

### Localized Electroretinograms from Isolated Poikilothermic Retinas with Macroelectrodes

What effect does the stimulation of some retinal elements have on the effect of stimulating others? Is there a real effect, or only an apparent one caused by stray light, chiefly from scattering in the dioptric media of the eye?

The interchangeable effects of stimulus area, duration, and intensity on the electroretinogram (ERG) of the intact frog eye were considered by Granit (1) to be evidence for interaction of retinal elements. Fry and Bartley (2), using the intact rabbit eye, showed that stray light could explain the apparent interaction and that, indeed, the ERG was mainly a response to stray light within the eye. Granit, Rubinstein, and Therman (3) obtained results apparently supporting interaction when they minimized stray light by using small stimuli of low intensity in an excised and opened frog eye. Recently, the stray light theory of Fry and Bartley has been revived and confirmed for the human ERG (4). This, together with the inconclusive character of the evidence for retinal interaction, raises several questions regarding the effects that stimulation of one retinal locus may have on the response from another locus.

We have recorded ERGs from isolated frog (*Rana pipiens* and *R. catesbeiana*) and terrapin (*Pseudemys elegans*) retinas. We removed the retina under subdued illumination and placed it flat on a black felt pad that had been soaked in Ringer's solution. The preparation was placed in a black box to minimize further any effects from stray light. Thread wicks from silver-silver chloride electrodes were led to any desired points on the surface of the retina, and another to the supporting pad. Two channels of alternating-current amplification led to a dual-beam cathode-ray oscilloscope and permitted simultaneous registration of potential changes that occurred between two pairs of electrodes, if desired, or between one pair of electrodes if the second beam was used for a time scale and stimulus marker.

Each stimulus was a 1-mm spot of light of adjustable intensity and duration.

Two such spots can be presented at any desired retinal locations and with any desired timing.

With a single spot of 50 msec duration and intensity as high as  $1 \times 10^8$  ft-lam, we have found the ERG to be extremely localized. When the supporting pad is the indifferent electrode, an ERG is registered only when the thread wick is at the locus illuminated. The response (*a*-wave plus *b*- or *x*-wave) may then be more than 100  $\mu$ v but generally is less, depending on the retinal locus and the age of the preparation. Moving either the light spot or the wick as little as 1 mm extinguishes the response to at least below the noise level of about 3  $\mu$ v.

By systematically moving the light spot together with the thread electrode to various retinal loci, we have been able to map the retina electroretinographically. Such a map reveals a functional outline of the optic disk, within which no response can be obtained. It also outlines the retinal margin where the response again falls to zero. Curiously, although we have found a definite increase in sensitivity from the periphery toward the center of the retina, it is not a smooth gradient. Instead, "peaks" and "valleys" of high and low sensitivity appear to exist. It is possible that these are artifacts caused by trauma of preparation, although no other evidence of physical injury can be found. Actual tears in the retina completely eliminate the ERG at the site of the injury.

Early investigators (5) cited by Granit reported rapid disappearance of the *b*-wave when the frog retina was removed from the bulb (although, to be sure, they were using less responsive apparatus). Our preparations have yielded apparently normal ERGs for more than 5 hours of experimentation, at times with little evidence of any deterioration or significantly decreased responses. Responses at a given locus are repeatable from one time to another within about 20 percent when the total height of the ERG is measured from the trough of the initial negative wave (*a*-wave) to the crest of the first positive wave (*b*- or *x*-wave). During an experimental session, the preparation was moistened occasionally with

Ringer's solution to prevent drying. The use of isotonic glucose does not appear to enhance the response or to prolong the usefulness of the preparation.

Despite the differences in technique, especially the localized recording described here, we have been able, although not consistently, to confirm the inhibition of Granit, Rubinstein, and Therman.

Using two stimulus spots spaced 2 to 3 mm apart on the retina, we did not find any effect of one stimulus on the ERG registered from the retinal locus of the other spot, regardless of the time relationship of the two flashes. With the spots very close together ( $\frac{1}{2}$  to 1 mm), however, evidence of interaction has been noted. Stimulating one spot alone produces no recordable response at the retinal locus of the other spot, but may inhibit the ERG response to stimulation of the second locus for many seconds afterwards. The recovery of the inhibited locus may be observed by repeatedly stimulating that locus and noting the progressive increase in potential throughout the ERG. Thus it appears that interaction of the ERG takes place over small distances on the retina but not over large ones.

Investigation of these and similar phenomena is continuing and a more complete report will be submitted for publication elsewhere. However, we wish to invite attention at this time to this relatively simple technique of registering localized ERGs without the use of microelectrodes.

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#### References and Notes

1. R. Granit, *Sensory Mechanisms of the Retina* (Oxford Univ. Press, London, 1947).
2. G. A. Fry and S. H. Bartley, *Am. J. Physiol.* 111, 335 (1935).
3. R. Granit, B. Rubinstein, P. O. Therman, *J. Physiol. London*, 85, 34P (1935).
4. R. M. Boynton, L. A. Riggs, *J. Exptl. Psychol.* 42, 217 (1951); H. Asher, *J. Physiol. London*, 112, 40P (1951).
5. F. Holmgren, *Uppsala Läkarefören. Förh.* 6, 419 (1870); W. Kühne and J. Steiner, *Untersuchen. Heidelberg Univ. Physiol. Inst.* 3, 327 (1880), cited by Granit (1), p. 85.

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