

## **Color electroretinography** *A method for separation of dysfunctions of cones*

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**Abstract.** Electroretinograms to white and color stimuli were recorded in four normal subjects and nine subjects with different cone dysfunctions, including protanopia, cone dystrophy, cone dystrophy with supernormal b-waves at dark adaptation, cone dystrophy with missing b-waves during light adaptation and rod-cone dystrophy with blue cone hypersensitivity. Color stimuli were obtained with Kodak Wratten filters in blue, blue-green, green, yellow and red. Electroretinograms to all stimuli were recorded during dark and light adaptation with different stimulus intensities and to 30-Hz flicker stimulation. In protanopia, responses to red during light adaptation and flicker stimulation were reduced. All cone dystrophies showed reduced amplitudes and prolonged implicit times to red when dark adapted. The light-adapted responses were equally reduced to all color stimuli in cone dystrophy and cone dystrophy with supernormal b-waves. Contrary to other cone dystrophies, in cone dystrophy with missing b-waves, responses to red were severely reduced and responses to green were preserved, indicating a predominantly red cone dysfunction. Blue cone hypersensitivity was clearly distinct from other dystrophies in having large responses to blue and blue-green and much smaller responses to all other colors in all stimulus conditions. The electroretinogram with color stimuli allowed separation of different cone dysfunctions and identification of new retinal dysfunction syndromes.

### **Introduction**

Cone dystrophies as a clinical entity with typical electroretinographic features were defined by Goodman and coworkers in 1963 [1]. Although photophobia, visual loss, central scotomas and defective color vision were characteristic clinical findings, Goodman et al. emphasized that an accurate diagnosis requires an electroretinographic (ERG) recording. The ERG showed reduced or missing responses at light adaptation and flicker stimulation and, to a lesser extent, reduced responses to bright stimuli at dark adaptation. The responses to lower stimulus intensities at dark adaptation were normal. Since then, several authors have described cone dystrophies with all kinds of inheritance, variable clinical findings, variable progression and similar ERG findings [2–4]. However, a separation of short-, middle- and long-wavelength-sensitive cones is not possible with an ERG elicited by white stimuli.

In 1929, Sachs [5] demonstrated that ERG responses to red stimuli were reduced in protanopes. Since then, congenital color vision deficiencies have been investigated by means of color stimuli in the ERG, and, in fact, a loss of green cone function in deuteranopia has been demonstrated in the ERG earlier than with psychophysical techniques [6]. Although the use of color stimuli in the ERG was common in color vision research [7, 8], they have been used only rarely in the examination of cone dystrophies. Some investigators used dim-blue stimuli at dark adaptation to separate rod responses [4, 9]. Matched stimuli have been used for detection of cone dysfunctions [3, 9]. These methods do not allow a separation of short-, middle- and long-wavelength-sensitive cones.

By recording ERGs to color stimuli, we detected patients suffering from cone dystrophies with different involvement of short-, middle- and long-wavelength-sensitive cones in the degenerative process. Therefore, we describe a simple method for a detailed examination of patients with cone dysfunctions.

### Methods

The ERG recording method has been described in detail [10, 11]. Recordings were performed after 30–40 minutes of dark adaptation with maximally dilated pupils. The recording protocol included all recordings according to the standard of ERG recording [12]. Six different light intensities increasing by 1 logarithmic unit in steps from the b-wave threshold of the normal eye were used for the dark-adapted recordings. The maximum light intensity was  $7.8 \text{ cm} \cdot \text{s}/\text{m}^2$ . The light-adapted recordings were performed under white light adaptation with  $4.5 \text{ cd}/\text{m}^2$  and with the light stimuli at the three highest levels. The 30-Hz flicker stimulus had a level 5 light intensity. White light from a filtered xenon light source served as stimulus in all examinations. Although this recording protocol included more recordings than the ERG standard [12], we will call it standard ERG in this report.

In addition to the recordings with white stimuli, we used Kodak Wratten filters for color stimuli (color-stimulated ERG). The filters used were Kodak Wratten No. 98 for blue with a maximum transmission at 450 nm, 44A for blue-green (492 nm), 61 for green (538 nm), 16 for yellow (589 nm) and 29 for red (629 nm). The same background as in the standard ERG was used for light adaptation. The 30-Hz flicker stimulus had a level 6 light intensity. Recordings were done in accordance with the same protocol as with white light. No averaging was done in either the standard or color ERG except for the oscillatory potentials (64 sweeps).

Normal values were obtained by examining one eye of four persons with normal visual acuity and refractive error less than 2 diopters. In all patients, both eyes were examined with standard and color ERGs. There was no

difference between the two eyes of each patient, and, therefore, oscillatory potentials were recorded in only one eye of each patient.

### **Patients**

Nine patients were examined. One patient suffered from protanopia, three had different types of cone dystrophies (cone dystrophy, cone dystrophy with supernormal b-waves at dark adaptation and cone dystrophy with missing b-waves at light adaptation) and five had rod-cone dystrophy with blue cone hypersensitivity. The clinical findings and results of the standard ERG recording in these patients are summarized in the following overview.

*Protanopia.* A 38-year-old man had normal visual acuity and normal ophthalmoscopic findings in both eyes. Color vision tested with Ishihara plates and Nagel's anomaloscope revealed a protanopia. The standard ERG was normal at all recording conditions.

*Cone dystrophy.* A 15-year-old girl complained of progressive decrease of visual acuity, which had been about 0.8 at the age of 8 years and was 0.05 in both eyes at our first examination. Funduscopy revealed a bull's-eye maculopathy. Central scotomas were found in the static visual field, and an achromatopsia was detected with Ishihara plates, panel D15 test, and Nagel's anomaloscope. The standard ERG showed mildly reduced responses when dark adapted and severely reduced responses at light adaptation and flicker stimulation.

*Cone dystrophy with supernormal b-waves at dark adaptation.* The clinical findings and standard ERG recordings in this man have been described before [10]. He was 23 years old when he underwent reexamination including color-stimulated ERG recording. He had reduced visual acuity of 0.2 in the right eye and 0.3 in the left eye. The fundus and visual fields were normal in both eyes. An achromatopsia was found by means of Ishihara plates, Farnsworth-Munsell 100-Hue test and Nagel's anomaloscope. The standard ERG was unusual in the dark-adapted recordings. The a-wave amplitudes and latencies were normal. The b-waves were markedly increased in amplitude and prolonged in implicit time. At light adaptation and flicker stimulation, all responses were reduced.

*Cone dystrophy with missing b-waves at light adaptation.* This man had been observed for 22 years; he was 40 years old when the color-stimulated ERGs were recorded. He complained of slowly progressive visual loss from 0.5 in the right eye and 0.4 in the left to 0.4 in the right eye and 0.3 in the left during follow-up. Mild pigment irregularities were seen at the fundus of both eyes, and central scotomas were present in the visual field. Color vision

testing revealed a protanomaly at first examination, which developed into achromatopsia over 18 years, tested with Ishihara plates, Farnsworth-Munsell 100-Hue test and Nagel's anomaloscope. The standard ERG showed normal a-wave amplitudes and reduced b-wave amplitudes when dark adapted. At light adaptation, a-waves were present, but b-waves were not detectable. The flicker responses were small.

*Rod-cone dystrophy with blue cone hypersensitivity.* Some of the clinical findings and standard ERG recordings of three of the patients suffering from this new retinal degeneration have been described before [13]. In this study, the results of all five patients studied in Germany up to now are included. Visual loss varied between 0.8 and 0.05. The visual fields were normal. Color vision, tested with Ishihara plates, panel D15 test, Farnsworth-Munsell 100-Hue test and Nagel's anomaloscope, was normal in all patients. The standard ERG showed similar responses to the same stimulus intensity at dark and light adaptation. The b-wave implicit time was constant and prolonged at all stimulus conditions. The b-wave threshold was elevated, and 30-Hz flicker responses were reduced.

## Results

Figures 1 and 2 summarize the data of all patients. The area within the dotted lines shows the mean normal value  $\pm 2$  standard deviations. The values given for the blue cone hypersensitivity are the means of all five patients.

### *Dark adaptation*

The a-wave amplitudes at dark adaptation at stimulus intensities 5 and 6 are shown in Fig. 1a and 1b. The a-wave amplitudes were normal for all colors in protanopia and cone dystrophy with supernormal b-waves. In cone dystrophy, all responses were severely reduced. In cone dystrophy with missing b-waves, a-wave amplitudes were reduced for red at both stimulus intensities and for yellow at stimulus intensity 5; all other responses were normal. Patients with blue cone hypersensitivity had normal responses to blue, slightly reduced responses to blue-green, and severely reduced responses to all other colors.

The b-wave amplitudes at dark adaptation are shown in Fig. 1c and 1d. The patient with protanopia had normal amplitudes at a low stimulus intensity and borderline responses at maximum stimulus intensity. The patient with cone dystrophy and supernormal b-waves showed increased amplitudes to all color stimuli except for normal amplitudes to red. In the other cone dystrophies, responses to all stimuli were reduced. The response was smaller to red in cone dystrophy and cone dystrophy with missing

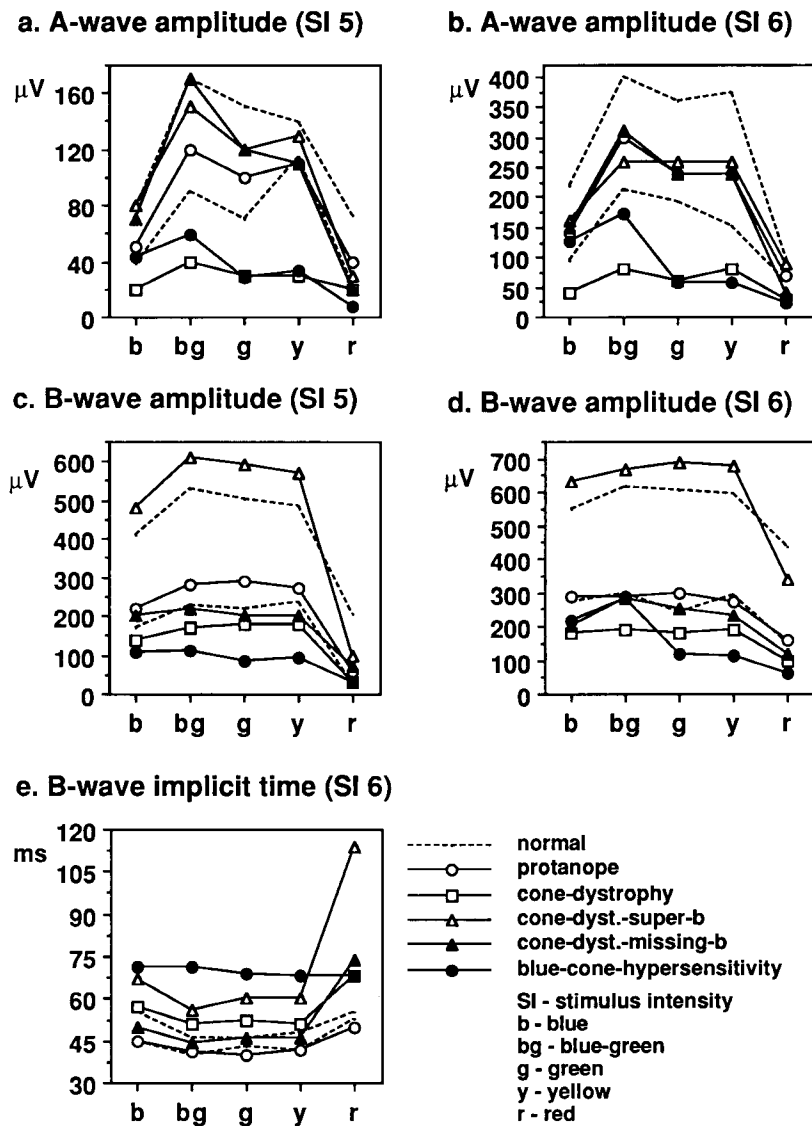


Fig. 1. Color ERG at dark adaptation. The dotted lines indicate the normal range including  $\pm 2$  standard deviations of the normal mean value. The results given for blue cone hypersensitivity are the mean of all five patients.

b-waves. In blue cone hypersensitivity, all responses were reduced, but responses to blue and blue-green were larger than to green, yellow and red.

The b-wave implicit times had a very small normal range (Fig. 1e). In protanopia, it was normal to all colors. A marked prolongation to red was found in all cone dystrophies. In contrast to the other cone dystrophies, the

implicit times to all other colors were normal in cone dystrophy with missing b-waves. In blue cone hypersensitivity, the implicit time was prolonged and constant for all colors.

*Light adaptation*

The a-wave amplitudes at light adaptation at stimulus intensities 5 (Fig. 2a)

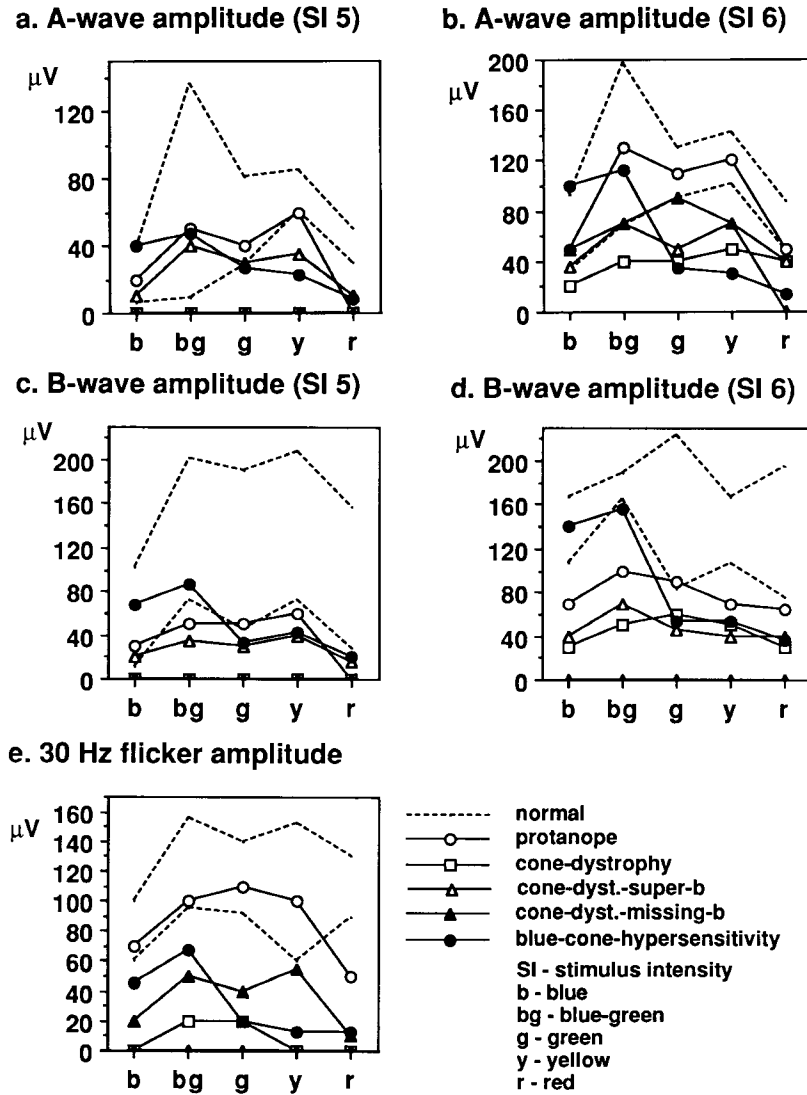


Fig. 2. Color ERG at light adaptation and 30-Hz flicker stimulation. For further details see Fig. 1.

and 6 (Fig. 2b) showed reduced values for most colors and patients. The patient with protanopia showed no response to red at lower stimulus intensity and a borderline amplitude at maximum light intensity. All of his other responses were normal. In cone dystrophy, no responses were measurable at lower stimulus intensity, and all responses were reduced at maximum stimulus intensity. In cone dystrophy with supernormal b-waves, responses were in the lower normal range or reduced at lower stimulus intensity and reduced to all colors at maximum stimulus intensity. The patient with cone dystrophy and missing b-waves showed no responses at lower intensities. At maximum light intensity, his responses were borderline for blue-, blue-green and green, reduced for yellow and missing for red. In blue cone hypersensitivity, the responses to blue and blue-green were normal and those to all other colors were reduced.

The b-wave amplitudes at light adaptation (Fig. 2c and 2d) were subnormal for the patient with protanopia. He had a response to red only at maximum stimulus intensity. In cone dystrophy, small responses were detectable only at maximum stimulus intensity. The patient with supernormal b-waves showed reduced responses to all colors. In cone dystrophy with missing b-waves, there were no measurable b-waves. Patients with blue cone hypersensitivity had normal responses to blue and blue-green and markedly reduced responses to all other stimuli.

### *30-Hz Flicker*

The 30-Hz flicker responses were at the lower-normal limit in protanopia, except for red (Fig. 2e). No responses were measurable in cone dystrophy with supernormal b-waves. In cone dystrophy, there were only small responses to blue-green and green. In cone dystrophy with missing b-waves, all amplitudes were reduced, but the response to red was much smaller than that to green. In blue cone hypersensitivity, responses to blue and blue-green were present in all patients. Only one of the five patients had a small response to red.

### **Discussion**

Most patients included in this study had unusual findings to white stimuli in the standard ERG. About 15 cases of cone dystrophies with supernormal dark-adapted b-waves have been described and reviewed recently [10]. Young et al. [14] mentioned one and Wakabayashi [15] three cases of cone dystrophies with missing b-waves at light adaptation. However, some of their findings were not in accordance with the clinical and electrophysiologic signs found in our patient. A rod-cone dystrophy with blue cone hypersensitivity is a recently identified entity [13]. The recording of ERGs to color stimuli explained the observed unusual standard ERGs and provided a

technique for easy identification of those patients [16,17]. The value of color-stimulated ERGs in blue cone hypersensitivity prompted this study of patients with different cone dysfunctions.

Color-stimulated ERG provided additional findings over the standard ERG in all patients. The patient with protanopia had a normal standard ERG. His color-stimulated ERG showed responses to red only at maximum stimulus intensity when light adapted. The red response showed a reduced amplitude, as did the red flicker response. The responses to all other colors were normal. This is in accordance with ERG recordings in patients with protanopia described previously [8].

In cone dystrophy, responses to all colors were moderately reduced with dark adaptation and severely reduced with light adaptation. The implicit times were prolonged to all colors with dark adaptation. The response to red showed a more reduced amplitude and more prolonged implicit time than with the other colors at dark adaptation. Flicker responses were small or not detectable. In cone dystrophy with supernormal b-waves, the color-stimulated ERG recordings showed features similar to those of cone dystrophy, except that the reduction of amplitudes was less severe. The similarity suggests that the functional defects involving the cones may not be different between these diseases. Therefore, the difference that results in the supernormal dark-adapted b-waves seems to be limited to the rod pathway. The ERG findings in cone dystrophy with supernormal b-waves are comparable to results in two similar patients examined with computer-assisted spectral electroretinography using four different color stimuli [18]. However, that report is the only one using more than two color stimuli for the evaluation of the cone receptors in humans, and only a comparison to normal subjects, not to other patients with cone dystrophies, was given.

In cone dystrophy with missing b-waves, the amplitudes to red stimuli were markedly reduced and the implicit times prolonged. The flicker response to red was smaller than to the other colors. Oscillatory potentials could be recorded to all colors except to red. In contrast to the other cone dystrophies, there was a difference between the responses to red and green. In cone dystrophy with missing b-waves, responses to green were larger than to red with light adaptation or with flicker stimulation, whereas in the other cone dystrophies responses to both colors were reduced to the same extent. Moreover, the implicit times with dark adaptation were prolonged for all color stimuli in cone dystrophy and cone dystrophy with supernormal b-waves, but only prolonged to red and normal to all other colors in cone dystrophy with missing b-waves.

The severe amplitude reduction to red and markedly prolonged implicit time with dark adaptation are characteristic findings in all cone dystrophies and are explained by the missing cone contribution to the response. The rods are much less sensitive to red than to all other colors, and therefore the remaining response is small and has a long b-wave implicit time. The prolonged implicit times to all other color stimuli are also due to the missing



cone contribution to these responses. The normal dark-adapted implicit times in cone dystrophy with missing b-waves indicate a cone contribution to all responses except to the one elicited by red. In cone dystrophy with missing b-waves, the red cones seem to be mainly affected, and the green cones seem to be relatively well preserved. These electrophysiologic findings correlate with the clinical history of a protanomaly slowly progressing to achromatopsia with a visual acuity remaining at 0.4 in the right eye and 0.3 in the left eye. The color-stimulated ERG findings in this patient could also be verified psychophysically. Spectral sensitivity measurement revealed normal blue and green cone sensitivity, but no red cone sensitivity. A detailed description of the family history, clinical, electrophysiologic and psychophysical findings will be presented in a subsequent article. The cone dystrophy with missing b-waves therefore may preferentially involve red cones. This retinal dystrophy can easily be distinguished from protanopia, where only the responses to red with light adaptation and flicker stimulation are reduced and the dark-adapted response to red shows a normal implicit time. Reichel et al. [3] described a patient with X-linked cone degeneration with predominant loss of red cone function and a molecular defect in the red pigment gene. However, family examinations in our patient indicated an autosomal dominant inheritance, and the clinical and electrophysiological findings were not identical.

The blue-cone hypersensitivity syndrome is clearly separated from all other diseases by large responses to blue and blue-green and small or no responses to the other colors at all stimulus conditions. The color-stimulated ERG, therefore, is the main diagnostic tool to detect this kind of retinal degeneration. The long b-wave implicit time found in all recording conditions is comparable to implicit times of blue cone responses measured with other recording techniques [19]. In addition to the long b-wave implicit times and the larger responses to blue than to red, a further clue for the blue cone origin of these responses is provided in the color-stimulated ERG. We found 30-Hz flicker responses to blue stimuli in all patients, but only a much smaller response to red in one patient. Higher-frequency flicker responses to blue stimuli have also been described in blue cone monochromatism [20].

The international standard for ERG recording recommends white light for all stimuli [12]. Several ERG recording techniques using color stimuli have been described. As stated by Reichel et al. [3], photopically matched stimuli [9] cannot sufficiently separate all three cone systems. Mehaffey and Berson [21] separated the short-, middle- and long-wavelength cones with color stimuli superimposed on bright chromatic adapting fields in monkeys. Computer-assisted spectral ERG with four different color stimuli [22] or a tricolor light source [23] was proposed for human ERG and applied in normal subjects [22, 23] and vitrectomy candidates [22]. None of the techniques mentioned before have been used in patients with different cone dystrophies.

One disadvantage of using Kodak Wratten filters for color stimuli is their

broad spectrum, which does not allow a selective stimulation of short-, middle- or long-wavelength-sensitive cones. Monochromatic filters would be preferable, but they require intensive light sources to obtain stimuli of sufficient strength. The advantage of Kodak Wratten filters is that they are inexpensive and easy to install. Using this simple setup with five different Kodak Wratten filters and a range of stimulus intensities, we could separate cone dysfunctions and detect cone dysfunction syndromes unknown before. We did not adjust the different color filters with neutral filters to have the same light intensity. Multiple stimulus intensities were necessary because some deficits showed up only at low or high stimulus intensities. We recommend the use of at least three color stimuli (blue, green and red) in patients with cone dystrophies for a separation of clinical entities on the basis of the functional retinal deficits found in the ERG.

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