Measurement of Binocular Alignment in Normal Monkeys and in Monkeys with Strabismus

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Accurate assessment of ocular alignment in monkeys is difficult because typical clinical methods require extensive cooperation by the subject or provide only a rough estimate of the misalignment. Recently, Brodie derived a geometrical model for determining the Hirschberg ratio in humans, and validated it photographically. In this study, we have applied these procedures in order to determine corresponding values for monkeys. We have found the average Hirschberg ratio for macaques to be approximately 14° of rotation per millimeter corneal light reflex displacement. We extended the model to binocular viewing conditions, which allows for a description of the visual axes in Cartesian coordinates in relation to the head. Fixation errors, computed in terms of lateral and axial error vectors from intended fixation targets, were then determined for one normal monkey and for three monkeys that have a naturally occurring strabismus. Assessment of the fixation errors was made at several different distances and angles in the horizontal plane. The standard deviation for our measurements averaged 2.1°. Our data indicate that measurements must be made at multiple locations throughout the visual field in order to accurately specify the pattern of misalignment. Finally, a procedure is demonstrated which specifies the misalignment in terms of a cyclopian eye, which is independent of the interocular separation. Invest Ophthalmol Vis Sci 30:1159–1168, 1989.

While animal models of strabismus are important in the search for the underlying processes responsible for binocular misalignment, the usefulness of such models in part depends on the ability to accurately assess the given pattern of misalignment. The clinical methods that are most commonly used to evaluate ocular misalignment in human patients are prism and cover tests. However, these tests are not well suited for assessing alignment in animals, and this lack of appropriate methodology has hindered animal studies of strabismus.1,2 For example, extended cooperation is required to complete a prism and cover test and this is often difficult to obtain from animals. Furthermore, these clinical tests provide information about the magnitude of misalignment only at the specific distances and positions tested, and are usually not performed for fixation targets positioned at several different locations in the visual field. The effort required to obtain an accurate estimate of deviations throughout an animal’s visual field by a prism and cover test would be prohibitive.

Another set of clinical tests that is sometimes used with human patients, especially children who are not able to undergo standard prism and cover tests, involve examinations of the corneal reflections produced by a small point source of light. The clinical procedures used to make this estimate differ but are usually variations on the Hirschberg test. Briefly, these procedures estimate the amount of rotation of an eye by the displacement of the corneal light reflections away from some landmark on the eye, typically the center of the pupil. As recently discussed by Brodie,3 various authorities, including standard ophthalmological textbooks, have disagreed concerning the appropriate value for the Hirschberg ratio, which is the estimated ocular rotation (in degrees or prism diopters) per millimeter of corneal light reflex displacement. Brodie derived an explicit geometrical model that predicts the value of the Hirschberg ratio when measurements are made from photographs of the corneal reflection. He then validated his model empirically by making calibration measurements of human subjects while they viewed fixation targets monocularly.

Brodie extended his model to provide a method for determining the relative misalignment between the eyes. He describes procedures that can be used to measure the angles formed by each eye with respect to the camera. However, he does not provide a method for determining alignment errors relative to fixation targets, nor does he calculate error terms that

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specify the magnitude of the discrepancy between where the subject is attempting to fixate and where the visual axes of the two eyes are actually directed. In the current report, we first apply Brodie’s geometrical model of the Hirschberg ratio to macaque monkeys, and demonstrate its validity by making calibration measurements of photographs taken while monkeys view fixation targets monocularly. Then, we extend the geometrical model to binocular viewing conditions and derive procedures that can be used to specify, from corneal light reflex photographs, where the visual axes are directed in relation to the head. We also describe procedures for calculating lateral and axial error vectors from intended fixation points. Finally, we apply these procedures to both normal macaque monkeys and to monkeys with a naturally occurring strabismus, and relate these results to clinical evaluations of oculomotor status made on the same animals.

Materials and Methods

Subjects

Macaque monkeys were used as subjects in all of these experiments. We made longitudinal measurements on eye parameters from six normal Macaca mulatta monkeys taken from our colony. Eye parameters and complete sets of photographs obtained under monocular viewing conditions were obtained for one normal control Macaca mulatta. Eye parameters and complete sets of photographs under monocular and binocular conditions were obtained from four Macaca nemestrina monkeys: one normal control and three monkeys that have a naturally occurring strabismus. These monkeys received complete ophthalmic examinations by a pediatric ophthalmologist including cover testing to evaluate the nature of their strabismus, and were anesthetized in order to examine their fundus and to measure any refractive error. All procedures with the monkey subjects conformed to the ARVO Resolution on the Use of Animals in Research.

Application of Brodie’s Geometrical Model to Monkeys

Our calculations in this paper are based upon a simplified model eye as shown in Figure 1.* The eye-

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* This model makes several simplifying assumptions that are known to be inaccurate (e.g., that the shapes of the cornea and sclera are spherical, that the eye rotates about its geometrical center, and that the optical axis is coincident with the pupillary axis). Measurements that are based on a methodology more sensitive than corneal light reflections may require a more exact model. However, as demonstrated below, the variability due to errors in the model are significantly less than the variability in our photographic methods.
ball is represented as the intersection of two spheres. The center of one sphere, \( R \), is the eye’s geometrical center of rotation. The intersection of the spheres defines the limbus, and points \( E \) and \( F \) designate its nasal and temporal limits. The line between \( E \) and \( F \) represents the plane of the pupil, and the bisection of this line, \( C \), is the pupillary axis. A line through points \( B \), the center of corneal curvature, and \( C \) defines the pupillary axis. As a further simplification, we are assuming that the pupillary axis is coincident with the optical axis of the eye. We are representing the visual axis in this simplified model by a straight line that connects the object of regard, \( O \), with the corneal center of curvature. The angle between the pupillary axis and the visual axis is designated as \( \kappa \).

Following Brodie,\(^3\) we can use this geometrical model to relate the measurement of the corneal light reflex displacement made from photographs of the eye taken under monocular viewing conditions, to the angle of rotation of the visual axis. From Figure 1, it can be noted that the perpendicular from the photographic plane that passes through \( B \) yields point \( P_1 \), the apparent corneal light reflex, which is the image in the photograph of the camera flash reflected off the corneal surface. For this model, the flash is assumed to originate at the plane of the camera lens, encircling the aperture, and the light rays from the flash are perpendicular to the photographic plane. The perpendicular that passes through \( C \) yields point \( P_2 \), the apparent pupillary center.

If the distance \( BC \) is known, then the distance between points \( P_1 \) and \( P_2 \) in the photographic plane can be related to angle \( \psi \) by trigonometry:

\[
\psi = \sin^{-1} \frac{P_1 P_2}{BC} \quad (1.0)
\]

where \( \psi \) is the angle between the pupillary axis and any perpendicular to the photographic plane.

If one knows the limbal radius \( CE \) and the radius of curvature of the cornea \( BE \), then distance \( BC \) can be calculated using the Pythagorean Theorem:

\[
BC^2 = BE^2 - CE^2 \quad (1.1)
\]

The value for \( CE \) can be measured by using calipers. The value for \( BE \) can be calculated using the formula:

\[
BE = \frac{N_2 - N_1}{D} \quad (1.2)
\]

where \( N_2 \) is the refractive index of the cornea and aqueous, \( N_1 \) is the refractive index for air, and \( D \), the corneal curvature, is measured using a keratometer.

The relationship between angle \( \psi \) and distance \( P_1 P_2 \) described in Equation 1.0 is approximately linear over a range of more than \( \pm 50^\circ \). The slope of the function within this linear range can be used as an estimate of the predicted Hirschberg ratio; that is, the slope can be used to specify the angular rotation of the pupillary axis that corresponds to each millimeter displacement of the corneal light reflex in the photographic plane.

Angle \( \psi \) in Equation 1.0 is specified in reference to the pupillary axis. The angle formed by the visual axis and any perpendicular is designated as angle \( \theta \) in Figure 1. Angle \( \theta \) is the sum of angle \( \psi \) and angle \( \kappa \). Therefore, Equation 1.0 can be restated in terms of angle \( \theta \):

\[
\theta = \sin^{-1} \frac{P_1 P_2}{BC} + \kappa \quad (2.0)
\]

Angle \( \kappa \) can be determined by measuring the distance between \( P_1 \) and \( P_2 \) while the subject views, monocularly, fixation targets at various locations. The angle between the camera flash and the fixation target at which the distance between \( P_1 \) and \( P_2 \) equals zero is our measure of angle \( \kappa \).

**Specification of the Location of the Line of Sight in Two-Dimensional Space†**

Figure 1 also shows the parameters that were measured and calculated in order to fix the Cartesian coordinates of the visual axis. Angle \( \psi \) and angle \( \theta \) are known based on the procedures described in the previous section. The film plane is parallel to the line \( y = 0 \). We must position the head such that the centers of rotation (\( R \)) of the two eyes are located at known positions \((-r, 0)\) and \((r, 0)\). Distance \( r \) is one-half of the distance between the centers of rotation of the two eyes. Once \( r \) is determined and the head is positioned properly, the bisection of the interocular distance, which we designate as the cyclopian center, will be located at \((0, 0)\).

Since the center of rotation of the eye is inside the eyeball, its position can be determined only if it can first be related to some landmark on the surface of the eye. The geometrical relationships that can be used to derive an estimate of the center of rotation in relation to external landmarks are also illustrated in Figure 1. To reiterate upon the earlier discussion, \( B \) designates the corneal center of curvature and \( R \) the center of rotation. The location where the pupillary axis passes through the front of the cornea is labelled A. It is possible to derive the distance from the front of the

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† We are making measurements only to the nasal and temporal limbus of each eye. If we were to extend our procedures to include measurements around the circumference of the limbus, it would be possible to use similar geometrical methods to specify the location of the lines of sight in three-dimensional space. We have not made such measurements to date and for this reason have not extended our model beyond two dimensions.
cornea to the center of rotation RA or from any position on the scleral surface to the center of rotation RS. Distance RA is specified by:

\[ RA = AT - HT \times \sec(\angle RTH) \]  

(3.0)

Distance AT is the axial length and can be measured using ultrasonography. The distance HT can be calculated by:

\[ HT = 0.5 \times (CT^2 + CE^2)^{0.5} \]  

(3.1)

given that HR is the height of triangle RTE. The length CE is the limbal radius and can be measured as described earlier. Distance CT can be calculated from:

\[ CT = AT - (BA - BC) \]  

(3.2)

where BA = BE, the corneal radius of curvature (from Equation 1.2), and BC is obtained from Equation 1.1. The angle RTH can be derived from trigonometry as

\[ \angle RTH = \tan^{-1} \left( \frac{CE}{CT} \right) \]  

(3.3)

The distance from the center of rotation to any location on the scleral surface, RS, can be derived from:

\[ RS = RT = RE = HT \times \sec(\angle RTH) \]  

(4.0)

The derivations just described allow us to determine the distance between an external landmark, the location of which can be specified in relation to our Cartesian coordinate system, and the center of rotation of an eye. We then use either of two procedures for positioning the head so that it is as depicted in Figure 1 with the centers of rotation of the two eyes located at (—r, 0) and (r, 0). One procedure is to shine a light source, positioned at the camera, onto the sclera while the animal is induced to take up fixation at extreme peripheral targets. The position in the x-y plane of the reflection on the sclera can be determined in relation to the camera. The position in the x-y plane of the center of rotation can then be estimated by extending the line that passes from the light source to the scleral reflection by distance RS, from Equation 4.0.

An alternative procedure is to determine the approximate location of point A, in relation to a light source that is positioned such that the Purkinje images are aligned. This can be done using a penlight. Once the position of A has been determined, geometry can then be used to extend a line from location A to point R, by angle ψ (from Equation 1.0) and distance RA (from Equation 3.0). It should be noted that the values for RA and RS are not necessarily the same for both eyes (eg, if the two eyes have different axial lengths). In that case the head would have to be rotated as well as translated in order to get both centers of rotation into exact position.

Once the head is positioned as shown in Figure 1, we can derive the location of each visual axis in the x-y plane. In general, the equation for a line can be specified by a point and a slope. For the visual axis, we can use point B (the corneal center of curvature), and derive the slope as a function of θ. Point B changes coordinates as the eye rotates about its center, but point B can be related to unchanging point R by knowing distance RB and angle ψ (From equation 1.0). The coordinates for point B given that point R has coordinates (—r, 0) are:

\[ (-r + \sin \psi RB, \cos \psi RB) \]

Therefore the visual axis can be specified as a line passing through this point with a slope equal to cot θ, or tan (90 — θ). Once the positions of the visual axes have been determined, they can be compared to the axes necessary for proper binocular alignment.

Photographic Methods

The camera used was equipped with a 55 mm lens and a ring flash. The animal was placed in a restraining chair and positioned such that the eye being photographed was positioned 60 cm in front of the camera. The opposite eye was occluded with an opaque contact lens. Photographs were taken while the animal was induced to take up fixation at each of seven fixation targets positioned at 0°, 10°, 20° and 30° to each side of the camera. The fixation targets were raisins or other small food items held in forceps during each photograph and then given to the subject to eat. A millimeter ruler, placed above the eyes of the monkey, was included in each photograph to serve as a reference for later corneal light reflex displacement measurements. This procedure was then repeated for the other eye.

Photographs for binocular viewing conditions were taken as illustrated in Figure 2. Photographs were taken with the monkey fixating target positions in front of, behind, and along the photographic plane (PP) which was positioned at a distance of 60 cm from the cyclopian center (CC). The locations of all fixation targets were specified in Cartesian coordinates along the x-y plane in relation to the cyclopian center (0, 0). The point (X, Y) represents the location of the fixation target and point (X', Y') represents the location (if it exists) where the visual axes cross. We can calculate three different error vectors from this comparison. The first error vector, Left Error, represents the error in left eye fixation and is denoted by the symbol L. Its magnitude can be expressed as the degrees of visual angle separating the fixation target.
and the nearest approach of the visual axis for the left eye. The nearest approach is defined as the intersection of the visual axis and the perpendicular to the visual axis that passes through the fixation target. The second error vector, Right Error (R), is a similar measure for the right eye. The magnitude of our third error vector, Cross Error (+), is the discrepancy in meter angles (calculated as 1/distance in meters from the cyclopean center) between where the visual axes cross in space and the fixation target. The direction of the vectors shown in Figure 2 represents the direction of the fixation errors compared to the fixation target.

Scoring of all photographs was accomplished by projecting slides onto a screen and measuring the distance of the corneal light reflex to the nasal and temporal limbus. Measurements of the photographic distances were converted to actual distances by use of the reference ruler in each photograph. To measure the reliability of our results, the standard deviation of the corneal light reflex displacement at each fixation target was computed for one monkey. There were no significant differences between the standard deviations measured across eyes, fixation target locations, photographs, or photographic sessions; therefore, all standard deviations were averaged together and the value of ±0.15 mm was obtained. Expressed in terms of ocular rotation, this corresponds to an average standard deviation of approximately ±2.1°.

Other Methods Used to Validate the Geometrical Models

Measurements of several parameters needed for numerical estimation of the geometrical model were obtained from ophthalmic examinations performed by a pediatric ophthalmologist. Keratometry was used to measure the radius of curvature (the keratometer was calibrated at a refractive index of 1.338), calipers were used to measure the corneal radius along the pupillary plane, and ultrasonography was used to measure axial lengths. Cover testing of the naturally strabismic monkeys while positioned in a primate restraining chair was used to obtain a clinical estimate of the magnitude and direction of the ocular deviations.

Additional measurements were made on one monkey for the purpose of validating our photographic estimates of angle $\kappa$. The monkey was first anesthetized with ketamine and then 40 mg of thiamylil was administered to minimize eye drift. The anesthetized monkey's head was positioned in front of a tangent screen. A reversing ophthalmoscope was then used to locate and project onto the tangent screen: (1) the pupillary axis by centering the corneal light reflex; and (2) the line of sight that connects the center of the pupil to the object of regard, with the object of regard being coincident with the image of the anatomical fovea as projected into space by the eye. The angle between these two axes, angle $\lambda$, was then computed by geometry.†

Results

Validation of Brodie's Geometrical Model: Monkey RU01

Table 1 shows the values of the parameters obtained by clinical examination needed to determine

† There is no direct way to estimate angle $\kappa$ using a reversing ophthalmoscope. However, angle $\lambda$, which can be estimated, would be expected to have a similar value. For a recent discussion of this distinction, see Uozato and Guyton.4
Table 1. Measurement of optical parameters and derivation of values for determining the Hirschberg ratio geometrically for Monkey RUO1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limbal radius (CE)</td>
<td>5.0 mm</td>
<td>Measured: calipers</td>
</tr>
<tr>
<td>Corneal curvature</td>
<td>52.0 D</td>
<td>Measured: keratometry</td>
</tr>
<tr>
<td>Radius of curvature (BE)</td>
<td>6.5 mm</td>
<td>Derived: Equation 1.2</td>
</tr>
<tr>
<td>Length (BC)</td>
<td>4.15 mm</td>
<td>Derived: Equation 1.1</td>
</tr>
<tr>
<td>Hirschberg ratio</td>
<td>13.9°</td>
<td>Derived: Equation 1.0</td>
</tr>
</tbody>
</table>

the Hirschberg ratio from the geometrical model for monkey RUO1. From keratometry and caliper measurements, the value of 4.15 was obtained for the distance BC in Figure 1 and in Equation 1.1. Using these values in Equation 1.0 yields a prediction that the Hirschberg ratio for this monkey should be 13.9° of rotation for each millimeter of corneal light reflex displacement measured in the photograph.

Figure 3 presents the photographic results for determining the Hirschberg ratio for monkey RUO1. Empirically, the angular rotation as a function of displacement in millimeters appears to be approximately linear over the 60° range that we measured. Least squares linear regression confirms that these data are adequately fit by a straight line (goodness of fit = 0.94). The slope of this line for RUO1 is 13.8, which is in good agreement with the value of 13.9 that was predicted from the geometrical model. Similar good agreement was obtained for the four additional monkeys included in this study, as is demonstrated in Table 2.

Also plotted in Figure 3 are the separate values for the distance of the corneal light reflex from the nasal limbus and from the temporal limbus. The values obtained for these measurements were also adequately fit by straight lines using the method of least squares. The angle at which the two lines cross provides our estimate of angle \( k \). The left eye of monkey RUO1 has an estimated angle \( k \) of 4.8° that is illustrated by the dashed line.

We used these methods to estimate angle \( k \) for eight additional eyes from four monkeys and the results are shown in Table 2. Note that our estimates of angle \( k \) for the two eyes from a single monkey sometimes differ by more than 1°. We do not know if this difference is real or just reflects measurement error. To date we have only attempted to validate our estimate on one eye of one animal. We used the reversing ophthalmoscope, as described in Methods, to estimate angle \( \lambda \) for the left eye of monkey F84115. This method estimated angle \( \lambda \) to be 1.75°, which is in reasonable agreement with the value of 1.4° that we estimated for angle \( k \) based on corneal light reflex photographs (see Table 2).

Average Parameter Values for Macaque Monkeys

The most precise estimates for the Hirschberg ratio and angle \( k \) can be determined for a given monkey if measurements of the limbal radius and keratometry measurements can be made for each animal, as we have done for the monkeys included in this paper. However, even if these measurements are not available for a particular monkey, it is still possible to use the photographic methods described in this paper to obtain an estimate of eye position. This can be done by making use of normative population data for monkeys. In Table 3 we show the values of BC for four rhesus (Macaca mulatta) monkeys from our colony, each of which was measured at each of six successive ages. It can be seen that the parameters that are used to estimate the Hirschberg ratio exhibit little variation across animals and regardless of age. These data indicate that a Hirschberg ratio of about 14°/mm is appropriate to use as an average value for rhesus macaques (Macaca mulatta). The same value is probably appropriate for pigtail macaques (Macaca nemestrina). Results from one normal pigtail monkey, PVH, are shown in Table 2.

Fixation Errors under Binocular Viewing Conditions

We obtained complete sets of photographs under both monocular and binocular viewing conditions for four monkeys: one normal control and three with...
naturally occurring strabismus. The ocular parameters and Hirschberg ratios based on monocular photographs are summarized in Table 2. We also summarize, in Table 2, the clinical evaluation of these monkeys that was based on an ophthalmic examination.

Results of binocular photographs for normal monkey PVH are shown in Figure 4. The Cartesian coordinate graph of the x-y plane in the center portion of the figure represents the monkey's visual field. The letters indicate the location of the fixation stimuli during the photographic sessions. The surrounding circular regions represent the results at each of the fixation targets. These results are based on an average taken from four or more photographs scored for each fixation target. Descriptions of the error vectors can be found in Figure 2 and in Methods. The direction of each vector indicates the direction of the visual axes away from the fixation target. The magnitudes of the vectors are drawn to the scales shown in the Figure. The radius of the inner circle corresponds to the standard deviations for the Left Error and Right Error (2° of visual angle), and for the Cross Error (0.32 meter-angles). For monkey PVH, the largest Cross Error vector was 0.26 meter angles and the largest Left Error and Right Error vectors were only 1.2°. The average magnitudes of the Cross Error, Left Error, and Right Error vectors were 0.13 meter-angles, 0.40°, and 0.36°, respectively.

Figures 5 through 7 are similar graphs for three monkeys with naturally occurring strabismus. Strabismic monkey F84115 exhibited large errors at near fixation targets (Fig. 5). The magnitude of the Cross Error vectors for the two nearest targets were 2.21 and 3.85 meter-angles, which is six times larger than the mean Cross Error term in our control monkey PVH. Similarly, the magnitude of the Right Error vectors for monkey F84115 were 20 times larger for the near targets than shown for the near targets by monkey PVH. Note that the Left Error vectors were in the normal range at all target locations. This pattern of results indicates that the left eye for this monkey is the preferred eye and that there is an esotropia during high accommodation. This conclusion is consistent with the clinical evaluation presented in Table 2.

Clinical evaluation of monkey T84151 indicated esotropia during high accommodation, variable to

| Table 2. Optical parameters, the Hirschberg ratio derived from both the geometrical and empirical models, angle $\kappa$, and clinical evaluation of the normal and strabismic monkeys |
|---|---|---|---|---|
| Monkey | PVH | F84115 | T84151 | M79434 |
| Eye Refraction (D) | os | +1.0 | os | +5.0 |
| Limbal radius (mm) | os | 5.75 | +7.0 | +6.0 |
| Keratometry (D) | os | 48.2 | +5.0 | +6.0 |
| Geom. H. R. (deg) | os | 14.5 | 49.2 | 14.8 |
| Emp. H. R. (deg) | os | 14.6 | 14.4 | 14.4 |
| Angle $\kappa$ (deg) | os | +1.3 | -2.5 | 14.0 |
| Clinical evaluation | Normal, no deviation | Accommodative esotropia 5-15° | Accommodative esotropia variable to 10° | Constant angle esotropia 12-15° |
| | | OS preferred | OS preferred | alternating |

Cross Error vector was 0.26 meter angles and the largest Left Error and Right Error vectors were only 1.2°. The average magnitudes of the Cross Error, Left Error, and Right Error vectors were 0.13 meter-angles, 0.40°, and 0.36°, respectively.

Table 3. The relationship between monkey age and the distance between the corneal center and the pupillary plane (BC) based on empirical data

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Corneal radius* (mm)</th>
<th>Keratometry* (Diopters)</th>
<th>Curvature radius† (mm)</th>
<th>Distance BC† (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.95 ± 0.13</td>
<td>58.2 ± 1.3</td>
<td>5.80</td>
<td>4.2</td>
</tr>
<tr>
<td>3</td>
<td>4.50 ± 0.13</td>
<td>54.8 ± 1.6</td>
<td>6.16</td>
<td>4.2</td>
</tr>
<tr>
<td>6</td>
<td>5.30 ± 0.06</td>
<td>51.0 ± 2.0</td>
<td>6.61</td>
<td>4.0</td>
</tr>
<tr>
<td>12</td>
<td>5.50 ± 0.05</td>
<td>49.5 ± 0.6</td>
<td>6.82</td>
<td>4.0</td>
</tr>
<tr>
<td>18</td>
<td>5.50 ± 0.13</td>
<td>49.2 ± 1.0</td>
<td>6.86</td>
<td>4.1</td>
</tr>
<tr>
<td>24</td>
<td>5.75 ± 0.25</td>
<td>48.0 ± 1.3</td>
<td>7.03</td>
<td>4.0</td>
</tr>
</tbody>
</table>

* Values for corneal radius and keratometry were measured on four monkeys at each age and are expressed as the Mean ± SE.† The curvature radius (from Equation 1.1) and distance BC (from Equation 1.1) were computed from these mean values. Using these values, the estimated Hirschberg ratio = 14.1° (from Equation 1.0).
Fig. 4. Measurement of binocular alignment for monkey PVH. The center of the Figure represents a graph of the monkey's visual field. The letters denote the locations where photographs were taken. The surrounding circles represent a magnification of each of these photographic locations. The vectors specify both the magnitude (see attached scale) and direction of the fixation errors as described in Figure 2. The magnitude for Left Error (L) and Right Error (R) vectors are expressed in degrees, while the magnitude of the Cross Error vector (+) is expressed in meter-angles. The inner circle represents the standard deviation for Left—Error and Right—Error (2°), and for the Cross—Error (0.32 meter-angles). Fixation errors less than these values are drawn as vectors with a length equal to the radius of the circle.

10°. Photographs taken during fixation to targets at 30 cm were consistent with this evaluation (Fig. 6). Eso deviations were consistently seen at both 30 cm target locations. However, it is interesting to note that our photographs taken during fixation to eccentric targets at 60 cm show that fixation preference switched between the left and right visual fields, and that the deviations at these locations were exo. This monkey shows only a moderate eso deviation straight ahead at 60 cm and has normal alignment for the straight-ahead target at 120 cm.

In Figure 7 we present the results for strabismic monkey M79434. Clinical evaluation with cover test indicated a constant angle esotropia of 12° to 15° at near and distance with alternating fixation. Photographs revealed that fixation alternated with the eye preference contralateral to the visual field. Eso deviations were seen at all distant targets, with the mean error at distant targets equal to 9.7°. There was no deviation present under the highly accommodative conditions at 20 cm.

Discussion

We have described a method for specifying, based on corneal reflection measurements, the location in two-dimensional space of the visual axis for each eye. We can determine the Hirschberg ratio and angle $\kappa$ from monocular photographs. We can determine the center of rotation based on measurements of the limbal radius, axial length and corneal curvature, which can be obtained from an ophthalmic examination. Once this is done, it is possible to make quantitative estimates of the magnitude of binocular misalignment based on measurements of photographs taken while the subject fixates, binocularly, targets at various locations in space. Our methods allow us to determine the location of the visual axes for the two eyes with reference to both the cyclopian center and the fixation target.

The Hirschberg ratio for a specific subject can be measured directly by taking photographs during monocular fixation of several targets at known angles. The slope of the line which plots the fixation angle versus displacement of the reflection from the center of the pupil provides this information directly. If these monocular measurements cannot be performed on a particular monkey, then our group data indicate that for an average macaque monkey a 1-mm displacement of the reflection corresponds to a 14° change in the direction of gaze. We have also found that the results obtained by the photographic method are highly reproducible. At least four photo-
graphs were taken of each monkey at each target location and duplicate conditions were repeated on different days. The standard deviation obtained across the measurements of each corneal light reflex displacement was ±2.1°.

Our methods for determining where the visual axes are directed have two general sources of variability. The first source, as mentioned previously, has to do with inaccuracies in the assumptions made by our geometrical model. For example, we are assuming that the eye rotates about its geometrical center of rotation. In fact, the center of rotation deviates slightly from the geometrical center and furthermore, the eye translates slightly during rotation. Even if we assume a worst case condition where our estimate of the true center of rotation is in error by as much as 2-mm, the error introduced at the 60 cm camera distance would be only 0.2°. Assuming that the cornea is spherical also misrepresents the slight flattening that occurs in the periphery. We have restricted our measurements to the central 60° of the visual field and, as we demonstrated in Figure 3, over this range the flattening is not a problem. In humans, Brodie has found that plots of reflex position are nearly linear over the entire 100° range that he measured. Our model also assumes the eye to be a centered optical system, with the optical axis coincident with the pupillary axis, and the corneal center of curvature a good estimate of the anterior nodal point. The potential errors introduced by these assumptions limit our ability to specify the exact locations at which the optical and visual axes pass through the pupil. However, given an average pupil size of 6 mm, the worst case magnitude of error introduced at the 60 cm camera distance would be about 0.5°.

We have named the second general source of error procedural variability, of which we have three types. The first is variability occurring during each photographic session that results from not having the position of the head exactly the same for each photograph. We used a primate restraining chair to hold the animal still, and had them fixate food targets. One could further reduce this source of variability by using some other method to restrain the head more completely. Also, scatter occurs by failure to present the fixation target consistently at the proper location. The second type of procedural error is measurement variability that occurs during the scoring of each photograph. The third type is the trial-to-trial variability as to where the subject chooses to fixate. Under conditions in which normal trained monkeys are highly motivated, this fixation error has been shown to be quite small, on the order of minutes of arc for fixation in the horizontal plane. All sources of variability combined, including inaccuracies in our geometrical model and procedural sources of scatter, lead to standard deviations in our repeated measurements of about 2°.

Our study demonstrates the importance of deriving estimates of the ocular deviation at many locations throughout the subject's visual field. Often, either due to time constraints or to the uncooperativeness of the
subject, estimation of the magnitude of the deviation is made only at one near location and at one far location. The pattern of results that we have seen for our animals suggests that had we measured at only two locations we would have generated a very incomplete description of the misalignment. A more complete description may be important when attempting to construct theoretical models that simulate the deficits that occur in strabismus. A limitation of our methods in this regard is that the position of a deviated eye may reflect either the resting position of a non-fixating eye during suppression, or the position to which the eye turns in order to eccentrically fixate. Our current methods do not provide a way of distinguishing between these two types of deviation.

We have chosen our coordinate system such that it is fixed, with the origin located at the bisection of the interocular distance. By incorporating the centers of rotation of both eyes into the one coordinate system, we obviate the need to translate coordinates depending upon the eye being studied. Therefore, not only can we specify the angles that the eyes rotate, but we can also determine the angles through which the eyes should rotate in order to fixate the proper target.

Other procedures have been devised that allow one to make an assessment of the relative misalignment between the eyes without taking into account the locations in space of the two centers of rotation, eg, subtracting the difference between the corneal light reflex displacement in the left eye from that in the right eye. The problem with these procedures is that part of the magnitude of the estimated deviation is simply a function of the interocular distance. This can be demonstrated by a simple example. Suppose that a normal subject has an interocular separation of 8 cm and both eyes are fixating on a target 60 cm in front of the nose. Now suppose one eye refixates to a target 60 cm from the nose but 30° to the right. If the other eye rotates through an equal angle (Hering’s law), then that eye will show an “apparent” deviation of 1.6°. A subject with a different interocular separation viewing this same target, or the same subject viewing this same target, or the same subject viewing targets at different locations would exhibit different patterns of “apparent” deviations that are solely a function of the interocular separation. Direct comparisons across subjects of different sizes or longitudinal studies of infants are impossible unless methods are used that take interocular separation into account.

In this paper we have devised methods that can be used to specify the magnitude of a misalignment, for any location in space along the horizontal plane, that is independent of interocular separation. Thus it may be helpful to think of the error vectors produced by our methods (Left_Error, Right_Error, and Cross_Error), in relation to a cyclopian eye. This can be done by transformation of the angle \( \theta \) from each eye to the cyclopian center (CC) where:

\[
\theta_{CC} = \tan^{-1} \left( \frac{h}{k} \right)
\]

where \((h, k)\) are the Cartesian coordinates of where the visual axes cross in space, or their closest approach to the fixation target. The difference between this cyclopian value of angle \( \theta \) and the angle \( \theta \) that is measured photographically during binocular fixation is determined by a single parameter, \( r \), and has the general form:

\[
\theta = \tan^{-1} \left( \frac{h \pm r}{k} \right)
\]

where \(2 \times r\) is the distance between the centers of rotation. The value of \( \theta \) for the cyclopian center can be derived from the values for \( \theta \) obtained for each eye measured by corneal reflection. From trigonometric substitution using the two previous equations:

\[
\theta_{CC} = \tan^{-1} \left( \frac{h \times \tan \theta}{h \pm r} \right)
\]

In other words, \( \theta_{CC} \) is the angle that would be necessary to fixate a target using a cyclopian eye. For normal subjects, the cyclopian value for angle \( \theta_{CC} \) should be the same for both eyes. In strabismic subjects, this value will be different for the fixating and deviating eyes, and it is independent of the interocular distance. 

**Key words**: ocular alignment, strabismus, Hirschberg test, corneal light reflex, monkey

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**References**