ISCEV extended protocol for the light-adapted full-field ERG luminance-response function (the photopic hill)

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Abstract

The International Society for Clinical Electrophysiology of Vision (ISCEV) standard for full-field electroretinography (ERG) describes a minimum procedure involving dark-adapted (DA) and light–adapted (LA) recordings. This extended protocol provides a template for additionally exploring the light-adapted ERG transfer function, commonly referred to as the luminance-response function. The LA ERG a-waves, arising from cones and cone off-bipolar cells, show a saturating function. The LA ERG b-waves, which arise primarily from activity of retinal bipolar cells, show an amplitude peak followed by a non-zero plateau (the 'photopic hill'). LA ERG i-waves, associated with retinal off-responses, show a peak for intermediate flash strengths and are non-recordable for strong flashes. This protocol provides methods of investigating these ERG characteristics, to assess change or generalised dysfunction affecting the LA retina and to help distinguish between the on- and off-responses of the cone system. The LA ERG transfer or "luminance-response" functions for ERG a- b- and i-waves are recorded with a standard 30 $cd.m^{-2}$ background and adequately interpolated with a sequence of nine flash stimuli from 0.03 to 300 $cd.s.m^{-2}$. A short protocol is also presented to measure the mid-range of the 'photopic hill' using 4 flash levels.

Keywords

Clinical Standards. Electroretinogram (ERG). Full-field ERG. International Society for Clinical Electrophysiology of Vision (ISCEV). Light-adapted (LA). Photopic Hill. Retinal dystrophies

Introduction

The International Society for Clinical Electrophysiology of Vision (ISCEV) standard for full-field electroretinography (ERG) describes a minimum procedure involving dark-adapted (DA) and light–adapted (LA) recordings, but encourages the use of additional protocols for clinical ERG testing(1). This extended protocol describes the LA ERG transfer function, commonly referred to as a luminance response (LR) series, a specialized 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 applications

The series of ERGs recorded for the ISCEV standard full-field ERG enables the distinction between generalized outer and inner retinal dysfunction and predominant rod or cone system involvement and can help differentiate between a wide range of retinal disorders (13). However, characterization may be improved by additional LR analyses, potentially enabling detection of more subtle changes such as early progression of disease or toxicity. For example, luminance-response functions may reveal abnormalities to weaker and/or stronger flashes that are not evident in the Standard LA 3.0 ERG, or the LR function may reveal the extent of an abnormality detected with the Standard (8, 14-16).

This extended protocol is designed to provide additional characterisation of cone system function, particularly in retinal disorders with dysfunction that is post-phototransduction or involves the inner nuclear layer of the retina. For brief, full-field flashes, the LA ERG LR series for a-wave amplitudes can be interpolated using a saturating (Naka-Rushton) function (2). The b-wave series shows a peak followed by a non-zero plateau, also called the “photopic hill” (2, 3). The reduction in the b-wave amplitude at high flash luminance levels is associated with reduction in the d-wave amplitude(4) and therefore relates to cone off-responses (2, 5, 6). Primate studies have shown two primary factors underlying the ‘photopic hill’ phenomenon: reduced on-response amplitude and a delayed and diminished off-response at higher luminance levels(2).

The LR series for the LA b-wave amplitude may be informative in retinal disorders affecting primarily the cone system, particularly in conditions where cone-driven on- and off-responses are affected differently (6-11). ISCEV has also established an extended protocol for the photopic On–Off ERG to long duration light stimuli can also probe such conditions (12). Saturating luminance levels are more readily generated for brief, than for extended flashes and they are less demanding for patients who may be unable to suppress blinking during longer light stimulation. Thus brief flashed are more suitable for an extended protocol describing an ERG luminance-response function. The method described here utilises brief flashes and extends ISCEV-standard light-adapted testing incorporating the LA 3.0 ERG.

Patient population

This protocol is suitable for patients of all ages able to tolerate ganzfeld stimulation and referred for investigation of possible generalised cone system dysfunction, especially those with suspected dysfunction occurring post-phototransduction or at the level of the inner nuclear layer.

Technical Issues

This extended protocol for the LA ERG LR function follows the basic patient preparation and requirements of the ISCEV Standard full-field light-adapted ERG [1]. Commercial systems for clinical ERG stimulation and recording accommodate the full protocol if they can deliver the required flash stimuli. Additional considerations are outlined below.

a) Light stimulators

Typical light sources include light emitting diodes and Xenon lamps. Typical Xenon lamps will not discharge at the low voltages needed to produce weak flash levels; calibrated attenuation systems are required to use discharge lamps. Commercial systems with LED based flash can provide accurate repeatable flashes at low luminance but may not be capable of achieving the strongest flash levels. All stimuli are brief flashes, ≤5 ms, as defined in ISCEV standards.

b) Photopic background

The LA ERG LR series can be recorded across a number of luminance ranges and with a variety of backgrounds. In general, stronger backgrounds shift all functions to the right (i.e. stronger backgrounds require stronger flash stimuli to produce similar amplitudes). Stronger backgrounds also enhance the peak amplitude and lower the saturated plateau of the b-wave 'photopic hill' (2, 6, 17).

c) Range of flash strengths

To define the full LR function for both the a- and b-waves, the stimulation system must be capable of accurately delivering white flashes across a range from sub-threshold flashes (< 0.1

cd.s.m^{-2}) to saturating flash strengths. LA ERG b-wave saturation is typically achieved for flashes greater or equal to 100 cd.s.m^{-2}; a-wave saturation requires stronger flashes of 300 cd.s.m^{-2} (18, 19). In addition, many ERG systems do not to generate the stronger flash levels, restricting the upper limits of testing. For interpolation, stimuli are presented at half log-unit intervals; clinics or clinical trial protocols may be unable to accommodate the time required to record ERGs for many flash levels.

d) Signal averaging. Averaging is not usually required for generating waveforms for IR analysis providing responses are reproducible. Responses to weak stimuli may be prone to intrusion of blink and eye movement artefacts.

e) Interpolation of ERG amplitudes

Amplitudes from individual ERG waveforms may be affected by eye movement artefact, eye position and/or changes in the patient's position. Interpolation across the LR series s takes advantage of all of the amplitude data the series and may provide more reliable measures and facilitate inter-laboratory comparisons. Plotting the LR function may facilitate identification of inconsistencies and outliers, particularly if the data are noisy.

f) Ocular pigmentation

There is evidence that full-field ERG waveforms may have larger amplitudes in those with lower levels of ocular pigmentation; the differences in amplitude associated with pigmentation have been shown to be greatest for LA b-waves in some studies (20-22).

g) Nomenclature of the function. In seminal studies and in older publications the term ERG "luminance-response" function is used to describe the DA stimulus-response series or ERG transfer function. It is acknowledged that brief flashes should be described in terms of strength rather than luminance but the widely used term "luminance-response" (LR) function is retained for descriptive purposes and in reference to historical studies.

Calibration

Stimulation and recording systems should be maintained and the calibration verified at regular intervals following the current ISCEV standard for clinical full-field ERG and the ISCEV clinical guidelines (1, 13, 25)

Protocol Specifications

Patient preparation, electrodes and recording conditions for the LA ERG LR extended protocol parallels the methods detailed in the ISCEV Standard full field ERG(1) including mydriasis and 10 minutes light adaptation prior to testing. This protocol may be used as a stand-alone method or integrated into the ISCEV Standard full-field ERG protocol by incorporating additional flashes of appropriate strengths.

a) Photopic background

This protocol uses the ISCEV standard light-adapted background to facilitate integration with ISCEV standard full-field ERG testing (30cd.s.m^{-2}).

b) Flash Strengths

Table 1 lists the sequence of nine brief flash stimuli (≤ 5ms) used to define the LR functions for a-waves, b-waves and i-waves of LA ERGs. For efficiency and patient convenience and comfort, a short protocol is also specified, with five flash stimuli within the range available from most commercial ERG systems. The short protocol is sufficient identify the peak of the LA b-wave LR function in a typical retina.

Table 1: Full-field flash stimuli for light-adapted luminance-response (photopic hill) protocol†.

#	Flash Strength* ($cd \cdot s \cdot m^{-2}$)	Log flash strength (Log $cd \cdot s \cdot m^{-2}$)	Artefact –free ERGs† N (min-typical)
1	0.03	-1.5	10 flashes
2	0.1	-1.0	10 flashes
3*	0.3	-0.5	5-10 flashes
4*	1.0	0.0	3-10 flashes
5*	3.0	+0.5	3-10 flashes
6*	10	+1.0	3-6 flashes
7	30	+1.5	3-6 flashes
8	100	+2.0	3-6 flashes

9	300	+2.5	3-6 flashes

† ERGs recorded at ≤ 2Hz. with at least the minimum number of artefact-free ERG waveforms. Record at least the 'typical' number in the range to establish presence/absence of a detectable ERG. More ERGs should be added to the averaged result if there is a low signal-to-noise ratio.

* Units are time-integrated luminance for brief flashes.

** Stimuli included in the short protocol. Stimulus 5 is the ISCEV Standard light-adapted 3.0 ERG.

c) Inter-stimulus interval

Flash stimuli for this protocol are presented from weak to strong with at least 0.5 seconds between flashes.

d) Signal averaging

Single flashes may be adequate to characterise the ERGs to moderate or strong flashes, but averaging for each stimulus may reduce variability and the effects of background noise, particularly for small responses. For weak stimuli 3-10 sweeps, without blink or other artefact, is usually sufficient to establish whether there is a detectable ERG as defined by this protocol. Individual ERG waveforms should be assessed for repeatability and inconsistent waveforms eliminated from the average.

Response evaluation

The peaks of the LA ERGs are measured according to the ISCEV standard ERG methods [1] Specifically, a-waves are measured from the trough to the average pre-stimulus baseline, b-waves are measured from the a-wave trough to the b-wave peak and i-waves are measured from the preceding trough to the i-wave peak as shown in Fig 1.

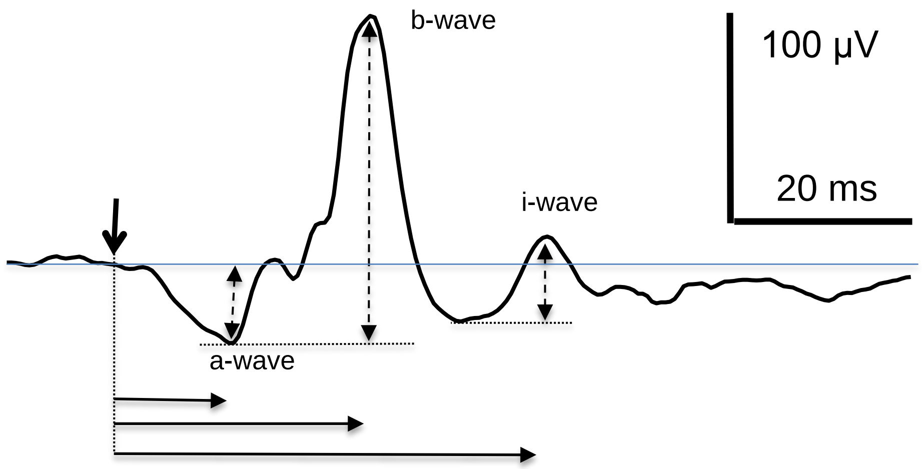

Figure 1: A typical averaged ERG from a light-adapted series is illustrated for a 3 $cd \cdot s \cdot m^{-2}$ flash with standard measurements. Peak times are measured from the flash (solid horizontal arrows), a-wave amplitudes from the pre-stimulus baseline and the b- and i-waves amplitudes are measured from the preceding troughs (dashed vertical arrows).

Light-adapted luminance-response functions are illustrated in Figure 2 for a typical healthy adult and for a patient with the incomplete form of congenital stationary night blindness (CSNB2). Note the LA ERG LR series is not needed for the diagnosis of CSNB2; data are shown to illustrate one type of abnormality. In the typical adult, the ERG a-waves increase with luminance and saturate; b-waves increase to a maximum for mid luminance ranges then are delayed and diminished for stronger stimuli, reaching an amplitude plateau. The i-wave is recorded with a maximum for mid-luminance stimuli and is not detectable for weak or strong flashes. Patients with CSNB2 have abnormal signal transmissions from photoreceptors to on- and off-bipolar cells(26). In this LA ERG LR series, the a-waves are similar to typical a-waves for weak stimuli but are prolonged for strong stimuli. In CSNB2, b-waves are recordable only in the mid luminance range due to the impaired signal transmission from photoreceptors resulting in reduced on and off bipolar cell activity.

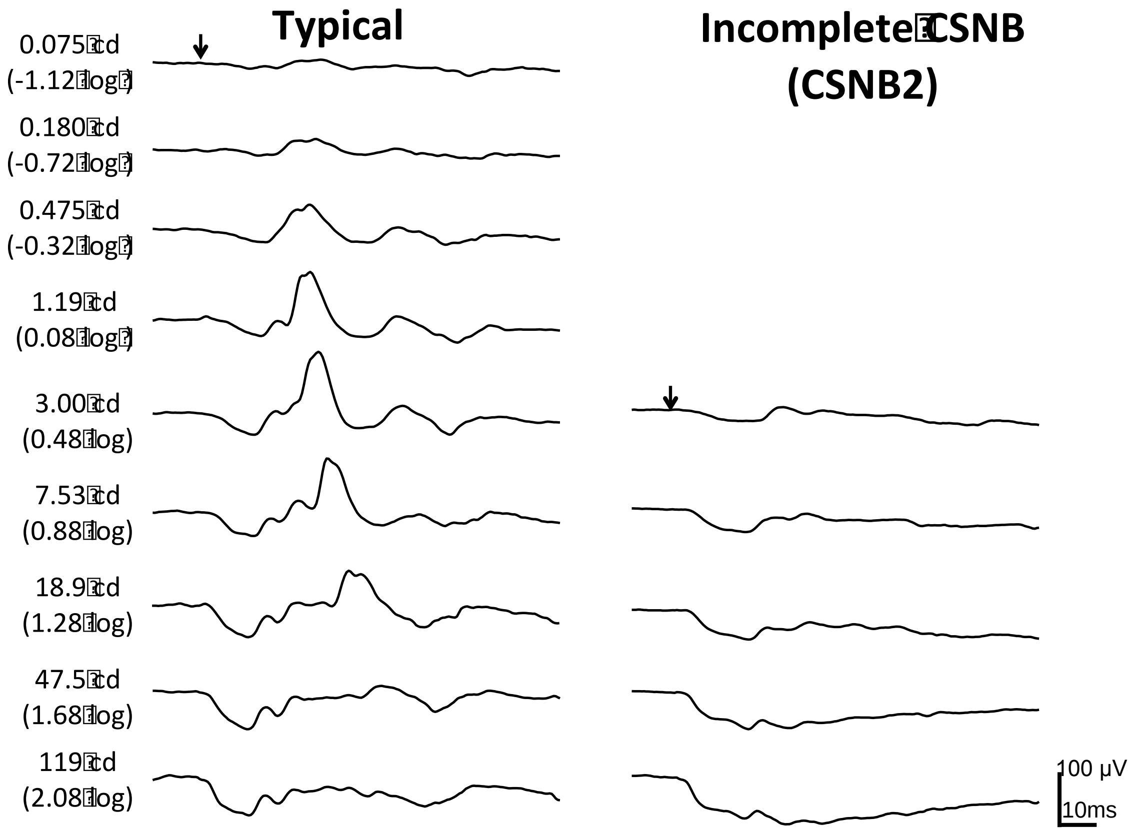

Figure 2. Light-adapted ERG LR series obtained from one participant with a healthy retina (left) and a patient with CSNB2 (right). The recordings were obtained using DTL electrodes to LED flashes on a constant photopic background. Flash luminance (phot cd s m^{-2}) is shown beside each ERG.

Interpolation: This extended protocol specifies plotting the averaged amplitudes of artefact-free ERG waveforms and using linear interpolation. Least squares fitting of suitable curves produces parameters that can be objectively reproduced, which may further improve intra-laboratory comparisons. As discussed in Appendix 3, curve fitting requires sufficient, good-quality data and saturated amplitudes; misleading parameters can result from insufficient or poor-quality data. Figure 3 illustrates interpolated b-wave amplitudes for typical light-adapted ERG LR series.

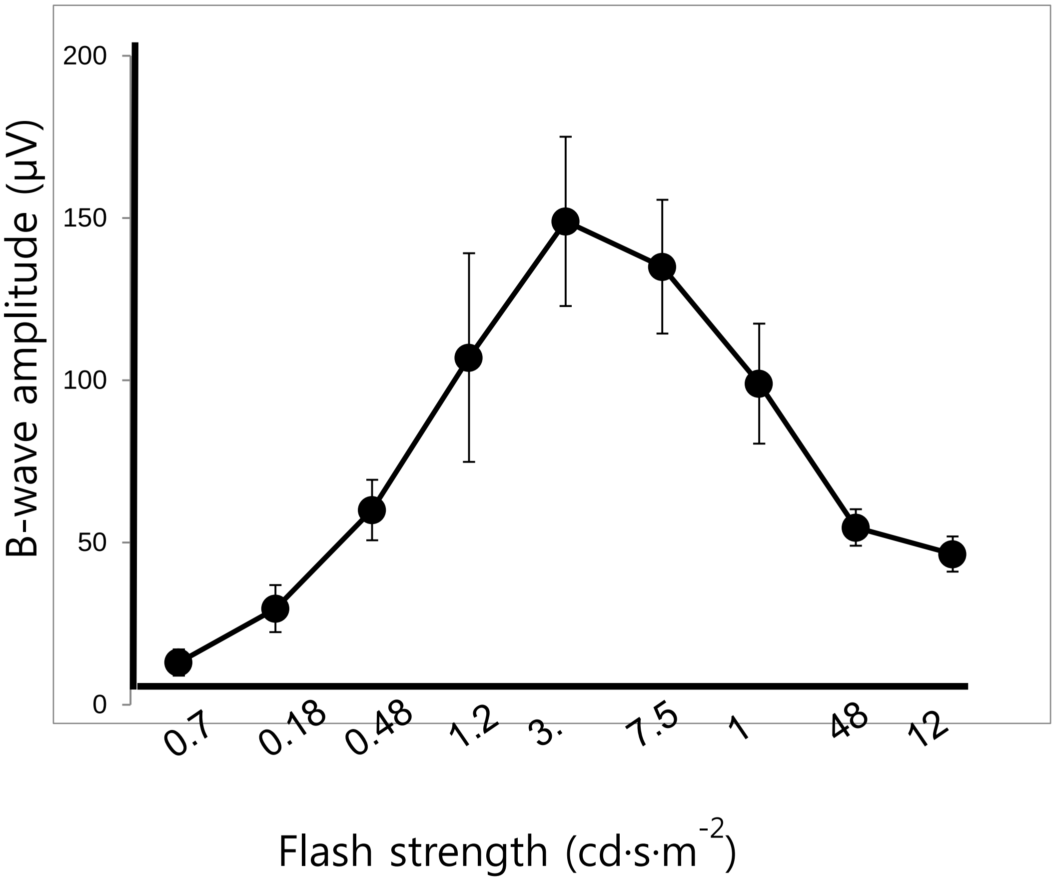

Figure 3: B-wave amplitudes for the light-adapted ERG LR series are shown. The data are from five typical young adults (mean ±SD) with liner interpolation.

B-wave amplitude; short protocol: Data obtained using the short protocol (Table 1**) will typically include the peak b-wave amplitude but reliable estimation of the "photopic hill" plateau is precluded. Linear interpolation is recommended; a Gaussian curve fitting for the short protocol is discussed in Appendix 3.

Reporting

Use of the ISCEV extended LA ERG LR protocol should be acknowledged and the waveform components that have been analysed must be specified (a-waves, b-waves and/or i-waves). Reports should specify whether the full or short protocol was used and any departures from ISCEV standard ERG methods and extended protocol acknowledged. Unless already stated as part of ISCEV standard ERG, reports should note time of testing, pupil diameter and type and position of the corneal electrode.

Ocular pigmentation and high refractive errors can impact ERG amplitudes and patients with characteristics that differ from the reference data should be noted. As iris colour is a good proxy for pigmentation of the ocular fundus (23, 24), iris colour may be recorded. Each laboratory should establish or confirm typical reference values for its own equipment, recording protocols and patient population giving attention to appropriate sample sizes. Any technical or compliance difficulties such as excessive eye movements or eye closure should be noted in the report.

Users of this light-adapted ERG LR series should report individual measures of amplitude and peak time for a-, b- and i-waves for the ISCEV standard light-adapted 3.0 ERG and for the ERG to the maximal flash stimulus in their protocol. Interpolated amplitude parameters for the LR series may be noted from linear interpolation (Fig 3) or from curve fitting procedures (Appendix 3). These include the saturated a-wave amplitude and an estimate of the flash luminance at half maximal amplitude (also called semi-saturation or sensitivity). For B-waves and i-waves, report the interpolated peak amplitude along with the flash luminance at the peak. The method of interpolation should be specified in the report. Table 2 lists the interpolated values to be reported for comprehensive characterisation. For some clinical applications interpolation of the LR function will not be possible (for example if amplitudes are small). A qualitative description of the LR function is sufficient in such cases. Note that the reporting in this protocol does not include interpolation of peak times.

Table 2: Interpolated key points for LA ERG LR series

Waveform	units	Description
a-wave	µV	Maximal a-wave amplitude (note whether saturation was reached)
	$cd.s.m^{-2}$	Sensitivity (flash strength at half V_{MAX})
i-wave	µV	Peak amplitude
	$cd{\cdot}s/m^2$	Sensitivity (flash strength at the peak)
b-wave (complete protocol)	µV	Maximal b-wave amplitude ("photopic hill")
	$cd{\cdot}s/m^2$	Sensitivity (flash strength at B_{MAX})
	µV	Saturated b-wave amplitude (b-wave plateau)
b-wave (brief protocol)	µV	Maximal b-wave amplitude ("photopic hill")
	$cd{\cdot}s/m^2$	Sensitivity (flash strength at B_{MAX})

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Appendix 1: Experimental procedures excluded from this extended protocol

The LA ERG LR extended protocol described here excludes the following considerations:

1. Interpolation or ERG amplitudes by least squares fitting of mathematical equation(s) is not a requirement of this extended protocol. We have included a discussion of fitting procedures in Appendix 2 as these are optional methods for describing data from luminance series.

2. Interpolation of peak times, whether linear or non-linear, is not included in this protocol.

3. This protocol specifies a single standard background and light adaptation of ≥10 minutes. LR series with a range of background levels and with partial light adaptation are beyond the scope of this protocol

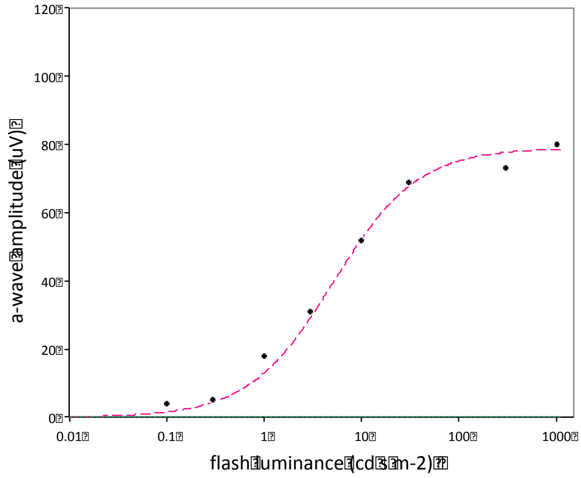
4. Chromatic variations in flashes stimuli are excluded from this extended protocol (i.e. ISCEV standard 'white' is use).

5. Studies and techniques for modelling LA ERG a-waves based on modelling the leading edge are excluded from the current considerations.

Appendix 2. Justification for the protocol details

The working group considered the methods published for recording LA ERG LR series to brief flashes. Studies with long flashes or chromatic flashes were excluded. This currently comprises a relatively small literature. The ISCEV standard recording methods and light-adapting background of 30 $cd.m^{-2}$ are not only convenient for clinical testing but are also consistent with virtually all publications (although some published studies used a range of backgrounds). Chromatic stimuli were excluded and are not widely used, thus the use of brief white flashes is specified, as defined by the ISCEV Standard full-field ERGs. The final consideration for the stimulus protocol was the luminance range and number of stimuli. To define a LR function, sub threshold and saturated stimuli are required and there must be adequate sampling for interpolation. Nine flash stimuli at half log unit intervals are adequate to define a typical LA ERG LR function for a-waves, b-waves and i-waves. Research studies tend to present more flash levels (i.e. sampling at a third or a quarter log unit intervals). More stimuli provide more samples interpolation, thus providing greater reliability. However, the underlying LR function will be the same. For those who have capacity to carry out a longer test additional flash levels are compatible with this extended protocol.

The method of amplitude interpolation is addressed in several ways in publications. Curve fitting can improve reliability because all data are used to determine the parameters. However, curve fitting can result in spurious values if data are incomplete or affected by noise. The working group agreed to propose linear interpolation, which is accessible and straightforward and has prepared Appendix 2 as a discussion of curve fitting methods. Note that interpolation methods are independent of the testing and ERG measurements. Specifying linear interpolation in this extended protocol does not preclude curve-fitting strategies and indeed users of this extended protocol are encouraged to use curve fitting in addition to linear interpolation.



Appendix 3: Curve fitting: interpolation of light-adapted ERG amplitudes

Luminance-response series for the light-adapted, full-field ERG (the photopic hill)

A-wave amplitude interpolation: A-waves are well described by a logistic growth function (1, 2) in the form:

$$V = \frac{V_{\max} I}{I + \sigma}, \qquad \text{Equation 1}$$

Where V is amplitude expressed in μV, I is time-integrated luminance with units of cd·sm^{-2}, V_{max} is the maximum saturated amplitude (μV), and σ is the semi-saturated flash strength (the time-integrated luminance evoking a half-maximal response in cd·s.m^{-2}).

As shown in Figure A3-1, light–adapted a-wave curve fitting is generally successful with a-wave amplitude data for the range of stimuli given in Table A3-1 (up to 300 cd·s·m^{-2}) (3). However, the curve is better defined by ensuring saturation of the a-wave with the addition of a strong flash at 1000 cd·s·m^{-2}. In patients with abnormal retinal function, such very strong flash stimuli might be required for successful curve fitting.

Figure A3-1. Luminance-response function for the a-wave of light adapted ERGs from a typical healthy eye fitted with a logistic growth function (dashed line)

The short protocol uses flashes up to 10 $cd.s.m^{-2}$, which does not saturate the a-wave; therefore the short protocol is sufficient for a-wave curve fitting. strengthThose using the short protocol should evaluate the a-wave amplitudes and implicit times separately for each stimulus.

I-wave amplitude interpolation: I-wave amplitudes are well described by a Gaussian function given in equation 2 below (see Figure A3-2):

$$V = G\left[\left(\frac{I}{\mu}\right)^{\frac{\ln(\mu/I)}{B^2}}\right]$$

Equation 2

Where V is amplitude (µV), I is the time-integrated luminance ($cd{\cdot}s/m^2$), G is the maximum amplitude of this Gaussian function (µV), B is a measure of the width of the Gaussian component ($cd{\cdot}s/m^2$), and µ is the flash strength at the peak G ($cd{\cdot}s/m^2$). *(Note that B is typically close 1 in healthy adults, which simplifies the fitting.)*

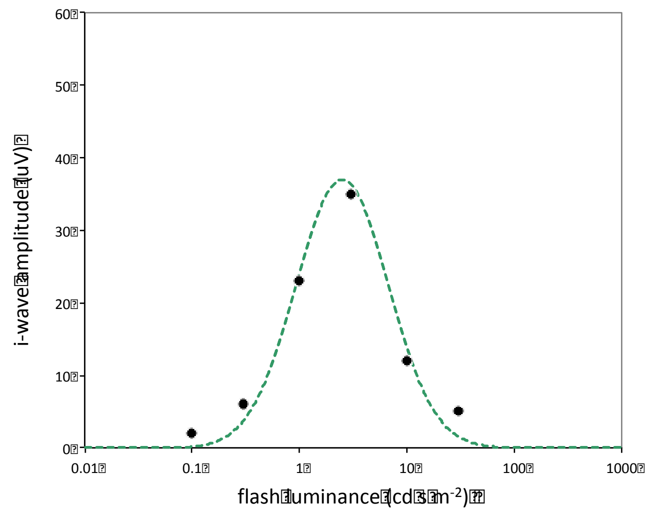


Figure A3-2: An example of representative i-wave amplitudes for the light-adapted ERG LR extended protocol. The curve shown is the best Gaussian fit (Equation 2)

The short protocol is not sufficient for interpolation of i-wave amplitudes. Users of the short protocol should measure and report i-waves separately for each stimulus.

B-wave amplitude interpolation – 'photopic-hill' protocol: The LR function of light-adapted b-wave amplitude is complex; curve fitting using the sum of two equations, Gaussian and logistic, is a method to fit the entire function. This requires sufficient data to define the 'hill' and plateau regions of the curve (4). Other options include fitting selected regions of the function, either the rising phase using a logistic curve (5) or the 'hill' portion of the curve using a Gaussian curve.

The entire light-adapted LR function can be fit using the photopic hill equation 3, comprising the sum of a logistic growth function and a Gaussian function:

$$V_b = G_b\left[\left(\frac{I}{\mu}\right)^{\frac{\ln(\mu/I)}{B^2}}\right] + \frac{V_{b\max} I}{I + \sigma_b} \qquad \text{Equation 3}$$

Where V_b is b-wave amplitude (μV), I is time-integrated luminance (cd·s/m^2), G_b is the maximum amplitude of the Gaussian component (μV), B is a measure of the width of the Gaussian component (cd·s/m^2), μ is the flash strength at the peak of the Gaussian component, G_b, (cd·s/m^2), V_{max} is the maximum saturated amplitude (μV) of the logistic component, and ▯ is the semi-saturated time-integrated luminance (the flash strength that evokes a half-maximal response) for the logistic component (cd·s/m^2). The fitting can be simplified by fixing the Gaussian width, B, to a value of 1 without any substantial change to the fitting parameters (4).

Figure A3-3 illustrates the photopic hill curve for a representative light-adapted b-wave LR function and Figure A3-4 shows additional individual examples. Table A3-1 gives the parameter descriptions for Equation 3.

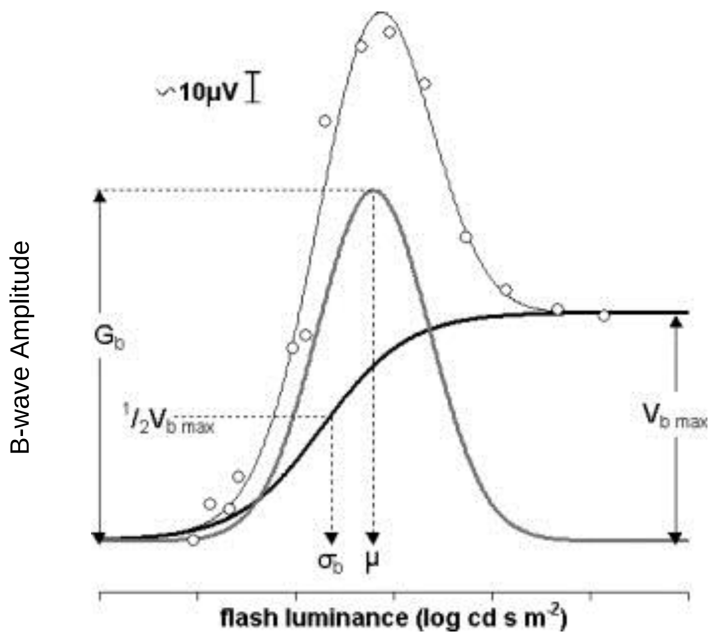


Figure A3-3: Graphical representation of Equation 3 with a representative set of ERG b-wave amplitude data (open circles). Thick grey line: Gaussian function. Thick black line: logistic growth function. Thin line: Equation 3: the Gaussian and logistic growth functions, which sum to create the photopic luminance–response curve or "photopic hill". [Reproduced with permission Hamilton et al 2007 (6)]

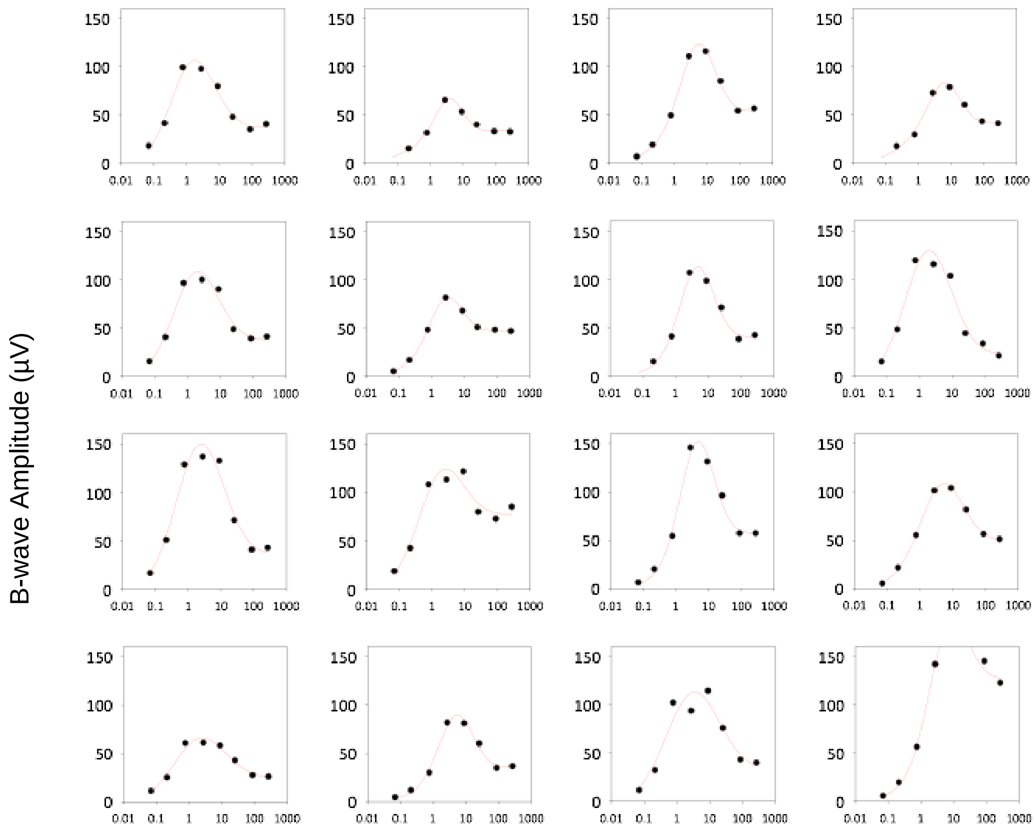


Figure A3-4: Variation in the fitting of equation 3 to light-adapted ERG b-wave amplitudes for sixteen healthy individuals.

A variety of software programs can be used to produce the optimum least squares fitting of the sum of two non-linear equations; see https://iscev.wildapricot.org/standards including a convenient template programmed in Excel®, which allows users to enter fitting parameters and b-wave amplitudes in µV (adjacent to the appropriate flash luminance levels in cd·s.m^2 to display data and curves for equation 3: LA_Bwave_breakdown_v4.xls

Equation 3 will result in spurious fitting if there are insufficient points to clearly define the location of the b-wave peak and plateau regions of the "photopic hill". Either the Gaussian function or the logistic growth function can inappropriately dominate the fitting of equation 3 if amplitudes from stronger flasher are not included. Figure A3-5 illustrates how truncated stimulus ranges can generate spurious fitting parameters.

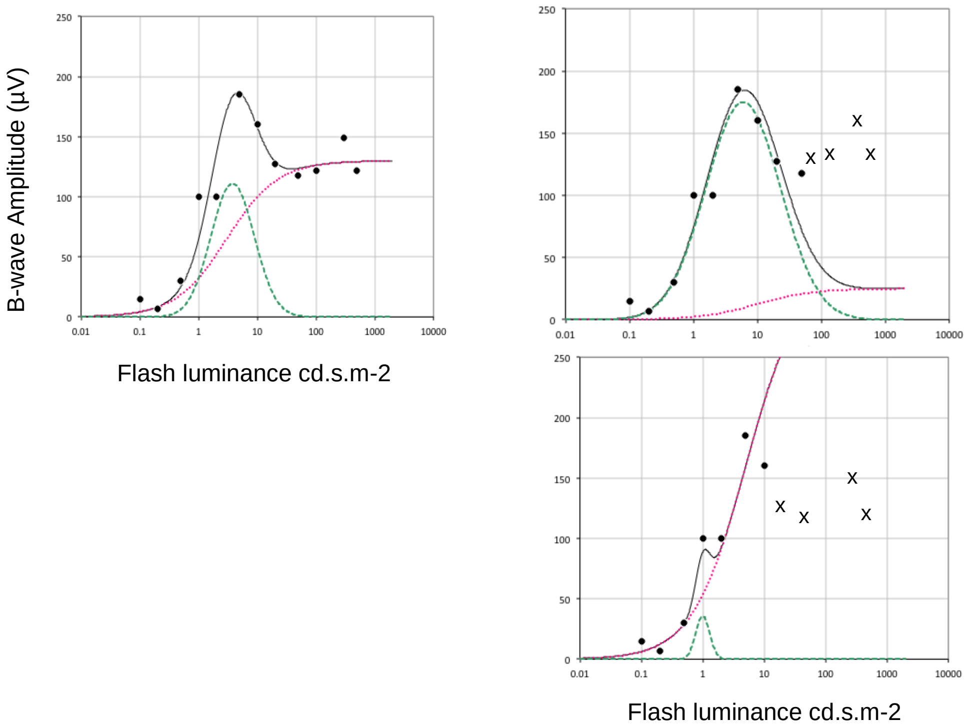


Figure A3-5: Graphical illustrations of curve fitting with equation 3. (Red line: logistic growth curve, dashed green line: Gaussian curve). The same b-wave amplitudes are shown for different ranges of flash stimuli (X indicates points excluded from the fitting). Equation 3 fits the data well for the complete range of stimuli (upper left); for 0.03 to 30 cd·s.m^{-2} (upper right) the Gaussian curve dominates the fitting; for the short protocol 0.1 to 10 cd·s/m^2 (lower left) a spurious logistic growth curve is produced by least squares fitting. Note that any sub-threshold (noise) data should not be included in curve fitting procedures. In this example, there are no light-adapted b-waves observed for flashes below 0.1 cd·s.m^{-2}.

For the short version of this extended protocol (flashes from 0.3 to 10 cd·s/m^2), we recommend fitting b-wave amplitudes using equation 2, a Gaussian curve without a logistic growth component. The Gaussian curve is symmetric so the descending portion will mirror the ascending portion and the ascending and peak portions will be suitably interpolated. It is important that the Gaussian fitting does not include b-wave amplitudes in the 'plateau' range of the luminance-response function as this provides a poorer fit and shift the peak of the Gausian curve to the left (Figure A3-6).

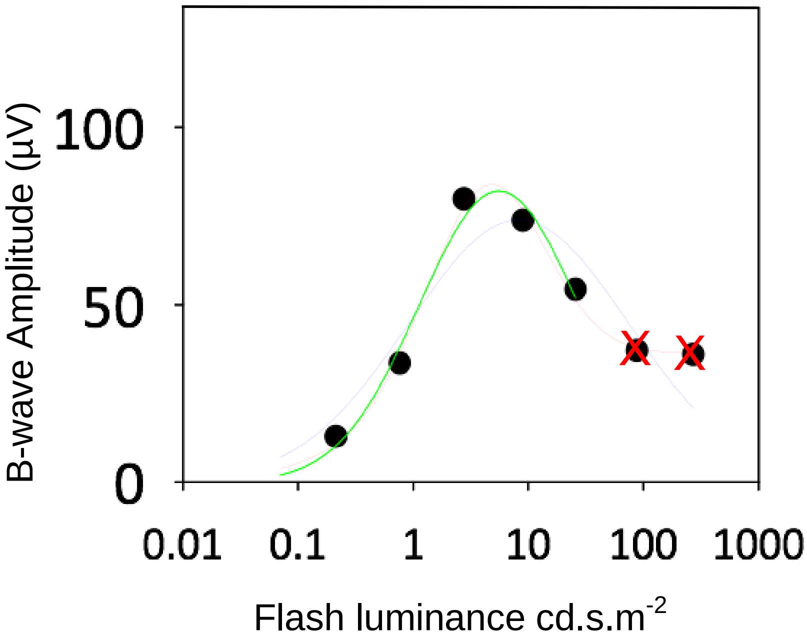


Figure A3-6: A representative illustration of curve fitting b-wave amplitude with equation 2, a Gaussian curve (green line). It is important to exclude data points in the plateau region of the light-adapted LR function (red X) prior to fitting.

While curve fitting provides robust and repeatable interpolation for ERG amplitude data, it may not be desirable under all circumstances. In particular, for clinical abnormalities with very small b-waves. If normative ranges are established either with curve fitting or by linear interpolation,

Table A3-1: Curve fitting parameters for logistic, Gaussian and "photopic hill"[1] equations

Waveform	Parameter symbol	Units	Description
a-wave	V_{MAX}	μV	Saturated amplitude
	σ	cd·s/m²	Sensitivity (flash strength at half V_{MAX})
i-wave	G_i	μV	Peak Gaussian amplitude
	μ	cd·s/m²	Sensitivity (flash strength at the peak, G_i)
b-wave (complete protocol)	G_b	μV	Peak amplitude Gaussian component
	μ	cd·s/m²	Sensitivity Gaussian component (flash strength at the peak, G_b)
	V_{bMAX}	μV	Saturated amplitude
	$σ_b$	cd·s/m²	Sensitivity Logistic component (flash strength at half V_{bMAX})
b-wave (short protocol)	G	μV	Peak amplitude Gaussian component
	μ	cd·s/m²	Sensitivity Gaussian component (flash strength at the peak, G)

1 The "photopic hill" equation is the sum of a logistic growth curve and a Gaussian curve when used to fit b-wave amplitudes of the extended protocol for light-adapted, full-field ERG LR protocol (Equation 3).