The ELECTRORETINOGRAM - ERG

eng. ***Hassanain Ali Lefta Mossa*** * , HasAli76@yahoo.com

The ***Electroretinogram*** ERG is an electrical response of the eye. The global or full-field ERG is a test used worldwide to assess the status of the retina in eye diseases in human patients and in laboratory animals used as models of retinal disease.

Researchers and clinicians who are interested in objective assessment of retinal function need to become familiar with the ERG waves. With proper analysis, the functional integrity of different retinal structures can be dissected out and we are able to understand information processing mechanisms and/or the sites of retinal disorders. The ERG originates from extracellular currents that are generated in response to a light stimulus.

1. Historical view.

As early as 1865 Holmgren found that a light stimulus could cause a change in the electrical potential of the amphibian eye. Shortly afterwards, similar findings were reported by Dewar from Scotland. He showed that light illumination through the pupil, which had previously been covered, caused a slight movement of a galvanometer, suggestive of a positive electrical change in the cornea relative to the back of the eye. This light-induced electrical activity of the eye was called the electroretinogram. Nowadays the electroretinogram response is commonly abbreviated to the ERG.

Gotch (1903) was the first to report that the response of the eye to a light flash consisted of two waves; first the cornea became negative and then a positive wave of larger amplitude appeared. Later Einthoven and Jolly (1908) separated the ERG response into three waves. The first wave to appear immediately after turning on a light stimulus was negative on the cornea. It was followed by a positive wave and a final slower wave that was also positive. Einthoven and Jolly (1908) suggested that the light stimulus triggered a chain of reactions leading to the formation of products A, B and C, and that every electrical wave indicated a change in a 'relevant' product. These authors' work was the foundation for the form of analysis of the ERG used to the present day. The waves are called a-, b- and c-waves. An additional corneal-positive wave that is more rarely recorded at the termination of the light flash is called the d-wave.

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* B.Sc. in Biomedical Engineering from Baghdad University, Iraq 2000.
M.Sc. in Medical Engineering from Al-Nahrain University, Iraq 2004.

Figure 1 shows ERG responses from different species. These responses are to bright light stimuli applied in the dark-adapted state. The ERG of the turtle eye (Fig. 1A) as elicited by a long (900 ms) step of light, shows an a-wave and b-wave complex separated from the d-wave which is generated at stimulus offset. A bright light stimulus of 40 sec duration is used to record the ERG of the bullfrog in figure 1B. The a-wave and b-wave are followed by the slow corneal-positive c-wave. After termination of the stimulus, a d-wave develops. The ERG responses of the rabbit (Fig. 1C) and the human (Fig. 1D) are elicited by fast bright flashes (50 or 100ms in duration) and therefore, only the a-wave and b-wave are seen. In the human response (Fig. 1D), fast oscillations can also be seen on the ascending limb of the b-wave. These ERGs in the different species clearly differ in amplitude and pattern. Some of this variability is due to species differences, particularly, the relative densities of rods and cones, while technical factors such as duration and intensity of photostimulation and method of recording also affect the waveform. Nevertheless, ERG responses of turtle, bullfrog, rabbit and human (Fig. 1), in addition to those recorded from other vertebrate species, are characterized by the basic features of a negative a-wave followed by a positive b-wave.

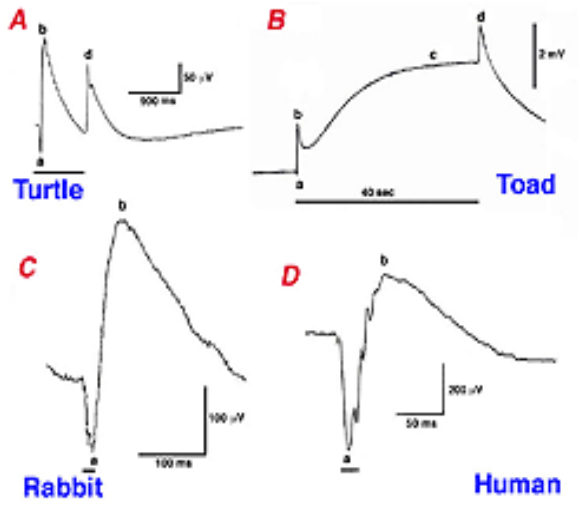

Fig. 1. (A) ERG response of turtle Pseudemys scripta elegans elicited by a 900ms light stimulus in order to separate the a-wave and b-wave from the d-wave. (B) The ERG of the bullfrog elicited by a long (40sec) light stimulus in order to show the c-wave in addition to the a-, b- and d-waves (Oakley, 1977). (C) The ERG response of a rabbit to a flash (20_s) flash of white light. (D) The ERG response from a human as typically recorded in the clinic. Note the fast oscillations on the ascending limb of the b-wave. Calibration bars are denoted separately for each ERG response.

In 1911, Piper published his analysis of the ERG. He divided the ERG into three components: I, II and III. Unlike Einthoven and Jolly who suggested that the waves reflected transient chemical processes, Piper suggested that all the ERG components lasted for the duration of the light stimulus. According to Piper, the first two waves, I and II, were characterized by different latencies and temporal properties so that the interaction between them resulted in the formation of the a- and b-waves.

Wave III was equivalent to the c-wave. Although Piper's analysis was very speculative and based only on a few facts, this interpretation together with that of Einthoven and Jolly's has set the basis for the idea that the ERG is the result of a few components. In 1933, Ragnar Granit published a more detailed study of the components of the cat ERG as illustrated in figure 2. He recorded the ERG from the anesthetized cat using corneal electrodes and observed the gradual removal of the different components as the level of anesthesia was deepened. Granit termed the different components in sequence of their disappearance: P-I, P-II and P-III. The P-I component is a slow cornea-positive wave. P-II is also a corneal-positive wave that rises relatively fast to peak amplitude and then recovers to an intermediate potential while the light stimulus is still on. The last component, P-III, which was the most resistant to the level of anesthesia, is a cornea-negative wave that develops faster than the other two and remains as a negative potential for as long as the light stimulus is on. The component analysis of Granit has been modified slightly over the years but remains the basis for our understanding of the ERG. For his work on the ERG, Ragnar Granit won the Nobel Prize for Physiology and Medicine in 1967.

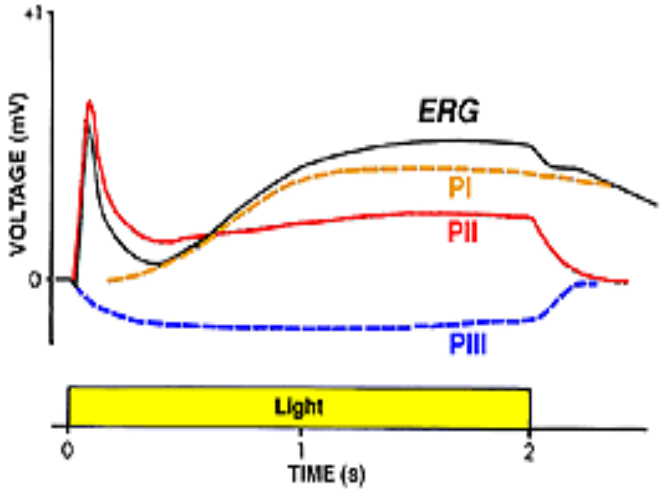

Fig. 2. The ERG of a cat in response to a 2 sec light stimulus. The components, P-I, P-II and P-III, have been isolated by deepening the state of anesthesia.

2. Electrical Basis of ERG Recordings.

ERG responses are recorded with an active extracellular electrode positioned either on the cornea, in the vitreous or at different levels inside the retina. Extracellular recording of electrical activity of living tissue is rendered possible when electrical currents spread along an extracellular matrix with electrical resistance. An example of extracellular electrical current in the vertebrate retina is the 'dark' current spreading from the inner segments to the outer segments of the photoreceptors.

In the vertebrate retina, the photoreceptors are arranged in parallel and therefore, their 'dark' currents are in parallel and sum up, giving rise to a strong radial extracellular current flowing away from the inner nuclear layer towards the pigment epithelium. Similarly, extracellular currents from all retinal cell types will sum up only if they are directed radially. In contrast, lateral currents will cancel each other since the retinal lateral arrangement is completely symmetrical. Therefore, when a homogenous light stimulation is directed at the whole retina, only radial extracellular currents are formed. We assume that a light stimulus elicits extracellular electrical currents that flow from sources to sinks. These currents will flow through different pathways including local and remote ones. We can divide these currents into two principal pathways, the local one (A) and the remote one (B) as shown schematically in figure 3. The current in pathway A, flows through a local route remaining entirely within the retina, while the current flowing through pathway B leaves the retina through the vitreous and anterior ocular tissue and returns to the retina through the sclera, the choroid and the pigment epithelium layer. The light-induced current flowing through pathway B can be recorded in a noninvasive manner, with extra-ocular electrodes, as illustrated in figure 3.

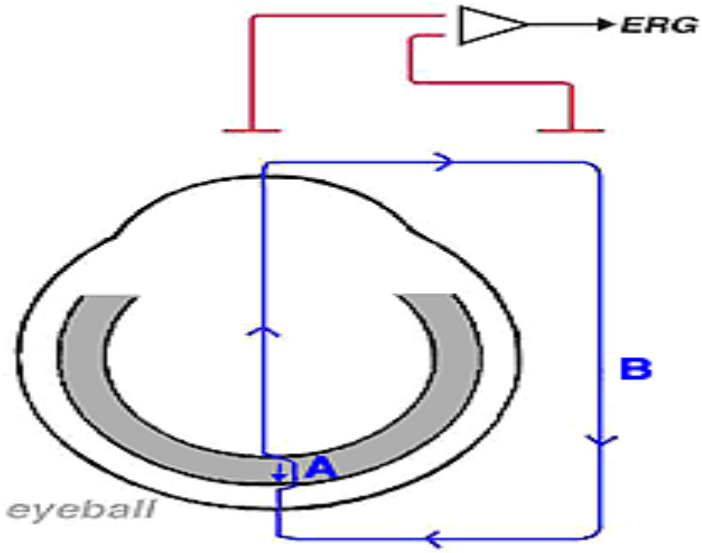

Fig. 3. A schematic representation of the extracellular currents that are formed following light stimulation. Pathway A represents local currents within the retina, while pathway B shows the currents leaving the retina through the vitreous and the cornea and returning to the retina through the choroid and the pigment epithelium. ERG recording in human is done along the B path.

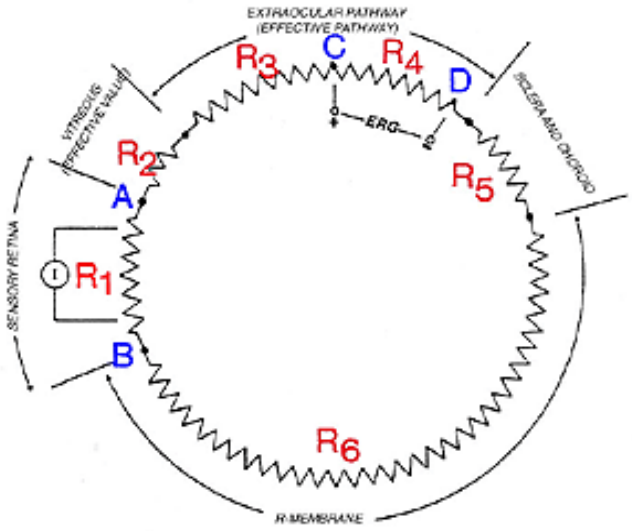

Fig. 4. An electrical scheme of the resistances through which currents IA and IB (figure 3a) flow when the retina is stimulated with light. The current source I, represents the electrical current that is generated in the retina in response to a light stimulus. Pathway A is the local intra-retinal route of current flow and pathway B is the remote route going from the retina and through the vitreous, lens, cornea, extra-ocular tissues and back to the retina through the sclera, choroid and pigment epithelium.

According to Ohm's law in electricity, when an electrical current flows through a resistor, a gradient of electrical potential is formed that equals the product of multiplying the magnitude of the current by that of the resistance. By applying this law, we can derive the relationships between the currents, IA and IB (Fig. 3), the resistances of the ocular tissues and the measurements of potential differences. Figure 4 shows an equivalent electrical circuit of the eye. A light stimulus elicits an extracellular current (source I) that divide into two pathways; one flowing through the retina (local pathway, IA in Fig. 3) and the other through extra-retinal and -ocular tissues (remote pathway, IB in Fig. 3). Each tissue (e.g. retina, vitreous, sclera, choroid, pigment epithelium) is represented in figure 4 by an electrical resistor. According to Ohms' law, the potential difference between two points is independent of the pathway through which the current is flowing. Therefore, the voltage difference between points A and B can be calculated for the local or remote pathways.

$$I_A R_1 = I_B (R_2 + R_3 + R_4 + R_5 + R_6) \quad \text{..... (Equation 1)}$$

Since the sum of the resistances on the right side of the equation $(R_2 + R_3 + R_4 + R_5 + R_6)$ is larger than R_1, the current I_A in the local pathway must be greater than current I_B.

When using two electrodes to record light-induced electrical activity of the retina, the largest light-induced potential change will be monitored if the measurement is done between points A and B, which are on the two sides of the cells producing the electrical response.

However, when the electroretinogram is recorded from humans or from laboratory animals during chronic experiment, the electrodes cannot be inserted into the retina. The alternative is to record from extraocular sites by placing both active and reference electrodes outside the eye. If these electrodes are placed at the loci designated in figure 4 as C and D, the voltage gradient between them is given by

$$V_C - V_D = I_B * R_4 \quad \text{..... (Equation 2)}$$

or

$$V_C - V_D = I_A R_1 - I_B(R_2 + R_3 + R_5 + R_6) \quad \text{..... (Equation 3)}$$

This is the ERG: the light-induced potential change that is related to light-induced electrical activity within the retina. In general, when retinal function deteriorates, the light-induced electrical activity in the retina reduces. The currents I_A and I_B will be smaller and the ERG will be smaller too thus, indicating retinal pathology

However, we have to remember that the magnitude of the different resistances and more so, the relationships between them can also affect the ERG that is measured with extra-ocular electrodes. The division of the current originating from the light-induced retinal activity into the local and remote pathways depends upon the relative resistances of the two pathways. From Equation (1), we can derive the following relationship

$$I_A/I_B = (R_2 + R_3 + R_4 + R_5 + R_6)/ R_1 \quad \text{..... (Equation 4)}$$

Any change in one of the resistances will cause a change in the magnitude of the current in the extraocular pathway (I_B) and the ERG ($V_C - V_D$) can change irrespective of retinal function. Therefore, knowledge of the different resistances and understanding the factors that affect them is needed for proper use of the ERG for clinical and/or research purposes.

The pigment epithelium layer (R-membrane) offers the highest resistance to electrical current along the ocular tissues as denoted by a large resistor R6 in figure 3b. Therefore, any change in the magnitude of this resistor will affect the distribution of currents between the retinal pathway (I_A) and the remote pathway (I_B). This change will be reflected in the ERG responses that are measured with extra-ocular electrodes. Such changes in the distribution of resistances may account for species differences in the magnitude of the ERG responses and for intra-subject differences within a given species. The importance of the resistances of the ocular tissues has been recognized by Arden and Brown (1965). They replaced the vitreous humor of cats with heavy oil in order to abolish current flow from the retina to distant sites and thereby ensured large potential recordings of local ERG from the retinal surface. In the clinical environment, it is well documented that the ERG can be reduced significantly in patients with giant retinal tears who have undergone vitrectomy surgery and injection of silicon oil into the vitreous.

Since silicon oil does not conduct electric currents, the resistance of the vitreous increases by several folds causing the current I_B to be so reduced that the ERG becomes very small in amplitude.

3. Origin of the Major ERG Waves.

Granit (1933) divided the cat's ERG into three components; P-I, P-II and P-III (Fig. 5). From his analysis, we know that the negative a-wave is the leading edge of the negative P-III component; the positive b-wave reflects the summation of P-II and P-III while the slow c-wave is the summation of P-I and P-III. However, the cellular origin of the different components needs to be understood. Basically, two types of approaches, physiological and pharmacological, have been used to dissect out these cellular origins. The physiological experiments are based on the assumption that the generators of specific ERG components are located in specific retinal layers and therefore, when these are passed by the intra-retinal microelectrode, the polarity of the specific ERG waves will reverse. These current source-density analyses have indeed revealed the anatomical location within the retina of the different ERG components. The pharmacological approaches to ERG analyses are based on retinal physiology and biophysics. In these experiments, specific agonists and antagonists of cellular mechanisms are applied and their effects on the ERG then analyzed.

The basic method of recording the electrical response known as the global or full-field ERG is by stimulating the eye with a bright light source such as a flash produced by a strobe lamp. The intense flash of light elicits a biphasic waveform recordable at the cornea similar to that illustrated below (Fig 5). The two components that are most often measured are the a- and b-waves. The a-wave is the first large negative component, followed by the b-wave which is corneal positive and usually larger in amplitude.

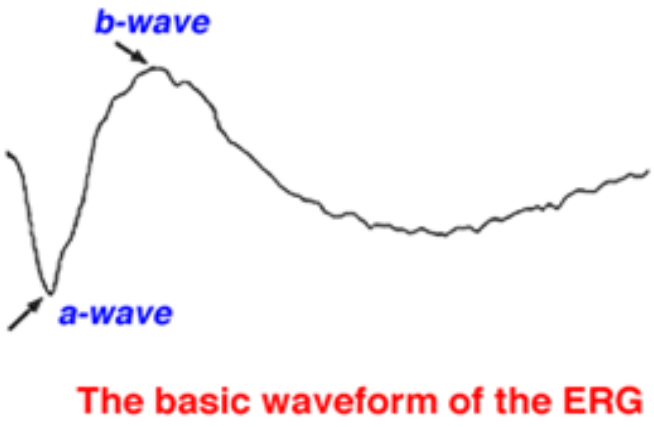

Fig.5. The biphasic waveform of the typical normal patient.

Two principal measures of the ERG waveform are taken: 1) The amplitude (a) from the baseline to the negative trough of the a-wave, and the amplitude of the b-wave measured from the trough of the a-wave to the following peak of the b-wave; and 2) the time (t) from flash onset to the trough of the a-wave and the time (t) from flash onset to the peak of the b-wave (Fig. 6). These times, reflecting peak latency, are referred to as "implicit times" in the jargon of electroretinography.

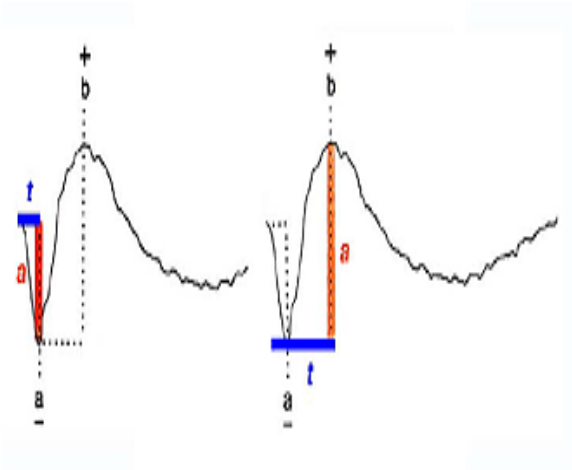

Fig.6. Amplitude and implicit time measurements of the ERG waveform.

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. Two other waveforms that are sometimes recorded in the clinic are the c-wave originating in the pigment epithelium and the d-wave indicating activity of the OFF bipolar cells (see Figure 7).

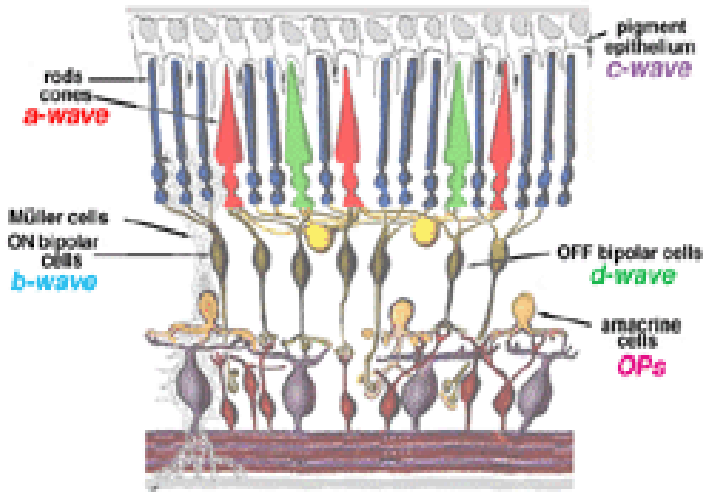

Fig.7. Retinal origins of the major components of the ERG.

The ERG of a normal full-term infant looks similar to a mature ERG. 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. Below are two figures illustrating how the b-wave attenuates in amplitude with age and slows in its implicit time (Fig. 8). There is considerable variation among individuals but the linear regression line in each figure indicates the trend of aging affects on the ERG.

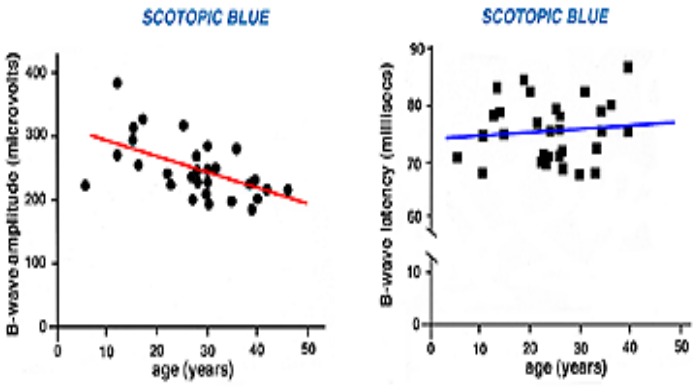

Fig.8. Scatter plot of b-wave samplitudes and latencies with age with regression lines to show the aging effects.

3. ERG Recording Electrodes.

The ERG can be recorded several ways. The pupil is usually dilated. There are a number of corneal ERG electrodes that are in common use. Some are speculum structures (Fig. 9) that hold the eye open and have a contact lens with a wire ring that "floats" on the cornea supported by a small spring. Some versions use carbon, wire or gold foil to record electrical activity. There are also cotton wick electrodes.

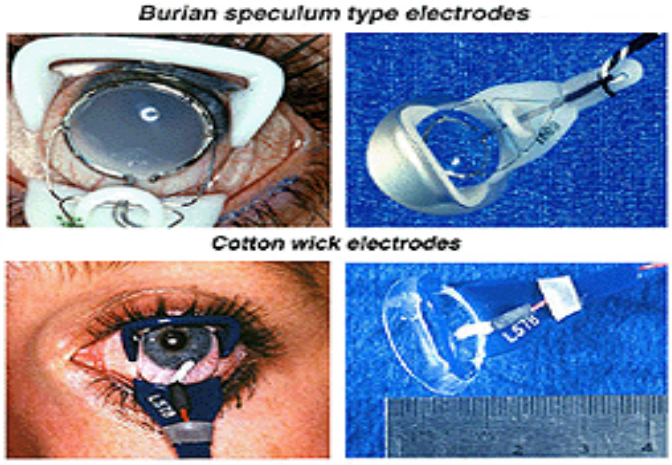

Fig.9. Speculum or Burian type electrodes used to record the human ERG.

There are yet other simpler ERG recording devices (Fig. 10) 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.

Fig. 10. Other simple types of electrode used to record the human ERG.

Each of these electrodes record large voltage responses directly from the cornea and each have advantages and disadvantages. Sizes are available down to a size that fits in the eye of most full-term babies. When the eye is too small for speculum recording electrodes we use the ERG Jet type most of the time. When the eye is very small such as in some microphthalmic eyes or cases of trauma to tissue surrounding the eye, we use a carbon wick or gold Mylar tape.

The ERG can also be recorded using skin electrodes placed just above and below the eye, or below the eye and next to the lateral canthus. Since skin electrodes are not in direct contact with the cornea there is significant attenuation in amplitude of the ERG, so a number of individual responses to flash stimulation must be averaged by computer. Pictured in figure 11 is a comparison of bright white flash ERGs recorded from the same person using three types of recording devices and an averaged ERG from skin electrodes.

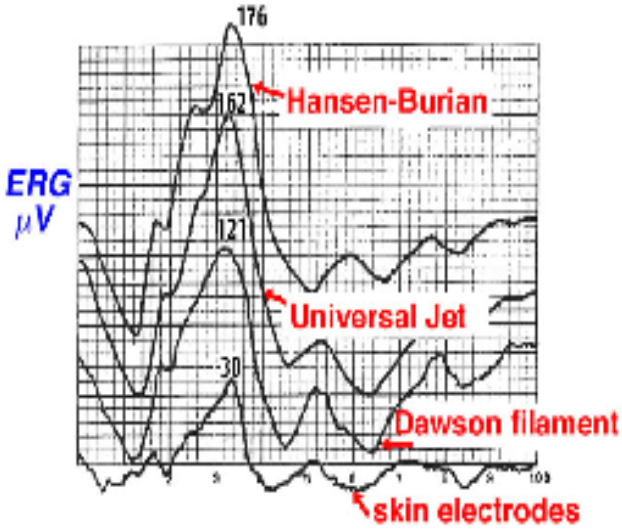

Fig. 11. Typical ERGs as recorded with different electrodes.

If electrodes are to be reused, they must be sterilized with a solution that neutralizes prion-transmitted diseases such as Creutzfeldt-Jakob disease (CJD). We use household bleach, e.g. Chlorox (active ingredient sodium hypochlorite), diluted to a 10% solution with distilled water. The electrodes need only be submerged in this solution for a minute. Do not leave electrodes in this solution more than a few minutes.

4. 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 (Fig. 12). The mobility of a strobe lamp or an array of LEDs is a necessity in some situations such as at the hospital bedside or in the operating room.

Fig. 12. Portable strobe light source.

For patients over 5 years of age most laboratories use a Ganzfeld (globe) with a chin rest and fixation points (Fig. 13). 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. Clinical decisions can be made from ERGs generated by either methodology.

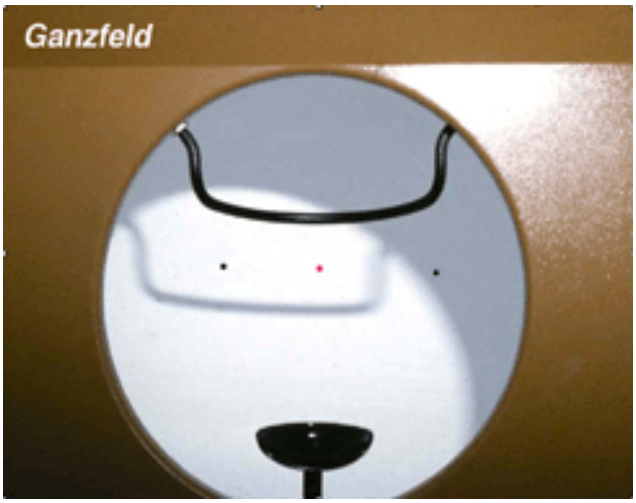

Fig. 13. The Ganzfeld stimulation globe.

Testing infants for ERGs

Infants up to about 2 years of age can usually be tested without sedation by the parent holding them bundled in a blanket. It is difficult to get a child less than 5 years of age to allow a contact lens or speculum recording electrode in their eye, so skin or scleral electrodes can be used, with their limitations. Alternatively, the child is sedated or anesthetized. Many clinics use chloral hydrate or the three-in-one "cardiac cocktail" to sedate pediatric patients. Chloral hydrate has several limitations including that dose restrictions limit the use to patients weighing less than about 15 kg. Both of these sedatives have little effect on the ERG. ERG testing is also sometimes performed as part of a more extensive exam under anesthesia (EUA). Few laboratories have Ganzfeld stimulators that can be tilted and placed over the face of a sedated patient and it is difficult to use such equipment in the operating room. Thus flash stimuli with sedated patients are usually delivered with a strobe lamp. In an ERG laboratory the sedated patient can be dark adapted and a more or less normal series of stimuli can be used although the length of effect of the sedative usually necessitates that the method be abbreviated to just 3 or 4 stimuli.

Dark adaptation even for a few minutes followed by single flashes of white or blue will assess scotopic ERG function. Photopic single flashes and 30 Hz flicker can be used to evaluate cone function.

It is usually not possible to completely darken the O.R. so abbreviated testing is accomplished under mesopic and photopic light conditions. Anesthesia affects the ERG varying with type and depth of anesthesia. Some anesthetics can attenuate b-wave amplitude as much as 50%. Light levels of anesthesia have little affected and most anesthetics do not usually affect a-waves or implicit times.

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, and brightness and color 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. By manipulating adaptation level and background illumination, flash intensity, color of the flash and rate of stimulation, rod and cone activity can be significantly isolated.

Using color stimuli

Peak wavelength sensitivity for rods is around 510 nm and the peak sensitivity of cones as a group is about 560 nm (Tennis ball yellow) (Fig. 14). By using color filters such as the Kodak Blue and Red Wratten series shown in figure 15, you can essentially isolate rod and cone ERGs using dim flash stimuli into photopic (cone)and scotopic (rod) signals as illustrated in Figure 10. Dim red analyses both rod and cone function by identifying b_x and b-wave. Rods are about three log units more sensitive than cones. However cones recover faster than rods.

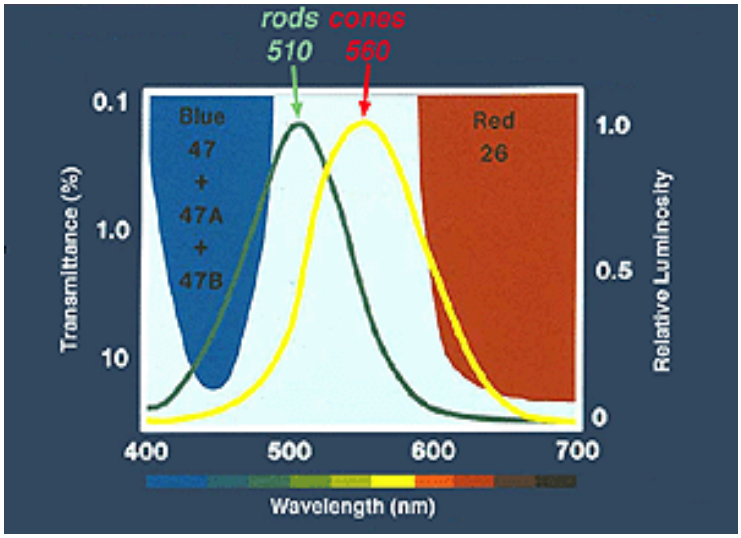

Fig. 14. Filter conditions used to isolate rod and cone components of the ERG using dim scotopic flashes.

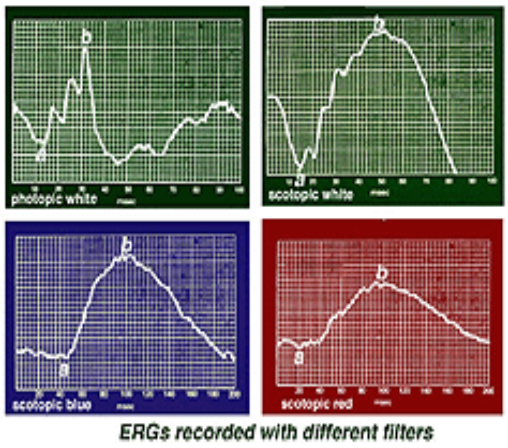

Fig. 15. Typical testing parameters used in our ERG recording set up.

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 (Fig. 16).

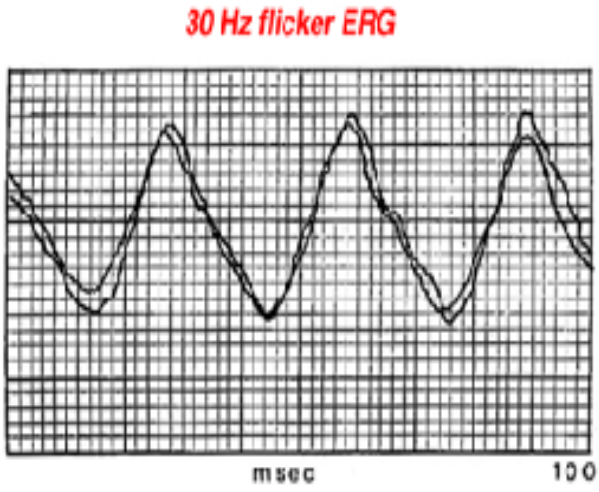

Fig. 16. Typical 30 Hz flicker ERG recorded in the clinic.