Theme section

Electrophysiology In Vision

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Electrophysiological tests in ophthalmology acts as an important clinical tool in evaluation, diagnosis and management of ophthalmic and neurological disorders. They give us information about functional integrity of the visual system and assess the disorders affecting visual pathway, retina, optic nerve and higher visual processing centres. Along with advanced imaging it gives an additional information of various ocular diseases. There are different types of electrophysiological tests which include electroretinography (ERG), electro oculography (EOG) and visual evoked potential (VEP). This article highlights various tests and test procedures in brief, their clinical implications and also recent advances in this field so that it can provide an aid in day-to-day ophthalmic clinical practice.

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Introduction

A highly ordered neural structure in retina originates from the photoreceptors and exits the eye through the ganglion cell axons.¹⁻³ ERG and EOG represent the function of different retinal layers and post-retinal visual pathway is represented by VEP. A simplified schematic diagram shows different retinal layers along with the electrophysiological test that best represents their functional integrity.(figure 1)

Electro Retinogram

The ERG is an electrical response of the retina to light stimulus which is useful in determining the clinical status of the retina. It is recorded by using a thin fiber electrode which is kept in contact with cornea or an electrode that is embedded within a corneal contact lens. These electrodes allow the electrical activity generated by the retina to be recorded at the corneal surface. ERG underwent various modifications in past. The ERG gives information about inherited and acquired retinal disorders, monitor disease progression and detect retinal toxicity due to various drugs or retained intraocular foreign bodies.



Figure 1: Schematic diagram showing retinal layers labelled according to ocular diseases that affect specific retinal layers. Various electrophysiological test indicated- Retinal Pigment Epithelium (RPE)function (Electro oculogram), photoreceptor to bipolar, horizontal and amacrine cell function (full-field ERG), ganglion cell function (pattern ERG) and retino-cortical pathway function (Visual Evoked Potential).

Full-Feld Electroretinogram

This electrical response is recorded in a dilated, dark-adapted eye using various corneal electrodes. 20 minutes of dark adaptation is required before dark adapted ERG recording and 10 minutes of light adaptation before light adapted ERG. Dark-adaptation test should be performed under dim red light. 5 min of extra dark adaptation should be allowed after insertion of contact lens electrodes. Low strength flashes followed by stronger flashes should be presented to avoid partial light adaptation from strong flashes. The patient should be steady as ocular movements may produce large electrical artifacts, may change electrode position or may cause blockage of light by the eyelids/electrode.

Types of Recording Electrodes

- Burian-Allen (BA): it has stainless steel ring surrounding a polymethylmethacrylate (PMMA) contact-lens core. It has a lid speculum that minimizes eye blink/closure. BA lenses are reusable and are available in various sizes.
- Dawson-Trick-Litzkow (DTL): these have conductive silver/nylon thread, are disposable and more comfortable for the patients.
- Jet: disposable plastic lens with a gold-plated peripheral circumference.(figure 2)
- Skin Electrode: the electrode is placed on skin over the infraorbital ridge near lower eyelid.
- Mylar Electrode, cotton wick and Hawlina-Konec Electrode (not in common)



Figure 2: Jet electrode. Source: Guru Nanak Eye Centre

Placement Of Electrodes(Figure 3)

- Recording electrodes: in contact with cornea, bulbar conjunctiva, or skin below lower eyelid after topical anesthesia.
- Reference on ipsilateral lateral canthus and ground electrode on forehead.

The scotopic measurements target rod-pathway function, whereas photopic measurements target cone pathway function.

The three major components of the ERG waveform are the a-wave, b-wave, and c-wave. The a-wave is the first corneal-negative wave, followed by corneal-positive b-wave and last corneal-negative c-wave.⁴ The amplitude of the a-wave is measured from baseline, whereas the larger b-wave is measured from peak to peak. Implicit time is the time between stimulus onset and maximum amplitude. Rods and cones in the outer photoreceptor layer generate the a-wave, whereas the b-wave is produced by bipolar and muller



Figure 3: Electrode Placement in ERG. Source: Guru Nanak Eye Centre.

cells. Although c-wave is generated by the retinal pigment epithelium but is a reflection of the interactions between the RPE and photoreceptors⁶ and so depends on the integrity of the photoreceptors. Photopic responses can be isolated under light adapted conditions using a stimulus wavelength more than 680 nm or 30 Hz flicker, while scotopic response is isolated by dark-adaptation for 45 min followed by a single flash stimulus or 10 Hz flicker.7 Rods cannot follow a flicker stimulus faster than 20 Hz, whereas cones can follow 30-50 Hz stimulus as they have faster recovery time.8 So, a 30 Hz stimulus with high background illumination isolates maximum rods and allows cone function to be recorded.⁴ As number of rods are more than cones, photopic conditions produces small b-wave amplitudes with short latency, whereas scotopic conditions produce larger b-wave amplitude with longer latency.9

The International Society for Clinical Electrophysiology of Vision (ISCEV) has introduced standards for various forms of ERG recordings which were most recently updated in 2015.

Indications

ERG is a useful in diagnosis of conditions like congenital stationary night blindness, congenital achromatopsia, pigmentosa, cone-rod dystrophies, retinitis cancerassociated retinopathy, melanoma-associated retinopathy and toxic retinopathies but not for localized pathologies.¹⁰⁻¹² In most retinal disorders, there is significant reduction in ERG amplitude and also implicit time.⁴ In retinitis pigmentosa, early disease affects the rods, thus produce reduced scotopic waves⁵ and in severe RP, all ERGs are extinguished and both scotopic and photopic b-wave implicit times are prolonged.5 Cone-rod dystrophy appears to involve only cones early in the disease, later the ERGs usually show attenuated rod physiology. ERG changes in cone-rod dystrophies are shown in (figure 4a,b) Completely extinguished ERGs can also occur in Leber's congenital amaurosis, retinal aplasia, total



Figure 4: (a) Advanced stage scotopic ERG of cone-rod dystrophy showing flat waves in right eye and decreased amplitude and prolonged latency. Source: Gurunanak Eye Center.



Figure 4: (b) advanced stage photopic ERG of cone-rod dystrophy having flat waves in right eye and decreased amplitude and prolonged latency in left eye. Source: Gurunanak Eye Center



Figure 5a: ERG showing patient having incomplete CRAO in the right eye. ERG pattern of CRAO eye (right eye) and the fellow uninvolved eye (left eye). There is slight decrease in b-wave amplitude of CRAO eye compared to normal eye in the dark-adapted 3.0 ERG. Also, PhNR amplitude is depressed in eye with CRAO compared to normal eye. Source: Correlation of electroretinography components with visual function and prognosis of central retinal artery occlusion¹⁸

Figure 5b: ERG of patient having subtotal CRAO in the right eye and normal fellow eye having decreased b-wave amplitude in both dark-adapted and lightadapted responses. Also PhNR amplitudes decreased considerably in the subtotal CRAO, compared to the fellow uninvolved eye.

Figure 5c: ERG of patient diagnosed with total CRAO in the right eye and normal fellow eye.Compared to the ERG pattern in Fig. 5a and 5b, more diminished responses in dark-adapted and light-adapted ERG, including PhNR amplitude, were observed in this case of total CRAO.

retinal detachment, and ophthalmic artery occlusion.^{4,13-15} ERG changes in patient with central retinal artery occlusion are shown in (figure 5a,b,c). In retinal detachments, ERG amplitudes correlate with the amount of healthy retina.¹³ Type 1 congenital stationary night blindness has abnormal dim scotopic ERGs and type 2 has abnormal dim and bright scotopic ERGs.¹⁶ The b/a-wave ratio is useful in diagnosing congenital stationary night blindness.¹⁷

Factors Affecting ERG

ERG responses can vary with age, sedation, sex and technique used by various laboratories. Attenuation of b-wave up to 50% can occur in anaesthetized children. Peak amplitude and implicit time of ERG occurs in young age and declines slowly with age especially after age 55–60.¹⁹ ERG responses may be more in women than men and reduced in high myopes.²⁰⁻²¹ Another limitation of the FERG is that, as it

is a mass potential from the whole retina, at least 20% of the retina has to be affected to see noticeable change.⁴

Photopic negative response (PhNR): The PhNR is a slow negative potential that follows the b-wave under light adapted conditions. It originates from retinal ganglion cells and thus useful in early detection of glaucoma.

Oscillatory Potential

Oscillatory Potentials (OP) are series of high frequency, low amplitude wavelets seen on the ascending limb of b-waves in both scotopic and photopic bright flash ERG recordings. They are thought to reflect activity initiated by the interactions between bipolar cells, amacrine cells, and ganglion cells in the inner retina. An OP abnormality means either delay of implicit time or reduction of amplitude, or both. A selective OP abnormality is observed in the early stage of diabetic retinopathy or diseases of retina such as central retinal vein occlusion.

Pattern ERG (pERG)

Full-field ERG is a diffuse retinal response and PERG is recorded for central stimulus subtending 30° (grey box) or 15° (black box) of the central retina respectively. The pattern ERG (pERG) uses contrast reversing pattern stimulus (checkerboards) to assess macular retinal ganglion cell (RGC) activity. Ocular media clarity, proper refraction and undilated pupils are important for pERG measurement. Over time, the dark checks become light, and the light checks become dark (at a rate of 4 reversals per second). It has three main components: N1 at 30 ms, P1 at 50 ms, and N2 at 95 ms (figure 6).²² Ganglion cell activity represents N2 and outer retinal activity represents P1. As the N2 signal originates from retinal ganglion cells and their axons, it helps in detecting optic nerve disease.23,24 PERG helps in differentiating anterior ischemic optic neuropathy from optic neuritis and also in differentiating retinal from optic nerve disease. Factors affecting PERG are same as FERG including sex and age.²⁵ The pERG is also abnormal in diabetic retinopathy and idiopathic intracranial hypertension.

Multifocal ERG

The multifocal ERG (mfERG) detects many local ERG responses, typically 61 or 103, within central 30 degrees. This detects dysfunction within the macula which may be missed by ffERG. mfERG responses are recorded under light-adapted conditions. The mfERG cannot replace ffERG. If diffuse retinal damage or rod pathway dysfunction is suspected, then ffERG should also be done.

Technique

Electrodes and their placement can be same as described for ffERG and eyes should be dilated with normal room illumination.²⁶ The retina is stimulated at 61or 103 hexagonal elements with a central fixation point of which 50% are illuminated on each frame and are displayed in 30° radius on liquid crystal display.²⁶ The hexagonal pattern is scaled in size to produce same amplitude across the retina with central hexagons being smaller than peripheral.²⁶ The hexagonal pattern alternates between black and white at a rate of 75Hz in a pseudorandom sequence, called binary m-sequence. Resulting waveforms are similar to the FERG consisting of an initial negative wave (N1 or a-wave), followed by a positive deflection (P1 or b-wave) and a second negative deflection (N2 or c-wave). mfERG has five concentric rings: ring 1 corresponds to the fovea, ring 2 to parafovea, ring 3 to perifovea, ring 4 to near periphery and ring 5 to the central part of the middle periphery. It also provides display of the signal amplitudes in a three-dimensional 'hill of vision' with the largest signals corresponding to fovea(figure 7).

Indications

mfERGs can be useful in detecting localized disease in the macular, paramacular or discrete peripheral retina. It is useful in diseases like age-related macular degeneration, macular holes, hydroxychloroquine toxicity, retinitis pigmentosa, branch retinal artery occlusion, fundus flavimaculatus, Stargardt's disease and acute idiopathic blind spot enlargement.²⁸ mfERGs are better than FERG in quantifying retinal toxicity due to ethambutol, chloroquine, or hydroxychloroquine (figure 8).^{4.27} The classic finding of chloroquine or hydroxychloroquine toxicity is a ring scotoma between 5 and 15^oof fovea.²⁹ mfERG may detect earlier progression of glaucoma than automated perimetry.³⁰

Pitfalls

Similar to ffERG. The presence of a depression in area of expected blind spot shows fixation to be adequate.²⁸ Decreased amplitudes are found in old age and high myopes.²⁸



Figure 6: Normal electrophysiological traces. P50 component and the larger N95 are seen in the normal PERG. The figures are using an intensity of 3.0 cd s/m2 for photopic ERGs, an intensity of 10 cd s/m2 for maximal response, and 80 mcd s/m2 for the rod-specific traces.



Figure 7: Normal multifocal ERG. Source: Evaluation of hydroxychloroquine retinopathy with multifocal electroretinography²⁷



Figure 8: Evaluation of hydrochloroquine toxicity with mfERG. Source: Evaluation of hydroxychloroquine retinopathy with multifocal electroretinography ²⁷

Visual-Evoked Potential

The VEP is a large positive polarity wave generated by occipital lobe in response to visual stimulation which begins at retina and ends at visual cortex.

Technique

The VEP measures one eye at a time with scalp electrodes placed over the occipital region. It is used to quantify the functional integrity of optic nerve, pathway to visual cortex and occipital cortex. The patient visualizes a display with a central fixation point and high contrast, equal sized and numbered black and white checkerboard-patterned stimuli which are placed 50–150 cm away, depending on the size of the display.³¹ A pattern reversal stimulus is most reliable with black and white checks reversing at rate of 2 per second (2 Hz)³¹ (figure 9). Large checks

should not be used as it may give false results. Three electrodes are placed: Oz (active/positive electrode placed at occiput), Fz (reference/negative electrode at forehead) and ground electrode placed at Earlobe/vertex/mastoid. Flash VEP using small flashes of light in a dim room are more useful than pattern stimulus in infants or patients with media opacities, poor cooperation, poor fixation or poor acuity.³¹ Pattern reversal VEP is done on undilated patients which are refracted for distance, whereas flash VEP is done on dilated patients.³¹ The signals of 100–200 responses are averaged and amplified to obtain peak, amplitude and latency.

Waveforms

The waveform is measured as an initial negative peak(N1), followed by a large positive peak (P1) and a second negative peak (N2). Pattern reversal latencies are recorded as N75 (N1), P100 (P1), and N135 (N2)(figure10). The components of the flash VEP are negative N2 peak (90 ms) and the positive P2 peak (120 ms)³¹(figure11). Pattern onset/offset VEPs have positive wave c1(75ms), negative c2(125 ms) and positive c3(150 ms).



Figure 9: Screen showing pattern reversal VEP. Source: Gurunanak Eye Center.



Figure 10: pattern reversal VEP waveforms showing N75 and N135 as negative peaks and P100 as positive peak.

Indications

Optic nerve function is best assessed by VEP, whereas it is not of much use in postchiasmatic disorders. With optic nerve dysfunction, P100 latency is prolonged most commonly but decreased amplitude can also be seen in optic atrophy. VEP is sometimes more sensitive than clinical assessment in



Figure 11: Flash VEP waveform is evaluated by measuring peak-to-peak amplitude between negative wave (N75) and positive wave near (P100). A significant decline in flash VEP is considered if there is decrease in peak-topeak distance between N75 and P100 by at least 50% from the reference amplitude.

both check widths, with only a small response to large check widths, and in F-VEP broad and slightly low amplitude present. The PhNR is relatively preserved, but does not fall below the a-wave. This shows severe bilateral retinal ganglion cell (RGC) and optic nerve dysfunction, with some preservation of peripheral RGC function. Optic neuritis: The PERG shows normal P50 components but mildly reduced N95 components in both check sizes. The PR-VEP is atypically delayed with normal amplitude and F-VEP is also normal. The PhNR is markedly reduced. Overall, there is optic nerve dysfunction with mild degeneration to RGCs centrally, with marked peripheral RGC dysfunction.

Macular dystrophy: The PERG P50 of 30° field is well defined, but that of a 15° field is absent showing severe macular dysfunction localized to central 15° field. The PR-VEP of 50' check widths has normal peak-time with borderline amplitude and loss of PR-VEP to small check widths. The F-VEP and PhNR are within normal limits.



Figure 12: Pattern reversal VEP of a patient of multiple sclerosis causing decreased amplitude in left eye compared to right eye showing more involvement of left optic nerve. Source:Guru Nanak Eye center.

detecting optic neuritis, especially once improvement has occurred clinically.³² Pattern reversal VEP is more useful in detecting conduction delay secondary to demyelination (figure 12).³³ The McDonald criteria for diagnosis of multiple sclerosis recommend use of VEP when MRI shows at least four but not more than eight T2 lesions consistent with MS.³⁴ VEP amplitudes in stroke patients are reduced in the ischemic area when compared with the nonischemic area of brain.³⁵ VEP can be predictive of visual recovery in traumatic optic neuropathy, with decreased amplitudes and increased latencies indicating worse visual acuity (figure 13).³⁶ It detects visual status in infants or young children and in cases of media opacities.37 P100 latency is better indicator than color vision and visual field in early stages of hydroxychloroquine maculopathy without ocular symptoms or fundus changes.38 It is useful in objective measurement of refractive error.³⁹

Pitfalls

VEP can detect optic nerve dysfunction but cannot explain the cause. It is best used as an adjunct to clinical history, examination and imaging. VEP detects the pathway from retina to area 17 of occipital cortex. Bilateral abnormal VEP can be due to chiasmal or retro-chiasmal lesions. An abnormal unilateral VEP suggests an optic neuropathy if ocular disease is ruled out. Other factors are electrode placement, scalp thickness, attention, fixation, mental activity, refractive error, pupil size, fatigue, state of dark adaptation and background illumination.⁴⁰⁻⁴⁴ VEP can be abolished by sedation or anesthesia and carbamazepine has been shown to prolong P100 latencies.⁴⁵

Electrophysiological findings of a normal person and in various diseases is shown in (figure 14).

The first row is showing normal findings of PhNR, PERG (to 30° and 15° fields), pattern reversal visual evoked potential (PR-VEP) (to 50′ and 12.5′ check widths) and flash visual evoked potential (F-VEP).

LHON: The PERG demonstrates normal P50 components but markedly abnormal N95 components which do not fall below the baseline and in 15° field the P50 is also reduced with early peak-time. The PR-VEP is severely declined in



Figure 13: flash VEP of a patient showing decreased amplitude and increased latency in both eyes after occipital trauma to 1year old having traumatic optic neuropathy. Source: Guru nanak eye center.



Figure 14: demonstrating the electrophysiological findings in a normal person, and in patients with Lebers Hereditary Optic Neuropathy, Optic Neuritis and Macular dystrophy respectively. Source: Clinical electrophysiology of the optic nerve and retinal ganglion cells.⁴⁶

Overall, it shows localized macular dysfunction in the 15° field with preservation of surrounding 15–30° field. The normal N95:P50 ratio and PhNR indicates normal RGC and optic nerve function.

Multifocal Visual Evoked Potential

The multifocal VEP (MfVEP) uses a 60-sector checkerboard that covers the central 22^o and electrical responses to pattern stimuli are recorded (figure 15).

Technique

Compared with conventional VEP, MfVEPs are made of electric responses from a wider region of the visual field (40–50^o radius), so it is capable of detecting a broad range of optic nerve damage.³⁷ It can detect local defects.³⁷

Indications/pitfalls

MfVEP is useful in optic neuropathies, such as glaucoma and correlates with defects seen on automated perimetry.⁴⁷ It is an objective topographic assessment of the visual field



Figure 15: The multifocal VEP stimulus display: the dartboard pattern consists of 60 sectors, each with a checkerboard pattern of 16 checks, eight white (200 cd/m 2) and eight black (3 cd/m 2). The entire display subtended a diameter of 44.5°, and the central 12 sectors fell within a diameter of 5.2° of the foveal center. Source: Study for analysis of the multifocal visual evoked potential. Korean J Ophthalmology³⁷

in glaucomatous and non-glaucomatous optic neuropathies and useful in patients with unreliable visual field.⁴⁸ Also, it shows defects not detected on automated perimetry in optic neuropathies.⁴⁸⁻⁴⁹ Pitfalls are same as that of standard VEP.

Sweep Vep

It is mainly useful for the examination of non-verbal children and malingering patients. Here, the program generates a pattern stimulus that is alternated at a high temporal frequency rate (5 to 15 Hz), producing a steady state visual evoked response. It detects a response very rapidly. For measuring visual acuity, the size of the pattern is reduced rapidly.20 different pattern sizes are presented in succession within 10 seconds. This sweep of the spatial resolution domain provides estimation of visual acuity by the smallest pattern size producing a response.

Technique

It starts by producing a cartoon to attract the attention of the child which is followed by the presentation of a checkerboard with large dimensions. After which, operator trigger sweep stimulations that generate a rapid succession of 20 different patterns of decreasing sizes.

Electro-Oculogram

The electrooculogram (EOG) measures the resting potential of cornea relative to the back of the eye. It gives transepithelial potential (TEP) of the retinal pigment epithelium that exists between the cornea and Bruch's membrane.

Technique

The skin electrodes are placed near the lateral and medial canthus and ground electrode on forehead. Inside a Ganzfeld, the patient fixates alternately on small red LED lights 30 degrees apart. The patient is light adapted in a well-illuminated room for at least 30min with dilated eyes. The patient keeps head still and moves eyes back and forth, alternating between the two red LED lights. The movement of the eyes produces a voltage swing of approximately 2-5mV between the electrodes on each side of the eye and producing a graph. After light adaptation, the lights are turned off and after every minute, a 10-s sample of eye movement is taken (figure 16). After 12-15min in dark, the lights are turned on and about once a minute, eye movements are recorded for about 10 s. Figure 16 shows segments of eye movements in various phases of EOG.

The voltage becomes smaller in the dark, reaching its lowest potential after 8–12min, the so-called dark trough. When the lights are turned on, the potential rises and reach peak in about 10min. Ratio of light peak to dark trough should be



Figure 16: Samples of eye movement in light-adaptation period, dark adaptation phase, and light-rise phase of EOG.

near 2:1. Ratio of less than 1.7 is considered abnormal between light peak and dark trough. Normal pigment epithelium and normal mid-retinal function, both are required for light rise of potential. Most common use of electrooculogram is confirmation of Best disease.

Identification of best disease is done by appearance of an egg-yellow fundus and is confirmed by recording both electroretinogram (ERG) and electrooculogram (EOG). The ERG will be normal whereas EOG will be abnormal. The EOG can also be used for tracking eye movement.

Conclusion

Various electrophysiologic tests are helpful in ophthalmology, each having different indications. The FERG is very useful in diffuse retinal disorders, whereas the MfERG is better in localized disease. VEPs is useful for diagnosing optic neuropathies, nonorganic visual loss, and evaluating retinal function of infants or children. MfVEP acts as an objective test for visual field defects in glaucomatous and non-glaucomatous optic neuropathies. Sweep VEP provides estimation of visual acuity in non-verbal children and malingering patients.

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