positions (difference between PI and PC) of the viewing eye was found to be less than 5° for each prism-reared monkey.

Table. Sex and Refractive Error Distribution (measured at ~6 months of age) of Normal and Prism-Reared Monkeys. Positive Numbers Indicate a Hyperopic Shift

Monkey	Sex	Right Eye Spherical Equivalent (D)	Left Eye Spherical Equivalent (D)
NM1	Male	1.00	1.00
NM2	Female	1.50	1.50
PM1	Male	6.75	6.75
PM2	Male	1.50	1.75
PM3	Male	3.75	3.50
PM4	Male	4.75	2.50
PM5	Female	8.50	8.50
PM6	Male	4.25	3.75

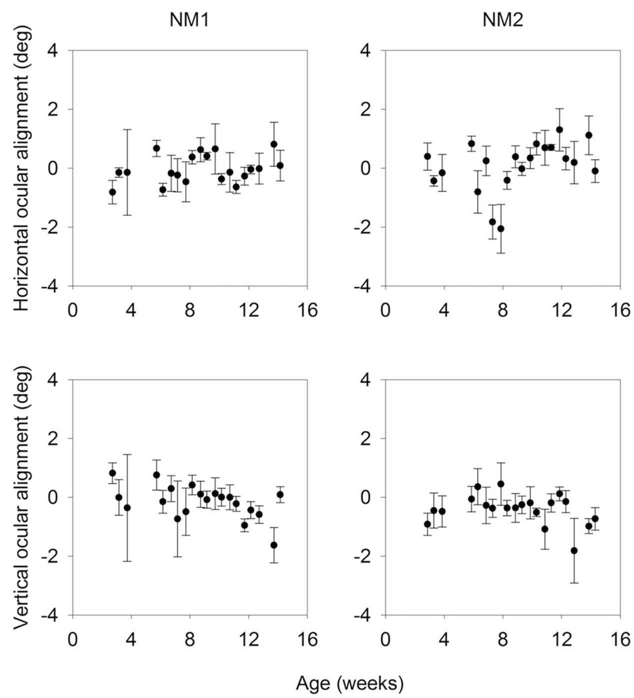

Figure 2. Longitudinal measurements of horizontal and vertical ocular alignment (after κ correction) in the two infant monkeys raised under normal visual environment. *Top row*: Positive values indicate esotropia and negative values indicate exotropia. *Bottom row*: Positive values indicate the left eye is above the right eye.

Alignment in Normally Developing Monkeys

The horizontal and vertical ocular alignment of normal monkeys during the 11-week measurement period is shown in Figure 2 (horizontal NM1: mean, –0.0° ± 0.1°; NM2: mean, 0.0° ± 0.1°; vertical NM1: mean, –0.1° ± 0.1°; NM2: mean, –0.4° ± 0.1°). Normal monkey data were not acquired beyond 14 weeks being that there was only nominal variation in alignment throughout the measurement period. Note that for each normal monkey, the average ocular alignment during the measurement period (NM1: 4.2° XT; NM2: 3.1° XT) was subtracted from each measured misalignment to account for the average value of angle κ. Note also that for the normal animals the viewing eye was not labeled because both eyes were fixating the target.

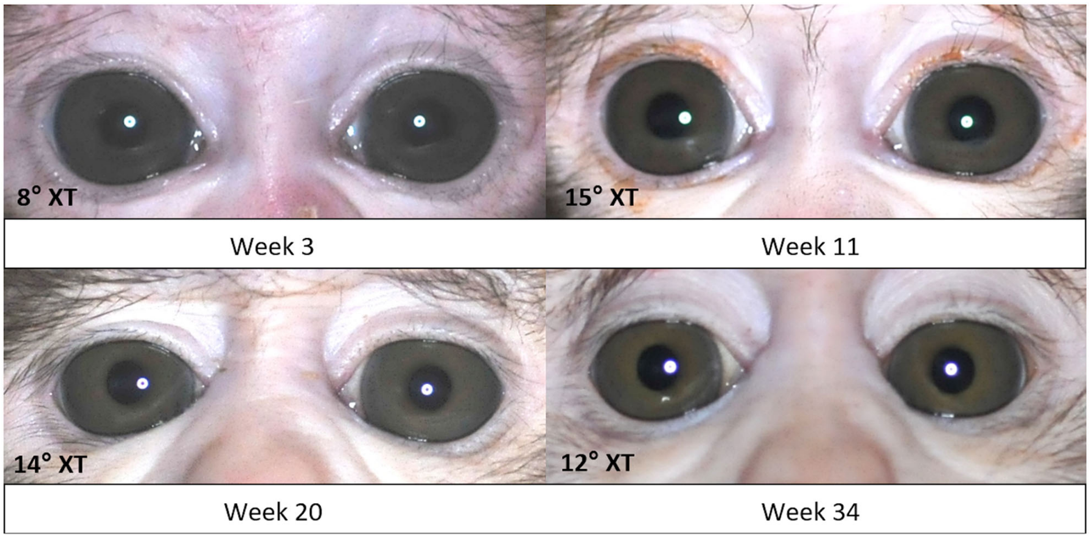

Figure 3. Photographs of prism-reared monkey (PM3) displaying ocular misalignment at 3, 11, 20 and 34 weeks of age.

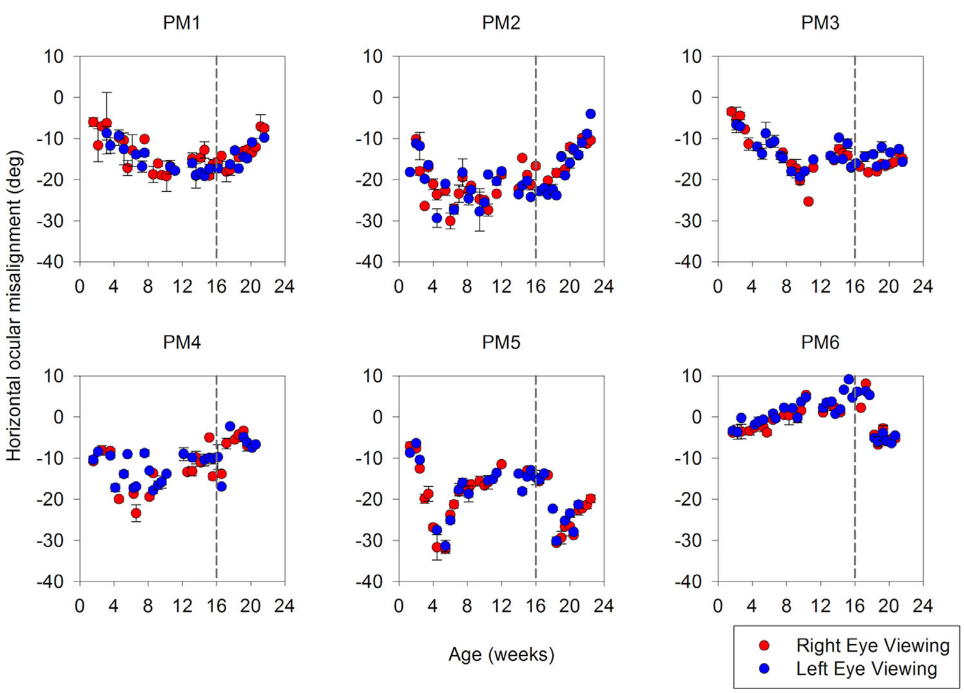

Figure 4. Longitudinal measurements of horizontal ocular alignment (mean and standard error) during and after prism rearing in six infant monkeys. Negative values denote exotropia and positive values represent esotropia. *Blue circles* indicate the horizontal ocular misalignment when the left eye was determined to be viewing the straight-ahead target, and *red circles* for right eye viewing. The *dashed vertical line* indicates the end of the prism-rearing period (16 weeks).

Alignment in Prism-Reared Monkeys

Horizontal Deviation. Rearing infant monkeys under prism-viewing conditions during the critical period of development disrupts binocular vision and induces strabismus. Figure 3 shows the photographs of prism-reared monkey PM3, illustrating the presence of ocular misalignment at 3, 11, 20, and 34 weeks of age. Prism-rearing induced gradual change in horizontal ocular misalignment. Figure 4 shows the horizontal ocular misalignment in all prism-reared monkeys plotted as a function of age. At 3 weeks of age, prism-reared monkeys were on average more exotropic than normal monkeys. Over the next few weeks, whereas normal monkeys displayed no change, prism-reared monkeys showed gradually increasing horizontal ocular misalignment, which stabilized at approximately

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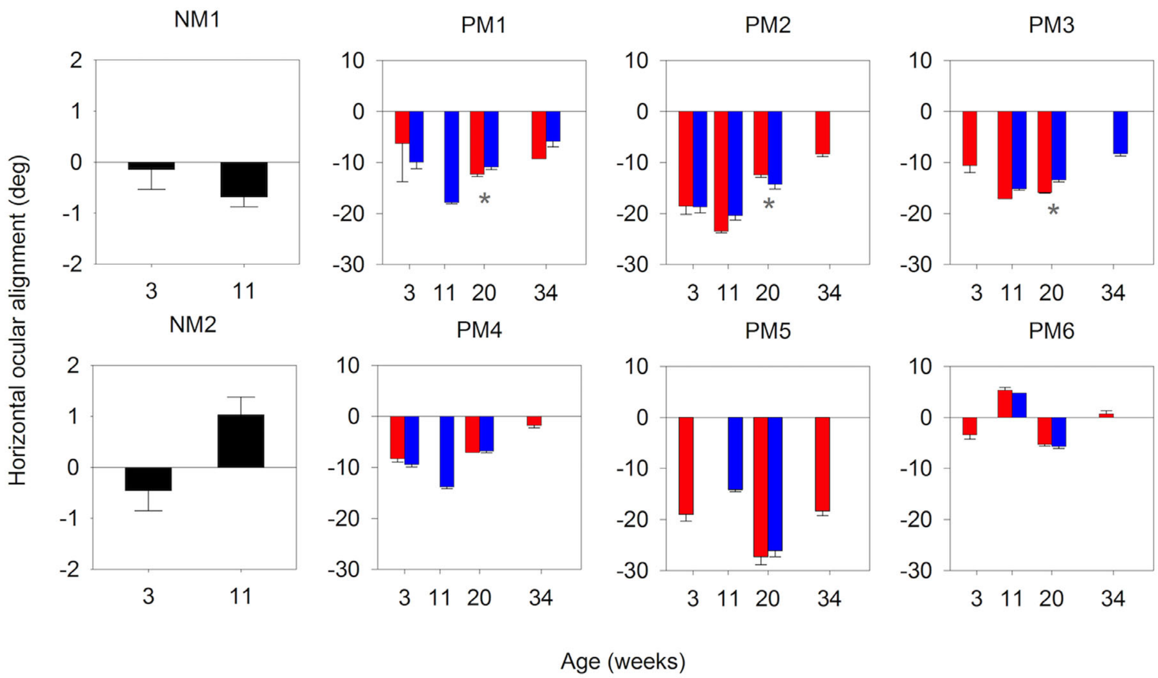

FIGURE 5. Bar plot showing the longitudinal change in horizontal ocular alignment (mean and standard error) in two normal monkeys, and during and after prism rearing in six infant monkeys. Bars in *black* denote ocular alignment in normal monkeys. Bars in *red* represent horizontal ocular alignment during right eye viewing, and bars in *blue* denote horizontal ocular alignment during left eye viewing. The *asterisk* marks the ages at which there was a significant difference in the horizontal ocular misalignment between right eye and left eye viewing ($P < 0.05$). Note the difference in *y*-axis scales between normal and strabismic monkeys.

11 weeks of age. Interestingly, after the prism-rearing period ended (week 16), a gradual reduction in horizontal ocular misalignment was observed, but at approximately 22 weeks of age, that is, 6 weeks after discontinuing prism-rearing, prism-reared monkeys were still strabismic. Note that in all these data, an estimated κ angle of 3.7° was subtracted while calculating horizontal misalignment.

Patterns of horizontal ocular misalignment were variable between monkeys (Fig. 4). Although PM1, PM2, PM3, and to a general extent PM4 displayed similar trends during and after the rearing period, PM5 exhibited a rapid development of horizontal ocular misalignment during the first 5 weeks, which then reduced and stabilized at 11 weeks. On removal of prisms at 16 weeks of age, an increase in horizontal ocular misalignment was observed in this animal before reaching a deviated position of approximately 20° XT at 22 weeks of age. PM6 appeared to gradually develop an esotropic strabismus during the rearing period but reverted to a small exotropia of approximately 6° XT at 20 weeks of age.

Figure 5 displays the horizontal ocular misalignment for right eye viewing and left eye viewing at various ages during and after the rearing period, namely, at 3 weeks when misalignment was first apparent, at 11 weeks when it appeared to stabilize, at 20 weeks, which was a few weeks after prism-rearing ended, and at 34 weeks, which was the end of the measurement period. Note that the monkeys were free to choose either eye for fixation. Therefore in some sessions, we observed that the animals viewed with the left eye in some photographs and viewed with the right eye in others (e.g., Fig. 5: PM1 at 3, 20, and 34 weeks of age) and in other sessions, the animal was always viewing with one of the eyes in every photograph (e.g., Fig. 5: PM1 at 11 weeks of age). Therefore some sessions yielded right and left eye viewing data, whereas other sessions did not. Whenever possible, right and left eye viewing data were compared. During the measurement period, horizontal ocular misalignment was significantly different between right eye and left eye viewing in three prism-reared monkeys at three different time points (PM1, PM2, and PM3; blue and red data in Fig. 5; $P \leq 0.05$ ANOVA repeated measures). Given this difference between right eye and left eye viewing was small (<5°), further analysis was performed on averaged data.

Data in Figure 5 shows that at 3 weeks of age, 5 prism-reared monkeys (PM1, PM2, PM3, PM4, and PM5) presented with horizontal ocular misalignment that was significantly different from normal monkeys ($P \leq 0.05$; Kruskal–Wallis test). At 11 weeks of age, 5 of the prism-reared monkeys were exotropic (mean, 16° XT; range, 13°–24°) and 1 prism-reared monkey (PM6) was esotropic (5° ET). On exposure to normal visual environment, horizontal ocular misalignment reduced in most prism-reared monkeys, with mean horizontal ocular misalignment at 13° XT (range, 4°–29°) at 20 weeks of age. At 34 weeks of age, i.e., 18 weeks after removal of prisms, 5 of the 6 prism-reared monkeys displayed a mean horizontal ocular misalignment of 9° XT (range, 2°–20° XT), which was still significantly different from the normal monkeys ($P \leq 0.05$; ANOVA).

Vertical Deviation. Prism-rearing induced a smaller magnitude of vertical ocular misalignment (compared with horizontal misalignment) in infant monkeys (Fig. 6) that was significantly different from normal monkeys in some of the prism animals at certain time points only. During the measurement period, vertical ocular misalignment was significantly different between right eye and left eye viewing in two monkeys at two time points (PM2 and PM4; blue and red data in Fig. 7; $P \leq 0.05$ ANOVA repeated measures). However, this difference was small in magnitude (PM2), and

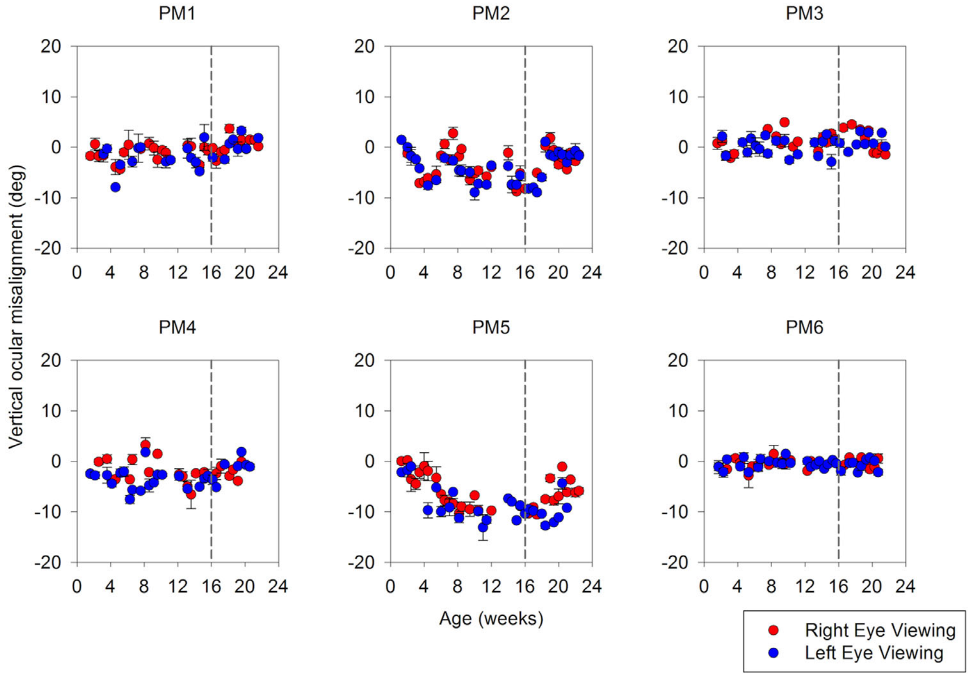

Figure 6. Longitudinal measurements of vertical ocular alignment (mean and standard error) during and after prism rearing in six infant monkeys. Negative values denote relative downward displacement of the left eye, and positive values represent relative upward displacement of the left eye. *Blue circles* indicate the vertical ocular misalignment when the left eye was determined to be viewing the straight-ahead target, and *red circles* for right eye viewing. The *dashed vertical line* indicates the end of the prism rearing period (16 weeks).

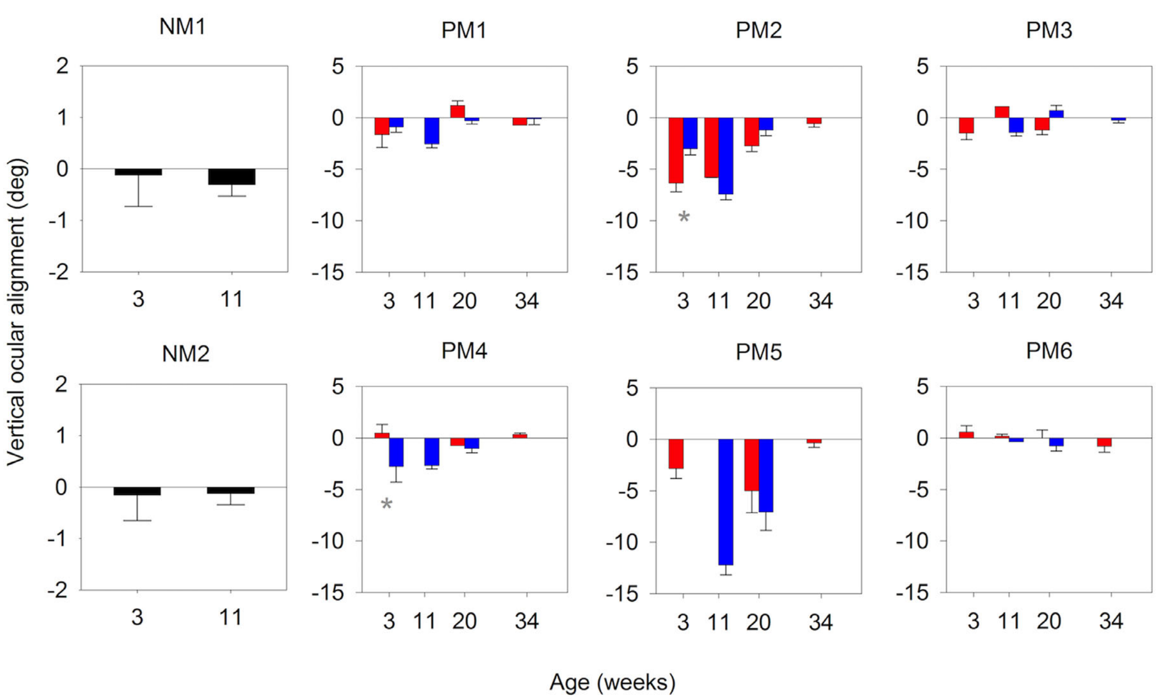

Figure 7. Bar plot showing the longitudinal change in vertical ocular alignment (mean and standard error) in two normal monkeys and during and after prism rearing in six infant monkeys. Bars in *black* denote ocular alignment in normal monkeys. Bars in *red* represent vertical ocular alignment during right eye viewing, and bars in *blue* denote vertical ocular alignment during left eye viewing. The *asterisk* marks the ages at which there was a significant difference in the vertical ocular misalignment between right eye and left eye viewing ($P < 0.05$). Note the difference in y-axis scales between normal and strabismic monkeys.

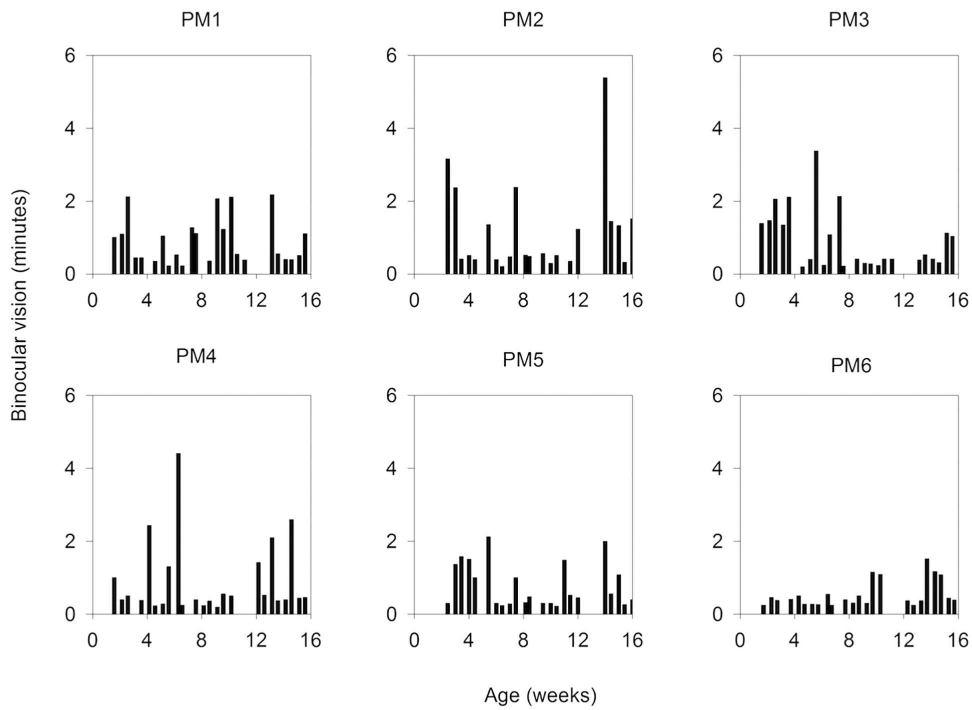

Figure 8. Bar plot displaying the time that prism monkeys spent without prism helmets, that is, under normal binocular vision, during the rearing period. Each bar shows binocular vision exposure from a single day that photos were acquired.

in the opposite direction (PM4), and therefore further analysis was performed on averaged data.

Data in Figure 7 shows that at week 11, PM2 and PM5 showed a robust vertical ocular misalignment response to prisms (mean 10°; range, 6°–18°; $P \leq 0.05$ Kruskal–Wallis). Because the vertical ocular misalignment measure is negative during both right eye and left eye viewing, this is indicative of a true vertical tropia as opposed to a dissociated vertical deviation (DVD). PM1, PM3, and PM4 displayed a small but variable magnitude of vertical ocular misalignment during both right eye and left eye viewing (mean 2°; range, 1°–4°; $P \leq 0.05$ Kruskal–Wallis) whereas, PM6 exhibited no statistically significant difference from normal monkeys during prism-rearing (PM6: mean 0°; $P \geq 0.05$ Kruskal–Wallis). After removal of prisms (~20 weeks of age), statistically significant vertical ocular misalignment was observed in only PM2 (mean 2°; $P \leq 0.05$ Kruskal–Wallis) and PM5 (mean 6°; $P \leq 0.05$ Kruskal–Wallis). At approximately 34 weeks of age, i.e., 18 weeks after removal of prisms, none of the prism-reared monkeys displayed a statistically significant vertical ocular misalignment (mean 0°; range, 0°–3°; $P \geq 0.05$ Kruskal–Wallis) as compared with normal monkeys.

Exposure to Binocular Vision

During the 16-week prism-rearing period, prism helmets were removed to measure ocular alignment. Because binocular vision during development impacts the development of strabismus,[38,39] we were careful to minimize this duration by working rapidly to acquire the photographs. Figure 8 plots the time spent under normal binocular viewing conditions (as a consequence of measuring the strabismus) during each week of the prism-rearing duration. On average, prism-reared monkeys were exposed to less than 2 minutes (range, ~20 seconds to ~6 minutes) of normal binocular vision per week during the photographic procedure. The total time spent under binocular viewing was approximately 30 minutes over the 16-week period.

Discussion

The purpose of the study was to investigate the longitudinal change in ocular misalignment during and after optical prism-rearing, and to compare these data to alignment data of infant monkeys raised under normal binocular vision. The main findings are as follows: (1) infant monkeys reared with optical prisms develop significant horizontal and mainly small vertical ocular misalignment; (2) characteristic changes in horizontal, but not vertical ocular misalignment, were noted in response to prism-rearing in the weeks after beginning prism-rearing; and (3) exposure to normal binocular vision after 16 weeks of prism-rearing (equivalent to 16 months in humans) resulted in reduction of ocular misalignment, but 18 weeks after discontinuing prism-rearing, prism-reared monkeys still presented with significant horizontal ocular misalignment. We discuss each of these findings in greater detail below.

Development of Horizontal and Vertical Ocular Misalignment

The presence of significant horizontal ocular misalignment and small vertical ocular misalignment as a consequence of prism-rearing is expected and was not a study goal. Rather, the goal of this study was to investigate the longitudinal evolution and progression of ocular misalignment. During the initial weeks, the horizontal ocular misalignment was variable, but a common feature among the animals was that horizontal ocular misalignment emerged soon after starting prism-rearing (~11 days after birth) and was noted to be different from normal animals as early as 3 weeks of age. Note that we are not making any assumption about alignment at birth other than the fact that alignment is similar across the normal monkey group and the prism-group before commencement of rearing. Horizontal strabismus angle tended to stabilize by approximately 10 to 11 weeks of age (~11 months in humans), which potentially could be indicative of the minimum necessary period of prism-rearing to produce strabismus. This timeframe supports studies in humans and monkeys that suggest early detection and intervention is essential to minimize the sensory and motor deficits associated with strabismus.[30,40–43] The choice of 16 weeks of prism-rearing, used commonly in many studies, is certainly sufficient and could even be more than the amount of time that is necessary to induce permanent strabismus in the animal model.[23,29–33]

Excluding a few instances, the strabismus angle seemed fairly similar between right eye and left eye viewing (comitant strabismus). In older prism-reared animals, we have often noted differences in strabismus angle between right eye and left eye viewing (possibly a dissociated horizontal deviation).[7] It is unclear whether the development of dissociated horizontal deviation occurs later than the current study duration (0–6 months), or if it is simply smaller than we could measure reliably using Hirschberg methods; we suspect the latter. Eye coil measurements when the study animals are adults will identify the presence of dissociated deviations.

Vertical ocular misalignment was significantly smaller than horizontal ocular misalignment and was only clearly present in two of the animals (PM2 and PM5), although the time course of its evolution was similar to that of horizontal ocular misalignment. In our convention, upward positions are positive and strabismus angle is calculated as the difference between the position of left eye and right eye; therefore a negative strabismus angle is equivalent to a right hypertropia. In previous studies in adult monkeys,[7,12] we have noted the presence of variable amounts of DVD in some animals. In the current study, a DVD would have manifested as a positive vertical strabismus angle during right eye viewing, and a negative vertical strabismus angle during left eye viewing. These data (Fig. 7) could discern a DVD in some of the animals, supporting the idea that prism rearing produces strabismus that mimics the human strabismus condition. Because DVD was observed only at specific time points, it is possible that DVD evolves later than 6 months of age or, as mentioned earlier, it was a limitation of the measurement method.

Factors Influencing Eye Misalignment

Prism-rearing of monkeys that begins after 3 weeks of age (in contrast to our animals who were reared with prisms from day 1) results in cortical changes, such as reduction in disparity sensitivity and higher binocular suppression, but does not result in development of ocular misalignment.[44] In infant monkeys, stereopsis emerges at approximately 3 to 4 weeks postnatal and matures to adult levels within 1 to 2 weeks.[45] Therefore to develop strabismus, there must be early decorrelation of binocular function even before the development of stereopsis. This temporal dissociation between the development of sensory (disparity) and motor (eye alignment) mechanisms could point to the presence of another signal that modulates ocular alignment. Additionally, the indication of the presence of a second signal is the variability in the evolution of horizontal strabismus between the animals in the current study. Although all the animals were reared using identical protocols, one of the animals tended toward esotropia, whereas the others were clearly exotropic. Note that the evolution of strabismus in the esotropic animal was monotonically biased toward esotropia from the onset of rearing, whereas it was biased toward exotropia in the other animals, also from the onset of rearing. In other words, disruption of binocular vision may not be the only factor that determines the evolution of strabismus. Identifying this other factor is purely speculative at this time, but it could involve any number of visual or nonvisual mechanisms, such as genetic coding, oculomotor proprioception, competitive neuronal interactions resulting in a convergent or divergent bias, accommodation, and interaction with refractive error mechanisms.

In this study, horizontal and vertical ocular misalignment was variable (i.e., continuously changing) during the initial few weeks of prism-rearing, coinciding with time course of stereopsis development, but stabilized at approximately 11 weeks, coinciding with when stereopsis normally reaches adult levels. Removal of prisms at 16 weeks resulted in a reduction in ocular alignment in the prism-reared animals, suggesting that perhaps visual-oculomotor development continues beyond the 4-month prism-rearing period. Additionally, there are other adaptive muscular or neurologic factors that play a role in the long-term maintenance of ocular alignment.[6,28]

In a study by Smith et al.,[46] the magnitude of ametropia and anisometropia was evaluated in monkeys with surgical and optical strabismus. The authors observed that in comparison to normal monkeys, monkeys with optical prism-reared strabismus (prism rearing started at 4 weeks of age) had a higher prevalence of hyperopic and myopic ametropia, as well as a significantly greater degree of anisometropia. In our study, the prism-reared monkeys displayed significant magnitude of hyperopia (PM: 4.69 ± 0.70 D; NM: 1.25 ± 0.14 D; $P \leq 0.005$, *t*-test) but the level of anisometropia was not significantly different (PM: 0.46 ± 0.37 D; NM: 0.0 ± 0.0 D; $P \geq 0.005$, Mann–Whitney *U* test). Thus although our study was not directed at refractive error, it supports the connection between hyperopic shifts and development of strabismus. Future studies that simultaneously assess longitudinal development of refractive error and misalignment in animals that are prism-reared from birth could provide additional insight into the correlation between these two factors.

Methodological Considerations

The Hirschberg method is a common clinical measure of binocular alignment and the conversion factor, used to convert the decentration of the first Purkinje reflex to angular eye position, is unique to each monkey because of variability in geometry. However, in a study by Boothe et al.,[34] limbal radius and keratometry (corneal curvature) were measured to estimate the Hirschberg ratio in monkeys of different ages. The authors observed that these parameters exhibited little variation across animals regardless of age,[15] and therefore we used the estimated Hirschberg ratio of 14°/mm as a conversion factor for all the monkeys in this study. Similarly, the angle κ is the angle between the pupillary axis and the visual axis, where the pupillary axis is the line perpendicular to the cornea that passes through the PC, and the visual axis is the line passing from the fovea through the nodal point of the eye. Because the fovea is structurally temporal to the pupillary axis, there is a typical nasal ward displacement of the first Purkinje light reflex (apparent exotropia) called positive angle κ. In a study by Riddell et al.,[47] 323 human infants were assessed and a rapid linear change in angle κ was observed within the first 150 days of age (at ~65 days: ~6.98° ± 0.75°; at ~160 days: ~5.50° ± 0.75°), following which minimal variation in angle κ were observed until it reached adult levels of 4.69° ± 0.25°. The closest study in monkeys was by Quick and Boothe,[15,35] in which κ angle was measured using the Hirschberg photographic in juvenile monkeys and the κ range was 0.6° to 4.8° in 3 normal monkeys and 11 strabismus monkeys. In our study, ocular alignment was measured in infant monkeys from approximately 3 weeks of age, which represents approximately 3 months (~120 days) in humans, that is, presumably in the range in which angle κ is constant and less than 5°. Further, the normal monkeys exhibited an average measured deviation of 3.7°, which is close to the reported value of angle κ.[15] This value was subtracted from measured strabismus angles in all monkeys (Fig. 4). Potentially some error in the prism-reared group could be introduced because of variability in angle κ or the Hirschberg ratio among the animals, but these errors are likely to be in the order of a few degrees at most, whereas the longitudinal development of misalignment due to prism-rearing is significantly larger in magnitude.

Small amounts of binocular vision during the developmental critical period may be sufficient to drive normal visual-oculomotor development, including alignment.[38,39] In this study, we strived to minimize the time of exposure to binocular vision (Fig. 8), and the total time of binocular vision experienced by the study animals on a weekly basis was significantly lower than the reported times that influence development.

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