

ISCEV guidelines for measurement and calibration of stimulus and recording parameters used in clinical electrophysiology of vision, 2020 edition

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1. Introduction

Clinical electrophysiology of vision comprises a range of non-invasive tests which assess visual function [1]. Retinal or cortical electrical potentials are evoked by visual stimuli and responses are amplified and filtered, digitized and typically require averaging to extract clinically useful information from background physiological noise. Stimulus and amplifier characteristics have substantial effects on the waveform, peak time and amplitude of responses and meaningful interpretation requires stimulators and recording devices to be accurate. Published standards for the full-field electroretinogram (ERG) [2], electro-oculogram (EOG) [3], visual evoked potential (VEP) [4], pattern ERG (PERG) [5], multifocal ERG (mfERG) [6], and several extended protocols [7–11] specify stimulus and recording parameters. Regular verification of stimulus and recording parameters is essential to ensure the reliability and stability of equipment and stimuli, to allow comparison with control data or for monitoring of responses. This document provides guidelines for the calibration and verification of stimulus and recording systems. Calibration refers to the process of measuring and, if necessary, adjusting a parameter to ensure it falls within a pre-specified range.

2. Stimulus calibration

2.1 Technical background

Visible light is the portion of the electromagnetic spectrum which can be absorbed by photopigment in the retinal receptors. This corresponds to wavelengths between approximately 380 and 750 nm in the human. Radiometry is the measurement of the energy contained in emitted or incident light. Photometry is a system of measurement that weights this energy by the nominal spectral sensitivity of the human eye, scaling the physical stimulus per unit wavelength to emulate its effect on a typical human observer. ISCEV Standards stipulate achromatic stimuli; pattern stimuli are specified as black, and white and light flashes and background are specified as white light. The white light may have a broad spectrum, or be derived from a combination of narrow-bandwidth sources producing light similar to the CIE standard illuminant C (coordinates near $x = 0.31$, $y = 0.32$). Standard photometric functions, spectral efficacy functions, describe the effect of light on the adult human eye as a function of wavelength under light-adapted (LA, photopic) and dark-adapted (DA, scotopic) conditions, $K(\lambda)$ and $K'(\lambda)$ respectively (Fig. 1) [12]. When normalized to their maximum values, these

become spectral efficiency functions, $V(\lambda)$ and $V'(\lambda)$, respectively [13, 14]. The scotopic peak sensitivity is shifted to shorter wavelengths compared to the photopic peak, with peak sensitivities at 507 nm and 555 nm, respectively

The photometric quantity of most relevance to clinical electrophysiology is luminance (L). Luminance is defined as the luminous flux per unit solid angle per unit surface area emitted or reflected from an extended surface, measured in a particular direction. It is independent of distance from source and depends on the viewing angle unless the surface is Lambertian (emitting light uniformly in all directions). The exit port of a ganzfeld dome (an integrating sphere used to deliver full-field light stimuli) acts as a Lambertian surface. Monitors displaying a checkerboard and the background of a ganzfeld bowl are examples of surfaces which should be calibrated in luminance units. The SI unit of luminance is the candela per square metre (cd/m^2). To convert between photometric units (e.g. $\text{candela}/\text{m}^2$ and footlambert) see [12, page 251]. A more in-depth discussion of photometry with respect to clinical electrophysiology can be found in [15].

If the stimulus emitted or reflected is much briefer than photoreceptor integration time (i.e. is a brief flash), the stimulus effectiveness is best quantified as time-integrated luminance (typically mean luminance \times flash duration) rather than its instantaneous luminance value. Flash time-integrated luminance should be calibrated in units of candela-seconds per square metre ($\text{cd}\cdot\text{s}/\text{m}^2$) and for brevity is termed “flash strength” in this document, as in the ISCEV standards and extended protocols.

Note that the scientific term ‘intensity’ applies only to point sources and is not suitable for describing any ISCEV standard visual electrophysiology stimulus [2, 15]. Similarly, illuminance (E), the luminous flux incident on a surface per unit area of the surface (in lumens per square metre, lm/m^2), is not useful for describing clinical electrophysiology stimuli as illuminance decreases with increasing distance from the source.

ISCEV standard protocols specify only stimulus luminance but retinal illuminance may be important when considering the influence of factors such as pupil size, eye size and ocular media opacity (media light transmission). Retinal illuminance is the luminous flux per unit area of retinal surface (E_r , lm/m^2). A conventional proxy for retinal illuminance is troland value (T , trol), an estimate of the effective stimulus at the retina, based on the assumption that pupil size is the major factor in calculating retinal illuminance. Troland value is the product of stimulus luminance and pupil area:

$$T = L \cdot a, \text{ where } L \text{ is the photopic luminance in } \text{cd}/\text{m}^2 \text{ and } a \text{ is pupil area in } \text{mm}^2.$$

Troland value (also termed conventional retinal illuminance) reflects luminance corrected for pupil size rather than true retinal illuminance, since neither pre-retinal light absorption by ocular media, nor eye size, nor the Stiles-Crawford effect etc. are accounted for.

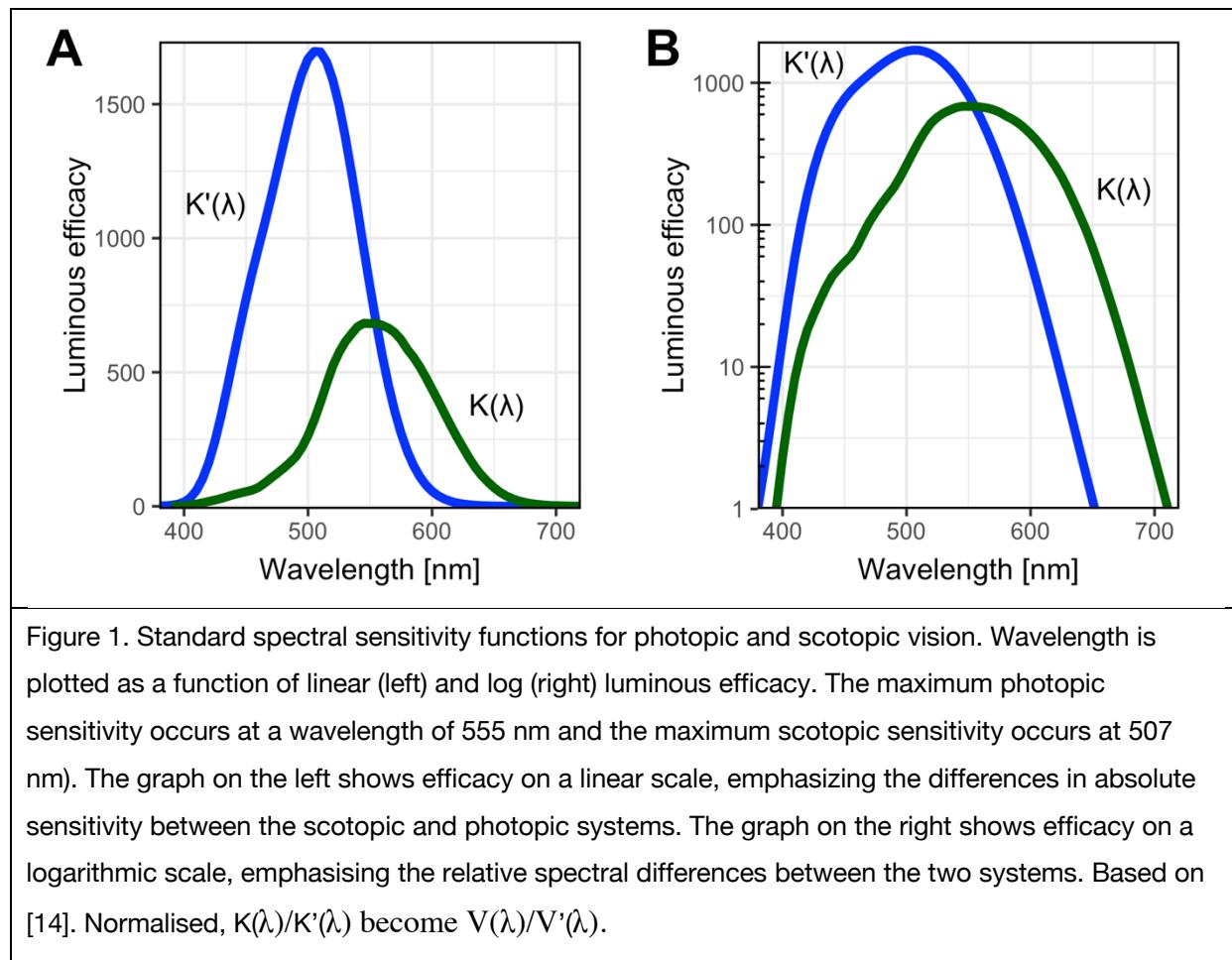
2.2 The photometer

Luminance is measured using a photometer. Photometers incorporate optical filters to match the spectral responsiveness of the nominal human eye (the $V(\lambda)$ or $V'(\lambda)$ function) and their output therefore indicates photopic or scotopic stimulus luminance. A radiometer, designed to measure energy across the entire electromagnetic spectrum, needs these filters added to achieve the appropriate output. Some photometers have a temporal integration option to measure pulses of light. Others have internal circuitry that integrates over time intervals longer than most strobe flashes. The protocol described in Table 1 applies in either of these cases.

Although luminance levels used for stimulation of rods are more accurately specified in scotopic units based on $V'\lambda$, the ISCEV ERG Standard [2] and this document use photopic units ($V(\lambda)$) as the primary specification for calibration of all stimuli. Note that photopic units are unsuitable if chromatic stimuli are used to stimulate a dark-adapted eye due to the different shapes of the $V(\lambda)$ and $V'(\lambda)$ functions. For example, a red and a blue flash with equal photopic luminance values will be very different if specified in scotopic units (blue flash stronger). If a short wavelength (blue) flash used to elicit DA, rod-system ERGs has a strength in photopic units of 2–3 cd·s/m²; its strength in scotopic units is approximately 4 scotopic cd·s/m².

The photometer should be capable of accurate measurement at the low luminance levels used in visual electrophysiology, e.g. the dark check of approximately 0.1 cd/m² used for the pattern VEP.

Response properties of photometers may vary with time and use. Photometer manufacturers typically recommend annual factory calibration using a source traceable to an international standard. This service may be offered by manufacturers and by national physics laboratories.



2.3 Protocols for measurement of visual stimuli

Commercial instruments do not necessarily provide the means for users to calibrate visual stimuli, but most manufacturers offer initial calibration and verification systems which may run prior to testing or with a follow-up schedule as part of ongoing maintenance, e.g. on an annual basis. In keeping with the previous version of this guideline, we recommended verification of calibrations at a maximum interval of 6 months [16] with re-calibration when indicated; more frequent checks may be essential to establish stability if values change substantially between successive measurements. The onus is on practitioners to ensure adequate calibration of stimuli and to interpret recordings with appropriate consideration of stimulus accuracy and stability.

2.3.1 Measurement of flash strength

The protocol for flash measurement is summarized in Table 1. The appropriate measure for brief flashes is time-integrated luminance (units $\text{cd}\cdot\text{s}/\text{m}^2$), here called flash strength. A

variability of up to 10% from flash to flash is typical of discharge lamps. LED sources produce flashes with more consistent strengths. If higher variability is found, then the manufacturer of the flash device (ganzfeld, hand-held flash or monitor) should be consulted.

2.3.2 Measurement of 30 Hz flicker strength

To calibrate the flicker stimulus, it is typically necessary to integrate the photometric measurement over a fixed number of flashes after the source output has stabilized. This is particularly important if using flash units based on discharge lamps, which may not have time to fully recover between flashes during 30 Hz flicker stimulation. For example, if using a discharge lamp, exclude the first 5 flashes and integrate over the following 30 flashes. The obtained measure can then be divided by the number of flashes (30 in this example) to obtain the strength of individual flashes in a flicker stimulus. Alternatively, the photometer may be used in continuous measurement ('steady-state') mode with a long time constant (if possible). Once the reading has stabilized to the flicker stimulation, divide by 30 to get the strength of a single flash.

Table 1. Flash measurement protocol

I. Preparation of equipment

- a. Prepare the radiometer to make *photometric* measurements (turning on the meter for sufficient time to stabilise and adding appropriate filters if indicated) in *temporal integration mode* ($\text{cd}\cdot\text{s}/\text{m}^2$).
- b. Place the detector at the position occupied by the eye during a test.

II. Making the measurement

- a. Darken the room, including turning off ganzfeld fixation and any background lights or infra-red monitoring devices e.g. lamps for cameras in the ganzfeld.
- b. Zero the photometer.
- c. Make at least five measurements.
- d. Select the median value as the flash luminance. If values differ by >10% consult the manufacturer or perform the required adjustments as appropriate.

2.3.3 Measurement of mean luminance of pattern stimuli

Visual display units (VDUs) presenting pattern stimuli are used in many clinical electrophysiological tests, including the VEP, PERG and mfERG. The mean luminance can be derived from the measurement of luminance of light (L_{max}) and dark (L_{min}) pattern elements (assuming equal size or duty cycle):

$$\frac{L_{max} + L_{min}}{2} \quad (1)$$

Most cathode ray tubes (CRTs) perform optimally with mean luminance settings of between 25 and 100 cd/m² since nonlinearities can occur with L_{max} of 200 cd/m² or greater. The ISCEV VEP Standard [2] requires mean luminance to be in the range of 40–60 cd/m², PERGs require mean luminance over 40 cd/m² and mfERGs require a mean luminance over 50 cd/m². The calibration of mean luminance is of the utmost importance for pattern stimuli as amplitudes and peak times of the responses are affected by mean luminance as well as by contrast.

For CRTs, luminance is likely to be greater in the centre than at the periphery. To maintain compliance with the current ISCEV mfERG Standard [6], there must be less than a 15% difference between the centre and periphery of the stimulus array. For a pattern VEP (less dependent on eccentric stimulus components), the maximum acceptable difference is 30% [4]. When recording pattern reversal or pattern onset/offset responses, there should be no transient changes in mean (space-averaged) luminance. Pattern onset/offset stimuli are particularly prone to unwanted luminance artifacts. A qualitative method of checking for luminance transients can be achieved perceptually. A sheet of white paper can be held in front of and parallel to the plane of the stimulus at a distance of ≈0.3 m, thus diffusing the pattern to a uniform field. The observer should stand next to the stimulus screen and view its reflection on the paper while the stimulus is modulated at a slow rate (e.g. 8 reversals per second). The room lights should be off to enhance sensitivity. The reflection from the paper should be constant over time, with no indication of when the pattern changes. If there is a transient or step luminance change with each pattern shift, then a luminance artifact is present which will contaminate the recorded potential. This situation must be remedied to obtain a valid pattern response. This method does not negate the need for regular quantitative photometry.

Table 2. The five major display techniques, with notes for their use as visual stimulators

Acronym	Description	Flicker	Advantages	Disadvantages
CRT	Cathode ray tube	yes	Continuous pixel size	No longer produced; loses contrast with small check sizes
LCD	Liquid crystal display (with TFTs)	no	Widely available. (Techniques can reduce luminance artifact)	Unacceptable luminance artifact on reversal if used without adaptations to correct.
Plasma	Plasma display	yes	Very bright	Pixels $\approx 3\times$ larger than LCD
OLED	Organic light emitting diode	no	Technology evolving. Has potential to optimise stimulus presentation.	Some have ‘picture processing’ detrimental to precise stimulation
DLP	Digital light processing	yes	Array of tiny mirrors, used for projection	Projection only; possible timing issues (colour wheel)

Table 2 lists the major types of visual display units (VDUs). In most applications, CRTs have been replaced by LCDs which, however, commonly produce major luminance artifacts on pattern reversal. A number of solutions have been proposed by manufacturers including inserting flicker at every frame, modulating a banded backlight, use of feedback loops, and feed-forward adjustments. LCDs may be superseded by OLEDs which are likely to offer increased quality with the caveat that built-in processing, intended to ‘improve’ the image quality for most consumers, may be detrimental to clinical applications that require stringent control and standardization of visual stimuli.

2.3.5 Measurement of contrast of pattern stimuli

Pattern stimulus contrast is defined as the Michelson contrast ratio:

$$\text{Contrast} = \frac{L_{max} - L_{min}}{L_{max} + L_{min}} \times 100\% \quad (2)$$

where L_{max} is the luminance of the light element and L_{min} is the luminance of the dark element. Thus, contrast ranges from 0% for a homogeneous field to 100% when the dark checks have a luminance of zero. Some VDUs may produce distortions in the stimulus at contrasts above 90%. The ISCEV VEP Standard [4] requires contrast to be at least 80%. For pattern VEPs, contrast level is less critical, because contrast has little effect on the VEP above

approximately 50%. However, mfERG and PERG amplitudes increase linearly with contrast. Thus, contrast calibration for mfERGs and PERGs is critical. The highest available contrast is specified in their standards, with a 90% minimum contrast for mfERGs and 80% minimum contrast for PERGs. It is advisable to secure access to or protect the ‘brightness’ and ‘contrast’ controls to prevent accidental adjustment.

A protocol for calibration of pattern mean luminance and contrast is presented in Table 3. It is suggested that a spot photometer be used for this calibration. A spot photometer is equipped with optics for measurement over a restricted field and usually has a means of monitoring the region that is being measured.

Table 3. Pattern stimuli measurement protocol**I. Preparation of equipment**

- a. Prepare the radiometer to make *photometric* measurements (turning on the meter for sufficient time to stabilise and adding appropriate filters if indicated) in cd/m²
- b. Put a large pattern element size (e.g., 2°) on screen
- c. Slow, or if possible, stop, pattern alternation

II. Making the measurement

- a. Adjust room lighting conditions to those used during testing
- b. Zero the photometer with the detector covered
- c. Where appropriate, focus the photometer optics
- d. Position the detector so that it is perpendicular to the screen and so that the measurement field is no more than half the size of either a bright or dark element
- e. Obtain stable measurement of light and dark elements at both the centre of the screen and, in separate measurements, near the edge of the screen.
- f. Make at least five measurements for both light and dark checks. Select the median value as the check luminance.

III. Calculations

- a. Calculate mean luminance using Equation (1) above with obtained measures of light element (L_{max}) and dark element (L_{min}) from the centre of the screen
- b. Calculate mean luminance for the periphery of the screen.
- c. Calculate stimulus contrast using Equation (2) above with luminance values obtained from the centre of the stimulus.
- d. If values differ from requirements of the standards, consult the manufacturer or perform the required adjustments as appropriate.

2.3.6 Measurement of element size

Pattern evoked responses show spatial tuning and thus are affected by the size or angular subtense of the pattern elements (e.g., check widths). Procedures for calculating visual angle for fixed viewing conditions and for choosing a viewing distance to obtain a desired visual angle are given below. For guidance, a pattern element of 1 cm subtends $\approx 1^\circ$ at a viewing distance of 57 cm.

2.3.6.1 Calculation of visual angle

(1) Measure the size of 10 elements (e.g. 10 check widths, whether black or white) across the centre of the screen and divide by 10 to obtain the mean element size. Do this horizontally and vertically to verify symmetry, e.g. square checks. (2) Measure the distance from the patient's eye to the centre of the screen. (3) Divide the element size by this viewing distance. (4) Determine the angle whose tangent is equal to this value by using the inverse tangent (\tan^{-1} on a calculator), converting from degrees to minutes of visual angle if necessary by multiplying by 60 (e.g., 0.25° equals 15 min.) Checksizes given in the ISCEV VEP Standard (1° and 0.25° , i.e. $60'$ and $15'$) refer to the width (equal to the height) of a check, not its diagonal: the diagonal is the size of 1 cycle of the fundamental spatial frequency of the pattern. See [17] for an explanation.

2.3.6.2 Calculation of viewing distance for desired element visual angle

(1) Measure the size of 10 elements (e.g. 10 check widths, whether black or white) across the centre of the screen and divide by 10 to obtain the mean element size. (2) Determine the tangent of the desired visual angle of one element (e.g. check width). (3) Divide the mean element size measured in step 1 by the tangent of the desired visual angle obtained in step (2) to obtain the viewing distance. Systems that allow input of the viewing distance to automatically calculate check size will have a monitor calibration protocol. Size calibration can be verified using the method above.

3. Recording equipment calibration

3.1 Technical background

Electrophysiological signals produced by the retina (full-field ERG, PERG, mfERG) and visual cortex (VEP) can be recorded non-invasively using standard, commercially available

equipment. Surface electrodes are typically used. These physiological signals have small amplitudes in comparison to external sources such as primarily AC line noise (60 or 50 Hz) and electrical noise from electronic equipment (often 120 Hz) and to the higher amplitude physiological ‘noise’ generated by muscle (e.g. heart, extraocular muscles) and brain (electroencephalogram, EEG). Differential amplifiers are used to reduce unwanted signals by amplifying the difference between the two inputs and rejecting signals common to both inputs, relative to an indifferent ('ground') electrode. The two inputs come from an electrode over the region responsive to stimulation, termed the active electrode, and an electrode over a more distant site, termed the reference electrode. Differential amplifiers’ ability to reject signals common to both inputs and amplify only their difference is governed by their common mode rejection ratio (CMRR). A high CMRR will help to eliminate much of the external line (mains) and physiological noise present in the recording environment. A CMRR of 100000:1 (100 dB) is reasonable for such recordings. For optimal common mode rejection, the active and reference electrodes must have closely matched impedances. Any impedance mismatch between electrodes would diminish rejection of unwanted signals even with perfect CMRR.

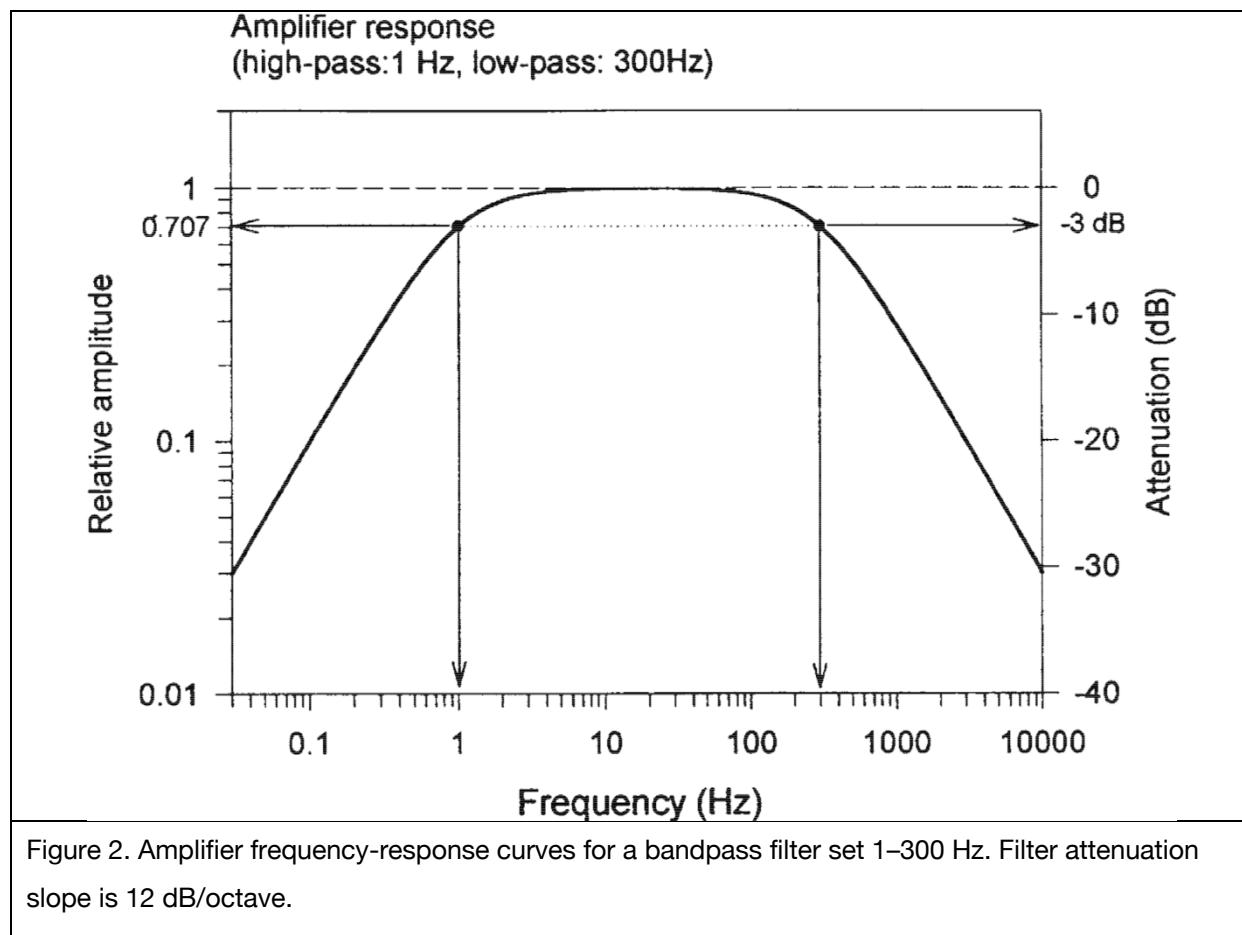
High amplitude artifacts, such as those generated by eye movements or blinks, can be excluded from the signal average on-line using an amplitude threshold criterion. The rejection threshold should be considerably higher than the expected amplitude of the physiological signal; for example, the VEP and PERG Standards [4, 5] suggest a $\pm 100 \mu\text{V}$ rejection threshold. This threshold may need to be higher when recording VEPs from children or patients with high EEG amplitudes.

Stimulus-locked signal averaging increases the signal to noise ratio by decreasing the random noise level by the square-root of the number of trials averaged. The choice of the number of averages should be governed by signal to noise conditions. For a small signal such as the pattern ERG or a reduced response, hundreds of averages may be needed to obtain a measurable signal, and more in a technically noisy or unfavourable environment. Signal averaging is generally not needed to record normal high amplitude full-field flash ERGs recorded with a corneal electrode, although all signals should be recorded at least twice to demonstrate reproducibility, and averaging may be required to record ERGs diminished by pathology. Averaging assumes a constant (stationary) response to the stimulus: if the patient’s state of adaptation or arousal alters over the duration of the recording, the averaged signal will not reflect these changes.

Typically, signals are converted from analogue to digital format using an analogue to digital converter (ADC) prior to averaging. Digitization should use a sampling frequency more than double the highest frequency contained in the signal, and with a minimum resolution of 12 bits (4096 levels). Amplifier gain should be matched to the ADC so that a sizable portion of its range is used to ensure high amplitude resolution, but staying below $\approx 90\%$ of the full amplifier range to avoid possible non-linearities at the extremes, e.g. clipping of the peaks.

The bandpass filtering characteristics of the amplifier should be tailored to the physiological signal as detailed in each ISCEV Standard and extended protocol. Amplifiers may use analogue high-pass and low-pass filters to attenuate low and high frequency components of the signal respectively. Filters are characterized by the frequency at which they reduce the incoming signal by 3 dB (29%), termed the corner or cut-off frequency, and by the slope of their attenuation in dB per octave (Figure 2). All analogue filters and digital emulations of analogue filters, especially low-pass filters which attenuate high frequencies, induce phase lag and dispersion which alters response peak times compared with unfiltered signals. Filter settings during testing should therefore be identical to those used to obtain reference data. Setting the low-pass filter to below 1/5th of the digitization rate will help to avoid substantial temporal aliasing due to production of artifactual low frequencies in the amplified signal. For example, for a sample rate of 1 kHz (1000 samples per second), the low pass filter cut-off should be no higher than 200 Hz.

Most current commercial equipment intended for clinical electrophysiology of vision use digital filters. Such filters are applied post-acquisition and have the advantage of improving the signal quality without waveform distortion.



3.2 Protocols for measurement of recording equipment

Calibration of recording equipment is essential to establish the accuracy and performance of recording devices and to allow appropriate interpretation of physiological signals. As for visual stimuli, manufacturers may offer ongoing maintenance protocols and annual calibrations but shorter intervals between checks may be necessary. Interpretation of electrophysiological data should be informed by an understanding of the accuracy and stability of recording parameters.

3.2.1 Measurement of electrode impedance

Impedance denotes resistance at frequencies other than direct current (DC), but at the relatively low frequencies used in clinical electrophysiology there is little difference between the two. It is measured by passing a low amplitude ($\leq 1 \mu\text{A}$) alternating current (AC, 10–100 Hz) through the tissue between a pair of electrodes. Impedance must not be measured using a DC ohmmeter since this will polarize the electrode, resulting in an unreliable measure and potentially resulting in a large standing potential between the electrode and the surface with

consequent drift. Impedance is equal to the ratio of the voltage between the electrode pair and the current, i.e. Ohm's law: impedance = V/I , where V is voltage and I is current. Typically, the active and reference electrodes are each measured against the indifferent (ground) electrode. If impedance is unacceptably high in both comparisons, the active and reference can be directly compared. If the impedance is low between active and reference, but each is high through the ground electrode, then the ground electrode is at fault.

Many commercial systems have internal impedance meters. Measurement of impedance of corneal or scleral ERG electrodes may produce phosphenes or may be dangerous to the eye, even with the very low current used in many devices. The circuits used to measure impedance should be fully isolated from both the line (mains) power and ground (earth).

3.2.2 Measurement of the amplification system

Calibration of amplifier gain is assessed by passing a known signal through the system and measuring system output. The known signal should pass through the entire system, beginning with the electrode-box. Many systems contain pre-amplifiers in the electrode-box, which go uncalibrated if the signal is passed only through the main amplifier. The amplitude of the input signal should approximate the amplitude of the physiological signal or response range. The amplitude of the output should closely resemble that of the input multiplied by the gain and taking into account the effect of the applied filters. Ideally, the system should be calibrated using both sine wave inputs of various frequencies and square wave pulses. Using sine wave inputs, both the amplification and filter settings can be readily assessed for accuracy. This technique requires the use of a signal generator capable of producing low amplitude output. Alternatively, simulated electrophysiologic signals can be used to determine the effects of the amplification system on measurable characteristics of the signal of interest. A squarewave pulse calibration signal will allow the detection of unwanted harmonic distortion or 'ringing' in response to an abrupt voltage change. The time constant of the high-pass filter can be assessed by measuring the duration that is required for a step change in DC level to be reduced to 37% of maximum. Using a 3-dB cut-off frequency (f_c), the time constant (τ) is related to f_c by the following equation:

$$\tau = \frac{1}{2\pi f_c} \quad (3)$$

Thus, a low frequency filter setting of 1 Hz is equivalent to $\tau=0.16$ s, and a setting of 0.3 corresponds to $\tau=0.53$ s. If amplifiers are not performing to specifications, or if distortions of

signals are observed, the equipment needs to be repaired. A useful check that all amplifier channels are working similarly is to pass an identical signal through all of the channels (setting all channels to the same input electrodes) with identical settings.

Table 4. Amplifier calibration protocol

- I. Preparation of equipment
 - a. Switch on amplifiers and signal generator and allow to stabilise (typically a minimum of 15 minutes)
 - b. Connect signal generator to amplifier inputs at the electrode box
 - c. Generate signals in the physiological amplitude range ($1 \mu\text{V}$ to 1 mV).
 - II. Making the measurements
 - a. Acquire and measure calibration signals as for a patient recording.
If signal averaging is used, the signal generator has to trigger the data acquisition system.
 - b. Measure multiple signal frequencies that begin below and extend above low-pass and high-pass filter settings
 - c. If values differ from nominal values, consult the manufacturer.
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The suggested protocol for amplifier calibration is given in Table 4. Amplifiers should be checked and if necessary recalibrated at a maximum interval of 12 months. Amplifiers must not be calibrated with a patient connected to the system.

4. Updates, revisions and links

ISCEV Standards, Guidelines and Extended Protocols are updated as necessary. Current publications can be found at <<https://iscev.wildapricot.org/standards>>. Clinical electrophysiology test results and publications using ISCEV procedures should cite the applicable current ISCEV Standards, Guidelines or Extended Protocols, if applicable stating how testing differed from or augmented the ISCEV procedure.

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