The Development of Scotopic Sensitivity

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PURPOSE. Test the hypothesis that the developmental increases in rod photoreceptor sensitivity and rod-mediated visual sensitivity at 10°, 20°, and 30° eccentric are concurrent. It is known that maturation of the parafoveal (10° eccentric) rod outer segments and visual sensitivity is delayed compared to that at 30° eccentric.

METHODS. Rod isolated electroretinographic (ERG) responses to full-field stimuli were obtained from dark-adapted subjects (n = 71), ranging in age from early infancy through middle age. Rod photoreceptor sensitivity was calculated by fitting a model of the activation of phototransduction to the a-wave response. Rod driven b-wave sensitivity was calculated from stimulus–response functions. A logistic growth model was used to summarize the developmental increases in sensitivity of the rod photoreceptors and the b-wave. Previously reported dark-adapted, rod-mediated visual sensitivities at 10°, 20°, and 30° eccentric, obtained using preferential looking procedures, were reanalyzed using the logistic growth model.

RESULTS. The logistic growth model accounted for 57% to 85% of the variance of each sensitivity parameter with age in normal subjects. The shape of the growth curve and the age at which sensitivity reaches 50% of the adult value is similar (10.0–13.5 weeks) for the rods, the b-wave, and peripheral visual sensitivity, but is significantly older, 19.5 weeks, for rod-mediated parafoveal visual sensitivity.

CONCLUSIONS. Rod photoreceptor sensitivity and peripheral, rod-mediated visual sensitivity develop concurrently. A parsimonious explanation is that rod photoreceptor sensitivity determines dark-adapted, rod-mediated visual sensitivity during development. (Invest Ophthalmol Vis Sci. 2000;41: 1588–1596)

The dark-adapted, rod-mediated visual sensitivity of young infants, according to most published data, is more than a log unit below that of adults. For instance, at ages 8 to 12 weeks, dark-adapted visual sensitivities, estimated using two alternative, forced-choice preferential looking procedures, are 1.1 to 1.7 log units lower than those of adults. Over the same limited age range in early infancy, sensitivities derived from the electroretinographic (ERG) a- and b-waves are barely half a log unit less than that of adults. The course of maturation of rod-mediated visual sensitivity, however, depends on the retinal region tested. The development of parafoveal (10° eccentric) sensitivity and rod outer segments is delayed compared to that in more peripheral retina. Furthermore, there is only a half log unit discrepancy between peripheral visual sensitivity for detecting 30° eccentric 2° diameter stimuli in 10-week-old and adult individuals. In other words, for some stimulus conditions, the immaturities in ERG and visual sensitivity are the same. The rods are thought to account for immaturities of the ERG a- and b-waves. Thus, despite some evidence to the contrary, the immature rods, which have short outer segments and low rhodopsin content with consequent low probability of photon capture, cannot be dismissed as the primary determinant of infants’ low, dark-adapted, rod-mediated visual sensitivity.

In developing mammalian retina, rod outer segments grow longer, following a course of logistic growth, and finally asymptote at adult length with equal synthesis and disposal of outer segment discs. Logistic growth also describes the developmental increase in rhodopsin content (Fig. 1) during outer segment development. The developmental increase in rhodopsin of several species including human, has also been summarized using a logistic growth model. By using this mathematical model of growth, the courses of development of the visual pigment and retinal responses could be compared. The courses of the developmental increase in rat rod photoreceptor and inner retinal sensitivity, derived from the electroretinographic (ERG) a- and b-waves, are indistinguishable from that of rhodopsin. That is, according to a logistic growth model, Age, the age at which a parameter is half the adult value, for rat rhodopsin, rod photoreceptor sensitivity, and b-wave sensitivity do not differ significantly.

In the present study, we tested the hypothesis that dark-adapted, rod photoreceptor sensitivity predicts dark-adapted, rod-mediated visual sensitivity during infancy. We have used contemporary ERG procedures to assess rod photoreceptor sensitivity, represented by the ERG a-wave, and b-wave sensitivity, as well as the developmental increases in the saturated amplitudes of the rod photoreceptor response and rod driven b-wave. Additionally, we have reanalyzed the developmental increase in rod outer segments, rhodopsin, and rod photoreceptor sensitivity in the rat and human by fitting a logistic growth model to the data.
The main data are from 71 normal subjects recruited for study of rod photoreceptor function. Healthy, term-born infants, ages 23 through 100 days (n = 47) were recruited by mail. a-Wave

range from those that evoked a small b-wave (<15 μV) to those that saturated the a-wave amplitude and slope. Also, stimulus–response functions to red (Wratten 29; λ > 610 nm) flashes were obtained. To isolate the rod function, the responses to phototopically matched red flashes were subtracted digitally from the responses to blue flashes.31 The unattenuated flash, measured with a detector (S350; United Detector Technology, Orlando, FL) placed at the position of the subject’s cornea, was 3.82 log μW/cm² per flash. Retinal illumination varies directly with pupillary diameter and the transmissivity of the ocular media and inversely with the square of the posterior nodal distance.6 The scotopic troland value25 of the stimulus was calculated, taking each subject’s pupillary diameter and the average axial length53 into account.50 Thus, the maximum intensity blue light produced about +3.6 log scot td sec retinal illumination in both infants and adults. If 1 scotopic troland produces ~8.5 isomerizations/rod/flash,34 the maximum intensity blue flash produced ~3,400 isomerizations per rod per flash.

All responses were differentially amplified (bandpass 1–1000 Hz; gain: 1000), displayed on an oscilloscope, digitized, and stored on disc for analysis later. An adjustable voltage window was used to reject records contaminated by artifacts. Two to 16 responses were averaged in each stimulus condition. The interstimulus interval ranged from 2 to 60 seconds and was selected so that subsequent b-wave amplitudes were not attenuated.

The rod photoresponse characteristics were calculated from the a-wave responses using the Hood and Birch24 formulation of the Lamb and Pugh25,35 model of the biochemical processes involved in the activation of phototransduction. A curve-fitting routine (MATLAB, fmins) was used to determine the best fitting values of S, a sensitivity parameter, and R_smp3, the saturated response amplitude, in

\[ R(t, I) = R_{\text{smp}}(1 - \exp[-0.5S(I(t-t_d)^2)]) \]

where I is the flash in isomerizations/rod/flash, and t_d is a brief delay. Fitting of the model was restricted to the leading edge of the a-wave response or to a maximum of 20 msec after stimulus onset. All three parameters were free to vary.

For the rod driven b-wave, which represents mainly the activity of the on-bipolar cells,26–36 the stimulus–response function

\[ V/V_{\text{max}} = I/(I+\sigma) \]

was fit to the b-wave amplitudes of each subject using an iterative procedure that minimized the mean square deviation of the data from the equation. In this Equation 2, V is the b-wave amplitude, V_{max} the saturated amplitude, I the stimulus in scot td sec, and σ the stimulus that evoked a half-maximum b-wave amplitude. Thus, 1/σ is a measure of sensitivity. The stimulus–response function was fit up to those higher flash intensities at which a-wave intrusion occurs.37

Subjects

The main data are from 71 normal subjects recruited for study of rod photoreceptor function. Healthy, term-born infants, ages 23 through 100 days (n = 47) were recruited by mail. a-Wave
data from seven were included in a previous report. All had been born within 7 days of their due date and were in good general health. Normal child and adult control subjects (ages 8–52 years, \( n = 25 \)) were also recruited; ERG data of 20 of these have been included as control data in a clinical report. No subject had a family history of eye or vision problems. Thorough ophthalmic examination disclosed no abnormalities. Written, informed consent was obtained from adult subjects and the parents of the infants and children. This study conformed to the tenets of the Declaration of Helsinki and was approved by the Children's Hospital Committee on Clinical Investigation.

The subjects described included few children. To obtain ERG data from children, we reviewed our clinical ERG database listing 1164 patients who had been referred for testing. We selected ERG data, obtained using the protocol described above, from the left eye of the 22 patients who were found by us to have perfectly normal eyes, including normal acuity for age, normal alignment and motility, normal pupillary responses, normal fundi, and little or no refractive error (spherical equivalents –2 to +4 diopters). Their ages were 6 months to 15.1 years. Vision and ocular features have remained normal 1 to 6 years (median, 3 years) after electroretinography. None of the 22 had a family history of eye disease or visual disorders.

**Growth Curve Analysis**

A logistic growth curve of the form

\[
y/y_{\text{max}} = \frac{\text{Age}_{\text{m}}}{\text{Age}_{\text{a}} + \text{Age}_{\text{m}}^n},
\]

where \( \text{Age}_{\text{m}} \) is defined as the age at which \( y \) is 50% of the adults’ value, \( y_{\text{max}} \); and the exponent, \( n \), indicating the steepness of the curve at \( \text{Age}_{\text{m}} \) was fit to each ERG parameter, \( S \), \( R_{\text{mp}3} \), \( V_{\text{max}} \), and \( 1/\sigma \) (expressed as percent of the adult mean). A least squares criterion was used to determine the values that minimized the deviation of the data from Equation 3. All parameters were free to vary. The b-wave response parameters, \( \sigma \) and \( V_{\text{max}} \), from an additional 71 normal subjects that we had previously studied were included in the growth curve analysis. Also analyzed were \( S \) and \( R_{\text{mp}3} \) values reported by Nusinowitz et al., in their Figure 2, for infants and adults. In this analysis, \( y_{\text{max}} \) was fixed at the adult values of \( S \) and \( R_{\text{mp}3} \) that were reported as means.
For comparison to the development of the ERG parameters, the logistic growth model was applied to the development of dark-adapted, rod-mediated visual sensitivity (the reciprocal of threshold). The psychophysical data from 169 dark-adapted, rod-mediated visual sensitivity (the reciprocal of threshold). These psychophysical data from 169 dark-adapted, rod-mediated visual sensitivity (the reciprocal of threshold) were gathered from 10-week-old infants (63–77 days; n = 27) and children and adults (n = 25). Consistent with a previous report, there is little change in the ERG parameters between age 8 and 52 years. The ERG parameters are plotted as a function of age in Figures 3 and 4. The parameters of the logistic growth model fit to the developmental increase in each parameter are listed in Table 1B.

If logistic growth is assumed to summarize the normal developmental increase in human rod photoreceptor sensitivity, the age at which S is half the adults’ mean value is 10.7 weeks (95% confidence interval [CI], 8.3–11.7 weeks) (Fig. 3A; Table 1B). Equation 3 accounts for 76% of the variation in S with age in normal subjects (n = 22). The age at which Rmp3 is half the adults’ value is 11.2 weeks (95% CI, 9.4–13.0 weeks). The shape of the growth curve, as indicated by the exponent, n (Table 1B), is similar for S and Rmp3. The values of S and Rmp3 at age 10 weeks, estimated by the growth curve analysis (Table 1B), agree well with the mean values at age 10 weeks (Table 1A), and values of ymax agree well with the mean observed values in adults (Table 1A). Inclusion of the data from the 22 patients with normal eyes in the analysis has little effect on the growth curves for S and Rmp3 (Figs. 3A, 3B; Table 1B). The Age50 values for the S and Rmp3 (Table 1B) are in reasonable agreement with those calculated for data reported by Nusinowitz et al. for which Age50 of Rmp3 at age 10 weeks, estimated by the growth curve analysis (Table 1B), agree well with the mean values at age 10 weeks (Table 1A), and values of ymax agree well with the mean observed values in adults (Table 1A). Inclusion of the data from the 22 patients with normal eyes in the analysis has little effect on the growth curves for S and Rmp3 (Figs. 3A, 3B; Table 1B). The Age50 values for the S and Rmp3 (Table 1B) are in reasonable agreement with those calculated for data reported by Nusinowitz et al. for which Age50 of 5 is 9.4 weeks (95% CI: 7.8–11 weeks) (r² = 0.39) and of Rmp3 is 12.5 weeks (95% CI: 9.4–15.6 weeks) (r² = 0.18). Thus, the Age50 values for the S and Rmp3 data reported by Nusinowitz et al. overlap one another and those for the subjects studied herein (Table 1B).

Assuming logistic growth describes the developmental increase in b-wave sensitivity, Age50 is 11.0 weeks (95% CI, 9.3–12.7 weeks) (Fig. 4A; Table 1B). The logistic growth curve fit to saturated b-wave amplitude, Vmax (Fig. 4B; Table 1B), reaches half of the adults’ mean value at 10.0 weeks (95% CI, 8.3–11.7 weeks). The values of ymax and Vmax estimated from the growth curve analysis at age 10 weeks are in reasonable agreement with those calculated for data reported by Nusinowitz et al. for which Age50 of Rmp3 at age 10 weeks, estimated by the growth curve analysis (Table 1B), agree well with the mean values at age 10 weeks (Table 1A), and values of ymax agree well with the mean observed values in adults (Table 1A). Inclusion of the data from the 22 patients with normal eyes in the analysis has little effect on the growth curves for S and Rmp3 (Figs. 3A, 3B; Table 1B). The Age50 values for the S and Rmp3 (Table 1B) are in reasonable agreement with those calculated for data reported by Nusinowitz et al. for which Age50 of 5 is 9.4 weeks (95% CI: 7.8–11 weeks) (r² = 0.39) and of Rmp3 is 12.5 weeks (95% CI: 9.4–15.6 weeks) (r² = 0.18). Thus, the Age50 values for the S and Rmp3 data reported by Nusinowitz et al. overlap one another and those for the subjects studied herein (Table 1B).

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agreement with the observed values; \( y_{\text{max}} \) and adult means are also in reasonable agreement (Tables 1A, 1B).

The mean dark-adapted visual threshold of 10-week-old infants is significantly higher (sensitivity lower) than that of adults at all eccentricities (Table 2A); the highest infant threshold is at 10° eccentric (parafoveal). In Figure 5, for each eccentricity, the sensitivities (left) and corresponding thresholds (right)\(^8,9,30\) are plotted as a function of age. Assuming logistic growth of peripheral visual sensitivity (Fig. 5A, left), the calculated value of \( \text{Age}_{50} \) is 10.7 weeks (95% CI, 8.9–12.5 weeks). See also Table 1B. The \( \text{dashed curve} \) is Eq. 3 fit to the \( \text{circles} \) (Fig. 5B, right) is 0.3 log unit above (or sensitivity is half) that of an adult’s threshold at 19.5 weeks (95% CI, 17.2–21.8 weeks). This is significantly older than \( \text{Age}_{50} \) for peripheral thresholds (Fig. 5A; Table 2B), and rod photoreceptor (Fig. 3A; Table 1B) or b-wave (Fig. 4A, Table 1B) sensitivity.

According to the logistic growth model, the parafoveal visual threshold (Fig. 5B, right) is 0.3 log unit above (or sensitivity is half) that of an adult’s threshold at 19.5 weeks (95% CI, 17.2–21.8 weeks). This is significantly older than \( \text{Age}_{50} \) for peripheral thresholds (Fig. 5A; Table 2B), and rod photoreceptor (Fig. 3A; Table 1B) or b-wave (Fig. 4A, Table 1B) sensitivity.
In Figure 5C (right), the dark-adapted, rod-mediated thresholds for detection of large, 10° diameter spots, presented 20° eccentric, are shown. According to the logistic growth curve, the age at which the threshold is 0.3 log unit above (or sensitivity is half) that of an adult’s, is 33.0 weeks (95% CI, 26.2–39.8 weeks).

**DISCUSSION**

The developmental courses of rod photoreceptor sensitivity, b-wave sensitivity, and peripheral rod-mediated visual sensitivity are statistically indistinguishable. Even though the ERG and psychophysical data were not gathered following a within-subject design, the 95% CIs overlap for the ages at which rod photoreceptor, b-wave, and peripheral visual sensitivity reach half the adult’s value (Fig. 6). Additionally, the shape of the summarizing growth curves, defined by the exponent $n$, is similar (Tables 1B, 2B). The developmental course for visual sensitivity mediated by parafoveal retina is relatively delayed, as is rod outer segment maturation in the parafoveal region.$^{10,14}$ Sensitivity measured with large (10° diameter) spots centered at the rod ring$^{14}$ is also relatively delayed. In infants, the large spots must test retina-containing rod outer segments in various states of maturation.$^{10,14}$

In developing rat retina, it has been possible to demonstrate that the developmental increase in rhodopsin content, rod photoreceptor, and b-wave sensitivity follow the same course. There is not broad overlap of the course of development of human rhodopsin and rod photoreceptor sensitivity (Fig. 6). As the large confidence interval for human rhodopsin indicates, the content of rhodopsin in human eyes is quite variable.$^{15}$ The variability is due not only to bleaching, but also likely depends on numbers of rods per retina and perhaps on long-term adaptations to ambient light.$^{15,22}$

The logistic growth curve has been fit empirically to the data herein. In other species, this model provides a satisfactory summary of developmental increases in rhodopsin (as shown, e.g., in Fig. 1) and other proteins involved in phototransduction processes as well as growth of outer segment length.$^{11,19,22}$ Furthermore, in developing rats, the amount of rhodopsin available for capture of light and the numbers of channels in the outer segment membranes available for closure by light are proportional to the rod photoreceptor response parameters, $S$ and $R_{mp}$.$^{11,15}$ In other words, during development, rod photoreceptor sensitivity is scaled by the amount of rhodopsin present, and the amount of rhodopsin present is proportional to the number of channels in the receptor membrane that are available for closure by light. As the present data for human development show, the rat b-wave response parameters, perhaps representing mainly the on-bipolar cells,$^{26,36}$ which take their input from the rods, have the same developmental course as the rod photoreceptor response parameters.$^{11}$

Immaturities of rod-mediated, human retinal function may depend on similar rod outer segment immaturities, although the anatomic data are sparse.$^{8–10,14}$ Infants’ higher visual thresholds at parafoveal than peripheral sites were reasoned to be consistent with shorter parafoveal rod outer segments.$^{8}$ The courses of maturation of the visual thresholds at parafoveal and peripheral sites appear to be a consequence of a later course of maturation of the parafoveal than peripheral rod outer segments.$^{9}$

Other results are also consistent with rods as the main determinant of 10-week-old infants’ lower scotopic visual sensitivity. Sensitivities derived from scotopic ERG and VEP responses to full-field stimuli were examined.$^{4}$ The differences between infants’ and adults’ rod (ERG a-wave), inner retinal (ERG b-wave), and VEP sensitivities were all the same,$^{3}$ and, in fact, similar to the infant–adult difference
subsequently found for peripheral visual sensitivity at age 10 weeks, namely approximately 0.5 log unit. Thus, it seemed likely that the determination of 10-week-old infants’ scotopic, dark-adapted visual sensitivity was primarily in the rods.

The present study evaluates sensitivity from early infancy through middle age. The concurrent courses of development of rod sensitivity, b-wave sensitivity, and peripheral visual sensitivity suggest that the maturation of the rod outer segments and quantum catch govern the developmental increase in dark-adapted scotopic visual sensitivity. Although recognizing the influence of receptive field immaturities on infants’ scotopic visual responses, the data herein as well as the results of analysis of the eigengrau in infants’ parafoveal and peripheral retina are evidence that rod outer segment immaturities, with consequent low probability of quantum capture, are critical determinants of dark-adapted infants’ low scotopic visual sensitivity.

**Figure 5.** For stimuli at 30°, 10° (parafoveal), and 20° eccentric in (A), (B), and (C), respectively, the dark-adapted visual sensitivity (left) or threshold (right) is shown as a function of age. On the left, the smooth curve is the logistic growth model fit to the data expressed as percent of adults’ mean sensitivity and displayed on log-linear coordinates. On the right, the data are plotted as log threshold, and the fitted growth curve is transformed for the log-log displays. In each panel, the square and error bars indicate the mean ± SD value in adults.


