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Development of the Cone ERG in Infants

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Abstract

Purpose. To assess cone photoreceptor and cone-mediated postreceptoral retinal function in infants.

Methods. ERG responses to a 1.8-log unit range of long-wavelength flashes on a white, rod-saturating background were recorded in 4-week-old (n = 22) and 10-week-old (n = 28) infants and control adults and children, 8 to 40 years of age (n = 13). A model of the activation of cone phototransduction was fit to the a-waves. Sensitivity (S_{CONE}) and saturated-response amplitude (R_{CONE}) were calculated. The amplitude and implicit time of the b-wave were examined as a function of stimulus intensity. The cone photoresponse parameters were compared to the rod photoresponse parameters (S_{ROD} and R_{ROD}) in the same subjects.

Results. S_{CONE} and R_{CONE} in infants were significantly smaller than in the mature control subjects. The mean S_{CONE} was 64% and 68%, and the mean R_{CONE} was 63% and 72% in 4- and 10-week-olds, respectively. The mean rod photoresponse parameters were considerably less mature, as the mean S_{ROD} was 35% and 46%, and the mean R_{ROD} was 39% and 43% of mature values at 4 and 10 weeks. The b-wave stimulus—response functions in the 4- and 10-week-old infants did not show the photopic hill that was characteristic of the children's and adults' photopic b-waves.

Conclusions. Peripheral cone function is relatively more mature than rod function in young infants. The lack of a photopic hill is hypothesized to result from immaturity in the relative contributions of ON and OFF bipolar cell responses.

During infancy, significant increments in both rod- and cone-mediated visual sensitivity are observed. By age 6 months, rod outer segment length, rhodopsin content, and consequent quantum catch have increased so that rod-mediated visual sensitivity has become equal to that of adults.[1] The improvement in acuity during infancy[2-4] is attributable, in part, to development of the foveal cones.[5-7] However, the foveal cones comprise less than 1% of all the cones in the retina.[8] The development of peripheral cone and cone-mediated function has been studied very little.

In keeping with earlier differentiation of cones than rods[9] and earlier maturation of peripheral cone than rod outer segments,[10] the cone-mediated ERG is relatively more mature than the rod-mediated response in single-stimulus conditions.[11] The cone photoreceptor response and the cone-mediated b-wave response to a range of stimuli have yet to be studied in infants. The goal of the present study is to investigate the activation of peripheral cone photoresponse and to compare the relative maturity of the cone and previously studied rod photoresponse. In addition, the stimulus—response characteristics of the cone-mediated b-wave are described.

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Methods

Subjects

Term-born infants, aged 4 weeks (23-39 days; $n = 22$) and 10 weeks (63-78 days; $n = 28$) were recruited by mail. All had been born within 10 days of their due dates and were in good general health. Normal control adults and children (ages, 8-40 years; $n = 13$) were also studied. There is no significant variation in the scotopic a- and b-wave response parameters over this age range.[12] No subject had a family history of eye or vision problems. Thorough ophthalmic examination disclosed no abnormalities. Written, informed consent was obtained from control 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.

General ERG Procedure

The left pupil was dilated with 1% cyclopentolate, and the subject was dark-adapted for 30 minutes. Parents stayed with infants throughout the procedure. After 30 minutes of dark adaptation, in dim red light, 0.5% proparacaine was instilled, and a bipolar Burian-Allen electrode was placed on the left cornea. A ground electrode was placed on the skin over the left mastoid.

Responses were differentially amplified (band-pass, 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.[12]

Cone ERG

Responses were recorded in a 1.8-log unit range (+1.4 to +3.2 log phot td · s) of full-field, long-wavelength (red Wratten 29 $\lambda > 610$ nm) strobe stimuli (Novatron, Dallas, TX) presented on a steady, rod-saturating background (~ +3 log phot td · s). This approach has been used to isolate a cone response in adults with little rod intrusion.[13] The stimuli were incremented in 0.3-log unit steps. On records such as those shown in Figure 1, cone photoresponse parameters were derived from the a-wave. The trough-to-peak amplitude of the b-wave and implicit time of the b-wave were measured and examined as a function of log flash intensity.

The initial portion of the a-wave depends on the photocurrent in the rods and cones.[14-19] We took care to restrict fit of the transduction model to the early portion of the a-wave, to minimize postreceptoral contamination.[19-22] The cone photoresponse parameters were calculated by fit of a modification of the Lamb and Pugh[16,18] model of the activation of phototransduction to the first 11 ms of the a-wave. The modification incorporates a cascaded low pass exponential filter that models the capacitance of the cone membrane[13,23] by numerical convolution of the filter output with the delayed Gaussian function used to model the rod response.[16,18] The equation[13] used was

$$R(i,\ t) = \left(\left\{1 - \exp\left[-0.5 I \mathbf{S}_{\mathrm{CONE}}\left(t - t_d\right)^2\right]\right\} \mathbf{R}_{\mathrm{CONE}}\right) * exp\left(-t / \tau\right) \quad (1)$$

where $\mathbf{R_{CONE}}$ is the saturated response amplitude (microvolts), $\mathbf{S}_{\mathrm{CONE}}$ the gain parameter (phot td^{-1} · s^{-3}), t_d a brief delay (ms), and τ the time constant of the RC filter (in milliseconds). The symbol * represents the convolution operation. In preliminary studies, according to the procedure of Hood and Birch,[13] the effect of varying t_d (1-5 ms) and τ (1-5 ms) on the root mean square (RMS) error of model fits was examined. For the final calculation of $\mathbf{S_{CONE}}$ and $\mathbf{R_{CONE}}$, τ was fixed at 1.8 ms and t_d at 3 ms for both infants and control subjects. These values produced minimum RMS errors and are similar to those used previously.[13]

Rod ERG

Responses to full-field, short wavelength (blue Wratten 47B; λ <510 nm) strobe stimuli (Novatron) were recorded as previously described.[12] Stimuli ranged from those that evoked a small b-wave (<15μV) to those that saturated the a-wave amplitude and slope. The rod photoresponse parameters ($\mathbf{S_{ROD}}$ and $\mathbf{R_{ROD}}$) were calculated by fit of the Hood and Birch[14] formulation of the Lamb and Pugh[16,18] model to the a-waves. The equation used was

$$R(i,\ t) = \mathbf{R}_{\mathrm{ROD}}\left\{1 - \exp\left[-0.5\mathbf{S}_{\mathrm{ROD}}I\left(t - t_d\right)^2\right]\right\} \quad (2)$$

where $\mathbf{R_{ROD}}$ (in microvolts) is the saturated response amplitude, $\mathbf{S_{ROD}}$ (scot $td^{-1} \cdot s^{-3}$) is a sensitivity parameter, I the flash in scotopic troland-seconds, and t_d a brief delay (in milliseconds). The rod and rod-mediated responses of these subjects have been presented in a previous report.[12]

Calibrations

The unattenuated flash, measured with a detector (IL 1700; International Light, Newburyport, MA) placed at the position of the subject's cornea, was 2.7 log phot cd/m^2. Retinal illuminance varies directly with area of the pupil and the transmissivity of the ocular media and inversely with the square of the posterior nodal distance.[24] We used direct estimation of each infants' dilated pupil, the published estimates of the ocular media density,[25,26] and measures of the axial length of the infant eye[27-29] to make this calculation. In summary, equal-intensity stimuli produced approximately equal retinal illuminances in both infants and control subjects.[24,30,31] The maximum-intensity, long-wavelength light produced a retinal illuminance of approximately +3.2 log phot td · s. For the rod studies, the maximum intensity short wavelength light produced approximately +3.6 log scot td · s in both infants and control subjects.

Results

Sample cone ERG records for a subject from each age group are shown in Figure 1. In Figure 2, the first 20 ms are replotted with the model of the cone photoresponse (equation 1) fit to the first 11 ms of the a-wave. The model of activation represented by equation 1 describes reasonably well the leading edge of the a-wave in both infants and control subjects. RMS errors did not vary significantly with age. The model parameters ($\mathbf{S_{CONE}}$ and $\mathbf{R_{CONE}}$) for each subject are plotted in Figure 3, and the means and standard errors are summarized in Table 1. Variability in $\mathbf{S_{CONE}}$ and $\mathbf{R_{CONE}}$ did not differ significantly between control subjects and either group of infants. Both $\mathbf{S_{CONE}}$ and $\mathbf{R_{CONE}}$ varied significantly with age ($\mathbf{S_{CONE}}$: F = 11; *df*: 2,62; $P < 0.001$; $\mathbf{R_{CONE}}$:F = 10.6; $df = 2{,}62$; $P < 0.001$). For both $\mathbf{S_{CONE}}$ and $\mathbf{R_{CONE}}$, the 4- and 10-week old infants differed significantly from control subjects (Scheffé test; $P < 0.05$) but not from each other. In the control group, there was no significant change in either $\mathbf{S_{CONE}}$ or $\mathbf{R_{CONE}}$ with age.

The cone sensitivity parameter ($\mathbf{S_{CONE}}$) was 64% ± 4.2% (SE) of the control mean at 4 weeks and 68% ± 4.8%) of the control mean at 10 weeks (Fig. 4). The average saturated amplitude of the cone response ($\mathbf{R_{CONE}}$) was 63% ± 5.1% and 72% ± 4.8% of the control mean at 4 and 10 weeks. These results contrast with the rod sensitivity parameter ($\mathbf{S_{ROD}}$) which was only 35% ± 3.7% of the control mean at 4 weeks and 46% ± 3.3% at 10 weeks (Fig. 4). The rod-saturated amplitude ($\mathbf{R_{ROD}}$) was only 39% ± 3.2% and 43% ± 2.2% of control subjects at 4 and 10 weeks. For both classes of photoreceptors, the relative immaturity of the sensitivity and saturated amplitude parameters were similar. Within individuals, those with more pronounced immaturities of cone photoresponse parameters had more pronounced immaturities of rod photoresponse parameters. Specifically, $\mathbf{S_{CONE}}$ and $\mathbf{S_{ROD}}$ correlated significantly ($r = 0.56$; $df = 62$; $P < 0.01$), and $\mathbf{R_{CONE}}$ and $\mathbf{R}_{\mathrm{ROD}}$ correlated significantly ($r = 0.45$; $df = 62$; $P < 0.01$).

Cone-Mediated b-Wave Amplitudes and Implicit Times.

The mean amplitudes and implicit times of the cone-mediated b-wave responses are plotted as a function of stimulus intensity in Figure 5. The control subjects' b-wave amplitude initially increased with flash intensity, peaked at approximately +2.3 log photopic td · s, and then decreased with further increments of stimulus intensity. At the brightest flash, the mean b-wave amplitude in control subjects was approximately half the peak amplitude. This “photopic hill” is a characteristic of the mature photopic b-wave stimulus response function.[32-37] A photopic hill was not seen in the 4- or 10-week old infants (Fig. 5) although the infants and control subjects had responses over the same range of stimuli and had maximum b-wave amplitudes that were similar. A simple shift by an amount proportional to the reduction in $\mathbf{S}_{\mathbf{CONE}}$ (~0.3 log unit) does not superimpose the functions. Analysis of variance showed significant main effects of age (F = 8.48, *df* = 2,8; *P* < 0.01) and stimulus intensity (F = 35.65, *df* = 6,8; *P* < 0.01), as well as significant interaction (F = 3.61, *df* = 12,414; *P* < 0.01).

The average implicit time of the b-wave did not change systematically with intensity at any age (Fig. 5). The average b-wave implicit times in 4- and 10-week old infants were similar (4 weeks, mean = 36 ± 3 ms [SD]; 10 weeks, mean = 38 ± 4 ms). The average b-wave implicit time in the control subjects (mean = 30 ± 3 ms) was significantly shorter.

Discussion

Peripheral cone function, as summarized by $\mathbf{S}_{\mathbf{CONE}}$ and $\mathbf{R}_{\mathbf{CONE}}$, underwent slight but statistically significant developmental increases after term but was relatively more mature than rod function, as indicated by the corresponding parameters $\mathbf{S}_{\mathbf{ROD}}$and $\mathbf{R}_{\mathbf{ROD}}$ (Fig. 4, Table 1). The sparse anatomic data also show relatively greater maturity of peripheral cones than rod outer segment lengths. For instance, at age 1 week, peripheral cone outer segment length is 91% and rod outer segment length is 42% of those in the adult.[10] The early maturity of peripheral cones sharply contrasts with the protracted course of development of the foveal cones.[5,6,10] For both classes of photoreceptors, at each age, the relative immaturity of the sensitivity ($\mathbf{S}_{\mathbf{CONE}}$ and $\mathbf{S}_{\mathbf{ROD}}$) and saturated amplitude ($\mathbf{R}_{\mathbf{CONE}}$ and $\mathbf{R}_{\mathbf{ROD}}$) parameters were similar. This suggests not only that peripheral cone outer segments are shorter in infants than in adults, but that sensitivity, which depends on the molecular mobilities of the transduction cascade proteins in the outer segment disc membrane up to channel closure, is proportional to outer segment length. It may also be taken as evidence that in infants' cones, the molecular composition of the disc membrane and the disc-to-channel relation are the same as in adults. The same can be said of the immature rod photoreceptors.[12]

The absence of a photopic hill in the infant subjects' b-wave stimulus/response function is an unexpected finding. The shape of the photopic b-wave stimulus—response functions of infants and control subjects differed (Fig. 5). In contrast, the scotopic b-wave stimulus—response function had the same shape in infants and control subjects.[12] The cone pathway includes both ON and OFF bipolar cells that make contributions to the b-wave. A decrease in the positive amplitude of the ON bipolar cell response and a delay in the peak of the OFF response with increasing stimulus intensity is thought to account for the photopic hill in the cone-mediated ERG.[36] Possibly, the shape of the infants' b-wave stimulus response function (Fig. 5) resulted from immaturities in the relative strength and/or timing of these ON and OFF bipolar cell contributions to the b-wave.

In summary, the present results are evidence of slight, but statistically significant, immaturities of the cone photoresponse in early infancy. The infants' b-wave stimulus—response function lacked a photopic hill. These data provide provisional norms against which the photopic ERG responses in young patients may be compared.

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FIGURE 1.
Sample cone-mediated ERG records in representative 4- and 10-week-old infants and a control subject. Responses to a 1.8-log unit range of long-wavelength (red) flashes presented on a steady, white, rod-saturating background are shown. The peak of the b-wave used to measure implicit time is indicated by the *tick marks*.

FIGURE 2.

The first 40 ms of the ERG records are plotted for representative 4- and 10-week-old infants and control subjects. *Dashed lines*: equation 1 fit to these records. $\mathbf{S_{CONE}}$ and $\mathbf{R_{CONE}}$ are shown for each subject.

FIGURE 3.
The activation parameters (**S_{CONE}** and **R_{CONE}**) for each subject are shown. *Horizontal lines*: means for each group.

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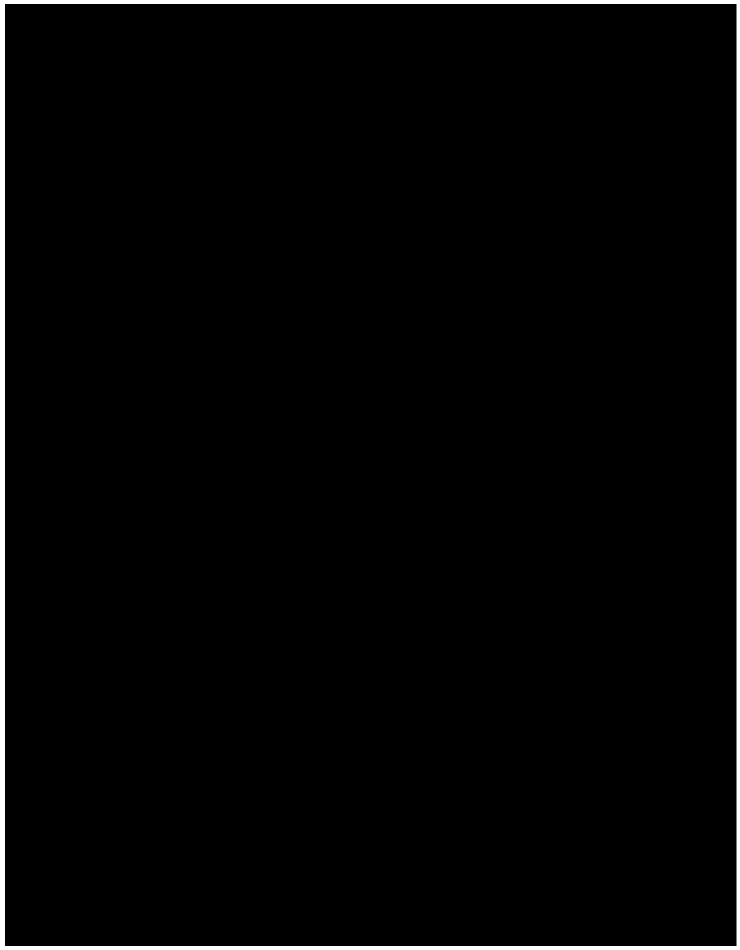

FIGURE 4.
The mean ± SEM sensitivity (**S_{ROD}**, **S_{CONE}**) and saturated amplitude (**R_{ROD}**, **R_{CONE}**) parameters in infants compared with control subjects. For each subject, the sensitivity and saturated amplitude was expressed as a percentage of the mean for control subjects.

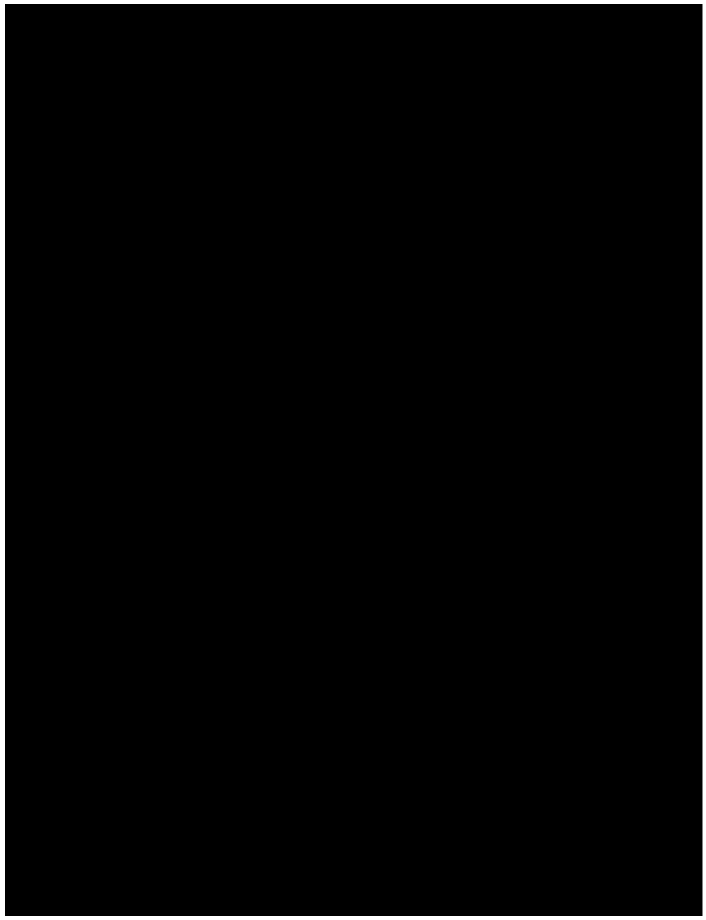

FIGURE 5.
Mean ± SEM b-wave amplitude and implicit time in 4- and 10-week-olds and control subjects. In control subjects, the peak of the photopic hill was at +2.3 log phot *td* · s. The infants' stimulus—response functions showed no photopic hill.

TABLE 1

Summary of Activation Parameters

Age	Cones		Rods	
	S_{CONE}(phot td^{-1} s^{-3})	R_{CONE} (μV)	S_{ROD}(scot td^{-1} s^{-3})	R_{ROD} (μV)
4 weeks	52 (3.5)	60 (4.9)	32 (3.3)	152 (12.4)
10 weeks	56 (3.9)	69 (4.6)	41 (3.0)	167 (8.7)
Control	81 (5.5)	96 (4.4)	90 (2.9)	389 (21.3)

Data are expressed as the mean ± SEM.