

Localized Electroretinograms from the Isolated Retina of the Frog

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A technique of recording localized electroretinograms (ERGs) with macroelectrodes is described. The localized ERG showed a typical *a* wave and *b* wave which confirmed the idea that the ERG can be a response to a focal as well as a nonfocal stimulus. An electroretinographic map of the retina is shown. At the optic nerve head the ERG is absent or is markedly reduced in amplitude. The change in the amplitude of the *b* wave from the central area to the periphery is not smooth; on the contrary, abrupt changes in the amplitudes were found. An area in the inferior half of the retina was found which gave large amplitude ERGs. This area was seen in all of the retinas plotted although its exact shape and size varied.

INTRODUCTION

THE electroretinogram (ERG) has been of considerable value in the study of retinal physiology. In order to study various characteristics of the retina, several kinds of preparation have been developed.

In the intact animal, the recording electrode is placed on the cornea and the reference electrode is placed on the skin. In the excised eye, the recording electrode is placed on the cornea or, with the anterior segment of the eye removed, directly on the retina, the reference electrode being on the back of the globe. In the "isolated" preparation, the retina is removed from the eye and the electrodes are placed on its inner and outer surfaces.

Fry and Bartley¹ reported long ago that the ERG from the intact eye is mainly nonfocal, i.e., it is aroused mainly by stray light. They hypothesized that there are actually two ways to evoke the ERG; with focused light (forming a spot on the retina), or with unfocused, general illumination. That the stray light is chiefly responsible for the ERG was confirmed by Boynton and Riggs,² and independently by Asher.³ No significant differences were found in the ERGs elicited by stimulating the fovea, the optic disk, or a peripheral area, which showed that the ERGs were responses to stray light. Boynton,⁴ by increasing a spot of light by steps from 1° to 100°, obtained bimodal responses from intermediate sizes. This led him to believe that with small spots (up to 15°) the response was largely to the stray light, while with huge "spots" the response from the (now small) stray-lit retina was finally masked.

With the isolated retina, any effect of stray light can be practically eliminated. On such preparations, Marg and Heath⁵ obtained localized ERGs, with characteristic *a* and *b* waves, from spots only 1 mm in diameter. Later but independently, Brindley,⁶ and Tomita and

Torihama,⁷ secured well-localized ERGs in the absence of stray light using microelectrodes.

By systematically recording ERGs from separate areas of the retina, one can obtain an electroretinographic map of the retina. Several questions immediately arise. Does the amplitude of the *b* wave vary systematically along meridians? What happens to the ERG in the region of the nerve head? Are the responses within the area centralis unique in any way?

The purpose of the present work is to extend that of Marg and Heath and to describe in more detail the technique used to record localized ERGs.

METHODS

The bullfrog (*Rana catesbiana*) was used. Each animal was dark adapted for at least one hour, then pithed and one eye was removed. An incision was made around the globe close to the limbus, and the retina was peeled out and flattened on a pad of black cloth soaked in Ringer's solution. This was then placed in a black, polystyrene box. The entire procedure was carried out, in reduced illumination, in less than 15 minutes.

The recording electrode consisted of a chloridized silver wire with a piece of Ringer-soaked black thread wound around it and held on with a bit of polyethylene tubing. The tip of the thread extended half a millimeter to a millimeter beyond the wire. In some experiments the wire alone was used. The responses obtained with these two types of electrodes were not greatly different, although the wick provided a steadier base line. The cloth pad served as the reference electrode.

The electrodes were connected with a capacity coupled amplifier operated with a decay time constant of 0.9 second. A dual-beam oscilloscope and a loudspeaker were connected with the amplifier. One beam was used to register the ERG while the other was used either to exhibit the duration of the stimulus, or as a time marker.

The stimulus was a 0.5-mm circular spot of light with an available illuminance of 250 ft-c, which could be reduced by a neutral wedge. A one-revolution motor

⁷ T. Tomita and Y. Torihama, *J. Physiol. (Japan)* 6, 118 (1956).

¹ G. Fry and S. H. Bartley, *Am. J. Physiol.* 111, 335 (1935).

² R. M. Boynton and L. A. Riggs, *J. Exptl. Psychol.* 42, 217 (1951).

³ H. Asher, *J. Physiol.* 112, 40P (1951).

⁴ R. M. Boynton, *J. Opt. Soc. Am.* 43, 442 (1953).

⁵ E. Marg and G. Heath, *Science* 122, 1234 (1955).

⁶ G. S. Brindley, *J. Physiol. (London)* 134, 360 (1956).

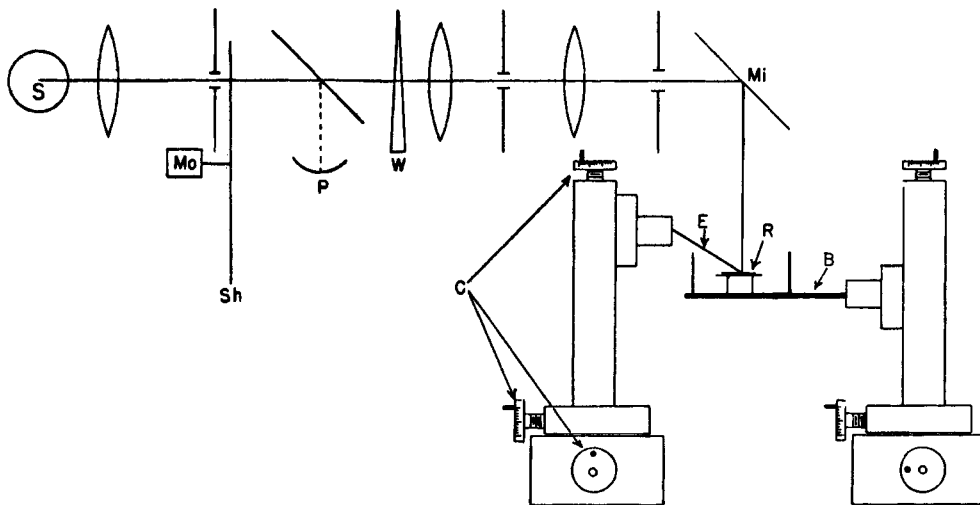


Fig. 1. Optical system of stimulator and stereomanipulators. *S*, source; *Mo*, motor; *Sh*, shutter; *P*, photocell; *W*, wedge; *M*, mirror; *E*, recording electrode; *R*, retina; *B*, box; *C*, controls for stereomanipulator.

driven shutter, whose speed and aperture were variable, controlled duration.

With the isolated-retina preparation, the chief source of stray illumination in the intact eye (scattering in the dioptric media) is eliminated. Great care was taken to minimize all other stray light. That from the optical apparatus was reduced by keeping its components to a minimum, and with appropriate stops (Fig. 1). With a piece of film in the position of the retina, the diameter of the stimulus and (with overexposure) the intensity of even weak stray light could be determined.

The box containing the retina was held by one stereomanipulator, and the recording electrode by another. This allowed linear movements of either in the three principal directions, which could be read to within one micron on vernier scales.

At the beginning of each experiment the recording electrode was adjusted to coincide with one edge of the stimulus-spot. The retina was then slowly raised until contact was made with the electrode. Contact was detected by noting a sudden decrease in the noise level seen on the oscilloscope and heard on the loudspeaker.

With the electrode and stimulus fixed, an ERG could be recorded from any locus by moving the retina. Thus an electroretinographic map of the retina can be obtained. The preferable procedure in determining the electrical sensitivity of the retina would be to choose a criterion response and determine the intensity of light required to produce it. This method is not practicable in the present experiments because of the time required in relation to the viability time of the preparation.

With the retina and stimulus fixed and the electrode moved, the specificity* of the recordings could be determined.

* This term is offered to designate the degree of localization of the ERG. If the ERG can be recorded only when the electrode is coincident with the stimulus, the specificity is excellent. If,

RESULTS

A. General Characteristics of the Localized ERG

The localized ERG showed the usual negative *a* wave followed by a positive *b* wave. The *c* wave and the *d* wave (off-response) were not apparent because of the short stimulus duration but were observed with longer stimulus duration.

The *a* wave was not always present. The presence or absence of the *a* wave did not appear to be correlated with any experimental procedure nor with retinal locus.

The amplitude† of the *b* wave depended on the retinal locus. In regions where the amplitude of the *b* wave was high, the latency and duration were short. The amplitudes were generally around 100 μv although values as high as 200 to 250 μv were not uncommon. In addition to differences from one locus to another in the same retina, there also appeared differences in the responsiveness of different retinas.

Typical spike potentials, distinct from the ERG, were seen even with the relatively large electrodes. These potentials were noted more often with the bare Ag—AgCl electrode than with the wick electrode and varied in amplitude and frequency.

B. ERG Viability

The electrical viability of the isolated frog retina was determined by recording ERGs from three different retinal loci at periodic intervals. To determine whether a reduction in the amplitude of the *b* wave was due to the cumulative effects of the light stimulus or due to a change in the vital state of the retina, the first retinal locus was stimulated every 5 minutes, the second every 15 minutes and the third every 30 minutes.

however, a response can be recorded at distances of 3 mm or more the specificity is poor.

† The amplitude was measured from the trough of the *a* wave or the peak of the *b* wave.

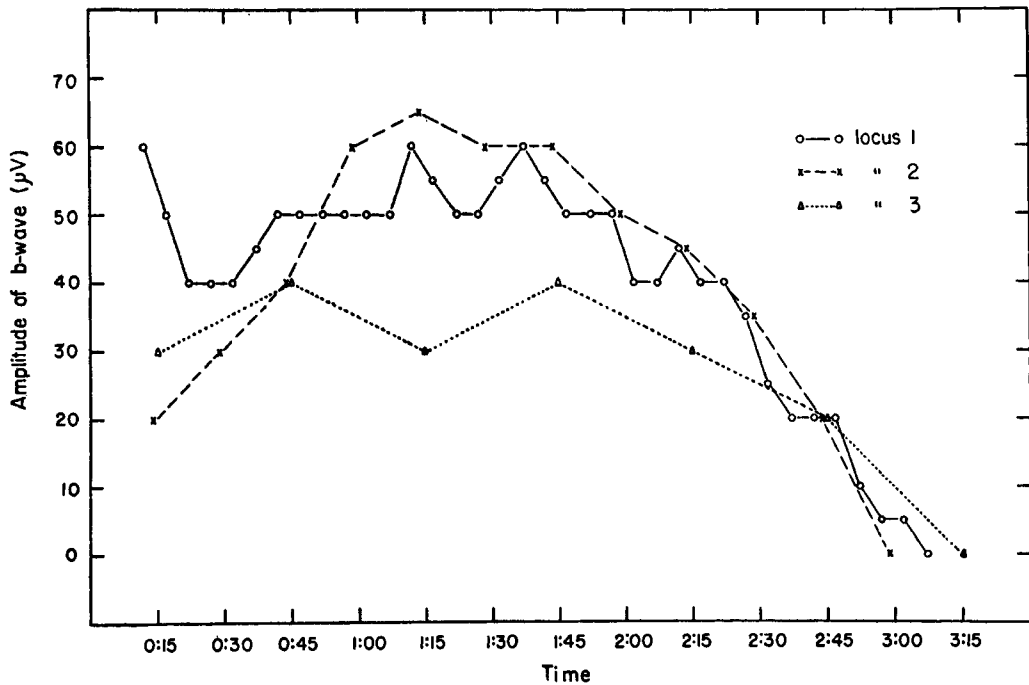


FIG. 2. Viability of the isolated retina of the frog. Locus 1 was stimulated every 5 minutes, locus 2 every 15 minutes and locus 3 every 30 minutes. Duration, 50 msec; illuminance, 8 ft-c.

The results are shown in Fig. 2. It can be seen that the amplitude of the *b* wave does not appear to show a general decline for two hours. This is seen at all three retinal loci which indicates that the frequent, short-duration stimulation itself does not induce any deterioration. After two hours, the amplitude gradually decreases and disappears after three hours. Some retinas retain their viability several hours longer.

C. Specificity

To determine the specificity, the recording electrode was moved systematically in 1-mm steps along Cartesian coordinates and a recording was made at each step.

In early experiments before the stimulator was adjusted and stray light reduced, the specificity was very poor. With stray light minimized and a proper selection of stimulus intensity and duration, good specificity was obtained along with good responsiveness. A stimulus duration of 50 msec and illuminance of 8 ft-c was used.

At a distance of 1 mm from the stimulus, the amplitude of the *b* wave is reduced to 52% (average of 40 measurements) of the value recorded when the electrode and stimulus are coincident. At 2 mm, the amplitude is reduced to 17%.

D. Electroretinographic Map of the Retina

The retina was moved in 1-mm steps and ERGs recorded at each point over its entire area. After the entire retina had been plotted, a recheck was made

on the first few loci to be sure no significant deterioration had occurred. Plotting of the retina took about 2 hours. At the end of the experiment, the exact location of the superior and inferior, nasal and temporal edges of the retina was recorded along with the coordinates of the optic nerve head. Notes were also made of any tears or folds in the retina.

Figure 3 shows a typical electroretinographic map of the retina. It can be seen that the amplitude of the *b* wave does not show a smooth gradient from the central area to the periphery. On the contrary, the responsiveness varied randomly along meridians. At the optic nerve head, the amplitude of the *b* wave disappeared or was greatly reduced. The disappearance of the ERG at the periphery and the observed edge of the retina usually coincided to within 1 mm.

The ERG also disappeared wherever tears in the retina were present. This was noted frequently in the region surrounding the optic nerve head. Folds in the retina resulted in either an inverted ERG or one of complex form.

E. Interaction

Interaction found but not consistently by Marg and Heath⁶ could not be found at all in the present experiments.

DISCUSSION

It is interesting that the spike potentials were recorded with the present technique. The works of

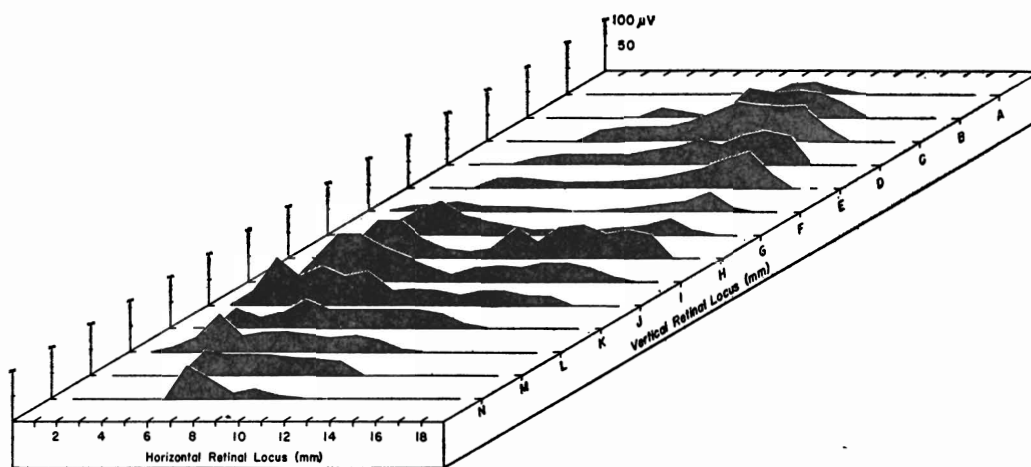


FIG. 3. Electoretinographic map of the frog retina. Optic nerve head located between F-9 and F-10. Duration, 50 msec; illuminance, 8 ft-c.

Rushton^{8,9} indicate that the spike potentials originate from the giant ganglion cells of the retina. In the frog, however, the evidence for giant ganglion cells is negative^{10,11} although Ramon y Cajal¹² has reported large, diffuse type ganglion cells. The investigations of Granit¹³ and others have shown that these spike potentials can be recorded with microelectrodes or in single fiber preparations. Our results show that they can be recorded with macroelectrodes although the isolation of single elements with microelectrodes may be more effective.

With regards to viability, Müller-Limmroth and Lemaitre¹⁴ have reported that the isolated frog retina showed a reduction of the *b* wave amplitude 20 minutes after decapitation. After 80 to 90 minutes the *b* wave had decreased to a point where it was slightly negative. As shown, our preparations yielded normal ERGs for at least 2 hours. The contradictory findings may be due to the relatively long stimulus duration (1 second) used by Müller-Limmroth and Lemaitre. In early

experiments we found that any long duration stimulation, even at low intensities, caused an appreciable deterioration of the amplitude of the *b* wave.

The recording of localized ERGs with good specificity supports Fry and Bartley's and also Boynton's concept that the ERG can be evoked either by nonfocal stray light or by a small focal beam of light. The *b* waves were of sufficient magnitude to indicate that a relatively small number of receptors can play an important role in the locally recorded ERG. If the ERG recorded between the cornea and an indifferent area represents the change in the entire retina or a large area of the retina, it may seem surprising that the amplitudes are not much greater than those of the localized ERG with its relatively few receptors, but the remoteness of the electrodes, the algebraic summation of the potential changes and possibly, retinal interaction may account for this phenomenon.

The electoretinographic map of the frog's retina illustrates two phenomena which cannot be explained at this time. One is the abrupt changes in the amplitude of the *b* wave along meridians and the other is the lack of correspondence of the area centralis (a band approximately 1 to 1.5 mm wide located about 1 mm above the optic nerve head¹⁵) and the area giving the greatest responses. Unfortunately there are no histological maps of the bullfrog's retina which could be used to correlate our findings with any local morphological differences.

⁸ W. A. H. Rushton, *Brit. Med. Bull.* **9**, 68 (1953).

⁹ W. A. H. Rushton, *Nature* **164**, 743 (1949).

¹⁰ H. B. Barlow, *J. Physiol. (London)* **119**, 58 (1953).

¹¹ L. Lipetz, *Electrophysiological studies of some properties of the vertebrate retina.* (Ph.D. thesis, University of California, Berkeley, California, 1953).

¹² S. Santiago Ramon y Cajal, *Die Retina der Wirbelthiere* (Wiesbaden, J. F. Bergman, 1894).

¹³ R. Granit, *Sensory Mechanisms of the Retina* (Oxford University Press, New York, 1947).

¹⁴ W. Müller-Limmroth and M. Lemaitre, *Z. Biol.* **105**, 348 (1953).

¹⁵ J. H. Chievitz, *Arch. Anat. u. Physiol.; Anat. Abt.* (1889).