Rod-Mediated Increment Threshold Functions in Infants

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PURPOSE. To obtain and analyze scotopic increment threshold functions to test the hypothesis that rod photoreceptor immaturity accounts for the elevation of infants' over controls' dark-adapted thresholds and elevation of parafoveal over peripheral thresholds in infants.

METHODS. Using a preferential looking method, thresholds for detection of 2", 50 msec, blue stimuli presented 10° (parafoveal) or 30° (peripheral) eccentric were measured in the dark and in the presence of steady red backgrounds. Ten 10-week-old infants and four control subjects (8–35 years) were tested. To evaluate pre- and postadaptation site determinants of threshold, a model of the increment threshold function was fit to the data, and the dark-adapted threshold (TD) and eigengrau (AO) were calculated. The values of TD and AO were compared between infants and controls and between parafoveal and peripheral eccentricities.

RESULTS. At both parafoveal and peripheral eccentricities, infants' values of TD and AO were significantly higher than those of controls. The loci of the coordinates (AO, TD) differed significantly between parafoveal and peripheral eccentricities. In every infant, the parafoveal value of TD was higher (by 0.3–0.6 log unit) and AO lower (by 0.2–0.5 log unit) than the peripheral value, whereas controls had no difference in TD and AO at the two eccentricities.

CONCLUSIONS. The results indicate that both receptor and postreceptor immaturities have a role in the elevation of infants' over controls' thresholds. In infants, rod photoreceptor immaturity before the site of adaptation accounts for elevation of parafoveal over peripheral thresholds. (Invest Ophthalmol Vis Sci. 2000;41:4347–4352)

The scotopic visual thresholds in young infants are significantly higher than in adults.1–2 For example, at age 10 weeks, dark-adapted thresholds in peripheral retina (30° eccentric) are approximately 0.5 log unit, and those in parafoveal retina (10° eccentric) are approximately 1.0 log unit above the average threshold in adult subjects.3,4 Rod outer segments are the last retinal structures to develop,5,6 and infants have short rod outer segments.7–9 This immaturity is greater in the parafoveal than the more peripheral retina.10–14 Short rod outer segments and low rhodopsin content,13 with consequent low probability of photon capture, are consistent with the elevation of the infants' visual threshold. Indeed, the developmental courses of peripheral dark-adapted threshold and rhodopsin content are statistically indistinguishable.16 However, postreceptor processes are not ruled out as determinants of infants' elevated dark-adapted thresholds.

Postreceptor immaturities have been identified and distinguished from receptoral (i.e., preadaptation site) immaturities by analysis of the effects of steady background lights on scotopic thresholds.1,17–19 Rod increment threshold functions obtained in studies of background adaptation have been used to test hypotheses about the receptoral and postreceptoral sites involved in development1,2,5,17,20 or disease.16,21–23 According to classical psychophysical theory, immaturity or disease in the rod photoreceptor before the site of adaptation reduces sensitivity equally for test and background stimuli. It is as though the stimuli were viewed through dark glasses.24 In this case, the increment threshold function displayed on log-log coordinates is shifted up and right along a diagonal (Fig. 1, left). A mathematical model18 specifies the increment threshold function with two parameters, TD, the calculated dark-adapted threshold, and AO, the eigengrau. Numerically, AO is the background that raises the threshold 0.3 log unit above the dark-adapted threshold and, in adults, approaches estimated values of intrinsic photoreceptor noise.25–27 The diagonal shift (Fig. 1, left) is produced by equal changes in TD and AO.18 According to classical theory, postreceptor immaturity, after the site of adaptation, causes only a vertical shift in the increment threshold function. The thresholds for the test stimulus in the dark and at every background are equally elevated.1,17,18 Only TD increases, whereas AO does not change (Fig. 1, right). The results of a previous study of rod increment threshold function development4 were consistent with a receptoral immaturity but could not rule out postreceptor immaturities.17

We studied background adaptation in 10-week-old infants and older control subjects. Following a within-subject design, scotopic increment threshold functions were obtained at parafoveal and peripheral retinal eccentricities. The model parameters TD and AO were analyzed for significant differences between infants and controls and between parafoveal and peripheral eccentricities.
METHODS

Stimuli

The test spots and the background field were rear projected onto a large screen (101 x 79 cm). Test stimuli were 50 msec, 2° diameter, blue (Wratten 47B, \( \lambda \approx 440 \) nm) spots presented 10° (parafoveal) or 30° (peripheral) from a central fixation stimulus. The fixation target was a red LED that flickered at 1 Hz and subtended 30-minute arc. The red (Wratten 29, \( \lambda \approx 610 \) nm) background field was 109° diameter and concentric with the fixation target. Calibrated neutral density filters controlled stimulus and background intensities.

Calculation of the retinal illuminance produced by the stimuli was based on luminance measurements made with a calibrated photodiode (UDT S-350; United Detector Technology, Orlando, FL) placed in the position of the subject’s eyes. Pupillary diameter was estimated by direct observation with an infrared viewer and comparison to the diameter of the cornea, which is 11 ± 0.5 mm (mean ± SD). Retinal illuminance varies directly with pupillary diameter and the transmissivity of the ocular media and inversely with the square of the posterior nodal distance. Similar to a previous report, infants’ average pupillary diameter was 5.4 ± 0.5 mm (mean ± SD) in the dark (no background condition) and remained approximately constant until background intensity exceeded 0 log scot td. The scotopic troland values of the stimulus and background were calculated, taking each subject’s pupillary diameter and the average axial length for age into account. Correction for light losses in the ocular media was applied.

Procedure

Thresholds were estimated using a two-alternative, forced-choice, preferential-looking method that incorporated a “fix and flash” procedure. The subject dark-adapted for 30 minutes. An adult held the infant 50 cm in front of the center of the screen. The subject viewed stimuli with both eyes. The infant’s gaze was attracted to a flickering, red LED fixation target at the center of the screen. A second adult watched the infant with an infrared viewer and reported when the infant was alert and looking at the fixation target. The fixation target was extinguished, and a test flash was presented. The observer reported stimulus location, right or left, based on the infant’s head and eye movements. The observer received feedback on every trial. In the procedure for the older subjects, the left or right position of the test flash was named.

Threshold was measured with a transformed up-down staircase that estimated the 70.7% correct point of the psychometric function. Testing continued until at least three alternations (median, 5; range, 3–6) were obtained. The average number of trials per staircase was 33 ± 6 (mean ± SD) for

\[ \text{FIGURE 1. Predictions of a model of the increment threshold function.} \]

Top: families of five increment threshold functions are shown in loglog plots. Curve 1 represents the most mature, and curve 5 the most immature condition. Two parameters, \( T_D \), and \( A_o \), specify the increment threshold functions. The horizontal asymptote for each curve is at \( T_D \). The oblique asymptote has slope +1.0. For each curve, the horizontal and oblique asymptotes intersect at \( A_o \). The predicted effect of a preadaptation site immaturity is to shift the curves up and right by equal amounts (top left). The predicted effect of a postadaptation site immaturity is to shift the curves up without horizontal translation (top right). Bottom: the loci of the coordinates (\( A_o, T_D \)) for preadaptation (left) and postadaptation site (right) immaturity are shown.
infants and 32 ± 5 for controls. The number of trials in each adaptation condition was similar.

Subjects were tested first in the dark (no background condition) and then while adapted to backgrounds that produced retinal illuminances of −4 to +1 log scot td. Half of the subjects were tested first at the parafoveal eccentricity and half at the peripheral eccentricity. Infants were tested in two to four sessions and controls in one session.

A model of the increment threshold function (Eq. 1)$^{17,18,35}$ was fit to the increment threshold data to minimize the sum of squared deviations from

$$\log T = \log T_D + \log \left[ \left( A_O + I \right) / A_O \right]$$  \hspace{1cm} (I)

where $T$ is the threshold at background intensity $I$, $T_D$ is the calculated dark-adapted threshold, and $A_O$ is the eigengrau, defined as the background intensity that elevates threshold 0.3 log unit above the dark-adapted level. Preadaptation site immaturities are postulated$^{17,18}$ to cause equal increases in both log $T_D$ and log $A_O$ (Fig. 1, left). An immaturity limited to a postreceptor site$^{18}$ increases only $T_D$ (Fig. 1, right).

The locus of the coordinates ($T_D$, $A_O$) was compared between infant and control subjects and between parafoveal and peripheral eccentricities. Preliminary inspection suggested the loci of infants’ parafoveal and peripheral coordinates differed. Parafoveal and peripheral data were compared using a parametric, second-order analysis, which tests for significant differences between the magnitudes and angles of two populations of vectors.$^{36}$

**Subjects**

A total of 13 infants, ages 64 to 71 days (median, 68 days) at the first session, participated. All infants were born within 10 days of term, were in good general health, and had normal eyes documented on thorough ophthalmic examination. Of these, 10 completed increment threshold functions at both eccentricities within 1 to 11 days (median, 3 days) of the first session. Their data are the basis of this report. The increment thresholds of the infants are compared with those of four previously reported$^{21}$ older control subjects (ages 8, 14, 21, and 35 years). The study conformed to tenets of the Declaration of Helsinki and was approved by the Children’s Hospital Committee on Clinical Investigation. Written, informed consent was obtained from the control subjects and parents of minor subjects.

**RESULTS**

Representative increment threshold functions of three infants and a control subject illustrate the fit of Eq. 1 to the data (Fig. 2). The parameters of the increment threshold functions fit to the data, $T_D$, the dark-adapted threshold, and $A_O$, the eigengrau, are shown in each panel. The observed and calculated values of the dark-adapted threshold are in good agreement. The three infants’ parafoveal (10° eccentric) increment thresh-
TABLE 1. Parameters of Increment Threshold Functions

<table>
<thead>
<tr>
<th>Subject No.</th>
<th>Age (1st Test)</th>
<th>( T_D ) (log scot td sec)</th>
<th>( A_D ) (log scot td)</th>
<th>( T_O ) (log scot td sec)</th>
<th>( A_O ) (log scot td)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Infants</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>71 days</td>
<td>-2.25</td>
<td>-1.91</td>
<td>-2.58</td>
<td>-1.47</td>
</tr>
<tr>
<td>2</td>
<td>69 days</td>
<td>-2.00</td>
<td>-1.69</td>
<td>-2.61</td>
<td>-1.17</td>
</tr>
<tr>
<td>3</td>
<td>70 days</td>
<td>-2.35</td>
<td>-2.14</td>
<td>-2.80</td>
<td>-1.61</td>
</tr>
<tr>
<td>4</td>
<td>71 days</td>
<td>-2.11</td>
<td>-2.80</td>
<td>-2.47</td>
<td>-2.43</td>
</tr>
<tr>
<td>5</td>
<td>71 days</td>
<td>-2.30</td>
<td>-1.48</td>
<td>-2.71</td>
<td>-1.86</td>
</tr>
<tr>
<td>6</td>
<td>69 days</td>
<td>-2.00</td>
<td>-2.78</td>
<td>-2.47</td>
<td>-2.43</td>
</tr>
<tr>
<td>7</td>
<td>70 days</td>
<td>-2.05</td>
<td>-2.53</td>
<td>-2.63</td>
<td>-1.72</td>
</tr>
<tr>
<td>8</td>
<td>66 days</td>
<td>-2.02</td>
<td>-1.53</td>
<td>-2.45</td>
<td>-1.06</td>
</tr>
<tr>
<td>9</td>
<td>68 days</td>
<td>-2.22</td>
<td>-1.87</td>
<td>-2.63</td>
<td>-1.53</td>
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<tr>
<td>10</td>
<td>64 days</td>
<td>-1.90</td>
<td>-1.40</td>
<td>-2.6</td>
<td>-0.98</td>
</tr>
<tr>
<td><strong>Controls</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
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<td>35 years</td>
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<td>-3.49</td>
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</tr>
<tr>
<td>3</td>
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<td>-2.46</td>
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<td>-2.28</td>
<td>-3.40</td>
<td>-2.31</td>
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* The \( r^2 \) values for the fit of Eq. 1 ranged from 0.97 to 0.99.

old functions are shifted up (\( T_D \) higher) and left (\( A_O \) lower) of their peripheral (30° eccentric) functions, as are those of the seven other infants. The parafoveal and peripheral increment threshold functions of the control subject are, as in all other control subjects, nearly superimposed. For controls, the values of \( T_D \) and \( A_O \) at the peripheral and parafoveal eccentricities do not differ. Table 1 summarizes \( T_D \) and \( A_O \) values for the 10 infants and 4 controls. Although the standard deviations of dark-adapted thresholds of infants at both the parafoveal (0.15 log unit) and peripheral (0.11 log unit) sites are significantly higher than those of the controls (0.03 and 0.04 log unit), they are similar to those of other 10-week-old infants (\( n = 27 \)) tested with the same stimuli (parafoveal, 0.16 log unit; and peripheral, 0.16 log unit).8,9

The parameters of the increment threshold functions in infants and controls are compared in Figure 3. The infants’ values of \( T_D \) and \( A_O \) are shifted up and to the right of those in the control subjects. Infants have higher dark-adapted thresholds (\( T_D \)) and higher eigengraus (\( A_O \)) than the controls at both the parafoveal and peripheral eccentricities. All the infants’ parafoveal points are above the diagonal line that represents the theoretical preadaptation site immaturity. All but one of the peripheral points are below the diagonal. The locus of the infants’ peripheral and parafoveal points8,9 differ significantly \((F = 6.80; \text{df}, 2,17; \text{P} < 0.01)\). The slope of the regression line through the peripheral points is 0.8 (95% confidence interval [CI], 0.68–0.92), which is close to the slope of 1.0 predicted by a preadaptation site immaturity. Relative to controls, the infants’ median shifts in \( T_D \) (0.86 log unit; range, 0.64–0.99 log unit) and \( A_O \) (1.06 log unit; range, 0.57–1.41 log unit) at the peripheral eccentricity are about equal. The slope of the regression line through the parafoveal points is steeper at 1.7 (95% CI, 1.45–1.99). Relative to the controls, the infants median shift in \( T_D \) (1.34 log unit; range, 1.08–1.48 log unit) is greater than the shift in \( A_O \) (0.71 log unit; range, 0.51–0.99 log unit) at the parafoveal eccentricity. Thus, the locus of parafoveal coordinates (\( A_O, T_D \)) lies between the pre- and postadaptation site predictions (Fig. 1). The elevation of the infants’ parafoveal threshold (\( T_D \)) above controls’ is greater, 1.34 log unit, than that at the peripheral eccentricity, 0.86 log unit. The half log unit difference in parafoveal and peripheral thresholds agrees with previous data.8,9

The parafoveal and peripheral values of \( T_D \) and \( A_O \) (Table 1) are compared in Figure 4. The regression line through the points with slope of \(-1.02 (\text{CI}, -0.80 to -1.22)\) is about
orthogonal to that shown in Figure 1. Every infant has a significantly lower (by 0.2–0.7 log unit; median, 0.4 log unit) parafoveal than peripheral eigengrau ($t = 5.96; df, 9; P < 0.01$). In every infant, the parafoveal, dark-adapted threshold is elevated over the peripheral threshold (median, 0.46 log unit; range, 0.3–0.7 log unit). This agrees with data from 20 additional 10-week-old infants.\(^8\)\(^9\) The coordinates of the controls’ points are close to (0,0). For individual control subjects, the parafoveal and peripheral dark-adapted thresholds differ by no more than 0.04 log unit and the eigengrau by no more than 0.07 log unit.

**Discussion**

Infants’ increment threshold functions are shifted up and right of those of controls. The higher $A_O$ values may be considered evidence that infants’ intrinsic noise is higher than controls’ at both eccentricities. The infants’ higher values of $T_D$ at both eccentricities confirm earlier reports.\(^2\)\(^5\)\(^8\)\(^9\)

According to the model summarized by Eq. 1, the shift of the infants’ peripheral increment threshold functions up and right from the controls (Fig. 3) indicates an immaturity at a preadaptation site, namely the rod outer segments. Both $T_D$ and $A_O$ (Fig. 3) are elevated by about 1 log unit and thus fall near the diagonal line (Fig. 1, bottom left) predicted by classical psychophysical theory.\(^17\)\(^19\)\(^24\)\(^35\)\(^57\) This perspective is consistent with the observation that the developmental courses of rhodopsin, rod photoreceptor sensitivity, and peripheral visual sensitivity are coincident.\(^15\)\(^16\) This is evidence that the rod photoreceptors control peripheral threshold development. As previously noted,\(^9\) anatomic measures show that infants’ peripheral rod outer segment lengths may be too long to be consistent with this conclusion. However, the rod outer segment length–threshold comparisons are frustrated by the paucity of anatomic data.

The shift of the infants’ parafoveal increment threshold function is also up and right from the controls (Fig. 3). However, the parafoveal points have a larger change in $T_D$ than $A_O$, and the regression line through the parafoveal points departs significantly from the diagonal line predicted by the preadaptation site model. In the context of the model (Fig. 1), this is evidence that outer segment immaturity alone is not sufficient to explain the elevation of infants’ parafoveal threshold over those in controls. Rather, immaturities at both pre- and postadaptation sites must cause the $(A_O, T_D)$ coordinates to fall between the pre- and postadaptation site predictions (Fig. 1). Interestingly, in a previous study\(^4\) of background adaptation in 4-, 10-, and 18-week-old infants and adults using large diameter ($10^\circ$) test stimuli that overlap the parafoveal region, the shift in $T_D$ exceeded that in $A_O$, and the regression line through $(A_O, T_D)$ coordinates fell between the pre- and postadaptation site predictions as do the present parafoveal results.\(^5\)

Thus, the comparison of infants’ and controls’ results, whether in the context of the model or by comparison to available rod outer segment length and rhodopsin data, indicates that rod immaturities alone are unlikely to account for the observed scotopic thresholds in 10-week-old infants. Studies of scotopic spatial summation in infants have led to the conclusion that postreceptoral processes must also be involved in determining infants’ scotopic visual thresholds.\(^3\)\(^7\)\(^28\)\(^39\) Studies of background adaptation in normal adults have demonstrated a postreceptoral component of adaptation.\(^9\) Thus, we suspect that postreceptoral as well as receptoral immaturities contribute to the elevation of infants’ dark-adapted thresholds.

The points representing the shift of infants’ parafoveal from peripheral increment threshold functions cluster about a diagonal (Fig. 4) that is orthogonal to the preadaptation site prediction (Fig. 1, bottom left). Remarkably, the infants’ parafoveal eigengrau is lower, not higher, than the peripheral eigengrau. There have been few studies of background adaptation in subjects with healthy, short rod outer segments. If rod outer segments are short due to disease, elevations of $A_O$ as well as $T_D$ are observed.\(^18\) The within-subject design of this study permits comparisons in infants with parafoveal rod outer segments that are shorter than their peripheral rod outer segments and in adults with equal parafoveal and peripheral rod outer segment lengths.\(^8\)\(^9\)\(^14\) If any within-subject difference in $A_O$ between the two retinal eccentricities were due to differences in rod outer segment length and the number of random events associated with the transduction cascade,\(^10\)\(^–\)\(^12\) the increment threshold functions mediated by the infants’ shorter parafoveal rod outer segments would have lower values of $A_O$ than their peripheral increment threshold functions.

Is the difference between infants’ parafoveal and peripheral eigengrau (0.4 log unit) reasonably explained by differences in rod outer segment lengths? The difference of 0.4 log unit (or 2.5 times) and the difference between infants’ parafoveal and peripheral dark-adapted threshold of 0.47 log unit (about 3 times) is plausible in view of available anatomic data. At the nearest available age, 5 days,\(^12\) parafoveal rod outer segments are about a quarter the length of the peripheral rod outer segments, predicting a difference of approximately 0.6 log unit. Thus, assuming continued growth of the rod outer segments,\(^13\) the 0.4 and 0.47 log unit differences observed at age 10 weeks are plausible.

The results herein lead to the conclusion that both receptoral and postreceptoral immaturities are needed to account for rod-mediated threshold elevations in infants over those in controls. Rod photoreceptor immaturity appears to be the main explanation for the elevation of the parafoveal over peripheral threshold in 10-week-old infants.\(^8\)\(^9\)

**Figure 4.** Differences between parafoveal and peripheral values of $T_D$ and $A_O$. For each subject, the differences between parafoveal and peripheral values of $T_D$ are plotted as a function of the differences in parafoveal and peripheral values of $A_O$. The regression line through the points has slope $-0.91$. The solid diagonal line, slope $+1.0$, is replotted from the preadaptation site prediction (Fig. 1, bottom left).
References