ISCEV extended protocol for the S-cone ERG

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Abstract

The International Society for Clinical Electrophysiology of Vision (ISCEV) standard for full-field electroretinography (ERG) describes a minimum procedure for testing generalised retinal function but encourages more extensive testing. This extended protocol describes a method of assessing the function of the short-wavelength-sensitive cone (S-cone) retinal pathway, using a short wavelength flash superimposed on a background that saturates the rods and adapts the L/M cones to elicit a response, known as the S-cone ERG. Stimulus parameters such as the strength and luminance of the flash and background respectively, and their spectral and temporal characteristics are specified. As a complement to the ISCEV standard, testing the S-cone ERG enables further characterization of light-adapted retinal function and may refine diagnosis of some retinal disorders. Typical applications are described including use in the diagnosis of rod monochromacy and S-cone monochromacy, identification and investigation of cone On-bipolar cell dysfunction and use of the technique to confirm the diagnosis of enhanced S-cone syndrome (ESCS).

Key words

Clinical standards; Electroretinogram (ERG); Full-field ERG; International Society for Clinical Electrophysiology of Vision (ISCEV); S-cone ERG; short-wavelength-sensitive cone ERG; Retinopathy; Retinal dystrophy

Introduction

The International Society for Clinical Electrophysiology of Vision (ISCEV) standard for full-field electroretinography (ERG) describes a minimum procedure for testing generalised retinal function but encourages more extensive testing [1]. This extended protocol describes a method of assessing the function of the short wavelength-sensitive cone (S-cone) retinal pathway, using a short wavelength flash delivered on top of a background light to elicit a response, known as the S-cone ERG. The S-cone ERG is a specialised procedure which is well-established and broadly accepted by experts in the field. The protocol was prepared by the authors in accordance with ISCEV procedures (http://www.iscev.org/standards/index.html).

Scope and application

The ISCEV-standard ERGs are recorded under dark-adapted (DA) and light-adapted (LA) conditions to assess both rod and cone system function. The standard LA ERGs are normally dominated by responses driven by the long wavelength-sensitive cones (L-cones) and medium wavelength-sensitive cones (M-cones). There are far less short wavelength-sensitive cones (S-cones) than L/M-cones, and their responses to light are of smaller amplitude and slower kinetics. Therefore, the S-cone-mediated retinal activity is not directly visible in the standard LA ERGs. This extended protocol describes parameters for the recording of the S-cone ERG, reflecting the activity of the S-cone pathways in the distal retina. The S-cone ERG is elicited using short-wavelength (blue) flashes on a long-wavelength background to saturate the rod system and to reduce contributions from the L/M-cone systems [2-4]. The ERGs to such stimuli normally vary in waveform shape according to flash strength. To dim stimuli there is typically a single low amplitude component of relatively long peak time (Figure 1). To stronger flashes an additional earlier peak occurs and grows with increasing flash strength. The action spectrum of the 1st peak has a maximum at 520 nm and that of the 2nd peak at 450 nm [3]. In S-cone monochromacy there is a later peak only [3], whereas in tritanopia there may be a normal 1st peak and a very small 2nd peak [4]. These and other studies are consistent with response contributions from the L/M cone system to the first peak and the S-cone system to the second peak.

The addition of S-cone ERG testing to the standard ERG protocol enables further characterization of LA retinal function, and can help determining the origin of abnormal standard ERGs. Typical clinical applications of S-cone ERGs are outlined below:

a) In inherited retinal disorders, a more detailed knowledge of the phenotype may refine the diagnosis and facilitate mutational screening. It can confirm the origins of the recorded signals in enhanced S-cone syndrome (ESCS) consequent upon mutation in *NR2E3* gene after the pathognomonic features have been revealed with conventional ISCEV standard ERG testing [5, 6]. Mutation in the NRL gene, the upstream gene to NR2E3, also cause a retinal phenotype similar to ESCS, but not identical in all ERG features [7, 8]. S-cone ERG recording is also important in the distinction between rod monochromacy and S-cone monochromacy. In both disorders, the rod-driven ERG components are normal or well-preserved, and the LA 30Hz flicker ERG is undetectable.

b) The LA 3.0 ERG is usually undetectable in rod monochromacy, but in S-cone monochromacy may be detectable as a small amplitude b-wave of long implicit time appearing at approximately 50ms, suggestive of an S-cone system origin that can be more accurately confirmed by S-cone ERG recording [2]. S-cones, like rods, transmit signals via an On-bipolar cell pathway, any disorder affecting global retinal On-pathway function, such as “complete” CSNB (congenital stationary night blindness), will have reduced S-cone ERGs [9].

c) Inherited tritanopia is rare, and such patients cannot be identified with Ishihara plates, but only by testing color vision in the tritan color confusion axis (e.g. HRR plates, Farnsworth-Munsell 100-Hue, L’Anthony D15, color contrast sensitivity etc.). Although many patients with tritanopic defects may have normal S-cone ERGs, some families show S-cone ERG abnormalities [4].

d) Elevated tritan thresholds can also occur in acquired disorders associated with reduction in S-cone pathway function. Examples include melanoma associated retinopathy characterized by an electrophysiological signature showing global retinal On-bipolar cell pathway dysfunction (electroretinographically indistinguishable from “complete” CSNB), diabetes, and vitamin A deficiency where some patients report seeing “white as green” as a result of loss of S-cone function. The recovery of S-cone function following vitamin A supplementation can be monitored with S-cone ERGs [10].

Patient population

Patients of all ages, able to tolerate Ganzfeld stimulation, referred for investigation of possible retinal dysfunction, retinal dystrophy, generalized cone or rod system dysfunction or patients with photophobia and/or nystagmus may benefit from the S-cone ERG, performed in addition to the ISCEV standard ERG.

Technical issues

S-cone ERG recording is performed following the specifications of the current ISCEV standard for full-field ERG [1]. Recordings of the S-cone ERG can be embedded within the standard protocol [1] or conducted as a separate protocol. Additional factors are outlined below:

a) Spectral characteristics and luminance of the background

The photopic background for the recording of the S-cone ERG must be of spectral composition and strength that will achieve 2 goals; (i) saturate the rod system and (ii) minimize the contributions of the L/M cones systems. The initial studies used white background, but later studies and the developments of new electrophysiological of vision systems, indicated that bright LEDs with spectral peaks between 570 nm and 620 nm (Yellow, Amber, Orange) were more suitable for the purpose of obtaining an S-cone ERG (Table 1). A high background luminance of appropriate wavelength may optimize isolation of S-cone mediated responses, but patient comfort, tolerance and possibility of photophobia must also be considered.

b) Spectral characteristics of the test flash

Short wavelength (blue) stimuli may be generated using Xenon light in combination with a suitable optical filter (Kodak #98, having a peak transmission at 450 nm), or by using a light emitting diode (LED) with a peak output of short wavelength, in the region of 440 – 470 nm (Table 1). Wavelengths longer than 470 nm typically generate ERGs that are dominated by the L/M-cones, and the contribution of the S-cones to the ERG may not be detectable. Light sources with wavelength shorter than 440 nm may not have sufficient strength to elicit a reliable S-cone ERG because its action spectrum peaks at 450 nm [3].

c) Strength of the test flash

Laboratories that use backgrounds of 300 cd.m^{-2} have used flashes of low strength, 0.03-0.05 cd.s.m^{-2}, while laboratories using bright backgrounds of about 500 cd.m^{-2}, use stronger flashes, 0.1-0.5 cd.s.m^{-2}, in order to elicit robust S-cone mediated responses (Table 1). Weak flashes may be more selective but elicit responses of relatively low amplitude (Figure 1), whereas stronger stimuli may be difficult for some patients to tolerate.

d) Units of flash strength and luminance

It is acknowledged that the strength of the colored flash requires consideration of the spectral sensitivity of the eye and is accurately described by radiant energy (Watts/steradian/m^2/nm), but for uniformity of clinical use and consistency with other brief flash stimuli it is defined in this protocol in units of cd.s.m^{-2}. For stimulus durations >10 ms stimulus strength is given in units of luminance (cd.m^{-2}).

Calibration:

Calibration in accordance with the ISCEV standard ERG is recommended [1]. A spectral photometer is required to determine the spectral characteristics of the short-wavelength flash. Care should be taken to measure a range of flash luminance levels as some Ganzfeld stimulators use different combinations and banks of LEDs for different luminance ranges and these may have different wavelength specifications.

Protocol specifications:

Patient preparation follows that for the current ISCEV standard ERG [1]. For routine applications, the S-cone ERG protocol may be added to the ISCEV standard protocol after the other LA ERGs. Additional specifications are outlined below and in Table 1.

a) Spectral characteristics and luminance of the background.

The background peak wavelength is between 570 nm and 620 nm (yellow, amber, orange). The background luminance is 300cd.m^{-2} Recordings obtained using higher luminance backgrounds may be added (see section c below).

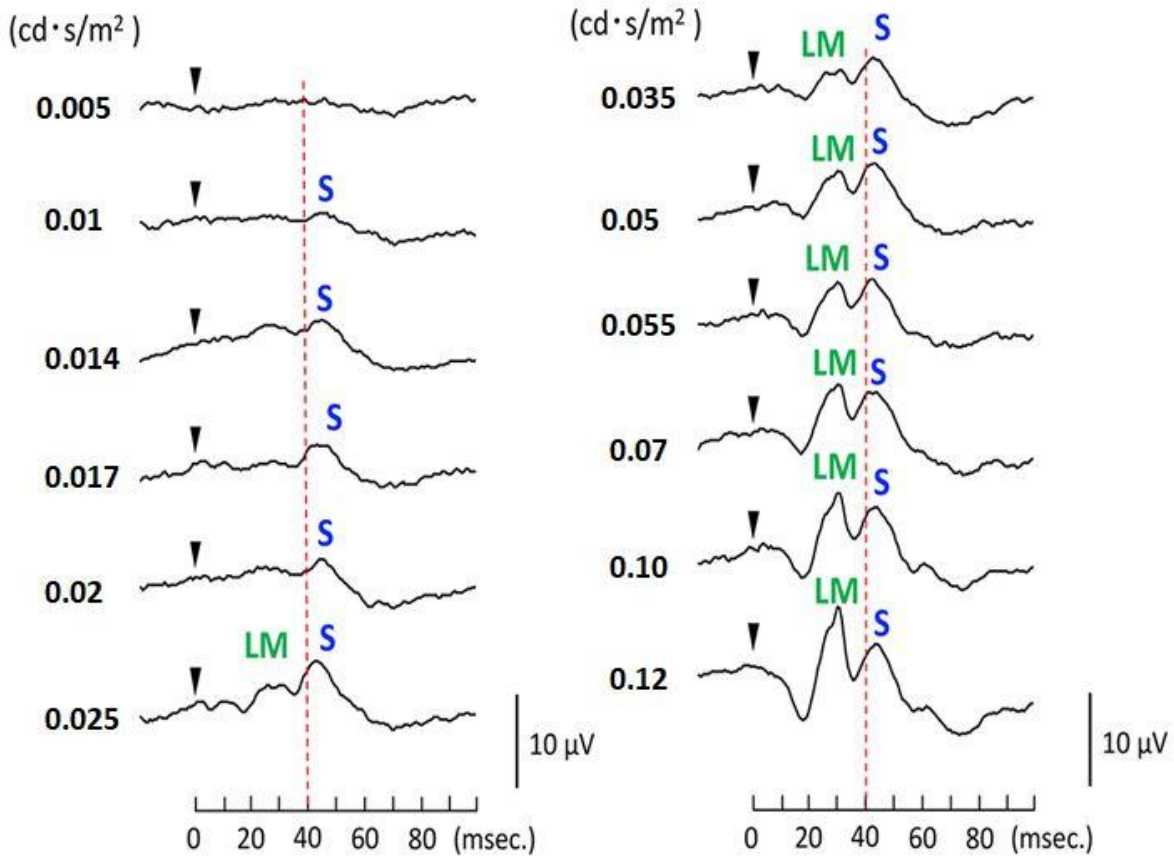

Figure 1: Stimulus-response series of the S-cone ERG recorded from a control subject using a Burian-Allen corneal electrode. An LED system was used to generate a 1msec blue (450 nm half-height bandwidth 20nm) full-field flash, superimposed on a yellow (570 nm) background (luminance 300 cd.m^{-2}). The stimulus frequency was 4.5 Hz. In this case 300 to 500 responses were averaged. At lower stimulus strengths (approximately of 0.01-0.02 cd·s.m^{-2}, single positive S-cone responses are observed. At middle and high stimulus strengths (approximately 0.025-0.12 cd.s.m^{-2}, two positive peaks are seen, the first (at approx. 30msec) reflects activity of the L/M cone system; the 2nd (at approx. 40msec) is the S-cone mediated ERG component. Red dotted lines are drawn at 40 msec.

b) Spectral characteristics of the test flash

For routine clinical diagnostics, LEDs with peak wavelength of between 450 nm and 470 nm are specified. If Xenon flashes and filters are used, a blue filter (Kodak #98 with peak transmission at 450 nm) is used. The method of stimulation (LED or optical filter) and the peak wavelength

and bandwidth at half-height of the stimulus should be stated.

c) Stimulus strength

The minimum S-cone ERG protocol includes a stimulus strength of 0.05 cd.s.m^{-2} delivered on a background luminance of 300cd.m^{-2}. This does not preclude the recording of additional S-cone ERGs or an S-cone ERG stimulus-response series e.g. stimuli ranging from approximately 0.025-0.2 cd.s.m^{-2} (see Figure1). Stronger flashes (e.g. 0.1-0.5 cd.s.m^{-2}) on a brighter background luminance (e.g. 500-560 cd.m^{-2}) can be used but may be difficult for some patients to tolerate.

d) Flash duration and frequency of presentation

Flash duration should be equal or less than 5 msec. The presentation rate is 2Hz (inter-stimulus interval 0.5s) as for the ISCEV standard LA3ERG.

e) Signal averaging. Small responses to dim flashes or abnormally reduced S-cone ERG components may require signal averaging of up to several hundred recordings.

e) Duration of light adaptation

The S-cone ERG may be embedded in the ISCEV-standard full field ERG protocol and conducted as a continuation of the LA ERG recordings. If the S-cone ERG protocol is used as a stand-alone protocol without prior dark adaptation, adequate light adaptation to the photopic background should be ensured e.g. 2 minutes.

Response evaluation:

. The waveform is evaluated as having a single or double peak. If there are two peaks the peak time is measured from stimulus onset to the 2nd (S-cone-mediated) peak. Amplitude is measured from baseline to the peak or may be measured from the trough that follows the 1st peak to the peak of the following positive wave (Figure 2), providing the method is stated.

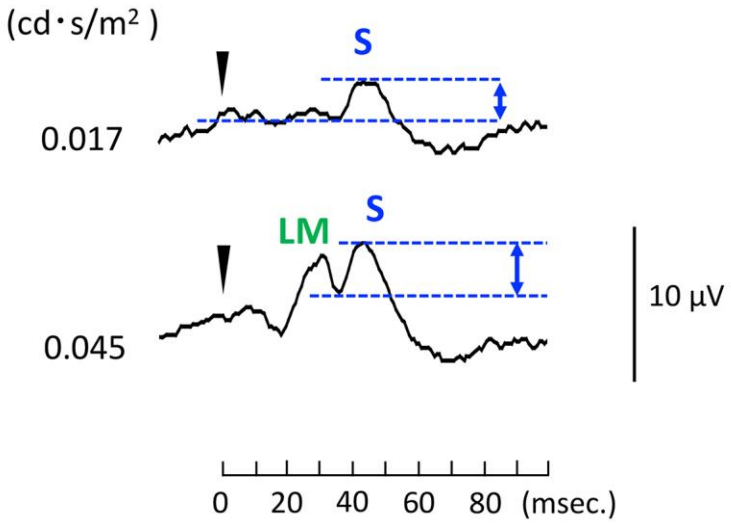

Figure 2: Measuring the amplitude of the S-cone ERG in a response to a dim stimulus that contains only one delayed peak, and in a response to a stimulus of moderate strength that contains 2 peaks, the 1st is the L/M cone ERG and the 2nd the S-cone ERG.

Reporting

Reporting the S-cone ERG should follow the recommendations of the ISCEV standard ERG [1]. The spectral characteristics of the stimulus and background should be specified. The strength and duration of the light stimulus and the luminance of the background should be stated. The presence or absence of a 2-peak ERG pattern should be acknowledged, and amplitude and peak time of the 2nd peak should be reported as S-cone ERG along with age-appropriate laboratory reference data. If not already documented as part of the ISCEV standard ERG, pupil diameters following mydriasis should be documented. It is acknowledged that for routine diagnostic reporting it may be enough to describe the S-cone ERG in qualitatively.

Table 1: Stimulus and background conditions for recording S-cone ERG

Reference	Stimulus wavelength	Stimulus strength	Rate of stimulus	Background wavelength	Background luminance
Norren et al. [11], [12]	441 nm (interference filter)	Not stated	Not stated	Yellow (>530 nm)	3.0-6.0 log Td
Yokoyama et al. [13]	440 nm (interference filter)	0.63W/m^2	Not stated	Yellow	13 Wm^2
Miyake et al. [14]	440 nm (interference filter)	7.58 μW/cm^2	3 Hz	Yellow	204 μW/cm^2
Gouras et al. [2], [3]	450 nm (Wratten 98)	approximately 0.02 μW	5.1 Hz	White	>5000-9000 Td (>100 cd/m^2)
Yamamoto et al. [15], [16]	450 nm (Wratten 98)	xenon flash of 5.0 cd.s/m^2 & Wratten 98	5 Hz	White	1.7 log cd/m^2 (50 cd/m^2)
Horiguchi et al. [17]	450 nm (LED)	long-flash of 2.8 log Td (12.6 cd/m^2)	3 Hz	Wratten 12 (Yellow)	4.8 log Td (=1262 cd/m^2)
Simonsen et al. [18]	440 nm (Wratten 98)	Xenon flash of 2.1 cd.s.m^{-2} & Wratten 98	4 Hz	Yellow	100cd.m^{-2}
Arden et al. [4]	440 nm (LED)	0.3-1.5 log Td-s (=0.04-0.6 cd.s/m^2)	Not stated	610 nm (orange)	18900 Td (=378 cd/m^2)
Maeda et al. [19]	450 nm (Wratten 98)	xenon flash of 5.0cd.s.m^{-2} &	5 Hz	White	1.7 logcd.m^{-2} (50 cd/m^2)

		Wratten 98			
Chiti et al. [20]	Combination of two filters of 430 & 450 nm	0.034cd.s.m^{-2} (=1.71 Td-s)	2 Hz	Yellow	10807 Td (=215 cd/m^2)
Marmor et al. [21]	440 nm (LED)	0.03-0.05 cd.s.m^{-2}	4 Hz	590 nm (orange)	300cd.m^{-2}
Tsuruoka et al. [22]	430 nm (LED)	0.03 cd.s/m^2 (-1.5 log cd.s.m^{-2})	1-5 Hz	White	2.0 log cd.m^{-2} (100 cd/m^2)
Audo et al. [5]	445 nm (LED)	0.08-0.8cd.s.m^{-2} (80 cd.m^{-2} with 1-10ms flashes)	Not stated	620 nm (orange)	560cd.m^{-2}
Sustar et al. [6], [23]	449 nm (Wratten 47B)	0.016-0.125 cd.s.m^{-2}	2 Hz	594 nm (amber)	100cd.m^{-2}
Rocha-Sousa et al [24]	440 nm	65 cd/m^2, 10 ms	Not stated	660 nm (orange)	350cd.m^{-2}
Cima et al. [25]	449 nm (Wratten 47B)	0.016cd.s.m^{-2}	2 Hz	594 nm (amber)	100cd.m^{-2}
Schatz et al. [26]	470 nm (LED)	0.1-0.5cd.s.m^{-2}	Not stated	594 nm (amber)	500cd.m^{-2}
Campi et al. [27]	blue 445 nm	0.4cd.s.m^{-2}	1 Hz	600 nm (orange)	560cd.m^{-2}
Littink et al [8]	Blue (445 nm) (LED)	2.0cd.s.m^{-2}	Not stated	594 nm (Amber)	15cd.m^{-2}

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Appendix: Justification for the protocol details and excluded methods.

A literature review was performed with PubMed search to find the publications that reported recordings of S-cone ERG using the following keywords: (S-cone or blue-cone) and (electroretinogram or ERG). Only the reports of S-cone ERG using a blue flash on strong adapting background illumination to suppress M- and L-cone activities were extracted. Animal studies were excluded, as well as studies using focal types of stimulation. Reports of S-cone ERG recording using silent substitution methods were also excluded. Studies from the year 1970 to 2018 were reviewed and those with specified stimulus parameters are summarized in Table 1.