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Guidelines for calibration of stimulus and recording parameters used in clinical electrophysiology of vision

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Abstract

In order to perform a technically adequate clinical electrophysiological procedure it is necessary to calibrate the stimulating and recording equipment. Published standards for the electroretinogram (ERG) [1], electro-oculogram (EOG) [2], visual evoked potential (VEP) [3], pattern ERG (PERG) [4] and guidelines for the multifocal ERG [5] specify stimulus and recording parameters. Yet most commercial instruments do not provide the means for calibration of these parameters. The goal of this document is to provide guidelines for proper calibration of stimulus and recording equipment. The need for such guidelines is clear on both clinical and scientific grounds. Stimulus and amplifier characteristics have substantial effects on the peak latency and amplitude measurements that are commonly used in clinical electrophysiology. Many review articles on clinical electrophysiology emphasize the need for establishing norms for each laboratory as a function of age and gender rather than relying on published norms. However, if stimulus and recording parameters are not calibrated periodically, then these norms may actually be misleading due to changes in stimulus or recording conditions induced by aging of equipment or inadvertent change in settings. This document is divided into two major sections. The first is concerned with calibration of the visual stimulus. It begins with background technical information on the physics of light and its measurement. This is followed by protocols for measurement of the luminous intensity of flash stimuli and the mean luminance, contrast, and visual angle of pattern stimuli. The second section is concerned with calibration of electrophysiological recording systems. It begins with a description of the characteristics of bioelectrical signals and their measurement. This is followed by protocols for measurement of electrode impedance and amplifier calibration. Although this document was prepared as guidelines for clinical electrophysiological testing, it should be noted that the techniques described are applicable to any studies that are dependent upon accurate measurement of luminance or electrophysiological signals.

Calibration of the visual stimulus

Technical background

Light is defined as that portion of the electromagnetic spectrum that can be absorbed by pigment in retinal photoreceptors. This corresponds to wavelengths between approximately 400 and 750 nm (10^{-9} m). Radiometry is the measurement of the electromagnetic energy contained in the emitted or incident light. Photometry is a system of measurement that weights the physical energy of the source by the nominal spectral sensitivity of the human eye. Thus, photometric measures scale the physical stimulus to the effect that it has on a standard visual system. Colorimetry characterizes the chromatic properties of light. Since ISCEV protocols involve only the use of achromatic stimuli, this guideline will be directed to the photometric measurement of broad spectrum white light. The Commission Internationale de l'Eclairage (CIE), founded in 1913, is the organization that has been responsible for the standardization of the measurement of light. In 1924, this organization published a standard function for the luminous efficacy of the human eye

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as a function of wavelength under light-adapted (i.e., photopic) conditions. Sensitivity to short wavelengths was subsequently modified by Judd. When normalized to a maximum value of 1.0 at its peak at 555 nm, this function is named V_λ. The standard scotopic observer ($V_\lambda{}'$), indicating sensitivity as a function of wavelength under dark-adapted conditions, was developed by the CIE in 1951. Scotopic efficacy is shifted to shorter wavelengths compared to the photopic function, with peak sensitivity at 507 nm. Spectral efficacy functions are illustrated in Figure 1. The graph on the left shows efficacy on a linear scale, emphasizing the differences in absolute sensitivity between the scotopic and photopic systems. The graph on the right shows efficacy on a logarithmic scale, illustrating the relative spectral differences between the two systems.

The photometric measurement of most relevance to clinical electrophysiology is luminance. Luminance is a measure of light per unit area emitted from an extended source or reflecting surface. This measure is independent of distance. The Système Internationale (SI) unit of luminance is the candela per square meter (cd m^{-2}). The relation between this measure and older measures of luminance is shown in Table 1. Use this table to convert from units in the column header to units in the row header. For instance, to convert 33 footlamberts to candela m^{-2} multiply 33 by 3.426 yielding 113.06 candela m^{-2}. For brief flashes of light, such as those typically used for the flash ERG and VEP, the luminance of the stimulus must be weighted by flash duration, since temporal integration of the neuronal visual pathways is longer than the duration of the flash produced by a xenon flash tube. Thus, the appropriate unit of time-integrated luminance for brief flashes of light is candela seconds per square meter (cd·s m^{-2}). Illuminance is a measure of the luminous flux incident on a surface per unit area of the surface. Unlike luminance, illuminance decreases with increasing distance from the source. The use of units of illuminance, such as lux, is inappropriate for measurement of ganzfeld flash or pattern stimuli.

Another measure of importance to clinical electrophysiology is 'retinal illuminance', an estimate of the effective stimulus at the retina.[1] The standard measure of retinal illuminance is calculated by multiplying stimulus luminance by pupillary area. The unit of retinal illuminance is the Troland (td). The Troland is defined as the retinal illuminance obtained when a stimulus of 1 cd m^{-2} is viewed through a pupillary area of 1 mm^2 (diameter of 1.128 mm). Scotopic Trolands (td) can also be measured using $V_\lambda{}'$ to calculate stimulus luminance.

Stimulus luminance is measured using a photoelectric device called a photometer. Since the spectral responsiveness of the photosensitive device usually does not match that of the human eye, an appropriate filter is placed in the instrument's light path so that its output indicates stimulus luminance. Because response properties of semiconductors and their amplifiers vary with time and use, it is important to have a photometer calibrated periodically (an interval of 5 years is recommended). Proper calibration of a photometer should be performed by the manufacturer or a specialist laboratory using a source with a luminous intensity that is traceable to an international standard. It is also imperative to ensure that the photometer is able to accurately measure the low luminance levels commonly used in visual electrophysiology (e.g., a dark check of 0.1–3 cd m^{-2} used for the pattern VEP). Care should be taken to insure that the photometer used is appropriate for the specific measurement.

Additional apparatus is required for calibration of visual stimuli. Because flash stimuli are brief, it is necessary to have a temporal integration mode for the photometer. When placed in this mode, the photometer simply sums the incoming light over the duration of the measurement. For measurement of pattern stimulus contrast, a spot photometer should be used. As the name implies, a spot photometer enables the measurement of luminance in a small area. It should also be equipped with a viewing system to enable identification of the precise area of measurement. Equipment manufacturers should provide instructions for proper calibration of their equipment.

Protocols for the calibration of visual stimuli

Calibration of ganzfeld strobe flash and background luminance

It is critical that your photometer is appropriately calibrated to international standards, as described above, and that the optics and electronic settings are appropriate for this calibration. Some photometers have options for special equipment necessary to measure pulses of light. Others have internal circuitry that integrates over time intervals longer than most strobe flashes. The following protocol applies in either of

[1] In reality this measurement reflects luminance corrected for pupil size rather than retinal illuminance since pre-retinal light absorption by ocular media are unaccounted for. However, this measure is traditionally termed retinal illuminance.

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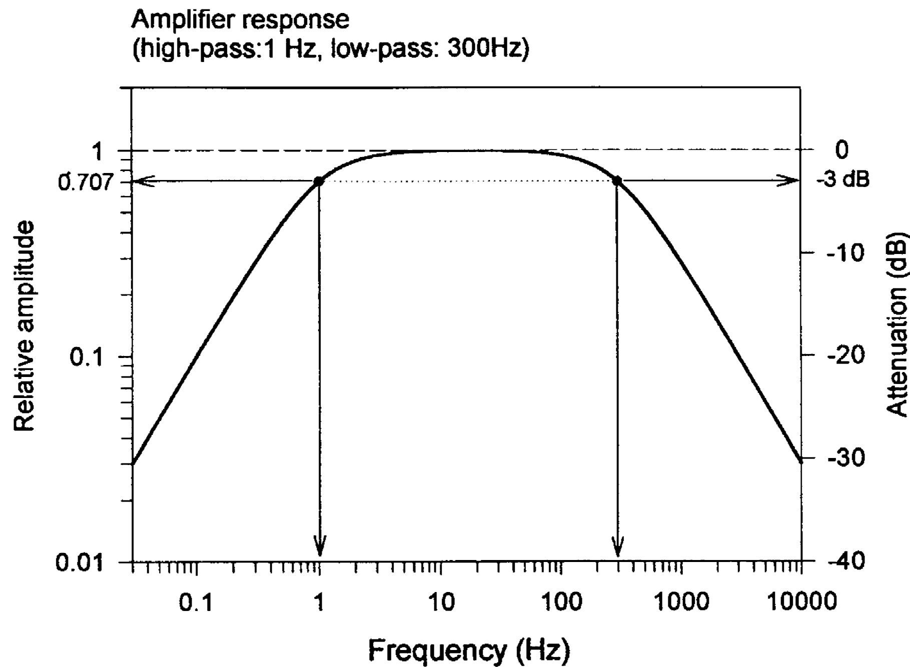

Figure 1. Standard spectral sensitivity functions for photopic and scotopic vision. Wavelength is plotted as a function of linear (left) and log (right) luminous efficacy. The maximum photopic sensitivity occurs at a wavelength of 555 nm and the maximum scotopic sensitivity occurs at 507 nm.

these cases.[2] It is recommended that ganzfeld luminance levels be calibrated at a maximum interval of 6 months. More frequent calibration is suggested if the equipment is used heavily, or if values change substantially between successive calibrations.

The protocol for flash calibration is summarized in Table 2. The appropriate measure for brief flashes is luminous energy, which integrates luminance over time (e.g., cd·s m^{-2}). A variability of up to 10% from flash to flash is to be expected. If a higher variability is measured, then the manufacturer of the ganzfeld should be consulted. It should be noted that this calibration measures the luminous energy of single flashes and may not be valid for flicker stimuli, since many flash units do not have time to fully recover between flashes during 30 Hz flicker stimulation. In order to calibrate the flicker stimulus it is necessary to integrate the photometric measurement over a fixed number of flashes after the output has stabilized (e.g., skip the first 15 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 flicker luminance. Alternatively, the photometer may be used in continuous measurement mode, with a long time constant (if possible). Once the reading has stabilized to the flicker stimulation, divide by 30 to get the energy for a single flash. This measurement is difficult with many systems. Therefore, one can assume that flicker luminance has not changed if single flash luminance has not changed, although the precise luminance of the flicker stimulus may not be known.

Photopic background luminance can be measured using a similar protocol to that for measuring the flash. To measure this continuous light level, the photometer should be out of temporal integration mode, so that the calibration should be in cd m^{-2} or some other luminance unit that is readily converted to this unit (see

[2] Although luminance levels used for stimulation of rods are most accurately specified in scotopic units, few photometers have scotopic correction filters available. Thus there is no convenient method for achieving this measurement. Therefore, the ISCEV ERG standard and this document suggest a photopic photometric calibration of this stimulus. Note that photopic units are unsuitable if bandpass chromatic stimuli are used for rod stimulation due to the difference between V_λ and V_λ'. For example, a blue flash calibrated in photopic units will have a much higher scotopic luminance than a red flash of equal photopic luminance. For those using a short wavelength rod flash, it should be noted that a xenon strobe at the recommended photopic luminance of 2–3 cd·s m^{-2} is roughly equivalent to 4 scotopic cd·s m^{-2}. Another difficulty with direct measurement of the rod flash luminance is that many of the commercially available photometers are quite variable at this low luminance level. Thus, it is recommended that the standard flash be used for calibration and that a calibrated neutral density filter be used to obtain the standard rod flash luminance.

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Table 1. Conversion of luminance units

Multiply Number of To Obtain Number of ↓	candela m^{-2} By Factor ↘	footlambert	millilambert	candela in^{-2}	candela ft^{-2}	apostilb	stilb
candela m^{-2}	1	3.426	3.183	1550	10.76	0.3183	10000
footlambert	0.2919	1	0.929	452	3.142	0.0929	2919
millilambert	0.3142	1.076	1	487	3.382	0.1	3142
candela in^{-2}	0.000645	0.00221	0.00205	1	0.00694	0.000205	6.45
candela ft^{-2}	0.0929	0.3183	0.2957	144	1	0.02957	929
apostilb	3.142	10.76	10	4870	33.82	1	31420
stilb	0.0001	0.00034	0.00032	0.155	0.00108	0.000032	1

1 nit = 1 candela m^{-2}

Table 2. Flash calibration

I. Preparation of equipment

- Prepare the photometer to make photometric measurements (adding appropriate filters if indicated) in temporal integration mode (cd·s m^{-2}).
- Place the detector at the position occupied by the eye during a test.

II. Making the measurement

- Darken the room, including turning off ganzfeld fixation and background lights and any infra-red monitoring devices (e.g. lamps for CCTV cameras).
- Zero the photometer.
- Make at least three measurements.
- Select the median value as the flash luminance
  - If values differ by $>10\%$ consult the manufacturer

Table 1). The background light should be turned on and the measurement should be checked over at least a 5 min interval to ensure its stability.

Calibration of mean luminance and contrast of a pattern stimulus

Pattern stimuli are used in many clinical electrophysiological tests, including the VEP, PERG and mfERG. Two parameters of pattern stimuli commonly measured are mean luminance and contrast. The mean luminance can be derived from the measurement of luminance of light (L_{max}) and dark (L_{min}) pattern elements:

$$\text{Mean Luminance} = \frac{(L_{max} + \mathrm{L}_{min})}{2} \qquad (1)$$

Most currently available video monitors perform optimally with mean luminance settings of between 25 and 100 cd m^{-2} since nonlinearities commonly occur with L_{max} of 200 cd m^{-2} or greater. The calibration of mean luminance is of utmost importance for the VEP since peak latency of the response increases significantly as mean luminance is decreased.

When recording pattern reversal or pattern appearance responses it is important to be sure that there are no transient changes in mean luminance at the time of the stimulus event. A quick and easy method to check 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 0.5 m. 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., two reversals per second). The room lights should be dimmed to enhance sensitivity. The reflection from the paper should be constant, 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 VEP. Pattern onset/offset stimuli are particularly prone to unwanted luminance artifacts.

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Table 3. Calibration of pattern stimuli

I. Preparation of equipment
- Set up the photometer for non-integrating luminance measurement in cd m^{-2}.
- Warm-up the monitor for a minimum of 15 min and put a large pattern element size (e.g., 2°) on screen.
- Slow, or if possible stop, pattern alternation.

II. Making the measurement
- Adjust room lighting conditions to those used during testing.
- Zero the photometer with the detector covered.
- Where appropriate, focus the photometer optics.
- 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
- Obtain stable measurement of light and dark elements at both the center of the screen and near the edge of the screen.

III. Calculations
- Calculate mean luminance (using Equation (1) above) with obtained measures of light element ($L_{\max}$) and dark element ($L_{\min}$) from the center of the screen
- Calculate mean luminance for the periphery of the screen. This value should be at least 70% of that obtained from the center of the stimulus according to ISCEV guidelines.
- Calculate stimulus contrast (using Equation (2) above) with luminance values obtained from the center of the stimulus.

Pattern stimulus contrast is defined as the Michelson contrast ratio:

$$\text{Contrast} == \frac{(L_{\max} - \mathrm{L}_{\min})}{(L_{\max} + \mathrm{L}_{\min})} \times 100\% \qquad (2)$$

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 commercial monitors may produce distortions in the stimulus at contrasts above 90%. When recording the pattern VEP, the calibration of pattern contrast is not as critical as the calibration of mean luminance. This is because contrast has little effect on the VEP for values above approximately 50%. Thus, as long as contrast is high, small changes in contrast will have no appreciable effect on the VEP. However, PERG amplitude increases with contrast without saturation. Thus, for this response, calibration of contrast is critical. The mfERG response depends on both mean luminance and contrast. It should be noted that for many of the monitors used to display pattern stimuli, contrast may vary at the extremes of pattern size. This is especially true for raster based systems in which contrast will decrease for small pattern elements.

It is recommended that the pattern stimulus be calibrated at a maximum interval of 6 months. More frequent calibration is suggested if the equipment is used heavily or if values change substantially between calibrations. It is advisable to cover or otherwise protect the 'brightness' and 'contrast' controls from 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.

Measurement of element size

Electrophysiological measures are affected by the angular subtense of the pattern elements. Procedures for calculating visual angle for fixed viewing conditions and for choosing a viewing distance to obtain a desired visual angle are given below. As a general rule, a stimulus of 1 cm subtends 1° at a viewing distance of 57 cm.

Calculation of visual angle. (1) Measure the width of 10 elements across the center of the screen and divide by 10 to obtain the mean element size. (2) Measure the distance from the patient's eye to the center of the screen. (Be sure to use the same unit of measure (e.g., cm) for both viewing distance and element size). (3) Divide the element size by the dis-

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tance. (4) Determine the angle whose tangent is equal to this value by using either a trigonometric table or the $\tan^{-1}$ function on a calculator. (Most calculators give results in decimal degrees. To convert degrees to minutes visual angle, multiply by 60 (e.g., 0.25° equals 15 min.)

Calculation of viewing distance for desired element visual angle. (1) Measure the width of 10 elements across the center of the screen and divide by 10 to obtain the mean element size. (2) Determine the tangent of the desired visual angle. (3) (3) Divide the element size as measured in step 1 by the tangent of the desired visual angle (as obtained in step 2) to obtain the viewing distance in the unit used to measure element size.

Calibration of recording equipment

Technical background

Electrophysiological signals produced by the retina (ERG) and visual cortex (VEP) can be recorded non-invasively using standard, commercially available equipment. Surface electrodes are used to record the electrical responses of the visual system. A number of special techniques are required to record these physiological signals since their amplitude is small in comparison to electrical noise generated by external sources (primarily generated by AC line noise of 60 Hz in the Americas and 50 Hz elsewhere) and high amplitude physiological 'noise' generated by muscle (e.g. heart; extraocular muscles) and brain (electroencephalographic activity). Differential amplifiers are used to eliminate many of these unwanted signals. Differential amplifiers amplify the difference between two inputs and reject signals that are common to both inputs (relative to a ground electrode). The two inputs come from an electrode over the region responsive to stimulation, termed the active electrode, and an electrode over a 'distant' site, termed the reference electrode. Differential amplifiers have a characteristic known as the common mode rejection ratio (CMRR). The CMRR describes the ability of a differential amplifier to reject interfering signals common to both inputs, and to amplify only the difference between the two inputs. A high CMRR will help to eliminate much of the line (mains) and physiological noise present in the recording environment. A CMRR of 100 000:1 (100 dB[3]) is reasonable for such recordings. For common mode rejection to work properly, the active and reference electrodes must be matched in impedance, and the impedance of each electrode connection should be maintained below 5 kΩ. An impedance mismatch between electrodes will diminish rejection of unwanted signals.

High amplitude artifacts, such as those generated by eye movements or blinks, should be excluded from averaged records. This can be done on-line using an amplitude threshold criterion. Artifact rejection algorithms are available on most commercial systems. The rejection threshold should be higher than the expected amplitude of the physiological signal and background physiological noise. VEP and PERG standard suggest a 100-μV rejection criterion. This setting should be higher when recording VEP from children since EEG amplitudes are higher in this population.

Finally, stimulus-locked signal averaging can be used to increase the signal-to-noise ratio. Using signal averaging, the random noise level is decreased by the square-root of the number of trials averaged. The choice of the number of trials to average depends on signal to noise conditions. For a small response, such as the pattern ERG, in an unfavorable noise environment, it may be necessary to average responses to 150–300 stimulus presentations to obtain a measurable response, whereas no signal averaging is generally needed to record the high amplitude dark-adapted bright flash ERG. It is important to remember that the principle of signal averaging assumes a constant (stationary) response to the stimulus. If repeated stimulus presentation changes the patient's state of adaptation or arousal, then signal averaging will yield erroneous results.

The nature of the signal that is being recorded affects the optimal amplifier settings. Large signals obviously require less amplification than small signals. Prior to averaging, signals are converted from analog to digital format using an analog to digital converter (ADC). Incoming signals should be digitized at a sampling frequency more than twice as high as 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 amp-

[3] In this document, dB = 20 log I/I_0, where I represents the measured value and I_0 represents a reference value (1 for most applications in vision). This differs from the definition used in automated perimetry where 1 dB s equals 1/10 of a log unit (i.e., dB = 10 log I).

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Table 4. Amplifier calibration

I. Preparation of equipment
- Warm-up amplifiers and signal generator until they are stable
- Connect signal generator to amplifier inputs
- Generate test signals in the physiological amplitude range (1 μV to 1 mv)

II. Making the measurements
- Acquire and measure calibration signals as you would do for normal recording. If signal averaging is used, the signal generator will have to be used to trigger the data acquisition system.
- Measure multiple signal frequencies that begin below and extend above low-pass and high-pass filter settings

litude resolution, but staying within 90% of its full range to avoid possible non-linearities at the extremes (e.g., clipping of the peaks).

The filtering characteristics of the amplifier must be set differently for optimal recording of signals with different frequency characteristics. For example, to properly record the ERG oscillatory potentials, which contain frequencies between 100 and 500 Hz and which can be obscured by a contemporaneous, high amplitude, low frequency b-wave, it is necessary to filter out low frequencies and pass relatively high frequencies. For slow oscillations, such as the pattern VEP, in which most of the response energy is contained between 3 and 30 Hz, low frequencies must be unfiltered and high frequencies are less relevant. Amplifiers contain analog high-pass and low-pass filters to attenuate low frequency and high frequency components of the incoming signal respectively. These 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 the slope of their attenuation in dB per octave. The effect of bandpass filtering is illustrated in Figure 2. The setting of analog filters, especially the low-pass (high frequency) filter, affects the obtained peak latency. Thus, it is important to maintain these settings at the same levels used to obtain normative data. Setting the low-pass filter to below 1/5 of the digitization rate will help to avoid substantial temporal aliasing. For instance, if the sweep duration is 256 ms and 256 points are sampled, then the sample rate is 1 kHz (1000 samples per second). The low pass filter should be set to no greater than 200 Hz to reduce production of artifactual low frequencies in the amplified signal (aliasing).

Calibration of amplifiers with a known input signal is necessary to know the relation between input and output voltage. Most modern systems have internal amplifiers that have been calibrated by the vendor. However, calibration of amplification and filtering should be performed at a maximal interval of 1 year. In the following sections, procedures for electrode impedance measurement and calibration of amplifiers will be outlined. This procedure requires a signal generator that can provide low voltage signals.

Protocols for the calibration of recording equipment

All equipment used for the recording of electrophysiological signals including impedance meters, amplifiers, electrode boxes and electrodes, should be specifically approved for use with humans and be marked as such. Failure to use appropriate equipment may put the patient at risk.

Measurement of electrode impedance

Impedance is measured by passing low amplitude (maximally 1 μA) alternating current, with a frequency of between 10 and 100 Hz, between a pair of electrodes, through the human tissue, with the electrodes *in situ*. The impedance is equal to the ratio of the measured voltage between the electrode pair and the input current (i.e., impedance = V/I, where V represents the obtained voltage and I represents the input current). Typically, the active and reference electrodes are each measured against the 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 it is the ground electrode that is at fault.

Many commercial systems have internal impedance meters. Impedance must not be measured using a direct current ohm meter since this will polarize the

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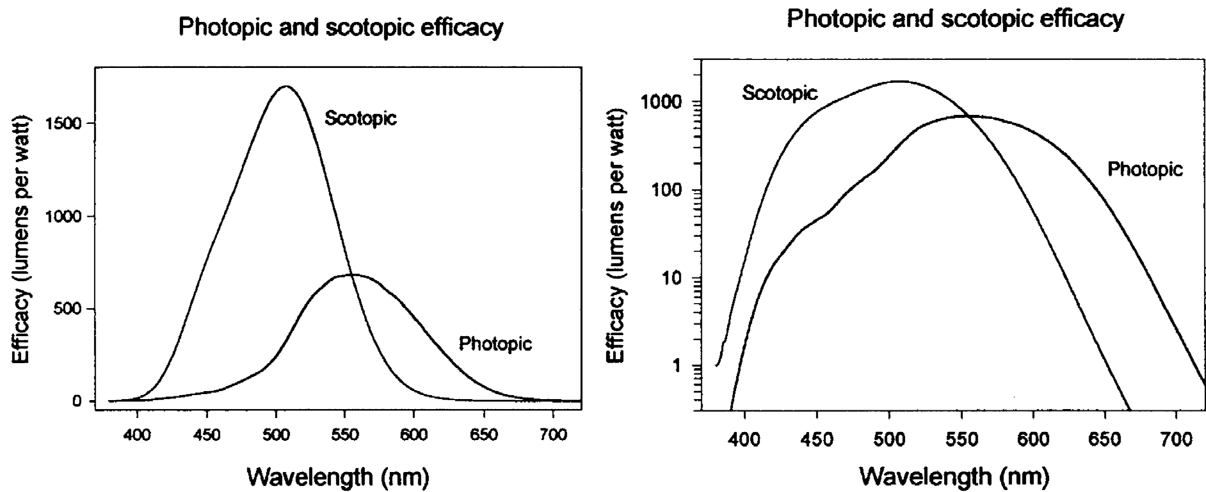

Figure 2. Amplifier frequency-response curves for a bandpass filter set 1–300 Hz. Filter roll-off is 12 dB/octave.

electrode, resulting in an unreliable measure and potentially resulting in a large standing potential between the electrode and the surface. One should use great caution in the measurement of impedance of corneal or scleral ERG electrodes, since even the low current used in many devices may produce phosphenes or may be dangerous to the eye. The circuits used to measure impedance should be isolated from both the line (mains) power and ground (earth).

Calibration of amplification system

Calibration of amplifier gain is achieved 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 often go uncalibrated if the signal is passed only through the main amplifier. Also, the electrode-box circuitry may influence the voltage reaching the amplifier. The amplitude of the input signal should approximate the amplitude of the physiological signal. The amplitude of the output should closely resemble that of the input multiplied by the amplification system gain factor. Ideally, the system should be calibrated using both sine wave input of various frequencies and square wave pulses. Using sinusoidal input, both the amplification and filter settings can be 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 square-wave 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:

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

Thus, a low frequency filter setting of 1 Hz will result in a time constant of 0.16 s, and a setting of 0.3 will result in a time constant of 0.53 s. If amplifiers are not performing to specifications, or if distortions of signals are observed, the equipment should be returned to the manufacturer for repair.

The suggested protocol for amplifier calibration is given in Table 4. If amplifiers are found to differ significantly from specifications, they should be returned to the manufacturer for adjustment or replacement. Amplifiers should be calibrated at a maximum interval of 1 year. Amplifiers should not be calibrated with a patient connected to the system. A quick and easy check of the amplifiers is to pass an identical signal through all of the channels (e.g., by setting all channels to the same input electrodes) with identical settings of all amplifiers. A difference in the output of one or more channels is suggestive of an amplifier calibration problem. This check may give early indication of the need for recalibration.

Acknowledgement

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