Rod and Rod-Driven Function in Achromatopsia and Blue Cone Monochromatism

Anne Moskowitz, Ronald M. Hansen, James D. Akula, Susan E. Eklund, and Anne B. Fulton

PURPOSE. To evaluate rod photoreceptor and postreceptor retinal function in pediatric patients with achromatopsia (ACHR) and blue cone monochromatism (BCM) using contemporary electroretinographic (ERG) procedures.

METHODS. Fifteen patients (age range, 1–20 years) with ACHR and six patients (age range, 4–22 years) with BCM were studied. ERG responses to full-field stimuli were obtained in scotopic and photopic conditions. Rod photoreceptor (S_{rod}, R_{rod}) and rod-driven postreceptor (log \( \sigma \), \( V_{max} \)) response parameters were calculated from the a-wave and b-wave. ERG records were digitally filtered to demonstrate the oscillatory potentials (OPs); a sensitivity parameter, log SOPA_{max} and an amplitude parameter, SOPA_{max}, were used to characterize the OP response. Response parameters were compared with those of 12 healthy control subjects.

RESULTS. As expected, photopic responses were nondetectable in patients with ACHR and BCM. In addition, mean scotopic photoreceptor (R_{rod}) and postreceptor (V_{max} and SOPA_{max}) amplitude parameters were significantly reduced compared with those in healthy controls. The flash intensity required to evoke a half-maximum b-wave amplitude (log \( \sigma \)) was significantly increased.

CONCLUSIONS. Results of this study provide evidence that deficits in rod and rod-mediated function occur in the primary cone dysfunction syndromes ACHR and BCM. (Invest Ophtalmol Vis Sci. 2009;50:950–958) DOI:10.1167/iovs.08-2544

Achromatopsia (ACHR) refers to a group of congenital, stationary retinal disorders in which there is an absence or a paucity of functioning cones.1–5 Complete ACHR, also called rod monochromatism, is an autosomal recessive condition characterized by reduced visual acuity, photophobia, nyctalops, deficits in color discrimination, and paradoxical pupillary constriction to dark.1–5 Hyperopia is common,1,6,7 though a broad distribution of refractive errors has been reported.8 Fundus appearance is typically normal,1–5 but exceptions have been reported.9,10 Blue cone monochromatism (BCM) is an X-linked condition that shares many of the characteristics of autosomal recessive ACHR, sometimes exhibited with reduced severity.1–5,11,12 Refractive error, however, is typically myopic.8,11,13–15 Clinically, Berson plates discriminate patients with BCM from patients with ACHR.16–18

In ACHR, rods are the only functional photoreceptor type, whereas in BCM, rods and short wavelength-sensitive cones are functional.12,19 ACHR and BCM are typically regarded as stationary conditions, but in both there have been reports of adults with progressive retinal disease.10,20–26

ACHR is understood to be a channelopathy of the cone photoreceptors. The most common molecular causes are mutations in the G-protein-gated cation channel genes CNGA3 (Online Mendelian Inheritance in Man [OMIM]600053) and CNGB3 (OMIM605080).1,7,27–29 Less frequently, a mutation in the transducin protein GNA12 (OMIM139340) has been associated with ACHR.30,31 The most common molecular causes of BCM are mutations in the opsin gene array of long wavelength– and medium wavelength–sensitive cone visual pigments located adjacent to the X-chromosome. (OMIM305700).29,31

In both ACHR and BCM, cone and cone-driven electroretinographic (ERG) responses to full-field stimuli are markedly attenuated or nondetectable, whereas rod and rod-driven responses are typically reported to be normal or near normal (Wiesen MH, et al. IOVS 2008;49:ARVO E Abstract 1267).3,4,6,9,21,26,27,35–39 Recently, however, abnormal rod-driven ERGs have been reported in some patients with CNGB3 ACHR10 and BCM.23

Our own clinical observations also indicated abnormalities in rod and rod-driven electroretinograms in pediatric patients with ACHR and BCM. Therefore, we undertook an analysis of rod photoreceptor and postreceptor ERG components. Our goal was to identify possible mechanisms underlying the abnormalities.

METHODS

Subjects

Twenty-one patients (Table 1), 15 with complete ACHR and six with BCM, who had been monitored by the Department of Ophthalmology, Children’s Hospital Boston, were studied retrospectively. ACHR patients exhibited typical features of ACHR, including low visual acuity, photophobia, paradoxical pupillary constriction to dark, and low-amplitude, high-frequency, ‘jelly-like’ nyctalops. All patients had normal fundus appearance. ACHR patients 1 and 8 are siblings. Clinical presentations of the patients with BCM were similar, though photophobia often appeared less severe. All were male, and all passed the Berson test.16 indicating that unlike patients with ACHR, they were able to distinguish a purple-blue (Munsell Color System 7.5 PB; dominant wavelength 468 nm) arrow from blue-green (5.0 BG; 491 nm) arrows. Two male patients who were classified as having ACHR (ACHR 4 and 7) were too young for color vision testing. Median ages at ERG were 2.7 years (range, 1–20 years) for the ACHR patients and 8 years (range, 4–22 years) for the BCM patients.

Visual acuity was measured in dim room light using age-appropriate tests (Teller Acuity Cards, HOTV, Lea, Feinbloom, or ETDRS). Refractive error was measured using cycloplegic retinoscopy.41 The most recent visual acuity and spherical equivalent values for each patient are

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reported in Table 1. Acuity was below normal for age in all patients. Twelve of the 15 ACHR patients were hyperopic; nine were outside the 99% prediction limit of normal for age.41,42 Two of the three myopic ACHRs were also outside the normal limit. All six BCM patients were myopic; four were outside the 99% prediction limit of normal for age.

Dark-adapted, rod-mediated visual thresholds, obtained in 11 ACHR patients and five BCM patients, were normal43 in all but one patient. Age.

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Dark-adapted, rod-mediated visual thresholds, obtained in 11 ACHR patients and five BCM patients, were normal43 in all but one patient (ACHR 7), who showed a mild (1.18 log units) but statistically significant threshold elevation. Four patients had repeat measurements with a 1.5-year (ACHR 5), 6.8 year (BCM 21), 7.8-year (ACHR 9), and 9-year (ACHR 12) intervals between tests, and none showed a change in threshold, suggesting a stationary condition.

ERG responses in the patients with ACHR and BCM were compared with responses in 12 healthy control subjects (median age, 23 years; range, 8–41 years). ERG response parameters in healthy subjects at the threshold, suggesting a stationary condition. (ACHR 12) intervals between tests, and none showed a change in threshold, suggesting a stationary condition.

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Electroretinography

Pupils were dilated with 1% cyclopentolate hydrochloride, and the patient was dark adapted for 30 minutes. All 12 control subjects and seven patients (four ACHR, three BCM) were tested awake (Table 1); 14 patients (11 ACHR, three BCM) had ERG testing under light inhalation anesthesia which has no significant effect on the ERG parameters studied herein.45 After dark adaptation, 0.5% proparacaine was instilled and, under dim red light, a bipolar Burian-Allen electrode (Hansen Ophthalmic Development Laboratory, Coralville, IA) was placed on the cornea. A ground electrode was placed on the skin over the mastoid. Responses were recorded from both eyes of the patients and from one eye of the control subjects. In patients, the eye with the larger scotopic amplitudes was selected for analysis.

The study was conducted over a period of several years. Thirteen patients (10 ACHR, three BCM) and all 12 control subjects were tested using an older electrophysiological recording system (Compact 4; Nicolet, Madison, WI), and eight patients (five ACHR, three BCM) were tested using a new system (Espion; Diagnosys, Lowell, MA). Despite differences between the two recording systems in the spectral composition of the stimuli (described below) and in data acquisition (256 Hz digitization rate for the Nicolet; 2000 Hz for the Espion), a previous comparison46 of rod and cone photoresponse parameters in normal adult subjects obtained using the Espion system (N = 7) and obtained earlier using the Nicolet system (N = 13)46-47 showed no significant differences. Therefore, the data obtained using the two systems have been combined.

Responses were differentially amplified, displayed, digitized, and stored for analysis. A voltage window was used to reject responses 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.47

Full-field stimuli were presented in an integrating sphere. Stimulus intensity was measured using a calibrated photodiode (IL1700: International Light, Newburyport, MA) placed at the position of the subject’s cornea. The troland values of the stimuli were calculated by taking each subject’s pupillary diameter into account. To test rod function, after dark adaptation, responses to brief (<3 ms), short-wavelength stimuli ranging from those that evoked a small b-wave (<15 μV) to those saturating the a-waves were recorded. In the Nicolet system, a filter (Wratten 47B, λ < 510 nm) was used; in the Espion

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* PPR, paradoxical pupillary response.
† Tested awake.
‡ Outside 99% prediction limit for normal.41,42
§ Grating acuity (Teller Acuity Cards).40
system, a 470-nm LED (half-bandwidth, 30 nm) was used. Flashes were presented over a >4 log unit range, starting with the dimmest and increasing in 0.3-log unit steps. The maximum intensity flash produced approximately 3.0 log scotopic troland seconds (scot td s) for an 8-mm pupil.

To isolate rod function in control subjects, dark-adapted responses to photopically matched long-wavelength flashes (Wratten 29 filter, λ > 610 nm) were recorded and subtracted from the responses to the corresponding short-wavelength flashes. In the Nicolet system, a filter (Wratten 29, λ > 610 nm) was used; in the Espion system, a 630-nm LED (half-bandwidth, 30 nm) was used. A 1.8-log unit range of red flash intensities was presented on a steady, rod-saturating background (−3 log photopic trolands). The maximum intensity flash produced approximately 3.2 log photopic troland seconds (phot td s) for an 8-mm pupil. Seventeen of the 21 patients were also tested with a 30-Hz flickering white stimulus (2.4 log phot td s).

Rod photoresponse characteristics were estimated from the a-wave by means of the Hood and Birch formulation of the Lamb and Pugh model of the biochemical processes involved in the activation of rod phototransduction. A curve-fitting routine (fmin subroutine; MATLAB; The MathWorks) was used to determine the best-fitting values of $S_{rod}$ (scot td)$^{-1}$ s$^{-1}$, $R_{rod}$ (μV), and $t_d$ (a brief delay, seconds) in the following equation:

$$P_a(t) = \left[1 - \exp\left(-0.51 \frac{t}{S_{rod} t_d^2}\right)\right]R_{rod} \quad \text{for} \quad t > t_d \quad (1)$$

In this equation, I is the flash in scotopic troland seconds. Assuming that the number of isomerizations of rhodopsin produced by the stimulus is known, the term $S_{rod}$ is related to the amplification constant, $A$, in the molecular models. In these models, $A$ summarizes the kinetics of the series of processes, initiated by the photoisomerization of rhodopsin, that result in closure of the channels in the plasma membrane of the photoreceptor. $R_{rod}$ is an estimate of the amplitude of the saturated response. Fitting of the model was restricted to the leading edge of the a-wave or to a maximum of 20 ms after stimulus onset.

The b-wave responses to short-wavelength flashes were also analyzed. The stimulus/response function

$$V(I) = V_{max} \frac{1}{1 + \exp \left(\frac{-I}{V_{max}}\right)} \quad (2)$$

was fit to the b-wave amplitudes of each subject. In this equation, V is the b-wave amplitude produced by flash intensity I, $V_{max}$ (μV) is the
saturated amplitude, I is the stimulus in scot td s, and \( \sigma \) is the stimulus that evokes a half-maximum b-wave amplitude. The function was fit only up to those higher intensities at which substantial a-wave intrusion occurred (\( \sim +1.0 \) log scot td s).\(^{54}\)

As established by Granit, the ERG waveform represents the algebraic sum of photoreceptor and postreceptor retinal responses.\(^{55,56}\) The isolated rod photoresponse, called \( P_r \), is modeled by equation 1. To evaluate postreceptor function, designated \( P_2 \), a putatively “pure” postreceptor response was isolated from the intact electroretinogram by digital subtraction of \( P_r \) from the record. \( P_2 \) primarily represents the bipolar cell response.\(^{57–61}\)

For \( P_2 \), the relation between flash intensity and the elapsed time between stimulus presentation and the instant at which the response reaches an arbitrary criterion voltage will be linear on a log-log plot with slope \(-0.2\) in normal retina, consistent with three stages of integration in the rod photoreceptor and three stages of integration in the rod bipolar cell.\(^{59}\) Departures from this relation indicate dysfunction of the ON bipolar cell G-protein cascade.\(^{59,60}\) We selected a 50-\( \mu \)V criterion and noted the latency at which the rising phase of \( P_2 \) reached that criterion. For a family of \( P_2 \) waves, we plotted the latency-versus-intensity relationship. To test for dysfunction, regression lines were fit, and the slope of the regression line (\( P_2 \) slope) in patients was compared with that in control subjects.

Oscillatory potentials (OPs) were extracted from the derived postreceptor response (\( P_2 \)), as described previously.\(^{62}\) In brief, \( P_2 \) was digitally filtered using a fifth-order Butterworth filter (butter subroutine; MATLAB; The MathWorks) with bandpass 75 to 300 Hz.\(^{63}\) The amplitude (\( \mu \)V) of each OP wavelet was defined as the difference between the peak and the trough immediately preceding it. To characterize the OPs, the summed amplitude of the OPs (SOPA) at each intensity was plotted as a function of stimulus energy, and the Michaelis-Menton equation

\[
SOPA(I) = SOPA_{\text{max}} \left[ \frac{P^*}{(P^* + SOPA_{1/2})^n} \right]
\]

was fit to the data. In this equation, SOPA(I) is the summed amplitude (\( \mu \)V) of the OPs in the response to a flash of I intensity, SOPA_{\text{max}} is the saturated amplitude (\( \mu \)V) of the OPs, and SOPA_{1/2} is the intensity at which the summed amplitude of the OPs is half SOPA_{\text{max}}.

**Statistical Analyses**

Preliminary analyses showed no significant difference between ACHR and BCM patients on any of the ERG parameters (\( S_{\text{rod}}, R_{\text{rod}}, \log \sigma, V_{\text{max}}, \log SOPA_{1/2}, SOPA_{\text{max}}, \) and \( P_2 \) slope; \( t \)-tests: \( df = 19; P > 0.2 \) on all tests). Furthermore, for each parameter, the range of values for ACHR and BCM patients was similar. Therefore, data from the two patient groups were pooled, and individual, independent sample \( t \)-tests for each ERG parameter were used to detect differences between patients and control subjects. The significance level for all tests was \( P < 0.01 \).

**RESULTS**

In Figure 1, sample ERG records from an ACHR patient obtained in scotopic (Fig. 1A) and photopic (Fig. 1B) conditions are shown. In all patients with ACHR or BCM, scotopic activity was observed over a \( \pm 3 \)-log unit range of intensities, whereas photopic activity was absent or markedly attenuated (\(<5\% \) of normal mean amplitude). Figure 1C shows sample fits of the model (equation 1) of the activation of rod phototransduc-
tion to the a-wave. Figure 1D shows the fit of equation 2 for determining the postreceptor (b-wave) response parameters. Note that lower values of log $\sigma$ indicate greater sensitivity; that is, lower intensity produces the half-maximum response.

Figure 2 shows $P_2$ responses derived from the records in Figure 1A and a plot of the latency-versus-intensity relationship. Scotopic OP records extracted from the $P_2$ responses shown in Figure 2 are displayed in Figure 3; OPs from a control subject are also shown.

Rod photoreceptor and postreceptor response parameters for the patients with ACHR and BCM and for control subjects are summarized in Figure 4. Amplitude parameters for rods, $R_{rod}$ ($t = -7.484$, $df = 31$, $P < 0.001$) and for postreceptor activity, $V_{max}$ ($t = -6.821$, $df = 31$, $P < 0.001$) and $SOPA_{max}$ ($t = -10.755$, $df = 31$, $P < 0.001$) were significantly lower in patients than in controls, with little overlap. For all sensitivity parameters ($S_{rod}$, log $\sigma$, and log $SOPA_{1/2}$), there was substantial overlap between patients and controls. However, b-wave log $\sigma$ in patients differed significantly from that in controls ($t = 3.152$, $df = 31$, $P = 0.0036$); in patients, higher intensity was needed to evoke a half-maximum response. In a previous study of young, healthy subjects with myopia (as high as −10 diopters), we found no differences in rod and rod-driven postreceptor response parameters between myopes and controls.64

The mean slope of regression lines fit to $P_2$ latency versus intensity plots was −0.19 (SD = 0.04) in the 12 control subjects and −0.20 (SD = 0.04) in the ACHR and BCM patients. These values were not significantly different from each other or from the normal mean slope of −0.2.59,60,65

**DISCUSSION**

In these young patients with ACHR and BCM, we have demonstrated significant deficits in rod and rod-driven function. Specifically, mean photoreceptor ($R_{rod}$) and postreceptor ($V_{max}$ and $SOPA_{max}$) amplitude parameters were reduced compared with those in normal controls (Fig. 4). Sensitivity parameters ($S_{rod}$, log $\sigma$, log $SOPA_{1/2}$) were less affected; only b-wave log $\sigma$ differed significantly from normal.

Although rod photoreceptors are not directly affected by the genetic mutations causing ACHR and BCM, it has been suggested that alterations in rod structure occur. High-resolution adaptive optics imaging of the photoreceptor mosaic in a subject with $CNGB3$ ACHR showed increased diameter of rod inner segments, possibly due to rods expanding into space that would normally be occupied by cones.66 In this subject, the density of rods at 10° eccentricity was reduced by about one-third compared with normal.66,67 Thus, the low values of $R_{rod}$ in our ACHR and BCM subjects may be attributed to a decrease in the total number of rods. Shorter rod outer segment length would also reduce $R_{rod}$. To our knowledge, rod outer segment length has not been measured in ACHR or BCM.

Only one other study10 quantitatively investigated rod activation in patients with ACHR. Khan et al.10 evaluated four

![Figure 3](https://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/933448/ on 11/06/2018)
adults with \textit{CNGB}3 ACHR who showed macular atrophy in middle age. Their rod photoreceptor and postreceptor amplitude parameters fell within the range of values observed in our patients. Our patients were younger than theirs (Table 1), yet most showed greater deficits. We observed neither fundus abnormalities nor progressive worsening in visual acuity or dark-adapted visual thresholds in our patients. We wonder, therefore, whether the alterations in rod and rod-driven function may indicate anomalies in rod pathway signaling rather than rod disease. Persons with ACHR and BCM prefer dim environments, which would increase the metabolic load placed on the rods. This, in turn, would result in more circulating current, which would require more energy with possible adverse long-term effects on rod function. Thus, the low calculated values of $R_{rod}$ could be due to fewer rods, shorter rod outer segments, or defective rod functioning.

In addition to the significant deficit in rod photoreceptor amplitude, we observed deficits in postreceptor response parameters ($V_{max}$, log $\sigma$, and $SOPA_{max}$). According to an explicit model, changes in $R_{rod}$ are predicted to alter b-wave sensitivity (log $\sigma$) but to have little effect on $V_{max}$ \cite{58,68}. In our patients, mean log $\sigma$ and mean $V_{max}$ were both approximately half the values in controls. The low $V_{max}$ could be caused by too few rod-driven bipolar cells. Although we are unaware of any anatomic evidence that the number of rod bipolar cells is reduced in ACHR or BCM, the reduced rod density found in the subject with ACHR\cite{66} may be accompanied by a proportionate reduction in rod bipolar cell density. In another system (immature simian central retina), the numbers of cones and cone bipolar cells are proportionately decreased.\cite{69} Another possible explanation for the reduction in $V_{max}$ that is consistent with the explicit model\cite{58,68} is a postreceptor change resulting from abnormal function of rod bipolar cells. However, the normal $P_2$ latency versus intensity slope (Fig. 2) indicates that, at the least, the G-protein amplification cascade in the rod bipolar cell was operational.

Our data do not allow us to exclude the possibility that there is some alteration of the rod-driven circuitry in ACHR and BCM. Reorganization of the postreceptor retina is a well-documented consequence in a number of photoreceptor disorders.\cite{62,70-73} The normal scotopic pathway is dominated by the rod-specific hyperpolarizing bipolar cell.\cite{74} In addition to this primary pathway, there are anatomic connections between rods and cones and some between rods and depolarizing cone bipolar cells.\cite{75-81} We speculate that the latter contacts may be more numerous in cone-deficient ACHR and BCM retinas. This
would allow substantial rod input to cone-depolarizing bipolar cells, with consequent reduction in the apparent postreceptor response from the primary rod pathway in ACHR and BCM. In a CNGA3−/− mouse model, anomalous synapses between rods and cone bipolar cells are documented.82

OPs are affected by inputs from both rods and cones.83,84 In ACHR and BCM retinas, cone input is absent or greatly diminished, possibly accounting for the dramatic attenuation in SOPs measured in our patients.

Whatever the actual mechanisms, the ERG data reported herein add evidence that deficits in rod and rod-mediated function occur in the primary cone dysfunction syndromes ACHR and BCM. Although it is well established that cones are adversely affected in primary rod disorders,82,85–91 there is less evidence that rods are affected in disorders with primary cone dysfunction.8,95,96

Each of the possible mechanisms for abnormal retinal function considered leads to hypotheses that can be tested by further ultra-high resolution imaging of persons with ACHR and BCM and by study of animal models.82,92–94 The new knowledge obtained will bolster efforts to design and evaluate effective therapies for cone dysfunction syndromes.95,96

References
Rod Function in Achromatopsia