ISCEV extended protocol for derivation and analysis of the strong flash rod-isolated ERG a-wave

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Abstract

The International Society for the Clinical Electrophysiology of Vision (ISCEV) standard for full-field electroretinography (ERG) describes a minimum set of tests, but encourages the use of additional protocols for clinical ERG testing. This extended protocol describes recording methods and derivations that will allow analysis of rod-driven components of the dark-adapted (DA) strong flash ERG a-wave, more closely related to rod phototransduction than ISCEV standard DA ERGs. The method involves recording ERGs to a flash strength equivalent to 30 cd.s.m^{-2} under conditions of dark-adaptation and additionally to the same stimulus following light adaptation (LA) and in the presence of a standard photopic background luminance of 30 cd.m^{-2}. The isolated rod-driven ERG a-wave is derived by subtracting the LA response from the DA ERG. The leading edge of the isolated rod-mediated a-wave is well described by a mathematical model of the biochemical steps involved in the activation of phototransduction from photon capture by rhodopsin to closure of the channels in the outer segment membrane. The method is likely to be of value in the characterization of retinal disorders which affect rod quantal catch, diseases that affect the dynamics of any component of the activation phase of rod phototransduction, or those affecting total numbers of rod photoreceptors.

Keywords

Introduction

The International Society for Clinical Electrophysiology of Vision (ISCEV) standard for full-field electroretinography (ERG) describes a minimum set of tests [1], but encourages the use of additional protocols for clinical ERG testing. This extended protocol describes extracting ERG components related to rod phototransduction from the a-wave response to a strong flash. The protocol was prepared by the authors in accordance with ISCEV procedures (http://www.iscev.org/standards/index.html).

Scope and applications

The normal human dark-adapted ERG to a strong brief flash is a mixed response with both rod- and cone-mediated contributions. Rod-isolated ERG components may be obtained by subtracting cone-mediated responses as detailed below. Information about the dynamics of rod phototransduction can be derived from analysis of the initial portion of the rod-isolated ERG a-wave [2-15]. This extended protocol describes the recording of ERGs to the same strong flash under both dark-adapted and light-adapted (LA) or photopic rod-saturating conditions. The cone-driven LA ERG is then subtracted from the mixed response obtained in the dark.

The protocol is applicable to patients in whom diseases affecting the rod photoreceptors are suspected. Specifically, the protocol enables derivation of parameters related to rod phototransduction, not obtainable from the standard ISCEV protocol, and thus allows for more detailed phenotyping and understanding of disease mechanisms. This includes characterization of diseases which affect rod quantal catch (e.g. shortening of the outer segment), diseases that affect the dynamics of any component of the activation phase of rod phototransduction, or diseases that reduce the total number of rod photoreceptors. This protocol has thus far only been used as a research tool and is not currently used in a clinical setting for the diagnosis of retinal disease.

Patient population

Patients of all ages able to tolerate Ganzfeld stimulation, referred for investigation of possible retinal or rod photoreceptor dysfunction, including those with reduced or delayed DA3 and DA10 ERG waves, consistent with abnormal rod function.

Technical issues

Technical issues

This protocol uses many of the recording parameters specified in the ISCEV protocol for the full-field ERG [1]. Additional considerations include the following:

a) Methods of estimating the dark-adapted cone system contribution to the scotopic ERG

The rod-isolated a-wave has been obtained by removing the cone-mediated contribution to the scotopic ERG a-wave using four main methods (4, 6, 7, 16-20; see appendix), outlined below.

1. An identical flash may be delivered shortly after the DA very strong flash, at a time interval sufficient for the cones to recover from the first flash, but prior to the onset of rod recovery. A practical consideration is that the use of such paired bright flashes in quick succession can be difficult for some subjects to tolerate.

2. A photopically matched red flash may be used to elicit an ERG mediated largely by L- and M-cones, to be subtracted from the mixed DA rod and cone system response. However, for strong flashes, it is likely the red flash would also stimulate the rods substantially, and so an estimation of the cone-derived component would be compromised [7]. It is possible to estimate the rod-derived component in the red flash response by delivering blue flashes that are scotopically matched to the red flashes [7,9], but this increases the range of stimuli required and consequent testing time.

3. A flash delivered immediately (300 ms) after extinction of a rod-saturating background [18]. Such stimulus delivery may not be readily achievable with standard equipment and also increases testing time, especially if averaging is needed.

4. An identical flash to that used under DA conditions, delivered in the presence of a steady rod-saturating background. Such stimuli may be conveniently integrated into the ISCEV standard ERG method, and this technique forms the basis of the extended protocol specified below.

b) Sequence and timing of scotopic and photopic ERGs

Suitable scotopic strong flash responses for a-wave analysis may be recorded after 20 minutes DA or after the ISCEV-standard DA ERG protocol. The ISCEV standard LA ERGs are recorded after 10 minutes light adaptation, to allow the cone-mediated responses to reach a steady-state amplitude. During the 10 minutes of light adaptation, the cone-mediated ERG grows in amplitude [21-23], and so a larger a-wave may be recorded. Thus, it could be argued that, for estimation of the cone component in the dark, the strong flash should be delivered immediately after onset of the photopic background rather than following adaptation to this background as the latter may lead to over-estimation. However, there is evidence that the ERG following a period of light adaptation may be closer to the dark-adapted cone-driven ERG than that obtained immediately following onset of the rod-saturating background [18]. Strong flashes presented immediately after onset of the photopic background can also be difficult to tolerate.

c) Spectral characteristics of scotopic flash

Shorter wavelength (blue) flashes preferentially stimulate the rods while minimizing stimulation of L- and M-cones and have been used in a number of studies [e.g. 2-9]. However, white flashes are consistent with ISCEV-standard ERG stimuli and have also been widely used to derive the rod-isolated ERG a-wave [e.g. 10, 12-14].

d) Scotopic stimulus flash strengths

Studies isolating and fitting models to the rod-mediated ERG a-wave have used a series of strong flashes [2-15]. However, shortened protocols based on one or two flashes have been proposed for clinical use [7,10]. The stimulus should be strong enough to saturate the rods and suitable to

probe changes in rod sensitivity. The flashes should be well-tolerated by most patients and chosen to avoid the flash artefacts that may be associated with the strongest flash stimuli.

e) Flash duration

The trough of the ERG a-wave to a 3.6 log sc Td-s flash occurs at approximately 10 ms. The duration of the flash must be considerably less than the photoreceptor integration time. Xenon flashes typically have flash durations of less than 1 ms and are ideal. Longer duration flashes produced by LED sources should be not more than 4 ms (i.e at least 2 times shorter than a-wave peak time).

f) Scotopic inter-stimulus interval

The interval between flashes should be a minimum of 30 s which allows for complete recovery of the rod ERG a-wave to a 3.6 log sc Td.s flash [17, 19].

g) Photopic background.

The photopic background is used to saturate rods, enabling recording of cone-mediated ERGs. A white background of 3.3 log sc Td (15 ph cd.m^{-2}) eliminates the rod a-wave and has a small effect on the cone a-wave [4, 19]. To minimize cone desensitization, a blue background which has a high scotopic, but low photopic luminance, has also been used [18,20]. The ISCEV standard for photopic ERGs stipulates a 30 ph cd.m^{-2} background which will produce a reasonable estimate of the cone-isolated ERG over the rising phase of the ERG a-wave (although there may be some desensitization of the cone photoreceptors).

h) Photopic flash strength

Photopic ERGs in this protocol are intended to estimate the cone-mediated contribution to the mixed DA strong flash ERG. To achieve this, stimulus strength should be unchanged from that used under scotopic conditions (see protocol specification below).

i) Photopic inter-stimulus interval

Cones recover within 2 s even in response to strong flashes [20]. Thus, an interval of ≥3 s between flashes is sufficient when averaging photopic responses. Flashes of this strength delivered at shorter intervals are less likely to be comfortable for subjects.

j) Signal averaging

Averaging may be used to improve the signal/noise ratio, and typically 3-10 responses have been averaged [5-18].

i) Response analysis and fitting

Once the LA (cone-driven) response has been subtracted from the DA (rod and cone-driven) response to obtain the isolated rod-driven a-wave, the equation describing the activation phase of phototransduction (given later, in Section 8) is fitted to the leading edge of the a-wave. The equation has three parameters R_{ROD}, S and t_d (see Section 8). Software capable of solving non-linear least squares algorithms (e.g. Levenberg-Marquardt or Gauss-Newton) is required to fit Equation 1 to the ERG recordings. Care should be taken in using non-linear curve fitting as a poor choice of starting values for each parameter could result in a sub-optimal fit. The following are two possible approaches used when fitting equation 1 to the ERG a-wave. First, non-linear curve fitting of equation 1 is applied for a wide array of starting values for each parameter e.g. S varied from 6 to 26 in steps of 2. Alternatively, t_d and R_{ROD} are set at fixed values and only S allowed to vary. With this second approach t_d is set at the mean value of healthy volunteers and R_{ROD} set at some pre-determined value, typically at either the peak of the a-wave or slightly higher (e.g. 10-15%) above the a-wave peak [9, 20].

As an alternative to using this model, one can simply measure the peak amplitude of the a-wave and the time to reach the peak or some fraction (e.g. 90%) of the peak amplitude [24]. Correlation has been demonstrated between the a-wave peak and R_{ROD}, and between time to peak and S, in healthy volunteers and patients with retinitis pigmentosa [24]. The time interval

between the response reaching 10% and 90% of peak amplitude is also likely to be an indicator of photoreceptor sensitivity [15]. The model, however, has the advantage of yielding parameters with an underlying theoretical basis.

The model described by Equation 1 only applies to brief flashes. If the flash duration is long (e.g. 5-10 ms or more), then the equation does not apply. An integrated form of the model is required to fit the response to long duration flashes [25].

Modeling of rod phototransduction becomes less reliable as the amplitude of the a-wave decreases and approaches noise levels. Application of the model under these conditions could lead to misleading results. Caution should be taken with interpretation of models based on isolated rod component of the ERG with very low amplitude responses (i.e. when the signal/noise ratio is low).

Calibration

The stimulus parameters are specified in scotopic trolands with conversion to photopic units for a white spectral light source with spectral composition similar to a xenon flash. If a source with different spectral composition is used, scotopic and photopic stimulus strength should be measured directly using appropriate filters. All other calibration issues are identical to those specified in the ERG standard [26].

Protocol Specifications

Patient preparation follows that for the current ISCEV standard ERG [1]. This extended protocol has the following additional specifications.

a) Sequence and timing of scotopic and photopic ERGs

Suitable scotopic strong flash responses for a-wave analysis are recorded after 20 minutes DA or following the standard DA ERG protocol. Photopic responses are recorded after 10 minutes light adaptation after the standard LA ERG protocol.

b) Scotopic stimulus flash strengths

This protocol specifies a flash strength equivalent to 75 scotopic cd.s.m^{-2}, or 30 photopic cd.s.m^{-2}. Assuming a pupil diameter of 8mm and a broad-spectrum white (7000 k) light source, this equates to a flash of 3.6 log sc Td.s. To be consistent with ISCEV stimulus notation this stimulus should be referred to as DA30. If the pupils are smaller a higher flash strength may be needed. If a range of flash strengths is used, the DA30 ERG should be included.

c) Flash duration

This should be a brief flash not longer than 4 ms.

d) Scotopic inter-stimulus interval

For the DA30 ERG, a minimum inter-stimulus interval of 30s is specified.

e) Photopic background

The photopic background luminance is 30 cd.m^{-2}, as for the ISCEV standard ERG protocol.

f) Photopic flash strength

The same flash strength should correspond to that used to elicit the DA strong flash ERG. The stimulus in this context is referred to as LA30, assuming it is delivered on the same background and after at least 10 minutes LA or after the ISCEV-standard LA ERG protocol.

f) Photopic inter-stimulus interval

The inter-stimulus interval is a minimum of 3 s. This allows cone recovery between flashes, is comfortable for most patients and an interval of 3 s allows efficient averaging if needed.

g) Averaging may be used to improve the signal/noise ratio, and typically 3-10 responses have been averaged in previous studies [5-18], although not always necessary.

Response evaluation

The averaged photopic response should be subtracted from the scotopic response to isolate the rod component of the ERG. The leading edge of the isolated rod-mediated a-wave is well described by a mathematical model of the biochemical steps involved in the activation of phototransduction [27,28] from photon capture by rhodopsin to closure of the channels in the outer segment membrane (Equation 1). The model is described by the following equation:

$$R(I,t) = R_{ROD} \{1 - \exp[-I \cdot S \cdot (t - t_d)^2]\}$$
(1)

Where I is the stimulus intensity (strength) in scot Td.s, S is a sensitivity parameter measured in (scot Td.s)$^{-1}$ s^{-2}, R_{ROD} (μV) is the saturated (maximal) response amplitude, and t_d (ms) is a brief delay. This model is based on consideration of the activation kinetics of each step of the phototransduction cascade [27,28] and returns parameters relating to rod phototransduction, although some caution should be taken in generalizing results from a human massed potential recorded from a surface electrode to parameters derived from recordings of single isolated rods. S is related to the amplification (or gain) of the biochemical steps in phototransduction. R_{ROD} is related to the number of channels in the rod outer segment membranes that are available for closure by light. t_d includes some delays in the biochemical cascade and those introduced by the amplifiers in the ERG recording system. To minimize intrusion of post-receptor potentials, fitting of the model should be restricted to the leading edge of the a-wave; i.e., from flash onset to the time at which the response reaches 90% of the peak amplitude.

Reporting

The dark-adapted (DA30) mixed response, the light-adapted (cone-mediated) response (LA30) and the rod isolated ERG should be shown along with the fit of the model to the a-wave of the rod isolated response. The time axis should be chosen so that the ascending portion of the a-wave can be clearly visualized as well as the pre-stimulus baseline (e.g. to show only the first 30 ms (Figure 1) or 60 ms of the response). Stimulus and recording parameters should be specified as for the ISCEV full field ERG. Pupil diameter should be reported. The derived parameters R_{ROD} and S should be displayed along with appropriate normative values for these parameters, which may need to take into account the effect of age [12].
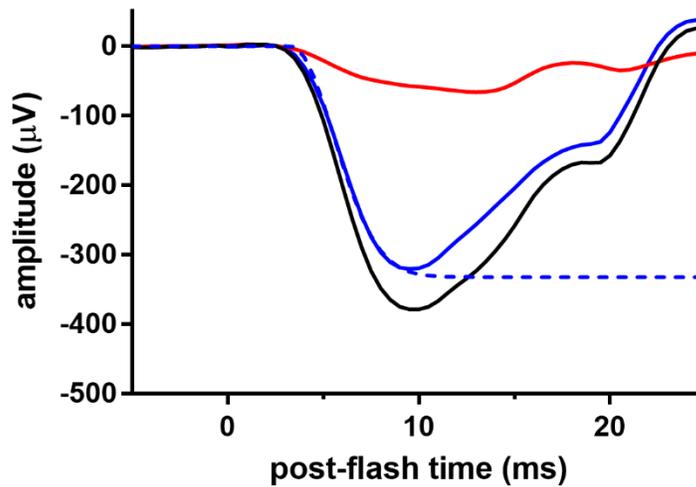

Figure 1: Recordings from a healthy volunteer using a Burian-Allen electrode in response to identical flash strengths (30cd.s.m^{-2}) under dark-adapted (DA30; black trace) and light-adapted conditions (LA30; red trace). The blue trace is the result of subtracting the LA30 response from the DA30 response and represents the rod contribution to the mixed response. The blue dotted line is the fit of equation 1. The derived parameters are $R_{ROD} = 332$ μV, $S = 26$ (scot Td-s)$^{-1}$ s^{-2}, $t_d = 3$ ms.

Appendix 1: Justification for the protocol details

A literature search was performed using the PubMed database (search date 4 Oct 2018) using search terms including "electroretinogram", "a-wave", "model" and "phototransduction". Studies relating to the rod-derived human ERG were primarily considered, although relevant findings from animal studies (including for example [17,18,24,27]) were also taken into account as these in many cases provide experimental evidence for origin of a-wave components. The search yielded a large number of studies, and selected key or exemplary references have been included in the References section and in Table 1. The literature search revealed that the most prevalent model in use in different laboratories for fitting the human rod a-wave was that based on the derivation of Lamb and Pugh [22], usually as formulated by Hood and Birch [4,10]. Hence this model was chosen.

A similar a-wave analysis to that given by Equation 1 has been applied in a number of studies to the cone-driven ERG [7,9,12,13]. The cone-driven a-wave is known to contain a substantial contribution from OFF-bipolar cells in addition to photoreceptors [18], making direct estimation of parameters relating to cone phototransduction less straightforward. Methods relating to cone-driven a-wave analysis were therefore excluded from this protocol.

Appendix 2. Limitations and further considerations

One limitation of the model is that, at high flash strengths, the very earliest portion of the predicted curve rises later, and more sharply, that the recorded a-wave. The fit can be improved by incorporating additional time constants [7,9,20,25]. However, as this introduces additional parameters, involves convolution operations, and renders the model not so easily described by a simple equation, this has not been included in the protocol. Figure 2 illustrates the effect of incorporating a capacitive time constant as described in previous studies [9,25].
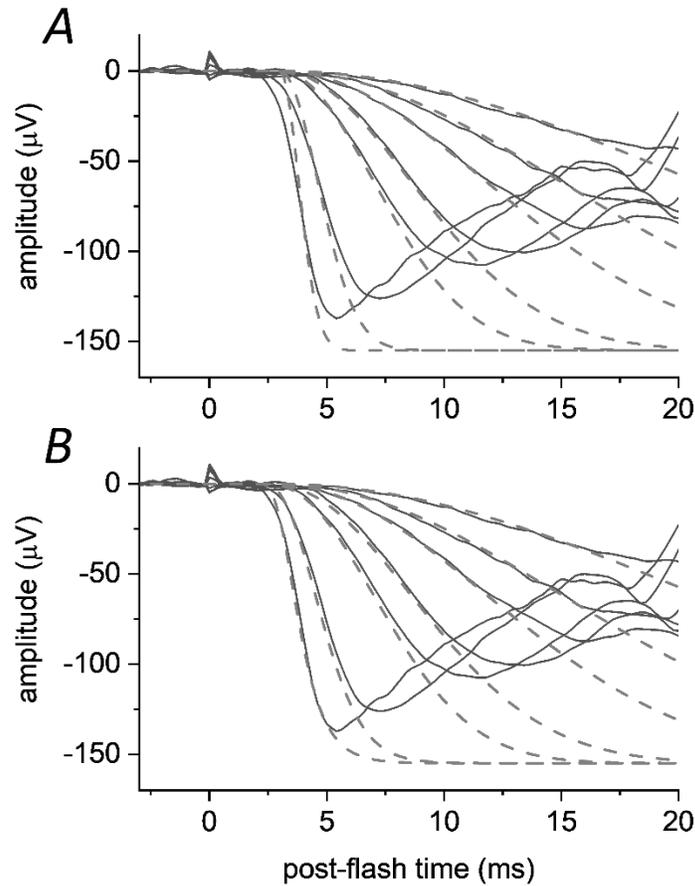
Figure 2: Effect of incorporating a capacitive time constant on model fit. *A*, Rod-system isolated ERGs (continuous traces) recorded from a healthy 31 year old male participant using a conductive fiber electrode in response to blue flashes (1.8 to 4.6 log scot Td.s), obtained by subtraction of responses obtained in the presence of a rod saturating blue background from those obtained in the dark. Dashed curves show the a-wave model (Equation 1, dashed curves). The fitting parameters are $R_{ROD} = 155$ μV, $S = 43$ (scot Td-s)$^{-1}$ s^{-2}, $t_d = 3$ ms. *B*, Responses and model fitting as in *A*, but the model takes into account an additional capacitive time constant (0.85 ms) by numerical convolution. The fit to the strongest flash responses is improved compared with *A*.

Robson and Frishman [15] have convincingly argued, based on a number of prior experimental studies (including [30,31]), that the early recovery following the a-wave negative peak in response to strong flashes originates from currents within photoreceptors (inner segments or axons, but not outer segments), with the portion of the response immediately prior to the peak also attributable to these currents (and not to the outer segment photocurrent). This portion of the response has been termed the "nose". They have proposed a more comprehensive model that also fits this part of the response [15] and may have greater validity (illustrated in Figure 3). However, as the model has a large number of parameters, is not easily described by a single equation, and is not in widespread use, we have not formally included their derivation. They also suggest a more accessible parameter, which is the time interval between the a-wave reaching 10% and 90% of the peak amplitude.
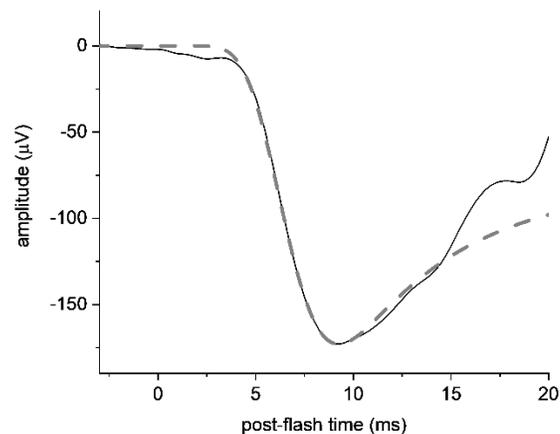

Figure 3: Alternative a-wave model. Rod-system isolated ERG recorded from a healthy participant using a conductive fiber electrode in response to white flashes (3.3 log scot Td.s). Dashed curve plots the model of Robson & Frishman [15] taking into account photoreceptor axonal currents, which may account for the peak and initial recovery of the response to strong flashes. The model fits this portion of the response (unlike Equation 1), but is not described by a single equation, and has a large number of parameters.

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	Authors and year	Participants	Flash characteristics (colour and strength)	Method of estimation of cone response for subtraction
1	Breton et al., 1994 [5]	6 healthy volunteers 1 patient with cone dysfunction with rod involvement 1 patient with sector RP	White and blue flashes <2.5 to 5.1 log scot Td s	None
2	Hood & Birch, 1994 [6]	15 healthy volunteers 11 patients with RP 4 patients with cone-rod dystrophy	White and blue flashes 1.9 to 3.8 log scot Td s	Photopically matched red flashes and 1.9 log Td white adapting background
3	Cideciyan & Jacobson, 1996 [7]	14 healthy volunteers	Blue flashes up to 4.6 log scot Td s White flashes up to 5.1 log scot Td s	Scotopically and photopically matched red flashes (and blue flashes scotopically matched to red flashes)
4	Fulton & Hansen, 1996 [8]	5 infants and children with ROP 7 term-born healthy infants 7 healthy young adults	Blue flashes. Max white flash strength 4.8 log scot Td.s (but attenuated by blue filter).	Photopically matched red flashes
5	Smith & Lamb, 1997 [9]	6 healthy adults	Blue flashes Up to 4.5 log scot Td.s	Photopically matched red flashes (and blue flashes scotopically matched to the red flashes)

6	Birch et al., 2002 [12]	100 healthy volunteers 24 patients with X-linked RP	White flashes 3.2 to 4.4 log scotopic Td.s	Identical flashes on 3.2 log Td white rod-saturating background (similar to ISCEV standard)
7	Tzekov et al., 2003 [13]	125 patients with usable rod a-waves (out of a total of 418 patients with RP)	White flashes 3.2 to 4.4 log scotopic Td.s	Identical flashes on 3.3 log scot Td white rod-saturating background (similar to ISCEV standard)
8	Mahroo et al., 2012 [25]	3 healthy adults	White flashes 1.0 to 2.4 log scot Td.s* Blue flashes 1.8 to 4.6 log scot Td.s*	Identical flashes on blue rod saturating background (c. 2.0 log phot Td and 3.1 log scot Td*)
9	Dimopoulos et al., 2013 [14]	25 patients with unilateral neovascular AMD 18 age-matched controls	White flashes 2.0 to 3.0 log scot Td.s	Identical flashes delivered on white rod saturating background 30 photopic cd m^{-2} (corresponding to ISCEV photopic background)

Table 1. Studies selected from published literature to exemplify different methods used to isolate rod a-waves for fitting the a-wave model in healthy human participants and various patient populations. In studies 3, 5 and 8, modifications were made, with explicit theoretical justification, to the fitting equation to improve the fit for the strongest stimuli. *Illuminance calculated assuming a dilated pupil area of 40 mm^2; values in Td are calculated by multiplying stimulus strength in cd.m^{-2} by pupil area in mm^2.