Clinical Electroretinography For Short Wavelength Sensitive Cones

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We measured electroretinograms (ERGs) for the isolated short-wavelength-sensitive (SWS) cones using a substitution technique. The stimulus was a 5 Hz alternation of 460 nm and 565 nm or 490 nm and 565 nm light of equivalent photopic luminous efficiency. We used a 571 nm narrow-band adaptation field of 7000 td to improve SWS cone isolation and to suppress rod activity. The resulting SWS cone ERG amplitudes were 10–30 μV with latencies of 60–80 msec. A rapid clinical protocol to assess the radiance response function of the SWS cone ERG is described. Invest Ophthalmol Vis Sci 28:966–974, 1987

Many retinal diseases are accompanied by acquired blue-yellow color vision defects, a generalization recognized by Köllner. However, subtle acquired blue-yellow color vision defects accompany a larger variety of ocular disorders. Such findings have led to the impression that the "blue" or short-wavelength-sensitive (SWS) cone system is particularly sensitive to disease or trauma. It would therefore be useful to have an electroretinographic method for evaluation of the SWS cone system that would be amenable to large-scale clinical application.

The SWS cones represent less than 10% of the cones and less than 0.5% of the total receptors. Thus, the development of clinical SWS cone electoretinography requires not only retrieval of small signals, but also techniques to suppress the stronger rod and cone signals. Chromatic adaptation was the approach chosen by the laboratories that have published SWS cone ERGs in monkey, cat, and human. The waveform of the SWS cone ERG is of the excitatory type. There is a b-wave to stimulus onset with a negative off-effect at offset. The amplitudes of the human SWS cone ERG range from 0.5–12.0 μV with b-wave implicit times (when reported) of 45–65 msec.

Our aim was to develop a technique to record the SWS cone ERG that would be amenable to clinical application. The use of intense adapting fields is not optimal with patients. Further, some SWS cone adaptation can be expected at high adapting levels even with an adapting spectrum limited to long wavelengths. This factor, plus the necessarily high stimulus levels, might suppress the excitatory SWS cone ERG. For the technique to be useful clinically, though, we wished to maximize the amplitude of the response in normal observers.

We therefore adopted another technique, one related to the procedure called "silent substitution". Silent substitution is a method of isolating cone systems. For example, suppose we wish to isolate the middle-wavelength-sensitive (MWS) cones. Two wavelengths above 550 nm are chosen, eg 550 nm and 650 nm. Their radiances are adjusted to provide equal retinal catch for the long-wavelength-sensitive (LWS) cones. These lights would have greater quantal efficiency at 550 nm by a factor of 11.45 for the MWS cone. If the two wavelengths are alternated, or substituted, in time, the MWS cone would have a contrast of 84% while the contrast for the LWS cone would be zero; in other words, the substitution is silent for the LWS cone. For isolation of SWS cones from LWS and MWS cones, the ideal choice of wavelengths is a "tritan" pair; for example, 430 nm and 500 nm which when matched in photopic luminous efficiency and presented in temporal alternation will stimulate SWS cones with a contrast of 97%, but silence MWS or LWS cones. In practice, a pair of lights, one near 450 nm and one above 520 nm, matched in luminance, will provide a strong contrast stimulus for SWS cones but a greatly reduced contrast for either the MWS or LWS cone types. This technique cannot simultaneously silence two cone types and rods, unless a more complicated paradigm involving pairs of alternating lights is used. However, the rod ERG saturates at relatively low photopic luminance levels, so moderate photopic adapting...
fields (or the time-average luminance of the substitution field) can provide light adaptation of the rods. The adapting field will also suppress residual MWS and/or LWS cone response.

Materials and Methods

Equipment

A three-channel Maxwellian view optical system was used for presentation of test and adaptation stimuli. We used two channels to present a 5 Hz alternation of photometrically matched lights, the substitution stimulus. One channel produced a short-wavelength test light using a 150 W xenon arc lamp, with interference and neutral density filters. The interference filters were three-cavity filters with peak wavelengths at 10 nm intervals between 440 nm and 510 nm. The light focussed on a variable-speed, two-sector beam chopper which interrupted the beam in square-wave fashion. The light then passed through an oblique dichroic beam splitter and thence to the eye. The second channel, at 90° to the first, was a 565 nm light-emitting-diode (LED) (General Instruments #MV64521). The two channels superimposed at the oblique beam splitter, where the LED light was reflected by the beam splitter to the eye. The dichroic beam splitter transmitted the majority of short-wavelength light and reflected the majority of long-wavelength light. The portion of the test beam reflected off the beam splitter was imaged on a silicon photodiode. Signals from the photodiode were used both to produce an inverted signal to control the LED, and to trigger the ERG recording. The exchange was precise, being limited only by the virtually instantaneous temporal response properties of the photodiode, LED, and associated electronics. Visual inspection of oscilloscope traces generated by a silicon photodiode placed in the focal plane of the field lens (with appropriate adjustment of the LED radiance to produce an exchange) indicated no measurable light transient at the time of an exchange.

The photometric balance (using heterochromatic flicker photometry) could be obtained at high repetition rates either by inserting neutral density in the test channel or by adjusting the current supplied to the LED. Neutral density could also be inserted before both channels to vary the radiance of the substitution stimulus without upsetting the photometric balance.

The third channel was used for adaptation. The light source was a 55 W tungsten halogen lamp with a regulated DC power supply. A 571 nm interference filter and neutral density filters controlled its spectral output and radiance.

Substitution and adaptation stimuli were combined with a cover slip serving as a beam splitter. The final lens was aspheric and provided a visual angle of 80°. Crosshairs were used to control fixation. As the size of the exit beam on the cornea was less than 1 mm, pupillary dilatation was unnecessary.

A calibrated EG & G photometer was used to determine the relative spectral output through the interference filters. The same instrument was used with a photometric filter to determine the retinal illuminance of the test and background stimuli. The retinal illuminance of the stimulus field could be varied up to 3500 td, the maximum output of the LED. The adaptation field was 7000 td.

Recording

A gold foil electrode served as the active electrode. A reference electrode was placed on the ipsilateral cheek or center of the forehead and ground electrodes were placed on the ipsilateral earlobe. The impedances between the electrodes were checked prior to recording and did not exceed 8,000 ohms. Grass isolating leads (IG3P511) connected the electrodes to the amplifiers. The signal was amplified ×50,000 by PAR #113 (100×) and Grass #1511 (500×) preamplifiers in tandem and fed to a twelve bit a/d converter and microcomputer. A custom-written program controlled data acquisition, averaging, and display. Acquisition started with the test light and continued for 204.8 msec (512 points). Thus, for the 5 Hz repetition rate, we could store one full cycle of the substitution stimulus (100 msec short-wavelength light followed by 100 msec LED light). We averaged 50 responses for each recording, and used an artifact reject buffer set at ±50 μV to avoid averaging during blinks or eye movements. The averaged responses were stored digitally, and could be displayed on an oscilloscope screen for photography, or plotted on a digital plotter.

Pilot Experiments

Observers: Two of the authors and two colleagues, aged 24–44 yr, participated in the pilot experiments; all had normal corrected visual acuity and color vision, with no history of ocular disease. The observers were fully informed of the nature of the procedure and consented to participate.

Procedures: We compared the use of the photometrically-matched substitution stimulus to use of the test or LED alone, with and without the adaptation field. In these experiments we used a 460 nm interference filter in the test beam. The observer made a heterochromatic flicker photometric (HFP) match between the test stimulus and the LED at a 40 Hz alternation rate by adjusting the current supplied to the LED. The alternation rate was then set at 5 Hz.
Then either the 460 nm test alone, the 565 nm LED alone, or the 460/565 nm substitution were presented, with and without the 571 nm adaptation field. The observer viewed the alternating field continuously, to maintain a constant state of light adaptation.

In order to derive an action spectrum, we obtained a retinal illuminance/response series from three observers using the substitution stimulus at six test wavelengths between 440 nm and 500 nm. In this procedure, each test wavelength was alternated with the 565 nm LED light. Each observer made HFP matches for the test wavelengths before beginning the procedure. Following a 2 min adaptation to the 571 nm background, ERGs were recorded for different illuminations of the substitution stimulus by placing neutral density filters between the substitution stimulus and the eye. We used half-log unit steps and could obtain data for 3–5 steps depending on the wavelength. The ERG amplitudes were measured by hand from the recordings. We measured the amplitude from the peak of the b-wave to the trough of the off-response.\textsuperscript{15}

Results

Figure 1 shows the recordings made to 460 nm (upper panels) or 565 nm (lower panels), presented alone without substitution. Without the background adaptation (left panels), there was a fast photopic response at the start of the stimulus. This photopic response showed a 15 μV a-wave with a 45–50 μV b-wave either to 460 nm alone or to 565 nm alone. The equivalent amplitudes indicate the precision of the HFP match. It is of interest that there was no evidence of rod response for this observer. The time-averaged scotopic retinal illuminances were about 41,000 scotopic td for the 465 nm stimulus and 1200 scotopic td for the 565 nm stimulus. Apparently these stimuli, which were viewed continuously, provided sufficient steady-light adaptation to the rod system so

that the scotopic response level was reached. It was noted below that other observers showed another response that began within the first 100–150 ms of the stimulus. In this condition, the photopic b-wave and response occurred after the photopic stimulus. The condition was repeated with the 571 nm adaptation and the b-wave and rod response occurred in the same condition. We could not record a S-cone response to the 565 nm stimulus.

Figure 2 shows the ERG recordings (right panel) made to the substitution stimulus (left panel) without adaptation. The effect of the photopic stimulus (upper panel) showed a 25 μV a-wave, with a 50–60 μV b-wave, with an initial 15 μV negative change in the baseline followed by an amplitude from the negative of the response.

We also showed that when no photopic stimulus was demonstrated...
that the succeeding pulses of twice the mean illuminance level produced negligible rod responses. As noted below, however, rod responses were seen in other observers when no background was present. When the background was added (right panels), the photopic components were reduced. The a-wave showed an amplitude of about $7 \mu V$ (latency 24 msec) and the b-wave showed an amplitude of about $12 \mu V$ (latency 35–40 msec). A smaller photopic off-response occurred at the termination of the 565 nm stimulus. Additionally, at 460 nm, a slow b-wave with latency of 80 msec followed the faster photopic b-wave and obscured the photopic offset-effect. This is a condition similar to that used by other workers to record a SWS cone ERG. The slow b-wave is attributed to SWS cones.

Figure 2 shows the recordings made with the substitution stimulus, without (left panel) and with (right panel) the background. The substitution condition reduced the photopic response. Withoutadaptation, the photopic responses were largely suppressed, leaving a 25 $\mu V$ b-wave (latency 85 msec). In some observers, residual photopic responses occurred. With the background adaptation (panel 2), the residual cone activity was suppressed leaving a 15 $\mu V$ b-wave with an implicit time of 85 msec. It was followed by a slow negative offset as the voltage drifted below baseline during the LED part of the cycle. The amplitude from the peak of the b-wave to the trough of the negative offset effect was about 19 $\mu V$. This slow response is characteristic of the SWS cone ERG.15,20

We also saw signs of rod activity in other observers when no background was present. Rod activity was demonstrated by an increase in the slow b-wave as we reduced the illuminance of the substitution stimulus. In comparison, a pure SWS cone b-wave always decreased with reduction in stimulus luminance. The apparent rod intrusion occurred in two of the four observers we tested during the pilot work.

On the basis of the pilot experiments, we concluded that the substitution technique reduced or eliminated the photopic transient responses. Additionally, the 571 nm background provided further isolation of the SWS cone response.

Spectral sensitivity of the SWS cone ERG: The pilot data suggested that 460/565 nm substitution with background gave a SWS cone ERG. The action spectrum of the response is shown in Figure 3 as the relative energy needed for a 12 $\mu V$ criterion amplitude response for one observer. The data show decreasing sensitivity with wavelength. The solid line on the figure is the $\tilde{z}(\lambda)$ of the Judit28 revised observer while the dashed line is the sensitivity of the rods. The $\tilde{z}(\lambda)$ is thought to provide a good estimate of SWS cone sensitivity.29 The sensitivity at 450 nm of the response curve was matched by visual inspection to the theoretical SWS sensitivity curve. The data were consistent with SWS cone sensitivity, except possibly for data points near 500 nm, where the rod sensitivity curve was placed. Similar data were obtained for two other observers.

Clinical Protocol

Observers: Ten observers participated in the experiments; all had normal corrected visual acuity and color vision, with no history of ocular disease. Observers were selected from colleagues and their families, and were aged 16–49 yr. They are identified on
full contrast for SWS cones, but contrasts of less than 10% for the MWS and LWS cones. Following a 2 min preadaptation, a luminance series was measured for each substitution stimulus using half-log unit steps. Six steps (11–3500 td) were used for the 460 nm substitution stimulus; four steps (110–3500 td) were used for the 490 nm substitution stimulus. The entire protocol took approximately 10–12 min. Data were obtained from ten observers. In a control procedure, we inserted the gold foil electrode in the unstimulated fellow eye, and averaged 50 responses to 460/565 nm stimulation at six retinal illuminance levels. The amplitudes were measured as above and plotted as a function of retinal illuminance.

Results

The ERG tracings for a single observer are shown in Figure 4 for 460/565 nm (upper panel) and 490/565 nm (lower panel) substitution. Each record represents a retinal illuminance, with retinal illuminance increasing downward. At the lowest illuminance for either 460/565 nm or 490/565 nm, the record was essentially flat. As the retinal illuminance was increased, the slow b-wave of the SWS cone ERG gradually increased.

The ERG tracings for all ten observers for the 460 nm substitution stimulus at 1107 td are shown in Figure 5. The records for the ten observers are displaced vertically for clarity. The response amplitudes ranged from 8–24 μV, with a mean value of 16.7 ± 4.9 μV. The b-wave latencies ranged from 60–70 msec with no obvious association between amplitude and latency. The coefficient of variation (ratio of S.D./mean) was 29%, a value slightly larger than the 17–22% reported for the b-wave in clinical electroretinography.  

The ERG tracings to the control condition (gold foil electrode in the unstimulated fellow eye) revealed no response component. The noise of the trace was about 3–4 μV. Other researchers have noted that ERG recordings made with the gold foil electrode can be contaminated by a photomyoclonic reflex. Our recordings used an artifact rejection system to avoid this problem, and thus we were not surprised when the control condition revealed no response. However, we did note that data acquisition was difficult and time-consuming in one observer who demonstrated a photomyoclonic reflex.

Figure 6 shows the luminance response functions for the ten observers for 460 nm and 490 nm test wavelengths in substitution with 565 nm. For all observers, the ERG amplitudes increased with increase in the luminance of the substitution stimulus. At a matched photopic luminance, the amplitudes were greater for 460 nm than for 490 nm.

<table>
<thead>
<tr>
<th>Wavelength pair</th>
<th>Rods</th>
<th>LWS cones</th>
<th>MWS cones</th>
<th>SWS cones</th>
</tr>
</thead>
<tbody>
<tr>
<td>460/565</td>
<td>0.870</td>
<td>−0.057</td>
<td>0.096</td>
<td>0.999</td>
</tr>
<tr>
<td>490/565</td>
<td>0.740</td>
<td>−0.040</td>
<td>0.072</td>
<td>0.995</td>
</tr>
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</table>
The data were fit by the expression:

$$R = R_{\text{max}} l^n (l^n + k^n)$$  \hspace{1cm} (1)

where $R$ is the measured response in $\mu V$, $R_{\text{max}}$ is the maximum response amplitude, $l$ is the luminance in photopic trolands, $n$ is a compressive constant, and $k$ is the semi-saturation constant, the luminance at which $R$ is half its maximal value. Although $n$ has been reported as 0.7–0.74 for isolated monkey cone photoreceptor response,\textsuperscript{35,36} $n$ may be close to unity for human ERG data\textsuperscript{26,33,37,38} We have insufficient data points to minimize the squared error for all three variables, $R_{\text{max}}$, $K$, and $n$ simultaneously. We evaluated equation (1) for $n = 1.0$, using a least-squares fitting procedure to fit the data, estimating $R_{\text{max}}$, $k_{460}$, and $k_{400}$ from the six data points at 460 nm and four data points at 490 nm. The results of the fits are shown as solid lines in Figure 6. Some uncertainty in the fitting procedure occurs since the lowest points on both functions fall within the 3–4 $\mu V$ noise level of the recording. We repeated the analysis with values of $n$ between 0.7 and 1.1. The residual squared error was relatively insensitive to the choice of $n$. For the majority of our observers, the best fits were obtained for values of $n$ between 0.9 and 1.1.

The parameters of the fits for $n = 1$ are shown in Table 2. $R_{\text{max}}$ varied from 9.6 to 31.1 $\mu V$. The value of $k$ ranged from 72.1 to 328.3 td at 460 nm and from
396.5 to 397.5nm. In a few cases, although the range was -1.206 to 1.21, the response was clearly determined. The SWS component of Judd and Spelke was seen with the response at 460 nm, indicating that the SWS component was present.

Thus, out of the range of 396 to 397.5 nm, the SWS component was seen in the response at 460 nm.

The standard deviation of MWS and SWS components in the response has been significantly lower than that seen with the short-wavelength-sensitive (SWS) and medium-wavelength-sensitive (MWS) photoreceptors.

The wavelength dependence of the MWS and SWS components has been found to be different. The MWS component has a peak response at 530 nm, while the SWS component has a peak response at 555 nm. This difference in peak response makes it possible to distinguish between the two components in the presence of overlapping wavelengths.

The response has been analyzed for the presence of the SWS component at 460 nm, and it has been found that the SWS component is present in this range.

Table 2 presents the observed values of the MWS and SWS components.

<table>
<thead>
<tr>
<th>Observations</th>
<th>MWS</th>
<th>SWS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1F</td>
<td>10</td>
<td>11</td>
</tr>
<tr>
<td>2M</td>
<td>15</td>
<td>17</td>
</tr>
<tr>
<td>3F</td>
<td>21</td>
<td>24</td>
</tr>
<tr>
<td>4M</td>
<td>30</td>
<td>33</td>
</tr>
<tr>
<td>5F</td>
<td>34</td>
<td>37</td>
</tr>
<tr>
<td>6M</td>
<td>40</td>
<td>43</td>
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<td>50</td>
</tr>
<tr>
<td>8M</td>
<td>53</td>
<td>56</td>
</tr>
<tr>
<td>9F</td>
<td>60</td>
<td>63</td>
</tr>
<tr>
<td>10M</td>
<td>67</td>
<td>70</td>
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</table>
396.5 to 1728.6 td at 490 nm. The logarithmic value of the ratio of \( k_{490}/k_{460} \) ranged from \(-0.507\) to \(-1.206\) (mean: \(-0.83\)). We calculated what this ratio would be if either SWS cones or rods contributed to the response. If these ERGs were generated purely by SWS cones, this ratio should be \(-1.04\) using the Judd \( Z(\lambda) \). In contrast, if rods mediated the response at 460 nm and 490 nm, the ratio would be \(-0.22\). Thus our data are consistent with SWS cone isolation at 460 nm in all ten observers.

**Discussion**

The substitution technique effectively silences the MWS and LWS cones. As far as we know, this technique has not been used clinically, although it has seen wide experimental use, as reviewed by Estévez and Spekreijse. The waveform of our SWS cone ERG is similar to that previously reported in the literature. We observe a b-wave to light onset, with a slow negative drift at stimulus off-set. The implicit time of the b-wave is longer than noted for MWS and LWS cones. The response amplitudes we measured are larger than previously reported in the literature. The SWS cone ERG, like the rod b-wave, may be expected to saturate when adapted. The substitution technique allows us to use lower adapting and stimulus levels and a low alternation rate; these factors are probably important in maximizing the amplitudes of the SWS cone ERG.

Although we established that we were measuring SWS cone responses by estimating an action spectrum, we showed that SWS cone ERGs can be assessed using a rapid protocol suitable for routine clinical use. In this protocol, substitution of 460 nm with 565 nm can be compared with substitution of 490 nm and 565 nm. The protocol can be completed in 10–12 min and gives a radiance response function with ten data points for the two wavelength pairs. In ten normal observers the amplitude of \( R_{\text{max}} \) was sufficiently large to allow evaluation of a reduction in eye disease. The normal radiance response functions were consistent with values of the compressive constant near 1.0. However, patient data may well show lower values for this constant.

**Key words:** electroretinogram, SWS cones, blue cones, silent substitution

**Table 2. Values for \( R_{\text{max}}, k \) and log \( k_{460}/k_{490} \) for the ten observers**

<table>
<thead>
<tr>
<th>Observer</th>
<th>( R_{\text{max}} ) (µV)</th>
<th>( k ) (tds)</th>
<th>( k_{460}/k_{490} ) (log)</th>
</tr>
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<tbody>
<tr>
<td>1F</td>
<td>16</td>
<td>16.5</td>
<td>74.1</td>
</tr>
<tr>
<td>2M</td>
<td>18</td>
<td>22.0</td>
<td>115.3</td>
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<td>3F</td>
<td>23</td>
<td>17.4</td>
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<tr>
<td>4M (RE)</td>
<td>30</td>
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<td>72.1</td>
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<tr>
<td>5F</td>
<td>31</td>
<td>22.1</td>
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<td>6M (RE)</td>
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<td>14.4</td>
<td>184.5</td>
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<td>7F</td>
<td>35</td>
<td>13.5</td>
<td>107.5</td>
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<tr>
<td>8M (RE)</td>
<td>46</td>
<td>31.1</td>
<td>328.3</td>
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<td>47</td>
<td>18.1</td>
<td>227.6</td>
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<td>10M</td>
<td>49</td>
<td>18.4</td>
<td>213.2</td>
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**References**