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THE PHYSIOLOGICAL SIGNIFICANCE OF THE RETINAL SYNAPSES

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ABSTRACT. Attention is drawn to the anatomical fact that the retina possesses the structure of a nervous centre in combination with a sensory end organ. It should therefore exhibit properties characteristic of nervous centres, such as the two fundamental synaptic reactions of summation and inhibition. The author then proceeds to review some recent experimental evidence establishing the presence of both these reactions in the retina. The paper is concluded by a few remarks on the necessity of including the synaptic factors in theories of perception.

EVEN if the meeting were composed mainly of biologists it would be necessary to-day to commence this review with an introduction on the histology of the retina. It may safely be stated that most of the workers interested in retinal function look upon the task facing us as if it were mainly one of identifying the properties, photochemical and others, of the rods and cones. And yet the work of histologists like Tartuferi, Ehrlich, Dogiel, and, last but not least, Ramón y Cajal has provided us with a very complicated picture of the structure of this organ. It hardly seems possible to assume that formations such as the horizontal cells, the amacrine, the ganglion cells, and the bipolars were present in order to behave only as simple conductors adding nothing to the visual process, and still less does it seem probable that the sensations, the outcome of a process that has passed through at least three synaptic junctions between neurones, would always be proportional to the initial photochemical reaction to light. At present it is impossible to state precisely how all these structures interact to bring about the discharge through the optic nerve, but we may derive some hints as to how our research should be directed by considering the nature of the structures concerned. Of fundamental importance then is the fact that ontogenetically the retinal layers are parts of the central nervous system, from which they have grown out during the course of embryological development. Those parts of the eye are made up of neurones similar to the multipolar and bipolar neurones of the higher centres. As a matter of fact the retina is a nervous centre projected to the surface of the body, and the optic nerve a central tract connecting two centres. One of the fundamental characteristics of organization in centres is well illustrated by the following words due to Sherrington: "Over and over again in the arrangement of the conducting paths of the nervous system there are places where two or more such paths converge and run to one. There two or more of the convergent paths, when active, will interact"⁽¹⁶⁾.

Just as in other nervous centres, so in the retina there are confluent paths. There are, in point of fact, two principal directions of retinal conduction, with three crossing-points, where conceivably interaction might take place. Firstly, impulses are conducted from the receptors through bipolars and ganglion cells to the optic nerve. But impulses may well be carried laterally too, crossing the paths leading upwards. The horizontal cells form a lateral system under the basal ends of the rods

and cones. Another lateral system consisting of the amacrine cells is connected with the bipolar cells. Finally, the ganglion cells branch laterally. Thus points of convergence are provided at three separate places in the retina, and by considering the functional aspects of convergence we obtain a programme of research which, as we shall see, already has found its own justification. For the sake of completeness it should be added that the optic nerve also contains fibres which lead downwards, towards the retina, but their function is still obscure, whereas at least a beginning has been made with the lateral pathway.

The two principal reactions at points of convergence are summation and inhibition, both of which have attracted considerable attention in physiology. Most important work in this field has been done by the Oxford school headed by Sir Charles Sherrington. Their joint work has recently appeared in book form⁽⁶⁾ and should be consulted for detailed information. It deals with reflexes of the spinal cord and thus with effects of interaction in terms of motor responses, but there is evidence to show that sensory and motor neurones behave in a similar way with regard to interaction⁽¹⁾. Theories of summation and inhibition need not concern us here: we shall only utilize the main fact that at points of convergence of several paths there may result an augmentation of the response, which is termed "summation," or a diminution, termed "inhibition."

The first experiment to be discussed is one reported by Adrian and Matthews⁽²⁻⁴⁾. Using valve-amplification and a capillary electrometer they recorded for the first time the actual impulses in the optic nerve of the conger eel. Kühne and Steiner⁽¹⁴⁾, with a slower instrument, had previously observed the main features of the optic-nerve response, integrated roughly as to total number of impulses in the fibres concerned. The frequency of discharge from the retina was found by Adrian and Matthews to rise with the intensity of stimulation. Owing, however, to the great number of fibres involved, this index of the intensity of the physiological process in the retina was less reliable than another one, namely the shortening of the latent period of the outburst of discharge in opticus following increased intensity of stimulation. Using this index they demonstrated conclusively that an increase in stimulated area affected the retinal response just as an increase in intensity did. Still more interesting was the following experiment, which at the same time provided a first step towards an analysis of the spatial effect which previously had been observed but not so conclusively demonstrated. They illuminated the retina with four light-spots some distance apart, and determined the latent period for the reaction to each light alone. Then all four were illuminated simultaneously, and the latency was found to be shorter than the average obtained with the individual stimuli. Clearly an interaction of some kind had taken place and, since the rods and cones are isolated from one another and since, on the other hand, there is in the eel's retina, as in all vertebrate retinæ, a dense net of internuncial neurones, the natural conclusion is that lateral effects accounted for the result. Recollecting that the shortening of the latent period was an effect in the direction of increased physiological activity in the retina, we should be justified in concluding with the authors that the process discovered was one of summation over lateral channels. Con-

firmatory evidence of a different character bore out this supposition. Evidently such a process, if present, must be at work when the four areas are brought closer together, when ultimately the experiment becomes identical with a comparison of latent periods for small and large areas. The interaction between the four separated spots took place over a still larger area when strychnine was applied to the retina, a substance known to facilitate central summation.

Let us now ask whether this experiment can be repeated with a sensory criterion as an index of increased retinal activity. Such a criterion is suitably provided by the rise of the point of fusion of an intermittent light following increased intensity of stimulation. It has long been known that larger areas give higher fusion-frequencies. An inquiry into the nature of this function showed that the fusion-frequency was proportional to the logarithm of the area stimulated⁽¹²⁾, just as previously it had been shown to be proportional to the logarithm of the intensity, the so-called Ferry-Porter rule. The true relations tend to be S-shaped over a large range, but for our immediate purpose we may combine the two formulae into one, thus

$$n = \alpha \log I \log A + \beta \log I + \gamma \log A + \delta$$

where n is the fusion-frequency, I is intensity, A is area and $\alpha, \beta, \gamma, \delta$ are constants.

The significant point here is the fact clearly brought forth by table 1, that the constants vary in a definite manner when central and peripheral vision are com-

Table 1

	α	β	γ	δ
Periphery at 10°	1.68	4.87	4.28	14.03
Central fixation	0.90	4.76	1.79	15.40

pared. One term contains $\log I$ alone and it is evident that the constant β is approximately the same in the centre and 10° towards the periphery. But in the terms containing the factor $\log A$ the peripheral constants α and γ are definitely greater than the central ones. This indicates a fundamental difference between central and peripheral vision, viz. that spatial factors play a greater rôle in the latter part of the field of vision.

Let us now apply a four-spot test to these conditions⁽⁹⁾. The principle is simply to determine the fusion-frequency of four separated areas individually illuminated, then to illuminate all of them simultaneously and to note whether the fusion-frequency has altered. The result of such an experiment is given in table 2. The four

Table 2. Fusion frequencies with single and four-spot stimulation

Intensity (metre-candles)	Central fixation		Peripheral fixation at 10°	
	Singles	Fours	Singles	Fours
94.0	45.7	46.1	39.8	45.5
0.94	26.6	26.6	24.2	25.4

Note. The fusion-frequency in all tables is given in flashes per second. In some of the originals referred to, revolutions per second were given.

spots were 1° in diameter and were lying symmetrically on an imaginary circle of 3° in diameter. Whereas in the centre of the field of vision the fours give only a just perceptible increase in the fusion-frequency over the values obtained with the singles, there is a very definite effect in the same direction in the periphery. Evidently then some kind of interaction has taken place, and because this interaction is indicated by an increase in fusion-frequency we interpret it as summation. The analogy with the results of Adrian and Matthews is only one of the reasons for localizing this effect in the retina rather than in the higher centres, in spite of its being obtained with a sensory criterion as an index of the state of excitation in the visual apparatus. A striking correspondence between the two lines of evidence is found in the fact that the human periphery is anatomically similar to the eel's retina, possessing a great many lateral connections besides a convergence of many receptors upon single ganglion cells. In the human fovea the path between receptor and ganglion cell is more isolated. Further, there is some evidence that the fusion-frequency is determined by retinal conditions. Thus Adrian and Matthews⁽⁴⁾ found the fusion point, at which the pulsating discharge in opticus became continuous, to behave with respect to area and intensity as the seen fusion-point of the visual experiment. Sachs⁽¹⁵⁾ tried to record the action-potential of his own eye (first successfully done by Hartline⁽⁷⁾) in response to intermittent stimulation, and claims to have observed that the retinal action-potential showed flicker as small waves of potential upon the main wave, as long as flicker was perceived, but that the impression of fusion corresponded to a normal, i.e. a fused, wave.

At the time when the experiments reported above were being conducted, Dr G. P. McCouch and I also tried to see whether strychnine would affect the rise in the fusion-frequency of the four spots over the value obtained with the singles. The experiments were very unpleasant because of the large amounts of strychnine taken subcutaneously, so much so that twice an injection of bromide had to be given immediately after the test had been performed. The attempts were therefore discontinued. The results, though preliminary in nature, are reproduced in table 3. Strychnine appears to enhance interaction.

A natural objection to these experiments, partly removed by the results obtained with strychnine, is the possible scattering of light in the periphery owing to defects in the optical system. Now at 10° this effect cannot be serious, as is shown by the old experiments by Aubert and Foerster⁽⁵⁾, who observed through the sclera an image formed by two points on the retina of the excised eye of an albino rabbit, and came to the conclusion that the diminution in visual acuity towards the periphery was chiefly conditioned by physiological factors and not by the physics of the optical system. But it is also possible to compensate for irradiation by giving the background a fairly high brightness against which the diffusion circles around the illuminated spots become negligible. This, as a matter of fact, was done in some cases, and the result was positive, as before. Thus it seems necessary to conclude that the peripheral increase in interaction as evidenced by the four-spot experiment and by the greater effect of area in peripheral vision is a sensory index of physiological properties of the receptive system and not an artefact.

The same type of interaction is also present with central fixation, but the separate areas must be brought nearer in order to exhibit it. A simple way of demonstrating this effect is to take two semicircular fields some distance apart and gradually bring them nearer to one another⁽¹²⁾. As they approach each other the two together will gradually give a higher fusion-frequency than either of them alone. The truly physiological nature of this result is perhaps best illustrated by the following modification of the experiment. Instead of both the semicircular spots being kept flickering, one is kept steadily illuminated at an intensity giving the brightness possessed by the flickering area when fused. The question is then whether the illumination of the non-flickering area influences the fusion-frequency of the flickering area. This in fact is the case⁽⁸⁾. But the increase, though in the same direction, is less than when the second area also is flickering. This experiment is of interest

Table 3. Fusion frequencies with and without strychnine

	Observer C			
	Experiment 1		Experiment 2	
	Control	Strychnine 1 grain	Control	Strychnine 0.9 grain
Singles	27.7	25.4	27.4	25.2
Fours	30.6	29.3	31.6	30.8
Increase of fours (per cent)	10.5	15.4	15.3	22.2

	Observer G			
	Experiment 3		Experiment 4	
	Control	Strychnine 1 grain	Control	Strychnine 0.6 grain
Singles	32.5	29.8	30.8	30.3
Fours	34.7	33.2	33.9	34.0
Increase of fours (per cent)	6.8	11.4	10.0	12.2

in connection with recent work on the rhythmic properties of neurones, but that question must not detain us here. The experiment is mentioned merely to show that it is impossible to obtain any explanation of foveal interaction in terms of irradiation.

In interpreting the types of interaction described above as summation we implicitly assume that impulses from adjacent areas impinge upon synapses which they have in common. How then is it possible in central vision to perceive two areas as separated if there is summation? As a matter of fact the visual acuity increases with illumination and so does summation⁽¹²⁾. On the face of it the difficulty in harmonizing these two facts appears unsurmountable, especially as it is possible to show that the maximal interaction in the experiment with the semicircular fields is reached before the approaching edges touch, and before there is any interference with the separability of the two semicircles⁽¹²⁾. These and similar considerations suggest that the interaction may take another form when the adjacent fields are at different levels of brightness, as, indeed, must always be the case when brightness-

discrimination in any form is called for. An experimental test is shown in table 4. Two semicircular fields were used. When both were equally bright there was a summation for either of them amounting to 8.6 per cent. Then the right field was made 50 per cent darker than the left and its fusion-frequency was determined, (a) when it was illuminated alone and (b) when the adjacent brighter field also was present. In the latter case there was no trace of the normal summation. In fact, the averages of four observers gave a diminution of the fusion-frequency of 1.5 per cent which, however, was chiefly dependent upon the readings of one observer. With the others only the summated excess was removed. The same experiment was repeated with the brighter semicircle, that is to say the readings were taken with the brighter instead of the darker field, and summation was found as before, but somewhat less in degree. The darker semicircle thus summed in the usual way on

Table 4

Conditions: Left semicircle always at 1.39 millilamberts, background at 0.012 millilambert. Both flickering and about 4 minutes of visual angle apart. Central fixation.

Singles	Doubles	Difference (per cent)
Both equally bright		
55.8	60.6	+ 8.6
Right 50 per cent darker than left. Fusion-frequency of right determined		
54.7	53.9	- 1.5
Right 50 per cent darker than left. Fusion-frequency of left determined		
55.8	58.8	+ 5.4

the brighter half of the field. This means that when adjacent areas are at different levels of brightness the interaction is of such a nature as to exaggerate the difference between them. There is summation for the brighter and inhibition for the darker area, the latter showing itself as absence of the summation which would otherwise occur. This inhibitory component could not be demonstrated in the periphery⁽⁸⁾, where summation alone was always found in similar experiments. The visual acuity also diminishes towards the periphery, a fact which must be attributed in part to the increase in convergence of many receptors upon individual ganglion cells, from which arise the fibres leading upwards, in part to the defects in the optical system at oblique angles of incidence, and, finally, in part also to the diminution in inhibitory interaction towards the periphery. It should be noted that the visual acuity already begins to fall off at 10 minutes of visual angle from the centre and is reduced by almost 50 per cent at about 85 minutes⁽¹⁷⁾.

Though out of the series of papers from which I have quoted freely I could multiply the number of experimental demonstrations of similar phenomena, intelligible enough if interpreted physiologically as above, these examples may suffice to demonstrate the necessity of directing both thought and experimental research to the elucidation of retinal interaction and the synaptic functions in general. It

will be recalled that interaction in the form of contrast, to take the best known example, has been recognized as a fact for a very long time and also that functions have been assigned to the synapses by different authors. Yet, rather than give a historical review of scattered suggestions and well-known facts, I have chosen to refer to quite recent work in which, as we have seen, more conclusive evidence for the occurrence of synaptic processes in the retina has been obtained. To analyze this evidence and to indicate the general direction of some of the research stimulated by the concept of "the retina as a true nervous centre" (Cajal's words) has been my chief purpose. Time does not permit me to discuss the bearing of this concept upon many visual problems⁽¹⁰⁾, nor will it be possible to indicate where it links up with the duplicity

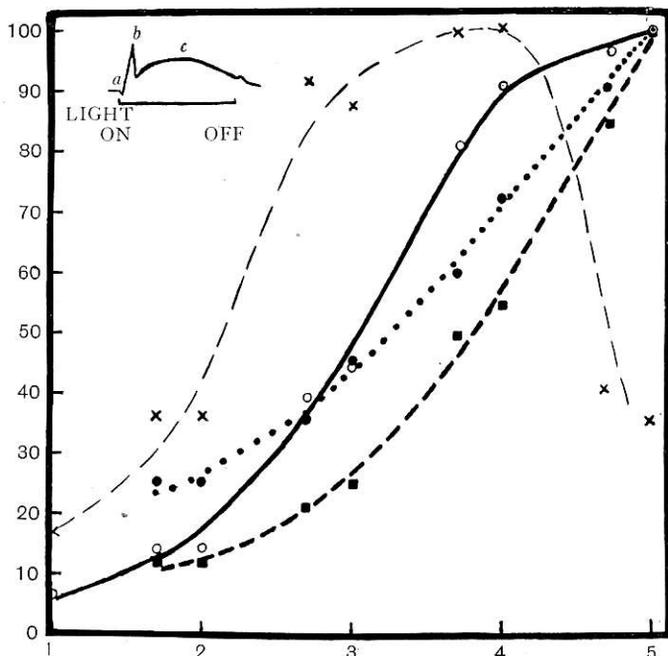


Fig. 1. Inset: schematic picture of retinal action-potential: ○—— maximal potential of *b*-wave (mV. \times 291); ■----- average rate of rise of *b*-wave (mV. \times 17,500/ σ); ●..... inverse value of latent period ($\sigma \times$ 1800); x— — maximal potential of *c*-wave (mV. \times 746). Illuminated ground glass area just in front of animal's eye.

theory and with the conclusions so lucidly derived by Hecht⁽¹³⁾ from photochemical considerations. Evidently it is necessary to take into account the fact that not only the relative distribution of rods and cones but also the synaptic arrangements alter as one passes out from the fovea towards the periphery. The difference between central and peripheral vision is therefore not merely one of rod-and-cone vision. The photochemical interpretations so far advanced presuppose proportionality between the initial and the final processes which in some cases may, in others may not, be present. The advantage of this kind of approach lies in its utilization of concepts which already may be subject to quantitative treatment. Figure 1 may serve to illustrate the difficulties met with. It refers to an as yet unpublished experiment on

the retinal action-potential recorded from the dark-adapted eye of a decerebrate cat by means of a directly coupled amplifier and a string galvanometer. In the inset there is a schematic picture of the retinal action-potential, from which it appears that the following quantities are measurable: (1) the latent period from the beginning of illumination till outburst of potential; (2) height of first positive wave (*b*-wave); (3) rate of rise of *b*-wave; (4) height of slow secondary rise of potential (*c*-wave). The latter wave is apt to vary more than the *b*-wave, which in the best preparations may be repeatable within 4 or 5 per cent. Not knowing which of the functions is the correct index of the photochemical process, we may call the maximal value for all functions 100 and then plot them against a common abscissa of log (intensity) in arbitrary units. This has been done in figure 1. Except for the *c*-wave at high intensities, all the curves rise with log *I*, but they are not proportional to one another and clearly represent different aspects of a very complicated process. This shows how difficult it is to theorize already at what we may call a "sub-sensational" stage. Incidentally all these curves may be taken to illustrate the Weber-Fechner law, on the assumption that any sensory phenomenon which throughout some range is proportional to the logarithm of the stimulus represents the Weber-Fechner law, an assumption which appears to be taken for granted!

The difficulties of the sub-sensational stage do not disappear when the sensations are introduced into the picture. Thus it has been shown⁽¹¹⁾ that the fusion-frequency, a recognized index of brightness, and the actual brightness perceived alter in a different direction at certain short exposures if both are measured as functions of time of exposure.

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DISCUSSION

Mr W. D. WRIGHT. I feel that the most promising method of solving many visual problems is by the combination of results such as those described by the author with accurate quantitative data on the subsidiary phenomena of colour-vision, less attention being paid to the straightforward data of colour-mixture. Personally I find Dr Granit's paper of particular value as a warning against the too ready acceptance of a simple photochemical reaction as the basis for many visual phenomena. It is, however, to be hoped that physiologists will, in the near future, be able to state with some certainty the relation between the intensity of the stimulus and the magnitude of the nerve impulse. It is very difficult to analyse experimental observations without a reasonably clear mental picture of the processes involved.

Dr H. E. ROAF. I can fully substantiate the author's statement about the spread of effect from one part of the retina to another. In a recent paper* I have shown that with foveal vision a surrounding field of illumination has little effect on the threshold to light, but that with extra foveal vision there is a marked influence which seems too great to be due entirely to scattering of light in the eye media.

AUTHOR'S reply. In reply to Prof. Roaf's remarks I wish to say that there will probably always be some scattering in the periphery. The increase in fusion frequency with the four spots is with higher intensities almost large enough to be equivalent to the increase obtained when the area of one of the spots is made four times larger†. There is little reason to expect so much scattering at 10° towards the periphery. I need, however, not discuss this question any further since Prof. Roaf seems to agree with me about the main findings and their interpretation. I might add that I have recently repeated the experiment of Adrian and Matthews with the decerebrate mammalian preparation using the latent period of the action potential as an index of the interaction between separated areas.

* *Proc. R. S. B.*, 110, 448 (1932).

† See reference (9).