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EXCITATION AND INHIBITION IN THE RETINA AND IN THE OPTIC NERVE.

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In the course of their pioneer work on the impulses in the optic nerve of the conger eel, Adrian and Matthews [1927 *a*, *b*; 1928] also made some valuable observations bearing on the relation between the retinal action potential and the discharge through the nerve. Thus they found that the well-known off-effect of the retinal response was accompanied by an equivalent outburst of impulses in the nerve, and this observation has since been confirmed by one of us [Granit, 1933] with the cat's eye and optic nerve. With regard to the other phases of the retinal response their evidence was less definite, apparently owing to the fact that none of the analyses of the complex retinal action potential could be relied upon to offer a sufficiently definite starting-point for a comparative study of the processes in retina and nerve. But they found the discharge to follow after a constant interval from the beginning of the initial negative *a*-wave of the retinal response.

Our attempt to analyse the nervous discharge in terms of the components of the retinal action potential presupposes knowledge of the work previously carried out by one of us [Granit, 1933] as well as by Granit and Riddell [1934]. It has been shown that in the cat's eye the retinal response consists of two positive components, P I and P II, which in various ways can be removed selectively from the complex action potential. P I together with P II is responsible for the slow secondary rise of the galvanometer following the fast initial phases, of which the positive *b*-wave is due to P II. The initial negative *a*-wave is part of the negative component P III, which then continues hidden under the positive slower phases, and by its rapid return towards the base line at cessation of illumination causes the off-effect or *d*-wave by releasing the more slowly disappearing positive components, particularly P II.

P I could not be shown to be associated with the discharge in the nerve, but when P II was removed the impulses disappeared. As the off-effect was found accompanied by a renewed outburst of impulses in the nerve, and as the positive P II was found associated with excitation, it was concluded that the release of positivity and the equivalent release of impulses at "off" meant that the negative P III during stimulation somehow had curbed excitation. P III was accordingly held to be the potential sign of inhibition and the off-effect by definition described as a "post-inhibitory rebound." (The analysis of the electrical response of the light-adapted frog's retina is given in Text-fig. 5.)

In substance this argument was deduced from a line of thought that has proved to be correct in reflex work [see *e.g.* Creed, Denny-Brown, Eccles, Liddell and Sherrington, 1932]. But as long as the negative component was only obtained as the final stage in the activity of agents removing P I and P II, it was difficult to put the argument to a direct experimental test. This difficulty has now been disposed of since Granit and Riddell [1934] showed that a flash falling early in the normal off-effect of the frog's eye first selectively activates P III and causes a deep negative "notch" in the record. Actually the flash also stimulates P II, unless the interval of darkness is very short, but, owing to the fact that after stimulation with light P II recovers at a slower rate than P III, it is possible for a short while after cessation of illumination to activate in a normal eye a practically pure P III. As the negative notch means partial or complete removal of the off-effect, the test of P III is carried out against a background of excitation in the nerve, and it should be easy to find out whether activation of P III implies diminution or cessation of the discharge at "off" [see a preliminary report by Granit and Therman, 1934]. The results to be reported below centre around this question, but at the same time we have taken up the more general problem of equivalent phases and components of the response in retina and nerve. "White" light has been used throughout this work. A study with monochromatic light is in preparation.

Method.

The large Hungarian frog was found to be the most suitable preparation for comparing retinal action potentials with the discharge in the nerve. A longer optic nerve may be had with fishes such as *Anguilla vulgaris* and *Lota vulgaris*, but their retinæ and optic nerves die very quickly, even though care be taken to adjust the osmotic pressure of the salines used around values given as maxima and minima for various

fishes[1]. This was noted already by Kühne and Steiner [1881]. The large and rigid eye of *Esox lucius* is easy to handle, but also dies within a very short time. The retinal action potential of the dark-adapted freshly excised eel's eye (*Anguilla*) looks like the retinal response of a frog and changes with light-adaptation as does the latter, but in order to observe this change it is necessary to use several eyes. Within a few minutes the brisk positive phases become sluggish and small; the initial phase is then dominated by a large negative *a*-wave; the negative component again comes to sight after the greatly diminished *b*-wave, and the off-effect, as a rule, takes place chiefly below the base line. This type of response, which clearly is abnormal, may be had for a considerable time after excision of the eye. Impulses may then be recorded from the optic nerve.

Both retina and optic nerve of frogs can be relied upon to give reasonably constant responses for half an hour or more, and to remain active for a considerably longer period. The optic nerve is short, about 7 mm., but the slower retinal change can nevertheless be filtered out by suitably chosen condensers in the amplifier. The dissection of the preparation must be carried out in good light, but if dark-adaptation is wanted the eye may be left to dark-adapt on the electrodes.

The preparation was put into a small ebonite holder and the optic nerve drawn out through a slit in the holder and put on two silver-silverchloride pins covered with cotton-wool dipped in saline and leading to the input of a five-stage condenser coupled amplifier. Cotton wicks were taken to cornea and back part of the bulb and connected with silver-silverchloride wires serving as retinal electrodes. The ebonite holder was mounted inside a black shielded and earthed box. A lens of +8D was put into a separate holder in front of the eye, when the stimuli appeared as projections of light on a ground-glass screen at a distance of 50 cm. from the eye. No lens was used when the lamp was allowed to shine directly on the eye. The correction was determined by direct observation of an image through a hole in the sclera [see Adrian and Matthews, 1927 *a*], observed through a microscope. The ground-glass was used when a limited area on the retina was wanted in order to restrict the number of active fibres in opticus. The glass was covered on the back by black paper in which a hole was left for the beam of light. The diameter of the area stimulated could be varied from 1 to 6° of visual angle by means of a metal screen fitting close to the surface of the paper. This part of the apparatus was, in fact, similar to the one used by Granit and Harper [1930], and the lamp was the one used by Granit and Riddell [1934],

[1] See *Tabul. biol.*, Berl., **1**, 1925.

here set up to produce an intensity of about 1000 m.c. at the eye with the ground-glass removed.

The movement of the shutter, determining the exposures, was originally recorded as an electric artefact on the film recording the nervous discharge, but later the prisms, leading part of the beam to the camera serving the string galvanometer (for records of retinal potentials), were made adjustable so that the beam of light stimulating the eye could be photographed directly with either camera.

As chief recording instrument we used a Cossor cathode-ray oscillograph, Type C, coupled to the Cossor "power unit[1]." The latter was driven by a Rotax converter transforming the D.C. of the mains to A.C. for the unit. The filament was heated separately by a 2 volt accumulator. The output of the condenser coupled amplifier was taken to one pair of deflector plates within the tube. The other pair was connected to the gun. The cathode ray was focussed on to the film of an Edelmann camera. The retinal response could be recorded by a separate system comprising a directly coupled amplifier, a permanent magnet Edelmann string galvanometer and a small Cambridge camera. But as we were chiefly interested in the fast phases of the retinal response, the amplifier serving the cathode-ray oscillograph was made with three sets of condensers which could be rapidly exchanged by means of switches. With the slowest 0·2 μF. condensers the response to a constant change of potential fell to half its initial value in 0·45 sec. The corresponding values for the 0·02 and 0·005 μF. condensers were 0·015 sec. and less than 0·005 sec. respectively. The largest condensers were always used for retinal recording, the smallest generally for records from the nerve. The usual controls were made to exclude artefacts [see preliminary report, Granit and Therman, 1934]. At times, on illumination, minute deflections were observed with a killed nerve on the electrodes. These could be traced to the retina and easily distinguished from the nervous response. Often no leakage whatsoever was noticed. The limits of amplification and the effect of shunting within a small nerve containing numerous thin fibres have been discussed by Adrian and Matthews [1927 *a*] and need not detain us here.

Results.

The type of discharge. Synchronization.

Several observers [Einthoven and Jolly, 1908; Chaffee, Bovie and Hampson, 1923; Granit and Riddell, 1934; Smit, 1934] have noted with the frog's eye that, despite continuous illumination, there

[1] Messrs Cossor, Catalogue 3232.

may appear small extra waves on the *b*-wave or on the off-effect of the retinal action potential. Some have seen them on either *b*- or *d*-wave (off-effect), some on both these phases. Chaffee, Bovie and Hampson even found it necessary to account for the extra waves on the *b*-wave by suggesting several theoretically possible solutions of the retinal action potential, whereas Granit and Riddell [1934] pointed out that the extra waves on the *b*-wave most probably were artefacts from the nerve.

Our experiments show that the typical discharge in the nerve at "on" and "off" is a very much synchronized one (see *e.g.* Pl. I), less marked in some eyes, but very obvious in others, particularly at high intensities of stimulation. The synchronized volleys are sometimes of large potential and are then easily picked up by the retinal electrodes. In such cases it is possible to record a wavy retinal response at "on" and "off," even with the smallest condensers in the amplifier. Sometimes the wavelets on the *b*-wave and the off-effect disappear when the nerve is cut or pinched with forceps near the bulb. They have then been artefacts from fibres outside the bulb, as suggested by Granit and Riddell [1934]. But at times they remain, despite cessation of activity in the nerve. The most probable explanation of the phenomenon would then seem to be that the retinal electrodes have been suitably located relative to the intraretinal fibres in the optic nerve. On the other hand, it will be demonstrated below that there is a definite relation between the retinal action potential and the discharge in the nerve. If large retinal areas act in unison with synchronous periods of rest and activity, it is, therefore, to be expected that synchronization in the nerve will be accompanied by synchronization in the retina. Our experiments cannot decide between the last two possible sources of wavelets on the retinal response. But we know enough nowadays about synchronous activity in nervous centres and the nature of the retinal response not to suggest a new analysis of the retinal action potential when a wavelet appears on its *b*-wave or off-effect.

Adrian and Matthews [1928] described synchronous activity in the eel's optic nerve, and state that the essential condition for obtaining a discharge of the rhythmic type is an even illumination of a very large part of the retina. The rhythm appeared after the light had been on for a few seconds. In our experiments there was generally some indication of a rhythm at "on," but it disappeared during prolonged illumination. Rhythm was generally best marked during the off-effect. It hardly ever failed to appear during this phase of the discharge, provided that the intensity of the stimulating light was sufficiently high. In Pl. I, record *D*, the off-effect can be seen to develop into a typical rhythmic discharge,

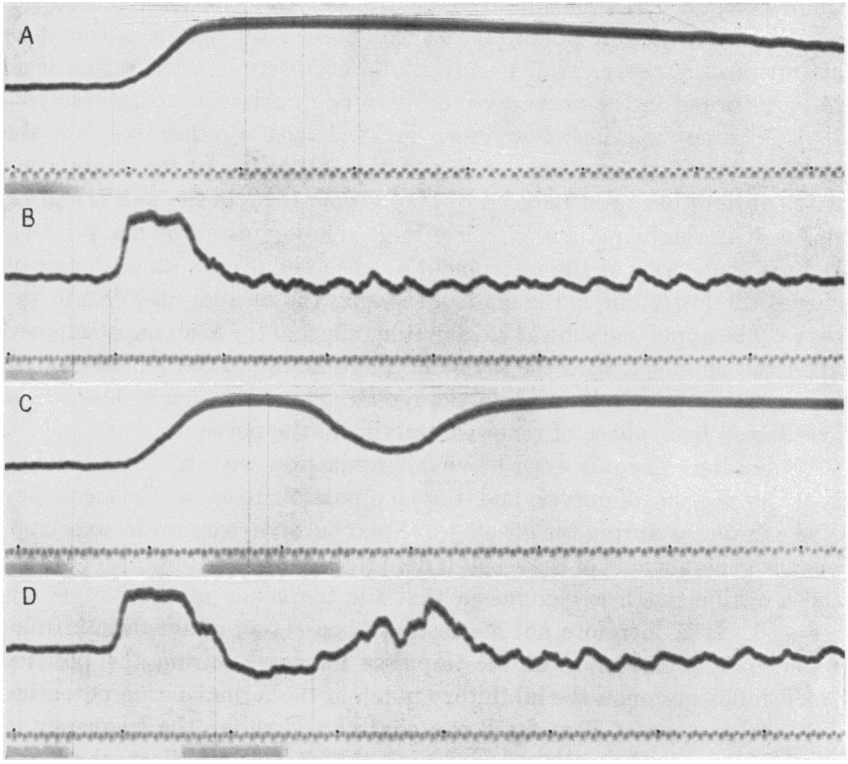
gradually damped out as the activity of the retina evanesces during darkness. The frequency of the rhythmic volleys diminishes with diminishing area and intensity. Thus, in the record *D* of Pl. I, the beats occur at a rate of about 29 per sec. with the 4° area used in this experiment. The record *B* of the same plate was taken a few minutes later with a 1° area. The synchronous volleys do not appear. The dependence of the synchronization upon area and intensity was also noted by Adrian and Matthews [1928]. We did not experiment with a very large evenly illuminated area, which probably explains our failure to see the rhythm appear in an eye left in the light, but quite often we removed cornea and lens and left the eye exposed to the full intensity of the stimulus. In such circumstances spontaneous rhythmic activity did not occur before the light was switched off.

The resemblance between the spontaneous rhythmic discharge and rhythmic volleys of impulses, caused by intermittent stimulation, was pointed out by Adrian and Matthews [1928]. At the back of it seems to be an inherent capacity of the retina to react rhythmically with a frequency dependent upon its own level of activity. The fusion frequency of intermittent stimuli increases with increase in area and intensity, as is easily demonstrated with the frog's optic nerve [cf. Adrian and Matthews, 1928, for the same result with the eel's optic nerve], and so does the frequency of the spontaneous rhythmic beats. The limiting rate of the rhythmical discharge with flicker is rarely above 25, unless the bulb is opened, when values about 30 may be obtained. Well-developed spontaneous volleys may occur at a frequency of about 30–45 per sec. when a forced rhythm (intermittent stimulation) would appear fused. In this respect our results differ from those of Adrian and Matthews [1928], who state that the limiting rates of flicker and spontaneous rhythms are the same. But then they also mention "occasional patches" of higher frequencies of spontaneous beats which are more likely to belong to the category of phenomena that we have observed.

As to the discharge between "on" and "off" of the stimulus, the most significant fact seems to be that, within very wide limits of area, intensity and state of adaptation, the adaptive mechanisms of the eye sooner or later set up a roughly constant level of activity, a fact which also characterizes the retinal action potential (constant level of potential). The same also holds good for human vision, as recently emphasized by Wright [1934] in some interesting experiments showing that the apparent brightness is gradually reduced to constancy no matter what the initial intensity happens to be. (See also below, section on P I.)

P III and inhibition.

In Text-fig. 1, *A* is the normal off-effect in the retina, recorded with the large condensers in the amplifier of the Braun tube. *C* shows how this off-effect is cut down by a flash of about 118σ, following at an interval



Text-fig. 1. *A* and *B*, off-effects in retina and nerve respectively; *C* and *D*, off-effects in retina and nerve respectively, but off-effects interrupted by flash of light. Stimulus photographed below record of tuning fork of 100 periods. Every 100σ marked by dot in Indian ink. Full intensity (no ground-glass). Note: speed of film varies somewhat from record to record. Explanation in test.

of about 100σ from cessation of illumination. This is the effect that was shown by Granit and Riddell [1934] to depend upon activation of the negative component P III. The negative notch is followed by a new positive phase. There is some amplifier distortion in the records, but this need not concern us here. *B* shows the discharge in the optic nerve at "off," taken at a fairly low sensitivity and slow speed of the film. There

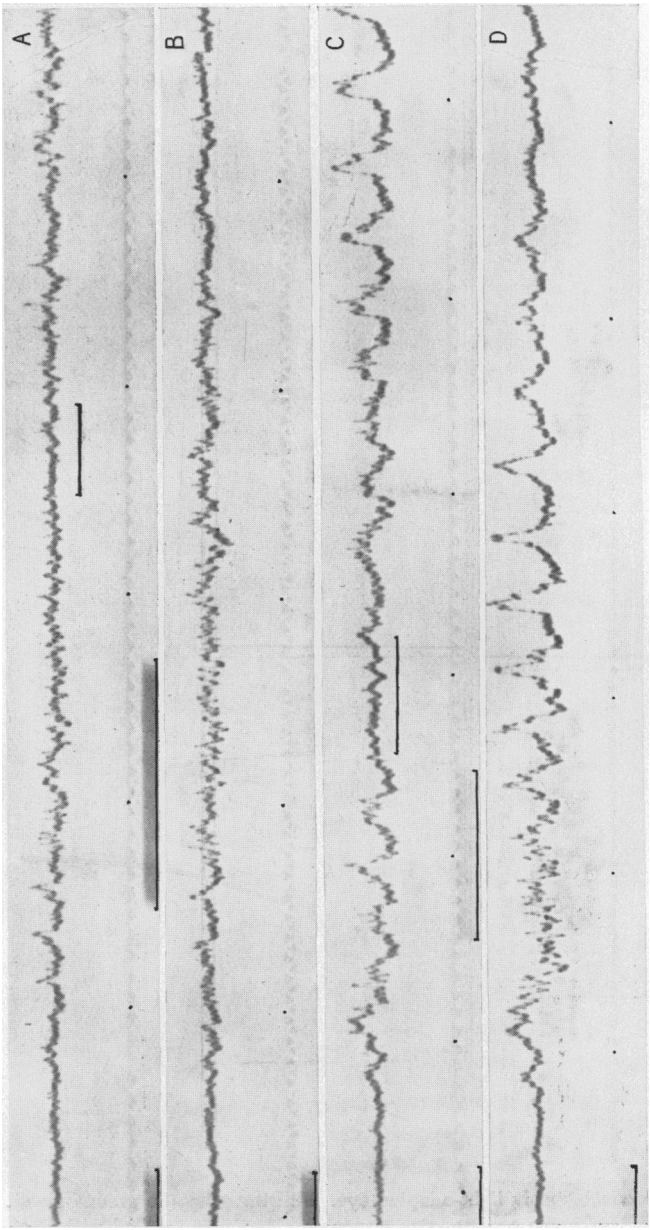
is a heavy initial discharge, followed by irregularly synchronized waves of impulses. In *D* a flash of about 120σ interrupts the normal course of the off-effect after an interval of 100σ. Intervals and flashes are within a few σ identical in retina and nerve. It is evident that the negative notch, signifying activation of P III in the retina, has its counterpart in the nerve in a period of diminished activity. Similarly the renewed rise of the retinal action potential is accompanied by a new outburst of activity in the nerve. Thus negativity and positivity in the retina seem to be mirrored in the nerve by inhibition and excitation respectively.

The same experiment is shown in Pl. I with another eye, but the ground-glass has now been put in and the diameter of the stimulating patch of light has been reduced to 4° of visual angle in the pair *C* and *D*, and to 1° in the pair *A* and *B*. The film is running faster (55 cm. per sec.) and the sensitivity of the instrument has been increased. In each pair of curves the lower one is the control showing the normal off-effect in the nerve. The upper one shows the inhibitory effect of the flash, superimposed upon the off-effect, with the period of maximal inhibition indicated by a horizontal line in Indian ink in the record. The inhibition in both cases is followed by a phase of renewed activity in the nerve.

Sometimes there is even less synchronization with the 1° area than in the upper pair of curves, and it is then possible to count the frequency of the impulses during the off-effect. Great accuracy cannot be expected, but the general trend of the result is definite enough: the inhibition during the negative notch is genuine in that the frequency of the impulses is reduced. It is therefore not a question of cessation of synchronization. Likewise the frequency of the impulses increases during the positive phase following upon the inhibitory notch in the retinal action potential. The upper curve of Text-fig. 2 is a control and shows the frequency of the impulses in the optic nerve during the normal off-effect, the lower curve (drawn in full) demonstrates the inhibition and the post-inhibitory excitation following a flash, the length of which is indicated by the horizontal thick line below the curve. The readings marked in the figure have been obtained from five successive exposures of the same eye to identical conditions, three of which refer to the control.

P II and post-inhibitory excitation. The nature of the latent period.

It was shown by Granit and Riddell [1934] that the positive rise following the negative notch in the off-effect varied in character with the interval between the flash and the end of the previous stimulus. At short intervals it never rose above the level of the off-effect and, in fact,



Same experiment as in Text-fig. 1 and similarly marked, but the area stimulated has been restricted to 1° (diameter of circular spot) in *A* and *B*, and to 4° in *C* and *D*. Full intensity and ground-glass. Fast film and high sensitivity. All records from optic nerve. Lines in Indian ink drawn through photographed stimuli and below inhibitory effect in *A* and *C*. Note: synchronization in *C* postponed by inhibition due to flash on the off-effect. Explanation in text.

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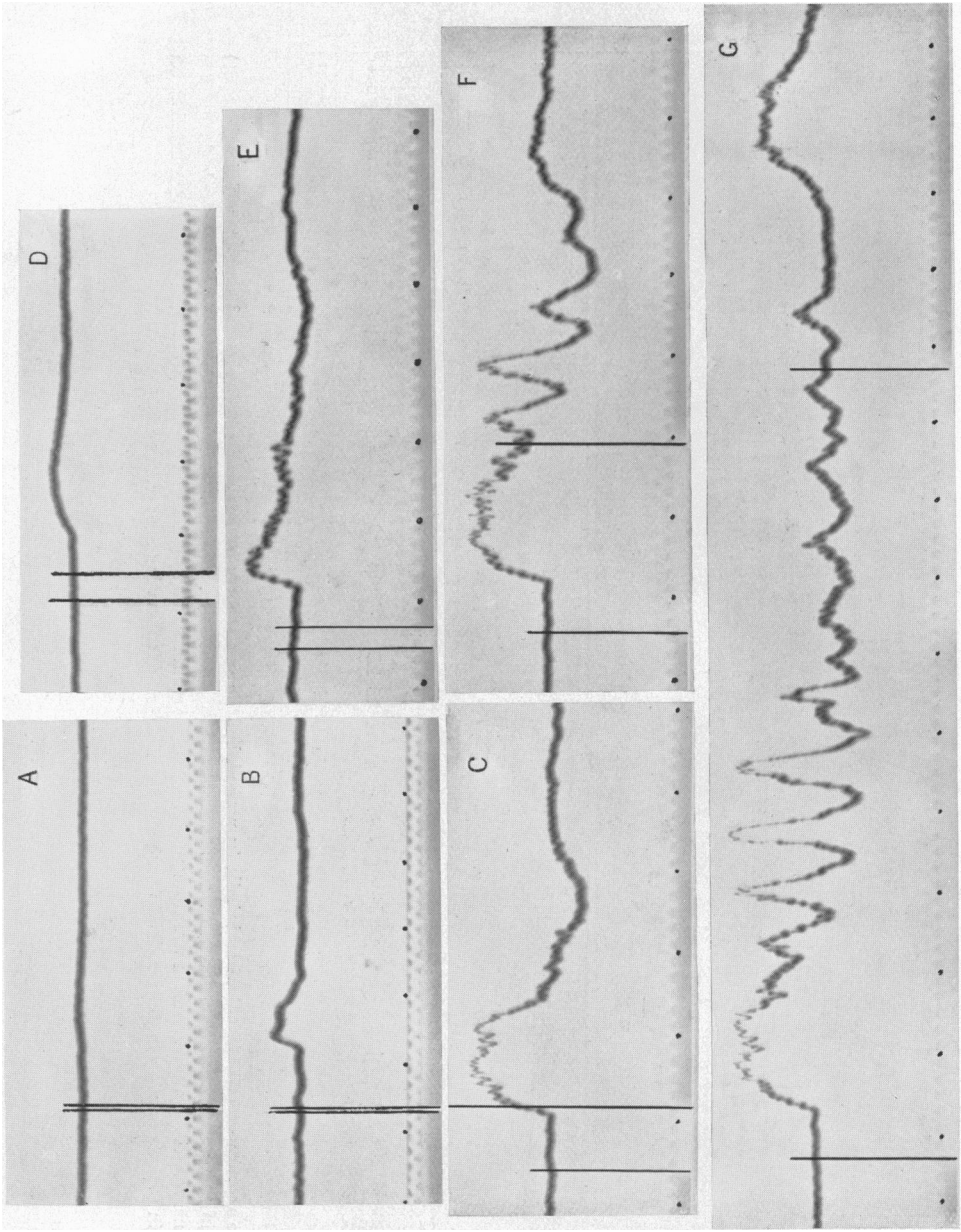

Gaps of darkness in a continuously light-adapted eye. Full intensity (no ground-glass). *A* and *D* retinal records, the rest nervous responses. Every 5σ marked with dots. Gaps marked by lines in Indian ink. Duration of gaps: *A*, below 5σ; *B*, below 5σ; *C*, 40σ; *D*, 18σ; *E*, 15σ; *F*, 115σ; *G*, about 500σ. *A*, *B* and *D* from the same experiment; *C*, *E*, *F* and *G* from another experiment. Note: synchronization as in *G* being inhibited in *F*. Explanation in text.

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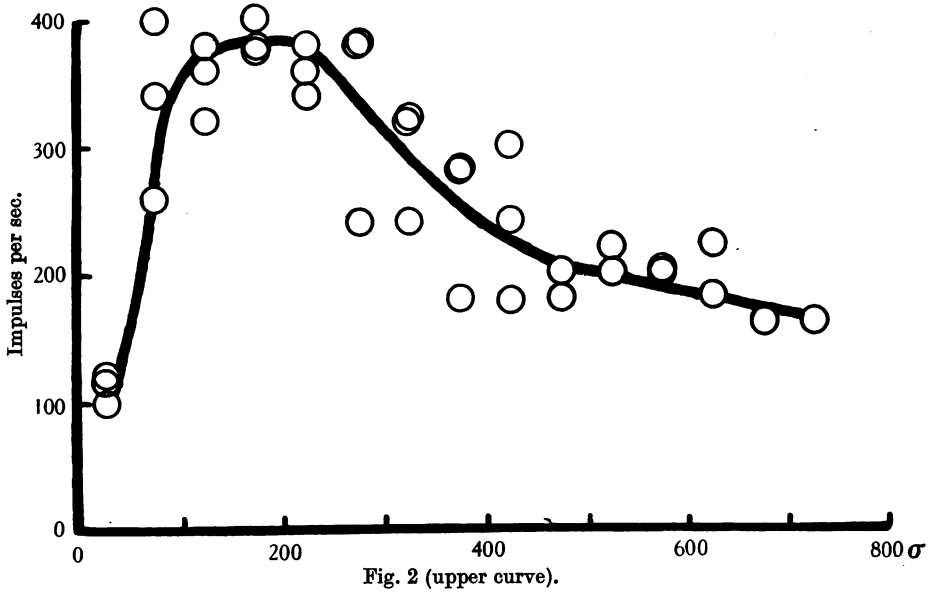


Fig. 2 (upper curve).

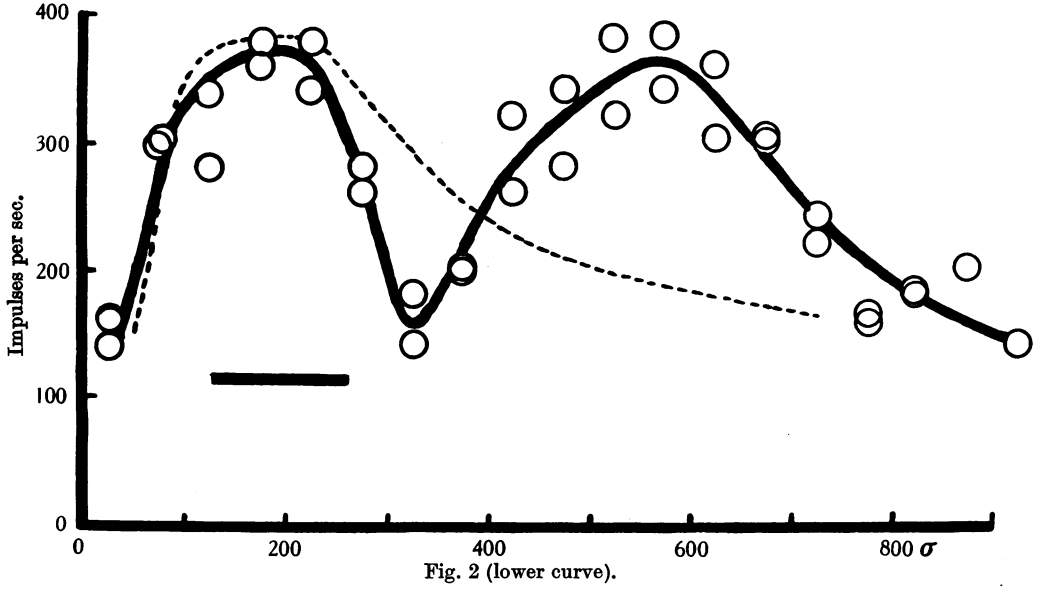


Fig. 2 (lower curve).

Text-fig. 2. Frequency of impulses during off-effect plotted against time in σ. Upper curve, off-effect; lower curve, same with flash superimposed as indicated by horizontal line. The dotted line is the upper curve re-drawn. Ground-glass. Diameter of area restricted to 1°. Full intensity.

represented mere resumption of the off-effect after the interruption caused by the reactivation of P III. At longer intervals there appeared in addition a positive peak above the level of the off-effect. This extra positivity was shown to depend upon activation of fresh P II. The off-effect itself is remnant P II, released by the return of P III towards the base line [see Text-fig. 5 below and also the analysis of the frog's retinal response, Granit and Riddell, 1934, p. 7]. As the flash is of the same intensity as the previous adapting stimulus, it can hardly be expected to elicit fresh P II before some recovery has taken place, *i.e.* before the remnant P II of the previous stimulus has fallen below plateau height. This is the earliest moment for a positive peak above the level of the off-effect to appear. Granit and Riddell [1934] found with strong stimuli that reactivation of P II required some 150–200σ to elapse from the cessation of previous illumination. The recovery of P II is thus a slow affair compared with the almost immediate recovery of P III, and by algebraical summation of potentials an off-effect ensues.

In order to determine the moment of reappearance of P II in the retina and compare it directly with events in the nerve, without recourse to the laborious process of counting the impulses, we modified the procedure of Granit and Riddell [1934]. The flash, thrown in at various points along the off-effect, was exchanged for a continuous light of the same strength as the previous adapting illumination, a method used already by Piper [1911], and which in practice amounts to systematic lengthening of a "gap" of darkness in an otherwise continuously illuminated eye. The stimulus, reappearing after the gap of darkness, reactivates the negative P III in the same way as the flash, used above, but whereas the latter was followed by a revival of the off-effect, sometimes (long intervals) mixed with fresh P II, the continuous light prevents the off-effect from reappearing after the gap of darkness. Thus positivity, activated by the stimulus after the interval of darkness, is due to reactivation of P II, and it is easy to determine the exact moment of its appearance.

Pl. II, *A* and *B*, shows what happens in retina and nerve respectively when the gap of darkness is very short. A small off-effect in the retina is accompanied by a group of impulses in the nerve, but both are cut down by reactivation of P III [Granit and Riddell, 1934] restoring *status quo ante*. The extraordinary rapidity of the light-adapted frog's eye should be noted. The gap of darkness is below 5σ. (With short intervals it is necessary to use high sensitivity in order to see anything at all in the retina; the impulses are easier to detect with moderate sensitivity

as apparently several impulses reach the electrodes simultaneously.) But the gaps of darkness need not be very much lengthened before restoration of *status quo ante* ceases to be the sole effect of the reappearing stimulus. Already with gaps of the order of magnitude of 10σ there are signs of post-inhibitory excitation. Curve *E* (Pl. II) shows a late secondary discharge in the nerve, and a new rise in the retinal record is indicated in curve *D*. The effect of further lengthening of the gaps of darkness is shown in records *C*, *F* and *G* (nerve). Inhibition of the discharge at "off" is followed by re-excitation, increasing in strength as the period of rest in darkness increases. In the retina the pure negative dip, caused by the reappearing stimulus, is followed by a positive swing. The negative dip and the positive swing were shown by Granit and Riddell [1934] to be the initial phases of the retinal action potential, known as the *a*-wave (due to P III) and the *b*-wave (due to P II), a fact easily understood when it is realized that a lengthening of the gap of darkness gradually liberates the retina from the after-effects of previous stimulation. The reappearing stimulus becomes an independent stimulus. By lengthening the gap of darkness it is thus possible to trace the origin and development of *a*- and *b*-waves, correlate them with events in the nerve, and analyse the latent period.

Returning now to the question of the moment of reappearance of P II (the *b*-wave), we find that the earliest signs of this phase in the retina and the equivalent discharge in the nerve fall around values of the order of magnitude of 150σ, counted from cessation of stimulation. In record *E* the post-inhibitory discharge, corresponding with the *b*-wave, appears after about 200σ. Shorter values may be had with enucleated eyes, longer by weakening the strength of stimulation. There seems to be an optimal duration of the gap of darkness for both retina and nerve of the order of magnitude of 30σ, characterized by post-inhibitory excitation appearing with minimal delay. Naturally, as the gap lengthens, its own duration postpones the moment of reactivation of P II. With very short gaps the inhibition of the off-effect dominates the picture. All these facts agree very well with the results obtained by Granit and Riddell [1934], and give the additional information that reactivation of P II, causing a new *b*-wave of positivity, is accompanied by a discharge in the nerve.

But the moment of reappearance of P II is more interesting still from another point of view: its latent period relative to the beginning of the new stimulus. The latter, as we have seen, activates an *a*-wave (negative P III) accompanied by inhibition, followed by a *b*-wave (positive P II) coincident with excitation. Accordingly the latent period of the excitatory effect

(*b*-wave and discharge) consists of two phases: (1) the duration of the inhibitory effect coinciding with the *a*-wave (P III); (2) the latent period of the excitatory process itself. It is hardly probable that the excitatory process should lack a latent period of its own, and that the whole of the interval between stimulus and excitation should be consumed in overcoming inhibition. The latent period of the excitatory effect, above rightly termed post-inhibitory excitation, decreases with a lengthening of the gap of darkness. This may be seen by comparing curves *E*, *C*, *F* and *G* of Pl. II. In *G* the latent period is about 100σ. By further lengthening of the gap of darkness the latent period ultimately reaches a value about 40σ. Is it then the duration of the inhibitory phase, or is it the latency of the excitatory process that shortens? The experiments provide no definite answer to this question, as it is difficult to know where the one begins and the other ends, but it seems probable that both phases of the total latency are shortened.

For the sake of completeness it should be added that the latent period also contains a third factor—the duration of the initial photochemical process, of which nothing is known.

From many points of view it is important to realize that stimulation with light elicits inhibition as well as excitation, and that the inhibitory process precedes the excitatory process. The fact that in the retina the corresponding phenomena are a negative *a*-wave and a positive *b*-wave means, in connection with latencies, that it is impossible to establish a precise correlation between the *b*-wave in the retina and the appearance of the discharge in the nerve. The recording instrument shows only the point at which the balance between negativity and positivity turns in favour of the latter (*b*-wave). In stating above that the reappearing P II (*b*-wave) is accompanied by a discharge in the nerve, we have therefore not been able to base this conclusion on measurements of the interval between *b*-wave and nervous discharge. It is only possible to state that they are approximately simultaneous. But the impulses quite often start during the *a*-wave [cf. Adrian and Matthews, 1927 *a*]. Of more importance is the fact that changes in the latent period of the *b*-wave are reflected by corresponding changes in the latent period of the retinal discharge. There is also some correspondence between retina and nerve with regard to size of *b*-wave and size of discharge: they both increase and decrease in the same fashion with changes in the experimental conditions.

In view of the fact that the latent period of the discharge involves some inhibition together with the time taken by the excitatory process

to reach threshold value, it is difficult to see what it means to measure the interval between the latent periods of the retinal action potential and the nervous discharge in order to obtain the interval between the processes in retina and nerve. This latter interval, shortened R.N. interval, can be of real significance only when a positive or negative change in the retinal action potential is unopposed by a change in the opposite direction. This is the case at "off." During the off-effect the negative P III swings back towards the base line releasing positivity, but not opposed by it. Thus a R.N. interval can be obtained which gives some indication of the time passed between a change in the retina (off-effect) and the outburst of impulses (off-discharge) in the nerve. A correct figure can only be obtained from experiments in which high intensity and high sensitivity have been used, as otherwise the starting-point, particularly in the retina, is too indefinite. Collecting values from our best experiments we find that in most cases the nervous discharge follows after the retinal effect; the R.N. interval is positive and lasts about 3σ. But in some 25 p.c. of the experiments the R.N. interval is negative, again averaging about 3σ. We conclude from this that the excitatory disturbance experiences very little, if any delay between the structures that give rise to the retinal action potential and the nerve. (With gaps of darkness shorter than the latency of the off-effect, the latter is delayed in both retina and nerve.)

Adrian and Matthews [1927 *a*] found a constant interval between the beginning of the *a*-wave and the beginning of the nervous discharge. We have seen above that the *a*-wave involves inhibition, and that the discharge, connected with the positive *b*-wave, follows after a latent period including an inhibitory phase. The R.N. interval, measured by Adrian and Matthews [1927 *a*], cannot therefore indicate "synaptic delay" between retina and nerve. The figures which they obtained ranged between $50–90\sigma$. The true "synaptic delay" is less than 3σ, judged by the off-effect.

Intermittent stimulation.

The frog's retinal reaction to intermittent light was analysed in detail by Granit and Riddell [1934], who showed that the intermittent ripples on the retinal action potential were produced by P III and P II interacting, remnant P II (off-effect) causing the positive and P III the negative phase of the intermittent waves. Connecting to the previous section we could describe the waves of flicker as elicited by a series of gaps of darkness in an otherwise continuous illumination. Each gap of darkness releases the off-effect which the next phase of light cuts down with a negative wave. At slow frequencies of stimulation, fresh P II may be

elicited in addition to the positivity given by the off-effect alone. From the results of the previous sections it follows directly that flicker is interaction between excitation and inhibition.

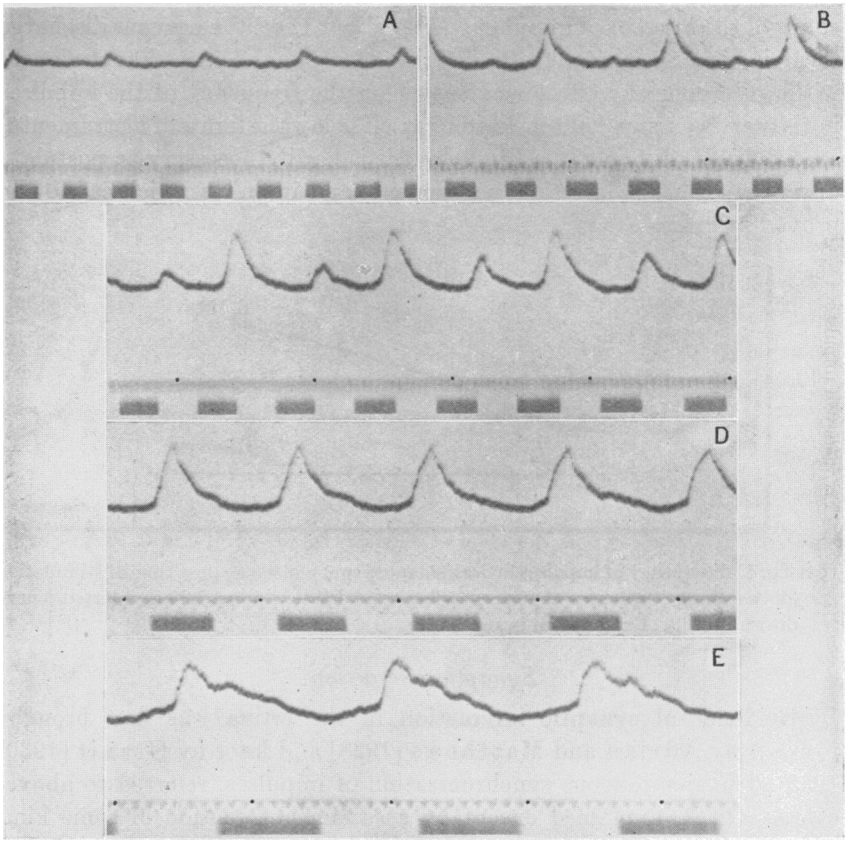
Some observations with flicker will here be used as further corroboration of the connections between the components of the retinal action potential and the discharge in the optic nerve. Granit and Riddell [1934] noted that, at high frequencies of stimulation, the retina sometimes responded with intermittent waves to every second stimulus, but did not mention it in their paper as further confirmation seemed desirable. Since then the phenomenon has been noted very often in this laboratory, both with the retinal action potential and with the discharge in the nerve. With high intensities and an enucleated eye it is almost the rule. Further analysis has shown that an increase in sensitivity generally reveals a smaller retinal wavelet or nervous discharge between the larger response to every second stimulus, as may be seen in Text-fig. 3. The phenomenon is interesting in itself, but is here chiefly mentioned as further corroboration of the fact that the size of the positive phases in the retina is roughly reflected by the discharge in the nerve.

Text-fig. 5 also shows another interesting fact: as the frequency of intermittent stimulation decreases, the position of the nervous discharge relative to the flash shifts, if the beginning of the flash be taken as the standard of comparison. Again the same change may be noted in the positive phase of the retinal action potential.

The fusion frequencies are identical in retina and nerve, provided that sufficient sensitivity be used in both cases. The fusion frequency increases in both retina and nerve with increase of area and intensity of stimulus.

The reaction to intermittent stimulation is being studied by one of us, and is mentioned here merely to supply further evidence with which to substantiate the conclusion that P II-positivity and P III-negativity in the retinal response are associated with excitation and inhibition respectively. In all our work we have never had reason to depart from this simple scheme in drawing inferences about the retinal action potential from the nervous discharge and *vice versa*. It is perhaps not necessary to burden this paper with a description of equivalent changes in retina and nerve in response to variations in area, intensity and state of adaptation. Changes in the latent periods of the retinal reactions at "on" and "off" have always been found associated with equivalent changes in the nerve, and there has also been a rough qualitative correspondence between the size of the *b*-wave or the off-effect and the frequency of the equivalent discharge in the optic nerve, judged by the displacement of the cathode

ray upwards in the pictures. On the other hand, it often looks as if the maximum of the discharge, judged by the same criterion, were reached before the corresponding positive phase in the retinal response is maximal. On account of the great number of fibres in the optic nerve it is difficult to compare corresponding events quantitatively.

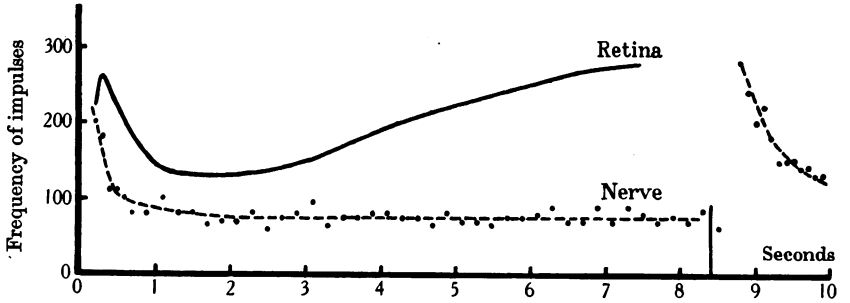


Text-fig. 3. Synchronized beats of impulses in light-adapted eye reacting to intermittent stimulation. Marked as Text-fig. 1. Enucleated eye, full intensity (no ground-glass). Frequencies of stimulation: *A*, 27·8; *B*, 21·8; *C*, 17·0; *D*, 10·3, and *E*, 6·7 flashes per sec. Explanation in text.

Is P I associated with activity in the nerve?

P I is responsible for the secondary rise of the retinal action potential of the dark-adapted eye. It disappears from the frog's eye after light-adaptation. In a well dark-adapted, freshly excised frog's eye, the

secondary rise or *c*-wave reaches a potential which quite often is higher than that of the *b*-wave. In Text-fig. 4 the ordinates of the retinal action potential, recorded with string galvanometer and directly coupled amplifier, have been plotted above the curve relating frequency of impulses to time of exposure for a period of about 8 sec. During the initial phases at "on" and "off" it has been impossible to make even an approximate count of the number of impulses. After about 1 sec. the nervous discharge is carried on at a constant rate. P I rises after about 2·5 sec., but obviously without having any effect whatsoever on the frequency of the impulses in the nerve. A small effect, keeping itself below the limits of instrumental sensitivity, is difficult to exclude, but it is hardly necessary to postulate any relation between retinal components and impulses which cannot be proved to exist.



Text-fig. 4. Frequency of impulses in dark-adapted eye, plotted against time of stimulation (dotted line). Ordinates of corresponding retinal action potential also plotted (curve drawn in full). Explanation in text.

Synaptic interaction.

Evidence of synaptic interaction in the retina was first brought forward by Adrian and Matthews [1928] and later by Granit [1930, 1933]. The spontaneous synchronization of impulses, referred to above, can hardly be explained except by assuming interaction of some kind forcing distant areas to discharge in unison. The facilitating influence of strychnine on synchronization [Adrian and Matthews, 1928] localizes this process of interaction to the synapses, and further support for this view is found in the fact that synchronization also is a feature of the activity of motor neurones [see *e.g.* Adrian and Bronk, 1928]. The shortening of the latent period of the discharge with an increase in area stimulated [Adrian and Matthews, 1927 *a*] must also be regarded as evidence in favour of nervous interaction, as Adrian and Matthews [1928] in a well-known experiment showed it to take place over areas

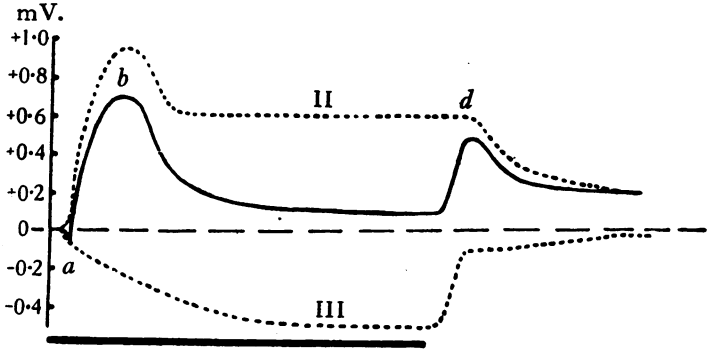
some distance apart. The same phenomena may be demonstrated with the retinal action potential [Granit, 1933], but they are absent in the eye of *Limulus* [Graham, 1932] which lacks the internuncial neurones of the vertebrate eye.

Interaction is clearly shown by the reaction to intermittent light. As a convenient index may serve the fusion frequency in retina and nerve. Adrian and Matthews [1928] showed that the fusion frequency in the eel's optic nerve rose with an increase in area or intensity; Creed and Granit [1933] obtained the same result with the cat's retinal action potential, and found the fusion frequency to be inversely proportional to the latent period. During the course of this work we have several times observed the fusion frequency in both retina and nerve in response to variations in area or intensity and been able to confirm the results referred to. The formally new point, however, is that the changes in the fusion frequencies are reflected similarly by retina and nerve. This justifies the conclusion that the events leading up to interaction have taken place in front of the locus responsible for the electrical reaction of the retina. From this fact follows directly that the electrical response of the retina takes place in the synaptic layers, as the individual rods and cones are known to be separated up to the point where their basal ends join with bipolars, lateral horizontal cells and other receptors.

As excitation and inhibition cooperate in producing the reaction to intermittent light in the frog's eye, inhibition (P III) in particular being responsible for the increase in the fusion frequency with light-adaptation, (Granit and Riddell, 1934) it is to be expected that the shortened latent period of the discharge (P II) with increase of the area stimulated should be accompanied by a similar shortening of the latent period of inhibition (P III). This, in point of fact, is the case. In Pl. I, showing the inhibitory effect of flashes on the off-effect, record *C* is obtained with a 4° area, record *A* with a 1° area. A glance at the figure shows that the inhibitory effect is very much delayed by a decrease in the area stimulated. With the 1° area maximal inhibition occurs after 200σ from the beginning of the flash; with the 4° area the maximal inhibitory effect has a latency of only 120σ. The intensity effect on the latent period of the inhibitory *a*-wave of the retinal action potential is well established by numerous observations since 1908 [Einthoven and Jolly]. As excitation (P II, *b*-wave) and inhibition (P III, *a*-wave) correspond in retina and nerve, further details need hardly be added.

Discussion.

Text-fig. 5 is a reproduction of the analysis of the retinal action potential of the light-adapted frog's eye [Granit and Riddell, 1934]. P II and P III are given in dotted lines. P I is lacking in this state of adaptation. For experimental identification with P II and P III respectively of the various phases of the retinal action potential, shown above to be associated with excitation and inhibition, the reader is referred to the work of Granit and Riddell [1934]. The complex positive potential, drawn in full, need only be regarded as a plot of frequency of impulses against time of exposure in order to represent a convenient, though somewhat schematic summary of the connection between the retinal component potentials, the complex retinal response



Text-fig. 5. Analysis of retinal action potential of light-adapted frog's eye [after Granit and Riddell, 1934].

and the discharge through the nerve. The initial negative *a*-wave then illustrates that part of the latent period which is due to inhibitory P III being elicited a few σ earlier than excitatory P II. In using shortenings as "inhibitory P III" and "excitatory P II" we do not intend to say that either component has been shown to cause the equivalent effect in the nerve; it has, strictly speaking, only been shown to be associated with it. Naturally one would like to think of the opposite potentials as causing the opposite effects of excitation and inhibition in the nerve, but there is no direct evidence on this point. Text-fig. 5 also shows that the off-effect is a post-inhibitory rebound, and that the low frequency of the discharge during continued stimulation partly depends on inhibition. Whether a plot of frequency of impulses against time of exposure actually would give the various phases in the same relative size as in the complex retinal potential is difficult to know, but there can be no doubt about the

broad qualitative resemblance. The discharge in the nerve at high intensities can definitely be seen before the *b*-wave rises from the *a*-wave, as noted already by Adrian and Matthews [1927 *a*] with the eel's eye which likewise has a large *a*-wave, and it is possible that the rate of change of potential is of some significance together with the absolute level of positivity above the base line. In view of these facts the diagram must be regarded as merely illustrative, inasmuch as it is intended to represent simultaneously the retinal response and the discharge in the nerve.

Locus of potentials. From the evidence on interaction preceding P II and P III, we concluded that the retinal action potential is localized to some post-synaptic point in the retina. From this point of view it is interesting to find slow potentials of opposite electrical sign connected with excitation and inhibition in a structure histologically equivalent to a nervous centre. Since the preliminary publication of our results, valuable confirmatory evidence has been reported by Eccles [1934], who in the superior cervical ganglion has found excitation and inhibition associated with respectively negative and positive slow potentials. If the inversion of the vertebrate retina be taken into account, the electrical signs of the potentials in the retina and in the superior cervical ganglion are identical for excitation and inhibition. His work, as well as ours, bears out the suggestion by Gasser and Graham [1933] that the negative and positive potentials, which they noted in the spinal cord, are similarly connected with central excitatory, and central inhibitory state.

As P II may be removed selectively from the cat's retinal action potential by means of asphyxia [Granit, 1933], it is obvious that the two components, shown to influence the discharge, are related in a very direct way to the stimulus. The one can hardly be held to elicit the other; they must be, as it were, in parallel, and somewhere act upon a common point of convergence. This differential sensitivity of P II and P III to asphyxia may yet prove to be of theoretical value.

Motor and sensory centres compared. Just as the motor centre requires excitation and inhibition for co-ordinated functions of a higher order, so does the retinal centre utilize both mechanisms for special purposes of its own, as when rapid and delicate adjustment to changes of illumination are required in flicker. The occurrence of synchronization and of post-inhibitory rebound in both types of centres also points to an inherent likeness in function between motor and sensory neurones, and serves to emphasize the analytical value of the retina for such problems.

Adaptation. Granit and Riddell's [1934] finding that P III increases during light-adaptation now appears in new light: the light-adapted eye

is a relatively more inhibited eye, and this factor is of prime importance for an understanding of the phenomena of adaptation, which hitherto have been discussed merely from the point of view of the bleaching and regeneration of visual purple. The state of adaptation is also defined by the balance of inhibition and excitation.

Flicker. The significance of the increase in inhibitory P III during light-adaptation is best understood with regard to the important rôle played by P III in increasing the speed and precision of the visual response. The increased fusion frequency and the briskness of the intermittent waves in the light-adapted eye chiefly depend on inhibitory P III [Granit and Riddell, 1934], inasmuch as this component is responsible for the large and rapid post-inhibitory rebound or off-effect and the likewise rapid inhibitory notches caused by light falling on this phase of the response. As pointed out above, an off-effect is elicited in the light-adapted eye by a gap of darkness shorter than 5σ. The dark-adapted eye is slow and cannot follow rapid changes in the illumination [Granit and Riddell, 1934].

In view of recent interest in the theory of flicker [Cobb, 1934; Hecht and Verrijp, 1933] it is necessary to emphasize the following conclusions (frog's eye): flicker is interaction between excitation and inhibition; the first effect of each new intermittent stimulus is inhibitory (a-wave); excitation is elicited as an off-effect, and not as a b-wave, at each interval of darkness; the long latency of the b-waves and the equivalent discharge under such circumstances shows that at fast rates of flicker they do not enter into the question.

Synchronization. The many similarities between the synchronization of the discharge in response to intermittent stimulation and spontaneous synchronization suggest that inhibition and excitation also may interact spontaneously to produce the latter group of phenomena. In favour of this hypothesis is the big difference between maxima and minima in a well-developed spontaneous rhythm, as well as the fact that clonus in motor centres is interaction between excitation and inhibition [Denny-Brown, 1929].

Vision. In the human periphery, which structurally is very similar to the frog's retina, the perceived fusion frequency changes with the state of adaptation just as does the fusion frequency in retina and nerve of frogs. The increased fusion frequency of the light-adapted human eye is simply explained by the results presented in this paper. We can hardly avoid the conclusion that in both cases similar mechanisms are at work. The presence of inhibition in the retina is thus bound to introduce a

number of new aspects into the experimental work with sensations. Most obvious are the consequences with regard to adaptation and flicker. On the authority of the duplicity theory, all changes coincident with changes in the state of adaptation have been deduced from real or imaginary properties of the "rod-cone—visual purple" system. The existence of an important inhibitory component, intimately connected with the state of adaptation, imposes serious limitations upon the applicability of the duplicity theory. Why, for instance, is there less inhibition in the dark-adapted eye though the stimuli are far above the thresholds for both rods and cones? Could it be that the rods, when sensitized by the presence of visual purple, were capable of suppressing an inhibitory component more marked in the system that begins with a cone. There are many questions of this kind still to be raised and answered. At any rate, it is clear that the change in the fusion frequency with the state of adaptation cannot be explained on the basis of a different photochemical system for rods and cones, but is a function of the rapid inhibitory P III.

Synchronization during the off-effect might offer a clue to an understanding of the rhythmic variations in the brightness of after-images, inasmuch as they are determined by the reactions in the sense organ.

Summary.

On the basis of the analysis of the retinal action potential, elicited by "white light" in dark- and light-adapted frogs' eyes [Granit and Riddell, 1934], the various components and phases of the retinal response have been correlated with events in the optic nerve (large Hungarian frog). This analysis is given in Text-fig. 5 for a light-adapted eye, lacking the component P I.

The retinal action potential has been recorded with a string galvanometer and a directly coupled amplifier, or (for fast phases and high sensitivity) with large condensers in a condenser coupled amplifier operating the deflector plates of a Cossor cathode-ray oscillograph.

The component P I of the retinal response, present only in the dark-adapted eye, cannot be shown to be connected with the discharge in the optic nerve.

The positive component P II (see Text-fig. 5) is associated with the discharge of impulses in the nerve ("excitatory P II"); the negative component P III is connected with inhibition of impulses "inhibitory P III"). The connection between P II and excitation has been demon-

strated by comparing the retinal and nervous responses to variations in area, intensity, state of adaptation, duration of "gaps of darkness" in an otherwise continuously illuminated eye, and rate of intermittent stimulation. Selective activation of P III during the off-effect, according to a method developed by Granit and Riddell [1934], has been used in order to demonstrate the inhibitory nature of P III against a background of excitation.

The off-effect, being a release of remnant P II by the return towards the base line of P III after cessation of stimulation (see Text-fig. 5), may be described as a "post-inhibitory rebound."

A short period of inhibition precedes excitation as the first effect of a new stimulus. Hence the latent period of the discharge in the nerve consists of two phases: (1) an inhibitory phase, (2) the latent period of excitation, both of which probably are preceded by a short latent period covering the duration of the hypothetical photochemical process initiating the response.

Inhibition increases during light-adaptation, and is chiefly responsible for the increased rapidity of the reactions of the light-adapted eye, evidenced *e.g.* by the increased fusion frequency of intermittent stimulation (flicker).

Flicker is interaction between excitation and inhibition, excitation being elicited as an off-effect during the intervals of darkness, inhibition again being activated by the intervals of light.

At high intensities the discharge is synchronized at "on" and "off." The frequency of the synchronized beats increases with an increase in area or intensity of stimulation. This is held to be evidence of synaptic interaction.

Further evidence of synaptic interaction, referring to both P II and P III, may be found on p. 17. The results of interaction are mirrored not only in the nervous discharge but also in the retinal action potential, which therefore must be held to be post-synaptic, *i.e.* localized to the retinal neurones.

Synchronized beats from the nerve may appear as artefacts on the retinal action potential in the shape of small extra wavelets.

General conclusions regarding the significance of excitation and inhibition for various aspects of retinal physiology and vision may be found on p. 19.

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