RAGNAR GRANIT

The development of retinal neurophysiology

Nobel Lecture, December 12, 1967

For some twenty years my main experimentation has not been within the field of retinal electrophysiology and my interests have shifted to problems of motor control, chiefly muscular sense organs and quantitative properties of firing motoneurones. For this reason my Lecture will be as retrospective as the Award and deal with the re-birth of retinal studies that was inspired by the increasing accessibility of electronic equipment from the mid-twenties onwards. Before this era vision was chiefly studied by psychophysical methods. These had led to well-established correlations between perceptions of *e.g.* colour, luminosity etc. and physically defined entities within the c.g.s. system of units. But in 1926 Adrian and Zotterman[1], using the afferent nerve of a stretch receptor in a frog muscle, had shown that it was possible by amplification to record the impulses in single nerve fibres and in 1927-1928 Adrian and Rachel Matthews[2,3] published their important experiments with the mass discharge in the long optic nerve of the *Conger* eel. Evidently the electrophysiological approach was the one now destined to pave the way for a deeper understanding of how this, our noblest sense organ, had organized its interpretation of the world of light, form and colour.

Frithiof Holmgren[4] (1831-1897) at Uppsala, who in 1865 discovered the electrical response of the retina to light, the electroretinogram (ERG) of the present day, had realized that many problems of vision could be analyzed by what he called his "method of objectivating the impressions on the retina", but amplification with the aid of the vacuum tube gave this general idea quite a different dimension. It was - Lord Adrian said - as if we had got a new very powerful microscope to work with.

The basic idea of my own approach was formed during my study of Ramón y Cajal's (1894) classicaldescriptions of the retina as a "true nervous centre", as clarified by his silver-stain preparations and likewise suggested by its embryological development from the brain. It seemed to me likely that psychophysical data might with some profit to the field be translatable into neurological equivalents, and so, in 1928 I went to the laboratory of Sir Charles Sherrington at Oxford in order to learn something about nervous centres. A

few years ago it gave me great pleasure to write an appraisal6 of this eminent physiologist and the concepts he bequeathed to posterity. Sherrington and Adrian shared the medical Nobel Prize in 1932.

At the Johnson Foundation for Medical Physics of the University of Pennsylvania I made an attempt to put this general program into practice by a psychophysical technique and to this end chose the fusion frequency of a flickering light that is proportional to the logarithm of stimulus intensity (Ferry-Porter law). This was done because I wanted an absolute measure of whether an excitatory state rose or fell instead of the relative one by the customary psychophysical technique comparing two half-fields. It was thus assumed that at least to a first approximation the intensity of retinal excitation would be measured by the fusion frequency. Very much later (1952) Chris:-tina Enroth[7] in a thesis from the Nobel Institute showed that the fusion frequency of the impulses from single retinal ganglion cells was in fact proportional to their impulse frequency. However, at the time (1929-1932), by use of this hypothesis, it proved possible to demonstrate facilitation between small flickering light spots separated by a weakly illuminated background[8,9] and to show that the fusion frequency within limits was proportional to the area illuminated. These effects corresponded to the excitability changes measured by Adrian and Matthews[10] in similar experiments using the mass discharge from the eel's optic nerve as indicator. With adjacent fields at different levels of brightness the fusion frequency rose in the brighter and fell in the darker field[11], a fact interpreted as demonstrating a mechanism of contrast. "The theoretical significance of the observation seems to be that the inhibitory system is excited relatively more and more as the intensity of the excitatory process in a group of neurones increases. Consequently the inhibitory effect passing from the more stimulated area to the adjacent less stimulated area will be greater than the inhibitory effect passing in the opposite direction" (p. 671). Today this sounds like a description of Hartline's (ref.12 and this volume, p. 269) lateral inhibition in the *Limulus* eye or of recurrent inhibition in the motoneurones, as measured by Granit and Renkin[13].

By these findings I was convinced that psychophysics could be translated into neurology but psychophysics was as strange a subject to neurologists as was neurology to psychophysicists and so the implications of this work fell between two chairs. Only the physiologists were understanding listeners.

I next decided to take up the electroretinogram and thereafter to remain within physiology. The complex ERG had to be split into components, a piece of work begun at Oxford[14], and the analysis established by various

means, since greatly improved upon, served as a starting point for much work up to the present day (see summary by Brown's). In a thesis from the Nobel Institute co-operating with the Ophthalmological Clinic of the Caroline Institute, Karpe[16] made electroretinography a useful clinical method, nowadays considerably developed and employed all over the world. Frithiof Holmgren[4], who always was very keen on applied physiology, would have been pleased if the had lived to see this development.

Although the ERG was a mass response from the whole retina it proved possible with its aid to take a few definite steps forward on the road towards a retinal neurophysiology. A slow cornea-positive component (PI) was found not to cause any impulse activity in the optic nerve, another, faster component of similar sign (PII) mimicked the mass dischargewith its rise at onset (on-effect) and cessation (off-effect) of illumination. Very definitely it could be shown[17] that the ERG altered with light and dark adaptation in such a manner that it was impossible to understand the processes involved in adaptation on a purely photochemical basis, the prevailing notion of the day. After some further work I came to the conclusion that the light adapted eye makes more use of inhibition, which was assumed to be relatively more important for cone projections within the retina.

However, to me at the time (in Helsingfors) the greatest problem of all was how to prove inhibition to be present in the retina itself. Both the design of this organ as well as the findings with the flicker method, mentioned above, suggested that under some, as yet unknown, circumstances impulses in the optic nerve might be stopped by light. The ERG suggested an approach to this question. Since the discharge correlated with a cornea-positive electrical deflexion, might not the opposite cornea-negative deflexion, obtained when a flash was superimposed on the off-effect, indicate inhibition? This effect had been studied by Granit and Riddell in great detail. By recording the mass discharge in the optic nerve together with the ERG, this surmise was verified. There was the postulated inhibition[18,19]. Very few, if any, later experiments have given me such delight. With my background in the physiology of the central nervous system, acquired in Sherrington's laboratory, I now knew for certain that the details of the visual image were elaborated by the interplay of excitation and inhibition in the nervous centre of the retina itself. Hartline (ref. 20,21 and this volume, p. 269), by his elegant method of splitting single fibres in the optic nerve, soon found any amount of inhibition and so did we, when, somewhat later (see below), our microelectrode studies of the retina permitted isolation of single fibres. With this technique I later went on to

show that the <on> and <off> components of the discharge were mutually exclusive when they were made to clash[22,23] and held this to be a "belated vindication of the essential truth of Hering's contention that there are two fundamental processes of opposite character in the retina" (ref. 24, p. 78).

The following quotation from "Sensory *Mechanisms of the Retina*"[25], written in 1943, shows how the problem of form discrimination was formulated at that time: "The accurate appreciation of contour, in particular, must be due to minute fluctuations of the eyeballs resulting in on- and off-effects as well as sudden inhibitions of the latter" (p. 168). The important role of the eye movements[26,27] has since been demonstrated experimentally in several papers. Off-impulses do not require complete cessation of light. A diminution of brightness suffices. Thus, because of she minute fluctuations of the eyebulb, the contour between two different levels ofbrightness acquires a life of its own created by the interplay of excitation and inhibition at <on> and <off>. No more need be said in order to show why the experimental establishment of retinal inhibition seemed so decisive a step towards a retinal neurophysiology capable of interpreting visual events. It is well known that for several years the leading theme of single-fibre work with the retina has been the effects of the interplay of excitation and inhibition.

So many things appeared tempting to study at the time that it was difficult to choose. I next fell for the temptation to analyze the relation between the amount of rhodopsin and the sensitivity of the dark-adapted eye. In his valuable thesis from my laboratory at Helsingfors Zewi[28] had shown that the total amount of rhodopsin could be extracted from a pair of frog eyes with an average accuracy of 4% when left and right eyes of g animals were compared. Our basic experiment[29] demonstrated that spectral lights between the wavelengths 4700 and 5850 Å, which were so weak that they could not bleach a rhodopsin solution tested at 5000 Å, nevertheless reduced the electroretinographically measured sensitivity of eyes exposed to them by as much as 74 to 56% depending upon the wavelength chosen. The non-exposed eye then served as control. The recent work of Donner and Reuter[30] suggests an explanation: they have shown that metarhodopsin II, one of the intermediary products of bleaching rhodopsin (see Wald's Lecture, this volume, p. 292) is likely to trigger a negative feedback depressing the sensitivity of the rods. This effect is probably responsible for the unduly neglected findings of Elenius[31] with short-lasting light adaptations of the rabbit's eye.

Another temptation was, of course, the mechanism of wavelength reception. This problem was then merely a branch of colour psychophysics and

Hecht[32], who had the enviable gift of always being plausible, had developed a trichromatic theory according to which there were, to be sure, three types of cones, but overlapping very closely in spectral distribution of sensitivity, as shown in Fig. 1. The element of plausibility came from his thorough mathematical elaboration of the theory to explain a number of well known, precise psychophysical observations on colour. To a neurological approach, however, the idea of wavelength discrimination being based on the differences between the narrow fringes of the three curves of Fig. 1, appeared to raise such formidable demands on the internal machinery of the retina that it seemed more reasonable to assume Hecht to be wrong. As a matter of fact, our earliest experiments with the ERG elicited by Spectral lights[33] showed in unmistakable terms that there had to be substances in the retina with-absorption spectra in different bands of wavelength widely apart.

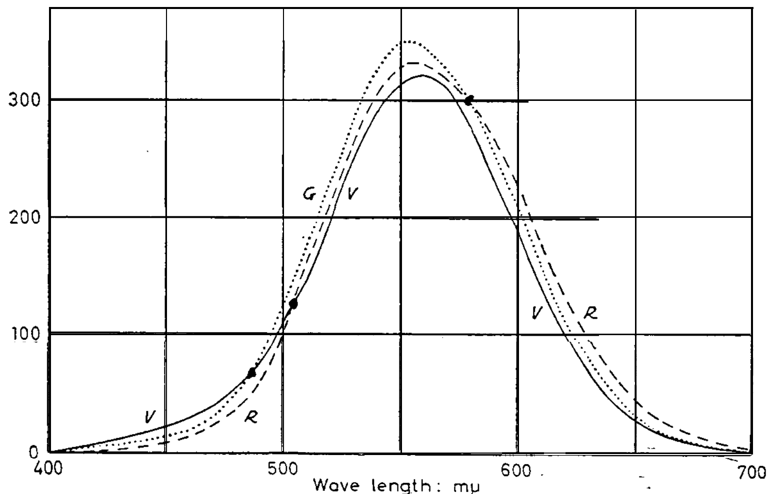

Fig.1. Spectral distribution of the primaries *V*, *G* and *R*, according to the hypothesis of S. Hecht (Bulletin No. 4 of the Howe Laboratory of Ophthalmology, Harvard University Press, Cambridge, Mass., 1931).

At Helsingfors[34] we then changed to microelectrodes: glass tubes with silver cores pulled out in a flame to fine tips, applied directly on to the retina of frogs. In Stockholm, after 1940, these were replaced by platinum wires insulated in molten glass, because the silver tips had a number of undesirable properties. It should not be overlooked that the first experiments on the frog retina were carried out under micro-illumination. The spectral lights were reflected internally from a silver-coated glass rod drawn out into a fine tip.

When this light spot and the microelectrode were adjusted relative to one another in order to obtain a precise response, the distance between the two tips was often so great that only the optic nerve fibres themselves could be considered as sources of the spikes obtained. Although I have often pointed out this circumstance, our results are generally being presented as though they all referred to the giant ganglion cells [35], studied some years later in the cat retina.

The main method was to illuminate the retina with spectral lights calibrated with respect to energy and listen to the threshold response of spikes heard in the loudspeaker. The spectral sensitivity is defined by the inverse value of the energy required for the threshold response and this figure was afterwards corrected for equal quantum intensity in every-wavelength. Optimal signal/noise ratio was established, partly by adjusting the microeleckodes, partly by working at the turning point of the characteristic curve of the amplifier.

With this method isolation of single fibres need not necessarily be perfect. Choice of the threshold response as index implies in itself functional isolation of the wavelength to which the most sensitive response is obtained. The type of response used was photographed but the threshold was determined acoustically. When an eye is <mixed>, *i.e.* contains both rods and cones in large numbers, it is necessary to maintain its state of light adaptation by special, regularly recurring controls. Another advantage of light adaptation is that threshold responses of even a small group of fibres then are very precise and sharply delimited. However, pure cone eyes (grass snake, tortoise) also were studied.

These experiments, carried out on many species of animals, lasted about five years, the four last ones after my move to Stockholm. They showed that there were two main types of message to the higher centres: *(i)* either the spectral distribution of sensitivity was wide, comprising the whole visible spectrum, with a maximum around 5600 Å (Fig. 5) which shifted to about 5000 Å after dark adaptation or else *(ii)* there were narrow bands, restricted to three main regions, as shown in the samples of Fig. 2. In the dominantly rod eye of rats an occasional narrow band after light adaptation was seen at 5000 Å and ascribed to rhodopsin. This is not included in the figure. I called the broad bands <dominators> and the narrow bands (modulators). Especial care was devoted to proving that a single, well isolated spike could deliver the photopic 5600 Å dominator in light adaptation as well as the 5000 Å scotopic dominator after dark adaptation. This is the electrophysiological version of the Purkinje shift from photopic to scotopic vision of which the dominator was held to be the carrier fibre.

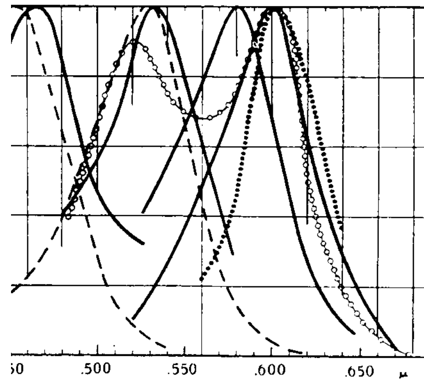

Fig. 2. Distribution of sensitivity of modulators from the eyes of rat (dots), guinea pig (broken line), frog (line drawn in full) and snake (interrupted by circles). The narrow rhodopsin curve at 5000 Å left out from original figure *(Nature,* 151 (1943) 11).

Since the same nerve fibre can deliver both rod and cone messages these experiments also showed that there is convergence of rods and cones towards the same final common path, as Cajal's silver stains had shown anatomically. They proved one more point that seemed to me most important. Clearly, since a single fibre could carry both rod and cone messages in the form of a dominator band destined for the same upper station, the visual message would have to be the same. Now, since luminosity is the sensory equivalent common to rods and cones-indeed, the rods are likely to record very little else - the brain must receive a special message of luminosity as distinct from colour specification. Wavelength discrimination was therefore held to be dependent on the narrow modulator bands. Similar bands were later beautifully isolated by Donner[36] in the pigeon's eye with the aid of considerably improved micro-electrodes.

There was not at the time enough photochemical work to exclude the possibility that narrow spectral bands might represent absorption spectra of photochemical substances. I therefore limited my theoretical interpretation[25, 37] to stating that wavelength discrimination was based on the modulator type of response. Later on, my own work (summarized in Granit[38]) and work by others (summarized in Granit[39] and in Dartnall[40]) led to the view that the narrowness of the modulator bands was a product of interaction between overlapping broad-band absorption spectra whose form in man soon began to emerge from the valuable ophthalmoscopic work of Rushton[41] and Weale[42]. Modulation thus came to illustrate a principle common in the neural

organization of most afferent systems and signifying a crispening of the information by interaction, probably largely inhibitory in nature.

A great deal of experimental labour was invested in the measuring of dominator curves in various eyes and proving their composite nature with respect to elements sensitive to different wavelengths (see e.g. Donner and Granit[43]), a theme ten years later developed by Donner and Rushton[44,45]. It is of some interest to observe in retrospect how relatively easy it was to discover by our methods special sensitivity to blue light in the eyes of several mammals compared with the difficulties it later encountered in demonstrating a blue-absorbing substance in human eyes.

The retina of the cat, to which my final work[46] in this field was devoted, has a considerable number of cones. It surprised me at first that, in order to demonstrate specific sensitivity to several different regions of the spectrum in the cat's light-adapted eye, it was necessary to have recourse to selective adaptation. The explanation is probably that the cat, compared with pigeon and monkey, has relatively few optic nerve fibres. The idea of the adaptation experiment was to bleach away a sizeable amount of rhodopsin by red, green and blue lights. Since this is a homogeneous substance, the relative effects of the bleaching lights, whatever their colour, could only be along proportional ordinates as determined by the ordinates of the absorption spectrum of these bleaching lights for rhodopsin. Remaining peaks of differential sensitivity in different spectral regions would then have to be caused by cone substances. On the basis of 4000 observations the three curves of Fig. 3 were obtained.

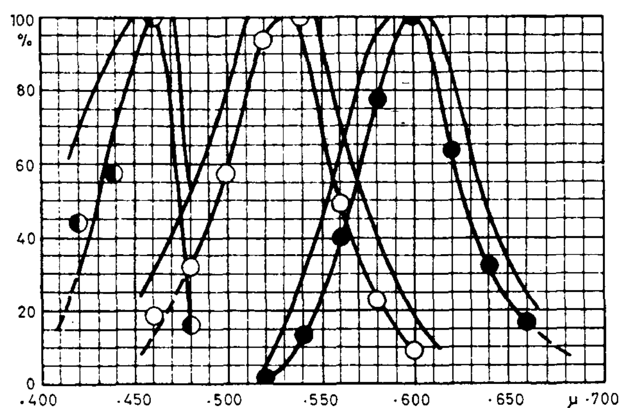

Fig. 3. Averages of individual modulators from cat eye after selective adaptation with blue, green or red filter. Outer contours indicate dispersion. (*J. Neurophysiol.*, 8 (1945) 195).

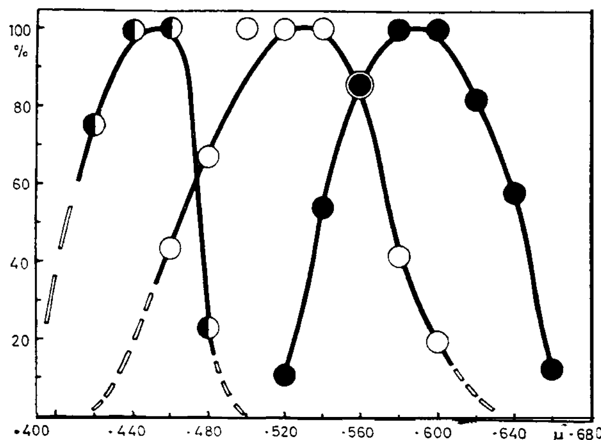

Fig. 4. Extreme values obtained in the experiments summarized in Fig. 3. (*J. Neurophysiol.*, 8 (1945) 195).

Fig. 4 shows the extreme values. Averaging these curves to add up to the photopic dominator with maximum around 5600 Å, the theoretical curves of Fig. 5 were drawn for the basically similar human eye (its periphery) which do not differ very much from curves later obtained by various means (see Wald, this volume, p.292).

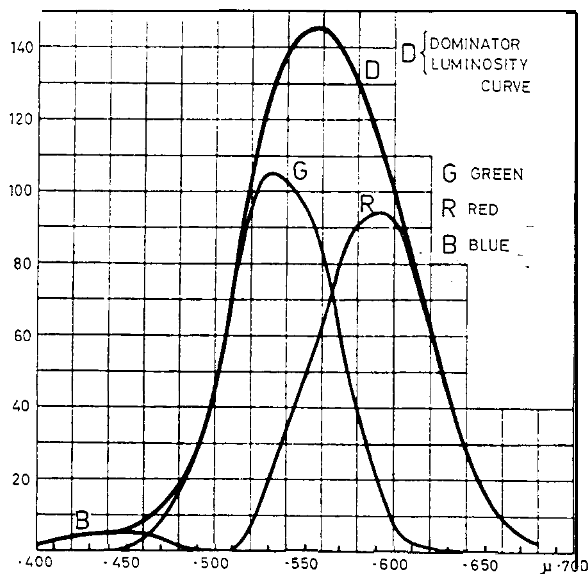

Fig. 5. Synthesis of human photopic luminosity curve (identical with photopic dominator) on the basis of three fundamental sensation curves *B*, *G* and *R*. Modulators indicated in original left out. (*J. Neurophysiol.*, 8 (1945) 195).

These results were criticized on the grounds that the cat does not see colours, as such an utterly irrelevant attack on retinal data, and since also proved to be in error. With the highly developed training methods of the present day it has not proved too difficult to make the cat respond to wavelength differences as distinct from differences of luminosity (Mello and Peterson[47]; Sechzer and Brown[48]).

In my Thomas Young Oration at the Royal Institution in London in 1945 I felt it safe to state: "The mechanism of colour reception is organized by the peripheral visual apparatus, the number of colour-sensitive elements is relatively limited, and these elements represent widely different regions of the visible spectrum. Those were Young's three fundamental assumptions. He was right even in assuming three main types of colour-receiving apparatus.- These are the three preferential regions within which modulators are found." I also made some attempts to relate these findings to colour psychophysics[25,37,46,49] but the somewhat monotonous work of recording spectral sensitivities and the absence of photochemical data, gave me a distaste for the whole field, and later on the microelectrode approach was taken over and greatly improved by fresh minds. I finished the Thomas Young Oration with the statement that I thought myself right but "further experience may nevertheless necessitate modifications. I can only hope that I shall not have to make these experiences myself, but that somebody else will try his hand at the optic nerve. I also feel just now that it would be interesting to see for a while what photochemistry and colour psychophysics could do for this field before any further labour is invested in electrophysiological work" (ref. 37, pp. 462-463).

In the form of an extensive review completed in 1959, I paid a leave-taking visit to the field in Volume II of *The Eye* (Granit[39]) and found it occupied by a large number of very competent workers. There was no need to return to it.

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