Introduction

Electroretinography (ERG) is the mainstay of clinical ophthalmic diagnostic testing. The ERG provides an objective, quantitative measure of retinal function and allows the clinician to monitor the function of rod cells, cone cells, and ganglion cells in each eye. It uses electrodes placed on the cornea or adjacent to the orbit to monitor changes in the electrical potential of the eye in response to specific stimuli. Careful manipulation of the stimulus and testing conditions allows the clinician to investigate different cell types and layers of the retina. The ability to distinguish between different cell layers and cell types means that ERG’s can be used to discern between myriad inherited retinal disorders and dystrophies that may otherwise prove clinically indistinguishable. Several different types of ERG test provide specific information about the patient's visual function. The full-field erg, or fferg, is the most common form of electroretinographic testing. It provides an assessment of general retinal function and can distinguish between the various cell types, revealing the function of photoreceptors, bipolar cells, ganglion cells and amacrine cells, but cannot provide specific information about individual sectors of the retina. The most recent advance in ERG technology is the multifocal electroretinogram (mfERG) which provides a detailed assessment of the health of the central retina and measures the response in each of a large number of small sectors, typically either 61 or 103, of the retina. It thus provides a map that allows the clinician to locate specific areas of malfunction. The pattern ERG, or pERG, measures the response to a temporally changing pattern of contrast at a constant level of luminance, providing information about ganglion cells and generalized macular function.

Basic Principle

Photoreceptors and downstream neurons in the retina maintain a non-neutral electrical “resting potential” by manipulating the intracellular and extracellular concentrations of positive sodium, potassium, and calcium ions and negative chloride ions, as well as larger electronegative molecules. Human rod cells present a model system of phototransduction. The chromophore, or light sensing pigment, in rods is 11-cis-retinal, which is bound to an apoprotein called opsins, forming rhodopsin. When a photon strikes 11-cis-retinal, it causes it to isomerize into all-trans-retinal. This conformational change causes rhodopsin to activate transducin, a heterotrimeric G protein. Activated transducin binds to the inhibitory subunits of phosphodiesterase 6 (PDE6), thereby de-inhibiting it. The newly active PDE6 hydrolyses cyclic Guanosine monophosphate (cGMP), reducing intracellular cGMP levels and closing cGMP-gated cationic channels (CNG) in the rod cellular...
membrane. This reduces the influx of NA+ and Ca2+ into the cell, thereby hyperpolarizing it. The hyperpolarization of the cell causes it to cease transmitting glutamate across synapses to bipolar cells, inducing changes in their polarization. Bipolar cells transmit this signal either directly to ganglion cells, each of which has an axon proceeding out of the orbit along the optic nerve, or to amacrine cells, which then activate ganglion cells or alter the output of other bipolar cells. Photoreceptors, bipolar cells, and amacrine cells operate via graded potentials, but ganglion cells generate action potentials in response to incoming signals from bipolar and amacrine cells; these action potentials help to propagate the information along the optic nerve. The function of each of these cell types can be measured precisely using various electroretinographic techniques.

**Components of ERG**

**a-wave:** Initial corneal-negative deflection, derived from the cones and rods of the outer photoreceptor layers

This wave reflects the hyperpolarization of the photoreceptors due to closure of sodium ion channels in the outer-segment membrane. Absorption of light triggers the rhodopsin to activate transducin, a G-protein. This leads to the activation of cyclic guanosine monophosphate phosphodiesterase (cGMP PDE) eventually leading to a reduction in the level of cGMP within the photoreceptor. This leads to closure of the sodium ion channels resulting in a decrease of inwardly directed sodium ions, or a hyperpolarization of the cell. The a-wave amplitude is measured from baseline to the trough of the a-wave.

**b-wave:** Corneal-positive deflection; derived from the inner retina, predominantly Muller and ON-bipolar cells

The hyperpolarization of the photoreceptor cells results in a decrease in the amount of neurotransmitter released, which subsequently leads to a depolarization of the post-synaptic bipolar cells. The bipolar-cell depolarization increases the level of extracellular potassium, subsequently generating a transretinal current. It is this transretinal current that depolarizes the radially oriented Muller cells and generates the corneal-positive deflection. The b-wave amplitude is generally measured from the trough of the a-wave to the peak of the b-wave. This wave is the most common component of the ERG used in clinical and experimental analysis of human retinal function.

**c-wave:** It is derived from the retinal pigment epithelium and photoreceptors

The c-wave is a reflection of the resulting change in the transepithelial potential due to the hyperpolarization at the apical membrane of the RPE cells and the hyperpolarization of the distal portion of the Muller cells. The c-wave generally peaks within 2 to 10 seconds following a light stimulus, depending on flash intensity and duration. Due to the c-wave response developing over several seconds, it is susceptible to influences from electrode drift, eye movements, and blinks.

**d-wave:** This is produced from the off bipolar cells.

The a-wave, sometimes called the “late receptor potential,” reflects the general physiological health of the photoreceptors in the outer retina. In contrast, the b-wave reflects the health of the inner layers of the retina, including the ON bipolar cells and the Muller cells.

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**Fig. 1:** Basic waveform of ERG

**Fig. 2:** Showing from where the major components of ERG originate.

Two principal measures of ERG waveform are-
Amplitude
- a-wave- from the baseline to the negative trough of the a-wave.
- b-wave measured from the trough of the a-wave to the following peak of the b-wave.

Time
- \( t_a \) from flash onset to the trough of the a-wave.
- \( t_b \) from flash onset to the peak of the b-wave.

These times, reflecting peak latency, are referred to as “implicit times” (Figure 3).

The ERG of a normal full-term infant looks similar to a mature ERG. A normal ERG in a newborn infant can be small amplitude the first couple of months. The ERG attains peak amplitude in adolescence and slowly declines in amplitude throughout life. After age 55-60 years the amplitude of the ERG declines even more. Implicit times slow gradually from adolescence through old age as well.

ERG recording electrodes
1. Recording electrodes/Active Electrode
   - Over cornea, bulbar conjunctiva or skin of lower eyelid (Figure 4).
   - Protect corneal surface with non-irritating ionic conductive solution (artificial tears or contact lens solutions containing sodium chloride and no more viscous than 0.5% methyl cellulose) & topical anesthesia for contact lens electrodes.
   - Conjunctival sac – used in pattern ERG
   - Cornea (contact lens electrode) in flash ERG

2. Reference electrodes
   - These electrodes connect to the negative input of the system.
   - Bipolar electrode- incorporated within the contact lens-speculum assembly- may produce lower amplitude than the monopolar electrode at a separate area.
   - Monopolar electrodes may be attached to skin near each temporal orbital rim, avoid placing over muscle masses.

3. Common electrodes/ Ground electrode
   - Connected to common input of the system.
   - Placed over earlobe/mastoid/forehead.

Fig. 4: Burian & Cotton Wick electrodes.

There are yet other simpler ERG recording devices using gold Mylar tape that can be inserted between the lower lid and sclera/cornea. Most electrodes are monopolar, i.e., are referred to another electrode site most commonly on the forehead. Some are bipolar with the reference electrodes built into a metal surface on a speculum (Figure 5).
If electrodes are to be reused, they should be sterilized with a solution that neutralizes prion-transmitted diseases such as Creutzfeldt-Jakob disease (CJD).

**Types of ERG**

The focal ERG (fERG; also known as the foveal ERG) is used primarily to measure the functional integrity of the fovea and is therefore useful in providing information in diseases limited to the macula. A variety of techniques have been described in the literature for recording fERGs. Differing field sizes varying from 3 degrees to 18 degrees and light stimulus frequencies have been used in the various methods, however each technique deals with the challenge of limiting amount of light scattered outside the focal test area. Focal ERG is useful for assessing macular function in conditions such as age-related macular degeneration, however requires good fixation from the subject. The full-field ERG (Ganzfeld; ffERG) measures the stimulation of the entire retina with a flashlight source under dark-adapted (scotopic) and light-adapted (photopic) types of retinal adaptation. This is useful in detecting disease with widespread generalized retinal dysfunction i.e. cancer associated retinopathy, toxic retinopathies, and cone-rod dysfunction. Due to the massed retinal electrical response, small retinal lesions may not be revealed in ffERG recordings. The multifocal ERG (mfERG) simultaneously measures local retinal responses from up to 250 retinal locations within the central 30 degrees mapped topographically. This new technology was developed by Erich Sutter in the early 1990s and involves powerful computers and high-intensity display monitors. The light stimuli are presented on a video monitor in one of a large number of arrays consisting of hexagonal elements. The hexagonal elements in the array are distributed so that the focal retinal responses have an approximately equal signal-to-noise ratio. The central hexagons are smaller than those in the periphery. The elements are stimulated in a pseudo-random sequence of light and dark, called a maximum length sequence (or m-sequence). The resulting waveforms are similar to those of the ffERG: initial negative deflection (N1 or a-wave), followed by a positive deflection (P1 or b-wave), and a second negative deflection (N2 or c-wave). MfERGs are useful in detecting localized abnormalities within the retina in conditions such as retinitis pigmentosa, branch retinal artery occlusion, fundus flavimaculatus, and Stargardt's disease. Degree of retinal toxicity related to certain drugs such as hydroxychloroquine or ethambutol is better detected using mfERG compared to ffERG. Early visual field defects due to glaucoma may also be detected sooner using mfERG compared to automated perimetry. The pattern ERG (PERG) uses pattern-reversal stimuli similar to VEP testing and captures retinal ganglion cell activity predominantly in the N95 waveform component. It is used to detect subtle optic neuropathies. In demyelinating optic neuropathy, the PERG is relatively normal, while it may be abnormal in ischemic optic neuropathies. P50 evaluates the macular function.

**Light stimulation for ERGs.**

There are also several methods of stimulating the eye. Some laboratories use a strobe lamp that is mobile and can be easily placed in front of a person whether sitting or reclining. The mobility of a strobe lamp (Figure 6) or an array of LEDs is a necessity in some situations such as at the hospital bedside or in the operating room.

For patients over 5 years of age most laboratories use a Ganzfeld (globe) with a chin rest and fixation points (Figure 7). The Ganzfeld allows the best control of background illumination and stimulus flash intensity. Either strobe lamp or Ganzfeld methods of flash presentation can be used to record the ERG following a single flash or to average responses to several flashes with the aid of a computer.
Fig. 7: Ganzfield Stimulation Globe

ERG recording guidelines according to ISCEV 2015 guidelines-

- Maximally dilate the pupils.
- Before dark adapted protocols- 20 min of dark adaptation.
- Before light adapted protocols- 10 min of light adaptation.
- Present low strength flashes before stronger flashes- so that the partial light adaptation due to bright light does not occur.
- Insert corneal contact electrodes (when these are used) under dim red light after dark adaptation period. Avoid strong red light. Allow 5 min of extradark adaptation after insertion of contact lens electrode.
- Allow at least 30 min recovery time in ordinary room illumination after use of strong light for retinal imaging (fundus photography, fluorescein angiography and others).
- Request the patient to fix and not move eyes. Ocular movements can change the positions of electrodes, can cause blockage of light by eyelids or electrode and may induce electrical artifacts.

Separating rod and cone ERGs

Most disorders of the retina are detected by an attenuation of amplitude. Implicit times, of both a- and b-waves are also affected in some conditions. Implicit times and amplitudes vary depending upon whether the eye is dark adapted or not, background illumination, brightness, rate of stimulation and colour of the light stimulus. These parameters allow separation of rod and cone activity in any duplex retina. Rods and cones differ in number, peak color sensitivity, threshold and recovery. There are about 120 million rods in each retina and about 6-7 million cones. Because of sheer numbers, the ERG following a white flash is dominated by the mass response of the rods. Peak wavelength sensitivity for rods is around 510 nm and the peak sensitivity of cones as a group is about 560 nm.

Using different rates (flicker) of stimulus presentation also allows rod and cone contributions to the ERG to be separated. Even under ideal conditions rods cannot follow a flickering light up to 20 per second whereas cones can easily follow a 30 Hz flicker, which is the rate routinely used to test if a retina has good cone physiology.

ERG recording methods

This includes 6 protocols named according to the strength of the stimulus in candela. second/square meter (time integrated luminance).

1. Dark-adapted 0.01 ERG (a rod-driven response of on bipolar cells).
2. Dark-adapted 3 ERG (combined responses arising from photoreceptors and bipolar cells of both the rod and cone systems; rod dominated).
3. Dark-adapted 10 ERG (combined response with enhanced a-waves reflecting photoreceptor function).
4. Dark-adapted oscillatory potentials (responses primarily from amacrine cells).
5. Light-adapted 3 ERG (responses of the cone system; a-waves arise from cone photoreceptors and cone Off- bipolar cells; the b-wave comes from On- and Off-cone bipolar cells).

Factors affecting ERG responses
- Duration of stimulus
- Size of retinal area illuminated
- Interval between stimuli
- Size of pupil
- Development of Retina
- Clarity of Ocular Media
- Age, Sex, and Refractive Error- Adult ERG by the age of 2 years, Women > Men.

Abnormal ERG responses

There are 4 types of abnormal responses.\textsuperscript{21-22}
- Supernormal Response – Amplitude of both a & b wave is 2 standard deviation above the normal. Eg. Albinism, Early Siderosis Bulbi.
- Subnormal Response- Both a & b wave have amplitude less than 2 standard deviation beneath the mean normal. Eg. Early RP, HCQ Retinopathy, Retinal Detachment, Vitamin A deficiency etc.
- Negative Response- Large a wave with small or no b wave (b/a ratio <1) Gross disturbance in retinal circulation. Eg. Arteriosclerosis, Giant cell arteritis, CRAO, CRVO etc.

Clinical applications of ERG
1. ERG in Retinitis Pigmentosa- like disease- Retinitis pigmentosa shows minimal or sub-normal a- and b-wave amplitudes (response primarily from cone system) and can even result in completely extinguished ERG waves in severe retinitis pigmentosa. There are a number of central nervous system syndromes with RP-like ocular involvement. Prominent among these are the mucopolysaccharidoses such as the Hurler, Scheie and Hunter syndromes & neuronal ceroid lipofuscinoses such as Batten's disease which have abnormal ERGs.
Other syndromes that may include retinitis pigmentosa are Bassen-Kornzweig syndrome (a-beta-lipoproteinemia), Alagille syndrome, Cystinosis, Kearn's-Sayres syndrome, Laurence-Moon-Bardet-Biedl syndrome, Myotonic dystrophy, Refsum's disease, Usher's syndrome etc.\textsuperscript{23}
2. Congenital stationary night blindness (CSNB) - Schubert-Bornschein type can vary in ERG appearance but the classic form has reduced b-wave amplitudes & Riggs type CSNB the a- and b-wave ERG amplitudes attenuate proportional to degree of expression.
3. Enhanced S-cone syndrome, sometimes called Goldman-Favre syndrome - ERGs show a poor rod photoreceptor response and increased ERG responses to blue flashes.\textsuperscript{24}
4. X-linked juvenile retinoschisis - ERG has a specific abnormality showing a normal a-wave but no b-wave that is a negative ERG.
5. Cone Dystrophies - Markedly depressed photopic response and less affected scotopic response.
6. ERGs in retinal vascular disease - Vascular occlusions such as central retinal artery thrombosis produce a characteristic avascular appearance to select areas of the fundus and an ERG with no b-wave. Ophthalmic artery occlusions usually result in unrecordable ERGs. Central retinal vein occlusion shows attenuation of b-wave amplitude and delay in 30 Hz flicker implicit time to beyond 35 milliseconds. In general, focal disease including due to vascular insufficiency, detachment, trauma, or focal toxicity reduces the full-field ERG amplitude proportional to amount of area affected.
7. Foreign bodies and Trauma - In Siderosis a transient supernormal response then negative pattern followed by non-detectable response in severe cases (rod function more affected than cones; reduction of b-wave amplitude more than a-wave) however a small piece of stainless steel or plastic outside the macula may have a minor effect on the retina. In general, if b-wave amplitudes are
reduced 50% or greater compared to the fellow eye, it is unlikely that the retinal physiology will recover unless the foreign body is removed. In traumatic cases ERG changes depends upon the extent of retina involved.

8. Drug toxicities - Several drugs taken in high doses or for long periods of time can cause retinal degeneration with pigmentary changes like thioridazine, chlorpromazine, Vigabatrin, and chloroquine or hydroxychloroquine. The American Academy of Ophthalmology guidelines recommend a baseline examination for patients starting these drugs to serve as a reference point; and to rule out maculopathy an annual screening after 5 years of use unless there is suspicion of toxicity or presence of unusual risk factors.

9. Systemic disorders and the ERG - Systemic metabolic disorders are reflected in retinal physiology. Liver and kidney disease and drugs that affect those organ systems, usually reduce ERG b-wave amplitudes, particularly in scotopic dim flash ERGs. For example, deferoxamine, an iron chelating drug used to reduce iron overload, can be toxic to the retina. Vitamin A deficiency shows reduced ERG amplitudes particularly under scotopic conditions.

Conclusion
Electrophysiological recording is a valuable asset for the clinician. Because ERG tests can measure the function of different cell types and cell layers, they can aid the clinician in distinguishing between symptomatically similar diseases. Furthermore, because they provide an objective measure of retinal function, they can help clinicians evaluate very young patients, very old patients, and others that may otherwise be difficult to diagnose. The quantitative results that ERG tests provide make them useful as tools for both prognosis and disease monitoring.

References
14. Guidelines for basic multifocal electroretinography (mfERG) Michael F. Marmor1, Donald C. Hood2, David Keating3,


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