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ISCEV STANDARDS

ISCEV standard for clinical visual evoked potentials: (2016 update)

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Abstract Visual evoked potentials (VEPs) can provide important diagnostic information regarding the functional integrity of the visual system. This document updates the ISCEV standard for clinical VEP testing and supersedes the 2009 standard. The main changes in this revision are the acknowledgment that pattern stimuli can be produced using a variety of technologies with an emphasis on the need for manufacturers to ensure that there is no luminance change during pattern reversal or pattern onset/offset. The document is also edited to bring the VEP standard into closer harmony with other ISCEV standards. The ISCEV standard VEP is based on a subset of stimulus and recording conditions that provide core clinical information and can be performed by most clinical electrophysiology laboratories throughout the world. These are: (1) Pattern-reversal VEPs elicited by checkerboard stimuli with large 1 degree (°) and small 0.25° checks. (2) Pattern onset/offset VEPs elicited by checkerboard stimuli with large 1° and small 0.25° checks. (3) Flash VEPs elicited by a flash (brief luminance increment) which subtends a visual field of at least 20°. The ISCEV standard VEP protocols are defined for a single recording channel with a midline occipital active electrode. These protocols are intended for assessment of the eye and/or optic nerves anterior to the optic chiasm. Extended, multi-channel protocols are required to evaluate postchiasmal lesions.

Keywords Visual evoked potential · Pattern-reversal visual evoked potential · Pattern onset/offset · Visual evoked potential · Flash visual evoked potential · Standard

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Introduction

Visual evoked potentials (VEPs) are visually evoked electrophysiological signals extracted from the electroencephalographic activity in the visual cortex recorded from the overlying scalp. As visual cortex is activated primarily by the central visual field, VEPs depend on functional integrity of central vision at all levels of the visual pathway including the eye, retina, the optic nerve, optic radiations and the occipital cortex.

This document updates the ISCEV Standard for clinical VEP testing and supersedes the 2009 VEP Standard [1]. The major changes in this current standard compared with the previous VEP standard are, a recognition of the variety of stimulus displays available, an increased emphasis on the need for manufacturers and users to ensure that there is no luminance change during pattern reversal or pattern onset/offset and edits to bring the language of this VEP standard into closer harmony with other ISCEV standards. Reports of VEP recordings performed to the standard method given here should cite this 2016 standard. Where a method is used which deviates from the standard method, the deviations should be stated, together with any normative or reference data. Where the method used conforms to a previous ISCEV standard for clinical VEPs, that previous standard should be cited.

This Standard presents minimum protocols for basic clinical VEP recording. Three standard stimulus protocols are defined. All ISCEV standard VEPs are classified as transient VEPs, that is, the stimulation rate is sufficiently slow that the waveform consists of a number of discrete deflections.[1]

The ISCEV standard VEP protocols are defined for a single recording channel with a midline occipital active electrode. These protocols are intended for assessment of prechiasmal function (i.e., function of the eye and/or optic nerves anterior to the optic chiasm). Extended, multi-channel protocols are required to evaluate postchiasmal lesions. Following a principle established in earlier standards [2–6], ISCEV has selected a subset of stimulus and recording conditions which provide core clinical information that can be performed by most clinical electrophysiology laboratories throughout the world. These are:

1. Pattern-reversal VEPs elicited by checkerboard stimuli with large, 1 degree (°) (acceptable range of 0.8° to 1.2°), and small, 0.25° (0.2° to 0.3°) checks.
2. Pattern onset/offset VEPs elicited by checkerboard stimuli with large, 1° (0.8° to 1.2°), and small, 0.25° (0.2° to 0.3°) checks.
3. Flash VEPs elicited by a flash (brief luminance increment) which subtends a visual field of at least 20°.

The standard does not require that all the three protocols are used for investigation on every patient nor does it preclude adding additional protocols. In many circumstances, a single stimulus protocol will be appropriate. Pattern reversal is the preferred stimulus for most clinical purposes. The pattern-reversal VEP is less variable in waveform and timing than the VEP elicited by other stimuli. The pattern onset/offset stimulus is more effective for the detection of malingering and for use in patients with nystagmus. The flash VEP is useful when poor optical quality, poor cooperation or poor vision makes the use of pattern stimulation inappropriate. To comply with this Standard, at least one standard protocol should be included in every clinical VEP recording session so that all laboratories will have a common core of data that can be shared or compared.

ISCEV recognizes that VEPs can be elicited by a wide range of stimulus protocols that are not specified in the standard and that other tests are valuable to identify lesions or answer specific clinical questions. By limiting this standard to three protocols, the intention is that standard VEPs will be incorporated universally into clinical VEP testing along with any additional tests and extended protocols a laboratory may choose to use. ISCEV actively encourages the use of additional protocols for clinical testing and for research and recommends that commercial recording equipment has the capability of allowing extended protocols to accommodate comprehensive and specialised; testing in addition to the standard VEP protocols.

ISCEV publishes and maintains other standards for clinical electrophysiological testing of the visual system: specifically, full-field electroretinograms (ERGs) [2], multi-focal ERGs [3], pattern ERGs [4] and electro-oculograms [5] as well as technical and

[1] A major determinant of the waveform of a VEP is the temporal frequency of the stimulus. At rapid stimulation rates, the waveform becomes approximately sinusoidal and is termed steady-state VEP. VEPs recorded with stimulation at lower temporal frequencies are termed transient VEPs.

calibration guidelines for clinical electrodiagnostic testing [6]. The ISCEV web site can be consulted for current updates (www.ISCEV.org/standards).

Basic technology

Electrodes

Skin electrodes such as sintered silver–silver chloride, standard silver–silver chloride or gold cup electrodes are recommended for recording VEPs. The skin should be prepared by cleaning, and a suitable paste or gel used to ensure good, stable electrical connection. The electrode–skin contact impedances should be below 5 kΩ as measured between 20 and 40 Hz. To reduce electrical interference, electrode–skin contact impedances should differ by no more than 1 kΩ between electrodes.

Electrode placement

The scalp electrodes should be placed relative to bony landmarks, in proportion to the size of the head, according to the International 10/20 system [7] (See Fig. 1A). The anterior/posterior midline measurements are based on the distance between the nasion and the inion over the vertex. The active electrode is placed on the occipital scalp over the visual cortex at O_z with the reference electrode at F_z. A separate electrode should be attached and connected to the ground. Commonly used ground electrode positions include the forehead, vertex (C_z), mastoid, earlobe (A1 or A2) or linked earlobes.

VEP stimulation

There are two main classes of standard VEP stimulation, pattern and flash. Standard stimulus and recording conditions are described below and are summarized in Table 1. The reader may refer to the current ISCEV guidelines [6] for guidance regarding the measurement and definition of stimulus parameters. All stimulus parameters should be calibrated either locally or by the manufacturer, and regular recalibration is advised [6].

Pattern stimulation

All standard pattern stimuli are high-contrast, black-and-white checkerboards consisting of squares with

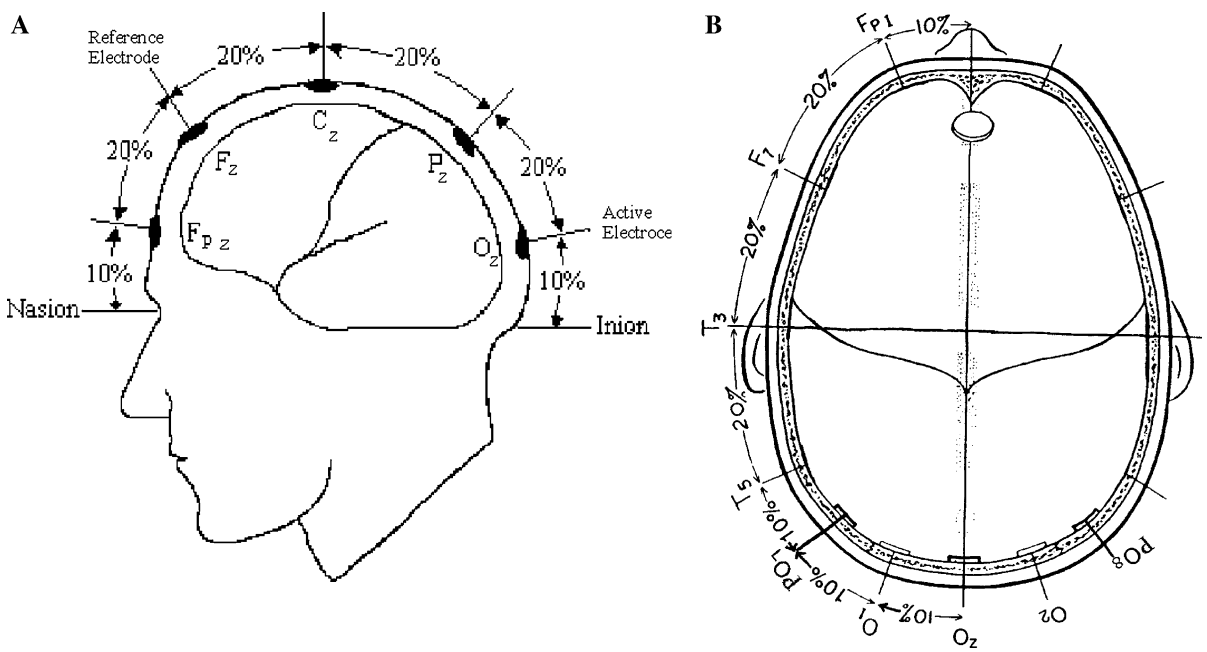

Fig. 1 Electrode locations. **A** Location of active and reference electrodes for standard responses. The active electrode is located along the midline at Oz. The reference electrode is located at location F_z. The subscript z indicates a midline position. **B** Locations of the lateral active electrodes, O_1, O_2, PO_7 and PO_8 are indicated along with the midline active electrode location, O_Z

Table 1 ISCEV standard for VEP assessment

(a) Standard stimuli

Stimulus type	Field size (minimum)	Presentation	Stimulus	Mean luminance ($cd \cdot m^{-2}$)	Michaelson contrast (%)	Presentation rate
Pattern reversal	15°	Monocular	Check widths: 1° (0.8°–1.2°); 0.25° (0.2°–0.3°)	50 (40–60)	≥80	2 (1.8–2.2) reversals/s
Pattern onset/offset	15°	Monocular	Check widths: 1° (0.8°–1.2°); 0.25° (0.2°–0.3°)	50 (40–60)	≥80	1.67 Hz. (1.4–1.67 Hz) (200 ms on; ≥ 400 ms off)
Flash stimulation	≥20°	Monocular	Flash ≥ 20°	3 $cd \cdot s \cdot m^{-2}$ (2.7–3.4)	–	1 (0.9–1.1) Hz

(b) Standard recording

	Electrode montage (international 10/20 channel system)		Filters (−3 dB)		
	Active	Common reference	Low freq	High freq	Sweeps averaged
Pattern stimulation	Oz	Fz	≤1	≥100	≥50
Flash stimulation	Oz	Fz	≤1	≥100	≥50

equal sides whose corners meet. The stimuli may be generated on a screen, with the viewing distance typically between 50 and 150 cm, adjusted to obtain the required check sizes and a suitable field size for any physical size of display screen. Optical systems may be used to produce checkerboard dimensions that are equivalent to those described for a freely viewed display screen. Stimulus changes, whether pattern reversal or onset/offset, must be achieved without a change in the average luminance of the stimulus. Both transient and step changes in luminance (luminance artifacts) can evoke VEPs associated with the luminance artifact in those who cannot resolve the pattern stimulation. There are simple ways for users to observe whether a luminance artifact is present; for instance, one may place a translucent paper between the screen and the eye or turn the monitor toward a wall and observe whether the diffused or reflected light has visible flicker.

Field and check size

Patterned stimuli are defined by the visual angle subtended by the side of a single check in degrees (°) or minutes of arc (min) subtended at the eye (1° = 60 min). For standard pattern VEPs, two check element sizes should be used: 1° (with an acceptable range of 0.8° to 1.2°) and 0.25° (0.2° to 0.3°) of arc per side. All checks should be square, and there should be an equal number of light and dark checks. It is not necessary to use a square field, but the aspect ratio between width and height should not exceed 4:3 and the field size should be at least 15° in its narrowest dimension. A fixation point, when used, should be positioned at a corner of the four checks located at the center of the field.

Luminance and contrast

A mean photopic luminance of 50 $cd \cdot m^{-2}$ (with an acceptable range of 40–60 $cd \cdot m^{-2}$) is required. Contrast between black and white squares should be high (as defined by Michelson contrast[2] ≥80 %). The mean luminance of the stimulus screen must be constant during checkerboard reversals (i.e., no transient luminance change). This is easily achieved with classical CRT (cathode ray tube) stimulators. Note that typical current liquid crystal display (LCD) screens present a brief luminance artifact during pattern reversal, rendering them unsuitable for VEP recording unless special precautions are taken. The luminance and contrast of the stimulus should be uniform

[2] Michelson contrast = $\{[L_{max} - L_{min}]/[L_{max} + L_{min}]\} \times 100$ %, where L = luminance, max = maximum of the white squares and min = minimum of the black squares.

between the center and the periphery of the field. However, many optical and electronic systems do not provide truly uniform fields. We encourage those following the standards to use stimulus displays that are as uniform as possible; variation from center to periphery of up to 30 % is acceptable.

Background illumination

The luminance of the background beyond the stimulus field is not critical when using standard VEP techniques, provided dim or ordinary room lighting is used. Ambient lighting should be the same for all recordings. Care should be taken to keep bright lights out of the subjects' direct view.

Pattern-reversal stimuli

For the pattern-reversal protocol, the black and white checks change phase (reverse) abruptly (i.e., black to white and white to black) with no overall change in the luminance of the screen. To meet this requirement, there must be equal numbers of light and dark checks in the display. Displays used for standard VEP testing must be synchronized with the averager and designed to avoid transient luminance artifacts. Standard pattern-reversal VEPs should be obtained using a reversal rate of 2.0 ± 0.2 reversals per second (rps) (this corresponds to 1.0 ± 0.1 Hz, as a full cycle includes two reversals). Reversal rate must be reported in rps, not in Hz. For a specific standard pattern-reversal VEP test, users should specify check width (for both large and small checks), stimulus rate (in reversals per second), number of reversals averaged, mean luminance, Michelson contrast[2] and field size.

Pattern onset/offset stimuli

For pattern onset/offset, the checkerboard pattern is abruptly exchanged with a diffuse gray background. The mean luminance of the diffuse background and the checkerboard must be identical with no change of luminance during the transition from pattern to diffuse blank screen. This is difficult to achieve with cathode ray tube (CRT) displays, and it is not possible with unmodified liquid crystal displays (LCDs).

Pattern onset duration should be 200 ms separated by 400 ms of diffuse background. This temporal pattern ensures that the VEP waveform to pattern onset is not contaminated by the pattern offset response. The ISCEV standard is based on the onset portion of the VEP waveform to onset/offset stimulation. The data acquisition system must indicate the appearance of the stimulus. At least two pattern element sizes should be used: checks of 1° and 0.25° per side.

Flash stimulus

The Standard flash VEP is elicited by a brief flash ($\leq$ 5 ms) that subtends a visual field of at least 20°, presented in a dimly illuminated room. The strength (time-integrated luminance) of the flash stimulus should be 3 photopic candelas seconds per meter squared ($cd \cdot s \cdot m^{-2}$). The acceptable range for the standard flash strength is 2.7 to 3.4 $cd \cdot s \cdot m^{-2}$, which matches the ISCEV standard flash for full-field ERG testing [2]. For VEPs, the standard flash may be presented on a flashing screen, by a handheld stroboscopic light or by positioning an integrating bowl (ganzfeld), such as that used for ERG tests [2], in front of the patient. The flash rate should be 1 per second (1.0 Hz, range 0.9 to 1.1 Hz).

Calibration

All stimulus parameters including luminance and contrast should be calibrated either locally or by the manufacturer. Regular recalibration is advised [6].

Recording parameters

Amplification systems

DC amplifiers or AC-coupled amplifiers with a minimum input impedance of 10 MΩ in the 50–60 Hz range may be used. Amplification systems must be electrically isolated from the patient complied with current safety standards for medical recording systems. The recording frequency band of bandpass amplifiers should include the range from 1 to 100 Hz. Notch filters (that suppress signals at the mains line frequency) are contraindicated, as they may reduce or distort the signal. Some users may encounter severe electromagnetic interference from the stimulus display that makes it difficult to obtain satisfactory recordings with these filter settings. Ideally, such interference should be eliminated by shielding or by

modifying the equipment. Rearranging the electrode leads may also be of benefit.

Sampling rate

A minimum sampling rate of 1000 Hz (1 ms per point) is recommended. See the ISCEV guidelines [6] for further information.

Averaging and signal analysis

Time zero is defined as the time of an abrupt flash, abrupt pattern onset or pattern reversal. However, flash duration may be up to 5 ms, and screen refresh rates vary and may take up to 17 ms. To avoid variations in peak times based on differences between stimulators, peaks should be measured from time zero at the midpoint of the stimulus onset. Time zero is defined differently in some systems (typically at the beginning of a screen refresh rather than the midpoint). This is acceptable but should be noted on reports as it has a systematic effect on the peak time recorded. The number of stimulus presentations (sweeps) per average depends upon the signal-to-noise ratio between the VEP and the background noise. In most clinical settings, the minimum number of sweeps per average should be 50. At least two averages must be recorded to verify the reproducibility of each VEP. A smaller number of sweeps per average may sometimes produce a clearer response because the longer recording time required to increase sample size may introduce increased variability due to loss of attention and/or increased movement. This is especially true for infants and young children.

Analysis time

The minimum analysis time (sweep duration after the stimulus) for all adult transient flash and pattern-reversal VEPs is 250 ms poststimulus. For onset/offset VEPs, the analysis time (sweep duration) may be extended to 500 ms if the examiner wishes to have the option of analyzing both the pattern onset and offset responses. The VEP in infants has later peak times; a sweep duration of at least 500 ms is often necessary to adequately visualize typical VEP pattern or flash waveforms in those under 1 year of age.

Clinical protocol

Preparation of the patient

Pattern stimuli for VEPs should be presented when the pupils of the eyes are unaltered by mydriatic or miotic drugs. Pupils need not be dilated for the flash VEP. Extreme pupil sizes (miosis or dilation) and any anisocoria should be noted for all tests. For pattern stimulation, the visual acuity of the patient should be recorded and the patient must be optimally refracted for the viewing distance of the stimulus. With standard electrodes and any additional electrode channels attached, the patient should view the center of the pattern field from the calibrated viewing distance.

Monocular stimulation is standard. This may not be practical in infants or other special populations; in such cases binocular stimulation may be used to assess visual pathway function from both eyes. When a flash stimulus is used with monocular stimulation, care should be taken to ensure that no light enters the unstimulated eye. Usually this requires a light-tight opaque patch to be placed over the unstimulated eye. Care must be taken to have the patient in a comfortable, well-supported position to minimize muscle and other artifacts.

The ISCEV standard VEP waveforms

VEP waveforms are age dependent. The description of standard responses below reflects the typical waveforms of adults aged 18–60 years of age. The time from stimulus to the maximum positive or negative deflection or excursion of the VEP will be referred to as the peak time. Historically, the term latency has been used in VEP studies to indicate the time from stimulus onset to the largest amplitude of a positive or negative deflection. However, in most other physiological recordings including electroretinography, the time from stimulation to the peak of a deflection is called the implicit time, with latency defined as the time from stimulus onset to the beginning of a response. To avoid confusion, with the latent period before the onset of the waveform, the ISCEV VEP standard uses the term ‘peak time’ as a synonym for ‘implicit time’ or ‘time to peak’ [1], because the meaning is more immediately apparent.

Pattern-reversal VEPs

A typical pattern-reversal VEP waveform consists of N75, P100 and N135 peaks. These peaks are designated as negative and positive followed by the typical mean peak time (see Fig. 2). The standard measure of VEP amplitude is the height of P100 from the preceding N75 peak. P100 is usually a prominent peak that shows relatively little variation between subjects, minimal within-subject interocular difference, and minimal variation with repeated measurements over time. P100 peak time is affected by non-pathophysiologic parameters such as pattern size, pattern contrast, mean luminance, signal filtering, patient age, refractive error, poor fixation and extremely large or small pupil sizes.

Pattern onset/offset VEPs

In those with good fixation, pattern onset/offset VEPs show greater intersubject variability than pattern-reversal VEPs. Pattern onset/offset stimulation is more effective for detection or confirmation of malingering and for evaluation of patients with nystagmus, as this technique is less sensitive to confounding factors such as poor fixation, eye movements or deliberate defocus. VEPs elicited by Standard pattern onset/offset stimulation typically consist of three main peaks in adults; a positive peak at approximately 75 ms, a negative peak at approximately 125 ms and a positive peak at approximately 150 ms (Fig. 3). The nomenclature of these three peaks is customarily termed C1, C2 and C3, respectively, although other naming conventions are also acceptable if clearly defined by the user. Amplitudes are measured from the preceding positive or negative peak.

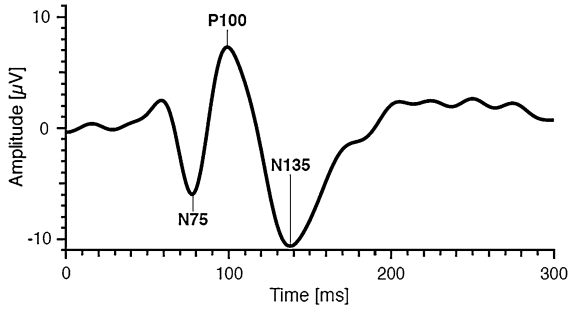

Fig. 2 A typical pattern-reversal VEP

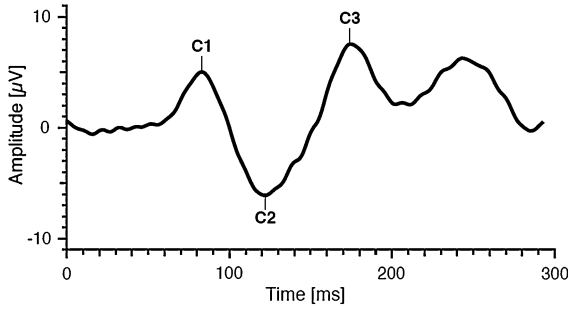

Fig. 3 A typical pattern onset/offset VEP. Note that with a 300-ms sweep only the pattern onset response is recorded

Flash VEPs

Flash VEPs are more variable than pattern VEPs across typical subjects, but are usually similar between eyes of an individual subject. They are useful with patients who are unable or unwilling to cooperate for pattern VEPs, and when optical factors, such as media opacities, prevent the valid use of pattern stimuli. Flash VEPs can give useful clinical information to complement that available from pattern VEPs.

The typical VEP to Standard flash stimulation consists of a series of negative and positive waves. The earliest detectable component has a peak time of approximately 30 ms poststimulus, and components are recordable with peak times of up to 300 ms. Peaks are designated as negative and positive in a numerical sequence (see Fig. 4). This nomenclature is recommended to differentiate the flash VEP from the pattern-reversal VEP. The most consistent and robust components of the flash VEP in typical adults are the N2 and P2 peaks. Measurements of P2 amplitude

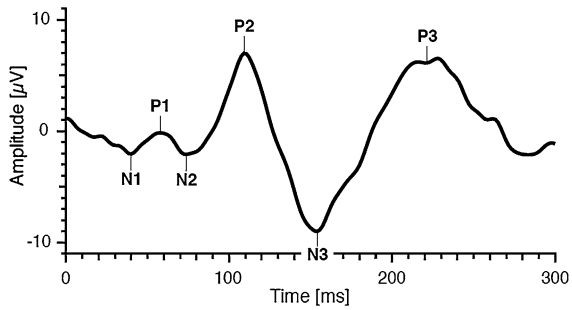

Fig. 4 A typical flash VEP

should be taken from the positive P2 peak at around 120 ms to the preceding N2 negative peak at around 90 ms.

VEP measurement and reporting

Normal values

Standardization should ensure similar VEP waveforms across laboratories. Manufacturers are encouraged to develop normal values for their systems. Nonetheless, each laboratory must establish or verify normative values using its own stimulus and recording parameters. The construction of a sample for laboratory norms should include the factors of age, and interocular asymmetry. Adult normative data cannot be generalized to pediatric or elderly populations. Interocular comparison of amplitude and peak time increases the sensitivity of VEPs for detecting monocular conditions. Laboratory normal ranges should use descriptive statistics, for example percentiles, which do not assume a normal distribution.

VEP reporting

A minimum of two recordings of each VEP condition should be acquired, measured and displayed to confirm reproducibility of the data. Reports using the standard VEP protocols should specify the following stimulus parameters: the field size of the stimulus, the strength (time-integrated luminance) of the flash or mean luminance of the pattern, the pattern element sizes, the contrast of pattern stimuli, the frequency of stimulation and the eye tested. In addition, reports should contain the following recording parameters: the filter settings and the locations of the positive (i.e., active) and negative (i.e., reference) and ground electrodes.

Traces should have a clear indication of polarity and calibration of time in milliseconds, and amplitude in microvolts. We recommend that VEP traces be presented as positive upwards. All VEP reports, including those for nonstandard responses (whether for local records or for publication), must include the peak time and amplitude measurements along with their reference intervals (i.e., normal values, limits of normal and the statistical criterion for the interval). Reports should indicate whether the tests met all conditions of this ISCEV Standard.

VEP interpretation

VEP abnormalities are not specific and can occur in a wide variety of ophthalmological and neurological conditions. The interpretation should include statements about the normality and abnormality of the result in relation to normative data as well as comparison between the eyes or with previous records. The type of abnormality in the VEP should be described, and this should be related to the clinical picture and to other visual electrodiagnostic results if available.

Specialized procedures

Pediatric VEP recording

In principle, the stimulation and recording methods recommended in the ISCEV standard can be applied to all populations. However, in infants, young children and in people with disabilities, modifications to VEP recording methods and testing strategies may be required to optimize the quality of the result and the pertinence to diagnosis and to visual assessment.

All VEPs in children must be compared with appropriate age-related normal values. When recording the VEP in young infants, the sweep duration should be of sufficient length to record the full VEP waveform. In typically developing children with good vision, the peak time of the main positive peak of the pattern-reversal VEP for large checks (1°) is usually within 10 % of adult values by six months of age.

Pediatric VEPs should be recorded when the infant or child is alert and attentive. Direct interaction with the child can help maintain attention and fixation, and two testers are beneficial; one to work with the child and the other to control data acquisition. Data quality and reliability will be improved if recording can be paused or interrupted when fixation wanders and then resumed as the child resumes adequate fixation. To facilitate compliance, an infant may view the stimulus while held on a lap or over the shoulder. The order of stimulus presentation should be flexible and selected to ensure that responses most critical to the diagnostic question are obtained within an individual child's attention span. Binocular pattern stimulation, which facilitates attention and fixation, may be useful to evaluate overall visual function. Monocular testing to at least one stimulus is desirable to assess the function

of each eye. It is particularly important to replicate VEPs in children to assure that the response measured is a reliable signal and not an artifact. Reports should note the degree of cooperation and arousal of the child. As for adults, additional channels of recording may be important for diagnosis of chiasmal and postchiasmal dysfunction. When pattern VEPs cannot be reliably recorded, flash testing, which is less dependent upon fixation, can usually be achieved.

Multi-channel recording for assessment of the posterior visual pathways

Multi-channel VEP recording is not required for a basic ISCEV standard clinical VEP. However, assessment of the chiasmal and postchiasmal visual pathway dysfunction requires multi-channel recording for accurate diagnosis. With dysfunction at, or posterior to, the optic chiasm, or in the presence of chiasmal misrouting (as seen in albinism), there is an asymmetrical distribution of the VEP over the posterior scalp. Chiasmal dysfunction gives a 'crossed' asymmetry whereby the lateral asymmetry obtained on stimulation of one eye is reversed when the other eye is stimulated. Retrochiasmal dysfunction gives an 'uncrossed' asymmetry such that the VEPs obtained on stimulation of each eye show a similar asymmetrical distribution across the hemispheres. We suggest that pattern stimuli for multi-channel investigations of visual pathway dysfunction should be presented with a field of 30 degrees (double the minimum size required by this standard). A minimum of two channels is needed to detect lateral asymmetries. We recommend a minimum of three active electrodes; two lateral electrodes placed at O1 and O2 and a third midline active electrode at Oz. All three active electrodes should be referenced to Fz. Additional electrodes placed at PO7 and PO8, also referred to Fz, may increase sensitivity to lateral asymmetries. The positions of the lateral electrodes are illustrated in Fig. 1B. For all stimulus conditions, normative data should include amplitude and peak time comparisons between homologous left and right occipital channels. Particular caution is needed when interpreting multi-channel pattern-reversal VEPs because of paradoxical lateralization. This phenomenon, in which the response recorded at a lateral scalp location is generated by activity in the contralateral hemisphere of the brain, occurs with a large field, large check reversal stimulus and common reference recording to Fz.

Acknowledgments ISCEV's standardization process requires the active participation of individual ISCEV members who act as consultants to the committee which writes the standard.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest

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