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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], and guidelines for the Pattern ERG (PERG)[4] 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 electrophysiologic 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 more generally applicable to studies which are dependent upon accurate measurement of luminance or electrophysiological signals.

Key words: electroretinography, luminance, photometry, visual evoked potentials

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 nanometres (10^{-9} metres). Ra-

diometry 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 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 protocol 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 efficiency of the human eye as a function of wavelength under light-adapted (i.e., photopic) conditions. Sensitivity to short wavelengths was subsequently modified by Judd. This function, termed V_λ, describes the relative *photopic* sensitivity normalized to a maximum value of 1.0 at the peak of the function at 555 nm (see Figure 1). It is closely approximated by the sum of sensitivities of the long and middle wavelength cones. The standard *scotopic* observer (V'_λ), indicating sensitivity as a function of wavelength under dark-adapted conditions, was developed by the CIE in 1951. V'_λ is shifted to shorter wavelengths when compared to V_λ and is scaled to unity at the scotopic peak sensitivity of 507 nm (see Figure 1).

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. Intuitively, one can think of luminance as roughly equivalent to brightness, and as an object is approached, its brightness does not change appreciably. The Système Internationale (SI) unit of luminance is the candela per square metre ($cd \cdot m^{-2}$). The relation between this measure and older measures of luminance is shown in Table 1. For brief flashes of light, such as those 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. Thus, the appropriate unit of time-integrated luminance for brief flashes of light is $cd\ s \cdot m^{-2}$. 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. The standard measure of retinal illuminance is calculated by multiplying stimulus luminance by pupillary area. The unit of retinal illuminance is the Troland (td).

Table 1. Luminance conversion factors

Multiply number of → To obtain by number of	Candela·m^{-2}	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}.

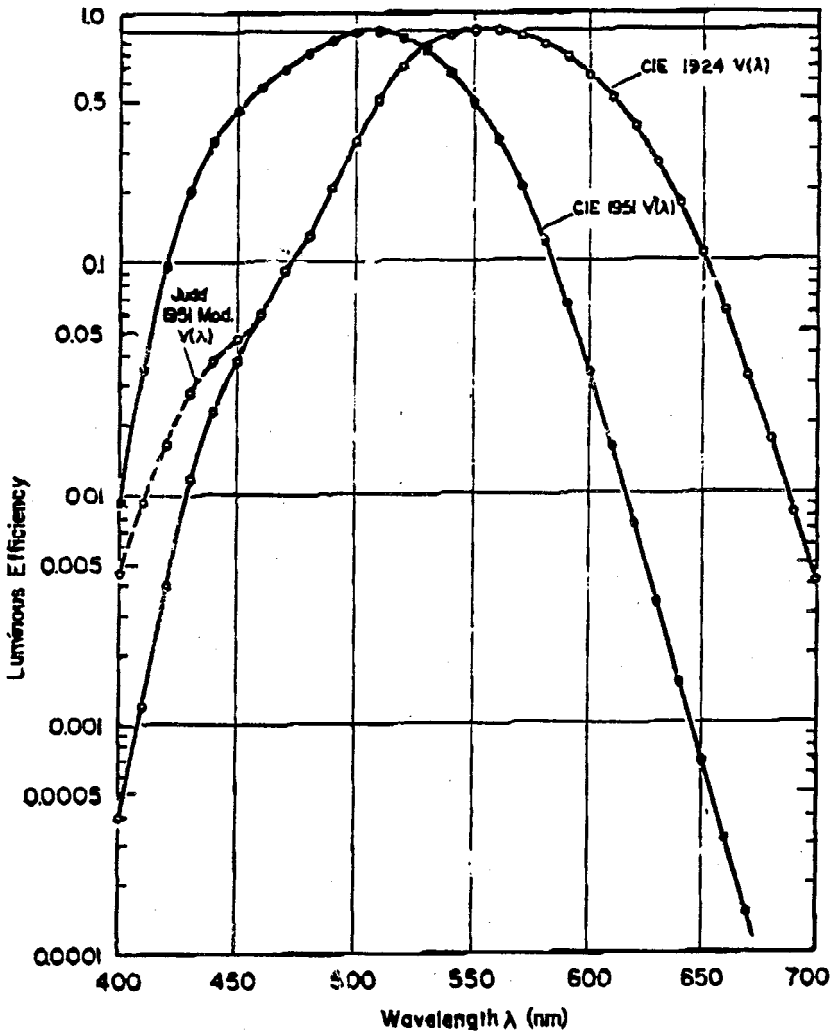

Figure 1. Standard spectral sensitivity functions for photopic (V_λ) and scotopic (V'_λ) vision. Each function is normalized to have a maximum value of unity which occurs at λ_m = 555nm for V_λ and at λ_m = 507 nm for V'_λ. (Adapted from Wyszecki G. & Stiles W.S. *Color Science*, New York:Wiley, 1982 with permission).

The Troland is defined as the retinal illuminance obtained when a stimulus of 1 $cd{\cdot}m^{-2}$ is viewed through a pupillary area of 1 square mm (diameter of 1.128 mm). Scotopic Trolands (td′) can also be measured using V'_λ to calculate stimulus luminance.

Stimulus luminance is measured using a photoelectric device called a photometer. Since the spectral responsiveness of the photosensitive substance

in a photometer is known, an appropriate filter is placed in the instrument's light path so that its output indicates stimulus luminance. Two types of photoelectric devices that are commonly used are photovoltaic devices, in which a current is produced in a semiconductor by incoming photons; and photoconductive devices, in which a biasing voltage is applied and the resulting current is modulated by the effect of light. Silicon is the most widely used photoelectric substance due to its desirable properties of high quantum efficiency, a relatively wide spectral response, high response speed, and little fluctuation with time. Cadmium sulfide and cadmium selenide cells are also used in photoconductive devices, but have considerably slower response times. 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 1 to 3 $cd \cdot m^{-2}$ used for the pattern VEP).

Additional apparatus is beneficial 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.

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 it, the optics and electronic settings are appropriate for this calibration. A listing of many available photometric devices can be found at the ISCEV web site www.ukl.unifreiburg.de/aug/iscev/. 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 these cases.

Although luminance levels used for stimulation of rods are most accurately specified in scotopic units there is no convenient method for achieving this measurement. Thus, the ISCEV ERG standard and this document suggest a 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 to 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.

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. It should be noted that this calibration measures the luminance 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. This measurement is not possible with many existing systems. Therefore, in many cases 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 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 checkerboard or square-wave grating pattern

The calibration of mean luminance of a stimulus for the pattern VEP is of utmost importance, since the peak latency of the response increases significantly as mean luminance is decreased. The mean luminance can be derived from the measurement of luminance of light (L_{max}) and dark (L_{min}) pattern elements:

Table 2. Flash calibration

I.		Preparation of equipment
	A.	Warm-up photometer as recommended in its manual and until its output is stable.
	B.	Add appropriate attachments.
	C.	Record in temporal integration mode (cd s · m^{-2}).
	D.	Place the detector at the position occupied by the eye during a test.
II.		Making the measurement
	A.	Darken the room, including turning off ganzfield fixation and background lights.
	B.	Zero the photometer.
	C.	Make at least 3 measurements.
	D.	Select the median value as the flash luminance.

$$\text{Mean Luminance} = (L_{max} + L_{min})/2 \tag{1}$$

Most video monitors perform optimally with mean luminance settings of between 25 and 50 cd·m^{-2}.

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. 2 reversals per second). The room should be dimmed to enhance sensitivity. The reflection off of 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.

Pattern stimulus contrast is defined as the Michelson contrast ratio,

$$Contrast = \frac{L_{max} - L_{min}}{L_{max} + L_{min}} 100\% \tag{2}$$

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. Many commercial monitors 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. It should be noted that for many of the monitors used to display pattern stimuli, contrast may vary as pattern size is changed. 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. *A listing of many available photometric devices can be found at the ISCEV web site www.ukl.uni-freiburg.de/aug/iscev/.* 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.

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 distance.
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 decimal values to minutes visual angle, multiply this value by 60 (e.g. 0.25^0 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.

Table 3. Calibration of pattern stimuli

I.		Preparation of equipment
	A.	Warm-up photometer and add optical attachments as recommended in the manual.
	B.	Record in non-integration mode, to measure luminance in $cd \cdot m^{-2}$.
	C.	Warm-up the monitor and put a large pattern element size (e.g., 2^0) on screen.
	D.	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.	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.
	D.	Obtain stable measurement of light and dark elements at both the center of the screen and near the edge of the screen.
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 center of the screen.
	B.	Calculate mean luminance for the periphery of the screen. This value should be $\geq$ 70% of that obtained from the center of the stimulus according to ISCEV guidelines.
	C.	Calculate stimulus contrast (using equation 2 above) with luminance values obtained from the center of the stimulus.

3. Divide the element size as measured in step 1 by the value 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 line AC fields and high amplitude physiological 'noises' 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. 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. Differential amplifiers have a characteristic common mode rejection ratio (CMRR) which specifies the ratio of differential input to common input amplification. The CMRR should be high to ensure that signals common to both inputs are only minimally transmitted whereas differences between inputs are highly amplified. This characteristic will eliminate much of the line and physiologic noise present in the recording environment. A CMRR of 100,000:1 (100 dB) 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.

A method to eliminate transient high amplitude artifacts, such as those generated by eye movements or blinks uses on-line artifact rejection. These algorithms usually use voltage amplitude as a criterion to designate trials as artifactual.

Finally, stimulus-locked signal averaging can be used to increase the signal amplitude relative to random physiologic activity. 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 single 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 and 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 also affects the optimal amplifier settings. Large signals obviously require less amplification than small signals. Many modern digital electrophysiological systems adjust recording sensitivity by changing the voltage range over which the digital to analog converter is scaled. In these systems, analog signals are sampled and digitized prior to averaging. Incoming signals should be digitized at a frequency

of at least twice as high as the highest frequency contained in the signal. A/D converters must have a minimum resolution of 8 bits (256 levels). A/D converters with a resolution of 12 bits or more are now commonly available and are preferable. The use of an 8 bit converter is adequate for recording visual electrophysiologic signals provided that the voltage of the incoming physiologic signal uses a relatively high portion of the digitization range. If sensitivity is set too high (e.g. 0.05 μV/bit resulting in a range of 12.8 μV for an 8 bit A/D converter), many of the incoming signals will either be clipped (flattened at the peaks) or automatically rejected (artifact rejection criterion is commonly a deflection of greater than 90% of the A/D range). Alternatively, if the sensitivity is set too low (e.g. 1.0 μV/bit resulting in a range of 256 μV), then a VEP signal of 15 μV will use only a small portion of the A/D range resulting in substantial distortion of the waveform. In practice, if an 8 bit A/D converter is used, the sensitivity should be set to reject up to 20% of incoming sweeps to ensure full use of the digital scale without unduly prolonging the procedure. If the A/D converter has a higher resolution, then sensitivity can be set to reject only trials that contain high amplitude interference (e.g. muscle activity).

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 (30%) or 6 dB (50%), termed the corner or cut-off frequency, and the slope of their attenuation in dB per octave. 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. With digital systems, setting the low-pass filter below 1/5 of the digitization rate will be sure to prevent 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 more than 200 Hz to avoid production of artifactual low frequencies (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 impedance measurement and calibration of amplifiers will be outlined.

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. Failure to use appropriate equipment puts the patient at risk of severe electrical shock.

Measurement of electrode impedance
Impedance is measured by passing low amplitude ($<$20 μA) alternating current, with a frequency of between 10 and 100 Hz, from the active (or from the reference) electrode, through the human tissue, towards the ground electrode with the electrodes *in situ*. The impedance is equal to the ratio of the measured voltage between active (or reference) electrode and ground and the input current (i.e. impedance = V/I; where V represents the obtained voltage and I represents the input current). Many commercial systems have internal impedance meters. Impedance must not be measured using a direct current ohm meter 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. Electrode impedance should be maintained at below 5 kΩ.

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 result in phosphenes or may be dangerous to the eye. A current of no more than 1 to 2 μA should be used to measure impedance of ERG electrodes. The circuits used to measure impedance should be isolated from mains power.

Calibration of amplification system
Calibration of amplifier gain is achieved by passing a known signal through the system and measuring system output. Ideally, 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 generated internally and 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

Table 4. Amplifier calibration

I.		Preparation of equipment
	A.	Warm-up amplifiers and signal generator until they are stable.
	B.	Connect signal generator to amplifier inputs.
	C.	Generate sinusoidal test signals in the physiologic amplitude range (100 μV to 1 mv).
II.		Making the measurements
	A.	Acquire and measure calibration signals as you would do for normal recording.
	B.	Measure multiple signal frequencies that begin below and extend above low-pass and high-pass filter settings.
	C.	Plot the amplitude of output as a function of input frequency to check the accuracy of amplification and filter settings. The graph should look something like that shown in Figure 2, with a flat range in the middle and the specified low-pass and high-pass frequencies at about 70% of input amplitude.
	D.	Input a square-wave signal and examine output for evidence of unwanted ringing (oscillations at the corners of the square-wave).

amplification and filter settings can be assessed for accuracy. 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:

$$\tau = 1/(2\pi f_c) \tag{3}$$

Thus, with a low frequency filter setting of 1 Hz, the time constant should be 0.16 s, and a setting of .3 will result in a τ of 0.53 s.

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, if performed weekly, will give early indication of the need for recalibration.

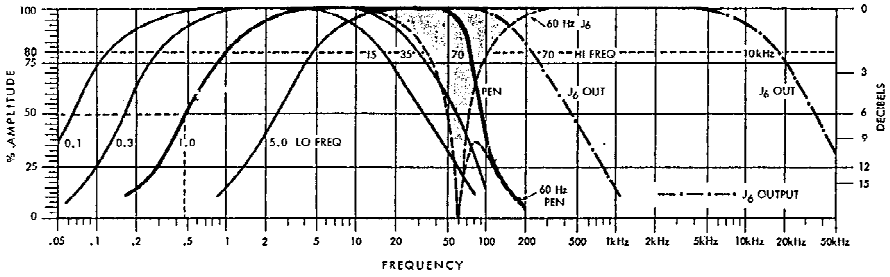

Figure 2. Amplifier frequency-response curves for various band-pass filter settings.

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