THE EFFECT ON THE FROG'S ELECTRORETINOGRAM OF VARYING THE AMOUNT OF RETINA ILLUMINATED

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Adrian & Matthews (1927) found, for the frog and conger eel, that the latency of the electroretinogram (e.r.g.) lengthened as the area of retina illuminated decreased; and this finding was confirmed for the cat by Granit (1933) and Creed & Granit (1933). If, in these experiments, the responses recorded really came from the areas of the retina intentionally illuminated, the results would provide very strong evidence that the earliest part of the e.r.g. is not generated by the rods and cones, for a rod or cone presumably cannot during the latent period be influenced by whether or not its neighbours are being illuminated. Evidence has however been brought forward that for the rabbit (Fry & Bartley, 1935), and for the human eye (Boynton & Riggs, 1951; Asher, 1951; Boynton, 1953), the e.r.g. recorded for a retinal image within the range of sizes known to have a large effect on latency is mainly due not to the image itself, but to stray light falling on the rest of the retina. This important conclusion, deduced elegantly but from indirect evidence, has not been universally accepted (Granit, Rubinstein & Therman, 1935; Granit, 1947, 1955); and the experimental results of Wirth & Zetterström (1954) in the cat may be difficult to reconcile with it. The present experiments on opened excised eyes of the frog strongly support, for this species, the conclusions of Fry & Bartley and of Boynton & Riggs, and at the same time show that, with the method here used to eliminate the effects of stray light (a steady illumination of the whole retina upon which the test stimuli were superimposed), the electroretinographic responses to illumination of different areas of the retina are to a close approximation simply additive. It may thus be unnecessary to postulate any lateral interaction in the production of the frog's e.r.g.

RESULTS

The apparatus used is described in the preceding paper (Brindley, 1956a). Test stimuli were of 0·63 sec duration, the opened excised eyes were light-adapted, and the e.r.g. was recorded with a condenser-coupled amplifier of over-all time-constant 0·77 sec. The anterior electrode was a saline-filled micropipette whose tip lay in the vitreous.

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The effect of a steady background, upon which the test stimulus
is superimposed

The e.r.g. resulting from a flash superimposed on another which precedes
it by less than one second varies in a complex manner with the time-relations
of the two flashes (Andrée & Müller-Limmroth, 1954). We are here concerned
only with the effects of *steady* backgrounds, with which the retina has come,
at least approximately, into equilibrium, and in particular with the following
two questions:

(1) How strong must such a background be to abolish the response to
a superimposed stimulus?

(2) How weak must it be to have little effect on the response to a super-
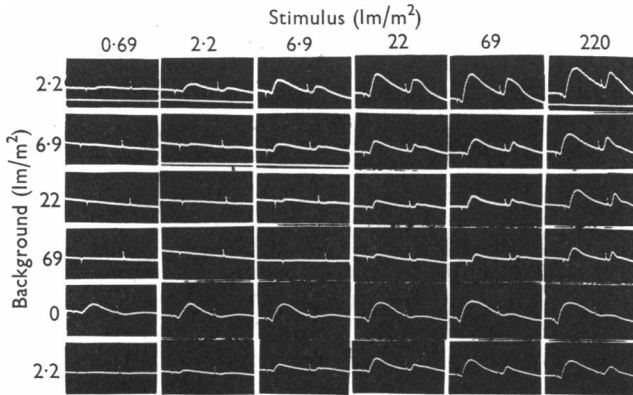imposed stimulus?

Fig. 1. Effect of a steady background on the e.r.g. provoked by a superimposed stimulus.
Column headings, intensity of stimulus in lm/m^2; row headings, intensity of background in
lm/m^2: each stimulus was of duration 0·63 sec, and its beginning and end are marked by
downward and upward artifacts respectively.

The results of one experiment designed to answer these questions are shown
in Fig. 1. The backgrounds were applied in the order of the rows of the figure,
and for each background, after an interval of one minute for the retina to come
approximately into a steady state, the test stimuli were given in ascending
order of brightness at intervals of about 20 sec. Comparison of the last row with
the first shows a decrease in size of the responses during the sixteen minutes
that the experiment occupied. Such a decrease was sometimes observed in
other experiments where no light fell on the preparation during the interval. It
was usually greater when the vitreous humour was left in place (as in the
experiment of Fig. 1) than when it was removed. It is thus likely that it
depends more on lack of oxygen or accumulation of carbon dioxide than on
light-adaptation.

Our two questions may be answered from Fig. 1 (and other experiments gave substantially the same result) as follows: Weber's law is approximately valid for the frog's e.r.g. over the range tested. A steady background ten or more times as bright as a superimposed stimulus abolishes the response to it. The effect of a steady background one-tenth as bright as the superimposed stimulus is small, though not undetectable.
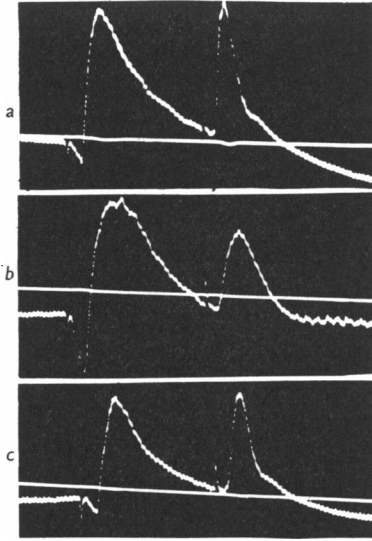

Fig. 2. Effect of a steady background of 22 lm/m² on the e.r.g. provoked by a superimposed stimulus of 220 lm/m². *a*, eye equilibrated with the background for 5 min; *b*, 5·1 sec after extinguishing the background; *c*, 2·3 sec after restoring the background, which had been extinguished for 6·7 sec.

In the remaining experiments of this and the subsequent paper (Brindley, 1956*b*), test stimuli of 220 lm/m² were used; and to prevent stray light from the stimulating beam from provoking a response from areas of retina not intentionally illuminated, it was superimposed on a steady background of 22 lm/m². Further information on the effect of such a background on the response to such a stimulus is presented in Fig. 2. In the responses obtained in the presence of the background, the b-wave is of about the same height but shorter, and the d-wave higher but shorter, compared with that obtained in the same long-term adaptional state but without background. These differences, though not large, were consistently found in similar experiments, and can be seen also in Fig. 1, although the long-term adaptational state was there not the same. The other difference seen in Fig. 2, the exceptionally large a-wave of the response obtained without background, is a feature peculiar to responses recorded within a few seconds of extinguishing an

adapting light. It is very similar to the effect of brief stimulating lights on the response to re-illumination, first described by Granit & Riddell (1933).

The relation between the area illuminated and the size of response

The preceding section shows that a steady background of 22 lm/m² has no very large effect on the response to a test stimulus of 220 lm/m² when the test stimulus occupies the whole retina, and provides some basis for the supposition that its effect may also be small when the test stimulus occupies only a part of the retina. The evidence that it achieves its other aim of abolishing the response to stray light is as follows.

(1) In a photometric experiment, when the test field was focused on white blotting-paper, the stray light, even for the largest test field used, nowhere exceeded 1/200 of the field brightness. The retina is less favoured than blotting-paper in having vitreous in front, but more favoured in having black pigment behind. If, as seems almost certain, the stray light on the retina does not exceed $\frac{1}{100}$ of the field brightness, the experiment of Fig. 1 shows that it will cause no response.

(2) At the end of experiments, perhaps as a result of oxygen lack, eyes in which much of the vitreous had been retained were sometimes found to respond very poorly, and in one case not at all, to illumination of the central part of the retina (background being present), though the peripheral part still responded well. If the stray light from the test field on the central retina had been strong enough to stimulate the periphery, presumably it would have done so.

The responses to circular test stimuli of diameters 0·343, 0·59, 0·80, 1·56 and 2·07 mm and to illumination of the whole of the same retina are shown in Fig. 3. The smallest stimulus gave no detectable response, and the responses to the larger stimuli are very nearly identical one with another if scaled in proportion to the areas illuminated. Similar experiments on three other eyes gave results almost identical with those of Fig. 3.

The additivity of the response for large areas

For fields of more than 22 mm diameter, curvature of the eye makes the area of retina illuminated depart substantially from the calculated area of the image; so to test for additivity of the response to stimuli of greater area, a square field stop was made, to be placed at H_2 in fig. 1 of Brindley (1956a), by means of which nine contiguous non-overlapping regions, together covering the whole retina, could be separately illuminated, and the sum of the responses to each of the nine separately compared with that to illumination of the whole retina at once. The results of a typical experiment are shown in Fig. 4. The responses to illumination of the separate regions are similar in shape to the
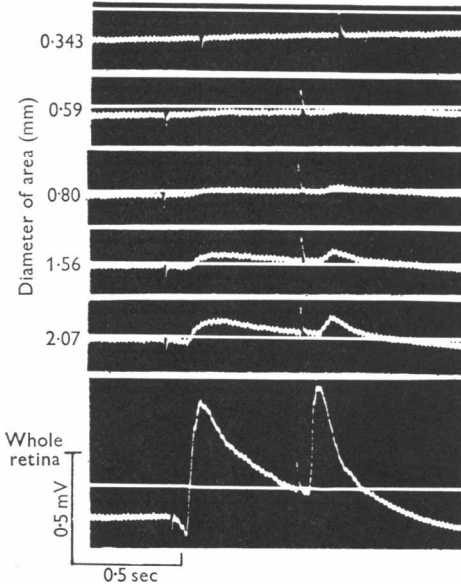
Fig. 3. Relation between area illuminated and the size of the e.r.g. produced.
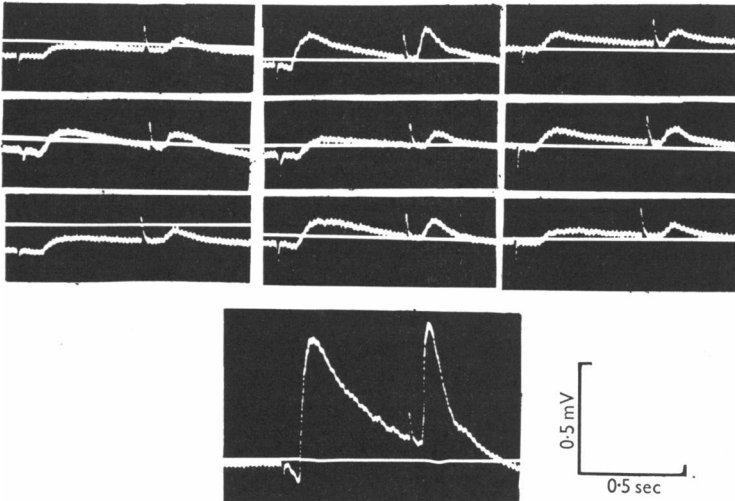Stimuli were circular, diameters in mm.

Fig. 4. Responses to illumination of different parts of the retina, and all of them together. The retina was divided by a square grid into nine parts (not equal, because of curvature and because the outer ones overlapped the edge of the eye). Above are shown, in their correct anatomical relation, the responses to illumination of each part separately; and below, that to illumination of all nine together (i.e. of the whole eye).

large-field e.r.g., and their sum reproduces it fairly well, except that it is about 20 % too large. Since it was the geometrical images that were to touch without overlapping, and any imperfections in the images due, for example, to refraction at the air-vitreous interface must cause some overlap, this result is not inconsistent with perfect additivity of the responses, and it establishes that under the conditions of the present experiments they are at least approximately additive.

DISCUSSION

Responses to the illumination of different areas should be additive if the retina and its conducting environment behave as linear circuit elements, the impedance of the sources does not change during activity, and there is no physiological interaction in production of the e.r.g. between the retinal areas concerned. The preceding paper shows that the first two of these conditions hold sufficiently accurately, since the probable decrease by 0·1 % in the retinal resistance during activity is too small to affect the present results. In so far, then, as these results demonstrate additivity in the frog's e.r.g., they make it unnecessary to postulate any interaction between different parts of the retina in its production.

We cannot be certain, though the results of the first two sections of this paper make it probable, that properties of the e.r.g. in the presence of a steady background are valid for the e.r.g. obtained without background. But if this extension *is* valid, then all stimuli of less than about 0·5 mm diameter give an e.r.g. too small to be detected, and the effects of area on the latency of the frog's e.r.g. found by Adrian & Matthews are due to stray light, and can be discounted as evidence for interaction. Strong support for this conclusion was obtained by applying to the frog's eye a modification of one of Fry & Bartley's experiments. Two spots of plane-polarized light of diameter 0·343 mm were projected on to a frog's opened eye, their axes of polarization being perpendicular. In the absence of background, switching either alone on or off by rotating an analysing polaroid in front of the eye caused an electrical response exceeding 150 μV; but if in this same way one was switched on and the other simultaneously off, then by suitably adjusting their relative brightness, rotation of the analysing polaroid could be made to give no trace of response.

Experiments like that of Fig. 4, besides demonstrating that the responses of different large areas are, at least approximately, additive, show that there is no substantial difference in the frog between the e.r.g. produced by central and peripheral retina. This is of interest in connexion with the hypothesis of Ottoson & Svaetichin (1952) that the positive components of the e.r.g. originate in rods and the negative in cones, since the principal evidence for this hypothesis, which is unacceptable on other grounds (see Brindley, 1956 b), was derived from differences between the responses of central and peripheral retina as recorded with microelectrodes.

SUMMARY

1. In the frog, the e.r.g. is abolished by superimposing the test stimulus on a steady background of ten times its brightness, and is little affected by superimposing it on the one of one-tenth of its brightness. Weber's law holds approximately over the range tested (backgrounds from 2·2 to 69 lm/m²).

2. In the presence of background, the e.r.g. obtained on illuminating a small area of retina is simply proportional to the area illuminated. The large responses obtained by illuminating small areas of the retina in the *absence* of background are probably due to stray light.

3. The e.r.g. obtained on illuminating the whole retina is, at least approximately, the sum of the responses obtained on illuminating all parts of it separately. There is no substantial difference, in the frog, between the e.r.g. produced by central and peripheral retina.

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