Guidelines

Electroretinoraphy

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1. Physiological Essence of Electroretinography

Electoretinogram (ERG) is a graphic imaging of bioelectrical activity changes of various cell types in the retina in response to photic stimulation. Actually it is an evoked potential of retina. ERG is recorded using electrode placed on the cornea.

The full-field (global) ERG shows electrical activity of almost all retinal cells and depends on the number of normal cells. ERG is generated when K+ is spread from depolarized retinal neurons to intercellular space in response to photic stimulation. Müller cell membrane, permeable for K+, is depolarized locally. It is the main source of ERG generating together with bipolar and amacrine cells. Bioelectrical activity is spread from outer cell layer to retina surface and correlates with the number of neurons in distal and proximal parts of retina.

Different ERG types demonstrate the variety of retina structure.

Embryologically the retina is a part of the brain. It consists of ten layers. Retinal pigment epithelium (retinal pigment epithelium (RPE)) (Fig. 1, I) absorbs and transforms light rays. Layer of rods and cones (neuroepithelial layer) is a photosensitive one (Fig. 1, II). It is the first-order retinal neuron. The external limiting membrane (Fig. 1, III) supports the retina. The outer nuclear layer (Fig. 1, IV) consists of rod and cone granules. Outer plexiform layer (Fig. 1, V) consists of a dense network of the proximal endings of rod and cone cells, and also bipolar cells.

Inner nuclear layer (Fig. 1, VI) is made up of bipolar, horizontal and amacrine cells, and also nuclei of Müller's fibers.

The second-order retinal neuron is originated from the bipolar cell layer. It ends in the seventh or inner plexiform layer (Fig. 1, VII).

The eighth layer (ganglion cell layer) consists of retinal ganglion (multipolar) cells (Fig. 1, VIII). These cells function as third-order retinal neuron.

The cone and rod cells (up to 500) are connected with one bipolar cell. In turn, the bipolar cells interact with one ganglion cell. The exception is macular area where each cone is connected with one bipolar cell having its ganglion cell.

The nerve fibers (Fig. 1, IX) are derived from ganglion cells and make up the optic nerve. The external limiting membrane separates the retina from the vitreous humor (Fig. 1, X).

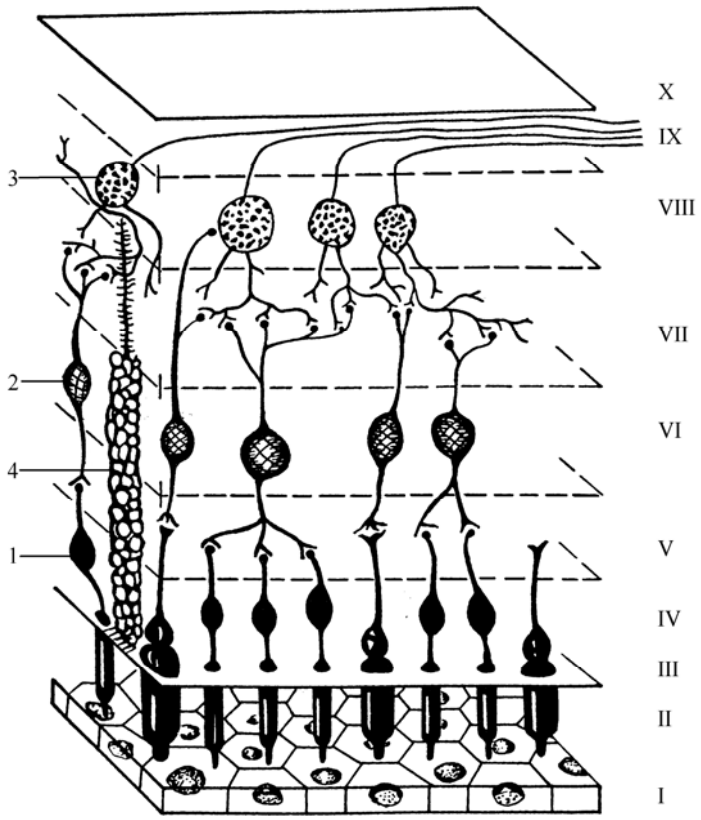

Fig. 1. Peripheral part of visual analyzer. Three retinal neurons: 1 — neuroepithelial layer, first-order neuron; 2 — bipolar cell, second-order neuron; 3 — multipolar cell, third-order neuron; 4 — framework, Müller's fibers.

The first two retinal neurons, photoreceptors and bipolar cells are involved when the global ERG is recorded. Although the horizontal, amacrine and Müllller's cell participating in trophic and supporting function are also required to record ERG. The third-order retinal neuron and ganglion cells are required to record pattern ERG (PERG).

In case the retina functions abnormally, it is indicated in ERG trace and the temporary and amplitude parameters of electroretinogram are changed. The causes impacting the normal retina functioning are choroidal and retinal circulation, state of pigment epithelium, cones and rods, bipolar, horizontal and amacrine cells, cone pigment and rhodopsin, metabolism, state of inner and outer layers involved in synaptic and chemical transmission of visual excitation, interaction between the neurons and glia cells, state of ion transport.

The electroretinogram is a valuable diagnostic tool.

ERG allows to detect the pathological process running in inner and outer layers of retina, in is central and peripheral parts. The technique makes it possible to study the state of rod and cone systems separately.

The important application of this technique is the diagnostics of initial changes in retina. The electroretinogram changes are characteristic for many retinal diseases and they allow to assess the injury level.

ERG is successfully used when the optic media is clouded or it is impossible to make ophthalmoscopy to assess the retina functions and predict the post-operational recovery of visual functions in patients suffering from cataract and hemoftalm.

The repeated ERG recording makes it possible to assess the dynamics of pathological process, the treatment efficiency, make prognosis for a disease.

ERG is also required to make differential diagnosis of retina and visual nerve. In this case it is done together with visual evoked potential (VEP) study.

Electroretinography as other neurophysiological techniques is nosologically non-specific. It is impossible to detect the cause of ERG change using the trace. However the right choice of ERG recording conditions allows to solve the wide spectrum of diagnostic tasks. It makes this study very important in ophthalmological practice.

ERG is the most informative objective technique to assess the functional activity of retina. The clinical value can be increased if you use it together with other ophthalmological techniques such as perimetry, ophthalmoscopy, ultrasound, Doppler, etc. Each technique contributes to the diagnostic process.

2. ERG Types and Applications

The waveform the recorded electroretinogram is different when using different types of stimulation. The reason is that the retina structures responding to stimulation depend on the stimulus type. The right choice of the stimulation allows enhancing the diagnostic capabilities of electroretonography.

The main ERG types (the classification according to the ISCEV standard is given in parentheses) are:

1. Rod ERG (dark-adapted 0.01 ERG).
2. Maximal ERG (dark-adapted 3.0 ERG).
3. Oscillatory potentials (dark-adapted 3.0 oscillatory potentials).
4. Cone ERG (light-adapted 3.0 ERG).
5. Flicker ERG (light-adapted 3.0 flicker ERG).
6. Focal ERG (Focal or macular ERG).
7. Pattern ERG.
8. On/off ERG (long-duration light-adapted ERG).

See the detailed information on ERG types and used stimulators on ISCEV site: http://www.iscev.org

Almost all ERG types can be recorded using Ganzfeld stimulator. This stimulator should illuminate evenly the whole visible field. The exceptions are the focal ERG where the stimulation of the macular area is done with LED penlights and pattern ERG where the stimulation is done with the use of reversal pattern monitor.

When ERG is analyzed, the peak latency and wave amplitude are measured.

The peak latency is the time from stimulus onset up to wave peak. It indicates the pulse velocity. The latency is measured in milliseconds (ms).

The wave amplitude is measured from previous wave peak. If it is the first wave, it is done from the isoline. The amplitude depends on the number of cones and rods participating in the response. It is measured in microvolts (μV).

Please, pay attention that recording conditions such as dark adaptation duration, intensity and duration of light stimulus, stimulation frequency, stimulus wavelength (stimulus color), angular size of stimulus, electrode placement type, etc. may impact ERG amplitude. ISCEV recommends each laboratory to collect its own normal values for each stimulation type in different age-groups.

Except recording conditions, other peculiarities can impact the amplitude. They are pupil size, clarity of the optic media, anaesthesia, nystagmus, etc. These changes should be included in the report in case they are detected during the exam.

To increase the informativeness of the study results, it is required to compare the data of different tests. For example, to identify the cone activity, you can use the following techniques: cone ERG, focal ERG and flicker ERG. The different types of cone pathologies result in sometimes pathognomonic changes of parameters in ERG tests listed above. The changes in all ERG tests evidence the last stages of pathological process.

2.1. Full-field ERG (Ganzfeld)

While stimulating the retina with single flashes (1 Hz), we record a potential. It consists of two main elements: negative a-wave and positive b-wave (Fig. 1).

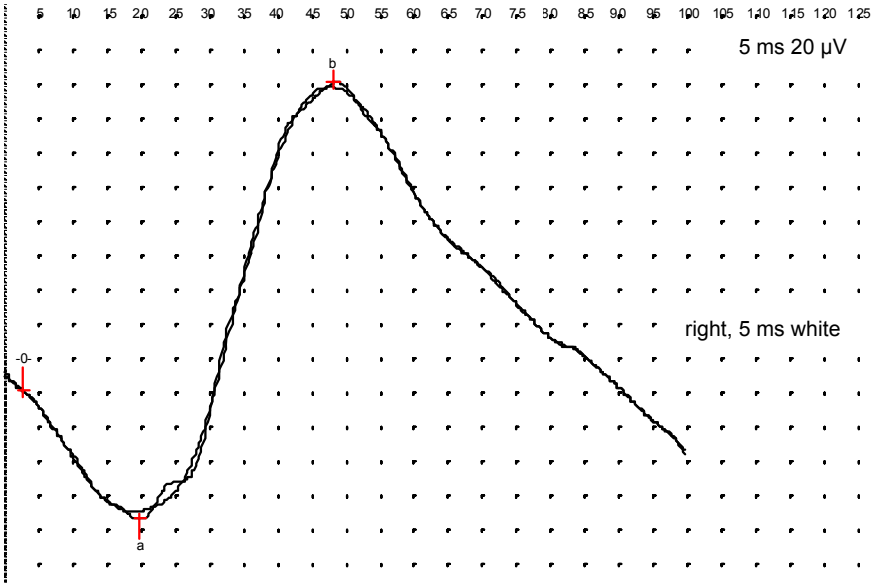

Fig. 2. ERG when stimulated by white flash with 5 ms duration recorded from healthy subject under scotopic conditions.

The normal values for cone and rod ERG are given in Table 1 and Table 2 correspondingly.

Table 1. The Normal Values for Cone ERG*

	Cone ERG			
	Latency, ms		**Amplitude, µV**	
	a	**b**	**a**	**b**
norm	17.7	35.8	13.4	51
min	15	30	7	30
max	21	40	25	70

** The data were obtained by Natalya Shubina, medical specialist, when using **Neuro-ERG** digital ERG system manufactured by Neurosoft Ltd.*

Table 2. The Normal Values for Rod ERG*

	Rod ERG			
	Latency, ms		**Amplitude, µV**	
	a	**b**	**a**	**b**
norm	23.8	48	23.3	124
min	20	43	6	90
max	27	54	42	190

** The data were obtained by Natalya Shubina, medical specialist, when using **Neuro-ERG** digital ERG system manufactured by Neurosoft Ltd.*

Each ERG component is generated by different anatomical structures. The electrical potential evokes in the first-order retinal neuron and initiates ERG a-wave. A-wave indicates the photoreceptor function. Then a potential spreads to bipolar cells (with participation of Müller cells) and generates b-wave. The horizontal and amacrine cells also participate in b-wave generation. The ganglion cells may also contribute to b-wave generation, although they mostly respond to pattern checkerboard reversal when pattern ERG is recorded.

The a-wave amplitude is measured from baseline to a-wave trough, the b-wave is measured from a-wave trough to b-wave peak. The b-wave amplitude is supposed to be more labile and clinically significant. The ERG value depends directly on the number of the normal cells.

The number of ERG abnormalities characterizes the stage of retina involvement in pathological process. The slight amplitude decrease evidences the initial or functional changes. The abrupt amplitude decrease occurs when the retina functions are disturbed as a result of organic pathology. At that the ophthalmic presentation of eye-ground can be unchanged.

If ERG can not be recorded, it is an electrophysiological indication of irreversible retinal changes.

The evident ERG amplitude increase shows the irritation of retina at acute hypoxia, intoxication, sympathetic ophthalmia, trauma or optic nerve atrophy as a result of conduction disturbances in inhibitory fibers.

Along with ERG amplitude and temporal parameters, b/a wave amplitude ratio is calculated. Normally it is ≥2. As a rule, the b-wave value of normal subject is twice more than a-wave one.

If this parameter value is less, it evidences the inner layer lesions. Such changes occur, for example, at acute circulatory injuries in retina. It is considered that the decrease of a/b wave amplitude ratio indicates the stage of retinal ischemia and makes prognosis of visual function recovery.

The physiological peculiarities of cones and rods allow to separate them from the general activity of retina. To do this, the conditions specific for cone or rod stimulation are provided.

After 7^{th} minute of dark adaptation, the bioelectrical activity of rods (scotopic ERG) starts prevailing in ERG. Besides, the rods respond better to low luminance stimulus (white or blue). That is why when we record full-field ERG trace (stimulation with white or blue flash under scotopic conditions), we mainly obtain the response of rod cells.

To estimate the cone function, 5-10 min of light adaptation (photopic conditions) are required. At that room light level is enough to suppress the rod activity. It is recommended to use more bright stimuli of long-wave part of spectrum (red or white).

The cone function can be estimated by red/white flash ERG recorded under photopic conditions.

The green flash ERG and blue flash ERG performed under photopic conditions supplement the cone studies.

2.2. Focal ERG

The focal ERG is done to assess the cone activity in macular area. This test is recorded with not more than 15° stimulus spot. Correspondingly the normal values for focal or macular ERG are less in comparison with full-field ERG (Fig. 3). The waveform and the composition of focal ERG and full-field ERG (while stimulating with Ganzfeld stimulator or penlight without concentrator) are identical.

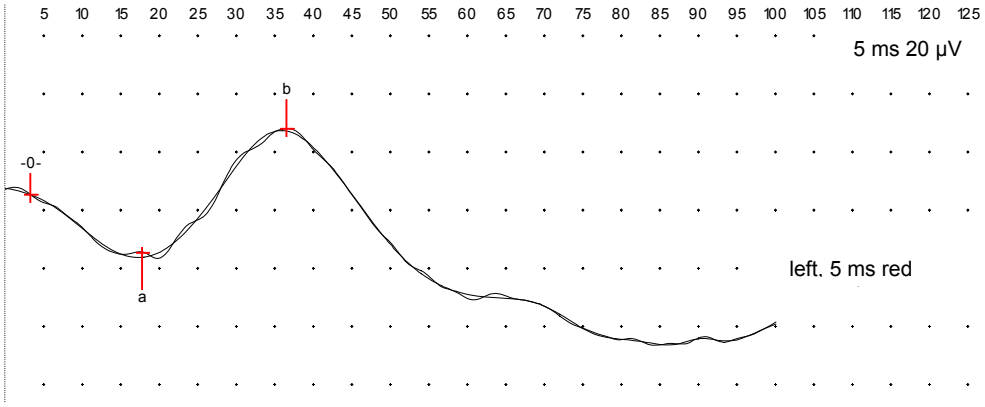

Fig. 3. The focal ERG at red penlight stimulation recorded from healthy subject under photopic conditions. The stimulus spot is 12°.

The normal values for focal ERG are given in Table 3.

Table 3. The Normal Values for Focal ERG*

	Focal ERG			
	Latency, ms		**Amplitude, µV**	
	a	**b**	**a**	**b**
norm	18.8	38.4	6.5	21
min	16	32	2	18
max	23	45	15	26

** The data were obtained by Natalya Shubina, medical specialist, when using **Neuro-ERG** digital ERG system manufactured by Neurosoft Ltd.*

The focal ERG is used to detect the macula pathologies. The red stimulus is usually applied for the stimulation. Although green and blue LEDs (with concentrators) are also used to estimate accurately the functional activity of cones in macular and paramacular areas. The focal ERG is always recorded in photopic conditions.

2.3. Flicker ERG

The flicker ERG is recorded when the retina is stimulated by flashes with 10-50 Hz frequency. This technique is applied to make differential testing of photopic and scotopic systems. The technique is based on different abilities of rods and cones to sense the flicker frequency.

The maximum flicker frequency perceived by rods does not exceed 20 Hz. Thus, only cones respond to 30 Hz stimulation (and higher). The graphic visualization of retinal response to high flicker frequency is sinusoid (Fig. 4).

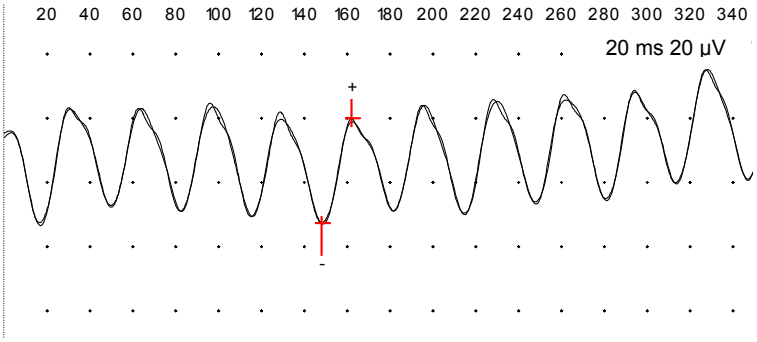

Fig. 4. The light-adapted flicker ERG at 30 Hz white flash stimulation recorded from healthy subject under photopic conditions.

The normal values for flicker ERG are given in Table 4.

Table 4. The Normal Values for Flicker ERG*

	30 Hz Flicker ERG
	Amplitude, µV
norm	37.8
min	20
max	51

** The data were obtained by Natalya Shubina, medical specialist, when using **Neuro-ERG** digital ERG system manufactured by Neurosoft Ltd.*

Except other parameters, the response amplitude is calculated. The amplitude decrease (at 30 Hz flicker frequency) (Fig. 4) evidences the decrease of the number of the functioning cones.

Sometimes changes in ERG trace obtained after 30 Hz flicker stimulation are not detected in other cone tests (red, green, blue flash ERG, focal ERG). The difference in these changes shows the different nature of response generation and allows to estimate the cone function more accurate. The changes detected after a certain test may evidence some retinal diseases.

The test conditions may impact the flicker ERG amplitude. According to ISCEV standards, it is recommended to use white flash for 30 Hz flicker stimulation and make the test under photopic conditions.

The 10-20 Hz flicker stimulation is a polyphasic response of mixed nature where the rods dominate (Fig. 5). It is a supplementary rod test and is done under scotopic conditions.

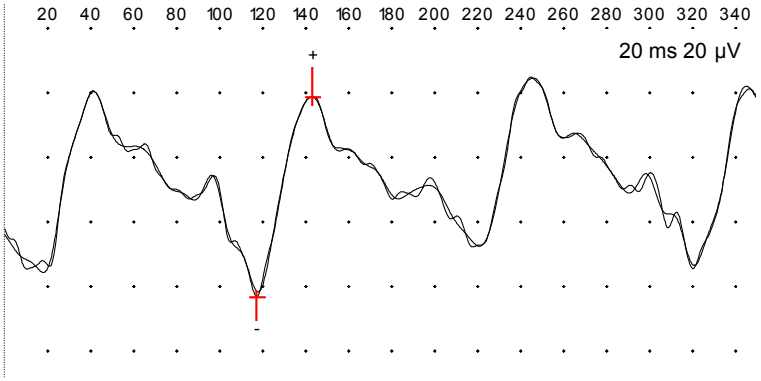

Fig. 5. The flicker ERG at 10 Hz white flash stimulation recorded from healthy subject under scotopic conditions.

The physiological peculiarities of the flicker ERG are very important. The Müller glial sells of the retina can not perceive more than 2-4 Hz flicker frequency. That is why at single-flash stimulation the b-wave of ERG shows the activity of inner layers of retina. However, the flicker ERG indicates the total activity of only neuronal elements of the retina (not after glia). It allows to estimate indirectly the glial and neuronal changes in retina. Thus, for example, when the trophic changes in retina take place, the amplitude is decreased primarily in single flash tests and then in flicker ERG.

2.4. Pattern ERG (PERG)

The pattern ERG is a retinal response evoked by viewing an alternating checkerboard. At that the white checks of checkerboard are replaced by black ones with 1 Hz frequency and vice versa. The size of checkerboard checks may vary, however ISCEV standards recommend to use 40' in size.

PERG waveform is characterized by first negative (N) component, at approximately 35 ms, referred to as N35. Is it followed by positive (P) component at 45-60 ms usually referred to as P50. This positive portion of the waveform is followed by a large negative component at 90-100 ms (N95) (Fig. 6).

The main physiological peculiarity of PERG is the following. The P50 peak is generated by all inner layers of retina. At that N95 is mainly generated by ganglion cells. That is why PERG is applied to detect the disorders connected with an injury of the third-order retinal neuron (Fig. 6, Table 5, Table 6).

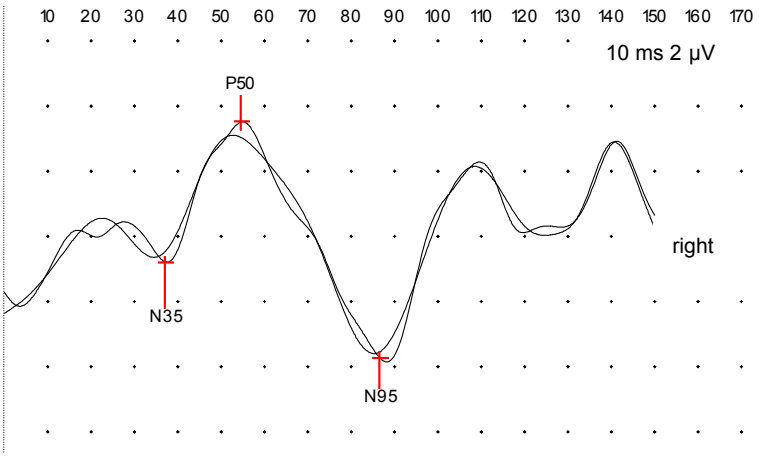

Fig. 6. The PERG waveform (check size 40') with maximal visual acuity (corrected).

Table 5. The Latencies

Stimulus	Channel	Component	Latency, ms
Right eye, 40'	1	N35	37.1
		P50	54.5
		N95	86.6

Table 6. The Amplitudes

Stimulus	Channel	Component	Amplitude, µV(/)
Right eye, 40'	1	P50	4.29
		N95	7.24

To assess the PERG waveform, the amplitude of P50 is measured from N35 trough and N95 is done from P50 peak.

The PERG amplitudes: P50 and N95 are measured in a different way in patient with different diseases. In case of macular diseases, P50 amplitude is decreased. When the optic nerve is injured, N95 amplitude is decreased. PERG supplements other techniques intended for macular area study as far as PERG waveform is changed in some cases in comparison with the full-field and macular ERG tests. The peak latencies in PERG waveform change rarely. The PERG is used to detect the glaucoma, the optic nerve disorders, the amblyopia.

The PERG parameters depend considerably on the vision acuity of a patient. Even if the defocus of 0.5 D takes place, the negative peak amplitude is decreased by 50%. Also PERG amplitude may decrease significantly in elderly patients, what is explained by the reduction in ganglion cell number in retina.

2.5. Oscillatory Potentials

The study of the oscillatory potentials is recommended by International standards as a supplementary test characterizing the retinal function. This test is done under scotopic conditions with the use of white flash. In this test the signal is filtered within 100-300 Hz frequency range to select the high-frequency peaks in full-field ERG waveform (Fig. 7, Table 7).

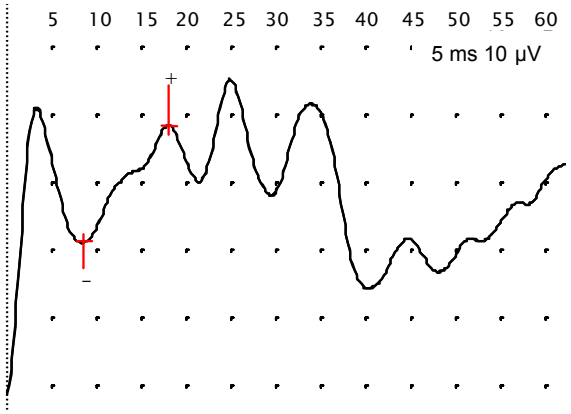

Fig. 7. The oscillatory potentials at white flash stimulation recorded from healthy subject under scotopic conditions.

Table 7. The Amplitudes

Stimulus	Channel	Amplitude, µV(/)
Right eye, white, 5 ms	1	17.2

Still there is considerable debate regarding how to interpret the oscillatory potentials. Anyway, there are usually three large positive peaks followed by a fourth smaller one (in amplitude). Their appearance evidences the normal function of the retina.

3. Basic Technology

3.1. Electrodes

The potential is recorded between the active (positive) and the reference (negative) electrodes. The active electrode is placed at cornea. It is not recommend to fix the active electrode at lower eyelid skin as far as the results may be doubtful. The reference electrode is placed near the orbital rim (most preferable) or at the earlobe of ipsilateral ear. The ground electrode is placed at the contralateral ear or the forehead. Please remember that the site type can impact the response amplitude. That is why use those sites where normal values were obtained. It will allow to compare the results at clinical study.

3.2. Active Electrode

The silver electrode constructed in the form of hook or loop is used as an active one. The electrode is placed at lower eyelid, thus, it contacts the cornea. Then it is fixed on the skin (under the orbit) with the adhesive tape.

The cleaning, the disinfection and the sterilization of the electrodes are done according to national standards and national guidelines for cleaning, disinfection and sterilization of semicritical items in healthcare facilities. The chemicals applied should be cleared for use in the user country and utilized according to norms stated by governing authorities and these guidelines.

Before the test instill 2% lidocaine solution (or other anesthetic) into patient's eye. Then the cornea should be protected with eye gel (placed under the lower eyelid). As soon as the test is finished, drop any anti-inflammatory solution into patient's eye (for example, gentamicine, laevomycetin eye drops, etc.).

3.3. Reference and Ground Electrodes

Use ear electrodes to record from the earlobe. In case the recording sites located on the head are required, use cup electrodes that can be fixed with the rubber tape or helmet.

To decrease the impedance, process the skin under electrodes with abrasive paste for skin preparation and alcohol. The ear electrodes are moistened in the saline solution. The cup electrodes are wetted with the conductive paste or gel. As soon as you placed the electrodes, measure the electrode impedance. It should not be more than 10 kΩ.

3.4. Recording Conditions

To differentiate the retinal function of cone and rod systems, different recording conditions are used.

The cone activity is mainly evokes in photopic conditions. At that the rod function is suppressed. To provide the photopic conditions, the room light level is quite enough (it is about 20 lx). If the dark-adapted test was performed first, 5-10 min of light adaptation is required. It is recommended to use the photopic conditions to test the functional state of cone system, i.e. red flash ERG or other color one, focal ERG, 30 Hz flicker ERG and higher, pattern ERG.

The scotopic conditions stimulate the rod activity and suppress the cone activity. It is stated that the rod activity starts dominating after 7^{th} minute of dark-adaptation. The maximal decrease in cone activity and domination of bioelectrical activity of rod system in ERG waveform is observed after 15^{th} min of dark adaptation. The rod system function is tested in scotopic conditions with the use of low-intensity white or blue flash. The oscillatory potentials and 20 Hz flicker (and lower) are also obtained from the dark-adapted eye.

3.5. Stimulators

The ganzfeld stimulator, set of penlights: red, green, blue and white and checkerboard pattern can be used for stimulation.

The ganzfeld stimulator is used to perform most tests. The background luminance across the full-field allows to record the maximal retinal response. The response of rod system can be obtained in the scotopic conditions with the use of low-intensity flash.

As far as the rods respond also to blue stimulus, examination with the use of blue penlight without concentrator (25º angle) can be performed as a supplementary scotopic test.

To study the cone activity in general, it is allowed to use red, green and blue penlights without concentrators under photopic conditions.

The focal ERG is done with the use penlights with concentrators which reduce the angular dispersion up to 12º.

The brightness of all penlights with concentrators is identical. The accuracy is 10% at 100 mCd level (Table 8).

Table 8. The Parameters of Penlights

	Angle		**Brightness, mCd**	
	with concentrator	without concentrator	with concentra-tors	without concentrator
White	—	120º	—	5200
Blue 475 nm	12º	25º	100	6500
Green 525 nm	12º	25º	100	6200
Red 660 nm	12º	25º	100	7400

The recommended distance from the corneal surface up to the penlight with concentrator (stimulator) is 10 mm, up to the penlight without concentrator is 10-30 mm.

To record pattern ERG, use checkerboard pattern reversal with 40' check size. The recommended distance to the screen is 1 meter. At this distance the visual angle is 14°. This value corresponds to ISCEV standard.

To save eye accommodation, the pupillary dilatation is not required for PERG recording. Please, pay attention that a patient should look at fixation point. The vision is corrected as far as the PERG amplitude decrease is observed at defocusing.

4. Test Report

The appointment card to electrophysiological study of vision should include the provisional diagnosis, the visual acuity level and the eyeground exam results to select the optimal set of diagnostic techniques.

Each test should be performed at least twice to confirm the obtained results.

The main conditions to acquire the quality record are the immobility of patient's eyelids during ERG recording and the look fixation at the light source.

The electrode impedance is checked regularly during the test ("Electrode impedance measurement" button).

Save test data after each test ("Save" button).

The test report should include the following data: patient's name, age, study date, provisional diagnosis. Specify also the peculiarities of patient's state such as anaesthesia, dilated pupil, nystagmus, etc. that may impact the results.

Each report should contain the normal values established for its own equipment within the laboratory.

The conclusion of the report provides the electrophysiological assessment of obtained results. The functional state of retina is estimated and rod and cone systems are tested. If the focal ERG is recorded, the macular area is also assessed.