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THE DISTRIBUTION OF EXCITATION AND INHIBITION IN SINGLE-FIBRE RESPONSES FROM A POLARIZED RETINA

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The electrical theory of retinal excitation and inhibition by slow potentials has been based on a number of correlations between such potential changes in the retinal cells and characteristic excitatory and inhibitory effects reflected by corresponding changes in the impulse activity of the optic nerve. The arguments for the theory have recently been summarized in monographic form (Granit, 1946). Now, if currents, generated within the retina itself in response to illumination or sudden darkness, may excite or inhibit, so that the discharge of single optic nerve fibres can become blocked or accelerated at both 'on' and 'off', then how would such fibres respond to externally applied currents? Granit & Helme (1939) experimented along these lines with the frog's eye but did not succeed in recording from single fibres *during* polarization. The electrical field, established by the polarizing current, caused uncontrollably large oscillations of the base-line at the level of amplification necessary for single-fibre work. However, they managed to prove that the components of the electroretinogram and the discharge of the *whole* nerve could both be either increased or decreased by a change in the direction of a current traversing the retina.

In order to understand why it is necessary to isolate single fibres, let us consider in what manner individual optic nerve fibres might have their properties obscured by the behaviour of the whole nerve. Assume, for instance, that a certain percentage of the fibres were excited at onset and inhibited at cessation of polarization, and that another group of fibres behaved in an opposite manner; assume, further, that reversal of the direction of the current exactly reversed the properties of the two fibre types with respect to onset and cessation of polarization, then, as a rule, the effects in the two groups of fibres would cancel. Actually, when I ultimately succeeded in performing this experiment with single fibres, the earlier average results were found to have been obscured by just this source of error. The new experiments now to be described reveal details which it would have been impossible to discover with the aid of any less discriminative method.

TECHNIQUE AND PROCEDURE

As before, it proved impossible to record with micro-electrodes from a frog's eye during polarization. Nor was the experiment successful with a mammalian eye—in which single spikes are more easily obtained—if one polarizing electrode was inside and the other one outside the eye. Recording was prevented by the disturbed base-line. But it could be reasonably well performed with the cat's eye if the polarizing electrodes—well-chlorinated silver-silver chloride rods—were placed nasally

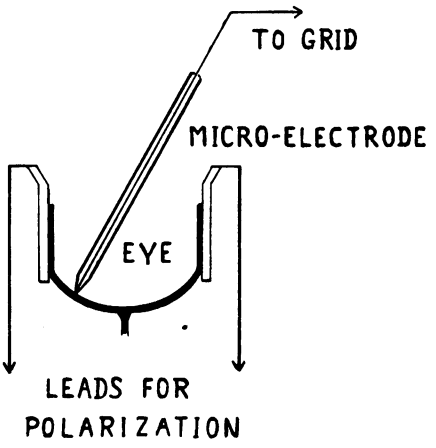

Fig. 1. See text.

and temporally on the outside of the eye as in Fig. 1. The animal was decerebrated, lens and cornea removed and the micro-electrode inserted, as illustrated by the figure. The polarizing device contained a commutator, a 50,000 Ω resistance in series with the eye and a galvanometer for measuring current strength. The impulses were led off across condensers to a balanced amplifier.

RESULTS

General. Ten dark-adapted cats were used, and in twenty-five experiments on single fibres the polarizing currents elicited effects. Many more fibres were picked up, but it often happened that a successfully isolated fibre was outside the region in which the density of the currents sufficed to stimulate its receptor or synaptic connexions. It will be shown below that—at moderate current strengths—these structures and not the nerve fibre itself caused the effects obtained. Lack of an effect could not be remedied by increasing current strength indefinitely, since above 2 mA. base-line disturbances began to be troublesome. As a rule, however, fibres picked up on the inside in the neighbourhood of either outside polarizing electrode showed effects. Threshold effects were obtained with currents around 0·2 mA. The maximum strength used was 2·5 mA. Nearly all fibres studied were picked up at the nasal polarizing electrode.

The experiments were begun in dark-adaptation by investigating the effects of current strength, direction and duration. The fibre properties were next analysed by means of observations on the effects of variations in the intensity

and duration of illumination. Polarization and illumination were then made to overlap, and finally a number of similar observations were repeated after light-adaptation.

Survey of fibre properties. All but one were of the on-off type so characteristic of the cat's optic nerve, i.e. they responded to both onset and cessation of illumination. Sometimes the off-effects were replaced by post-excitatory inhibition in the high-intensity range, sometimes they were maintained. (For a description of the properties of cat optic nerve fibres, see Granit (1945 *a*, *b*).) In order to be able to demonstrate inhibitory effects it is desirable to have spontaneously active fibres. The majority of fibres were of this type. In silent fibres it is often impossible to be certain as to whether absence of a response to polarization signifies inhibition or merely means lack of an effect.

In Fig. 2 are shown the typical antagonistic effects of polarization. The figure refers to a silent fibre. In record *a* the fibre responds to onset of polarization. The current was then reversed, and it is seen that in record *b* the fibre

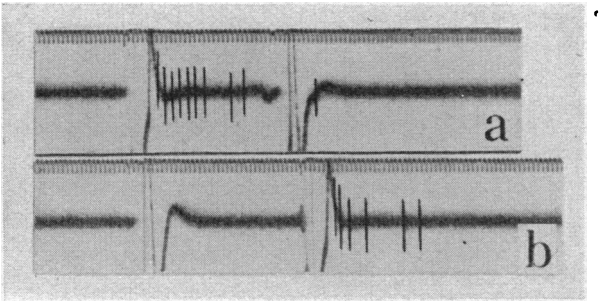

Fig. 2. Effects of polarization. Time in $\frac{1}{50}$ sec. in this and all records to follow. Current strength 1·0 mA. See text.

merely discharged when the polarizing current was broken. Thus on- or off-effects—to use the usual terms describing the responses to light and darkness instead of the terms make- and break-effects—were elicited according to the direction of the polarizing current. There was no absolute correlation with the polarity of the current in the sense that a micro-electrode in the nasal part of the retina would only pick up fibres responding with, say, on-effects when the nasal electrode served as cathode or off-effects with the same electrodes as anode. A couple of millimetres from a fibre discharging merely at the make of the current could be found another one discharging merely at the break of the same current. Both, however, reversed their properties when the current was reversed. The fundamental result of this work is therefore contained in the general statement that opposite currents, irrespective of the sign of the external polarizing electrode, as a rule, have opposite effects at make and break. Does this also hold for inhibition?

Before answering this question let us first recall that it is known from the work with single-fibre preparations responding to illumination that the off-effect presupposes pre-excitatory inhibition or inhibition at 'on' and that post-excitatory inhibition, i.e. inhibition at 'off', is commonly seen in certain fibres. The simplest way of demonstrating either form of inhibition is by using a spontaneously active fibre. The spontaneous rhythm is then blocked by inhibition at 'on' or at 'off'. Often both types of inhibition are mixed in the same fibre of the cat's retina, in a manner depending upon the strength of the stimulating light. Very few fibres in this eye are pure in the sense that they would respond only at 'on' or at 'off' (see Granit, 1945*a*).

Fig. 3 illustrates a spontaneously active fibre in which the spontaneous discharge is inhibited in *a*, at the make of the current, and accelerated into a vigorous off-effect at the break. In *b* is shown the response to the reversed current. There is now an on-effect at the make: a long pause of post-excitatory

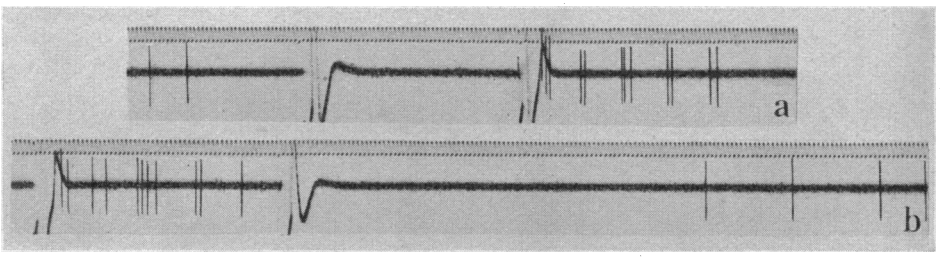

Fig. 3. Effects of polarization at 0·5 mA. See text.

inhibition follows at the break, after which the spontaneous activity is resumed. Calling excitation *plus* and inhibition *minus* we have thus a perfectly symmetrical series: current passed one way leads to make +, break −; the other way, to make −, break +. This suggests that inhibition was present in originally silent fibres too though not demonstrable in them. The work on illumination has led to the conclusion that off-effects always are a consequence of pre-excitatory inhibition (see Granit, 1946). It can thus be said that polarization has provided a sensitive test by means of which a hitherto unknown property of the retinal sensory mechanism, a kind of reciprocal relationship, has been revealed.

The opposite effects of opposite polarizing currents with respect to on- and off-discharges have not always occurred throughout the whole range of current intensities (see below). In Table 1, however, all fibres that *within some range* of current strength have given symmetrical opposite effects have been marked by + and − as above. Uncertain effects have been marked by ±. Zero signifies that the fibre has been silent so that it has not been possible to demonstrate inhibition directly.

TABLE 1. Effects of polarization of eye

No. of fibres	Current one way ↑		Current reversed ↓	
	Make ('on')	Break ('off')	Make	Break
6	+	−	−	+
5	−	+	+	−
2	+	0	−	+
2	+	0	0	+
2	0	+	+	0
1	+	0	−	0
2	+	±	±	+
1	+	−	−	±
1	+	−	0	±
1	±	±	+	±
1	±	+	+	+
1	−	−	−	−
25				

The table shows that eleven fibres (6+5 in the two uppermost horizontal rows) have shown the symmetrical distribution of excitation and inhibition, illustrated in Fig. 3. Seven more fibres (2+2+2+1) have also responded with opposite effects to opposite polarizing currents, but in them the spontaneous activity has either been lacking or else so slow and irregular that it has been impossible to be certain of the inhibitory block. Of the seven remaining fibres four have shown an indication of responding to opposite currents with opposite effects, two (two last rows) have merely given excitation *or* inhibition, independently of the direction of the current.

Effect of current strength and duration. The fully symmetrical opposite effects of opposite polarizing currents were most noticeable at low and medium strengths, i.e. from threshold values to 1·5 mA. With stronger currents the inhibitory blocks were often broken up by excitation. In many fibres, however, symmetry persisted up to the maximum, 2·5 mA. The impulse frequencies of nearly all responses were measured and plotted as a function of current strength, but the variations noted are best illustrated by a few sample records. Cases in which the full symmetry was preserved and an increase of current strength merely led to an increase in frequency of the on- or off-spikes, elicited by opposite currents, have not been included. Figs. 4 and 5 are chosen to illustrate samples in which the response also changed type. In Fig. 4 the opposite effects are clearly visible at 0·5 mA. (records *a* and *b*). But, at 2·5 mA., inhibition at 'on' still prevails in *c*, corresponding to record *a* with respect to current direction, whereas in *d*, corresponding to record *b*, an off-effect has added itself to the on-effect. At the end of the latter are shown the typical base-line irregularities interfering with recording at this strength of stimulating current.

Fig. 5 also begins with two records, *a* and *b*, at 0·5 mA., illustrating opposite effects of opposite currents. With respect to direction of current, record *c*, at 1·5 mA., corresponds to *a*, and record *d*, at 2·0 mA., to *b*. Both on- and off-excitations are seen in the latter records. Thus, above a certain current

strength, it may happen that the inhibitory effects are superseded by excitatory ones. Similar variations in response type are also seen with lights of various strength.

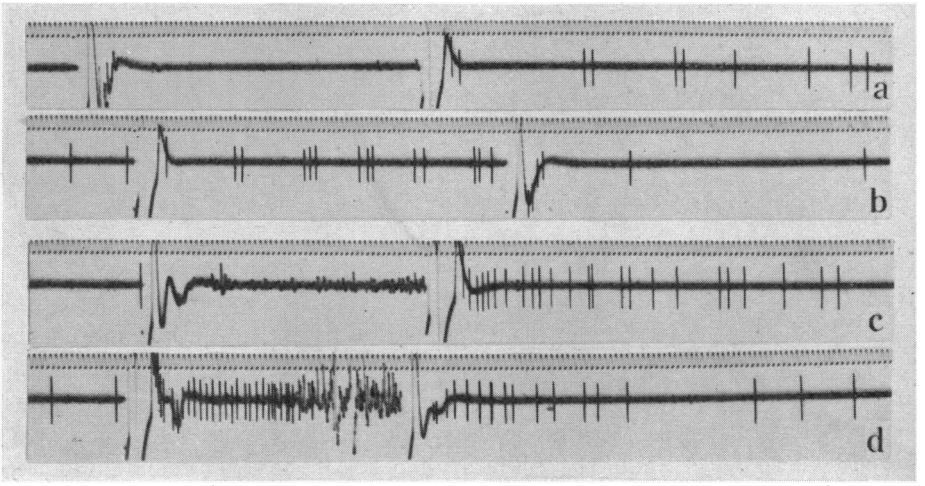

Fig. 4. Effects of polarization, fully explained in text.

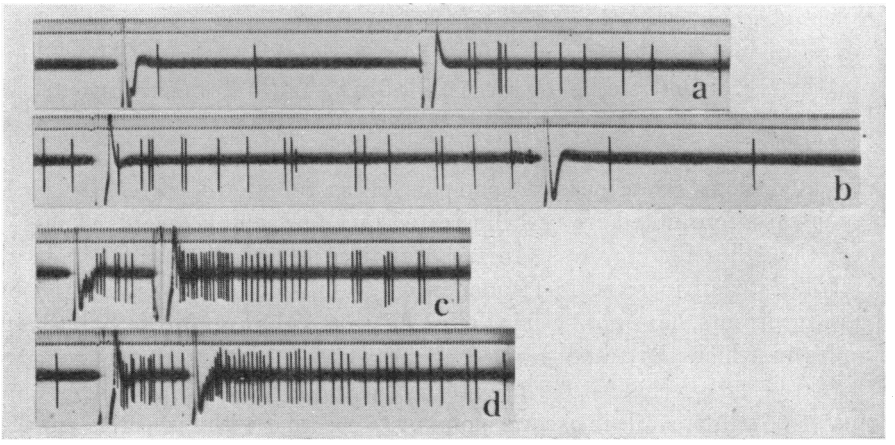

Fig. 5. Effects of polarization, fully explained in text.

The polarization device was operated manually and so did not permit shorter periods of stimulation than about 0·5 sec. From this duration to about 5 sec. there were very insignificant variations in the effects at 'off'. Sometimes the off-effect was slightly favoured, sometimes slightly diminished in frequency by an increase of stimulus duration. Mostly the off-response was independent of

stimulus duration. Parallel experiments were made with light stimuli of different durations and a general similarity was noted.

It is well known that in most cold-blooded animals the average off-effect, within limits, increases with stimulus duration, and Hartline (1938) has drawn attention to the similar behaviour of off-discharges in single fibres of frogs. In cats, however, there have been no systematic observations on the effect of stimulus duration upon the off-discharges of single fibres. Within the limits used for durations of polarization, the observations here recorded, in which the duration of illumination was varied, show that in this animal the off-effect after illumination also is relatively insensitive to this particular variation, and that different fibres behave differently. On the whole it seems as if stability of type and reaction with respect to the intensity and duration of the stimulus were a feature of the frog's optic nerve fibre in contradistinction to the variability characterizing the fibres isolated in cats.

Interference between effects of illumination and polarization. If the light stimuli are too strong relative to overlapping polarizing currents or vice versa, then the stronger stimulus breaks through and elicits its own response. But by suitably adjusting the strength of the overlapping stimuli relative to one another interference effects can easily be demonstrated. When polarization series for different strengths of current were taken in dark- and light-adaptation it was often noted that the curves correlating impulse frequency and polarization strength (in mA.) were different in the two states of adaptation. Since the optic nerve may legitimately be held to be independent of state of adaptation, these results were taken to signify that the polarizing current had stimulated structures in the retina itself.

This conclusion received further support from the experiments in which stimulation by polarization overlapped with stimulation by light. In many of these experiments it was found possible to adjust the stimuli so that excitatory or inhibitory effects, caused by illumination, modified the effects of subsequent overlapping polarization. Thus, for instance, an excitatory effect, caused by polarization, was weakened if it fell into an inhibition that had been set up by light; or, again, if polarization caused excitation, this effect could be facilitated by a similar moderate effect of preceding illumination. Such experiments proved beyond doubt that—at least at moderate current strengths—in the first instance the retina itself and not the nerve fibres had been stimulated by the polarizing current (cf. also, Granit & Helme, 1939, for direct demonstration of effects of polarizing currents of the same order on the components of the electroretinogram). A priori it would also seem probable that the threshold of the retina to polarizing currents would be lower than that of the optic nerve fibres.

CONCLUSION AND DISCUSSION

If the general aspects of the electrical theory for generation of excitation and inhibition in the retina by its own component illumination potentials are isolated from specific assumptions, the remaining view is contained in the statement that opposite slow potentials, generated by illumination, have opposite effects. Component potentials of opposite sign have actually been demonstrated in the electroretinogram (see Summary, Granit, 1946), and it has been shown that pre-excitatory inhibition in many respects parallels the behaviour of the so-called negative component PIII, whereas the positive component PII coincides with excitation both at 'on' and 'off' (Granit & Therman, 1937). Later, post-excitatory inhibition was found (Granit, 1945*a*, *b*). This has hitherto been an isolated phenomenon, but the polarization test has now shown that pre- and post-excitatory inhibition in the same discharging structural element are connected in a way that throws new light on the functional organization of the retina.

In the assembly of fibres from a given region some respond to a current which traverses the bulb in a given direction with on +, off −, others with on −, off +. These effects are reversed with a reversal of current direction. Thus there will always in any given region be fibres responding to any given current with opposite effects at 'on' and 'off'. Either or both directions of current may be represented by component potentials of the electroretinogram. At the moment we need not consider all the consequences of both alternatives. Let it suffice to point out that there has turned up in these experiments a connexion between excitation and inhibition which for the sensory field could do what Sherrington's reciprocal innervation is doing for locomotion. In addition, there is also now a demonstration of a physical process that would be capable of accomplishing the desired result with regard to reciprocity.

This reciprocity mechanism for on- and off-effects must be a matter of internal organization of the retina, but one may well ask what purpose it would serve if it were activated by light stimuli. Sherrington (1906) himself raised and replied to this question when, long ago, as if in anticipation of the present results, he pointed out that visual contrast or induction provided a striking parallel to reciprocal innervation and that both in the retina and the spinal cord such mechanisms might be analogous with the 'make' and 'break' responses to galvanic stimulation of peripheral nerve. As to recent ideas for explaining contrast, etc., along the same lines, see Göthlin (1943) and Piéron (1943). The analogy seems more perfect than could ever have been anticipated before the actual discovery of the two forms of inhibition in the retina had been made. However, unless supplemented by the information provided by the delicate polarization test, the off-effects and the two forms of inhibition would have remained totally disconnected bricks in a puzzle game. Now one is

beginning to see some physiological purpose in all these complexities. Further experiments along new lines with illumination will be necessary in order to complete the picture.

As to the comparison with peripheral nerve, recent results from this laboratory by Skoglund (1945) have shown that for nerve in catelectrotonus weak cathodal test stimulation leads to on (make) +, off (break) −, weak anodal test stimulation to on −, off +. The responses to both directions of test current are reversed for nerve in anelectrotonus. *Thus the retina behaves as if certain structures had some inherent differential polarity equivalent to that produced by the two types of electrotonus in peripheral nerve.* It is well known that there is a resting current across the intact bulb so that this idea is by no means inherently improbable.

SUMMARY

1. The eye bulb of the cat has been placed between two polarizing electrodes, and, with a micro-electrode, single-fibre discharges have been picked up from the optic nerve in response to polarization of the bulb with currents from 0·2 to 2·5 mA. Polarization has been shown to influence the retinal cells themselves. The effects, however, have always been picked up from the nerve.

2. Most fibres respond to the 'make' and 'break' of the polarizing current in an opposite manner: excitation at make, inhibition at break or vice versa. The fibres acting in this manner respond to a reversal of the current with a reversal of the effects at make and break.

3. Polarization is thus a sensitive test of properties inherent in the organization of the retinal cells. These properties suggest the presence of reciprocal innervation and thus throw new light on the general significance of the existence of pre- and post-excitatory inhibition in the retina.

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