Development of Electroretinogram and Rod Phototransduction Response in Human Infants

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Purpose. To describe and analyze developmental change from birth through maturity of human electroretinogram (ERG) response, especially in terms of rod phototransduction as represented in the ERG a-wave.

Methods. Electroretinograms were recorded from 16 human infants from 5 to 270 days of age, two children 1.9 and 3.4 years of age, and 13 older subjects between 10 and 43 years of age. A range of full-field, white-light flashes up to intensities sufficient to saturate a-wave and b-wave amplitudes and a-wave rate-of-rise was used. The a-wave leading edge, the a-wave and b-wave amplitudes, and the b/a-wave ratio at maximum intensity were analyzed using a model of the activation steps of the G-protein phototransduction cascade. This model, applied to a-waves, provides three parameters interpretable in terms of rod phototransduction: \(a_{\text{max}}\) (a-wave maximum amplitude, proportional to circulating dark current), \(A'\) (estimated constant of transduction amplification), and \(\tau'_{\text{ef}}\) (sum of brief delays associated with the cascade steps).

Results. Both \(a_{\text{max}}\) and \(b_{\text{max}}\) (maximum b-wave amplitude) increased rapidly from birth. \(b_{\text{max}}\) reached apparently mature values by approximately 6 months, but \(a_{\text{max}}\), and thus \((b/a)_{\text{max}}\) (b/a ratio at maximum intensity), did not reach mature values until sometime after the third or fourth year. Similarly, \(A'\) was immature at birth at approximately 25% to 50% of adult levels at intensities below rate-of-rise saturation. For the youngest infants, rate-of-rise saturation appeared to occur at lower effective isomerizations per rod compared to that of the adult. Following a time course similar to that of \(a_{\text{max}}\), full maturity for \(A'\) probably was not reached before 5 years of age.

Conclusions. Results from the a-wave analysis are consistent with immaturities in the rod photoreceptors early in life. The difference from those of the adult may be explained by lower neonatal concentrations in one or more of the transduction substrates, decreased outer segment length, and, possibly, decreased density of some membrane proteins mediating the cationic dark current. Early adultlike b-wave amplitudes suggest early maturity for the inner retinal elements (rod bipolar and Müller cells) underlying b-wave response, compared to the photoreceptors. Invest Ophthalmol Vis Sci. 1995;36:1588-1602.

Retinal potentials in the form of the electroretinogram (ERG) have long been recorded in human infants.\(^1\)\(^-\)\(^3\) The human ERG waveform is recordable at term birth and undergoes developmental change during at least the first year of life. At birth, the waveform is reported to be immature in amplitude and timing for the major a-wave and b-wave features.\(^4\)\(^,\)\(^5\) Prenatally, cone outer segments appear and begin to develop before rod outer segments appear,\(^6\) whereas at term birth, rod outer segments have developed sufficiently\(^7\) that they may be represented in the white flash ERG response, although the relative contribution of rods and cones may not be adultlike.\(^8\)\(^,\)\(^9\) Waveform amplitudes and measures of b-wave sensitivity subsequently develop toward adult values for at least 6 months after birth,\(^10\)\(^,\)\(^11\) possibly longer. The nature and timing of photoreceptor functional development, especially at the level of biochemical mechanisms, has remained largely unknown.

The a-wave of the ERG originates in biochemical events related to photoreceptor transduction. Extra-
cellular ionic currents generated in the photoreceptors, flowing through ocular and periorcular tissues, are measurable at the corneal surface by means of a contact lens electrode. Analysis of the ERG a-wave by Hood and Birch and others, recorded under dark-adapted conditions that favor rods, shows the time course of the a-wave rising phase is consistent with an origin in the summed photocurrent response of individual rods.

An analytical model of the activation steps of the phototransduction G-protein cascade proposed by Lamb and Pugh has been applied to interpretation of ERG a-wave response. The analysis by Breton et al. applied to adult rod a-waves, provides parameters interpretable in terms of total rod dark current \( (a_{\text{max}}) \), a constant of transduction amplification \( (A) \), and a brief delay associated with cascade molecular interactions \( (t'_{\text{eff}}) \).

The ERG a-wave provides an opportunity for the study of photoreceptor development. We report here a biochemically based analysis, similar to Breton et al., applied to a-waves recorded from 16 human infants younger than 1 year of age, two children 1.9 and 3.4 years of age, and for comparison with the younger subjects, 13 older subjects ranging in age from 10 to 43 years. These data are unique for infants and younger subjects concerning ERG response series up to very high stimulus intensities, where the leading edge of the a-wave may be studied with a minimum of b-wave interference and analysis of transduction amplification and kinetics may be carried out. Such analysis may be expected to yield information on the development of the photoreceptors and the phototransduction mechanism.

**METHODS**

**Subjects**

Full-field ERG data were recorded from 16 infants younger than 1 year of age (age range, 5 to 270 days) and two children 1.9 and 3.4 years of age (see Table 1, INF 1 to 18). All patients in this group were referred for clinical evaluation, and the ERG data were recorded as part of a diagnostic workup. The clinical nature of the testing in infants and children sometimes precluded recording the full range of stimulus intensities. On the basis of a complete ophthalmologic examination consisting of patient and family history, external examination, indirect ophthalmoscopy and other clinically indicated specialized testing, and a recordable ERG, all 18 patients were thought to have visual problems not related to the retina and, therefore, were assumed to have normal retinal function (see listing of diagnosis in Table 1). Additional ERG results are included in Table 1 as subjects A1 to A13 (age range, 10 to 43 years). Most subjects in this category were recruited as normal volunteers. Some subjects were selected retrospectively from our patient population because retinal function in both eyes or in the eye selected for study was deemed normal after ophthalmologic evaluation. Informed consent was obtained from all subjects or their legal guardians after an explanation of the procedure and its risks and possible benefits. For some subjects, data recorded during routine diagnostic examination were used retrospectively. Complete anonymity was maintained for all subjects. The tenets of the Declaration of Helsinki were followed. Data were recorded at the Scheie Eye Institute and The Children’s Hospital of Philadelphia.

**Electroretinogram Recording System**

The ERG-recording system was an LKC Systems (Gaithersburg, MD) EPIC-1000 Instrument, consisting of a ganzfeld for full-field stimulus presentation, two xenon flash units (for low- and high-intensity ranges), an interface for computer and stimulators, and recording amplifiers. The low-intensity flash unit (Grass Photostimulator; Grass Instruments, Quincy, MA) was used for single flash and repetitive stimulation (20-μsec duration), whereas the high-intensity unit (Vivitar Photoflash; Vivitar, Santa Monica, CA) was used only for single flashes (1.5-msec duration) separated by intervals (minimum, 15 second recycle time). Stimulus flashes were delivered through an aperture into a ganzfeld sphere 40 cm in diameter with an interior surface coated with a highly reflective matte white paint. Infants were tested supine in a nursery basinette with the ganzfeld viewing port positioned horizontally over the head. A small inspection port was cut in the back surface of the ganzfeld to allow viewing of the infant during testing. Toddlers were tested either supine on an examination table or sitting up and looking through the viewing port in its usual upright position. For toddlers and older patients, a chin and headrest were provided to maintain head position, and a dim red light-emitting diode at the equator opposite the viewing port was used to maintain fixation.

A 10-second minimum time between flashes was used at intensities lower than the maximum intensity of the Grass Stimulator (2.4 cd-sec/m²). Progressively longer interstimulus intervals were used for higher intensity flashes to maintain dark adaptation (as long as 2 minutes between flashes of 600 cd-sec/m²). Repeat flashes at higher intensity were avoided. In general, the total number of single flashes approached the minimum required to cover the intensity range. On-line signal averaging for single flashes was not
TABLE 1. Infant and Adult Parameter Values

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age*</th>
<th>a_max</th>
<th>b_max</th>
<th>(b/a)_max</th>
<th>A' (avg)</th>
<th>K'</th>
<th>Diagnosis After ERG and Other Testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>INF1</td>
<td>5 days</td>
<td>81 (109)</td>
<td>234</td>
<td>2.89</td>
<td>3.62 (2.04)</td>
<td>14.7</td>
<td>Congenital syphilis; no pigmentary retinopathy</td>
</tr>
<tr>
<td>INF2</td>
<td>8</td>
<td>110 (183)</td>
<td>316</td>
<td>2.87</td>
<td>3.58 (1.36)</td>
<td>14.7</td>
<td>Congenital syphilis; no pigmentary retinopathy</td>
</tr>
<tr>
<td>INF3</td>
<td>16</td>
<td>131 (165)</td>
<td>285</td>
<td>2.18</td>
<td>1.87 (1.78)</td>
<td>14.5</td>
<td>Microphthalmia OS; nl OD</td>
</tr>
<tr>
<td>INF4</td>
<td>46</td>
<td>252</td>
<td>592</td>
<td>2.02</td>
<td>1.09</td>
<td>14.0</td>
<td>Hepatitis; poor fixation; normal retina</td>
</tr>
<tr>
<td>INF5</td>
<td>57</td>
<td>305</td>
<td>686</td>
<td>2.25</td>
<td>0.61</td>
<td>13.8</td>
<td>Congenital glaucoma; normal retina</td>
</tr>
<tr>
<td>INF6</td>
<td>63</td>
<td>312</td>
<td>419</td>
<td>1.34</td>
<td>1.34</td>
<td>13.7</td>
<td>Cephalohematoma; poor fixation; normal retina</td>
</tr>
<tr>
<td>INF7</td>
<td>76</td>
<td>334</td>
<td>566</td>
<td>1.69</td>
<td>1.48</td>
<td>13.6</td>
<td>Optic nerve hypoplasia; normal retina</td>
</tr>
<tr>
<td>INF8</td>
<td>104</td>
<td>332</td>
<td>604</td>
<td>1.81</td>
<td>1.49</td>
<td>13.1</td>
<td>Central visual impairment; normal retina</td>
</tr>
<tr>
<td>INF9</td>
<td>127</td>
<td>169</td>
<td>410</td>
<td>2.42</td>
<td>2.48</td>
<td>12.9</td>
<td>Oscillopia; normal retina</td>
</tr>
<tr>
<td>INF10</td>
<td>128</td>
<td>454</td>
<td>644</td>
<td>1.42</td>
<td>1.79</td>
<td>12.9</td>
<td>Nystagmus; normal retina</td>
</tr>
<tr>
<td>INF11</td>
<td>131</td>
<td>253</td>
<td>537</td>
<td>2.12</td>
<td>1.87</td>
<td>12.8</td>
<td>Crouzon’s; normal retina</td>
</tr>
<tr>
<td>INF12</td>
<td>148</td>
<td>254</td>
<td>443</td>
<td>1.74</td>
<td>2.06</td>
<td>12.8</td>
<td>Cleft palate; normal retina</td>
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<tr>
<td>INF13</td>
<td>142</td>
<td>215</td>
<td>351</td>
<td>1.63</td>
<td>—</td>
<td>12.7</td>
<td>Hypoplastic ON; normal retina</td>
</tr>
<tr>
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<td>205</td>
<td>356</td>
<td>722</td>
<td>2.03</td>
<td>3.08</td>
<td>12.1</td>
<td>Cerebral palsy; normal retina</td>
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<tr>
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<td>210</td>
<td>331</td>
<td>514</td>
<td>1.55</td>
<td>2.18</td>
<td>12.0</td>
<td>Optic nerve hypoplasia; normal retina</td>
</tr>
<tr>
<td>INF16</td>
<td>270</td>
<td>424</td>
<td>892</td>
<td>2.1</td>
<td>5.23</td>
<td>11.5</td>
<td>Nystagmus; normal retina</td>
</tr>
<tr>
<td>INF17</td>
<td>1.9 years</td>
<td>291</td>
<td>565</td>
<td>1.94</td>
<td>2.87</td>
<td>9.7</td>
<td>Family history retinitis pigmentosa; normal retina</td>
</tr>
<tr>
<td>INF18</td>
<td>3.4</td>
<td>497</td>
<td>575</td>
<td>1.16</td>
<td>1.04</td>
<td>9.1</td>
<td>Central visual impairment; normal retina</td>
</tr>
<tr>
<td>A1</td>
<td>10.2</td>
<td>598</td>
<td>704</td>
<td>1.18</td>
<td>5.44</td>
<td>8.6</td>
<td>Normal subject</td>
</tr>
<tr>
<td>A2</td>
<td>12</td>
<td>602</td>
<td>694</td>
<td>1.15</td>
<td>5.57</td>
<td>8.6</td>
<td>Normal subject</td>
</tr>
<tr>
<td>A3</td>
<td>14</td>
<td>606</td>
<td>888</td>
<td>1.47</td>
<td>4.01</td>
<td>8.6</td>
<td>Family history retinitis pigmentosa; normal retina</td>
</tr>
<tr>
<td>A4</td>
<td>15</td>
<td>527</td>
<td>731</td>
<td>1.39</td>
<td>—</td>
<td>8.6</td>
<td>Optic nerve tumor OS; normal OD</td>
</tr>
<tr>
<td>A5</td>
<td>17</td>
<td>546</td>
<td>576</td>
<td>1.05</td>
<td>6.10</td>
<td>8.6</td>
<td>Normal subject</td>
</tr>
<tr>
<td>A6</td>
<td>20</td>
<td>497</td>
<td>546</td>
<td>1.10</td>
<td>8.33</td>
<td>8.6</td>
<td>Family history retinitis pigmentosa; normal retina</td>
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<tr>
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<td>20</td>
<td>572</td>
<td>771</td>
<td>1.35</td>
<td>5.77</td>
<td>8.6</td>
<td>Normal subject</td>
</tr>
<tr>
<td>A8</td>
<td>22</td>
<td>530</td>
<td>740</td>
<td>1.40</td>
<td>5.30</td>
<td>8.6</td>
<td>Normal subject</td>
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<tr>
<td>A9</td>
<td>23</td>
<td>497</td>
<td>452</td>
<td>0.91</td>
<td>6.01</td>
<td>8.6</td>
<td>Normal subject</td>
</tr>
<tr>
<td>A10</td>
<td>26</td>
<td>428</td>
<td>574</td>
<td>1.34</td>
<td>7.10</td>
<td>8.6</td>
<td>Normal subject</td>
</tr>
<tr>
<td>A11</td>
<td>28</td>
<td>619</td>
<td>752</td>
<td>1.21</td>
<td>5.12</td>
<td>8.6</td>
<td>Normal subject</td>
</tr>
<tr>
<td>A12</td>
<td>29</td>
<td>488</td>
<td>630</td>
<td>1.29</td>
<td>6.20</td>
<td>8.6</td>
<td>Normal subject</td>
</tr>
<tr>
<td>A13</td>
<td>43</td>
<td>337</td>
<td>483</td>
<td>1.43</td>
<td>7.30</td>
<td>8.6</td>
<td>Normal subject</td>
</tr>
</tbody>
</table>

* Age for patients INF1 to INF16 is listed in days, and for the remaining patients it is listed in years.

The values listed for \( a_{\text{max}} \) and \( b_{\text{max}} \) are maximum \( a \)-wave and \( b \)-wave amplitudes, respectively, in \( \mu \text{V} \). The \( h_{\text{max}}/a_{\text{max}} \) or \( (b/a)_{\text{max}} \) ratio provides a measure of relative change in photoreceptor \( (a_{\text{max}}) \) compared with inner retina potentials \( (h_{\text{max}}) \). Values for \( A' \) are corrected for the increased retinal illumination that results from smaller infant eyes compared with adult eyes by applying a size-corrected value of \( K'(K') \). Thus, \( K' \) is the number of isomerizations per size-corrected scotopic troland. Numbers in parentheses for INF1 to INF3 are for upper bound estimates of \( a_{\text{max}} \). Derivation of parameter values is explained in the text. The last column lists each infant’s presenting problem and final diagnosis, if different.

used. Off-line averaging of single-flash responses taken simultaneously from the right and left eyes was performed to reduce noise in data from normal volunteers. For some subjects, independent flashes in the same eye were averaged when repeat data were available. The number of responses averaged off-line, when performed, ranged from two to four responses.

Flash intensity was varied over a range of approximately 6 log units by means of the two flash units and neutral-density filters, available in steps as small as 0.2 log units. Filter attenuation and flash intensity in the ganzfeld were calibrated with an EG&G DR2550 photometer (Gamma Scientific, San Diego, CA). The maximum intensity flash corresponded to 1553 cd-sec/m² in terms of luminance at the ganzfeld surface or 141,000 scot td s in terms of scotopic retinal illumination. Pupil size was limited to a 7-mm diameter by the aperture of the gold laminate of the recording contact lens. Dilation to more than 7 mm was confirmed before recording. The scotopic troland value converts flash luminance to retinal illumination adjusted only for pupil size and the difference in cone versus rod spectral sensitivity. A complete ganzfeld and dual stimulators (Grass and Vivitar) were available at each location. Each instrument and its filters were independently calibrated. All data taken above approximately 300 scot td s (the majority of data analyzed in this study) were recorded with the Vivitar flash unit. The time interval between flashes was controlled closely for the upper two log units of intensity to ensure complete recovery of \( a \)-wave response amplitude.

A personal computer compatible with Tecmar LabTender (Tecmar, Solon, OH) IO-board was used for control of the visual stimulator and for analog-to-digital conversion. The recording amplifiers were AC.
ERG and Rod Phototransduction Response in Infants

FIGURE 1. Jet contact lens electrode fitted with custom plastic tube speculum.

coupled and low-pass filtered (two-pole Butterworth). Filter settings were low-frequency cutoff at 0.3 Hz and high-frequency cutoff at 1500 Hz. Some data were taken with a high-frequency cutoff of 500 Hz. The electrical signals were digitized by an 8-bit or a 12-bit (more recent data) A/D converter, with sampling at 0.5-msec intervals and were stored for further processing. A prestimulus baseline of 20 msec was recorded for all subjects.

Procedure

Full-field ERGs were recorded from infants, children, and adults without general anesthesia or sedation in a room completely darkened except for a dim red light illuminating the keyboard. Each subject was dark adapted for at least 30 minutes, and recordings were made from both eyes simultaneously for most subjects. Neosynephrine (10%) and tropicamide (1%) drops were used to dilate the pupil. Topical anesthetic was instilled on the cornea, and 2.5% hydroxypropyl methylcellulose gel was used on the inner contact lens surface to protect the cornea. The recording lens was a Jet (Universo SA, Switzerland) unipolar disposable type with an annular gold laminate on the inner surface for electrical contact. A custom-made plastic tubular speculum was press-fitted to the anterior surface of the Jet electrode for use with infants (see Fig. 1). This tube speculum allowed easy manipulation of the electrode for insertion into the eye and prevented lid closure during testing. Pupil position could be monitored using red light illumination within the ganzfeld by looking through the long axis of the tube. The reference electrode was placed on the temple, and a ground electrode was placed on the forehead.

Basis of A-wave Analysis

The adult ERG waveform to a flash of moderate intensity has two major features, a negative going a-wave followed in time by a positive going b-wave (see Fig. 2, 10-year-old subject). These features result from the addition of opposite polarity components underlying the a-wave and b-wave. Under scotopic conditions, the a-wave component represents primarily the summed rod photocurrent response, whereas the b-wave is generated at the level of bipolar cells in the inner retina.

The rod photocurrent response underlying the a-wave originates as a decrease in the circulating rod dark current in response to light. The dark current is

FIGURE 2. Electroretinographic amplitude growth with age. Electroretinographic response over a range of intensities is shown for four subjects 46 days (INF4), 104 days (INF8), 205 days (INF14), and 10 years (A1) of age. Across waveform sets, the lowest and highest rate-of-rise waveforms are in response to the same intensity flash (incident at the cornea), except that the highest flash for the 104-day-old is 0.6 log units less intense than for the other three subjects. A-wave amplitude for the three infants is substantially lower than for adults, as represented by the 10-year-old (A1). B-wave amplitude, on the other hand, is adultlike by at least 205 days (INF14) of age. The maximum a-wave response indicated for this infant (396 μV) occurs for a less than maximum intensity flash and is different from the value for the highest intensity flash used for estimating A' and reported in Table 1 (356 μV).
carried by sodium (Na\(^+\)), potassium (K\(^+\)), and calcium (Ca\(^{2+}\)) cations between the rod inner and outer segments in the dark-adapted state. Because it represents a shutting off of current flow, the maximum amplitude of the photocurrent response is limited by the magnitude of dark or circulating current available.

The current analysis and interpretation of a-waves of infants and children in terms of the G-protein phototransduction cascade is based on the quantitative model of Lamb and Pugh,\(^\text{20}\) as adapted to the study of human adult a-waves by Breton et al\(^\text{19}\) and similar to the one by Hood and Birch.\(^\text{21}\) The transduction analysis involves fitting an analytic expression of the time course for rod G-protein cascade activation to a-waves.\(^\text{19,20}\) The activation rates for the first three cascade steps (Rh*, G*, and PDE* production) can be combined and approximated by a delayed ramp function.\(^\text{20}\) This approximation allows a simple solution for the differential equations describing change in cyclic guanosine 3,5'-monophosphate (cGMP) cytoplasmic concentration and cGMP-gated channel closure:

\[
F(t) = \exp[-(1/2)\phi A(t - \eta_{\text{eff}})^2], \quad t > \eta_{\text{eff}} \quad (1)
\]

where \(F(t)\) is the cGMP-activated current expressed as a fraction of its dark value, \(\phi\) is the number of isomerizations per rod delivered by the flash at \(t = 0\), \(\eta_{\text{eff}}\) is a brief delay, and \(A\) is an "amplification constant" expressible in terms of biochemical parameters. When applied to the a-wave, \(F(t)\) is equivalent to the normalized a-waves transformed to go from 1.0 to 0.0, as is the convention for photocurrent:

\[
F(t) = 1 - a(t)/a_{\text{max}} \quad (2)
\]

where \(a(t)\) is a-wave amplitude for each response and \(a_{\text{max}}\) is the maximum a-wave amplitude for the highest intensity response. For isolated rods, the delay, \(\eta_{\text{eff}}\), is the sum of brief delays associated with each cascade step. For ERG recordings, this term includes contributions from the recording apparatus and the capacitance of the (nonvoltage-clamped) photoreceptor membrane and is renamed \(t'_{\text{eff}}\) (approximately 1 to 3 msec in adults using the same equipment and techniques as in the current study\(^\text{19}\)). Similarly, only an estimated amplification constant, \(A'\), can be obtained by fitting equation 1 to ERG recordings (approximately 6.9 sec\(^{-2}\) in adults\(^\text{19}\)). \(A'\) is affected by physiological factors external to rods, such as preretinal absorption and lower effective rhodopsin density on the rod disk membrane. The expression for \(F(t)\) has a Gaussian form scaled by the product of \(\phi\) and \(A\) (or \(A'\)). If \(\phi\) is known, \(A\) (or \(A'\)) can be estimated from fitting the expression to the rising phase of the rod response, or, in the case of the ERG, to the rising phase of the summated rod response, as represented in the a-wave leading edge.

Within limits placed by certain restrictive assumptions extraneous to the transduction model (similar recording pathway impedance in adults and infants, comparable physiological optics except for eye size, identical biochemical properties for transduction proteins), changes in \(a_{\text{max}}\) and in \(A'\) can be related to possible changes in receptor characteristics, such as outer segment length and concentrations of the protein substrates underlying the transduction cascade.

**Curve Fitting**

Equation (1) was fit to individual a-wave responses with a least-squares minimization procedure based on the simplex algorithm in the Matlab (Mathworks, Natick, MA) package, with both \(A'\) and \(\eta_{\text{eff}}\) varied to obtain the best fit. Because the b-wave intrudes on the a-wave in a complex time- and light-dependent manner, it was necessary to restrict the time range of the fitting. For each response, the maximum time of the fitting was set to approximately 10% to 20% before the point of obvious b-wave intrusion (see Fig. 3).

**Conversion to Isomerizations Per Rod**

Calibration readings from the photometer in units of time-integrated photometric luminance (cd-sec/m\(^2\)) were first converted to units of rod retinal illuminance (scotopic troland seconds, or \(Q\)) and then to units of rod photon absorption (isomerizations/rod, or \(\phi\)) for a typical adult rod. The conversion from flash intensity at the cornea to units of photoisomerizations per rod per flash is outlined in detail in Breton et al.\(^\text{19}\) The conversion takes into account eye size, preretinal media transmissivity, the dimensions and physical optics of the absorbing rod, photopigment optical density, and isomerization quantum efficiency. The conversion factor, \(K\), from retinal illuminance to rod absorption for adult human rods was estimated to be \(K = 8.6\) photoisomerizations per rod. Thus,

\[
\phi = QK \quad (3)
\]

Our maximum unattenuated flash of 1553 cd sec/m\(^2\) produced a retinal illuminance of 141,000 scotopic td s in a typical adult and, therefore, corresponded to a \(\phi\) of 1.21 \times 10\(^6\) photoisomerizations per rod per flash. Taking the total rhodopsin content of an adult human rod as \(7 \times 10^7\) molecules,\(^\text{22}\) our maximum flash would have bleached approximately 1.7% of the rhodopsin.
FIGURE 3. (A) A-waves, normalized to $a_{\text{max}}$, for subject INF4, 46 days (same as in Fig. 4, top data set). Best fits of equation 1 are shown for each waveform. Solid fit lines show the extent of response included in the fit. Long dashed lines show predicted photocurrent time course beyond the point of b-wave interference. The short dashed line to the left of the highest intensity response shows the predicted trace for the average value of $A'$ determined from the lower intensity fits, with $\phi$, the number of isomerizations, set to the appropriate value if incident quanta continue to be absorbed and to activate the transduction cascade as they do at lower intensities. It rises too rapidly to fit the highest intensity a-wave response, indicating that response rate-of-rise saturation has occurred. (B) Normalized a-waves and model fits to individual waveforms for subject INF8, 104 days of age. (C) Normalized a-waves and model fits to individual waveforms for subject INF14, 205 days of age. (D) Normalized a-waves and model fits to individual waveforms for subject A1, 10 years of age.

Correction of Isomerizations for Size of the Eye

Smaller eye size in infants should increase retinal illumination, and, therefore, isomerizations per rod for a given flash intensity at the cornea, assuming other rod factors are the same as in the adult (which, of course, they may not be). Given this assumption, the increase in retinal illuminance will be proportional to the reduced retinal area over which an image of fixed angular subtense is spread. Infant eyes measured at birth by ultrasound biometry have an axial length of approximately 17 mm compared to approximately 23 mm in adults, and they grow to adult size during approximately the first 5 years of life.23 Because direct measurements of eye size were not made on our infants, eye size was estimated from its correlation with age, and a difference in retinal illumination from adult was based on corrected eye size. A correction factor was applied directly to the adult value of $K$ to yield a size-corrected value, $K'$ (Table 1). Values of $A'$ derived from fitting equation 1 to a-wave data were then corrected by $1/K'$.

RESULTS

Electroretinographic waveforms recorded over a range of intensities are shown in Figure 2 for representative patients in the study. Two important trends are...
apparent as a function of age. The first is the increase in a-wave maximum amplitude from the 5-day-old infant (the youngest subject) to the 10-year-old child. Responses of the 10-year-old child were indistinguishable from those of the adult. The second is the increase in b-wave amplitude. This is most apparent in the 205-day-old infant, whose b-wave maximum amplitude (722 μV) exceeded the mean for young adults (672 μV for subjects A1 to A12) but whose maximum a-wave amplitude (356 μV) was approximately 65% of the young adult mean (543 μV). Data for maximum amplitudes are summarized in Table 1.

Figure 3 shows normalized a-waves and theoretical model fits for the same four subjects in Figure 2. The solid smooth lines and their long dash extensions are model fits to the individual a-waves (see Methods).19,20 The solid portion indicates the time domain included in the fit. The dashed portion is not included in the fit because it is in the region of b-wave interference. For each subject, there is an intensity range over which a single value of $A'$ tends to fit all the waveforms. For normal adult subjects, this range is from approximately 2.5 to approximately 4.5 log isomerizations per rod.19 Values for $A'$ reported in Table 1 are the average from the individual fits in the intensity range that tend to produce a constant value for $A'$. Also shown in Figure 3 are short dash traces (leftmost model trace) calculated for the average value of $A'$, but with $\phi$, the number of isomerizations, set to the appropriate value if incident quanta continue to be absorbed and to activate the transduction cascade as they do at lower intensities (in other words, as if saturation did not occur). It can be seen that theory predicts a rate-of-rise too rapid to approximate the actual response recorded for that intensity (leftmost data trace).

Figure 4 shows raw waveforms from the youngest three infants studied (ages, 5, 8, and 16 days; INF 1 to 3). Only the fit of the model to the highest intensity response in each set is shown (dashed lines), although all waveforms were fit. For these youngest infants, the theoretical traces fit only the initial portion of the a-wave leading edge. Interference from b-wave and other inner retinal potentials, possibly including cone-driven potentials that may have greater weight in the response of the youngest infants, was evident beyond this point (see Fig. 4). The proportion of a-wave fitted by the model predicted time course increases with age, reaching near perfect fits by at least 46 days, as seen in Figure 3A.

Figure 4. Waveforms and transduction model fits to a-waves of the youngest infants, ages 5, 8, and 16 days. For the youngest infants, the theoretical traces fit only the initial portion of the a-wave leading edge. The proportion of a-wave fitting the model predicted time course increases with age, achieving near perfect fits by at least 46 days, as seen in Figure 3A.

slope, more distinct for the 16-day-old infant than for the 5- or 8-day-old infant, was chosen as the most probable value of $a_{\text{max}}$. A second, or upper-bound, estimate was derived from the lowest point reached by the a-wave, even though this occurred later in time and did not correspond to the model shape when the fit with the larger value of $a_{\text{max}}$ was made. The values derived from the upper bound estimates are shown in parentheses in Table 1 and as open diamonds in Figures 5A and 7 to distinguish them from the lower-bound estimates (open circles in Figs. 5 and 7).

$a_{\text{max}}$ and $b_{\text{max}}$ amplitudes were measured for the highest intensity flash, which saturated a-wave rate-of-rise and minimized b-wave interference.18,19 $a_{\text{max}}$ and $b_{\text{max}}$ are shown in Figure 5A as a function of age for each of the 18 subjects younger than 4 years of age and for 10 older subjects with adultlike response, whose ages ranged from 10 to 26 years. Amplitude and age are shown as logarithmic values to emphasize the results for the lower ages. Viewed in double log coordinates, a rapid growth trend in the first year for
FIGURE 5. (A) Maximum a-wave and b-wave amplitude as a function of age. Data are shown in double log coordinates to emphasize values for younger infants. A-wave amplitude (filled circles) increases steadily in the first year, reaching adultlike values sometime before the second decade. B-wave amplitude (open squares) starts at a higher value than a-wave compared to adult and reaches adultlike values by approximately 6 months. Second-order regressions are fitted by a least squares procedure through the first two decades of a-wave and b-wave data to help visualize trends. Both a-wave and b-wave amplitudes show substantial individual variation. (B) Maximum b/a wave ratio. For the youngest subjects, (b/a)_{max} ranges from approximately 2.9 to 1.5. The mean value declines to approximately 1.30 as a-wave amplitude increases to adultlike values. The first-order regression line fitted to the data suggests a steady decline in (b/a)_{max} with age that extends beyond the first year.

both \( a_{\text{max}} \) and \( b_{\text{max}} \) is apparent. Based on the results from infants 1 to 3 (Table 1), \( a_{\text{max}} \) shortly after birth is approximately 100 \( \mu \text{V} \) (20% of that of the young adult). Near the end of the first year, based on results from subjects A1 to A12 (Table 1), it increases to approximately 70% of that of the young adult. By 10 years of age, and possibly as early as 5 years of age, \( a_{\text{max}} \) values indistinguishable from those of the young adult are reached. The initial \( b_{\text{max}} \) values are closer to those of the adult (approximately 40%) than are \( a_{\text{max}} \) values. \( b_{\text{max}} \) reaches (or exceeds) adultlike values by approximately 6 months, whereas \( a_{\text{max}} \) is not mature by the end of the first year (see also Table 1). Second-order regression lines fitted to the double logged data with a least squares procedure indicate the early rapid increase for both a-wave and b-wave amplitude and the later slowing of growth. The growth slowdown appears somewhat earlier for b-wave than for a-wave amplitudes and even begins to trend downward in early adulthood.

Figure 5B, also in double-logged coordinates, shows the b-/a-wave ratio obtained by dividing \( b_{\text{max}} \) by \( a_{\text{max}} \), or (b/a)_{max}, for each subject. In newborns and through at least the first year of life, (b/a)_{max} tended to be large when compared to adults. Maximum b-wave amplitude shortly after birth, at approximately 300 \( \mu \text{V} \), was closer to mature values than a-wave maximum amplitude and increased rapidly to adultlike values (500 to 700 \( \mu \text{V} \)) by or before approximately 6 months. The (b/a)_{max} ratios for the youngest infants were frequently 2 or greater, but with considerable individual variation (1.34 to 2.42 for 19 infants in the first year). These ratios tended to decline through the first year of life, as indicated by a first-order regression line fitted to the double-logged data. Values for (b/a)_{max} from a total of 49 adult subjects between 10 years and 80 years have a mean of 1.30 with a standard deviation of 0.18 (Breton, unpublished data, 1995). For these adults, the upper bound for 95% confidence that an individual value is within the adult spread is 1.60. Of the infants younger than 1 year of age, 13 of 16 had (b/a)_{max} values greater than 1.60. A t-test for (b/a)_{max} for these 16 infants versus the values for the 49 adults was highly significant (t = -8.86, \( P < 0.001 \)). The two children between 1 and 4 years of age had one (b/a)_{max} ratio lower than 1.60 and one higher.
Amplification constants for adults and infants are shown in Figure 6 as a function of log isomerizations per rod per flash. The top panel (Fig. 6A) shows values of $A'$ determined from fits of the model to the individual waveforms of six normal adult subjects (as in Breton et al\textsuperscript{19}). A best-fitting “Weber” saturation function is shown as a smooth solid line (see footnote for derivation). The lower panels (Figs. 6B to 6D) show amplification constants for infants of descending age within the first year of life. In Figure 6B, the age range is 204 to 270 days ($n = 3$), in Figure 6C it is 63 to 142 days ($n = 6$), and in Figure 6D it is 5 to 57 days ($n = 5$). Within each panel, a Weber saturation function is fitted to the combined data to show a general trend for that age range. The adult trend is shown for comparison (dashed line), and a line of slope $-1$ (dashed line at $45^\circ$) indicates saturation of the adult transduction response at high intensities (see Breton et al\textsuperscript{19} for a detailed explanation). In infants, $A'$ values were low compared with adult values (approximately 1 to 3.5 sec$^{-2}$ versus 6 sec$^{-2}$), and they increased with age similar to $A_{\text{max}}$.

Weber saturation functions for the two oldest groups of infant data (Figs. 6B, 6C) are positioned down and to the right, roughly along the line of slope $-1$. However, the Weber function for infants younger than 60 days of age gives some suggestion that response saturation is occurring at a lower effective number of isomerizations, such that the saturated values for $A'$ at high intensities for some of these infants do not fall along the slope $-1$ line defined by the adult values.

Weber saturation functions are calculated on the assumption that $A'$ values are constant at lower intensities, consistent with the results obtained for individual amphibian\textsuperscript{22} and mammalian rods,\textsuperscript{24} but they decline at higher intensities as the rate-of-rise of the photocur-

### Weber Saturation Function Calculation: Best-fitting Weber functions to the amplification constant data were calculated according to the equation:

$$A_0 = A_{\text{max}}/(1 + \phi/\phi_{1/2\text{SAT}})$$

where $A_{\text{max}}$ is the maximum value for $A$ in the intensity range in which it is a constant, $\phi$ is the flash intensity in isomerizations per rod, and $\phi_{1/2\text{SAT}}$ is the half-saturation constant for the function.\textsuperscript{19} The choice of this function to fit the amplification data is for convenience and does not imply a theoretical basis for the fit.

Correction Factor for Eye Size: Eye length at ages from birth to 120 months as measured by ultrasound biometry and listed in a table by Grignolo and Rivara\textsuperscript{23} was used to calculate relative differences in retinal area at the ages listed. The graph of difference factors was then smoothed by fitting an exponential regression to the data. The regression equation was:

$$CF = 1 - \exp(-0.056(M + 6.53))$$

where $CF$ is the correction factor for age applied to $A'$, and $M$ is infant age in months.
ERG and Rod Phototransduction Response in Infants

FIGURE 7. Estimated amplification constant, $A'$, versus age. The value of $A'$ plotted is an average of values determined by fits of equation 1 to individual a-waves of a given subject in the lower intensity range, where $A'$ is expected to be constant. The increase over the first year and the point at which adultlike values begin may be similar for $a_{\text{max}}$ and $A'$ (see Fig. 5A).

**DISCUSSION**

Results of our transduction-based analysis of infant ERG a-waves are consistent with maturational change in photoreceptor physical and functional attributes during and beyond the first year of life. Reasonable candidates for maturational change include outer segment length, outer segment organization (disk orientation and vacuole inclusion), density of transmembrane ion channel proteins, cytoplasmic cGMP concentration in the dark, or other factors affecting the proportion of channels open in the dark. Reduced Amplification Constant, $A'$

Results for infant ERG a-waves are consistent with a reduction in the rate of photocurrent generation per photon incident at the retina (but not all photons necessarily absorbed). The reduction is by a factor of approximately 2 to 4 relative to adult, as reflected in the estimated rod amplification constant, $A'$. $A'$ does not appear to reach fully mature values within the first year of life. At least part of the reduction in $A'$ may be accounted for by reduced effective density of rhodopsin. In addition, if it is accepted that the $A'$ versus isomerizations function saturates at a lower effective number of isomerizations per rod for the youngest infants than for adults, this result suggests a difference in a second transduction substrate density, specifically in the effective density of phosphodiesterase. It is important to confirm this result on a larger number of normal infants within the first month of life.

How should differences in $A'$ between infants and adults be interpreted? The value of $A'$ depends on the value of $\phi$ calculated from knowledge of the physiological optics of the adult eye. The conversion factor, $K'$ (from adult scotopic retinal illuminance to rod photon absorption in the infant), incorporates physiological factors aside from phototransduction cascade kinetics, such as eye size, preretinal media transmissivity, the dimensions and physical optics of the absorbing rod, photopigment optical density, and isomerization quantum efficiency. When disease at the biochemical level is not a factor, as in this study, assumptions concerning the efficiency of photon absorption by rhodopsin and fundamental biochemical properties affecting rates of molecular interaction (other than effective species substrate density) for the cascade proteins appear safe. Errors of overestimation of absorbed quanta for a flash of fixed intensity at the cor-
sea (for example, decreased rate of photon capture caused by decreased density of rhodopsin molecules on the rod disk) will move the calculated values of $A'$ down and to the right along a line of slope $-1$, as shown by the arrow on the right side of Figure 8. On the other hand, positioning of the Weber saturation line, or half saturation point ($\phi_{1/2\text{SAT}}$), down but not to the right in this diagram is consistent with a limitation in rate-of-rise for transduction imposed at the level of phosphodiesterase activation.

These points are illustrated by plotting $A'$ versus isomerizations per rod for three subjects in the study (A1, indistinguishable from adult; INF14, 205 days of age; INF5, 57 days of age) in Figure 8. The data from INF14 are shifted down and to the right of the adult data (A1), and they coincide along the slope $-1$ line. This is consistent with less sensitivity for INF14 but approximately equal saturated rates of response for INF14 and A1. In comparison, the data from subject INF5 are apparently shifted down, but not to the right, relative to the other two data sets. These data summarize a trend that may be present in our results: for very young infants, a positioning of the $A'$ versus isomerizations function down and not to the right compared to adults, and for older infants younger than 1 year of age, positioning down and to the right with the degree of shift depending roughly on age.

In excised neonatal rat rods, Ratto et al. attribute a decreased sensitivity to light to a lowered concentration of functional chromophore in the neonate photoreceptor, which lowers the effective density of rhodopsin able to be activated by light. Our results for $A'$, showing lower average values in the (lower intensity) constant region, are consistent with this result and with other studies suggesting that rhodopsin concentration in the retina is likely to be low at birth compared to adults and to reach mature levels at a later, but unknown, time. Reduced effective rhodopsin concentration in infant rods will reduce the efficiency of photon absorption for a given photon density. A value of $K'$ not adjusted for this lower efficiency will then be an overestimate, and the fitting procedure for the transduction model will compensate by using a smaller value of $A'$ to achieve a fit. If the correct value for $K'$ were known, the value of $A'$ would approximate more accurately the actual amplification constant of the participating rods. Thus, if a reduction in effective rhodopsin density were the only difference from adult and other transduction substrate densities were similar to those in the adult, the actual rate of transduction response from the point of photon capture onward would be adultlike.

Effect of Receptor Size on $\phi$ and $A'$

Experimentally, $A$ (or $A'$) is determined in isolated rods, or for the massed rod response of the ERG, by normalizing response amplitude and estimating its rate-of-rise from the fit of equation 1 to the response trace. The value of $\phi$ used in equation 1, and on which the value of $A$ or $A'$ depends, is estimated from assumed dimensions of a typical rod of the type under study. In our infant studies, the variation of rod dimensions (and therefore rod volume) from the adult cannot be measured directly, and $\phi$ is that estimated for a typical adult rod, corrected only for the increase in retinal illuminance resulting from smaller infant eye size.

In accord with convention, the value of $A$ estimated for excised rods is reported as the amplification per photon absorbed per rod. For individual rods, $A$ is inversely proportional to cytoplasmic volume (see Lamb and Pugh); thus, with all substrate densities constant, $A$ must increase as outer segment volume decreases. This is not observed for ERG recordings because photon absorptions vary proportionately with rod volume (and, thus, length or diameter) in the uniformly illuminated field. Under the conditions of
ERG recording, estimated values of $A'$ will appear not to vary with photoreceptor size (or with retinal rod density).

For this reason, differences in our estimates for $A'$ between infants and adults are not accounted for by differences in photoreceptor size or spacing. Estimates of $A'$ will remain comparable to those in adults unless something other than rod volume is changing, such as reduction in photon absorption efficiency because of reduced rhodopsin density or lower efficiency in the transduction pathway. In effect, the value of $A'$ determined experimentally from the ERG a-wave is thought of more accurately as an estimate of the amplification per photon absorbed per (some) unit volume of disk membrane, cytoplasm, outer segment plasma membrane, and their associated complements of cascade substrates and conductance proteins.

**Recording Pathway Impedance**

An important assumption made in comparing amplitudes between infants and adults is that the impedance of the recording pathway is essentially the same for both. The large b-wave amplitudes achieved early in development, along with the smaller a-waves (see Table 1, Fig. 2), argue that impedance is not an important factor in determining the actual recorded amplitudes because impedance change would affect a-wave and b-wave amplitudes alike.

**Differences in $a_{\text{max}}$**

Although in vivo measurements of $A'$ are insensitive to photoreceptor size changes, $a_{\text{max}}$ is not. Outer segment length for rods and cones is reported to increase after birth. Before birth, cone outer segments develop ahead of the appearance of rod outer segments. From histologic studies in human fetuses, rod outer segments first appear at approximately 8 weeks before term, and rod outer segment development over much of the retina overtakes cone several weeks before term.6,7 Approximately 1 week after birth, rod outer segments in the midperiphery, where rod density is maximal, are clearly longer than cone outer segments, although both are much shorter than adult length.7

Maximum a-wave amplitude, $a_{\text{max}}$, is proportional to the magnitude of the total dark current generated in all the rod photoreceptors,10 which is determined by the total cationic conductance held open in the dark. Thus, in normal adults, $a_{\text{max}}$ may be interpreted as proportional to total rod outer segment length. However, in infants (and in patients with photoreceptor disease), additional factors may contribute that can modify this interpretation, including the proportion of cGMP-gated channels held open in the dark-adapted state, their density per unit area of outer segment membrane, and the impedance of the ERG current path. To the extent that these other factors are similar to those in the adult, the growth in $a_{\text{max}}$, as shown in Figure 5A, may be interpreted as a parallel growth in infant rod outer segment length. Available anatomic evidence indicates that infants at approximately 1 week after full-term birth have rod outer segment lengths approximately 30% to 50% of adult length, suggesting a factor of 2 to 3 increase for rod outer segment length between birth and maturity (at approximately 10 years of age).7 This compares to a factor of approximately 4 to 5 increase in $a_{\text{max}}$ over a similar period shown by our results.

Thus, growth in outer segment length alone may not be enough to account for the entire observed increase in $a_{\text{max}}$. If current path impedance is not a significant factor, as it appears not to be (see discussion of $(b/a)_{\text{max}}$ amplitude ratio below), only two factors, the proportion of cGMP-gated channels held open in the dark and cation channel density, need be considered. The proportion of cGMP-gated channels open in the dark is determined by cytoplasmic dark concentration of cGMP, which, in turn, is determined by the relative rates of cGMP hydrolysis and regeneration and by the cell’s buffering power for cGMP. If the proportion of conductance open in the dark is constant, dark current magnitude will be proportional to the density of cationic channels. Our results may be explained if the density of cGMP-gated conductance protein is less than adult levels and matures along with outer segment length and other transduction proteins.

**Decline in $(b/a)_{\text{max}}$ Ratio**

Shortly after birth, $(b/a)_{\text{max}}$ starts out at high values compared to the adult value and declines steadily during the first year of life, but it does not reach adult values until sometime later (see Fig. 5B). After birth, a-wave and b-wave mechanisms are immature, as indicated by reduced amplitudes. However, the high $(b/a)_{\text{max}}$ ratio could be explained if elements of the b-wave mechanism (photoreceptor–bipolar synapses and bipolar–Müller cells of the inner retina28) are more mature than the mechanism underlying the a-wave shortly after birth and the b-wave mechanism proceeds to full maturity at an earlier time. This hypothesis is supported by anatomic results in neonate primates showing inner retinal structures, including synaptic microstructure, relatively mature compared to the photoreceptors, especially outer segments, which are not of adult length.7,29,30 On the other hand, the presumed inner retinal mechanism(s) responsible for oscillatory potential generation do not appear to progress to early maturity in the same way as the b-wave because the amplitudes for these wavelets riding
on the b-wave remain low, at least through the first year (see waveforms, Fig. 2).

The finding that \((b/a)_{\text{max}}\) is not constant during the first year, but starts out high and declines toward adult values, may imply something about photoreceptor resting potential in the dark. Less than (normal) adultlike photoreceptor depolarization in the dark would not produce a full hyperpolarizing excursion at the receptor–bipolar synapse, and the scaled down synaptic signal would be expected to elicit a proportionately smaller b-wave. On the other hand, adultlike resting potentials for developing photoreceptors imply that a saturating response should produce a synaptic hyperpolarization similar in magnitude to that in adults, in spite of shorter outer segments and reduced magnitude dark current per cell (implied by small \(a_{\text{max}}\)). From this reasoning, an adultlike b-wave response could be elicited from very young infants, provided the b-wave and synaptic mechanisms were mature in terms of generating response amplitude and an adultlike photoreceptor resting potential were present in the dark. The observation of large b-waves in conjunction with small a-waves at intensities that appear to produce a-wave amplitude and rate-of-rise saturation suggest to us that adultlike photoreceptor depolarization in the dark may be present soon after birth and be maintained during the process of development. It does not seem impossible that changes in transmembrane conductance protein density supporting the dark current may “balance” between the inner and outer segments to maintain a photoreceptor-typical state of dark-adapted depolarization during development.

Cone Response

Rods dominate cones numerically in the adult retina by a ratio of at least 20:1.31 In spite of rod domination at the photoreceptor level, cones can influence scotopic white flash a-wave response because they elicit an amplified, negative inner retinal potential. This was shown convincingly by Bush and Sieving32 for monkey ERGs in which rod response was suppressed by the presence of a constant background, and cone-driven inner retinal response was selectively blocked pharmacologically. A cone-driven, negative potential was described; at low to intermediate intensities, it was attributable mostly to inner retinal response and appeared to saturate in amplitude at an intermediate flash intensity equivalent to approximately 3 log isomerizations per rod. At higher intensities, the proportion of the negative response attributable directly to cone photocurrent increased.

Thus, for adults and infants, white light stimulation has the potential of recruiting a cone-driven negative response that could interfere with accurate measurement of rod a-wave kinetics. Hood and Birch33 argue that cone contamination in the white flash response may bias transduction parameter estimates to higher values, especially if these are based on responses to lower stimulus intensities. This problem was considered by Breton et al19 in applying the Lamb and Pugh model to human a-wave analysis. The Breton et al19 study concluded that a-wave response at higher intensities (more than approximately 3000 isomerizations per rod) was determined principally by rods. This conclusion was reached, in part, because the values of \(A'\) plotted against intensity showed the expected behavior if the response was determined from one class of receptors and because, in one well-studied subject, the estimates obtained from blue flash response, where cone contribution is diminished, were comparable to those estimated from white flash. Data recorded from two additional normal adult subjects (Breton, unpublished data, 1995) confirms the Breton et al19 finding that estimates of \(A'\) coincide for white and blue flash responses (uncorrected for cone contribution) at high stimulus intensities (above approximately \(10^4\) isomerizations per rod). In the same data at lower intensities, \(A'\) estimates for white flash exceed those for blue, in agreement with the conclusions of Hood and Birch33 for moderate intensity flashes.

White flash intensities for the current study range up to \(10^6\) isomerizations per rod, where estimates of \(A'\) are relatively less affected by cone contamination. Absolute estimates of \(a_{\text{max}}\) may be moderately affected by cone response (15% to 25%, based on the Hood and Birch33 results). However, relative comparisons across age should be much less affected, especially for the all but three infants older than 1 month of age (see next paragraph for discussion of the youngest three infants).

Infants Younger Than One Month

For the three youngest infants (INF 1 to 3), the model shape failed to fit the full extent of the highest intensity a-waves, although for each it provided a good fit to the early part of the response (Fig. 4). These response shapes, obtained at high intensity, resemble (at least superficially) cone responses recorded by Hood and Birch34 in humans under light-adapted conditions that suppress rods and by Bush and Sieving32 in primates under similar light-adapted conditions and with pharmacologic isolation of cone receptor from inner retinal response. This similarity may be at least partly coincidental. For human infants, even at 1 week after birth, the available anatomic evidence indicates the presence of rod outer segments that are already longer than cones.7 Thus, it appears that the anatomic substrate is sufficient to provide a substantial, if not dominant, rod photocurrent response. Nevertheless, the
cone-driven response may have a greater influence on a-wave shape in our youngest infants than it seems to have in older infants and adults.

Although it is unclear why the shape of the a-wave response to white light in our youngest infants does not match that expected of rods alone, it seems reasonable that at least some of the change could be accounted for by changing rod–cone weighting. The development of cone-driven components is not addressed directly in this study, but it could be explored further by changing the rod–cone ratio of the response using flashes of different spectral makeup or by isolating cone response using a two-flash technique. It is important to note that the distorting influence on the a-wave, whatever its origin, grows rapidly less with age and is not evident in the responses of the 46-day-old infant (INF4) and, thus, has little impact on the major conclusions of this study.

**Key Words**
a-wave, development, electroretinography, photoreceptor–transduction, rods

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