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AN ANALYSIS OF THE RESPONSE FROM SINGLE VISUAL-PURPLE-DEPENDENT ELEMENTS, IN THE RETINA OF THE CAT

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Hartline (1935) first showed that, on functional grounds, the optic nerve fibres of vertebrates could be divided into three main classes, according to whether they respond to the onset or the cessation of illumination or to both. He showed the existence of 'on-', 'off-' and 'on-off'-elements. Granit (1947), Granit & Tansley (1948) and Gernandt (1949) have studied the nature of such elements in the eyes of mammals, particularly of the cat, and have shown that elements may behave differently according to the wave-length and the intensity of the stimulating light. The recent work of Donner & Granit (1949), in which the light stimulus has been measured not so much in terms of absolute energy units, but in terms of its effects on the bleaching of visual purple, opens up new lines for investigation.

Comparison between the incidence of on-off-elements in the almost pure rod retina of the guinea-pig and their occurrence in the cat, in whose retina cones are more numerous, led Granit (1947) to the opinion that cones must participate in the production of the majority of the on-off-elements, and that the latter resulted from a stimulated element being antagonized or inhibited by another. Granit & Tansley (1948) came to a similar conclusion from studies of the 'off-on' ratio, and again considered that the inhibition was largely, though not exclusively, associated with cones. Donner & Granit (1949) have since shown that in the cat there are also 'on-off-elements' which act as if they are pure visual purple elements.

It is possible that off-effects may not only arise as the result of release from inhibition by another element; for it is theoretically possible that they could also arise, for example, from the effects of adaptation within a single cell or fibre. Against this idea, however, it has to be noted that certain elements which show adaptation quite strongly do not show any off-effects. Secondly, there are some elements which, at threshold, appear to depend only on visual purple but which, at higher intensities, show off-effects, and these off-effects are sometimes more potent at the red end of the spectrum, even though the light stimuli are equal in terms of their effects on visual purple (equal V.P. stimulation). Such elements therefore must receive contributions from elements other than those dependent only on visual purple and it seems probable that, at least in these cases, a visual purple-dependent receptor is inhibited by another element which depends on some other photosensitive pigment, and which is probably a cone. The strong suggestion is, therefore, that off-effects do, in fact, mean interaction between two groups of cells and the occurrence of inhibition; but it would be a mistake in the present state of knowledge to lose sight of the other possibilities. For example, off-effects occur in the eye of *Pecten* (Hartline, 1937; Shoepfle & Young, 1936), though the probability of interaction between different neural elements appears to be somewhat remote on structural grounds. It may, however, occur. For the present, it will be assumed as a working hypothesis that off-effects are symbolic of inhibition of one element, or group of elements, by another, but it is not intended at this stage to suggest that all off-effects must be due to this cause. A reduction of the on-discharge, compared with the on-discharge of a pure on-element, particularly when coupled with the presence of an off-effect, will, similarly, be assumed to be evidence for inhibition.

The introduction of the equal V.P. stimulation simplifies the investigation of these elements which show off-effects, and the experiments reported in this paper were designed to study the nature of the response from those ganglion cells, or optic nerve fibres, in the retina which were activated directly or indirectly by receptors dependent on visual purple only. The results are of interest, partly in demonstrating the very great variation in the responses from different ganglion cells, partly in emphasizing that this variation may occur even though the photoreceptors themselves are all activated by visual purple and partly in providing further evidence that inhibitory processes are involved. Because of the very great differences between the responses from the ganglion cells, the results must be considered as indicative rather than exhaustive.

METHODS

All experiments were performed on isolated elements in the retinae of decerebrate cats, prepared by the standard technique (Granit, 1947). After decerebration, the cat was left in total darkness for about 90 min. so that apart from the effects of the test stimuli the retina was in the dark-adapted state and the preparation was in a fairly stable condition. After decerebration, 4–6 c.c. 20% urethane were given, to abolish small movements of the eye.

Each element, after isolation with the micro-electrode, was tested (equal V.P. stimuli) at $\lambda=460$ mμ. and at $\lambda=620$ mμ., sometimes also at $\lambda=500$ mμ. and other intermediate wave-lengths. The intensities used were adjusted to have an equal bleaching efficiency on visual purple. For that purpose the curve determined by Donner & Granit (1949) for pure on-elements was used. The Wright colorimeter was used as the source of these monochromatic radiations. If the response was not demonstrably different at these different wave-lengths when tested at two or three different levels of intensity (multiples of the equal V.P. units), then it was assumed that visual purple was the only photosensitive substance involved in the sensory layer. In the analysis of some of the elements, complete curves have been plotted from records obtained at several different wave-lengths and these were not significantly different. In these cases, therefore, there was certainly no material contribution made by any elements other than those sensitized by visual purple. Indeed for all the elements from which the data are discussed in this paper there was no evidence to indicate that anything besides visual purple was sensitizing the element or modifying its activity. Except in one or two series of experiments, each element was subjected to flashes of light lasting for 3 sec. The response as seen, after amplification, on the cathode-ray tube was photographically recorded for 1 sec. before illumination, for 3 sec. during illumination and for sufficiently long after the cessation of the stimulus for the majority of the after-effect, if any, to be recorded. The frequency of discharge of impulses in each 0·2 sec. period was then determined and the results plotted in graphical form. During the first 0·2 sec. period after both the onset and cessation of the stimulus the frequency changed very rapidly and the impulses were counted in 0·1 sec. intervals. The results are expressed as frequency per sec. The curves as shown have all been reduced by subtraction of the spontaneous discharge of the element under investigation, the spontaneous activity being determined before each test.

RESULTS

On-elements

In order to be able to determine whether or not, or to what extent, inhibitory processes are contributing to the response from an isolated element, it is necessary first to study those elements which give pure on-discharges, as these are almost certainly the simplest type of element, and, in them, interference from other elements is probably minimal.

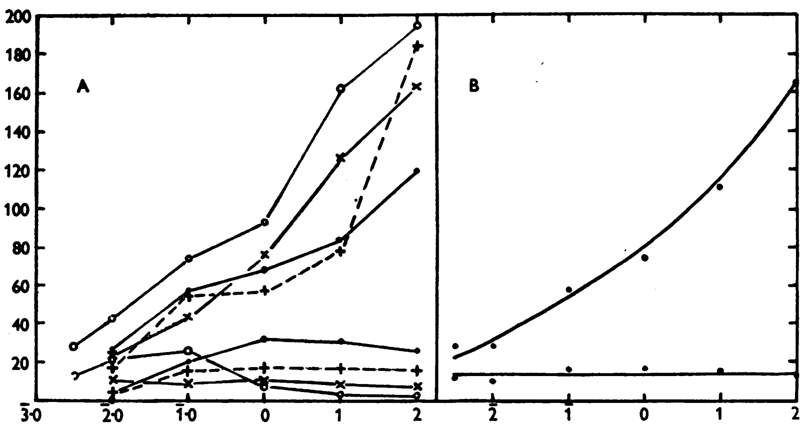

Fig. 1. Relationship between the frequency of impulses during the on-discharge and the light intensity in four different elements. Ordinates: impulses per sec. Abscissae: light intensity in logarithmic units. Upper curves: initial discharge. Lower curves: discharge after 3 sec. A, individual elements; B, mean values.

The responses from four such elements have been recorded and the following conclusions can be drawn. The magnitude and the character of the response vary with the intensity of the stimulus, but with equal V.P. stimuli, they are independent of wave-length. The initial outburst of impulses when the stimulus is applied shows a frequency which varies almost linearly with the logarithm of the intensity of the stimulus (Fig. 1). After 0·2–0·3 sec., however, the frequency declines rapidly, and at the end of the 3 sec. period of stimulation is much more nearly independent of the intensity of the stimulus, though the elements differ to some extent in the magnitude of this delayed or 'equilibrium' response. A stimulus lasting only 3 sec. is rather short and may not allow a precise determination of the asymptote and so of the exact value of the 'equilibrium' response. Moreover, although some elements may behave as pure on-elements at low intensities, they often show a change of character at high intensities, and the final response at high intensities may be partly determined by inhibitory processes. Certainly, in some cases an off-effect may suddenly appear at high intensities. Fig. 2 shows the frequency of response from one element at five different intensities and also the final phase of the response from another similar on-element at a relatively high intensity. This last clearly shows an off-effect.

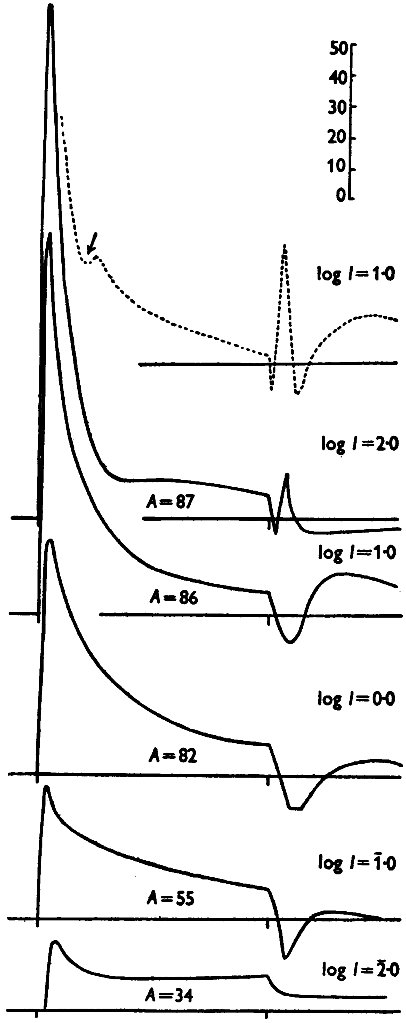

Fig. 2. Impulse frequency in the discharge of on-elements at different light intensities. Ordinates: impulses per sec. Abscissae: time; the duration of the stimulus being 3 sec. The continuous curves are all from the same element; the dotted curve is from another element. *A*, area enclosed by the curve representing the on-discharge. The arrow indicates the beginning of a notch in the curve which in some elements becomes, at high intensities, much larger than the one shown.

It seems likely that the rapid decline in frequency while the stimulus is maintained is more of the nature of an adaptation process than one caused by inhibition from neighbouring elements, but in a complicated structure like the cat's retina the possibility of inhibition cannot be eliminated. In general, the higher the initial frequency the more rapid is the decline, and it may be of interest to observe that in the element for which the frequencies are recorded in Fig. 2 the area enclosed by the curve depicting the frequency during the on-discharge is almost identical for the three highest levels of intensity. It is as though a maximum amount of activity can be evoked, and if this activity occurs early, i.e. in response to a strong stimulus, then it must decline quickly. On the other hand, a less intense initial discharge can be maintained for longer at a relatively high level. The area within the curve, of course, represents the total number of impulses in the discharge.

When the stimulus is removed, there is generally an immediate decline in frequency, and the latter may fall below the level of the spontaneous discharge, only to recover again quickly and then slowly decline once more. It seems probable that some sort of inhibitory influence is at work to cause this depression of the response at 'off', but it must clearly be of a transitory nature so that the element almost immediately begins to discharge again and the 'after-discharge' continues to abate slowly. As already indicated and shown in Fig. 2 if the light stimulus has been very strong then, instead of a 'silent' period at 'off', a brief off-effect may occur within the period during which, at lower intensities, the discharge was suppressed at the cessation of the stimulus. The appearance of such an off-effect is sometimes correlated with appearance of a notch (see Fig. 2) in the curve depicting the frequency during the on-discharge. How much these effects are due to inhibitory processes from other cells and how much they depend on the properties of the receptor, bipolar cell and ganglion cell of the direct path cannot at present be determined.

On-off-elements

While there is a general similarity in behaviour of the four on-elements studied, on-off-elements show much greater differences in their responses to the same stimuli.

The on-discharge. From what has been said in the section on 'on-elements', it is clear that even the so-called pure on-element is by no means certainly a simple element and may become an on-off-element if the stimulus is sufficiently intense. In the elements investigated the necessary intensity to produce an off-effect has in some cases been more than one thousand times the intensity necessary to produce an on-discharge. Possibly even higher values may sometimes be required. On the other hand, Fig. 3 A shows an element which at low intensities gives a pure on-effect without even an after-discharge, but whose response is converted into a definite on-off type by raising the intensity of the stimulus only one hundred times.

Furthermore, elements which begin as on-off-elements at low intensities may have their on-discharges entirely suppressed at higher levels and so become off-elements (Fig. 3 B). It appears therefore that the differences between on-, on-off-, and off-elements are largely determined by the balance set up in the elements by excitatory and inhibitory processes. As the latter becomes more prominent so the off-effect becomes more pronounced. In a typical on-off-element, and while the intensity of the stimulus is low, the on-discharge increases progressively with intensity at about the same rate as that of a pure on-element, but, as the intensity is raised, there comes a time when the on-discharge ceases to increase and may even fall (Fig. 4). Presumably the depressant effects of inhibition becomes greater at that point than the augmenting effects of the direct stimulus. Although the inhibitory process is eventually effective in preventing an increase or causing an actual decrease in the initial on-discharge it gives the impression of being more effective in reducing or even eliminating the delayed or 'equilibrium' response. Fig. 5 shows clearly how the delayed response is reduced at the higher intensities while the initial discharge

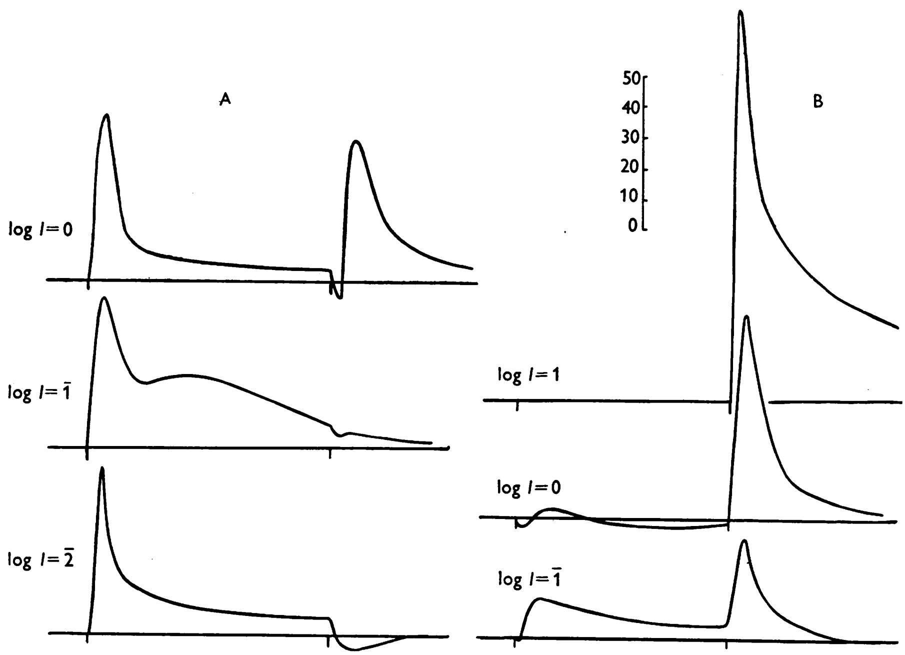

Fig. 3. A, discharge of an element which changes from the 'on' type to the 'on-off' type as the intensity of the stimulus is raised. B, discharge of an element which changes from the 'on-off' type to the 'off' type as the intensity of the stimulus is raised. Ordinates: impulses per sec. Abscissae: time, the duration of the stimulus being 3 sec.

may be still on the increase. The point, however, is difficult to assess since the delayed response of a pure on-element becomes relatively and sometimes absolutely less important the higher the intensity of the stimulus. As a result of this effect of inhibition on the delayed response, some on-off-elements only discharge for a short time at 'on' and then again at 'off', with a long silent period while the stimulus remains in action (Figs. 5, 6).

An attempt has been made to analyse how the inhibitory process acts on the on-discharge by comparing the average discharge of several on-off-elements at different intervals after the onset of the stimulus, and at different levels of light intensity with the corresponding discharges in 'pure' on-elements. The highest intensities have not been used because, with these, the so-called 'pure' on-elements may suffer some degree of inhibition. Since there is very wide varia-

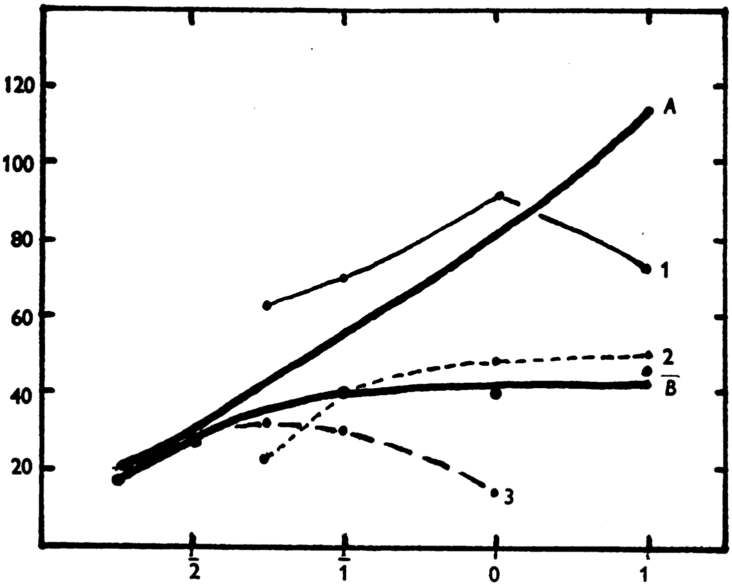

Fig. 4. Relationship between the initial frequency of impulses at 'on' and the light intensity in on-off-elements, as compared with the same relationship in on-elements. *A*, frequency of impulses in on-elements (mean curve, four elements). *B*, frequency of impulses in on-off-elements (mean curve, nine elements). 1, 2 and 3, curves for individual elements, as examples. Ordinates: impulses per sec. Abscissae: light intensity in logarithmic units.

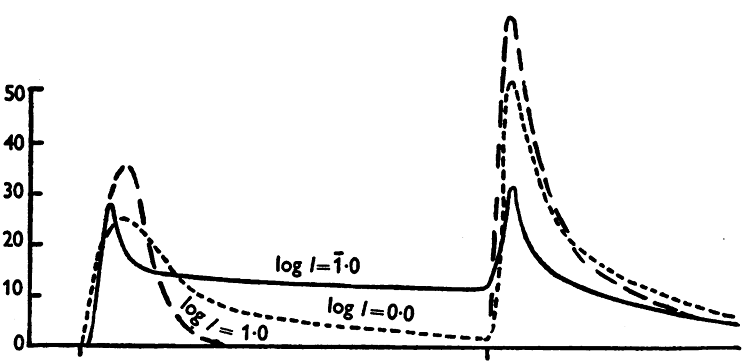

Fig. 5. Impulse frequencies at three different intensities of the light stimulus in an on-off-element showing how the delayed response may be completely inhibited while the initial discharge remains. Ordinates: impulses per sec. Abscissae: time, the duration of the stimulus being 3 sec.

tion in the responses from the on-off-elements, probably indicating different proportions of inhibition to stimulation, the pooling of results is unsatisfactory, but is unfortunately the only way of treating the data. Although the actual numerical values obtained are probably of little significance the results seem to indicate fairly clearly the sort of way in which the inhibition counteracts the

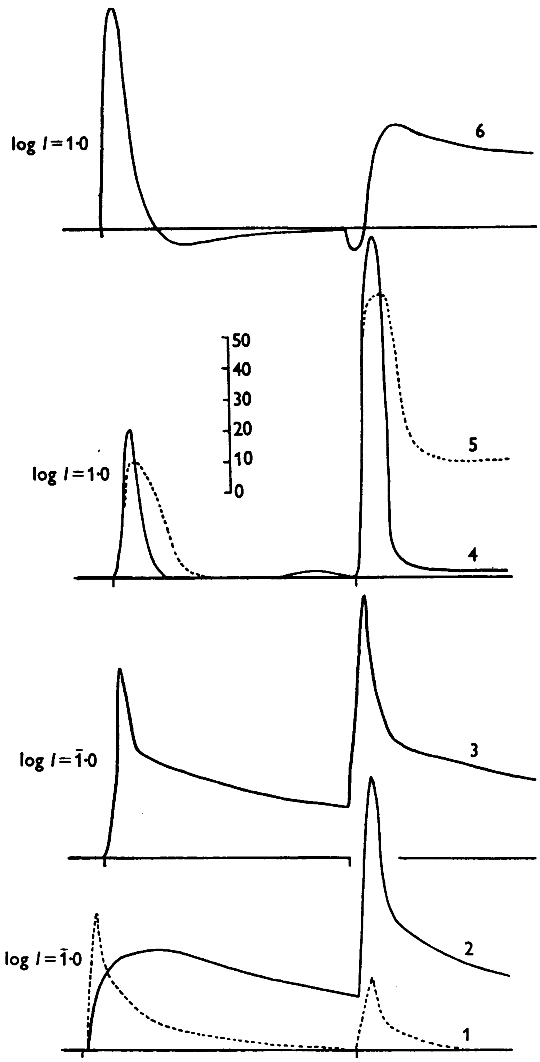

Fig. 6. Various types of discharge from on-off-elements. 1, 2 and 3 at intensity 1̄·0; 4, 5 and 6 at intensity 1·0. Ordinates: impulses per sec. Abscissae: time, the duration of the stimulus being 3 sec. Note the delayed on-discharge in 2, which corresponds with a type of discharge described by Hartline (1935) for the frog. Note also the character of the off-discharges in 4 and 6.

excitation. The continuous curves in Fig. 7 A–D show the mean frequency of response from the pure on-elements 0·2, 0·8 and 1·6 sec. after the onset of the stimulus and at four different intensities. The dotted curves show the mean frequencies of response for the on-off-elements under the same conditions. At the lowest intensity both curves are much the same, and there is no evidence for any inhibition, but at higher levels the curves for the on-off-elements are all

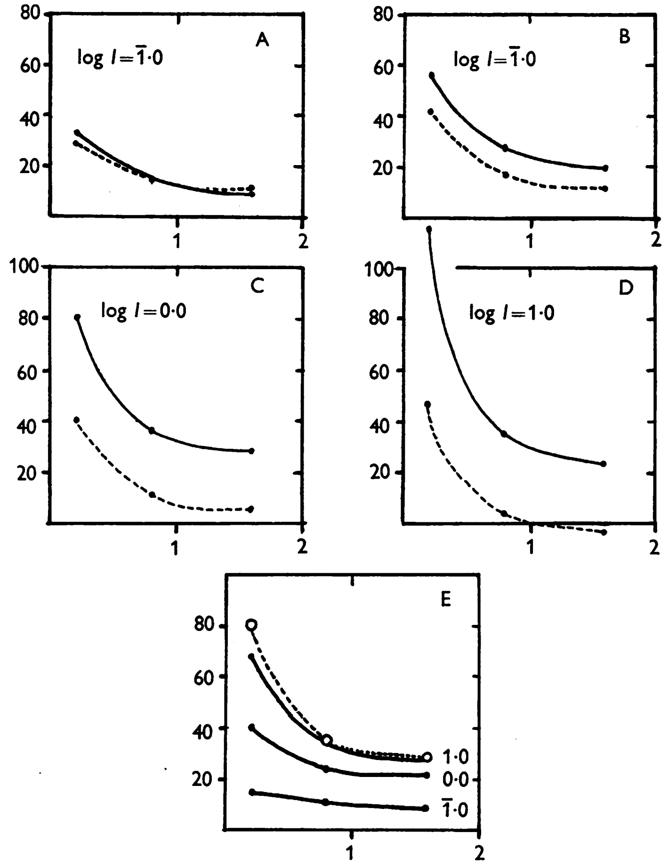

Fig. 7. A–D, curves indicating impulse frequencies of (*a*) pure on-elements (continuous curves), and (*b*) on-off-elements (dotted curves) at different time intervals during the on-discharge and at different intensities of stimulation. The curves are based on the mean values for four on-elements and nine on-off-elements. Ordinates: impulses per sec. Abscissae: time after the onset of the stimulus in sec. E, the differences between the discharges of the on-elements and those of the on-off-elements in B, C and D, as measures of the amount of inhibition which occurs at the three higher intensities, are plotted as continuous curves. The dotted curve represents the mean response of the four on-elements at the same time intervals after stimulation and at intensity 0·0. The amount of inhibition at intensity 1·0, thus corresponds closely to the response of an on-element to a stimulus of one-tenth the strength.

lower, and indicate a reduction or inhibition of the on-discharge. The extent of the inhibition increases steadily with the intensity of the light and acts to depress the magnitude of the response throughout its whole time course, the initial response being actually depressed rather more than the delayed response, especially at higher brightnesses. When the differences between the 'on' curves and the 'on-off' curves of Fig. 7 are plotted, the resulting curves (Fig. 7 E) may be considered to represent the magnitude of the inhibition which has taken place. The inhibition falls off during the time interval of the stimulus at much the same rate as the discharge of a pure on-element declines under the same conditions, so it would seem possible that the inhibitory process may be a purely subtractive process; the inhibiting element, which in general responds less intensely than the 'stimulated' element, directly antagonizes the latter so that its response is reduced in direct proportion to the response of the former (inhibiting) element. Owing to the nature of the data these results should be regarded with caution, but they do at least indicate an approach which may clarify the relationship between excitation and inhibition.

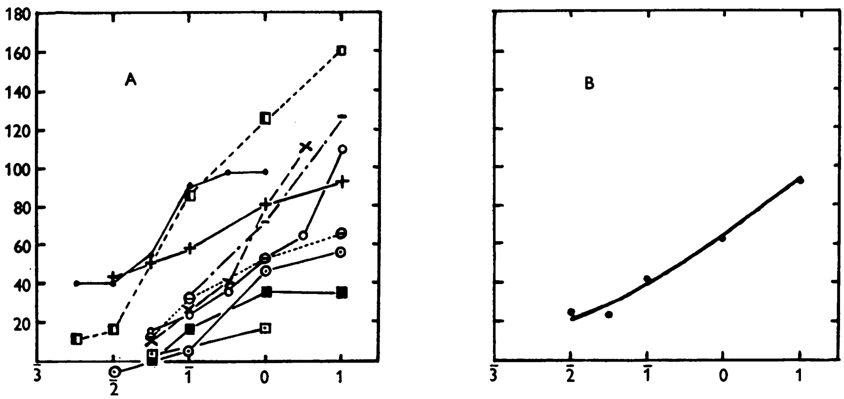

Fig. 8. A, initial discharges at 'off' in individual on-off-elements. B, mean initial discharge at 'off' in on-off-elements. Ordinates: impulses per sec. Abscissae: logarithm of the intensity of the stimulus.

The off-discharge. In most cases the off-effect increases progressively with the increase in intensity of the light in much the same way as the on-discharge of an on-element (cf. Fig. 8 B and Fig. 1 B). At very high light intensities the maximum frequency at 'off' may be reduced and the discharge may become delayed or prolonged. There are also further complicating factors in the off-discharge, for in some elements the discharge is sudden and rapidly dies down again, while in other elements it develops a conspicuous 'tail' which becomes higher and longer, the greater the intensity of the original stimulus. In this connexion the results of two experiments may be mentioned, as they may be important in the interpretation of off-effects in general though, since only two elements have so far been examined from this point of view, the results are mentioned with some diffidence. It was found that at one level of light intensity (about 100 times threshold) the duration of the stimulus between the limits of 2 and 5 sec. made no significant difference to the size or duration of the off-effect. At one-tenth of this intensity the off-effect was rather small, and the duration of the stimulus only contributed rather doubtfully to its character. If there was any effect at all, then the longer the duration of the stimulus the less intense the off-effect. Probably the off-effect here was more in the nature of an after-discharge, and the longer the duration of the stimulus the more adaptation has taken place and so the smaller the after-discharge. On the other hand, when the intensity of the stimulus was high (about 1000 times threshold) the size of the off-effect definitely increased with the duration of the stimulus. Both the immediate discharge was somewhat greater and the 'tail' was conspicuously increased and prolonged (Fig. 9). In another element, in

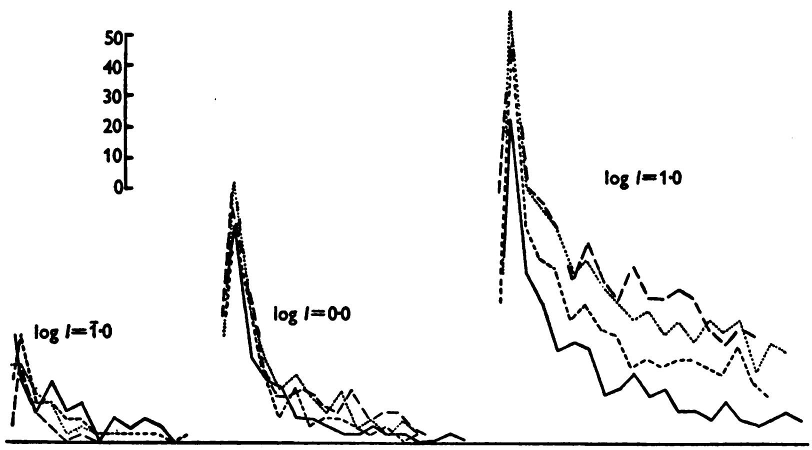

Fig. 9. The off-discharge of an on-off-element at three different intensities, showing the effect of the duration of the stimulus in increasing and prolonging the discharge at the high intensity only. Duration of stimulus: ——, 2 sec.; - - - -, 3 sec.; . . . ., 4 sec.; – – – –, 5 sec.

which the tail of the off-effect was almost absent, the duration of the stimulus had practically no effect, at any intensity level, on the character of the off-discharge.

DISCUSSION

The first point to be emphasized on examination of the records discussed in this paper is the great variation and complexity of the elements, for it must be remembered that all the receptor elements which contribute to the picture under discussion are activated by visual purple only, i.e. the receptors belong to the rod family only, and there was no evidence that any of the elements analysed behaved differently at different wave-lengths as they would do if cones were contributing to the effects. The stimulating lights, when of different wave-lengths, were always such as to cause equal stimulation of receptors dependent on visual purple only, so that there is no need to assume that any other than visual purple-dependent receptors were involved.

The elements whose electrical behaviour was under investigation were probably ganglion cells or their fibres and it is important to remember that between the receptor itself and the ganglion cell there are, at the very least, two synapses (see Fig. 10 A). The actual arrangement of the cells and their synaptic connexions is represented diagrammatically in Fig. 10 B, which itself is probably a great over-simplification of the real state of affairs. At present it is impossible to attribute any of the effects observed to particular elements in this complex, except the initial photoreception to the rods, conduction to the bipolar cells and

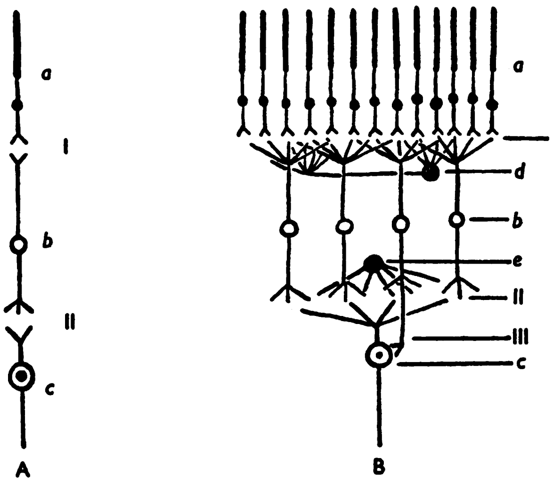

Fig. 10. The structural basis from which the impulses are recorded. A, the simplest possible system. The electrode picks up discharges from *c*. B, diagram showing the main groups of cells which may be concerned. Again the electrode picks up from *c*. *a*, Rod; *b*, bipolar cell (probably of more than one type); *c*, ganglion cell (probably of more than one type); *d*, horizontal cell; *e*, amacrine cell; I, synapses between rods and bipolar cells; II, synapses between bipolar cells and the dendrites of ganglion cells; III, synapse between bipolar cell and the cell body of a ganglion cell.

the observed electrical discharges to the ganglion cells or their fibres. Comparison with results from the eye of *Limulus*, as recorded by Hartline & Graham (1932), indicate that some of the effects may be inherent in the receptor or, at most, in a single synapse. For example, the initial discharge, followed by a period of adaptation, is exactly comparable with the discharge in the fibres of *Limulus*, including even the 'notch' which develops at high intensities immediately after the initial outburst of impulses and which in the elements in the cat's retina seems to be associated with the appearance of an off-discharge. Practically none of the ganglion cells observed in these experiments has ceased to discharge immediately after the light has been turned off and has then remained 'silent'. There is generally a momentary pause of so-called post-excitatory inhibition (Granit, 1944) in the discharge at 'off', followed by what appears to be an after-discharge. Are these changes observed at 'off' to be considered as properties of the receptor, or the synapses, or are they caused by some actively inhibiting agent or elements?

Off-effects proper are probably the result of inhibition. In the examples discussed here they must be caused by an element or elements sensitized by visual purple. Such may be identical with or include the same element as is causing the initial on-discharge, but presumably working along some parallel pathway or, alternatively, neighbouring elements may be involved. The latter suggestion is decidedly the more attractive, since some such mechanism may be presumed to occur in the retina in order to account for the phenomena of simultaneous and successive contrast. The observation that some off-effects attain a high initial frequency but are short-lived, while others are less severe but more prolonged, may indicate a difference in their causation. To complicate the issue still further, it is not impossible that off-discharges may themselves be inhibited by other fibres discharging at 'off'.

It is quite clear that the off-effects are not simply the result of the stimulated element recovering its own 'uninhibited' frequency on removal of the light stimulus. The initial off-effect is generally far too great for this, especially when the effects of adaptation are taken into consideration and it is remembered that the stimulus causing the discharge has ceased. It is evident that some cumulative process occurs during the inhibition while the light is on, so that when the light is removed there is an immediate release which evokes a corresponding high rate of discharge, and this peak discharge may sometimes be followed by a very prolonged 'tail' discharge or period of activity which only gradually dies away. When the light stimulus is intense enough, both the peak and the tail may be increased by prolonging the period of illumination, as if some chemical transmitter had accumulated while the period of inhibition was in progress, or as if the inhibiting adjacent element itself had suffered inhibition and thus ceased to influence the fibre picked up by the electrode. It is important, however, to note that the duration of the stimulus does not necessarily affect the character of the off-effect.

It will be interesting to compare this picture of stimulation and inhibition, which is dependent only on receptors sensitized by visual purple (rods), with that which occurs when elements other than those dependent on visual purple (cones) are involved.

SUMMARY

1. Impulse frequencies have been recorded from single elements (probably ganglion cells) in the retina of the decerebrate cat. All elements selected were sensitized by visual purple only.

2. A single element may behave first as an on-element, then an on-off-element and finally as an off-element depending on the strength of the stimulus used.

3. Different elements show responses which suggest different degrees of excitation and inhibition. Since the least inhibited elements, the so-called pure on-elements, show an initial outburst of impulses followed by rapid adaptation it is difficult to determine when inhibition proper begins to exert its effects. The appearance of a peak off-effect has generally been taken to indicate inhibition.

4. Inhibition reduces both the initial and the delayed frequency of impulses during the on-discharge. The effect is actually greater on the initial outburst but is relatively greater on the delayed discharge.

5. There is often a period of inhibition or reduced activity at 'off' in those elements which might otherwise be regarded as pure on-elements. An after-discharge follows.

6. The relationships between light intensity and peak frequency at both 'on' and 'off' have been plotted for both on- and on-off-elements, and also between light intensity and 'equilibrium' frequency (at 3 sec.) in on-elements.

7. In some elements the off-effect is intense and shortlived; in others it may be less intense initially but more prolonged.

8. With high light intensities, increase of the duration of the stimulus may lead to increased and prolonged off-effects, but does not necessarily do so.

9. Maximum peak frequencies up to 200 impulses per sec. have been recorded, but owing to the fact that the camera speed was rather too low for these frequencies, this figure is put forward with reserve.

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