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Clinical S-cone ERG recording with a commercial hand-held full-field stimulator

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Abstract

Our purpose was to explore S-cone ERG protocols for a commercial full-field hand-held stimulator that contains colored LEDs, and to see whether the test would be useful as a part of routine ERG testing. S-cone responses were elicited by blue flashes over a longer-wavelength background. With the standard stimulator containing blue (461 nm), green (513 nm) and red (652 nm) LEDs, we were unable to obtain satisfactory responses. Reproducible S-cone ERGs were obtained with a stimulator that had been custom-fitted with shorter-wavelength blue (440 nm) LEDs for stimulation, and orange (590 nm) LEDs for background adaptation. S-cone responses took only a few minutes to record, and the typical waveform showed a slow peak at 45–50 ms with amplitude 3–9 μV, but ranging from 0 μV to more than 10 μV. Larger waves appeared in a patient with enhanced S-cone syndrome. S-cone responses could also be obtained with an alternating blue-orange flicker protocol. We added the S-cone response to our regular ERG protocol for a number of months. Although most normal subjects and patients showed recognizable S-cone responses with this stimulator, the amplitudes were small and there was too much variability to make the technique effective for routine clinical testing. In general, the S-cone responses followed the standard cone ERG responses in disease.

Introduction

The blue-sensitive or short-wavelength sensitive cones (S-cones) comprise only about 10% of the cones in the human eye, and they differ functionally from the long- and medium-wavelength sensitive cones (L- and M-cones). S-cones have been shown to be affected early in glaucoma, diabetes, drug toxicity and some hereditary retinal dystrophies [1,2]. However, the S-cone ERG is difficult to record. The S-cones produce responses that are much smaller and considerably slower than L/M-cone responses, and it is difficult to isolate them without having them overwhelmed by the L/M-cone signals. A number of techniques have evolved for recording S-cone responses [2–10], but none of these protocols have found their way into routine clinical usage outside of the laboratory which initiated the technique. Our goal was to explore whether a clinical method for recording S-cone responses could be developed with commercially available Diagnosys instrumentation.

Methods

To develop the technique for S-cone recording, we worked with normal subjects who were studied according to the tenets of the Declaration of Helsinki, and who participated with full informed consent. After a clinical method had been established, recordings were made routinely from patients for whom S-cone information could be of relevance to their retinal disease.

All subjects were tested using the Diagnosys Espion Electrophysiology System, (Diagnosys LLC, Littleton, MA), which includes a hand-held, full-field stimulator (ColorBurst) that is powered by colored LEDs. We worked with two ColorBurst units: a standard commercial hand-piece, and a second hand-piece that had been custom-fitted with LEDs more appropriate to the wavelength requirements of S-cone stimulation (see Table 1).

For all recordings, the pupils were maximally dilated using 1% Mydriacyl and 2.5% phenylephrine, and the corneas were anesthetized with a drop of 0.5% Proparacaine. Burian–Allen contact lenses were placed in each eye, with a ground electrode on the forehead. Recordings of S-cone responses were made in ordinary room illumination.

Two methods of eliciting S-cone responses were used:

1. Single flash method: Blue flashes were presented on an L/M-cone-adapting background. Flashes were delivered at 4 Hz and 30 responses were averaged.
2. Flicker method: Steady blue light was alternated with steady orange or yellow light at a rate of 30 Hz while adjusting the blue intensity to minimize the response. Then the colors were alternated at 4 Hz, and 30 responses were averaged.

Table 1.

LED color	Standard unit (nm)	Custom unit (nm)
Blue	461	440
Green	513	–
Orange	–	590
Red	652	680

Results

Standard hand-piece

We first tried to isolate S-cone responses with the standard commercial Diagnosys hand-piece, using blue (461 nm) flashes against several different backgrounds that would adapt the L/M-cones (see Table 1). Backgrounds tried included red (652 nm) at 150–700 cd/m^2 and yellow (derived from a mix of green and red LEDs) at 300 cd/m^2. However, the responses to blue flashes were dominated by L/M-cone waveforms (even at the dimmest blue intensities). With intense blue flashes, we occasionally saw a mixed response that may have included a late S-cone component, but these waveforms were not consistent or reliable. Using the flicker method (alternating blue and yellow stimuli) also yielded variable responses with L/M-cone waveform predominance.

Custom hand-piece

Our custom-fitted hand-piece had different LEDs (see Table 1). In particular, the blue LED had a shorter wavelength (440 nm) closer to the peak sensitivity of the S-cones, and the orange LED (590 nm) could stimulate both L- and M-cones with relatively little effect upon the S-cones. With this unit we tried a variety of stimulus and background intensities, and found that relatively pure S-cone responses could be obtained reliably using dim blue flashes on a bright orange background of 300 cd·s/m^2 (Figure 1). The amplitude of these responses appeared to reach a maximum at a stimulus intensity near 0.03–0.05 cd·s/m^2, and if the stimulus intensity was increased further, faster components took on the characteristic wave form of an L/M-cone response and in fact appeared identical to the response to an orange or red flash. Our protocol for clinical practice was to begin with a blue flash of 0.03 cd·s/m^2 and increase intensity to yield a maximal S-cone waveform (without intrusion of L/M-waveform elements). The S-cone responses typically showed no *a*-wave, and a slow arching *b*-wave with amplitude 3–9 μV and implicit time 45–53 ms (Figure 2). However, the normal range was considerable (Figure 3) with some showing responses > 10 μV, while others showed virtually no

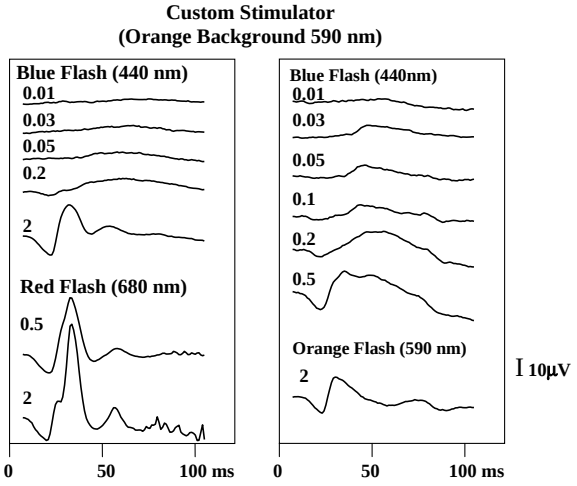

Figure 1. ERG responses with the custom hand-piece, in two different eyes, to flashes of different color and intensity over bright orange (590 nm) background. Deep blue flashes (440 nm) elicit an S-cone response at low intensities (numbers indicate cd·s/m^2). As stimulus intensity increases, the response appears to reach a maximum amplitude before the intrusion of faster L and M components (that match the response to an orange flash).

recognizable response under our recording conditions. The S-cone peaks were always very distinct from L/M-cone responses (that peaked at less than 35 ms).

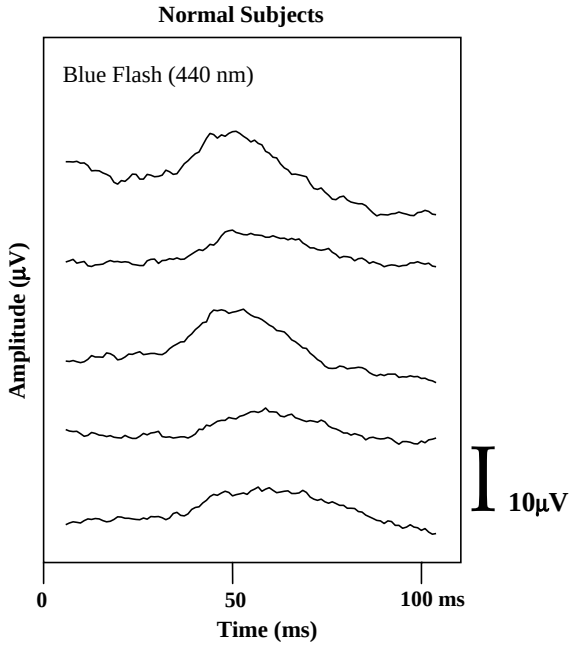

Figure 2. Maximal single-flash S-cone responses from several normal subjects. Stimulus intensities that maximized the response ranged from 0.01 to 0.05 cd·s/m^2.

To see whether the frequency of stimulation (4 Hz) was critical to these responses, we compared (in five normal subjects) the results of using 2, 6 and 8 Hz frequency of blue flashes. The results on average were within 15% of the 4 Hz amplitudes, and validated our choice of this frequency to produce stable responses. We also compared (in six normal subjects) our initial S-cone recording (which took 5–10 min to complete) with results from a second and third recording (that finished roughly 30 min later) to see whether longer periods of L/M-cone light adaptation would alter the responses. Relative to

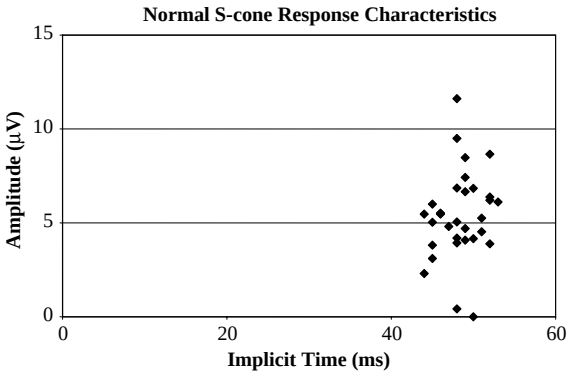

Figure 3. Single-flash S-cone response amplitudes from 32 normal subjects (ages 19–49). The amplitudes ranged from zero to > 10 μV (the symbol for the non-recordable response is placed arbitrarily at 50 ms implicit time).

the first response, amplitudes averaged 119% in the second recording and 104% in the third recording. Given signal noise and variation (see Figures 2 and 3), we felt that this showed no clinically significant effect.

The flicker method of S-cone isolation is shown in Figure 4. First, we recorded 30 Hz flicker responses using blue and orange stimuli, and varied the blue intensity to minimize the response. This sometimes took a number of tries, and the balance was never perfect. Using the blue and orange intensities that produced the lowest amplitude of 30 Hz response, we then flickered more slowly at 4 Hz and averaged the responses. The resulting waveform is characteristic of the S-cone signal and typically had slightly larger amplitude (10–15 μV) than the single-flash response. While this method was effective, it took time to balance the blue and orange intensities at 30 Hz before the S-cone response could be recorded, and we found it somewhat less efficient in clinical practice than the single-flash method.

Clinical recordings

For a number of months we incorporated S-cone recordings with the custom hand-piece into our routine ERG protocol (which includes the ISCEV Standard ERG and for most patients a multifocal ERG [mfERG]). Figure 5 shows single-flash S-cone responses from patients with different types of eye disease, illustrating a range of S-cone involvement. We also had the opportunity to evaluate one patient with enhanced S-cone syndrome (Figure 6), a disorder in which there is no rod function and the retina is dominated by cells that respond with the wavelength sensitivity of S-cones. A strong blue stimulus produced an extraordinarily large response, which nonetheless had *b*-wave latency characteristic of the S-cones. An orange stimulus did not elicit any L/M-cone response, but produced a weak S-cone response.

While these results are interesting with respect to individual patients, we noted earlier that normal S-cone responses spanned a rather large range from 0 to more than 10 μV. The smaller responses among normal subjects and perhaps some patients too, may relate to technical issues (lens comfort, cooperation, hand-piece position, etc.) as much as physiology, but in practice it

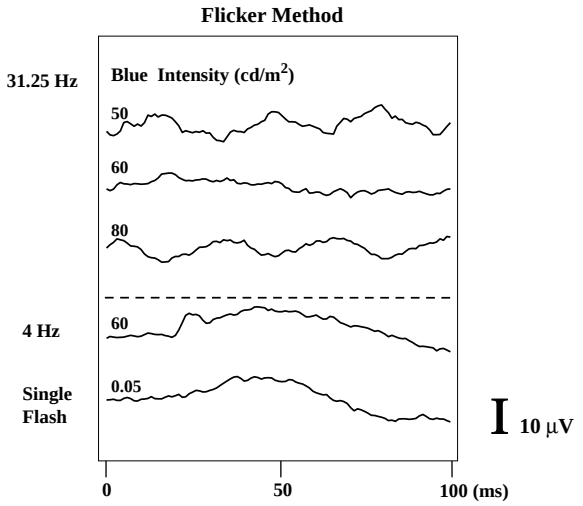

Figure 4. Flicker isolation of the S-cone response. The top sections show nulling out of the response to 30 Hz blue–orange alternation by varying the blue intensity (values shown in cd·s/m^2). The bottom section shows the averaged S-cone response to 4 Hz stimulation near the 'null' intensity, and a single-flash response for comparison.

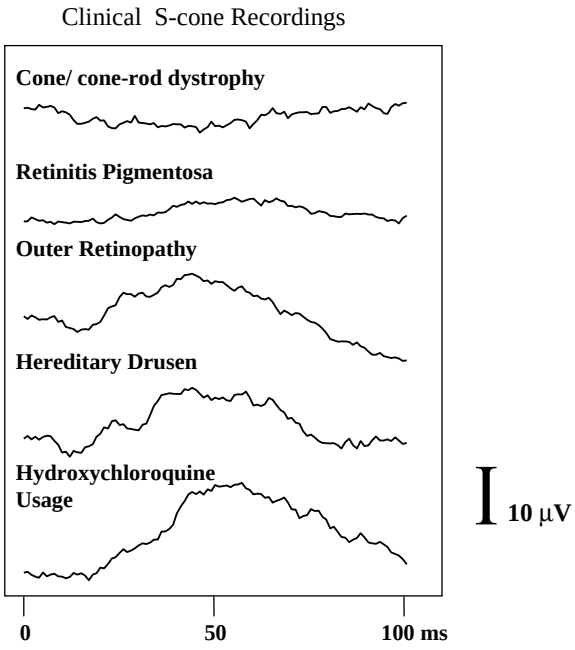

Figure 5. Examples of S-cone records in routine clinical practice. The full-field ERG and mfERG were reduced in the retinitis pigmentosa and cone–rod dystrophy patients; the mfERG was reduced in the outer retinopathy patient but was normal in the patients with hereditary drusen and hydroxychloroquine usage.

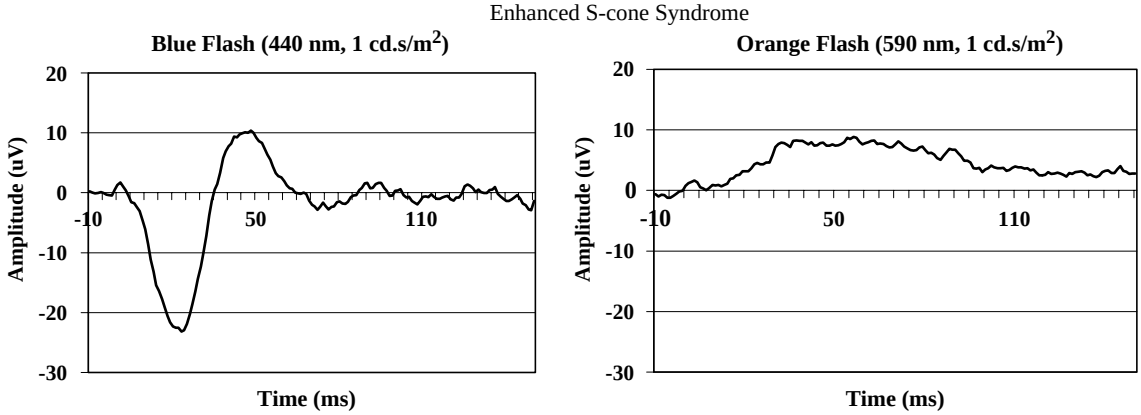

Figure 6. S-cone responses from a subject with the enhanced S-cone syndrome. A high intensity blue flash that would elicit an L/M-cone waveform from normal individuals produced a large *a*-wave and a slow *b*-wave with implicit time characteristic of the S-cones. A red flash of the same intensity elicited only a much weaker response that is similar to the normal S-cone waveform. These responses are consistent with a retina containing S-cones primarily.

was hard to define a meaningful normal range. Thus patient results were intriguing when surprisingly large, but were hard to interpret pathophysiologically for one individual when low. Looking at results from 33 patients, including retinitis pigmentosa (8), cone or cone–rod dystrophy (7), hydroxychloroquine usage (8), drusen (2) and other diagnoses (8), we found that the single-flash S-cone responses followed roughly the behavior of the full-field (L/M) cone responses with respect to amplitude (Figure 7). Flicker responses showed a similar correlation. Patients with very poor mfERG signals generally had poor S-cone responses (Figure 8), but there was a wide range of S-cone response amplitudes among patients with strong mfERG signals.

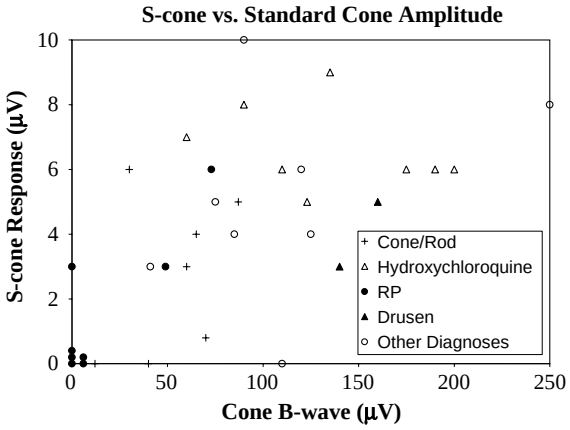

Figure 7. Single-flash S-cone and standard ISCEV cone responses compared in an unselected series of patients: retinitis pigmentosa (•), cone or cone/rod dystrophy (+), hydroxychloroquine usage (△), drusen (▲) or other diagnoses (○).

Discussion

There is reason to think that recording the S-cone ERG may have value for the evaluation of pathophysiologic processes in the retina, and for monitoring disease processes that may damage S-cones early or selectively. However, the S-cone ERG has been hard to record in a clinical context. A number of effective methods have been reported, and some are in regular use by the laboratories that developed them, but none have been easy to transfer into widespread usage because of the need for specialized equipment or conditions [2–10].

All of the single-flash methods for recording S-cone ERGs use a high intensity background to suppress the L- and M-cones, and a blue stimulus of varying wavelength and intensity [2–10]. Depending on the wavelength characteristics of the stimulus and background, the response may either be an isolated S-cone ERG (as we have demonstrated) or a composite waveform in which a late S-cone peak can be recognized after the L/M-cone peak [2,8]. Our S-cone response matches the waveform and timing of other published reports, and showed an appropriate and marked enhancement in a patient with the enhanced S-cone syndrome.

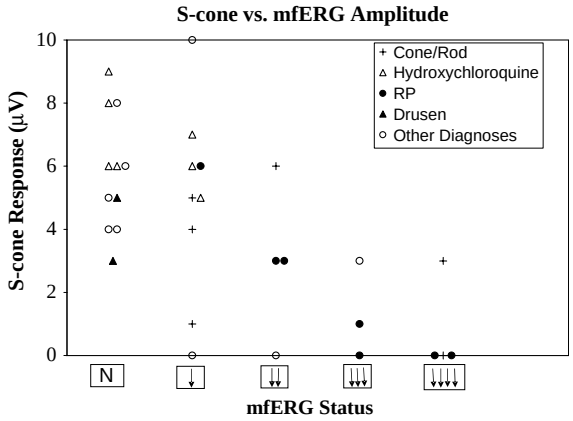

Figure 8. Single-flash S-cone responses relative to the multifocal ERG (mfERG). The mfERG is graded as normal (N), or as reduced with mild loss (↓), moderate loss (↓↓), severe loss (↓↓↓) or a minimal signal (↓↓↓↓). Patient diagnostic categories are labeled as in Figure 7.

Our difficulties with the standard Diagnosys hand-piece probably reflect two characteristics: (1) The 461 nm blue LEDs are closer in wavelength than those of the custom unit to the M-cone sensitivity curve, and thus probably produced a greater degree of M-cone stimulation. (2) The background adaptation wavelengths are problematic: the red (652 nm) LEDs used alone may not fully suppress M-cones, and the green (513 nm) LEDs (added to produce yellow or orange and suppress M-cones) have a short enough wavelength that they may suppress S-cones as well.

With the custom-fitted hand-piece, the blue stimuli were of shorter wavelength, and the orange LEDs (580 nm) allowed M-cone suppression at a wavelength that would have little effect on the S-cones. We found that our simple single-flash protocol could be performed within a few minutes. Our 'routine' procedure was to use weak blue stimuli (usually in the range of 0.01–0.07 cd·s/m^2) to maximize the isolated S-cone response, and also record one response to an orange flash for comparison. We tried 'routine' flicker S-cone responses, but found that in general it took more time to fiddle with the intensities and minimize the 30 Hz waveforms. The flicker stimuli were also annoying to some subjects.

Our results show that reasonable S-cone responses can be elicited from many normal subjects and patients, using a commercially available hand-held Ganzfeld stimulator (Diagnosys) that is custom-fitted with 440 and 590 nm LEDs. When we added this test to our Standard ISCEV ERG protocol, we found enough variability and inconsistency in the results to argue against its routine use. However, the methodology could be quite effective for studying S-cone responses in specific diseases, or for studies where data from a population of patients could be pooled. Another concern that argues against routine usage is that the recording time for S-cone responses extends the duration of ERG contact lens wear, which can already be quite considerable if multifocal as well as full-field ERGs are being performed. This can only be justified if the results contribute consistently to clinical evaluations, which has not been the case in our experience to date. Our S-cone responses followed, in general, the behavior of the standard cone *b*-wave and at least within our modest sample they did not show distinctive differences that would alter diagnoses. There was variability in S-cone responses relative to mfERG responses, but the same is true for the full-field cone ERG relative to the mfERG. Overall, we believe that S-cone ERG testing is a useful adjunct test for patients with certain diseases, and our simple and efficient protocol may encourage wider S-cone recording for appropriate indications.

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