Electroretinographic Responses of the Short-Wavelength-Sensitive Cones

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An electroretinographic response of the human short wavelength (S) cone system can be distinguished from that of the longer wavelength (L or M) cone system by using ganzfeld short-wavelength stimulation at relatively high levels of retinal adaptation. The S cone response has both an a- and b-wave component in its ERG, both of which are slower than those of the L or M cone response at the same level of retinal adaptation. Proof that this is the S cone response is obtained by action spectra and by examination of a sex-linked achromat who is known to have only S cone and rod vision. This approach allows the simultaneous and rapid assessment of both the S and the L or M cone systems in the human retina using conventional electroretinogram (ERG) equipment, ganzfeld blue flashes on a white background, and computer averaging. Invest Ophthalmol Vis Sci 31:1203–1209, 1990

This paper demonstrates a method to examine short wavelength (S)- and longer wavelength (L or M)-sensitive cone responses in the human electroretinogram (ERG) simultaneously and quickly with a ganzfeld stimulator using blue test flashes on a strong, white adapting field. Under these circumstances the rods are saturated and the ERG of the long (L)- or middle (M)-wavelength-sensitive cones is much earlier than that of the S cones so that both responses can be distinguished with the same flash.

Most of the other methods used to examine the S cone ERG have involved Maxwellian viewing systems. These require that attention be given to eye position so that the beam remains properly aligned in the pupil. Rod saturation is not always guaranteed because much of the retina is not covered by the adapting beam. The few studies using ganzfeld stimululi have used a small diffuser on or directly in front of the eye so that the ocular alignment must also be carefully monitored. Almost all methods have used a blue test flash on a yellow adapting field to selectively reduce the response of the other cones. Our method examines both major cone mechanisms in the human retina simultaneously and at the same state of retinal adaptation, with equipment that can be found in any ERG laboratory.

Materials and Methods

Our methods for recording the ERG have been described previously. A Burian Allen bipolar contact lens electrode, usually one in each eye, was used to record the responses. The pupils were widely dilated, 8 mm in diameter, and measured before the recording session to determine retinal illumination. Both ganzfeld test and adapting fields were used. Wratten filters were placed before the strobe (Grass Instruments, Quincy, MA) in order to obtain defined spectral stimuli. The following filters were selected for determining the action spectrum of the ERG: Wratten filters 36 (415 nm), 98 (430 nm), 50 (450 nm), 48 (460 nm), 45 (480 nm), 75 (490 nm), 61 (520 nm) and 74 (530 nm), 73 (570 nm), 29 (630 nm), and 72B (600 nm). The irradiance of the most frequently used blue test flash (Wratten 98) is approximately 0.02 µW/flash. The numbers in parenthesis indicate the nominal wavelength of maximum transmission of each filter. The energy transmitted by each filter was measured with a Tektronix (Beaverton, OR) digital photometer with a calibrated irradiance probe. These energies could be reduced stepwise by Wratten neutral density filters. The steady adapting light came from a 9-V, 80-W halogen projector lamp; the voltage and current across the lamp was monitored continuously and the luminance it produced on the white interior of the ganzfeld measured by the same digital photometer with a luminance probe. The retinal illuminance was expressed in photopic trolands although it was known that the retinal illuminance varies with

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pupillary aperture according to the cosine of the visual angle. Approximately 100–1000 responses to the same flash presented at 5.1 Hz are averaged with a Nicolet (Madison, WI) CA-1000 computer with artifact rejection and a band pass of 5–1500 Hz.

Thirteen normal subjects, ranging in age from 11 to 78 yr old, and one blue cone monochromat were studied. The blue cone monochromat was a 21-yr-old male with best-corrected visual acuity of 20/70 with −5.00 diopters (right eye) and 20/100 with −6.25 diopters (left eye). His photopic spectral sensitivity was maximum at 440–460 nm, with almost no sensitivity at longer wavelengths; his scotopic sensitivity was normal. The spectral sensitivity curves were determined with a 2° test spot covering the fovea on a 5000 photopic troland background (photopic) and no background (scotopic). He made numerous protan and deutan crossing errors when tested with the Farnsworth Panel D-15. A report on his spectral ERG has been published previously.

In measuring the b-wave of the S cone ERG, we used the lowest point between the falling phase of the L or M cone b-wave and the rise of the S cone b-wave (see Fig. 5). This is usually an easy measurement at high backgrounds but tends to be more difficult as the background is reduced. We have recently found that with our Grass strobe flash, a Wratten 72B (orange), produces approximately the same L or M cone ERG as the Wratten 98 (blue) but without generating an S cone b-wave. It may be possible to subtract the former from the latter response to isolate the S cone ERG under these conditions. However, this calculation would assume superposition of these responses, which is unproven.
Results

Figure 1 illustrates corneal ERG responses to a short (450-nm) and a long (600-nm) wavelength flash at different flash intensities in the presence of a white adapting field. The short wavelength responses had a large, later positive b-wave that was absent with long wavelength flashes. Both wavelengths produced an earlier b-wave, but this was much larger with long-wavelength than with short-wavelength flashes. If the energy of the long-wavelength flash was reduced to the point (Fig. 1, bottom trace) where the quicker b-wave was approximately the same amplitude as it was to the short-wavelength flash (Fig. 1, top trace), then the greater effectiveness of short-wavelength stimuli for generating the late b-wave rather than the early b-wave could be better appreciated.

In order to identify the mechanisms generating these early and late b-waves, we used seven spectral Wratten filters to determine the action spectra based on a constant response (2.5 μV) criterion (Fig. 2). The later b-wave had its lowest threshold at 450 nm and was undetectable at wavelengths longer than 500 nm, a pattern indicative of an S cone response. The early b-wave, on the other hand, could be elicited by all spectral lights but had its lowest threshold at 540 nm, a pattern indicative of an L or M cone response. The L or M cone response was about 1 log unit less sensitive to short wavelengths, implying that the S cones do not make a strong contribution, and probably make no contribution at all, to the early b-wave.

The slope of the intensity/amplitude function of the early b-wave was greater than that of the late
b-wave (Fig. 3), a characteristic of the S cone response seen in other retinas.\(^1\) \(^9\) The difference is believed to be due to the fact that the number of ERG generators is larger for the L or M than for the S cone mechanism. The larger the number of generators contributing to a response, the steeper will be the relationship between ERG amplitude and light intensity.

The implicit time of the S and L or M cone b-waves was measured in 15 normal subjects ranging in age from 11 to 66 yr; all were adapted to a retinal illumination of 9000 photopic trolands. The implicit time of the S cone ERG b-wave was 38.2 msec (SD = 2.5); that of the L or M cone b-wave was 23 msec (SD = 1.1). There was no overlap between these implicit times.

This S cone ERG could be distinguished only at relatively high levels of retinal adaptation. Figure 4 illustrates how the ERGs of the L or M and S cones emerged from a mixed cone/rod ERG, as the strength of the adapting field was increased. Two discrete positive b-waves, labeled L/M and S, could be identified at the higher levels of retinal adaptation. The separation of the S from the L or M cone response became greater as the adaptation level increased.

Figure 5 illustrates how the S cone b-wave was measured. Parallel lines are drawn between the S cone b-wave and the lowest point after the peak of the L or M cone b-wave. The amplitude and implicit time were measured at the peak of the S cone b-wave. This measurement tended to become more difficult at dim backgrounds. Figure 6 illustrates this phenomenon in another subject. This figure shows that as the strength of the white adapting light increased, the amplitude and the implicit time of the L or M cone response decreased proportionately more than those of the S cone response.

Figure 7 shows the average amplitude (left) and implicit time (right) of the L or M and the S cone b-waves obtained with blue flashes in 13 normal subjects. The amplitude of the L or M cone b-waves

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**Fig. 5.** Electroretinograms obtained with a blue flash (Wratten 98) at different levels of retinal adaptation, indicated by photopic troland values on the left of each trace. The hatched lines shows how we measured the S cone b-wave. The calibration, lower right, signifies 5 μV vertically and 10 msec horizontally. Corneal positivity is upwards on the trace.

**Fig. 6.** Electroretinograms obtained with a blue flash (Wratten 98) at different levels of retinal adaptation. The retinal illumination is indicated by the photopic troland values to the left of each trace. The dashed lines connect the implicit time of the L or M and the S cone b-waves. The calibration, lower right, signifies 5 μV vertically and 13 msec horizontally. Corneal positivity is upwards on the trace.
increased, whereas that of the S cone b-waves decreased as the level of adaptation decreased. Part or all of this decrease may have been due to a swamping of this response by that of the L or M cone ERG. The implicit time of the S cone b-wave decreased slightly, but that of the L or M cone b-wave decreased considerably as light adaptation increased. This behavior of the L or M cone response matched that found with red flashes.\textsuperscript{13}

In order to clarify further our interpretation that this late b-wave response was due to the S cones, we tested a sex-linked achromat who had no L and M cones, but only S cones and rods. Figure 8 shows how light adaptation changed his ERG responses to a blue and a green flash, the latter approximately 0.6 log units more effective for rod vision. At a background level of 5000 photopic trolands, little to no response could be elicited with the green flash, whereas a late b-wave was produced by a blue flash. This must have been the response of the S cones, because at this point the action spectrum for this subject's vision matched that of the S cones.
Figure 9 illustrates the responses of a normal subject to red and blue test flashes at sufficient level of white light adaptation to saturate any rod response. The middle trace is the response of the sex-linked achromat to the blue flash under the same circumstances. The late b-wave of the achromat matched the late b-wave to blue light of the normal subject. The achromat's ERG revealed what was not as easy to demonstrate in the normal response: that there was an a-wave component to the S cone ERG, which was also later than that of the L or M cone ERG.

**Discussion**

The results show that a blue light flash (450 nm) on a bright white background produced two different cone responses in the normal retina, one relatively early, which was generated by the L or M cone mechanisms, and a later ERG generated by the S cones. It is therefore possible to examine both of these cone systems simultaneously in the same ERG as long as the retina is sufficiently light adapted. Light adaptation does at least two things to facilitate this phenomenon. It saturates the rod response, and it speeds up the L or M cone more than the S cone ERG so that these two distinct responses become more separated in time. The greater the light adaptation, the greater the separation between the S and the L/M responses. We believe that this is due to the fact that the L/M cones absorb a greater fraction of the white adapting light than the S cones, and therefore are proportionally more light-adapted as the strength of the adapting field increases. An alternate, or additional, explanation is that the S cones simply do not speed up as effectively as the L/M cones as background illumination increases, and in fact may approach saturation. The short wavelength flash is important because it is a strong stimulus for the S cones and a relatively weak stimulus for the L and M cones. This keeps both responses at similar amplitudes. Using longer wavelengths or white light as the test flash would generate much larger responses from the L and M cones because they are so numerous in the retina.

This technique resembles the technique used to separate rod from cone components in the dark-adapted human ERG. The rod response, like the S cone response, is slower than the L or M cone response. Using a long wavelength (red) flash favors the L and M cones over the rods because the latter are relatively insensitive to long wavelengths. Shorter wavelengths or white light would produce larger responses from the rods because they are so numerous in this retina.

The conventional way of detecting S cone responses in usually a strong yellow adapting field. This preferentially desensitizes the L and M cones as well as the rods, and leaves the S cones much less light-adapted and consequently more responsive. Our recent research on the effects of light adaptation on the ERG required stronger full field adapting lights than had ever been used in human electroretinography. It was this research that led to the finding that the S cone response in the mixed cone ERG is more exposed by stronger levels of white light adaptation, as demonstrated in the current paper. The use of a white adapting field obviates the need for any specific chromatic filter and is inherently stronger than any filtered field. Consequently, rod responses can be eliminated more effectively. We have recently used a Corning yellow glass filter in front of our adapting light to compare how much better it was than white light for exposing the S cone ERG. It was only slightly better but even with a yellow adapting field, it is impossible to eliminate the L or M cone response with suprathreshold flashes. The S cone ERG may be isolated at threshold, but this is much less relevant electrophysiologically. The most effective way to isolate the S cone ERG at suprathreshold levels of stimulation would be to substitute spectral stimuli, which are equivalent for the L or M but not for the S cones. However, this is technically difficult, especially with ganzfeld testing conditions. For clinical purpose we believe it is more informative and more rapid to examine both the S cone and the L or M cone response simultaneously with the same flash.

The greater delay in the S cone response is a noteworthy phenomenon. It is apparent not only in the flash ERG but also in the pattern ERG and in the
visually evoked response (VER).

This paper shows that the S cone ERG has both a- and b-wave components. The fact that both the a- and the b-wave exhibit this delay implies that the photoreceptor cell is responsible for it. There is evidence, however, that the outer segments of the S cones respond as fast as L or M cones. In addition, the PIII component to blue light in ground squirrel retina, considered to be generated by S cones, has the same time course as that generated by the longer-wavelength-sensitive cones, indicating that the delay is postreceptoral. Further research should clarify this intriguing point.

**Key words:** electroretinogram, cones, short wave sensitive cones

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