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OBSERVATIONS ON THE RETINAL ACTION POTENTIAL WITH ESPECIAL REFERENCE TO THE RESPONSE TO INTERMITTENT STIMULATION.

By R. S. CREED[1] and RAGNAR GRANIT[2].

(*From the Department of Physiology, Oxford.*)

In the publications of the early workers on retinal action currents, observations on the response to intermittent light are often reported. The first to undertake such experiments were, we believe, Kühne and Steiner [1880], who produced intermittent light by rhythmically compressing the rubber tube leading to their gas flame. But, apart from the finding that with this mode of stimulation the response also became rhythmic below a certain frequency of alternation, no really significant contributions were made to the problem until Piper in 1911 investigated the reactions of various types of vertebrate retinæ to an intermittent stimulus of given strength. He observed several interesting facts which, as occasion arises, will be referred to below in connection with our own observations. Some general conclusions based on Piper's work still summarize the state of knowledge. The retinal response to intermittent light consists of undulations during the slow phase of the retinal action potential (*c*-wave), which disappear above a certain frequency of stimulation. This critical frequency is low in eyes containing mainly rods (cat), and very much higher in eyes in which cones predominate (pigeon). Where the retinal action current shows a well-developed initial negative *a*-wave and a marked off-effect (*e.g.* frog), these two appear to be among the factors determining the troughs and crests of the waves. Some observations of a similar character on the eyes of fishes were made by Day [1915] in Piper's laboratory.

Since that time the retinal reaction to intermittent light has hardly been studied at all by direct methods. Renqvist [1924] noted that

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[1] Fellow of New College.

[2] Helsingfors University; Fellow of the Rockefeller Foundation.

flashes superimposed upon steady illumination of the frog's eye gave rise to small *a*- and *b*-waves on the background of the slow *c*-wave. Sachs [1929], recording from his own eye, found that intermittent potential waves were accompanied by a sensation of flicker, whereas a smooth potential curve corresponded to the sensation of continuous light. Adrian and Matthews [1928] recorded the impulses in the optic nerve of the conger eel and found that below a certain frequency, increasing with the area and intensity of the stimulus, the discharge waxed and waned at the same rhythm as the stimulation, but that above that frequency it was represented by a steady stream of impulses such as is given by continuous illumination.

Several problems have thus been raised which are of importance both from the point of view of the processes in the retina and in connection with the sensory phenomena of flicker and fusion. The experimental work presented below is concerned only with the retinal action potential and not with flicker. When the terms "fusion" and "fusion frequency" are used, they refer to the point at which the intermittent potential waves cease. Much remains to be done before a complete understanding of what occurs in the retina can be reached. We have attempted no more than a general survey of the ground in the light of recent additions to knowledge of retinal physiology. In particular, the new evidence [Granit, 1933] regarding the three components of the action-current complex necessitates a study of how responses deficient in one or other of these components behave when the stimulus is intermittent. In order to be in a position, however, to discuss the effects of repetitive stimulation, it seemed desirable first to investigate the responses to single flashes. Part I of this paper contains the results of this study.

METHOD.

Records have been obtained from some 25 cats, decerebrated 2 hours before beginning the observations. During this interval the preparation was shut up in a box so as to ensure complete dark-adaptation. The pupil was, in all preparations, widely dilated. The animal technique and the methods of stimulating and recording were the same as those described in a previous paper [Granit, 1933]. The only modification of the apparatus for purposes of this research has been the introduction of a sectored metal disc which could be rotated in a plane where the cross-section of the stimulating beam was narrow. The disc was driven by an electric motor, the speed of which could be controlled by a sliding resistance. Three discs were employed, with open sectors of 180°, 90°, and 45° respectively. The opaque sectors in each were of the same size as the open sectors. The third disc thus had four open and four opaque sectors.

The circular ground-glass sheet constituting the stimulus object was 46 mm. in diameter and was mounted 370 mm. from the cat's eye where it subtended 7°. The intensity of the light falling upon it was varied by inserting Wratten neutral-tint filters, with transmission factors of 0·5, 0·1, 0·01, and 0·001, in the beam. At full intensity the brightness of the ground glass was about 11 millilamberts. The area of the stimulus was varied by mounting an iris diaphragm just in front of the ground glass. Since we do not know how sharp was the image formed on the retina, it is impossible to state confidently what precise effect on the area of this image a known change in the area of the stimulus object would produce. On ophthalmoscopic observation, the decerebrate cat's eye appears to be approximately emmetropic. The duration of the stimulus was controlled by a Compur photographic shutter placed where the beam was narrow.

To obtain the "fusion frequency" with intermittent stimulation, the motor driving the sectored disc was slowed down from a high speed, or, less frequently, speeded up from rest. The point at which the string began to oscillate or became steady respectively was later determined from the tracing. Frequencies of alternation are always expressed in flashes per second.

The periods during which the eye was exposed to light were directly photographed on the moving strip of sensitized paper. The duration of each flash was thus readily measured with the aid of the time marker (Rayleigh wheel) simultaneously registering intervals of 20σ. The slight spread, or "irradiation," of photochemical effect on the paper could be accurately estimated by comparison of the apparent durations of the (actually equal) light and dark phases, as recorded by the signal when the motor was running steadily. Judged by this criterion, our measurements of latent period, always made with the paper moving at maximum velocity in the camera, are 3σ too long.

The use of valve amplification of the retinal action potential has enabled us to keep the string of the small permanent magnet string galvanometer (Edelmann's model) tense enough to follow rapid changes accurately and to give reliable information about the latent period. Variation of such factors as area and intensity of the stimulus could then be studied over a certain range. But the more sensitive the recording apparatus, the more difficult it is to obtain a base line sufficiently steady to record oscillatory responses of small amplitude from an eye *in situ*.

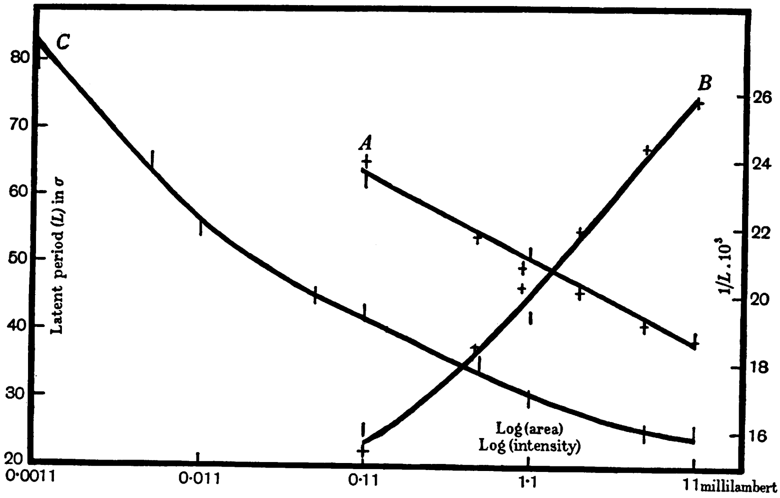
Slight movements of the eyelids and of the external ocular muscles are liable to confuse the picture. Moreover, the eye of the decerebrate cat, even when thoroughly dark-adapted, is an unsuitable preparation for investigation of the effects of either greatly diminished area or greatly diminished intensity of stimuli. When using low intensities of stimulation, we have rarely found it possible to work with initial *b*-wave deflections of less than 5 mm. Illuminations lower than about 0·01 ml. have only occasionally been used.

I. CONTINUOUS STIMULATION AND SINGLE FLASHES.

The electrical response of the retina may be affected by the area, by the intensity, and by the duration of the stimulus that evokes it. We have investigated the influence of each of these factors separately. The results may be stated very briefly because, in the main, they merely confirm and extend the conclusions of previous workers. Shortening of the latent period with increasing intensity of stimulus has been repeatedly observed [Einthoven and Jolly, 1908; Ishihara, 1906; Adrian and Matthews, 1927*a* and *b*; Sachs, 1929; Granit, 1932]. The similar effect of increasing the area of a stimulus of constant intensity has been studied by Ishihara [1906], Adrian and Matthews [1927*a* and *b*], and Granit [1933].

In Text-fig. 1, curve *A* represents the relation found in one experiment between latent period preceding the *b*-wave and either the area (crosses) or the intensity (vertical strokes) of the stimulus. Each value plotted is the average of five readings. With a constant area of 1660 sq. mm. placed 370 mm. from the eye, the intensity was first reduced to 0·1 and 0·01 of its original value (11 ml.). The filters were then removed, and the area reduced in several stages, again to 0·01 of its initial extent. The abscissæ are log area and log intensity plotted on the same scale, the lowest point on the curve being the common starting point for both sets of observations. In this experiment and within these limits, area and intensity evidently influence the latent period to precisely the same degree. They do not, however, invariably do so. Curve *B* is plotted to the same abscissæ, but the ordinates are the reciprocals of the latent periods in *A*. Curve *C* shows the results of another experiment in which a stimulus of the same area was placed about 70 mm. from the eye. The higher potential developed enabled measurements to be made over a ten-thousandfold range in intensity. Each point is a single reading. The latent period at the lowest intensity is somewhat uncertain on account of the gradual development of action potential. Similar results have on several occasions been obtained with this large stimulus. The points do not lie on a straight line over the whole range. When the reciprocal of the latent period is plotted against log intensity, an **S**-shaped curve is obtained.

The amount of potential, measured by the height of the *b*-wave, and the rate at which it develops have been found to vary as would be expected both with the intensity and with the area of the stimulus. The findings in this connection have not been analysed in detail, but are in rough agreement with previous work.



Text-fig. 1. The ordinate scale on the left gives the latent period of the retinal response for curves *A* and *C* in σ. That on the right gives the reciprocal of the latent period for curve *B*. Abscissæ are log brightness in millilamberts and log area in arbitrary units. For explanation, see text.

The duration of the stimulus is in our experience devoid of influence on the latent period. The shortest flashes we could obtain have been about 4σ in length. These have been tried on a number of different preparations, and the latent period preceding movement of the string has always been the same as that for prolonged stimuli. Thus, in Plate I, fig. 2, the latent period is 30σ both for a flash of 4σ and for one of 110σ. At very low intensities measurable records could not often be obtained with such brief exposures. A brightness of 1·1 ml. has sometimes been used. The results are then the same as with more intense stimuli. The latent period for a stimulus of 0·11 ml. is also unchanged by shortening the flash to 40σ. Briefer exposures are impracticable with so feeble a stimulus.

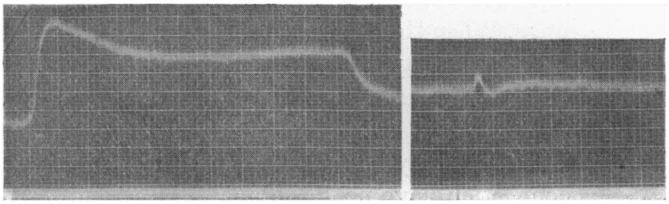


Fig. 1.

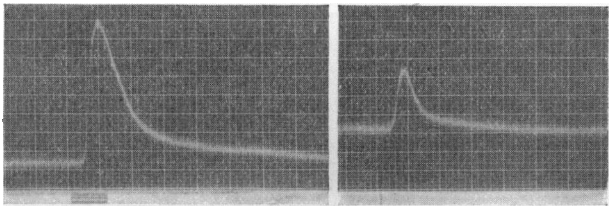


Fig. 2

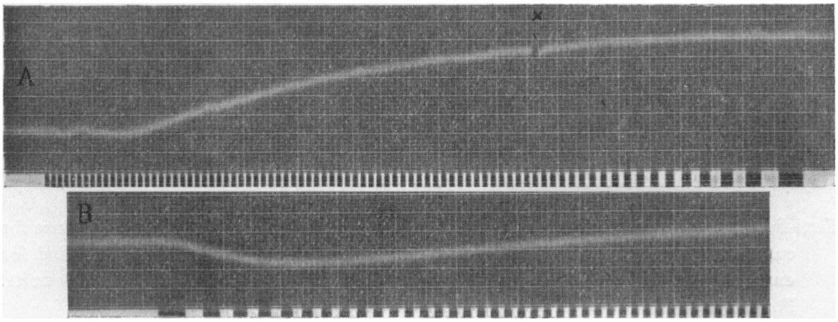


Fig. 3.

Plate I. Fig. 1. Records showing the effects of continued stimulation and of a flash lasting about 4 σ, both of intensity 1·1 ml. The signal at the bottom indicates the beginning and the end of the stimulation. The thin vertical strokes are 20 σ, and the thick 100 σ, apart. The interval between the horizontal rulings is 5 mm.

Fig. 2. Responses to two flashes, one lasting about 110 σ and continuing beyond the peak of the *b*-wave, the other lasting about 4 σ. Brightness of stimulus 11 ml. Both have the same latent period.

Fig. 3. Both records show responses to intermittent light of intensity 11 ml. In *A* the carotid artery has been ligated. P II is in consequence very small (minute *b*-wave). The cross marks an artefact produced by throwing a high resistance into the circuit of the motor driving the sectored disc. In *B* P II has been removed by ether, leaving only P I and P III. There is no trace of ripples.

The amount of potential developed is, however, generally diminished by making the flashes shorter (Plate I, fig. 2). With the more intense stimuli this was not always so. The *b*-wave might then be as high with a flash of 4σ as with a longer stimulus. But as a rule the *b*-wave became progressively smaller as the flash was shortened below 20σ (cf. Hartline [1928], on the eyes of arthropods). Piper [1911], who used strong stimuli, found that "within wide limits" the *b*-wave was independent of the time of stimulation.

In one experiment, illustrated in Plate I, fig. 1, the form of the curve resulting from very short flashes differed from that usually seen. To exclude chance variations in the base line, it was photographed several times, always with the same result. *A* is the response to a long-lasting stimulus of 1·1 ml. *B* is a flash of about 4σ. It shows a small initial negative wave, not visible in *A*, followed by the *b*-wave, a small negative deflection, and a secondary rise. It is reminiscent of the recurrent sensory images elicited by short flashes. It may indicate that for unknown reasons conditions were favourable in this experiment for some complicated kind of interaction between the processes P II and P III [Granit, 1933].

Discussion. It is not the purpose of this paper to investigate the relation between quantity of light and height (or area) of the curve of potential. On the other hand, the absence of any change in the latent period as a result of using the briefest flashes was so unexpected a finding that some discussion of it is demanded.

The effect of varying area or intensity is easily detected and is in no way surprising. At low intensities it is probably exaggerated artificially by the gradual ascent of the rise of potential. But Adrian and Matthews [1927 *b*] found that below a certain critical duration, the latent period preceding the discharge of impulses in the conger eel's optic nerve was lengthened by shortening the flash. It is probable that at the high intensities we were using 4σ is longer than the critical duration for the dark-adapted cat's eye, and that lengthening of the latent period would have been observed had we been able to obtain still shorter flashes. At low intensities too little potential may have been elicited with flashes below the critical duration. But it is also possible, though less probable in view of the finding by Adrian and Matthews of a constant retinal nerve interval in frogs and conger eels, that the latent period of the discharge in the optic nerve is not directly related to that of the *b*-wave. Our results cannot be explained by assuming that the initial negative wave disappears with short flashes and thereby allows the rise of the *b*-wave to be determined at an earlier stage. We have several high intensity curves in which the *a*-wave is to all appearance unchanged when the flash is shortened to 4σ. It is worth emphasizing that although the latent period would probably be affected by the length of exposure with very short flashes, this phenomenon does not appear comparable with the effects of area and intensity.

From the effects of diminishing area and intensity, and from those of narcotization and asphyxia [Granit, 1933], it might be inferred that every reduction in the height of the *b*-wave is accompanied by a lengthening of the latent period. The experiments with flashes, however, make it certain that no such correlation can be established.

II. INTERMITTENT STIMULATION.

Plate II, tracing *A*, shows the response to a stimulus of full intensity, intermitted at a constant rate of 10·8 per second. It will be noted that the well-developed *b*-wave, characteristic of intense stimulation, tends to take its own course and does not show oscillations in response to the first few flashes. Later, oscillations become obvious, but the earlier of them are smaller than those set up after the lapse of 1 sec. These facts were also observed by Piper [1911]. Finally a semi-stationary state is reached in which ripples of unvarying size are superimposed on the background of the *c*-wave. The oscillations show no sign of diminishing in amplitude towards the end of the 5 sec. for which the stimulus in one experiment was applied. The latent period from the beginning of the flash to the beginning of the upstroke also remains constant. The oscillations are remarkably symmetrical provided the frequency of repetition of the stimuli is high enough. In *A* the symmetry is to some extent caused by the slackness of the string. In *B*, however, the string is tense enough to justify the conclusion that the rise and the fall of the action potential itself are much alike in steepness. The upstrokes are perhaps slightly more steep than the downstrokes.

When the *b*-wave is smaller (as, for instance, with less intense stimuli), or when the frequency is low, ripples corresponding with the rate of stimulation appear in the records from the very beginning. It is only large *b*-waves that succeed in masking more or less completely the initial rhythm (see Plate II, *B* and *F*). In some tracings taken with stimuli of 0·011 ml. the ripples are actually larger and rise more steeply than the initial *b*-wave elicited by a continuous stimulus of the same intensity.

When the frequency is increased, the oscillations become smaller until no trace of them is to be seen (Plate II, *B*, *C*, and *D*). The fusion frequency can be determined from records like Plate II, *E* and *F*, during which the motor was slowed down and speeded up respectively. The former method, by which most of our results have been obtained, gives slightly lower values (2–3 flashes per second) than the latter. The highest

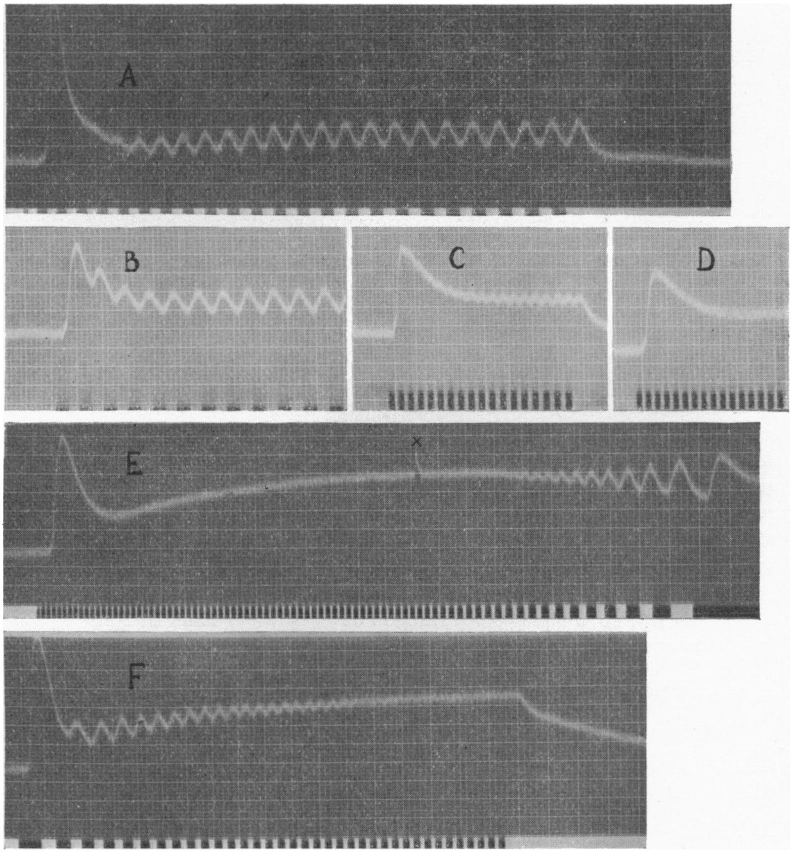


Plate II. Series of responses to intermittent light (intensity 11 ml.). *A*, string slack. Constant rate of stimulation of 10·8 flashes per second. The *c*-wave is unusually small. *B, C,* and *D* are from another experiment. They show the effect of increasing the frequency of alternation. *E*, from another experiment, is typical of the response when the motor is slowed down. The cross indicates an artefact as in Plate I, fig. 3. *F*, from another experiment, illustrates the effect of speeding up the motor. Note the *c*-waves in *E* and *F*.

fusion frequency that we have found for the dark-adapted cat's eye is 32 with a stimulus of about 11 ml. In most cats the maximum frequency is probably not higher than 25 or 30 [cf. Piper, 1911]. Considering that in our experiments the pupil has been widely dilated, and that the animal is nocturnal and thoroughly dark-adapted, we have every reason for believing that a stimulus of the order of magnitude of 10 ml. or 100 m.-c. is physiologically maximal.

Control experiments.

It might be expected that the apparent fusion frequency in the tracing would vary with the intrinsic frequency and sensitivity of the string. The slackest string used has been one taking about 50σ to reach its full deflection when a constant current is passed through it. About the same time is occupied by the rising phase of the fastest *b*-waves of the cat's retinal action potential with stimuli of our maximal intensity.

TABLE I. Fusion frequency of intermittent potential waves for a stimulus of constant strength, 11 ml. The values were obtained with the string at three different tensions. At each tension the sensitivity was varied by shunting the string through different resistances. The sensitivity is indicated by the deflection in mm. of the *b*-wave of the retinal action potential, which, throughout the experiment, was 0·58 millivolt. The string was unshunted only when maximally tightened (rising to maximal value in 2·5 σ); in the other cases the damping is considerable. String resistance 3100 ohms.

String aperiodic, rising in 57 σ		String just periodic, rising in 5 σ		String periodic rising in 2·5 σ	
Height of *b*-wave	Fusion frequency	Height of *b*-wave	Fusion frequency	Height of *b*-wave	Fusion frequency
21	24	9	22	30	23
21	21	11·5	24	30	22
39	25	18	25	30	23
40	23	18	22	—	—
—	—	57	24	—	—

Table I contains the results of one of the experiments made to test the influence of these factors. It is evident that considerable variation in string tension does not affect the fusion frequency. The values of the fusion frequency are typical of those obtained under the same standard conditions of stimulation in a large number of other experiments. The variations in the value are also typical. A high degree of accuracy in the determination of the exact point of fusion is unattainable even when, as in this and most other series, the motor is slowed down for every observation. The unlikely possibility is not altogether excluded that the variations may arise in part from the fact that in different records the fusion point occurs at different intervals after the onset of stimulation.

Just as the retinal action potential cannot be satisfactorily recorded when the stimulus, although easily visible with the human eye, is feeble, so no doubt there is a limit to the smallness of the oscillations that can be detected in the tracings. The stability of the base line varies inversely with the sensitivity of the recording apparatus. In other words, it is by no means certain that the retinal processes entirely lose their oscillatory character when the frequency of stimulation is above what is here called the fusion point. The true fusion frequency may be higher. Nevertheless, variations in the fusion frequency caused by changes in area and intensity of the stimulus, as determined from our records, are of real significance and must correspond with similar variations in the true fusion frequency. What the control experiments show is that our criterion of fusion frequency, even if, in a sense, a fictitious one, is unaffected by considerable variations in the tension and the sensitivity of the string.

Isolation of the process responsible for the oscillations.

The complex retinal action currents have recently been analysed into three components known as P I, P II, and P III [Granit, 1933]. The positive P I rises and falls slowly, and is principally responsible for the secondary rise or *c*-wave with strong stimuli. P II causes the *b*-wave and part of the *c*-wave at high intensities. With weak excitation the electrical response gives a maintained positive deflection of simple form, almost entirely made up of P II. P III is negative. To it alone is due the *a*-wave. By methods described elsewhere, we have removed one or other of these processes in order to determine their respective rôles in the production of the oscillations.

P I is readily abolished by giving the preparation ether to inhale. The ripples persist and the fusion frequency is unaffected.

P II is very minute as a result of asphyxia in Plate I, fig. 3 *A*. Although the intensity is 11 ml. and the *c*-wave is well developed, there is only the merest trace of rippling at the end of the record. Complete removal of P II causes total disappearance of oscillations, though no doubt the *c*-wave might be made to wax and wane if the periods of light and darkness were each of some seconds' duration. But this would clearly be a different phenomenon.

In Plate I, fig. 3 *B*, both P I and P II have been largely removed by a high concentration of ether. The remaining negative deflection (P III) traces a smooth curve.

Hence it is clear that P II is the component chiefly responsible for the ripples in Plate II. Moreover, at low intensities, P II is manifested in an almost pure form [Granit, 1933]. Oscillations are then readily recorded, and are so large that they cannot possibly be ascribed to the tiny effects of P I and P III. The conclusion is in keeping with the finding of Adrian and Matthews [1928] that the optic nerve discharge waxes and wanes with intermittent stimulation, coupled with Granit's inability to show that any component other than P II is concerned with the discharge of impulses [1933]. It also harmonizes with Renqvist's observation [1924] on the frog's eye (where *a*-waves are large) that flashes superimposed upon a steady stimulus give rise to small *a*- and *b*-waves without affecting the slow rise of the *c*-wave.

The possibility that P II and P III interact, when present together, is not excluded. No means is available of removing P III at high intensities, leaving P II isolated for study. Evidence will be furnished below that such interaction does in fact take place, but we cannot say whether it has any effect on the fusion frequency. It may be that the fusion point and the shape of the individual ripples would be altered were P II released from the modifying influence of P III.

Factors influencing fusion frequency.

In the upper part of Table II figures are given showing how, in one of many similar experiments, the fusion frequency, determined as described above by slowing down the motor, varies with the brightness and with the area of the stimulating object. The range of variation in each series is a hundredfold. As a rule a given increase in intensity has a greater effect than a corresponding increase in area. If the fusion frequencies are plotted against log brightness or log area, they lie on approximately

TABLE II. Effects of variation in area and intensity on the fusion frequency and the latent period of the intermittent potential waves of the retinal action potential. The values are averages of 3–4 measurements for the fusion frequency and of 10 or more for the latent period. Distance to cat's eye 370 mm. Maximal area of 1660 sq. mm. represented by circular disc with a radius of 23 mm. D is the duration in σ of the flashes at the fusion point. L is the latent period of the intermittent waves in σ.

	Experiments with constant area of 1660 sq. mm. Brightness varied			Experiments with constant brightness of 11 ml. Area varied			
Brightness	11	1·1	0·11	Area 1660	473	165	16·7
Fusion frequency	19·3	16·8	14·8	19·3	18·0	15·8	13·6
D	26·0	29·8	33·8	26·0	27·8	31·6	36·8
L	60	76	95	60	65	71	84
D expressed as percentage of L	43	39	36	43	43	45	44

straight lines. It is obvious from Table I that great accuracy cannot be expected in such experiments.

The series do not cover a wide enough range to permit the definite formulation of a general law relating fusion point and brightness or area, such as has been found to hold for sensory fusion of flickering stimuli. Adrian and Matthews [1928] similarly noted an increase in the fusion frequency for the optic nerve, as defined by the cessation of rhythmical waxing and waning of impulses, when either the area or the intensity of the stimulus was increased.

The lower part of Table II illustrates an empirical rule relating the fusion frequency with the latent period of the ripples in the curve of retinal action potential. By "latent period" we mean the interval between the beginning of a flash and its corresponding ripple. Each value given for the latent period is the mean of a large number of determinations and is therefore a fairly reliable figure. Many determinations can be made from every record, since the length of the latent period appears to be unaffected both by the frequency of intermission and, at any rate within our limits, by the total length of the exposure. (It will be explained in a later section, however, that it is not necessarily the same as the latent period for a continuous light.) The product of the latent period and the fusion frequency is approximately constant. This is equivalent to saying that, at the point of fusion, the duration of the flash (one-half the reciprocal of the fusion frequency) is a fixed fraction of the latent period. The last line of the table expresses this fraction as a percentage. In view of the difficulty of accurately measuring the fusion frequency, the variations in this figure cannot but be regarded as very small.

Results obtained with preparations other than the one from which Table II is constructed give much the same value. In all, 50 independent determinations of the fraction have been made, each representing the average of all the values obtained with the same area and same intensity on any given day with the motor slowing down. The arithmetic mean of these is 44 p.c., with a mean variation of 4·3 (= 9·8 p.c. of 44). When the flashes at the fusion point are so frequent as this, it is evident that each occurs shortly before any potential can have been developed by the one immediately preceding it. There is thus scope for interaction between the retinal processes set up by successive flashes.

The rule should not be regarded as more than a convenient first approximation to a formula relating length of flash at the point of fusion and latent period. The range of variation on which it is based is insufficient to justify far-reaching conclusions. It may well be that over a wider range some systematic deviation would be found, but within the limits that we have investigated it appears to be true that the fusion frequency varies inversely as the latent period of the oscillations.

Rapidly intermittent stimulation and the Talbot-Plateau law.

According to the Talbot-Plateau law, whenever intermittent stimuli follow one another with sufficient rapidity to give rise to a sensation of continuous light of uniform brightness, the apparent brightness is that which would have arisen had the amount of light intermittently reaching the retina been uniformly distributed over the whole period of stimulation. The law has repeatedly been verified over a wide range of intensities and over a wide range of variation in the relative lengths of the bright and dark intervals. Only at very high and at very low intensities have exceptions been reported. For all ordinary conditions the law is so reliable that many workers regard the episcotister as the method of choice for producing accurately known variations in the physical intensity of illumination. It becomes of interest, therefore, to know whether a similar relation holds when the retinal action potential, instead of the subjective brightness, is investigated.

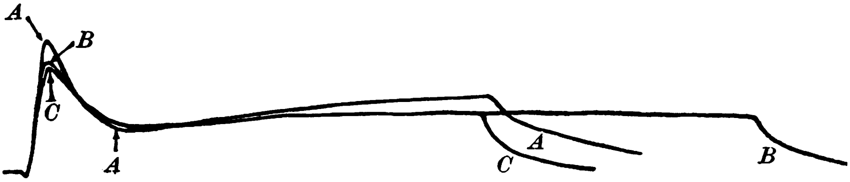
We have made no systematic series of records, but in the course of other enquiries we have compared steady tracings from retinæ intermittently illuminated at a fast speed of alternation, light and darkness being of equal duration, with others from the same retinæ continuously illuminated from the same source but with the intensity reduced to a half by means of a Wratten neutral-tint filter. That the fused curve from the intermittently illuminated retina is lower than that from the retina continuously illuminated at full intensity is clear from experiments in which the motor has been speeded up from rest with an open sector between the source of light and the eye. That its height is identical, within the limits of experimental error, with that from the retina continuously illuminated at half-intensity is shown in Text-fig. 2. So far, then, as the potential developed is concerned, a Talbot-Plateau relation seems to hold under these conditions. The latent period, however, may be different in the two cases and must be considered separately.

Since the latent period of the initial *b*-wave is the same for short flashes as for continued illumination, it might be expected that it would also be the same for stimuli rapidly alternating with darkness. This indeed is often so. But not uncommonly, as shown in Table III, the intermittent stimulus requires much longer than the continuous to produce any effect. Moreover, it will be noticed that the latent period with the continuous

TABLE III. Comparison between initial latent period in σ for continuous and for rapidly intermittent stimulation.

Brightness in ml.	*A*, continuous stimulus	*B*, intermittent stimulus	Ratio *B*/*A*
11	37	73	2
1·1	40	69	1·7
0·11	50	65	1·3

stimulus progressively shortens as the intensity is increased. Yet, in the same experiment, when the stimulus alternates at 30–40 flashes per second, a converse relation holds between latent period and intensity. And the higher the intensity, the more does the length of the latent period differ from that with continuous illumination. As has been stated above, the phenomenon cannot always be demonstrated, but it is clearly of great interest and must later be discussed in connection with our other findings.



Text-fig. 2. *A* is the response to continuous illumination at 11 ml., *C* to 5·5 ml. *B* is the response to an intermittent stimulus (40 flashes per second) of 11 ml. The rotating disc gives equal periods of light and darkness. The *b*-wave of *B* is slightly larger than the corresponding wave of *C*, although the two curves coincide in their further course.

At the moment it need only be pointed out that the initial latent period for a rapidly intermittent stimulus is often longer than that for a steady stimulus of half the intensity. The two forms of stimulation are therefore not equivalent in all respects, and a Talbot-Plateau relationship cannot be established for the latent period.

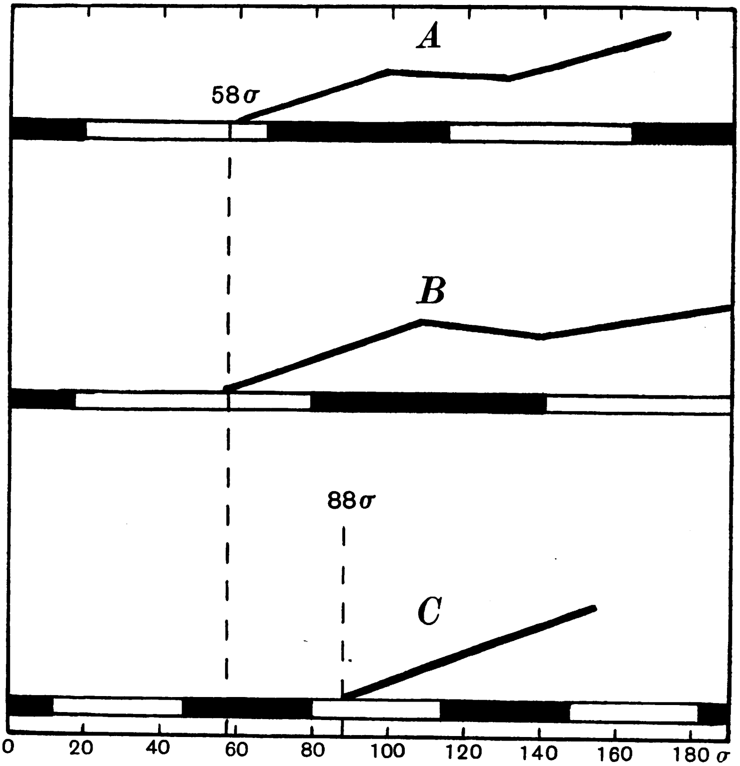
So far we have only considered the course of events with a rapidly alternating stimulus of considerable duration. This is perhaps the only condition that Talbot and Plateau and most subsequent investigators of their law have had in mind. But, with very short exposures of a bright intermittent light, when the rate of intermission is only just high enough to cause under these conditions a steady sensation, it has been found [Granit and Hammond, 1931] that the subjective brightness is greater than the law would allow. This is entirely in keeping with the fact recorded above and by Piper [1911], and illustrated in Plate II *A* and *C*, that at rates of alternation insufficient to produce smooth tracings, the initial *b*-wave may nevertheless reveal no trace of discontinuity until several flashes have occurred. In these circumstances the *b*-wave is larger than at higher rates. The Talbot-Plateau law, both as regards sensation and as regards retinal action potential, only holds when the rate of intermission is fast enough to cause fusion for a prolonged period.

The latent period preceding the oscillations.

When we have followed the usual procedure of slowing down the motor from a speed much above the fusion frequency, it has been our almost invariable experience that the latent period preceding the upstroke of the waves in the later part of the tracing has been longer than that preceding the initial *b*-wave. Sometimes it is twice as long. And it was explained in a previous section that the latent period of the initial *b*-wave itself is commonly longer than that for a steady stimulus of the same intensity. The initial *b*-wave, it is true, rises from a steady base line, whereas the ripples rise from the downstrokes of the preceding ripples. Instrumental inertia cannot, however, account for the phenomenon, for it can be made negligible by tightening the string and at the same time increasing the amplification. Nor can the long latent period be ascribed to fatigue, since the latent period of oscillations remains approximately constant when a regularly intermittent stimulus is continued for as long as 5 sec.

Some light is thrown upon the factors responsible for lengthening the latent period by an experiment illustrated diagrammatically in Text-fig. 3. In this the motor was driven at a slow speed from the beginning of each observation. The rising phase of the *b*-wave, occurring sometimes in two stages with such a slow rate of stimulation, is plotted against time in σ. The curves *A*, *B*, and *C* are all for the same intensity of stimulation and end at the highest point of the *b*-wave. In *A* and *B* the latent period (unsually long for a stimulus of 11 ml.) is about 58σ. In *C*, however, the latent period is 88σ and the string has not begun to move until some time after the second flash has been delivered.The *b*-wave here rises in a single step to the same height as is attained at the second step in *A* and *B*. It is evident that the first flash alone in *C* would have elicited a smaller *b*-wave, and therefore that the rise represents the sum of the effects of two flashes. But why was the beginning of the rise delayed for 30σ as compared with *A* and *B*?

The second flash occurred just before the expiration of the normal latent period of 58σ following the first. It seems that the delivery of the second flash has delayed the appearance of the *b*-wave caused by the first. For, although the first flash is briefer in *C* than in *A* or *B*, it was shown in Part I that the latent period is independent of the duration of the flash. The cause of the delay will be dealt with more fully in the discussion. Once the delay is over the effect of the second flash summates with that of the first. This is shown not only by the height of the resulting *b*-wave but also by the fact that the *b*-wave commences 42σ after the beginning of the second flash, which, if alone responsible for it, would have been followed by a latent period of 58σ. The whole experiment is a good illustration of how complicated may be the effects of repetitive stimulation.



Text-fig. 3. For explanation, see text.

Discussion.

When discussing the sensory phenomena of intermittent visual stimuli and the Talbot-Plateau law, many writers [*e.g.* Grünbaum, 1898; Parsons, 1924] have concluded that the individual flashes of light cannot be considered as merely reinforcing one another's action. Some factor, or factors, other than summation of the excitatory effects of the successive periods of illumination must come into play. The observations which have been recorded above fully confirm this view. Some might hope to find a solution to the problem of this additional influence by recourse to the possible stimulating effect of darkness following light. We propose, however, to confine our attention to certain other considerations which are based on a strong experimental foundation.

It has been shown that the latent period preceding the retinal potential changes may be longer with rapidly intermittent than with steady illumination, and that this is especially likely to be found with intense stimuli. With alternations not rapid enough to cause a steady response, it has further been shown that the latent period of the ripples is approximately constant, but is often very much longer than that of the initial *b*-wave with a fast motor. Lastly, it has been shown that the response to a single flash can be considerably delayed by interposing a second flash shortly before the deflection caused by the first is due to occur. How can these lengthenings of the latent period be explained?

Granit [1933] has produced evidence that of the three components of the retinal action currents the positive P II alone is associated with the discharge of impulses along the optic nerve, while the negative P III tends actively to inhibit the discharge. The inhibitory component P III increases with intensity of stimulation. Our records show that it is often well developed in the response to short flashes, where it manifests itself both as an *a*-wave and, in one experiment, by the more complex effect illustrated in Plate I, fig. 1. It seems probable therefore that in repetitive stimulation P III inhibits and counteracts for a time the rise of P II which would otherwise have occurred in response to the preceding flash.

P I is left out of account for reasons which have been given above. It appears to play no rôle in the production either of oscillations in the curve of retinal potential or [Granit, 1933] in the setting up of impulses in the optic nerve. The conclusion that the negative component P III has a share in the reaction to intermittent light was also reached by Piper [1911] and is in keeping with Renqvist's observation [1924] that flashes superimposed upon steady illumination give rise to both *a*- and *b*-waves in the frog's retina. Our own evidence, with an eye in which the negative component is small, is less direct.

With weak stimuli and a steady slow rate of intermission, the ripples may be larger and rise more steeply than the initial *b*-wave for a continuous stimulus of the same intensity. This can best be accounted for on the basis of interaction or mutual facilitation between the excitatory effects of the individual flashes.

Spatial summation is shown by the influence of area both on the latent period for steady illumination and on the fusion frequency for intermittent stimulation. Areal effects are also perhaps illustrated in another way. Measurement of the records in three long series of experiments with intermittent stimuli shows that a given change of area always has less effect than a corresponding change of intensity on the latent period of the ripples (see Table II). (It should perhaps be mentioned that in one of these the ratio D/L fell steadily from 54 to 45 p.c. as the area was decreased a hundredfold and in Table II it falls with decreasing brightness, but these are exceptional.) Now the latent periods of the ripples are, of course, all longer than those of the initial *b*-wave of a continuous stimulus would be. But since for a continuous stimulus the effects of change of area and change of intensity are much alike, and may be identical (as in Text-fig. 1 *A*), it would appear that the lengthening of the latent period resulting from repetitive stimulation increases with the area more than it does with the intensity. In other words, the interaction between the effects of successive stimuli which causes the latent period for repeated flashes to be longer than that for single flashes is more marked the larger the retinal image and the larger the number of active retinal neurones. This conclusion is no more than tentative. It could only be definitely established by making serial observations on the effects both of intermittent and of steady stimuli on one and the same preparation.

A complete explanation of all our findings in terms of a complex process of interaction between excitatory and inhibitory components would admittedly be speculative. In the case of the latent period, however, it appears not only plausible, but also highly probable. But to go further and formulate a full-fledged theory of fusion frequency would now be premature. If fusion be due, for example, to two processes of opposite sign just balancing one another, the time relations of the rise and fall of both P II and P III would have to be considered. The data on these points for short flashes are at present unprocurable. There is the further difficulty that the initial *b*-wave is different from the intermittent "*b*-waves" evoked by the later flashes.

At high intensities and a steady rate of alternation, the first few flashes cause no rippling of the large initial *b*-wave, and the earlier ripples are smaller than the later ones. Among several possible factors at work in this, the three following are worthy of mention. Some form of inhibition may at first constitute a block. Or there may be some "inertia" to be overcome before the semi-stationary state of balance between the processes co-operating to form the intermittent waves is reached. Or some response of an "all or nothing" character may at first be evoked. The last might take the form of a rapid utilization of some photochemical substance, which is only resynthesized after some time in sufficient quantity to be again capable of reacting. Whatever may be the true explanation, factors must obviously be taken into account which are unpredictable from a study of single flashes.

We have dealt rather fully with certain complicated effects. They must not, however, be allowed to distract attention from our main result, viz. that the ripples of intermittent stimulation are chiefly due to the rise and fall of P II during the bright and dark intervals. Piper [1911], at a time when much less was known about the nature of retinal action currents, came to essentially the same conclusion for eyes in which, as in the cat, the negative initial *a*-wave and the off-effect are small. For eyes in which they are large, he concluded, on the other hand, that the reaction to each flash was a negative *a*-wave, and to each dark interval a positive off-effect in which a small *b*-wave might be merged. His evidence for this view is partly derived from the order of magnitude of the latent period and partly from the responses to single flashes and to momentary interruption of a continuous stimulus. Such arguments do not appear conclusive. We have shown that the latent period of the intermittent waves in the cat's eye is identical neither with that of the *b*-wave for a single flash nor with that of the *a*-wave. Moreover, it seems unlikely that the retinæ of different vertebrates would differ fundamentally in their response to intermittent light.

Doubtless, when off-effects are large, they may contribute to the oscillations, thereby illustrating the interaction between P II and P III on which we have already laid emphasis. But the response to intermittent stimulation cannot, we believe, be immediately reconstructed from data provided by a study of single flashes and brief interruptions of a steady light.

With stimuli which are not intermittent, the most striking of our findings has been that whereas area and intensity have pronounced effects on the length of the latent period, a flash lasting 4σ gives a response after precisely the same interval as does a similar stimulus of indefinite duration. This can only be interpreted as evidence that the latent period is determined much less by the velocity of the initial photochemical reaction than by nervous interaction at more proximal levels in the retina. In contradistinction to this, the amount of potential developed is often less for a short than for a long flash.

Sensory flicker and fusion.

Hitherto we have deliberately avoided making more than the barest of reference to the sensory phenomena of flicker and fusion. The experimental enquiry has been devoted entirely to retinal processes. It remains to indicate briefly the obviously close analogies which these bear to the sensations which they may ultimately evoke. For technical reasons the fusion point as determined from records of retinal action potential may well be lower than that which is perceived. The retino-cerebral apparatus which forms the physical basis of events in consciousness is probably more sensitive, and more stable, as a recording mechanism than the amplifier and string galvanometer with which our results have been obtained. Nevertheless, the two criteria may be expected to resemble one another in broad outline. This is found to be the case [cf. Sachs, 1929].

Fusion frequency, expressed in flashes per second, is roughly proportional to log intensity and, within limits, to log area, no matter whether the criterion be sensory [Ferry-Porter law; Granit and Harper, 1930], or the retinal action potential (*supra*), or the impulses in the optic nerve [Adrian and Matthews, 1928].

For rod vision the fusion frequency is lower than for cone vision [Schaternikoff, 1902; Lythgoe and Tansley, 1929; Creed and Ruch, 1932]. The same is true of retinal action currents [Piper, 1911].

SUMMARY.

1. The action potential of the dark-adapted retina of the decerebrate cat has been recorded from the eye *in situ* with the aid of valve amplification and a string galvanometer.

2. The area and the intensity of the illumination of the object used as stimulus affect both the latent period preceding the development of action potential and the height and steepness of the *b*-wave.

3. The latent period for the shortest flashes (4σ) is the same as that for prolonged stimulation, although the amount of potential which is developed may be less. Shorter flashes could not be obtained with our apparatus. It is probable that with them some lengthening of the latent period would be found, but the effect cannot be comparable with that of diminished area or intensity.

4. With intermittent stimulation at slow rates, there are ripples in the curve of retinal action potential. If a large *b*-wave is present, the rhythm of the stimulus may not appear in the tracing until several flashes have been delivered. Thereafter the amplitude of the oscillations and their

latent period remain constant during a period of at least 5 sec., but the latent period is usually much longer than that following the commencement of steady stimulation. With weak stimuli the ripples may be larger and may rise more steeply than the initial *b*-wave of an otherwise similar steady stimulus.

5. Above a certain frequency of alternation, the ripples disappear. Control experiments show that the "fusion frequency" is not affected by the properties of the recording apparatus.

6. The fusion frequency varies directly as log brightness and log area of the stimulus over at least a hundredfold range. Variations in brightness usually have more effect than variations in area. The product of fusion frequency and latent period of ripples is always found to be approximately constant. The duration of the flash at the fusion point is about 44 p.c. of this latent period.

7. The retinal process chiefly responsible for the ripples is the component P II [Granit, 1933] of the retinal action potential. P III probably interacts with P II and may, in so doing, affect the fusion frequency.

8. With rapidly intermittent stimulation, the smooth curve of action potential approximates closely to that given by a steady stimulus of half the intensity. The initial *b*-wave of the former, however, often occurs after a longer latent period than that of the latter. This is especially true with intense stimuli.

9. A flash delivered shortly before the end of the normal latent period following a preceding flash may greatly delay the expected movement of the string. Once the delay is over, the effect of the second flash summates with that of the first.

10. In the discussion, the various lengthenings of the latent period which have been found in repetitive stimulation are attributed to the inhibitory influence of the negative component P III on the positive component P II. Evidence of temporal and areal summation of effects is also presented.

11. Piper's views are briefly discussed, and the impossibility of predicting the results of intermittent stimulation from a study of single flashes and momentary interruption of steady illumination is emphasized.

12. The resemblance is pointed out between findings obtained by the study of retinal action potentials and the sensory phenomena of flicker and fusion.

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