LECTURE 7

THE ELECTRORETINOGRAM (ERG)

The electroretinogram is a field potential recorded from the cornea of the intact eye in response to light. It represents the total electrical activity of all the retinal cell types in response to stimulation by light. It is used in research to study basic retinal functions and in clinical practice to diagnose conditions that affect the retina or to differentiate between those afflictions which undermine retinal function from those that undermine the function of the remainder of the visual pathway.

For all the complexity of the origins of the ERG, it is a very simple looking curve (Figure 7.1) with 2 main parts. The ERG in figure 7.1 shows the response to a standard bright flash after 30 minutes of dark adaptation by a healthy visually normal 29 year-old woman.

Understanding how the ERG is generated helps immensely to interpret what it means.

The ERG as described by Granit (1933) is composed of three major components – PI, PII and PIII – in order of their disappearance under anaesthesia.

PI	-	Forms the c-wave
PII	-	Forms the b-wave
PIII	-	Has an early fast component generated by the photoreceptors and a slow component generated by non-neuronal retinal structures, the Muller cells and the RPE in response to changes of extracellular potassium.

Key to understanding how the ERG is generated are two points:

- The pathway by which light signals are sent to the brain as electrical signals (once they have been converted from light to electrical energy) is basically a straight one. The simplest pathway to consider is that which exists at the fovea centralis. Here, electrical signals pass from one cone to one bipolar cell to one ganglion cell which then sends the signal to area 17 of the visual cortex via the rest of the visual pathway. So the direct pathway for the transmission of light on the retina consists of photoreceptor (first-order neuron) to bipolar cell (second-order neuron), to ganglion cell (third-order neuron). All other pathways are secondary to this direct pathway.

- The second key harks back to basic cell physiology. As far as a cell in the body is concerned, we have two bodies of fluid – intracellular fluid and extracellular fluid. Inside these two fluids are what the cells needs. It allows what it needs to pass through its cell wall while keeping everything else out and secreting what it does not need into the extracellular fluid. The important thing to note is that there are a set of mechanisms by which the cell takes what it needs from the extracellular fluid. One such mechanism is coupled transport. You normally have

different ions at very different concentrations inside the cell compared with outside. Potassium concentration is high inside the cell and sodium concentration is high outside it. So let's say some important ion such as chloride or bicarbonate is needed by the cell, the cell will let it in if it is coupled to a sodium ion. Also, the cell will only let this sodium ion in (traveling down its concentration gradient – which all the ions inside and out the cell are trying to do) if it has something the cell needs, otherwise it is kept out.

The rapid movement into the cell of sodium ions and the subsequent movement out of it by potassium ions are the most important things that occur during an action potential. The ERG measures the rapid changes in extracellular potassium in response to an action potential which is generated by the response of the eye to light. From this ERG, we can work out what must be happening inside of the neuronal (direct pathway) cells caused by their reaction to light.

The b-wave has been studied the most in part because it is the easiest to generate. The a-wave for instance requires 100 times more lighted to be elicited.

The b-wave is useful in the assessment of the retinal function. It is almost certainly generated from the retina from structures post-synaptic to the photoreceptors. We are sure of this because specific drugs which block transmission between the first- and second-order neurons block the ERG. We know it must be generated on the retina because it is fully generated even when the optic nerve is fully transsectioned (cut).

Currently, the most probable hypothesis on the generation of the b-wave is the Muller cell hypothesis which holds that ***the b-wave is an extracellular expression of radial current flow caused by a potassium ion-mediated depolarization of the Muller cells*** (Holder 1987; Baker et el, 1988).

Functions and Importance of a well-taken ERG

We can monitor the function and structural integrity by a variety of methods which we can demarcate into three broad groups.

- ***Visually*** *using tests such as ophthalmoscopy, fundus photography and fluorescein angiography.*
- ***Psychophysically*** *using such well-known tests as visual acuity, color vision, contrast sensitivity and visual fields.*
- ***Electrophysiologically*** *using the ERG, electro-oculography (EOG) and visual evoked potentials (VEP).*

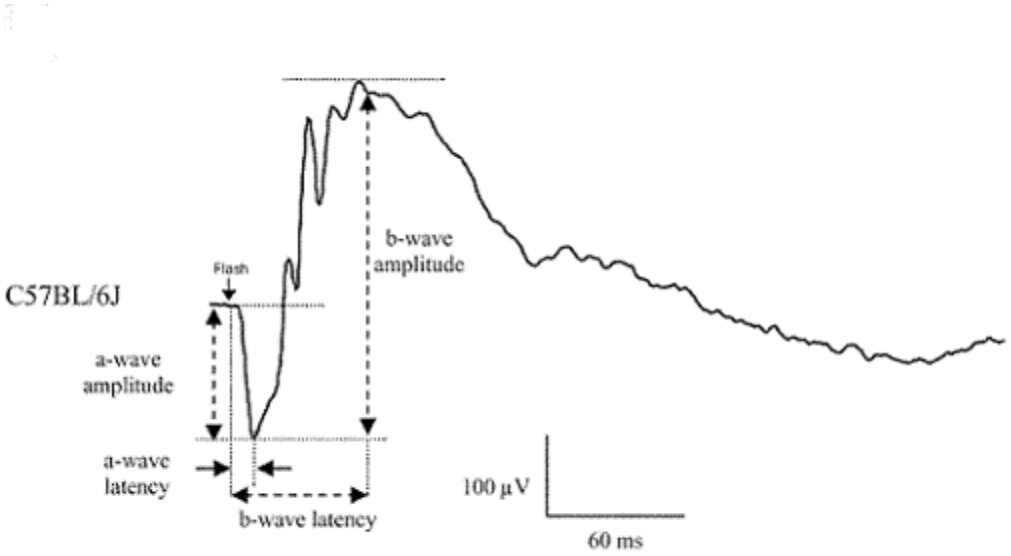


Figure 7.1 The Basic Waveform of the ERG (with OPs)

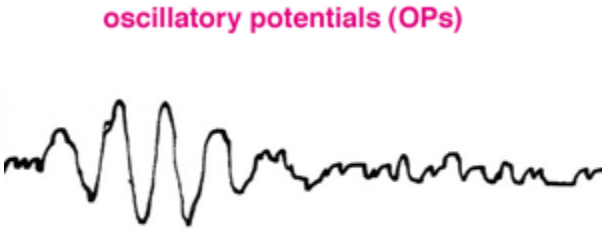


Figure 7.2 Oscillatory Potentials

Of all these tests, the single best objective tests which most accurately reflects the overall function of the retina, is the ***standard full-field flash ERG***.

The advantage of the electrophysiological tests is becoming more obvious with the passing of time. As any good doctor will tell you, 90% of any good remedy is prevention. Barring prevention of a disease, the next best thing is early detection. This is where the electrophysiological tests and other new tests such as retinal tomography and scanning laser polarimetry (e.g. GDx-VCC) come in. These tests if applied and interpreted properly can detect disease or malfunction of the retina very long before the signs of reduced visual acuity, reduced visual fields and reduced color vision or contrast sensitivity start to occur.
Also, remember that the damage on the retina happens to neurons. As we know from basic anatomy and physiology, neurons seldom regenerate after injury so once a neuron is damaged, it cannot be salvaged so the treatment for conditions that cause neuronal damage is primarily to prevent damage to more neurons – damage limitation essentially. Therefore early detection saves many more neurons from damage because treatment is started much earlier.

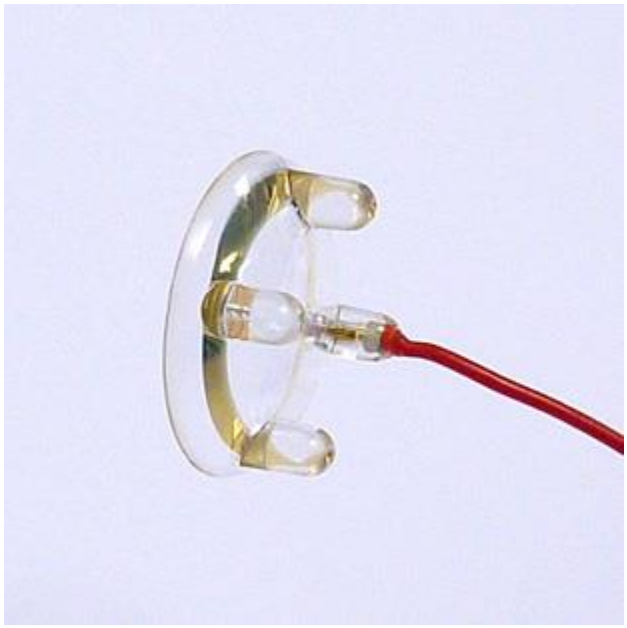
Damage limitation is how glaucoma is managed. Glaucoma cannot be treated! What constitutes glaucoma is damage of the RNFL cells. Once a cell is damaged it cannot be regenerated so the management for glaucoma is focused entirely on reduction of IOP (even when IOP is already within the normal range when glaucomatous damage has begun) to prevent damage to more RNFL cells.

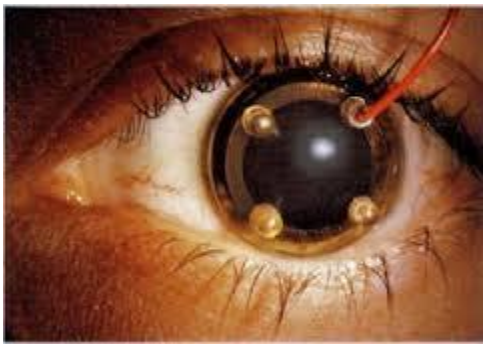
To be useful, the ERG must be able to do three things:

- Separate rod function from cone function
- Compare the activity of local retinal areas
- Test retinal function at different retinal depths.

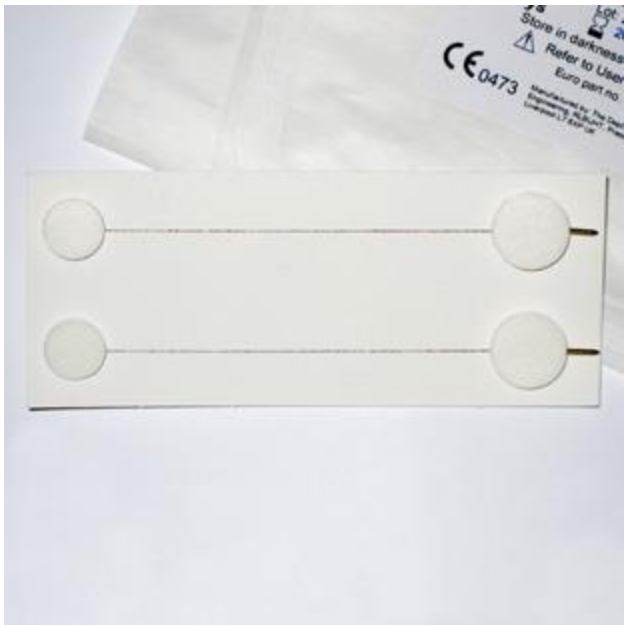
ERG protocols are usually designed by an international body called ISCEV (International Society for Clinical Electrophysiology of Vision) and they usually state whether the ERG response was measured under dark-adapted (scotopic) or light-adapted (photopic) conditions. But to isolate rod from cone function is a little more complex. The response to a dim white or blue light by a dark-adapted eye represents rod function. The response to a flashing red light in the light-adapted eye represents the cone response. The response to a standard bright flash in the dark-adapted eye represents the response of not just the rods but also the dark-adapted cones.

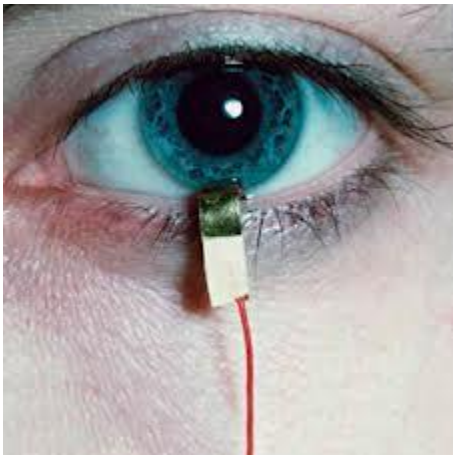
A normal ERG (flash and pattern reversal) means retinal function is fine. If it is coupled with an abnormal VEP, the problem lies somewhere else on the visual pathway.





Figures 7.3 (a and b) **The Jet Contact lens Electrode**





Figures 7.4 (a and b) **DTL (left - a) and Gold Foil Electrodes**

How is the ERG generated?

We already know about the positive ions that flow from the inner to the outer segment of the photoreceptor in the dark (dark current). Exposure to light increases the resistance between inner and outer segment, positive ion flow is therefore curtailed. As a result, the outer segment hyperpolarizes. This is the beginning of the action potential in the photoreceptors in response to light.

The dark current depolarizes (activates) the horizontal cells and the hyperpolarizing (off-center) bipolar cells.

Exposure to light hyperpolarizes (switches off) the horizontal cells and the off-center bipolar cells and depolarizes the depolarizing (on-center) bipolar cell which was hyperpolarized in the dark.

This activity causes different cells to alter their permeability to different ions according to the flow of electrical current in the retina. Generally, we have what are called current sinks and sources.

The ERG of a vertebrate is complex and consists of several waves, the a, b, c and d waves, as well as other minor components such as oscillatory potentials.

The ERG probably originates from the Muller cells which essentially act as potassium-sensitive electrodes that sense the concentration (and changes in concentration) of extracellular potassium.

Light causes a distal (in the region of the outer plexiform layer) decrease in K^+ concentration, followed by a small distal increase and then a larger proximal (in the region of the inner plexiform layer) increase. The distal increase is most responsible for the b-wave and is believed to be caused by the depolarizing (on-center) bipolar cells.

The b-wave has two current sinks, one in each plexiform layer and a current source which runs along the inner retinal margin. Light stimulation causes a K^+ increase in both plexiform layers which are sensed by the Muller cells causing them to depolarize. It is thus the extracellular current flow around the Muller cells, caused in particular by the distal increase in K^+ that give rise to the b-wave of the ERG.

a wave ***The a wave and the PIII component have an early fast component due to the photoreceptors and a slow component originating in the Muller cells. Slow PIII reflects rod activity.***

c-wave ***Generated by the RPE in response to a decrease in extracellular potassium in the subretinal space caused by light exposure.***

Oscillatory Potentials ***These are shown in figure 7.2 above. They are small wavelets superimposed on the b-wave arm. They arise most readily in mesopic conditions, elicited by repetitive bright full-field flashes with a constant stimulus interval. They are thought to be generated by feedback from either the Amacrine or Interplexiform cells or both. However their origins are unknown.***

Equipment and Use

Background and Stimulator

It is important to have a dedicated electrical circuitry for the ERG to prevent it from current surges from big equipment such as elevators.

The ideal conditions for an ERG should include a full-field dome (like that used for the visual field) of diffuse light stimulation (this dome is called a Ganzfeld dome).

The light source should be able to produce a full-field illuminance of 1.5 to 3 cd/m^2. In addition to which the stimulator (Ganzfeld) should be able to produce a steady and even background across the full-field. It must also be possible to adjust the illumination source relative to the background light (from the Ganzfeld) and separately from it.

The background illumination must have flexible adjustment but its color temperature must be constant over the range of intensity changes.

There must be an integrated light meter at the level of the eye to measure the amount of light that falls on the eye.

Electrodes

There are several types which need to be studied according to which one you will use. Essentially we need three electrodes for each measurement. **An active electrode, a reference electrode and a ground electrode**. The reference electrode is sometimes within the assembly containing the active electrode (such as in a contact lens). Such electrodes are ***bipolar electrodes***. The ground electrode could be a skin electrode attached to the orbital rim or forehead. Earclip or cuff electrodes may even be used.

Four of the electrode types (Figures 7.3 and 7.4) still in common use are discussed below:

Jet Electrode This is a small (12mm) contact lens electrode with prongs sticking out of it to prevent lid closure during measurement.

DTL Electrode This is made of silver-coated nylon fibres. If immersed in saline, it can be draped over the cornea. It is usually placed under the lower lid to improve recording stability.

Foil A simple electrode made of gold foil which requires anaesthetic. The recording stability is poor and with prolonged use, it becomes increasingly noisy. Should be used only for quick procedures where there is a risk of abrasion.

Skin These are simple conventional Ag/AgCl electrodes. They are usually used as reference and/or ground electrodes but can be used as actives as well. When used as active electrodes, their ERG amplitudes are much smaller than with other electrodes requiring a rigorous standardization with parametric measurements before application and averaging.

CLINICAL PROCEDURE

The ISCEV standard ERG procedure calls for the following five tests to be performed:

- Maximal Response in the dark adapted eye
- Rod response
- Cone response
- Response to flicker
- Oscillatory potential

The order in which the tests are performed is a matter of preference. Some clinicians prefer to perform the light-adapted responses first so that the retinal neurons start from a similar light-adapted state before dark-adapted responses. For dark-adapted responses, a minimum of 30 minutes of dark-adaptation is required. If for example either fluorescein angiography or fundus photography are performed first, then one hour of dark-adaptation is necessary.

Maximal dark-adapted response is generated by the Serial Block Face scanning electron microscopy (SBF) in the dark-adapted eye and is the response of rods and dark-adapted cones.

The rod response is generated under dim or no background light and the stimulus is a dim light lower than the SBF (the SBF is 1.5 to 3 cd/m^2 measured at the level of the eye).

The single flash cone response is recorded with a SBF on a background of 17 – 34 cd/m^2.

The flicker response is the cone response with the light source flickering at 30Hz. At the beginning the response to flicker varies so it must be averaged.

Oscillatory potentials are performed on the dark-adapted eye using the SBF and a high frequency filter which permits components between 100 and 1000Hz. It is usual to ignore the first oscillatory response and average the subsequent three or four measurements.

To perform the procedure

- **Explain test**
- **Dilate eye**
- **Patch dilated eye to start dark adaptation**
- **Remove patch under red light (Ganzfeld background light off)**
- **Check dilation and anaesthetize eye.**
- **Prepare electrodes: disinfect electrodes and rinse with artificial tears. If electrode is a contact lens, put two drops of methylcellulose into the concave part of the lens.**
- **Place reference electrode on forehead and ground electrode on ipsilateral earlobe.**
- **Insert electrode, connect it to machine and check its impedance (resistance).**

The clinical procedure of measuring ocular electrophysiology will be explained in details in Clinical Examination of the Visual System course (Opto 431)

LECTURE 8

THE VISUAL EVOKED POTENTIAL (VEP)

The VEP is a close relative of the ERG and it is the objective measurement of visual function in response to visual stimulation. Generally, electrical stimulation of the brain in response to sensory stimulation is referred to as an ***evoked potential*** or an ***event-related potential***.

The VEP can be recorded simultaneously with the ERG to provide a differential diagnosis. The VEP is a response that arises primarily from central retinal (macular) cones. The first reason the VEP arises primarily from the macula is because there is little or no convergence of the photoreceptors within the macula and thus though the macula represents about 5% of the retina, it represents 50% of the signals that reach the occipital cortex. The second reason is that the VEP electrode is positioned close to the inion and in this position it is closest to the macula signals which are sent to the back of the occipital cortex and thus closest to the skull. The third reason is that the VEP is measured under photopic conditions when the cones (which are stacked predominantly in the macula region) are more active.

So while the ERG measures ion changes on the retina in response to the activity of the retinal photoreceptors in general, the VEP measures a much smaller sub-signal which has managed to make its way to the occipital cortex. Therefore, there is a relationship between the ERG and the VEP although not a strong one.

With the VEP, the brain is treated as a ***black box*** with information being fed into it and its response analyzed with a view to attempt to understand how that response is generated.

Unlike the ERG, the clinical use of the VEP is vast which also means that analyzing the VEP response is vastly more complicated because of the myriad of factors that affect it. Some of the clinical uses of the VEP are itemized below:

- It provides an objective estimate of refractive error, visual acuity, accommodation, stereopsis and a prognostic assessment of amblyopia.
- It is valuable in the assessment of macular and optic nerve function.
- It greatly aids diagnosis in psychogenic problems and in demyelinating nerve diseases such as multiple sclerosis.
- It is valuable in assessing presurgical determination of postsurgical acuity in patients with media opacities.
- It provides objective visual field assessments.
- It is used in early detection of poisoning.
- It is used for the assessment of optic pathway misrouting in albinism.
- It is used in the establishment of brain death.

- It is used in the objective assessment of neurological development.

Theory

The mechanisms which underlie the VEP are vastly more complex than those that underlie the ERG as you might have guessed from just how versatile the VEP is. This complexity arises from two factors. The first one is the intrinsic brain rhythms which occur spontaneously in the cortex and are generated by the pacemaker generators of rhythmic activity in the thalamus. The second is that the effects of spatial and temporal summation come into importance here. There are about 100 billion neurons in the brain and thus each neuron has about 3000 to 5000 neighbors with which it is in direct contact.

Thus a neuron could fire or be inhibited by a stimulus which directly affects it or it could fire because it is connected to one or several other firing neurons (spatial summation). As if this were not messy enough, when the neuron fires and you try to measure the signal, you have to differentiate the signal from the intrinsic brain rhythms and the hundreds of thousands of other neurons which are firing in close proximity. This is like trying to whisper to a friend next to you in a class of 20 extremely noisy five-year olds – in short it is a daunting task!!

The problem can be managed in four steps:

- Make sure your signal is constant and fire it at regular intervals
- Assume the same response from your target neurons each time your signal is fired.
- Place your electrodes precisely (a placement error of 2cm invalidates your entire recording). The importance of electrode placement in VEPs cannot be overemphasized.
- Perform ***filtering*** and ***averaging*** to help remove the noise (from other firing neurons) and the intrinsic brain rhythms.

One important thing to note is that with VEPs much more than with ERGs, you need to isolate the circuitry into which your machine is plugged (Explain).

Figure 8.1 depicts a stimulus train (repetitive firing of a constant signal at regular intervals), noise, signal and the noise+signal (which is what you measure with the VEP).