SINGLE FIBRE ANALYSIS OF INHIBITION AND THE POLARITY OF THE RETINAL ELEMENTS

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(Received for publication April 23, 1947)

A retinal element is the sum total of receptors and neurones (bipolars, ganglion cells, horizontals, amacrines) which converge to form the "final common path" (Sherrington) that can be isolated by Hartline's (12) technique of microdissection or our own micro-electrode technique. Granit and Tansley (9) isolated 100 such elements in the cat's retina and found that the great majority of the pure on-elements consisted of converging rods whereas cones also were present in the large majority of elements containing off-components, i.e., pure off-elements and on-off-elements. Since off-effects are preceded by inhibition during illumination and again are inhibited by re-illumination, the threshold sensitivity for the off-components is a measure of inhibition which thus is somehow connected with cones (cf. also 8). The off/on-ratio at the threshold may be regarded as an index of inhibition and is a very important property of the on-off-elements which form about 80 per cent of all the elements in the cat's retina (9).

Now it has been found (7) that polarisation of the cat's retina reveals two types of on-off-element, the one responding to the onset of cathodal polarisation with an on-effect and to anodal polarisation with an off-effect, the other type having exactly opposite properties. The responses of both types of elements were completely reversed by current reversal. In general, on-responses were succeeded by inhibition at the opening of the polarising current and, vice versa, off-responses preceded by inhibition at the closing of the current. These facts raise the question as to how the properties revealed by polarisation are related to the pure on-, pure off-, or on-off-character of the elements (as defined by illumination) and, in case of the on-off-elements, to their threshold off/on-ratio. This question which is essential for an understanding of the mechanism of inhibition will be answered in the present work.

TECHNIQUE

The micro-electrode has been described in a previous paper in this journal (6) and, with more attention to detail, elsewhere (8). Decerebrate cats were used and given some 4–6 cc. of a 20 per cent urethane solution. They were left to dark-adapt with the micro-electrode inserted. Silver rods, coated with silver-chloride, served as polarising electrodes. These were thrust vertically into the nasal and temporal angular spaces outside the bulb and consequently tangentially to it. The simple polarising device, containing a 50,000Ω resistance in series with the electrodes, has been described elsewhere (7). The threshold responses to polarisation were read off on a micro-ammeter. The spikes from the retina were picked up at the inside of the eye through the opened cornea, always at the region near the nasal electrode. The terms "cathodal" and "anodal" will be taken to mean that the nasal polarising electrode has been respectively cathode or anode. The threshold currents varied a great deal, depending upon the position of the element relative to the electrode, but the absolute values are of little interest for the present problem. On an average they have been around 0.7 mA.
Results

General. Every experiment was begun by selecting a large, well-isolated spike giving a sharp report in the loudspeaker. The retina was then stimulated with one or two wave-lengths from our large Wright colorimeter, previously mentioned (6)—generally with wave-length 0.510μ (green), sometimes with wave-length 0.650μ (red) and in one experiment with 0.460μ (blue). The spikes were not photographed but the cathode ray screen was watched at the same time as the discharge was heard in the loud speaker. In this manner the absolute thresholds were determined, separately for the on- and off-components of an on-off-element in order to determine their off/on-ratio.

In our experience an accuracy of about 20 per cent can be reached in such measurements. This presupposes, on an average, 15 measurements for each threshold value, the actual number varying from 5 to 30, depending upon the preparation and the frequency of spontaneous activity of the isolated spike (9). The elements were considered to be pure with respect to their on- or off-character when, from the threshold to the full strength of the spectral light, they maintained their pure on- or off-character. With the present routine and technique it was found easy to pick up some 10–20 well-isolated spikes from each retina but some of them were lost before the necessary measurements had been completed, particularly if they were on-off-elements (80%) which required two sets of measurements.

When the visual thresholds had been determined the thresholds for anodal and cathodal stimulation (during 3 sec.) were measured. These values could be obtained with an accuracy of about 5 per cent. The present results are based on the analysis of 10 pure on-elements, 6 pure off-elements and 71 on-off-elements. They have since been confirmed in further similar measurements (4) from other points of view.

Pure on-elements. These elements all behaved similarly. They responded to cathodal polarisation at the threshold with an on-effect. When the polarising current was reversed it was necessary to use a relatively stronger current in order to excite. The threshold response to the anode was a pure off-effect. Calling the threshold current for the cathodal on-response – on, the corresponding current for the anodal off-response + off, the ratio – on/+ off = 0.65 ± 0.08, a figure illustrating the average electrical asymmetry of the on-elements. The extreme values were 0.48 and 0.78.

Pure off-elements. All the 6 off-elements were of opposite polarity. The cathode gave a threshold off-response, the anode an on-response. These elements thus behaved as if they had been “reversed” on-elements. But they differed from the pure on-elements in being perfectly symmetrical with respect to the threshold currents for “on” and “off.” The average ratio + on/– off = 1.01 ± 0.05, the extremes being 0.88 and 1.07.

On-off-elements. For these the light thresholds acquired special significance because of the off/on-ratio. On-off-elements may be highly off-sensitive or highly on-sensitive or, again, the on- and off-components may have almost
identical light thresholds. Granit and Tansley (9) discovered the enormous range of variation in their off/on-ratio. For the present material a distribution curve is given in Figure 1. The ordinates are number of elements of each type, grouped together in logarithmic steps on the abscissa, which illustrates that the actual off/on-ratios have varied between 0.001 and 10,000. Thus the highly on-sensitive elements are found to the left, the highly off-sensitive elements (containing marked inhibitory components) to the right, those of equal sensitivities for "on" and "off" being, of course, at the point marked 1. The distribution may to some extent be influenced by the fact that some of the off/on-ratios were determined with different wave-lengths. This, however, is not of great importance because Granit and Tansley (9) showed that the off/on-ratios for 60 elements, measured with blue and red light and plotted against each other, gave a correlation coefficient of 0.96 and a regression coefficient as high as 1.19.

In the graph of Figure 1 the black part of a column signifies that the on-responses were cathodal, the white part that they were anodal. This means, of course, also that for the black part the off-responses were anodal, for the white part cathodal, because on- and off-thresholds had opposite polarities. It is further seen that the extremely off-sensitive elements (to the right) behaved like pure off-elements (cathodal "off"). The vertical dividing line is drawn at the point for which the off/on-ratio = 1.4. This was the dividing line in the sense that all elements (except one) with a higher off/on-ratio responded with cathodal off-effects, to the electrical stimulus as if they had been pure off-elements (though this fact is somewhat obscured by the logarithmic grouping of the material). The on-sensitive elements, however,
were less homogeneous. The majority of them—32—reacted as pure on-elements in the sense that they gave cathodal on-responses but 13 behaved as the pure off-elements and gave anodal on-responses (= cathodal "off").

It will be recalled that the pure on-elements were electrically asymmetrical whereas the pure off-elements were symmetrical. The on-off-elements, by this criterion, have more in common with the pure off-elements since they too were symmetrical. The average ratio of the polarisation thresholds for "on" and "off" was 0.99 ± 0.047.

**Discussion**

In order to understand these results it is necessary to understand how the off-components, which presuppose inhibition during illumination, arise. An on-component does not present the same difficulties. All receptors produce on-responses to stimulation and the only known retina which undoubtedly is non-synaptic, that of *Limulus*, was long ago found by Hartline and Graham (13) to respond with pure on-effects. Pure on-effects also dominate in the rod-retina of the guinea pig (5, 8) which photochemically is wholly dominated by visual purple and does not possess the photopic dominator. Similarly the pure on-elements of the cat's retina are dominated by rods and visual purple (9). The rod-retina is apparently a simple system, summing up the direct effects of stimulation, responding to cathodal stimulation with an on-effect and being less prone to give the opposite anodal off-response. Such systems might be found anywhere in peripheral nerve and have been known since the days of Pflüger.

In the vertebrate retina the on- and off-effects are preceded by slow potential changes capable of doing what the electrical stimuli are doing in the polarisation test. Long ago Granit and Therman (10) showed that when an off-effect was inhibited by re-illumination this inhibition was accompanied by the abrupt onset of the component potential PIII of the retinal electrical response and, in an analysis of latent periods, Bernhard (2, 3) proved conclusively that the swing-back of this potential preceded the release of the off-effect. Previously Granit and Therman (11) had found that the off-effect itself (in frog eyes) was accompanied by a renewed rise of the opposite component potential PII, possibly elicited by the swing-back of PIII. Since PII also preceded the on-effect, this component therefore accompanied excitatory changes at "on" and "off," whereas the onset of the opposite PIII only could be shown to accompany inhibition; its release, however, gave excitation at "off" together with reactivation of PII. In the eye the inside is positive to the back of the bulb with PII, negative with PIII (see 8).

In the single axon preparation of Sepia Arvanitaki (1) has described what might be called the prototype of such systems. This nerve generates a local negative potential at the cathode and a local positive potential at the anode. In these local responses the nerve is actively engaged. If current strength is increased the local potentials increase until suddenly a discharge is elicited. This discharge occurs at the onset of the cathodal local potential
and at the cessation of the anodal stimulus as an off-effect when the positive anodal potential swings back, overshooting the zero line and in so doing sets up the necessary negative local response. (For details see 1).

In view of what is known about the components of the electro-retinogram it is not surprising that in our experiments the pure on- and off-elements should have opposite polarities. On the contrary, it is what one would expect. But new problems arise when we ask for the reasons why some systems are cathodal, other anodal. It would solve several difficulties if we accepted the attractive suggestion (7) that the systems might be differently polarised by the resting current of the eye. However, recent evidence has emphasized another and in many ways more satisfactory possibility which need not necessarily exclude differential polarisation. Granit and Tansley (9) and Gernandt (4) proved conclusively that the on- and off-paths of an on-off-element, picked up by the micro-electrode in the cat’s retina, can be initiated by different receptors and come together at some synaptic station in the retina. Similarly the enormous range of variation in the off/on-ratio (see also Fig. 1) can only be explained if the on- and off-components really are unrelated in the sense that their threshold properties are determined by different receptors joined to the common “element” by, for instance, horizontal and amacrine cells or other associational paths. An “element” thus consists of direct and indirect paths. The off-response is on this view the consequence of a preceding block or inhibition exerted by certain indirect paths upon other direct paths within the same element and the whole on-off-system part and parcel of the contrast interplay between various paths, as further illustrated by Gernandt’s (4) work on polarisation and colour thresholds.

These new experimental facts suggest explanations of our results in terms of anatomical orientation. To cathodal polarisation the pure on-elements were found to respond with an on-effect, the pure off-elements with an off-effect. We know from the earlier work on polarisation of the retina (7) that the opposite pole in all such tests is inhibitory. If it is a matter of orientation, then the receptors of both pure on- and pure off-elements are similarly stimulated at the onset of cathodal depolarisation, say, at the initial station where the local cathodal response is generated. The direct path always lets through this on-response. But the indirect path through horizontal and amacrine turns its anode, generating the opposite anodal local response, towards the direct path and blocks it. The response of the indirect path to cathodal stimulation as well as to light will therefore be inhibition succeeded by an off-response at the release of the block. Its threshold, however, will be determined by the real cathodal on-threshold just as if it had been an on-element. Current reversal reverses the poles and accordingly causes indirect excitation through the same horizontal and amacrines and is therefore really a cathodal threshold at the contact point of the associational fibres, though anodal when referred to the polarising electrodes. This could explain why the electrical off/on-ratios for cathodal and anodal thres-
holds for pure off- and for on-off-elements fall around 1.0. These elements are, as we saw, all symmetrical and in this respect differ from the pure on-elements which are asymmetrical. Horizontals and amacrine cells thus serve as "commutators," making use of the blocking properties of the anode to produce the inhibition-off-effect system combined with the component PIII of the electro-retinogram. The number of such commutators is one of the main factors determining the off/on-ratio. The on-off-elements represent different combinations of direct and indirect paths. They become pure off-elements when the number of indirect commutator paths is sufficiently high but by electrical stimulation we can always excite the blocked direct paths to give the on-effect that light does not succeed in eliciting. The on-off-elements may also possess a number of on-components always inhibited by light, which give anodal on-responses to polarisation.

The alternative explanation (7), based on differential polarisation with consequent differential sensitivity to "on" and "off," need not be excluded by the present theory. If the earlier suggestion (7) were correct it would mean that anodal on-effects need not be indirect nor anodal off-effects direct, as postulated above.

In his comprehensive work on the retina Polyak (14) also suggests that the retinal associational cells (he mentions the horizontals) might act as "countersynapses." We have here indicated how such a system might work in order to explain such fundamental facts as the opposite polarities of the on- and off-elements and their connection with, respectively, excitation and inhibition as well as the properties of the two types of on-off-element. It is worth while adding that the excitatory component PII of the electro-retinogram can be led off from the optic nerve whereas the inhibitory PIII cannot (2, 3). If PII is directed to or originated in the associational cells whilst PII electrotonically is conducted straight down to the ganglion cells, their different accessibility in the optic nerve can be understood.

**Summary**

Single fibre responses have been isolated from the nasal region of the cat's retina with the micro-electrode technique and tested by illumination and polarisation of the retina between fixed electrodes.

The pure on-elements respond to threshold cathodal polarisation with an on-effect. An increase of current strength by some 30 per cent elicits a threshold anodal off-effect.

The pure off-elements (inhibited by light) have opposite polarity. They respond to cathodal polarisation at the threshold with an off-effect, to anodal stimulation of the same strength with an on-effect (not elicitable by light).

The on-off-elements are either "cathodal" or "anodal," depending upon their off/on-ratio determined by illumination at threshold strength. They are held to be combinations of on-paths and off-paths.

The results form the basis of a simple explanation of the nature of inhibition in the retina in terms of the horizontal and amacrine cells.
ACKNOWLEDGMENT

The authors desire to acknowledge a grant from the Rockefeller Foundation to the Nobel Institute for Neurophysiology.

ADDENDUM

In a theoretical paper, of which an advanced copy was communicated personally to the senior author, J. C. Eccles has recently developed a theory of inhibition which is in essential agreement with the results and ideas reported above. The manuscript was received when this paper had been written but because of its importance it was felt desirable to draw attention to his views in this manner.

REFERENCES