Principles and Practice of Clinical Electrophysiology of Vision

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Other Protocols for Recording of Electroretinograms and Slower Potential Changes

Sven Erik G. Nilsson

The a- and b-wave electroretinogram (ERG) represents the initial events of a fairly long series of potential changes in the retina and the retinal pigment epithelium (PE) that are evoked by light. When the dark-adapted eye is stimulated by turning on a continuous light, the fast a- and b-waves are followed by the slow c-wave of the ERG and by fast and slow (light peak) oscillations, which are still much slower (Fig 42–1). If the light is turned off, a series of off-effects arise, including the "off c-wave," the "off fast oscillation," and the "off slow oscillation," (the dark trough). These off-potentials are of opposite polarity as compared with the corresponding on-potentials (Fig 42–1). Whereas the a- and b-waves represent the photoreceptor potential[1, 3] and interactions between the neural elements and the Müller cells in the inner retina[5, 16, 17] respectively, the slower potential changes reflect mainly PE changes in response to neuroretinal activity. It is of interest to study these PE responses clinically. The slow oscillations are generally investigated indirectly by means of the electro-oculogram (EOG).[2, 10, 11] For the c-wave of the ERG, however, a setup for corneal dc recordings must be used. Such equipment allows us to record the fast and slow oscillations as well. This section will provide a brief background regarding the generation of the three PE potential changes mentioned above as well as a description of equipment for corneal dc recordings of such slow responses in patients.

SLOW PIGMENT EPITHELIUM RESPONSES

The standing potential (SP) of the eye is a transocular potential built up by several components, e.g., from the cornea. The major contribution (approximately 10 mV) comes from the PE, however.[22, 23] In the dark, the apical PE membrane is more hyperpolarized than the basal one is.[14, 30] This voltage across the eye may be altered by several events in the retina and PE such as the ERG potentials, the fast oscillation, and the slow oscillation (the light peak).

The c-wave of the ERG (Fig 42–2) originates to a large extent in the PE.[23] It represents the sum of a positive component from the PE (PI) and a simultaneous negative (and generally smaller) component from the Müller cells (slow PIII). The positive component is generated as PE cell hyperpolarization (the difference in hyperpolarization between the apical and basal membranes) in response to the decrease in extracellular potassium concentration that occurs in the photoreceptor layer during light stimulation, and the negative component arises as Müller cell hyperpolarization in response to the same potassium change.[5, 8, 9, 14, 24, 25, 32, 38] Since the positive re-

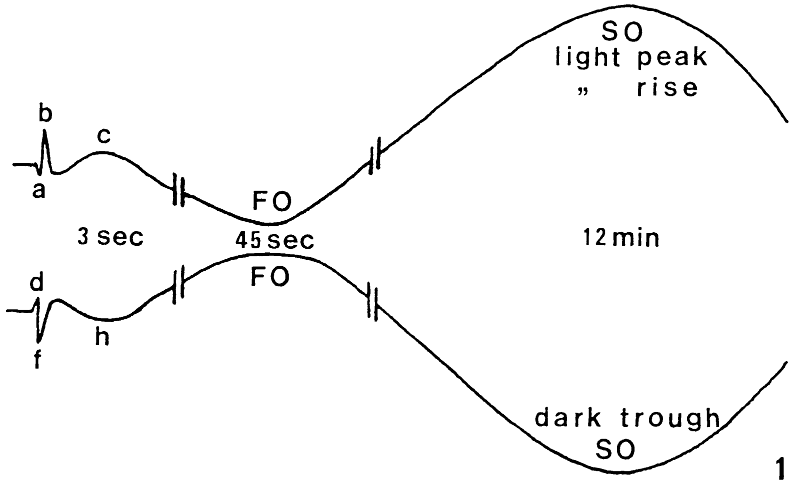

FIG 42–1.
Schematic representation of the light-induced changes in the voltage across the eye. In response to a prolonged light stimulus *(upper curve)*, the ERG with the a-, b-, and c-waves is first elicited. At about 45 seconds the negative "fast oscillation" is maximal, and at about 12 minutes the positive "slow oscillation" (light peak, light rise) reaches its peak. After light adaptation when the light is turned off *(lower curve)*, a series of off-effects occur. The "off-ERG" includes the h-wave or "off c-wave." The fast oscillation is now positive, and the slow oscillation (dark trough) is negative. The response is to a large extent a mirror image of the response to light. (From Nilsson SEG: *Acta Ophthalmol* 1985; 63(suppl) 173:22–27. Used by permission.)

sponse is generally the larger one, the c-wave of the ERG provides information on the health of the PE.

A direct corneal dc recording of the fast oscillation and the light peak (slow oscillation) from a normal, dark-adapted subject in response to continuous light stimulation is demonstrated in Figure 42–3. (With this slow time course, the ERG is seen only as a small upward deflection at light onset that represents the c-wave.) We showed long ago that these slow amplitude variations of the transocular potential can be recorded in the human without general

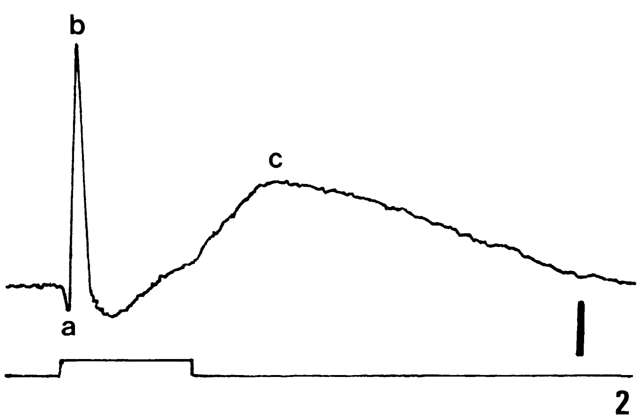

FIG 42–2.
The human dc recorded ERG. A 1-second light stimulus is indicated on the *lower line* (amplitude calibration, 100 μV).

anesthesia.[20, 21] The negative fast oscillation, peaking at 45 to 60 seconds in humans, is caused by a delayed hyperpolarization of the basal PE membrane. This response is related to the light-induced decrease in potassium concentration in the subretinal space mentioned above.[6, 12] The positive light peak has a maximum at 10 to 12 minutes in humans. It represents a depolarization of the basal PE membrane. It is not related to the potassium changes in the subretinal space but seems to depend on a "light peak substance" or a transmitter substance originating in the photoreceptors.[7, 13, 29, 31, 37] A dependence of the light peak upon the neuroretina was demonstrated long ago when it was found that it was abolished by experimental occlusion of the retinal circulation in the monkey.[35] Melatonin,[15, 33] synthesized in the photoreceptors, and dopamine[4, 26, 28, 34] have been thought of as tentative candidates for transmitter substances.

EQUIPMENT AND PROCEDURE FOR RECORDING AND LIGHT STIMULATION

Recording Equipment

There is no equipment commercially available for dc recordings of slow ocular potentials in patients. Each laboratory has to build its own equipment. Our setup and procedure, which have been improved

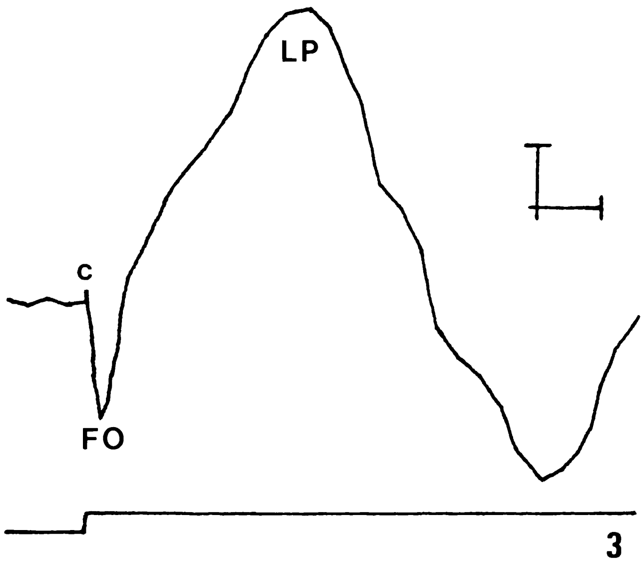

FIG 42–3.
Direct corneal dc recording of the ERG (c-wave only is seen), the fast oscillation *(FO)*, and the light peak *(LP)* in a normal patient (light stimulus, 16 lux; amplitude calibration, 1 mV; time calibration, 3 minutes).

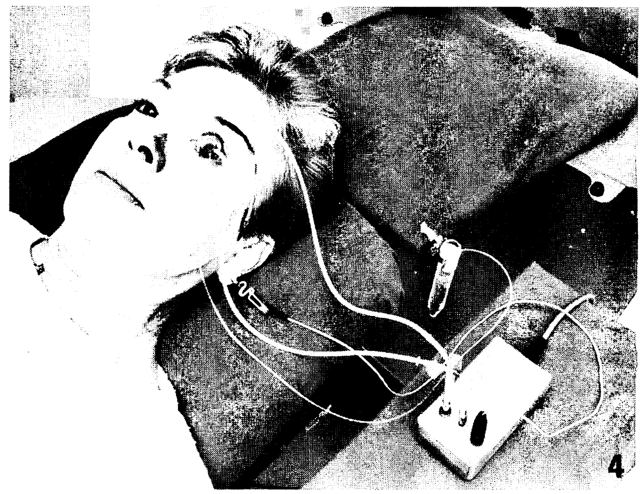

FIG 42–4.
A contact lens on the eye and a plastic chamber on the forehead are connected by means of saline-agar bridges to matched calomel half-cells (recording and reference electrodes) plugged into a preamplifier. To provide a well-defined suction the contact lens is equipped with a second tube ending in a test tube with saline, the surface of which is located 20 cm below the eye. The earlobe is grounded. (From Nilsson SEG, Andersson BE: *Doc Ophthalmol* 1988; 68:313–325. Used by permission.)

throughout the years,[18, 20, 21, 36] are described here.

The pupils of the patient's eyes are dilated with 0.5% tropicamide and 10% phenylephrine hydrochloride. Topical tetracaine anesthesia is used. A polymethylmethacrylate (PMMA) contact lens is placed on one of the eyes (Fig 42–4). The eyelids are held apart by means of a groove and a ridge along the edge of the contact lens. A chamber made of PMMA is attached to the forehead above the eye by means of a piece of ring-shaped, double-sided adhesive tape. Both the chamber and the contact lens are filled with a solution containing 2% sterile methylcellulose and 0.9% sodium chloride. If the recording session is intended to be long in duration, a few drops of tetracaine are added to the solution. Saline-agar bridges in polyethylene tubes are used to connect the contact lens and the chamber to matched calomel half-cells, which serve as recording and reference electrodes, respectively (Fig 42–4). Both electrodes are plugged into a preamplifier (impedance, 10^9 Ω). The saline-agar bridges are replaced with new ones for each patient to make certain that mercury ions will not reach the eye. The calomel half-cells are filled, from top to bottom, with saline, mercury chloride powder, metallic mercury and mercury chloride powder (mixed thoroughly by stirring in a mortar), and metallic mercury. The contact lens is prevented from sliding on the eye by means of a negative pressure of 20 cm of water that is created by connecting the lens through a saline-filled second polyethylene tube to a test tube with saline (Fig 42–4). The surface of this solution is placed 20 cm below the level of the eye. One earlobe is grounded. Silver–silver chloride electrodes, in the form of a freshly chlorinated silver rod in a contact lens and an electrocardiographic (ECG) electrode on the forehead, were tried for dc recordings. They were found to be less stable than calomel electrodes for long recording sessions.

From the preamplifiers, the signals pass to a two-channel, low-drift, differential-input dc amplifier built in our own department to meet very high demands regarding high impedance, low drift, and low noise (Fig 42–5). The common-mode rejection ratio (CMRR) is approximately 100 dB, which means that disturbing 50 Hz is attenuated sufficiently to allow us to record without the use of a shielding cage. Coarse offset adjustment is performed manually. Both amplifier channels are provided with low-pass filters, 12 dB per octave, with the high-frequency cutoff set to 300 Hz (with 100 Hz as an option). Each channel is in turn divided into two branches, one of which (gain set to 100) is used for recording very slow potential changes such as the fast oscillation and the light peak. The second branch, the gain of which is set to 1,000 (with 100, 200, and 500 as op-

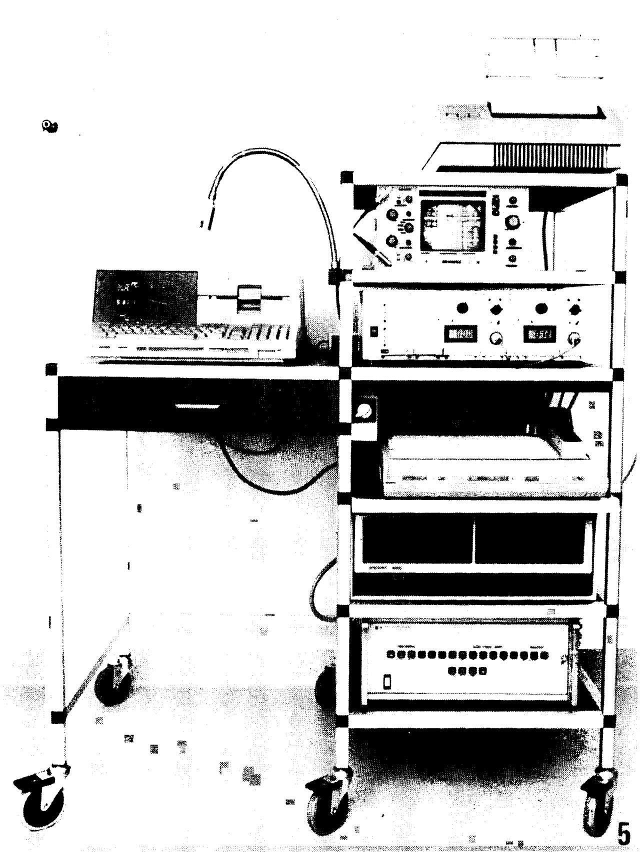

FIG 42–5.
Signal processing and display unit. *Left:* computer with display screen. *Right (top to bottom):* thermoprinter, oscilloscope, dc amplifier, plotter, flexible disk memory, and multiprogrammer. (From Nilsson SEG, Andersson BE: *Doc Ophthalmol* 1988; 68:313–325. Used by permission.)

tions), is used for ERG recordings. This second branch is equipped with an internal balance for final offset adjustment. It may be controlled manually, but it is generally controlled automatically from the computer. In such a case, the computer orders the amplifier just before each flash to balance the potential level against zero level.

On their way from the amplifier to a Hewlett-Packard (HP) 9826 computer (Fig 42–5) the signals pass an oscilloscope (showing the noise level) and an analoque to digital (A/D) converter (in an HP 6940B multiprogrammer). The computer analyzes ERG a-, b-, and c-wave amplitudes and implicit times and displays them digitally on the screen together with the curve. Selected recordings may be averaged. When the fast and slow oscillations are recorded via one of the channel branches, the computer samples the signal four times per minute during the first 2 minutes and then once per minute. The potential variations are displayed on the screen. The light peak may be elicited not only by turning on continuous light but also by using repeated flashes. In such a way, the ERG may be recorded repeatedly and simultaneously with the light peak. The computer analyzes the potential level just before every stimulus flash and displays the ERG traces superimposed on the light peak (see Chapter 68 on the clinical application of dc recordings). The size of the random access memory (RAM) of the computer is 1.2 megabytes.

An HP 9876A thermoprinter (Fig 42–5) prints out what is displayed on the computer screen when desired. An HP 9895A 8-in. flexible disk memory (size, 1.2 megabytes) (Fig 42–5) stores the information generated by the computer. Whenever required, the computer can retrieve such information. Graphs of higher resolution and quality are produced from recordings selected for further use by an HP 7225B plotter (Fig 42–5) controlled by the computer.

Light Stimulation

A 150-W halogen lamp (Osram) provides the stimulus light, which is first focused upon the entrance to fiber optics (Fiberoptic-Heim AG).[20] Neutral-density filters (Balzers) in a rotating mount (moved by a Philips stepping motor controlled by the computer via the multiprogrammer) allowing changes in light intensity over a total range of 7 log units in steps of 0.5 log units are interposed between the light source and the fiber optics. An electronic shutter (Uniblitz, Vincent Associates, Inc.), which is computer and multiprogrammer controlled, permits continuous variations in flash durations from 10 ms to infinity and in flash intervals from about 30 ms (flicker) to infinity. The exit of the fiber optics is connected to a hemisphere half a tennis ball in size that is approximately evenly illuminated by the stimulus light. In this way, Ganzfeld stimulation of one eye can be obtained (Fig 42–6).

Recording Procedure

For stable recordings of 5 seconds' duration (dc ERG with a-, b-, and c-waves), it is essential to ensure a steady eye position. This can be achieved by investigating one eye at a time and having the free eye fixate on a deep red light-emitting diode (LED) located about 1 m above the eye.[20] Five seconds before each flash, the computer tells the patient by means of an acoustic signal and by turning on the LED that a flash is to come. In this way, the patient

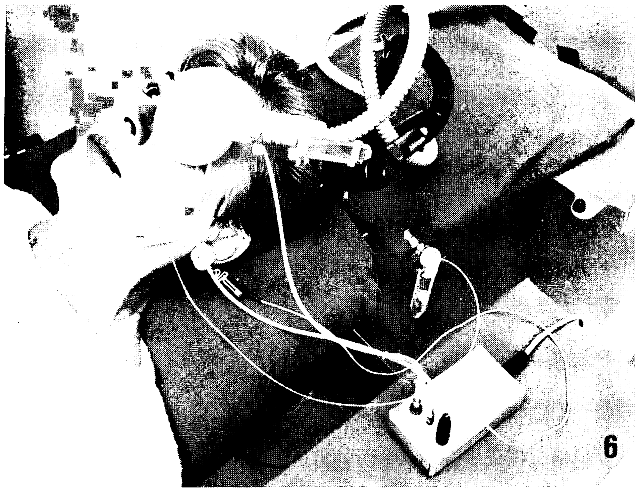

FIG 42–6.
Ganzfeld stimulation of the left eye. (From Nilsson SEG, Andersson BE: Doc Ophthalmol 1988; 68:313–325. Used by permission.)

is given sufficient time to fixate on the LED before light stimulation and, thus, obtain and maintain a steady eye position just before and during the recording. When the sweep is completed, the LED is turned off, and the patient can close the free eye and rest until the next signal comes (often 1- to 3-minute intervals between 1-second stimuli for dc ERGs). When general anesthesia is used for small children, it is possible to record from both eyes at the same time.

We have written programs for several kinds of recordings (a- and b-wave ERG; a-, b-, and c-wave ERG; 30-Hz flicker ERG; EOG; direct corneal recordings of the fast oscillation, the light peak, or the dark trough; light peak with ERGs superimposed), as well as for analysis and graphic plotting of information such as intensity-amplitude curves.

By using the technique described above it is possible to obtain stable dc recordings in most patients. Averaging is generally necessary for patients, whereas in volunteers with some previous experience, single recordings are sufficiently stable. For examples of clinical dc recordings, see Chapter 68.

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