An analysis of retinal receptor orientation

I. Angular relationship of neighboring photoreceptors

Alan M. Laties and Jay M. Enoch

The orientation of retinal receptors is important in the understanding of retinal physiology. The photoreceptors of spider monkey eyes tend to be oriented toward an anterior point in the eye rather than to the center of the retinal sphere. In this paper some of the implications of this finding are considered. Differences in cell orientation have been computed for both a center-pointing and an anterior-pointing hypothesis. The meaning of the derived values is considered in some detail.

Key words: retina, primate, photoreceptors, photoreceptor orientation (normal and abnormal), photoreceptor tilt (normal and abnormal), center-pointing hypothesis, graded differential orientation, histology of the retina, Stiles-Crawford effect.

A graded differential orientation (g.d.o.) of photoreceptors in the primate eye was demonstrated recently. While receptor structures were found to be oriented normally to the globe at the posterior pole of the eye, they were observed to tilt away from the normal by progressively larger angles in the retinal periphery (Fig. 1). These findings imply an ordered relationship of photoreceptors, one to another. Although the geometry of the orientation of receptors with reference to the globe has been described, the consequences of g.d.o. on the angular relationships of neighboring receptive structures have not been explored. In order to do so, it is our purpose to calculate the angular relationships of neighboring photoreceptors along a given meridian for two specific cases: (1) The center-pointing hypothesis, in which there is no g.d.o.; that is, a spherical eye in which all receptive structures are aligned so as to be segments along radii (Fig. 2, A and B) pointing toward the center of the scleral or retinal sphere. (2) A g.d.o. model in which the photoreceptive structures in a spherical eye are all aligned so that light rays which emanate from a point on the

*The term receptor is used to denote the inner and outer segments as a unit.
Fig. 1. This photograph demonstrates the tilt of rods and cones near the equator of the eye. \( \theta \), (see Fig. 3, A) is approximately 30°. Fluorescence microphotograph of freeze-dried Cebus monkey retina. (X600.)

...line of sight located anteriorly can enter them coaxally (Fig. 3, A and B). The effective deviation from parallel alignment of one receptor to another is compared for the two cases. It is important to note that parallel receptor alignment is not compatible with either alternative.

For the sake of uniformity, all measurements used in the following series of calculations have been standardized and are based on spider monkey eyes. As in a prior communication, the external limiting membrane of the retina of the spider monkey eye is assumed to be spherical and to have a radius of 7.2 mm. Measurements of the lengths and average separation of photoreceptors present problems of tissue artifact. Although every histologic method results in some artifacts, care was taken within the limits of modern histologic technique to lessen them; the measurements used are, we believe, reasonable and the best estimates.

The length of the inner and outer segments of photoreceptors varies with location; the longest receptors along any given meridian being the foveal cones and the shortest, the last few blunted cones at the ora serrata. Outside the fovea, where a mixed population of rods and cones is present, it is generally recognized that the rods are somewhat longer. For calculations presented in this paper, all receptors at a given locus are assumed to be the same length. The combined lengths of the inner and outer segments of the photoreceptor are used for the length of receptor elements in the calculations.

Average separation of photoreceptors is too small a quantity to be measured accurately in the material available. As in the case of receptor length, it varies with retinal locus. We believe that a 2.5\( \mu \) center-to-center cell separation at the external limiting membrane is a valid approximation. (Near the fovea it is 2.25\( \mu \) or slightly less.) For purposes of calculation, the assumption is made that the magnitude of photoreceptor separation is unchanged with change of retinal locus. In addition, an outer segment diameter of 1.5\( \mu \) is also assumed. With these two assumptions...
Fig. 2. (A) In the center-pointing hypothesis all receptors would point toward the center of the retinal sphere (O). Neighboring receptors are represented; the scale has purposely been distorted. (B) Anatomic-to-geometric translation for the center-pointing hypothesis. $\Delta \Phi$ represents the divergence angle between two neighboring photoreceptors; $r$, the radius of the retinal sphere; $\Delta r$, photoreceptor length; $A$, the center-to-center separation between neighboring cells at the external limiting membrane; $B$, center-to-center separation at the distal end of the neighboring photoreceptors. In the calculations (based on spider monkey determinations) $r = 7.2 \text{ mm.}$, $\Delta r = 50.6 \mu$, and $A = 2.5 \mu$.

Fig. 3. (A) In the graded differential orientation (g.d.o.) model all receptors are in line with an anterior point D. Again, these are supposed to be neighboring receptors and the scale has purposely been distorted. (B) Geometric consideration of the g.d.o. hypothesis. $\Delta \Theta_1$ represents the divergence angle of the neighboring photoreceptors. Note A, r, and $\Delta \Phi$ are the same as in Fig. 2 B, and $\Delta r$ in Fig. 2 B = $\Delta x$ in this figure. $DO = d$ was set = 8.7 mm., $r = 7.2 \text{ mm.}$, $A = 2.5 \mu$. At $\Phi = 4.2^\circ$, $\Delta x$ was set = 50.6$\mu$. 
operative, the interstitial matrix between two neighboring outer segments would be on the order of \( 1/x \).

**Case 1**

The center-pointing hypothesis. In order to understand the requirements of the center-pointing hypothesis (Fig. 2, A, B), it is important to determine the difference in center-to-center photoreceptor separation from its origin near the external limiting membrane (A) to its termination at the pigment epithelial cell "bed" (B). We seek to determine \( B - A \). In addition, the angular separation of the axes of neighboring cells needs to be determined.

For small angles, \( \tan \Delta \Phi = \Delta \Phi \),

\[
\Delta \Phi = \frac{A}{r} = \frac{B}{r + \Delta r},
\]

and \( r \Delta \Phi = A, \ (r + \Delta r) \Delta \Phi = B. \) (1)

Hence,

\[
B - A = \Delta \Phi \left((r + \Delta r) - r\right) = \Delta r \Delta \Phi. \] (2)

\( \Delta \Phi \) and \( B - A \) have been determined for the three retinal points indicated in Table I. These calculations assume \( r = 7.2 \) mm. (Reference 2) and that uniform orientation persists across the retina. In Case 1 all receptor elements are pointing toward the center of the retinal sphere and thus are segments of radii of the same sphere. Hence, the angular relationships for neighboring receptor elements are the same in the periphery as in the posterior pole, if one assumes the same separation cell-to-cell at the external limiting membrane. Similar geometric considerations would further imply that their distal separations \( B - A \) would be a simple function of their length. Thus, \( B - A \) is greatest for the longer foveal receptors (Table II). Computed values of \( \Delta \Phi \) and \( B - A \) are too small to be observed in the light microscope.

**Case 2**

The graded differential orientation hypothesis. For the second case, that in which receptor elements are assumed to be coaxial with an anterior point, a model previously described has been adopted. In this model, all inner and outer segments are coaxial with a point "D" along the line of sight in the region of the lens (Fig. 3, A and B). Selected values (Table I) of the pertinent parameters have been calculated for arbitrarily chosen positions (formulas and/or derivations follow). Sample retinal points selected were located 4.2°, 48.4°, and 78.6° from the fovea with the angle \( \Phi \) measured from the center of the retinal sphere. In each of these instances, a different combined length of the inner and outer segments was used in accordance with the values in Table II.

\[
\theta_1 = \cot^{-1} \left( \frac{r/d \sin \phi + \cot \phi}{} \right). \] (3)

One form of the first derivative of this relationship is given by the expression:

\[
\frac{d\theta_1}{d\phi} = \frac{r/d \cos \phi + 1}{(r/d)^2 + 2 (r/d) \cos \phi + 1}. \] (4)

With the values \( r = 7.2 \) mm. and \( d = 8.7 \) mm., this latter relationship is plotted in Fig. 4. From this relationship, it is possible to determine the rate of change of orientation of the receptors relative to the center of the retinal sphere.

In Case 2 we are interested in the values of \( \Delta \theta_1 \) and \( C - A \) (Fig. 3, A and B). Obviously:

\[
\Phi = \theta_1 + \theta_0. \] (5)

The relationship for the neighboring receptor elements is:

\[
(\Phi + \Delta \Phi) = (\theta_1 + \Delta \theta_1) + (\theta_0 + \Delta \theta_0). \] (6)

Subtracting expression (6) from (5):

\[
\Delta \Phi = \Delta \theta_1 + \Delta \theta_0. \] (7)

Dividing by \( \Delta \Phi \) and rearranging terms:

\[
\Delta \theta_0 = (1 - \frac{\Delta \theta_1}{\Delta \Phi}) \Delta \Phi. \] (8)

For the small intercellular changes involved, we may assume:

\[
\frac{\Delta \theta_1}{\Delta \Phi} = \frac{d\theta_1}{d\phi}. \] (9)

Hence:

\[
\Delta \theta_0 = (1 - \frac{d\theta_1}{d\phi}) \Delta \Phi. \] (10)

As the problem has been defined above, \( \Delta \Phi = A/r \). Both \( A \) and \( r \) are constant values, and hence, \( \Delta \Phi = 0.000347 \) radians. The expression \( d\theta_1/d\phi \) may be obtained from Fig. 4 for differing values of \( \Phi \). In Fig. 3B, \( \Delta \theta_1 \) represents the divergence angle for neighboring photoreceptors if all cells are pointing at the common point D. The difference in separation of neighboring cells at the external limiting membrane (A) and at the pigment epithelium (C) may be derived in a manner similar to that used in Case 1.

Let \( DP_x = x \) (Fig. 3, B), and the length of the combined inner and outer segment equal \( \Delta x \).

\[
\Delta \theta_x = \frac{A}{x} = \frac{C}{x + \Delta x}, \text{ and} \] (11)

\[
A = x \Delta \theta_1, \ C = (x + \Delta x) (\Delta \theta_1). \] (12)

\[
C - A = \Delta x (\Delta \theta_1). \] (13)

*See reference 2.
Table I. Primary relationships

<table>
<thead>
<tr>
<th></th>
<th>(Measured)</th>
<th>(Calculated)</th>
<th>(Calculated)</th>
<th>(Calculated)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Phi$</td>
<td>$\theta_1$</td>
<td>$\theta_2$</td>
<td>$d\theta_1/d\Phi$</td>
<td>$\theta_3$</td>
</tr>
<tr>
<td>4.2°</td>
<td>2.2°</td>
<td>2.3°</td>
<td>0.546</td>
<td>1.9°</td>
</tr>
<tr>
<td>48.4°</td>
<td>26.2°</td>
<td>26.6°</td>
<td>0.557</td>
<td>21.8°</td>
</tr>
<tr>
<td>78.6°</td>
<td>42.5°</td>
<td>43.7°</td>
<td>0.580</td>
<td>34.9°</td>
</tr>
</tbody>
</table>

Table II. Case 1 vs Case 2

<table>
<thead>
<tr>
<th>Measured values</th>
<th>Calculated values</th>
<th>Case 1</th>
<th>Case 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\Phi$</td>
<td>Length*</td>
<td>$\Delta\Phi$</td>
<td>Divergence angle</td>
</tr>
<tr>
<td>4.2°</td>
<td>50.6μ</td>
<td>1.2°</td>
<td>+0.0176μ</td>
</tr>
<tr>
<td>48.4°</td>
<td>39.1μ</td>
<td>1.2°</td>
<td>+0.0136μ</td>
</tr>
<tr>
<td>78.6°</td>
<td>27.6μ</td>
<td>1.2°</td>
<td>+0.0096μ</td>
</tr>
</tbody>
</table>

*Length of combined photoreceptor inner and outer segments (spider monkey).

(Note: In calculations used to derive the pertinent values for Cases 1 and 2, $\Delta r = \Delta \chi$.)

Three basic factors influence the sample calculations which appear in Table II. First, the distance $\chi$ shortens as $\Phi$ increases. Assuming a constant value $A$, one is surprised that $\Delta \theta_1$ does not increase as rapidly as $\Phi$ increases. However, the reader is reminded that the external limiting membrane is rotating about point $O$ at the center of the retinal sphere and the point $D$ looks increasingly oblique at the line segment $A$. It becomes foreshortened just as the iris aperture apparently narrows horizontally when viewed laterally.* This effect is greater than the decrease in distance $\chi$. Lastly, as in Case 1, the shortening of the receptors in the periphery serves to reduce $C - A$.

As in Case 1, neither the small angle $\Delta \theta_3$, nor the distance $C - A$ predicted for neighboring receptors (Case 2, Table II) would be visible in the light microscope.

Discussion

The angular divergence of neighboring photoreceptors can be seen to be greatest (Table II) with the center-pointing hypothesis. Simply stated, the divergence angle of neighboring receptors coaxial to a nearer point is greater than their divergence angle would be were they coaxial to a more distant point. At its shortest, $\chi$ in Fig. 3, $B$ is longer than $r$.

Table II also shows the calculated angular divergence to be of the order of one minute of arc for either hypothesis. This angle is so small that the typical drawing showing parallel alignment of neighboring photoreceptors in textbooks is clearly accurate within the limits implied. However, one need only consider the consequences of parallel alignment to realize that such a conception of receptor orientation must be erroneous. By definition, parallel photoreceptors would all be in line with the same point at infinity. If the photoreceptors are perpendicular to the retinal surface at the posterior pole, such an infinity assumption has the absurd requirement that they be flat against Bruch's membrane at the equator and actually have a reverse incline to the globe at the ora serrata!

In the above calculations, the assumption is made that change in orientation is continuous and gradual receptor-to-receptor. Since physical and histologic considerations render direct measurement of the angular divergence of neighboring photoreceptors beyond the reach of present technology,
only indirect means are possible to test this assumption. One such approach to the problem is to use psychophysical measurements. O'Brien and Miller's experiments on human observers, repeated and described in detail by Enoch, have shown by psychophysical techniques discontinuous local variance in orientation by groups of receptor elements in the human retina. The magnitude of the discontinuous local variance thus uncovered has not as yet been quantified.

Measurements of the Stiles-Crawford effect over small areas offers another possible approach to this problem. However, such studies are not without difficulty: In general, measurements of the Stiles-Crawford effect subtend areas rather large compared to the subgroups measured by the O'Brien-Miller method or those considered in this paper; ocular aberrations and instability of eye fixation make truly localized Stiles-Crawford measurements difficult to perform accurately; and lastly, psychophysical measurements of directional sensitivity also tend to reflect the integrated neural response of the area tested. In sum, these limitations have hindered detailed analysis of the Stiles-Crawford effect over small retinal areas.

As just noted, O'Brien and Miller's and Enoch's studies of local variation in directional sensitivity of groups of receptors are important indications that photoreceptor orientation may vary in discrete regions from the orderly models considered in this paper. In both receptor-orientation models discussed above, the differences in orientation cell-to-cell along a given meridian are on the order of one or two hundredths of a degree.

A comparison of this minute quantity to the theoretic maximum deviation of one cell relative to its neighbors is instructive. In order for either hypothesis (Case 1 or Case 2) to hold, extremely small differences in orientation are required per cell. If the receptors bend only at the external limiting membrane, it can be shown that the maximum change in angle (±) by a cylindrically shaped cell is given by the simple relationship, \[ \Delta \phi = \frac{q}{\Delta x} \] (Fig. 5). The separation
between cells is \( q \) (interstitial matrix), and \( \Delta x \) is the length of the combined inner and outer segments. If \( q \) is set at 1.0 \( \mu \) and \( x = 50 \mu \), \( \Delta \theta_i = 0.02 \) radians or 1\(^{\circ}9'\). This value might be compared with the value of \( \Delta \Phi \) = 1.2 minutes of arc derived for the center-pointing hypothesis. In the g.d.o. case, \( \Delta \theta_3 = \Delta \Phi - \Delta \theta_1 \) (where \( \Delta \Phi \) is also a constant 1.2'); \( \Delta \theta_3 \) here (in this special case of disturbed orientation of one cell) would be approximately equal to \( \Delta \theta_1 \). A value of \( \Delta \theta_3 = 1^{\circ}9' \) is much more than an order of magnitude greater than values computed for either hypothesis in Table II. This point requires some explanation and the figures, some modification.

The intimate relationship of a receptor to the pigment epithelium cells and to the fibrillar baskets of Mueller cells at the external limiting membrane limit lateral motion. Given the natural sheathing in rods and cones, it is possible that their inner and outer segments cannot bend as a unit without substantial distortion. Thus, it is doubtful that the derived theoretical maximum deviation angle (Fig. 5) is ever attained in the normal retina.

Due to differential shrinkage and subsequent deformation in processing, little is added to our knowledge of naturally occurring receptor distortion by electron microscopic studies. It is worthwhile to note, however, that given the limitation of the preparative techniques, photoreceptors are clearly separate in electron microphotographs (by 0.8\( \mu \) in the macaque). When preparations which are considered technically to be the very best are viewed in the light microscope, the outer segments appear parallel.

Besides supportive and nutritive functions, the space between neighboring receptors, the interstitial matrix, serves important optical requirements. For photoreceptors to function properly, they must be separated. Kapany \(^9\) and Enoch \(^10\) have demonstrated that frustrated total reflection begins to reduce resolution when the separation of neighboring photoreceptors is less than one wavelength of light. In the case of a tissue with the properties of the retina, the necessary separation would be approximately 0.3 to 0.5\( \mu \), the wavelengths of light in this medium. If receptors are separated by less than this amount, some portion of the luminous energy may leak into neighboring receptors and create a form of "cross-talk." Using the method of dynamic scanning, Enoch and Glismann \(^11\) recently found that measured resolutions of isolated, well-orientated photoreceptors approach the theoretical limit in fresh samples. These data suggest that the necessary minimum separation between receptors was present in their sample preparations. In addition, recent data suggest that the ability to regenerate and realign the photoreceptor may not be lost with maturity.\(^14\,15\)

If a g.d.o. model is accepted as a general description of the orientation of primate retinal photoreceptors, questions concerning the origin of receptor alignment and the control of orientation immediately come to mind. In addition, it would be of
value to know if g.d.o. is present in species other than primates. At present only partial answers to these questions are available. Preliminary work on the eyes of newborn rhesus monkeys \(^7\) which have never experienced light (taken by cesarean section) has revealed a marked tilt of the peripheral photoreceptors. Although the specimens were not of sufficient quality to allow accurate measurements, the receptor tilt was of the same order of magnitude and direction as that previously found in adult spider monkey eyes, thus implying that at least some degree of differential orientation is inborn. Practical inferences regarding the orientation of retinal receptors in the human can be obtained by studying the Stiles-Crawford effect in normal patients and in patients with pathologic conditions in which there are known disturbances of receptor orientation. Several reports of deviated receptor elements

Fig. 6. Example of differential orientation of photoreceptors at two points along the same meridian of a *Gekko gekko* retina. (Fluorescence micrograph. ×600.)
have now been presented. Although some study of such conditions has been undertaken, quantitative understanding has been elusive—a special pathology of receptor elements is needed. New developments in techniques for experimental retinal detachment and subsequent reattachment make improved evaluation of this problem possible.

No studies are available at present on photoreceptor orientation in eyes deprived of light for long periods of time or in eyes in which light enters eccentrically. Thus, it is not known whether the maintenance of orientation is dependent on the amount of light flux reaching the retina, the direction of the light flux, or on some other factors.

Some form of differential orientation of photoreceptors appears to be present in all species so far studied. Although quantitative studies are lacking, peripheral photoreceptor tilt has been found to be in the same sense as occurs in primates in all fish (Carassius auratus), amphibian (Rana pipiens), and reptile eyes (Gekko gekko) studied (Fig. 6). Among primates, peripheral photoreceptor tilt has been seen in all superfamilies so far studied from human to New-World monkey.

The establishment of a theory of g.d.o. leads naturally to speculation on its meaning to human vision. Specifically, it raises questions as to where the maxima of the Stiles-Crawford effect would be expected for peripheral points, and what would be the predicted orientation of photoreceptors if they are coaxial to a point in the line of sight at or near the exit pupil of the human eye. At present, very few studies deal with this subject, with the notable exception of that of Aguilar and Plaza. In a study now in progress, we are analyzing this question in greater detail.

The authors wish to thank the Misses Marilyn Jenkins and Judith Pfleuger, and Mr. Robert Paltzman for invaluable technical assistance.

REFERENCES

17. Laties, A.: Unpublished data, 1969. The eyes of newborn rhesus monkeys were made available through the courtesy of Dr. Ronald Myers of the Laboratory of Perinatal Physiology of the National Institutes of Health.