NORMALIZATION OF THE ERG: AGE GROUP 26 - 35

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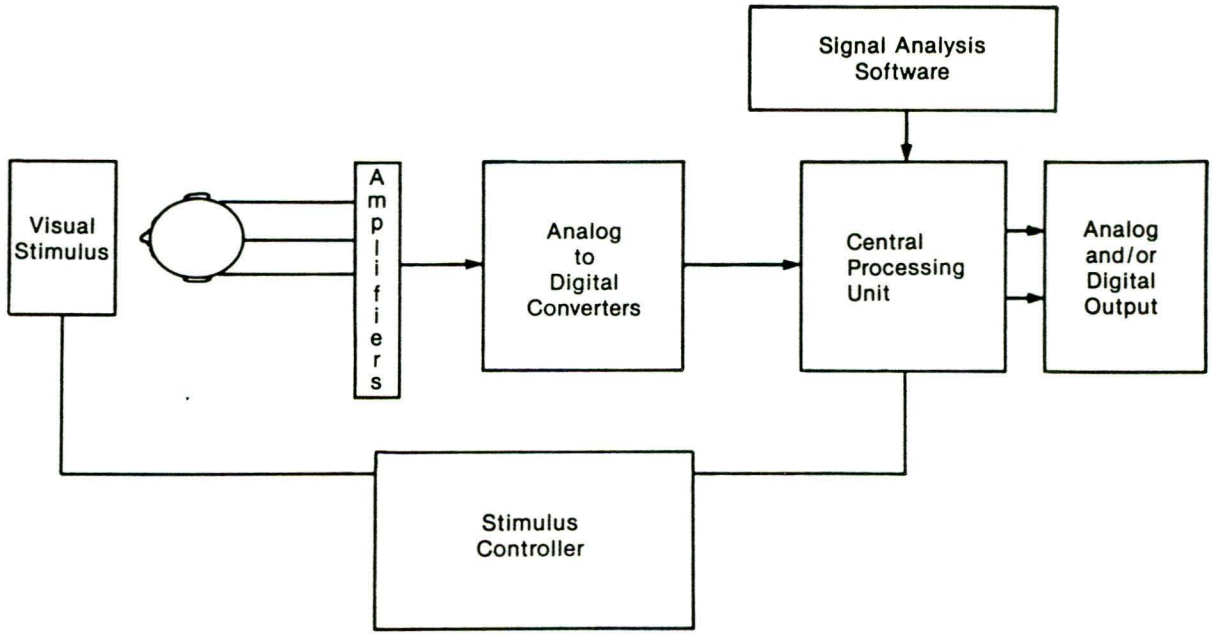
The science of electrophysiology is based upon the existence of a 6uV standing potential between the cornea and the back of the eye. This impulse was first discovered by DeBois-Reymond in 1849. The electrical impulse was later termed an "action potential." Holmgren, a student of DeBois, was later to reveal that the action potential could be illicited using a light stimulus. By 1873 the source of the impulse was identified as the retina. This was accomplished through the work of Holmgren as well as being independently discovered in the laboratory of DeWar and McKendrick. DeWar recorded the first electroretiniogram (ERG) in man in 1977.

Several advances were made in the following years: The ERG response was found to originate in the receptor layers, not the gang;ion cells. The amplitude of the response was reported to increase with increasing light stimulus intensity. Also, methods were developed and used to distinguish rod from cone responses.

Increasingly sophisticated technology, including the use of fast sensitive galvanometers and eventual amplification instruments made the use of fast recording devices possible. The introduction of the contact lens electrode (concurrently developed by Gosta Harpe in 1944 and Lauren Riggs, an American psychologist) and a better understanding of the major components of the ERG, made clinical ERG application possible.

TYPICAL ERG APPARATUS



Several variations of the ERG can be diagnostically useful to locate abnormalities in various retinal structures. These include the pattern, focal, and dc-ERG. The early receptor and oscillatory potentials will be discussed later. The electrooculogram measures slow light induced changes in the standing potential. These are recorded as the Arden ratio (Light rise/dark trough) which is about 180 in the normal eye. The visual evoked potential is generated in the occipital cortex in response to a pattern stimulus. This wave represents the end stages of visual processing. The potential may be used alone or in conjuntion with the ERG to localize abnormalities to a dysfunction along the optic nerve pathway.

The major ERG wave components have been named by letter by Eintoven and Jolly while Granit named processes for their sequence of disappearance under esther anesthesia.

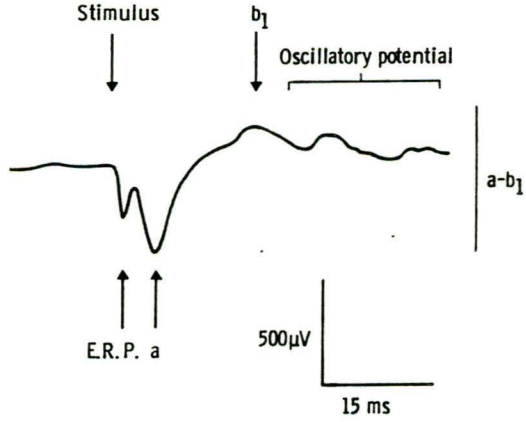
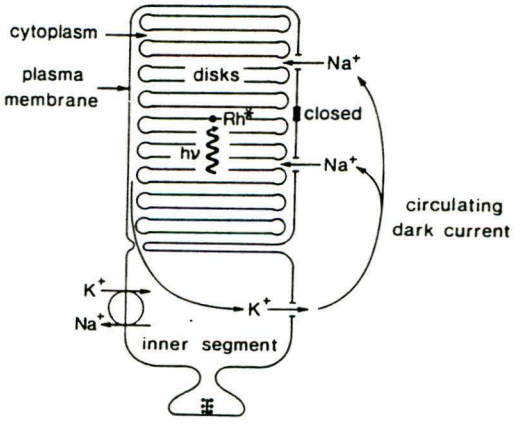


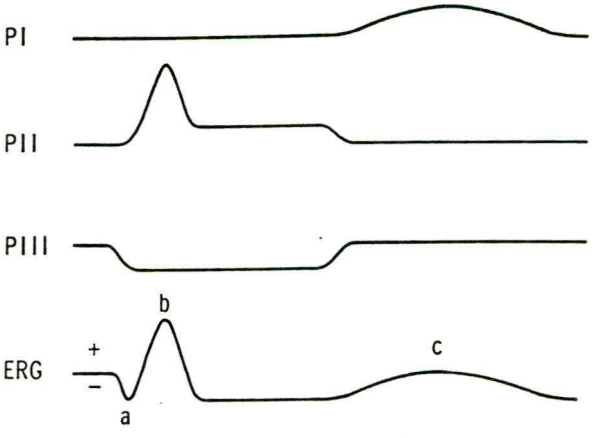
DIAGRAM OF ERG

The ERG is a mass response. Many cells add their signal to each portion of the wave. The signal emmitted by the photoreceptor cells arises from a complex series of chemical events. Rhodopsin, a photopigment found in the outer segments of rods and cones, is "bleached" in the presence of light. The breakdown of Rhodopsin into opsin (protein) and retinal (aldehyde of vitamin A) is facilitated by the enzymes, retinal reductase, alcohol dehydrogenase and retinal isomerase. The regeneration of rhodopsin occurs normally in the dark for 35-40 minutes. Vitamin A from the retinal pigmented epithelium is an essential element in this metabolic process. After the described photochemical events in the rods and cones, hyperpolarization of the cellular membranes creates an action potential which is transmitted along the nerve fiber layer and through the visual pathway. This hyperpolarization, producing an action potential via CLOSED Na/K channels is unique to the photoreceptors, thus in

the dark there is a Na+ dark current. Because different cells, each in their own way, contribute to the ultimate complete ERG wave, patterns may be difficult to interpret. Different methods of analysis, such as Fourier, kernal and signal analysis, have been applied to aid in the understanding of variations in the waveform.



ION MOVEMENTS ACROSS THE SURFACE MEMBRANE OF A ROD



ADDITIVE NATURE OF THE ERG WAVEFORM

The following is a list of ERG components whose origins and importance were shown through animal research:

A WAVE (P III): appears as a negative going wave which occurs prior to the B wave. The A wave can be divided into three sub-components: Distal, slow and proximal. The distal portion is the major contributor to the A wave. It is directly generated by responses of the rods and cones. Slow P III is thought to be generated by Muller cells and also contributes to the C wave. The proximal III is a rapid response preceding the B wave. Its cellular origin is unknown.

B WAVE (P II): This wave is thought to be generated bu Muller cells and is composed of the greater positve P II added to the negative P III. Studies found that the wave originated in the retina by current flowing through a radially oriented cell that acted as a dipole. Eventually identified (using current source density analysis or CSD, and intracellular recording) to be the Muller cell.

Important diagnostic characteristics of the B wave iclude the evoked voltage response to a light stimulus: amplitude, latency, time corse and response to flickering stimuli.

The dc component of the B wave is the only other positive wave of the ERG and has only been studied in mammals. It is a low amplitude response that emerges in dark adapted ERG at lower intensities then the B or C wave and continues until stimulus offset.

The oscillatory potentials of the B wave (or OPs) are 7-10 wavelets superimposed on the B wave. They have been found to originate in the inner plexiform layer and are probably responses of depolarizing amacrine cells.

The cone B wave (B 1) is a small response with a short latency, while the rod B wave (B 2) is a large reponse with a longer latency. These two waves have the same origin and look similar when recorded seperately under scotpic and photopic conditions.

Finally, the X wave is a sharp, small positive going photopic peak preceding the photopic B wave.

C WAVE (P I): This response originates in the retinal pigment epithelium and has 2 sub-components. A corneal negative response also referred to as slow P III, added to a larger corneal positive response generated in the RPE.
Physiologically it is a response to decreased extracellular K+ after a light stimulus. The positive RPE C wave component is generated by the hyperpolarization of the RPE apical membrane.

The C wave encompasses a fast oscillation trough and a light peak. These 2 characteristics are utilized in recording a slow oscillation of the EOG.

D WAVE: A positive going initial deflection in the photopic ERG at light offset. The response is very small in mixed retinas that have more rods than cones and is questionable in mammals.

E WAVE: A delayed off field potential which may be present in mammals but has only occasionally been recorded. It has a long latency after light offset (2-60) and may represent a scotopic D wave.

PNR: Proximal negative response indicative of proximal retinal activity. A sharp negative-going , trancient response at both onset and offset of a small well-centered light spot. May be associated with amacrine cells.

The clinically recorded ERG consists of only two major components. The negative A wave and the positive B wave. Taking advantage of the inability of rods to follow a fast flickering light can be used to isolate cone responses. A negative waveform (a well formed negative A wave with a small or absent B wave) can be seen in many diseases that effect both the scotopic and photopic ERG. Comparing results of the ERG, VEP and EOG can be helpful in localizing the dysfunctional system depending on the condition. Supporting clinical information on the disorder in question is essential to the final diagnosis, i.e.: fundus examination, visual fields, refractive state and family history. Flourescein Angiography has been used as a valuable adjunct to clinical evaluation.

Disorders With "Negative" ERG Waveforms*

	Scotopic ERG	
Disorder	a-Wave	b-Wave
Stationary defects		
Autosomal recessive CSNB†	N‡	↓↓↓
X-linked recessive complete CSNB	N	↓↓↓
X-linked recessive incomplete CSNB (Miyake)	N	↓↓
Åland disease (Forsius-Eriksson ocular albinism)	N	↓↓
Oguchi's disease	N	±↓↓
Retinal dystrophies		
Early or transitional forms of RP	±↓	↓↓
Infantile Refsum's disease	↓↓	↓↓↓
Goldmann-Favre vitreoretinopathy	↓↓	↓↓↓
X-linked recessive retinoschisis	N–↓↓	±↓–↓↓↓
Congenital blindness		
Leber's congenital amaurosis	↓↓	↓↓↓
Vascular disorders		
Ischemic central vein occlusion	±↓	±↓↓
Central retinal artery occlusion	±↓	±↓↓
Retinal Toxicity		
Quinine	±↓	↓↓
Vincristine	N	↓↓↓
Paraneoplastic melanoma	±↓	↓↓↓
Optic Atrophy	N	±↓↓
Degenerative myopia	±↓	±↓↓

DISORDERS WITH NEGATIVE WAVEFORMS

The skill required for ERG diagnosis is the ability to identify an abnormal response , versus a normal one. One must be able to compare a patient against a group of normal subjects of about the same age that have been tested under similar conditions.

Standards have been developed in regard to aquairing normal ranges for each individual laboratory. The International Society for Clinical Electrophysiology of Vision (ISCEV) have reviewed and updated these standards including instrumentation, recording procedures and measurement in clinical electroretinography. The most recent standard was set in 1987 and includes guidelines for Basic Technology, Clinical Protocol and description of the 5 standard ERG responses.

The following testing methods and results have been accomplished in order to comply with the ISCEV requirement that each laboratory establish normal values for its individual equipment and patient population.

METHOD AND MATERIALS:

Materials:

1) Venous Electrodiagnostic system:
   - venous model 1020
   - AST computer keyboard
   - AST premium 286 disc drive
   - princeton ultra 14 computer display screen
   - mitsubishi video display screen
   - panasonic KX p1180 multi-mode printer
   - grass RPS 107 regulated power supply
   - grass {S 22 photostimulator
   - model 7313 oscilloscope
   - Ganzfeld apparatus including ERG contact lens/electrode
   - computer interfacing box

2) Burian-Allen Bimodal contact lens electrode
3) Ground electrode
4) EEG conductive paste
5) alcohol pads
6) tape
7) sterile saline
8) proparacaine
9) celluvisc (carboxymethylcellulose 1.0%)
10) cellufresh (carboxymethylcellulose 0.5%)
11) eyepatch
12) red light lamp
13) dark adapting room

Method:

Ten individuals with no known retinal pathologies were selected for ERG analysis. Prior to testing, each subject waited 10 minutes in a dark room. This dark adaptation was also ensured by very dim ambient testing room illumination. The only illumination was a 40-Watt red lamp and the computer screen. The long wavelength red light is the visible light which produces the least rod activity. The patient's corneas and adnexas were anesthetized with 0.5% proparacaine. The blink response was reduced by also anesthetizing the non-tested eye which was subsequently patched. EEG conductive paste was put on the ground electrode and taped to the opposite cheek. Before inserting the scleral contact lens/electrode unit, the lens was filled with celluvisc to prevent a keratitis. Insertion into the palpebral fissure was done by instructing the patient to look up while the lower lid was depressed and the lens was placed in the lower

fornix. After guiding the patient's head into the ganzfeld apparatus, they were instructed to look straight ahead. Three types of flashes were presented to each eye being tested. The first light tested rod function at low light levels, and the third "flicker" test isolated a pure cone response. Cone and rod responses were tested separately by manipulating the light intensity and frequency of flash in the following manner:

OD1	intensity	1	5 hz	(5 flashes)
OD2	intensity	16	5 hz	(5 flashes)
OD3	intensity	16	30 hz	(30 sec. duration)

(sequence repeated for OS)

RESULTS:

Table 1

Int 1, 5 hz		Int 16, 5 hz		Int 16, 30 hz	
b-wave amplitude (uV)	implicit time (msec)	b-wave amplitude (uV)	implicit time (msec)	b-wave amp. (uV)	impl time (msec)
140.4	53	206.3	33	-18.0	15
155.9	52	232.6	44	-22.0	15
275.0	54	292.4	49	-27.9	19
254.4	56	280.2	47	-29.9	20
210.0	51	253.9	42	-18.2	18
227.5	52	268.0	44	-21.7	18
197.8	54	222.7	44	-21.4	18
161.1	54	193.5	52	-17.4	18
164.6	50	190.9	44	-15.0	17
132.8	50	192.5	44	-12.1	17
225.7	46	304.3	40	-21.4	17
223.1	47	301.4	41	-19.6	17
154.3	54	211.4	48	-15.5	18
198.6	61	253.9	58	-16.2	19
143.7	48	218.1	44	-12.2	18
165.5	49	270.0	42	-14.2	18
160.7	59	271.9	51	-14.2	20
226.1	52	210.2	43	-15.8	19
157.1	50	235.2	39	-22.3	17
157.3	45	233.6	39	-29.6	17

Table II
Mean, Standard deviation, and Normal ranges

	Int 1, 5 hz b-wave amp. (uV)	Int 1, 5 hz implicit time (msec)	Int 16, 5 hz b-wave amp. (uV)	Int 16, 5 hz implicit time (msec)	Int 16, 30 hz b-wave amp. (uV)	Int 16, 30 hz impl. time (msec)
$\bar{X}$	186.6	51.9	242.2	44.4	-19.2	17.8
1 std. dev.	41.1	1.4	36.9	5.4	5.3	1.3
2 std. dev.	82.2	2.8	73.8	10.8	10.6	2.6
Normal Ranges	132.8 - 275.0	45-61	190.9-304.3	33-58	12.1-29.9	15-20

Table III
Preveously established Norms for the ERG

	b-wave amplitude (uV)	implicit time (msec)
Rod only response	200 - 350	50 - 60
Rod/cone response	350 - 600	45 - 60
Cone only response	100 - 200	23 - 25

DISCUSSION:

ISCEV Standards:

1) Light diffusion - full-fiel ganzfeld dome stimulator
2) Electrodes - corneal contact exlectrode with conductive solution.
   - ground electrode attached at dif. site
3) Light sources - Stimulus 1.5 - 3 cd/ms (std flash), white light, 5 msec duration
4) Electronic recording equipment - amplifier with range of .3 - 300 hz, computer-aided display
5) Preparation of patient - pupil dilation, 20 min. dark adaptation, fixation straight ahead
6) Measurement of ERG - amplitude and implicit times
   a) a wave amplitude - baseline to trough
   b) b wave amplitude - a wave trough to b peak
   c) implicit time -flash onset time to wave peak
7) Reporting the ERG - representative waveform with std responses. Strength of stimulaiton with light adaptation.
8) Specific Responses
   a) rod response - first measurement, white flash below SF, 2 sec min. between flash
   b) maximal response - white SF with 5 min between stimuli both rod and cone response
   c) oscillatory potentials - white SF high-pass filter of 75 - 100 hz. 15 sec between flash 10 minute light adaptatiobn to background luminance 17 - 34 cd/ms (suppresses rods)
   d) flicker response - SF flicker with 17 - 34 cd/ms background. 30 hz

Comparing our experimental data with previously established normative data, it can be seen that the b-wave amplitudes from our study were consistently lower in all three trial periods. Trial I with low light intensity and low frequency had an average amplitude of about only 7% less than accepted values. With each consecutive trial, a precipitous drop can be seen in our b-wave amplitude values compared to the accepted normative values. The implicit time values that we found were very similar to the values in the previously established norms. Trial I, the rod only response, had a value within the established normative range. Our trial II average implicit time fell only 0.6 uV short of

the minimum accepted value. The flicker implicit time response that we averaged was over 5 uV short of the accepted minimum value. The lower b-wave amplitudes and slower implicit times that we found may be due to the fact that we dark adapted the 10 patients only 10 minutes versus the 20 minutes that were recommended in the ISCEV standards. Also, before the flicker cone response was obtained, our patients did not light adapt as the ISCEV standards recommended.

In conclusion, the ERG is a useful diagnostic tool for early detection of diseases which affect the retina and visual pathway. The ERG may best be used in conjuction with other clinical testing procedures such as flourescein angiography, ophthalmoscopic fundus exam, and other electrodiagnostic tests. New clinical uses of the ERG include monitoring gancyclovoir toxicity in AIDS patients and monitoring progression of glaucoma. The primary use of the ERG is in the detection of Retinitis Pigmentosa. Equipment used for ERG testing must be normalized so that test results may be compared against a normal age adjusted population. It can be seen how important it is to normalize each labs' equipment because of the variation we found from previously accepted normative data. Our equipment is now standardized for the ages 26 - 35 years old.

Table: Diseases which can be diagnosed and monitored with the ERG

Achromatopsia
Acute retinal necrosis syndrome
Amblyopia
Benign concentric annular macular dystrophy
Chloroquine retinopathy
Congenital stationary night blindness
Diabetic retinopathy
Dominant progressive foveal dystrophy
Familial foveal retinoschisis
Fenestrated sheen macular dystrophy
Fundus flavimaculitus
Glaucoma
Gyrate atrophy
Gancyclovoir toxicity (CMV treatment)
Ischemia
Lebers congenital amaurosis
Progressive rod/cone dystrohies
Retinal degeneration
Retinal detachment
Retinitis Pigmentosa
Rod monochromatism
Siderosis
Stargardts
Uveitis
Vitamin A deficiency
X-linked juvenile retinoschisis

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