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The Effect of Illumination upon the Sensitivity of Isolated Retinal Elements to Polarization.

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In the first observations on the effect of polarizing currents on the discharge of isolated elements in the cat's retina (GRANIT, 1946) it was noted that just as polarization influenced the threshold to light so also did illumination change the threshold necessary to elicit a discharge by polarization. Neither effect was seen with all elements. The influence of polarization upon stimulation with light has since been analyzed from various points of view by GERNANDT (1947) and GRANIT (1948 a, b). This paper will briefly describe some results obtained in the reversed experiment when the threshold of polarization was measured both in the dark and *during* illumination with light of known multiples of the threshold effect.

Four questions have been raised in the present experiments. (i) Can illumination depress the electrical threshold? (ii) Can illumination facilitate the electrical threshold? (iii) Can anodal elements become cathodal or, *vice versa*, cathodal ones anodal under the influence of illumination? (iv) Do the electrical and light thresholds after illumination return to normal along similar curves?

Method.

The fully dark-adapted eye of the decerebrate cat was used together with the micro-electrode technique for isolation of retinal elements in the manner developed in this laboratory and several times described (*e. g.* GRANIT, 1947). Polarization electrodes were inserted in the nasal and temporal corners of the eye. The anodal elements then respond to the make of the anodal current, the cathodal ones to the make of the cathodal current. The micro-electrode picks up the discharge on the retina just inside the nasal electrode which determines the polarity. Current reversal produces off-discharges in both types of elements at cessation of polarization, in both cases also preceded by inhibition. Similarly the on-effects to the make of the current, for both types of element, are succeeded by inhibition at the break (see particularly GRANIT, 1948 a).

The experiments were begun by determining the polarity of the element isolated, *i. e.* by finding out whether the on-effect was elicited by the anode or the cathode, as stated above. Then the thresholds in milliamperes for both anodal and cathodal effects were measured for 3 sec. of polarization. This must not be done too rapidly or the elements are liable to vary in sensitivity to the current. Sometimes the thresholds varied too much for quantitative work but generally they did not do so and could be used in the experimental analysis. The next step consisted in measurements of the light thresholds at "on" and "off" for 3 sec. exposures to some wave-length from our Wright colorimeter (WRIGHT, 1946), mostly 0.510 μ, but other wave-lengths were also tried. The off/on-ratio at the threshold varied from element to element as pointed out before (GRANIT and TANSLEY, 1948). When afterwards the eye was illuminated with a stimulus 1,000 times the threshold-light this factor was always based on the more sensitive of the two components. Occasionally stronger non-spectral stimuli were used, generally the Ilford spectral filters for red, green and violet, placed in a beam of 800 m. c.

The polarization device, with its commutator for changing the direction of the current and a high resistance in series with the eye, was switched on and off by hand and so it was impossible to measure the polarization thresholds *during* illumination very quickly. However, when large changes were obtained they were generally noted already within the first minutes of illumination. The element refused to respond to the polarizing current that had elicited a discharge in the dark-adapted eye.

The eye was kept illuminated for 10—20 min. during which final values were obtained for the change in the electrical threshold. Then followed recovery in the dark and measurements of the light and polarization thresholds until both had become normal. If the thresholds did not recover the results were considered unreliable even though, to all appearance, the spike was as perfect as in the beginning.

Results.

We can describe the results very briefly because their interpretation is so difficult that very little is gained by giving more details than are necessary for answering the questions raised

above. In all, successful measurements were carried out with 28 elements.

(i) Illumination at 1,000 times threshold strength can raise the threshold to polarization in all types of element but does not necessarily do so in all elements. Some remain uninfluenced but in many of the uninfluenced elements a definite decrease of electrical sensitivity was noticed when stronger lights were used (8—800 m. c. or Ilford filters in the 800 m. c. light beam). Others withstand strong illumination without change of electrical threshold. The amount of change varies so much from element to element that it proved impossible to unravel any correlations with respect to type of element or off/on-ratio. The typical effect of illumination was a depression of the electrical threshold which generally was instantaneous but sometimes the threshold decreased or increased during continued illumination. In this material the anodal elements proved to be more sensitive to the effect of illumination on the polarization threshold than the cathodal ones.

Some examples will show the order of magnitude of the maximal changes when both the on- and the off-component were affected symmetrically. The letters D and L will be used to denote the dark- and light-adapted states respectively. The values are given in milliamperes. 0.05D—0.1L, 0.075D—0.50L, 0.3D—0.7L. These three elements were anodal and illuminated at 1,000 times the threshold. There were four pure on-elements but in all of them the effect was small or absent though in one definite. The on-elements, as pointed out before, are cathodal (GERNANDT and GRANIT, 1947). The effects were generally small or absent in the cathodal on/off-elements too but it proved possible in most of them, by increasing the strength of illumination, to obtain definite and easily measurable drops in the electrical thresholds. The following are examples of such cathodal elements for which stimulus strength had been increased by using the Ilford spectral green filter: 0.075D—0.35L, 0.2D—0.35L, 0.25D—0.43L. The transmission of the 800 m. c. stimulus is reduced to about 10 % by the Ilford green filter.

(ii) Can facilitation of the electrical threshold be demonstrated? Whereas the polarization thresholds several times proved to be uninfluenced by illumination it was difficult to be quite certain about the existence of facilitations. It is true that there were some cases in which facilitation was noted but the effects were small. Perhaps the safest statement at the moment is that facilitation seems probable (see below).

(iii) Do shifts of polarity occur? Such shifts are sometimes regularly obtained, indeed, a modest degree of asymmetry of the effect of illumination on the opposite poles needed for make- and break-discharges is quite common. Greater degrees of asymmetry amounting to complete shifts of polarity have also been seen. Since these cases are very interesting some examples will be given.

An anodal element with an off/on-ratio of 1.8 at wave-length 0.510μ gave the characteristic anodal make- and cathodal break-discharge to polarization between 0.20—0.25 mA. Immediately after illumination with the test light (1,000 times the threshold) the element refused to respond to polarization with 0.5 mA. Somewhat later, during polarization, it showed reversed polarity and responded with a *cathodal* make- and break-discharge to 0.38 mA and occasionally with an anodal break-discharge to 0.25 mA. There was full recovery in the dark and the electrical threshold also returned to normal. The element again became anodal. Repetition of the illumination experiment, this time performed at wave-length 0.460μ at 1,000 times threshold strength. Complete reversal of polarity with cathodal make- and anodal break-discharge already for 0.15 mA after 4 min. of illumination. Afterwards complete recovery of light threshold and the polarity swung back to normal together with the return of the original polarization threshold. The element again became anodal and required 0.25 mA to respond. This is one of the cases where also a real facilitation of the polarization threshold seemed probable.

A cathodal element with off/on-ratio 16. Before illumination the polarization threshold was 0.2 mA. At this strength the element was giving a good cathodal make- and an anodal break-discharge. Illumination with Ilford spectral violet filter in the 800 m. c. light beam. The polarization threshold fell to 0.5 mA; there was now anodal make- and cathodal break-discharge Thus complete reversal of polarity. Full recovery afterwards in the dark. After 5 min. the element again became cathodal and responded to 0.25 mA with strong typical effects.

A third element that reversed its polarity did not return to normal after illumination and maintained its shift of polarity. The off/on-ratio also changed from 0.042 to 0.11 but since this

took place at a higher absolute level of sensitivity to light it was impossible to conclude that the element had been damaged in the course of the experiment. The spike was perfect all the time. There were no adjustments necessary to maintain the spike in the course of the experiment and a very large number of readings were taken. It was several times illuminated and always made a rapid recovery.

Since in addition several elements underwent minor shifts of polarity we conclude that illumination can re-balance certain elements suggesting very definitely that the on/off-element consist of anodal and cathodal components either of which may be suppressed or, perhaps, enhanced by illumination. To be noted is that all these effects are obtained with illuminations of relatively modest strength. With strong lights we have sometimes seen very large depressions of the electrical threshold.

(iv) The relative rates of recovery of light and polarization thresholds may vary from element to element but the general rule noted was that the polarization thresholds recovered very quickly and long before the light thresholds, particularly in cases were the latter underwent large depressions leading to delayed recovery (GERNANDT, 1948).

Finally remains to be mentioned that the changes of the polarization thresholds did not necessarily require changes in the spontaneous frequency of the discharge. The spontaneous rhythm did, of course, change very often as a consequence of illumination but in some cases this effect was modest or absent, in others the rhythm re-established itself later during illumination without concomitant changes in the polarization thresholds. The change in the latter were not therefore secondary to the variations in the spontaneous frequency.

Discussion.

Since light elicits large changes of potential in the retina it seems natural to compare the effects of illumination upon the polarization thresholds with the effects of polarization on the electrical thresholds of nerves. These are known to be considerable. It is well known from psychophysical work that the state of adaptation influences the electrical thresholds (see *e. g.* SCHAEFER, 1942) and the essential question raised by this work is therefore whether the analysis of single elements can add anything to our knowledge of such phenomena.

In this respect there is an interesting parallel with the reversed type of experiment dealing with the effect of polarization upon the light threshold. In these experiments too (GRANIT, 1948) the light thresholds of the anodal elements were highly sensitive to polarization, those of the (always) cathodal pure on-elements practically not at all, and those of the cathodal on/off-elements were generally difficult to influence, but a minority of the cathodal elements was as sensitive as some anodal ones. In the anodal ones it was particularly easy to elicit strong inhibition of the light threshold by polarization with the depressing (cathodal) pole.

All these results were interpreted to signify that the simple cathodal on-elements were deficient in structures sensitive to polarization, the anodal elements amply supplied with such structures, the majority of the cathodal on/off-elements being more like the pure on-elements whilst the minority behaved like the anodal ones and possessed these structures.

From this point of view it seems of fundamental importance that only a certain number of the elements, and particularly some anodal ones, are so designed that the modest light adaptation used in these experiments easily raises the electrical threshold. This result suggests that the direct path through receptor, bipolar and ganglion cell, shared by all elements, is deficient in structures which are capable of being influenced by modest light adaptation so as to make the element relatively more insensitive to electrical stimulation.

Clearly the direct path through receptor, bipolar and ganglion cell is neither very sensitive to polarization (GRANIT, 1948 a) when tested with the light threshold nor is its polarization threshold particularly sensitive to the changes set up by illumination. The former experiment was easier to perform and so it could be shown that the curves relating light thresholds to polarization strength in the case of the anodal elements differed fundamentally from those of the pure on-elements which could be regarded as prototypes of pure cathodal elements (GRANIT, 1948 a).

In both types of analysis the anodal on/off- and pure off-elements appear to be extremes representing one type of design whereas the majority of the cathodal on/off-elements tend to approach the cathodal pure on-elements as the other extreme.

The latter seem to be deficient with respect to some structures possessed in abundance by most anodal elements. The earlier experiments on the effects of polarization upon the light thresholds suggested that these structures must be internuncials. Accordingly these internuncial neurones of the retina appear as structures which, when polarized, easily set up potential changes influencing the light thresholds or *vice versa*, when illuminated, easily set up potential changes with the result that the electrical threshold is raised. Light and polarization are to some extent interchangeable with respect to the internuncials with which the anodal elements are particularly well supplied.

These results are thus interpreted to mean that certain internuncial cells represent an organ designed for the production of electrical potential serving the ultimate purpose of directing and controlling the internal switchboard and thereby also the off/on-ratio. This mechanism was used by one of us (GRANIT, 1948 b) to study the colour sensitivity of the retinal elements.

The fact, that the polarization thresholds returned to normal after light adaptation before the light thresholds did so, shows that what might be called "photochemical sensitivity" is something very different from what is being measured by the polarization threshold. Hence the depression of photochemical sensitivity may persist after the time when the electrical test shows that the "internal switchboard" is functioning normally.

The cases in which a shift of polarity were noted seem to us valuable as a warning against too much schematization. They show that some elements contain both anodal and cathodal components and that definition by polarity thus to some extent depends upon what components happen to be in the majority. There is apparently a *zone of overlap* of cathodal and anodal properties between the theoretically pure anodal and cathodal elements. Whether such shifts of polarity are due to blocking potentials or to active withdrawal of certain synaptic knobs under the influence of electrical forces we do not know. The second possibility should not be left out of sight.

Long ago one of us (GRANIT, 1938) introduced the concept "electro-adaptation" to account for the fact that the light-adapted frog's retina had a more prominent negative component than the dark-adapted one and that certain other potential changes characterized light adaptation. The idea that potential changes can re-balance an organ such as the retina is therefore not based on the present results alone. (For further discussion, see *e. g.* GRANIT, 1947.)

Summary.

Polarizing electrodes were placed in the nasal and temporal corners of the eye of the dark-adapted decerebrate cat and the threshold discharge of retinal elements to polarization and illumination was measured by studying the spikes isolated by a micro-electrode in the eye.

In certain types of element the threshold to polarization rose when the eye was illuminated at 1,000 times threshold strength, in others not.

Some elements that in the dark had a lower threshold for anodal currents became more sensitive to the cathode during illumination and, *vice versa*, some elements with lower thresholds to cathodal stimulation became more sensitive to anodal stimulation during illumination.

After illumination the polarization thresholds often returned to normal before the thresholds to illumination did so.

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References.

GERNANDT, B., J. Neurophysiol., 1947. *10.* 303.
GRANIT, R., Documenta Ophthalm., 1938. *1.* 7.
GRANIT, R., J. Physiol., 1946. *105.* 45.
GRANIT, R., Sensory Mechanisms of the Retina. London, Oxford University Press, 1947.
GRANIT, R., J. Neurophysiol., 1948 a. *11.* 239.
GRANIT, R., J. Neurophysiol., 1948 b. *11.* 253.
GRANIT, R. and K. TANSLEY, J. Physiol., 1948. *107.* 54.
SCHAEFER, H., Elektrophysiologie II, Vienna, 1942.
WRIGHT, W. D., Researches on Normal and Defective Colour Vision. Kimball, London, 1946.