

Isolation of Colour-Sensitive Elements in a Mammalian Retina.¹

By

RAGNAR GRANIT.

Since the days of SCHULTZE's classical work (1866) the rat's eye has been known to contain chiefly rods and a limited number of cones. From later contributions to this question based on differences between rod and cone nuclei it appears that the rods may outnumber the cones in a relation of 99:1 (MENNER, 1928). Very accurate figures cannot be obtained, probably because the cones themselves are very small and for this reason apt to be overlooked. But WALLS (1934) has shown very definitely that there are cones in the rat's eye.

Nevertheless I was surprised to discover in this retina an easily demonstrable "red" receptor coupled with a "green" one. Together they gave a spectral distribution curve of sensitivity which probably illustrates elementary principles of colour reception in a retina built less for this purpose than in order to integrate the weak stimuli of dusk and night vision. From many points of view the simple structure of this system offers analytical advantages. The results have been obtained with the microelectrode technique applied in the manner described by GRANIT and SVAETICHIN (1939).

Technique and Procedure.

The physical equipment, consisting of micro-electrode, amplifier, cathode ray oscillograph, spectrum controlled with respect to energy, and wedge of known transmission in the different wave-

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lengths in order to reduce the energy of the stimulus, has been described in detail elsewhere (GRANIT and SVAETICHIN, 1939) together with other aspects of the technique. Suffice it to mention that spikes of activity in response to onset or cessation of illumination are recorded photographically and listened to in a loudspeaker. The amount of energy for a threshold effect or for cessation of "flicker" of an intermittent stimulus is determined under repeated checking of the level of sensitivity of the preparation. Depending upon the nature of the experiment this is done either by running a regularly returning calibration wave-length and interpolating with reference to this calibration or else by light-adapting the preparation and following the recovery (dark adaptation) curves for a number of wave-lengths from which visibility curves then can be constructed.

The rats (all albinos) are anaesthetized with chloralose-urethane or urethane alone and tracheotomized. Some 10 animals received dial. Before this they have been in the dark for not less than two hours. The cornea is cut away in red light and, when afterwards the lens is gently pressed out from the bulb, there follows with it the greater part of the vitreous body. The eye gradually fills itself with a serous fluid containing more or less blood but probably never quite free from traces of blood.

Sources of Error.

As soon as the micro-electrode, inserted under a binocular preparation microscope, touches the retina, spontaneous firing is heard in the loudspeaker. The animals anaesthetized with dial were particularly prone to give a resting discharge of troublesome magnitude, interfering with the measurements. The reason for this almost certainly was disturbed breathing. Indeed, the retina could serve as a model of how a respiratory centre reacts to carbon dioxide. Dyspnoe is accompanied by a heavy spontaneous discharge, and, if the rat dies during the experiment, there is a roar of impulses from the loudspeaker, before complete silence signals cessation of function. Some of them may be extraretinal spikes. The retina is very sensitive to the oxygen supply and becomes silent long before the animal can be said to be dead. Increased secretion in the respiratory ducts or changes in the rate or efficiency of the respiratory mechanism for other reasons are immediately mirrored in the general level of sensitivity of the

preparation. It is important therefore to be on one's guard with respect to such sources of error and repeatedly to insert the reference wave-length as a check on the stability of the experimental conditions.

There is also a normal rotation of activity (GRANIT and SVAETICHIN, 1939, GRANIT, 1941) to be kept in check by similar means. Extra-retinal spikes are often noted but with some experience they can be recognized and, in most cases, even avoided.

Quite good and stable preparations are obtained from time to time, though relatively rarely animals lacking spontaneous discharge. If a silent spot is found in a normally active retina, it either begins to fire later in the experiment or else there are other spots in the same eye which discharge spontaneously. The threshold in most experiments has therefore been a just perceptible acceleration of an already present discharge. By running the grid of the loudspeaker valve at the bend of its characteristic curve the baseline noise is reduced to subthreshold intensity and the much larger spikes are thus sharpened by contrast to enable precise determination of the threshold.

Results.

Types of Discharge.

We owe to HARTLINE (1938) the first description of the different types of discharge obtained from single fibres, in his case from the frog's optic nerve. These types of discharge can all be seen also with the micro-electrode technique, and all of them I have actually seen in the rat's eye. Thus in fig. 2 there is an element which merely reacts with an off-effect, each time inhibited by re-illumination. Fig. 1 illustrates an "on"-off element having a slow spontaneous discharge which is accelerated at "on" or "off". The same element is responding to intermittent light in the lower curve. Re-illumination promptly inhibited the off-discharge. The response of the large active unit of fig. 3 is a pure "on"-discharge stopping at cessation of illumination but spontaneously firing in the intervals between the periods of illumination.

Finally there is the very interesting type of discharge illustrated in fig. 4, the two upper curves being from the eye of a white mouse. This is a very slowly adapting receptor reacting all the time during

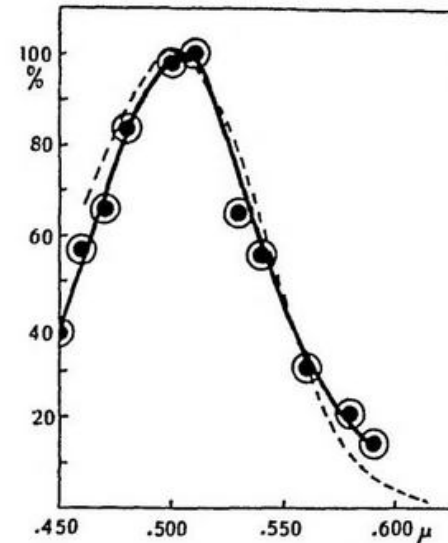
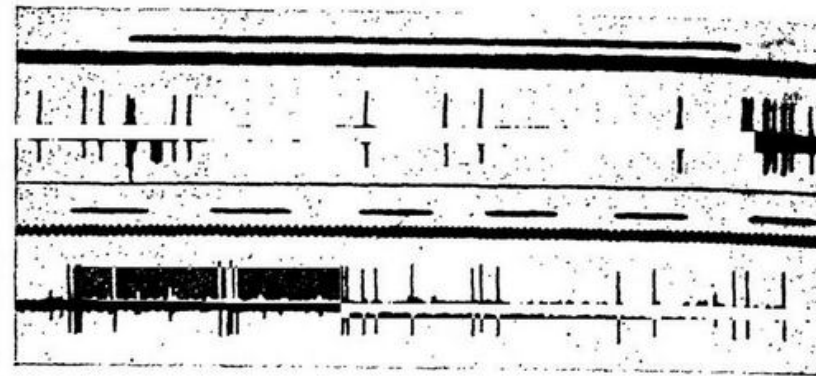


Fig. 1. *Above*: Single unit of rat's eye reacting to onset and cessation of illumination with rapid acceleration of spontaneous discharge. In the lower curve the same unit responds to "flicker". Light signal uppermost in all records. Immediately below it time in 1/50 sec. in this and all records. *Below*: Distribution of sensitivity to spectral light of the isolated unit illustrated above. The index was cessation of "flicker". The broken lines are tracing LYTHGOE'S (1937) absorption curve for visual purple.

illumination and followed at "off" by a silent period after which there is a gradually subsiding after-discharge. This type of firing is known from other sense organs. But it is a particularly interesting member of the retinal family of typical responses on account of the fact, shown in the same figure, that the after-discharge could not be inhibited by re-illumination. On-off-responses as

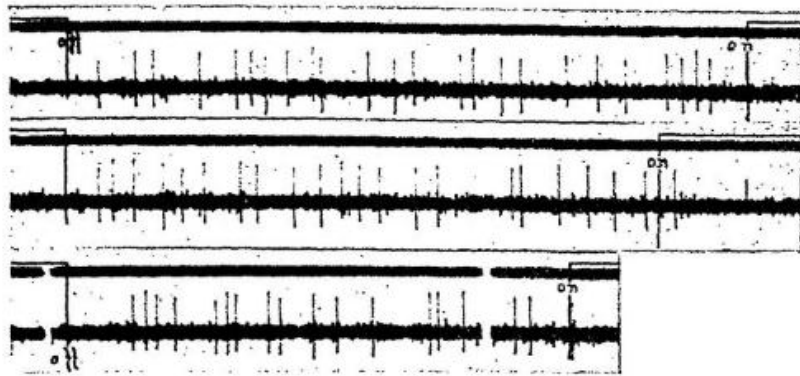


Fig. 2. A unit reaction merely to cessation of illumination and inhibited by re-illumination, which follows after an interval, shortening from above downwards. Its high threshold necessitated use of the microscope lamp (2.400 m. c.). No spontaneous discharge.

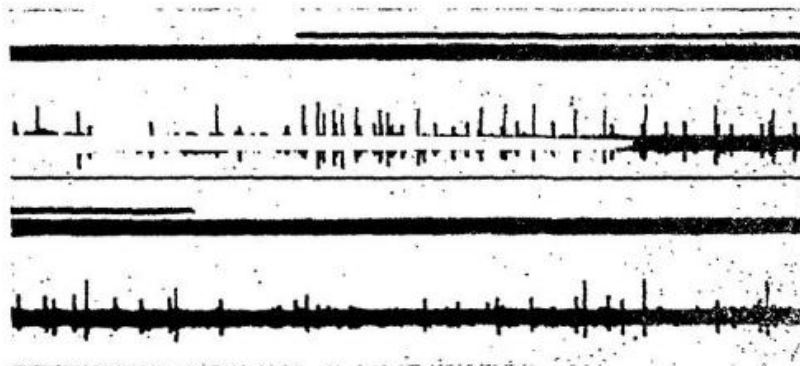


Fig. 3. Some spontaneous discharge preceding and following the activity of units merely reacting to the onset of illumination and lacking a definite after-discharge. The lower curve follows in direct continuation of the upper one and shows cessation of illumination.

well as pure off-responses are in my experience always coupled with complete inhibition upon re-illumination. For this reason it would seem natural to reserve the term "off-effect" for the type of discharge that is greatly accelerated at cessation of illumination and promptly inhibited by re-illumination. The term "after-discharge" should be used for the impulses following cessation of illumination with or without a silent period.

In the optic nerve of the horse-shoe crab (*Limulus*) HARTLINE and GRAHAM (1932) first found an element reacting with a

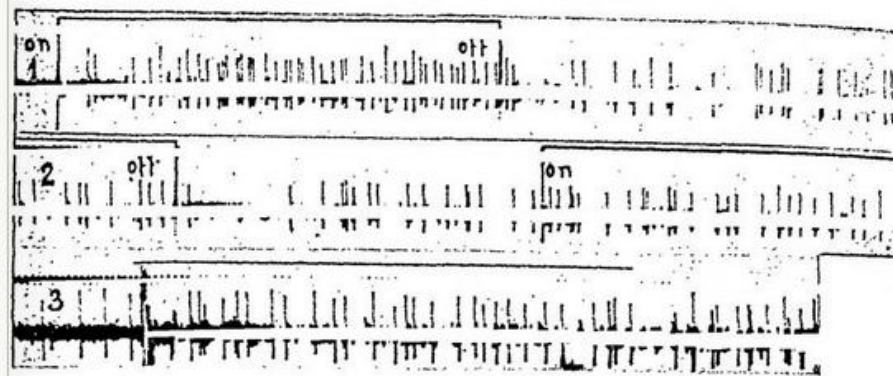


Fig. 4. Curves 1 and 2 refer to the retina of a white mouse and stimulation with the microscope lamp (2.400 m. c.). No spontaneous rhythm but a long after-discharge which is not inhibited by re-illumination (curve 2). Curve 3 illustrates a similar type of response from the retina of a rat.

rapid initial discharge followed by a silent period after which the discharge was resumed at a slower rate during illumination. This type of response I have also seen in the rat's eye.

I have not made any systematic observations on the relative distribution of the frequency with which the different types occur. But a comparison with the results obtained during long experience with the frog's mixed retina leads to the general conclusion that elements reacting *during* illumination are less common in the frog's eye where the great majority of types merely respond at the onset and (or) cessation of illumination. I have never seen an element with a non-inhabitable after-discharge preceded by a silent period (fig. 4) until this work with mammals was taken up. They must therefore be rare in the frog's retina. Pure off-elements, on the other hand, are far more common in frogs than in rats.

These observations may account for the fact that the frog has an I-retina and the rat an E-retina, distinguished by criteria which have been enumerated elsewhere (GRANIT, 1935, 1938, CHARPENTIER, 1936). Thus the E-retinae have a smaller off-effect in their electroretinograms than the I-retinae and this may signify a lesser degree of utilization of inhibition, as already suggested at that time (1935). But, despite this, the E-retinae may have a considerable after-discharge, simulating an off-effect (in the stricter sense of the term) in the discharge from whole nerve.

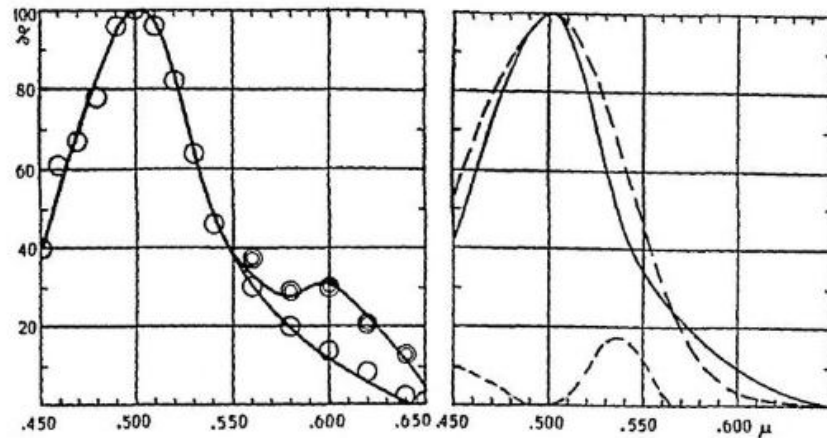


Fig. 5. Curves illustrating the average distribution of sensitivity to spectral light of 10 rats (81 averaged observations from individual experiments, based on in all 152 observations). *To the left:* Equal energy spectrum and curves drawn through the averaged data. *To the right:* The smoothed curve lacking the hump (left) replotted on the basis of an equal quantum intensity spectrum to be compared with LYTHGOE'S (1937) absorption curve for visual purple (broken lines). Below is plotted the difference between the two upper curves.

Distribution of Sensitivity to Monochromatic Light in Relation to the Absorption Curve for Visual Purple.

GRAHAM and RIGGS (1935) carried out some experiments with the electroretinogram as index of sensitivity which led to the conclusion that the sensitivity to monochromatic light could be explained by assuming it to be determined by visual purple.

Fig. 1 illustrates a single experiment, carried out with the on-off-element reproduced above the visibility curve, in which the values show good agreement with LYTHGOE'S (1937) absorption curve for visual purple, drawn in broken lines in the same figure. A correction from equal energy to equal quantum intensity (DARTNALL and GOODEVE, 1937) would have shifted the visibility curve a little towards the short wave-lengths.

Such results are obtained but I would not dare to state that they represent the most common finding. If the observations from 10 animals are averaged the curve to the left in fig. 5 is the result. It has seemed natural to split the values in the red end into two groups because there is often in the individual curves a hump around 0.600μ . In their present form the curves therefore illustrate the two alternatives obtained in the individual experiments.

To the right in fig. 5 is shown a comparison with LYTHGOE'S (1937) absorption curve for visual purple. My averaged data are here corrected for equal quantum intensity. The two curves differ in a systematic fashion. Below is plotted their difference in the regions where it must have some significance, a low hump in the greenish-yellow and a second increase towards the violet. These two difference curves correspond very well with rises in the absorption spectrum of a mixture of haemoglobin and oxyhaemoglobin. Considering that traces of blood hardly can be completely avoided and always were noted, the assumption that the visibility curves have been modified by a thin haemoglobin-oxyhaemoglobin filter is more than probable.

Analysis of the Properties of the Light-Adapted Eye.

In order to obtain further information about the "red" element an animal with the micro-electrode inserted is light-adapted to the lamp built into the microscope (Zeiss model) which represents about 2.400 m. c. This, of course, is a strong light for an opened, pigmentless eye. After 10 min. of light-adaptation the recovery of sensitivity (dark-adaptation) to the wave-lengths shown against the recovery curves of fig. 6 (left part) is studied with brief exposures at intervals of 15 sec. Two casual observations with wave-length 0.475μ are perhaps not very reliable. But the rest of the data follow consistent curves. The long wave-lengths are relatively more resistant to light-adaptation and their recovery curves are all of the same type. The curve for 0.500μ (maximum for visual purple) has a second rising phase which sets in when an asymptote or perhaps even a fall has been reached by the responses to the long wave-lengths.

From these curves the moment 30 min., at the broken vertical line, is selected for the plotting of the distribution of sensitivity in per cent of the maximum just before the fast rise of the curve for wave-length 0.500μ begins. This curve is shown to the right in fig. 6 where it is compared with the visibility curve *before* adaptation to 2.400 m. c. The large increase in the red is striking. The distribution of sensitivity of a light-adapted eye is also illustrated in fig. 7 with a greater number of points in the red. The sharp and narrow curve with a maximum in 0.600μ is merely the "red" receptor alone, given in per cent of its own maximum instead of in per cent of the maximum at 0.500μ . — During the

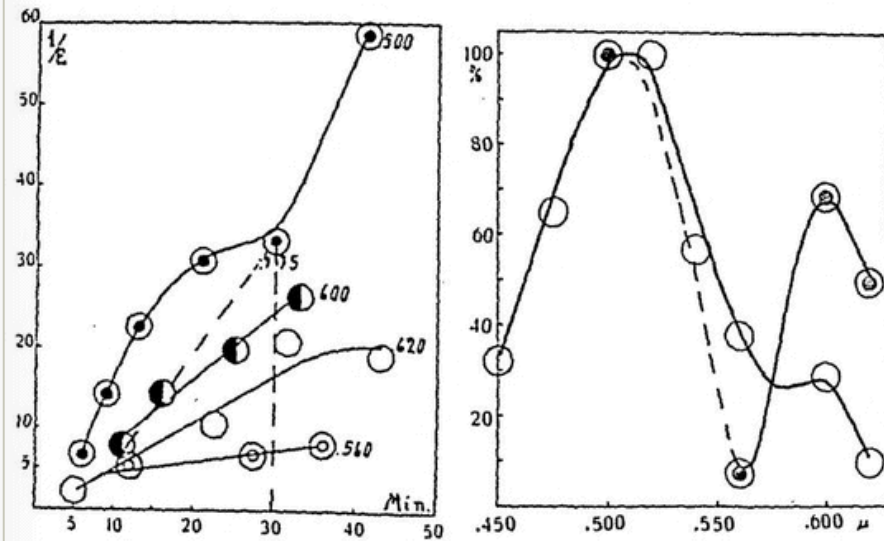


Fig. 6. *To the left:* Recovery curves for the wave-lengths indicated showing the return of sensitivity (inverse value of energy) against time, after cessation of 10 min. of light-adaptation to 2.400 m. c. *To the right:* Distribution of sensitivity to spectral light: \circ before, and \ominus after light-adaptation at the moment marked to the left by the vertical line through the recovery curves.

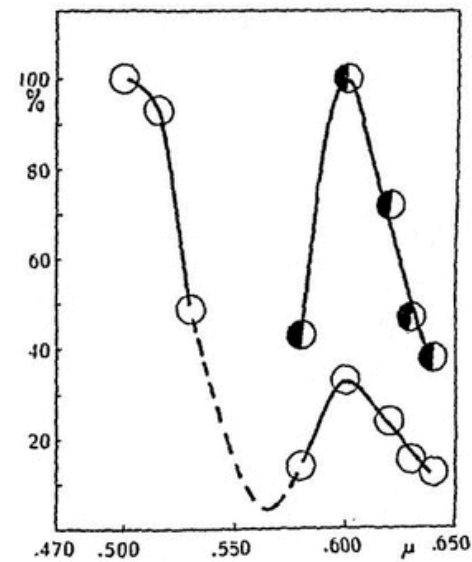


Fig. 7. Distribution of sensitivity to the equal energy spectrum after some light-adaptation to 2.400 m. c.: \circ in per cent of the maximum; \bullet in per cent of the maximum for the isolated "red" element.

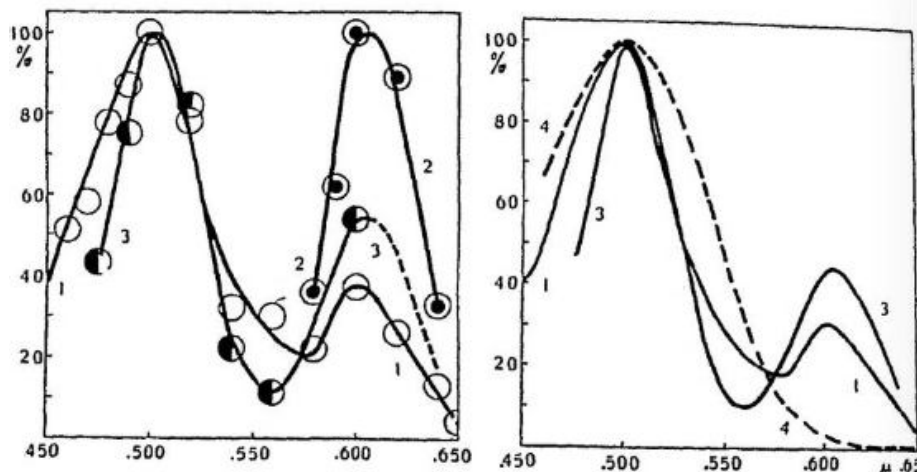


Fig. 8. To the left: Visibility curves of an active unit. 1 dark-adaptation, 2 immediately after light-adaptation, 3 somewhat later when the "green" substance is well above the threshold of the monochromator. To the right: The same curves similarly labelled corrected for an equal quantum intensity spectrum and compared with 4, the absorption curve for visual purple.

time devoted to following the recovery curves the sensitivity of the retina never returns to values near the maximal sensitivity at the beginning of the experiment, and, roughly, the level of sensitivity is about a 1 000 times below that of the completely dark-adapted animal.

Quite often it is impossible after light-adaptation to record anything but the narrow and precise "red" curve together with the top of the "green" one, because there is not enough energy in the monochromator to enable the intermediate low values to be measured. With some animals the red end may be seen in the isolated state. The reason for this also is that during light-adaptation the "green" substance becomes so insensitive that the energy of the monochromator does not suffice to elicit a "green" response. But the latter always returns during the early phase of dark-adaptation. And it always returns along a two-step curve.

The experiment of fig. 8 (left part) was of particular interest because to all appearance the same unit was followed all the time and complete curves were obtained for both the dark- and the light-adapted states. Despite 30 hours of previous dark-adaptation of the rat this unit gave the hump in the red already from the beginning (curve 1). The "green" curve was unusually narrow. After light-adaptation responses were first obtained only from the

red end. The rest of the spectrum was subthreshold. Thus, without further difficulties, the "red" substance was obtained in the isolated state (curve 2). Then the "green" substance gradually rose above the threshold, as shown by the recovery curves for the wave-lengths 0.600 and 0.500 μ in fig. 9, referring to the same experiment. A certain later stage in this recovery process is shown by curve 3 of fig. 8 (left). It is the moment when the "red" is about 50 % of the "green".

The characteristic and regularly recurring properties of the "red" substance are thus: (i) maximum around 0.600 μ , (ii) steep descent of the visibility curve towards either side, particularly towards the yellow and (iii) the important fact that it always appears coupled with the "green" substance and (iv) that it is more resistant to light-adaptation than the latter. At the moment it also seems probable that (v) the "red" and "green" receptors are coupled to the same final common path. To this question I shall return below.

To the right in fig. 8 some of the curves of the left half are plotted after having been corrected for equal quantum intensity. LYTHGOE'S absorption curve for visual purple is inserted for comparison. The "green" curve is narrow already in the dark-adapted state and becomes still narrower after light-adaptation. There is no definite reason to assume that the haemoglobin-oxyhaemoglobin filter had become increased in thickness during light-adaptation.

Is the "Green" Substance Identical with Visual Purple?

As is well known visual purple changes a great deal in concentration with state of adaptation, and this process has recently been followed quantitatively by ZEWI (1939). How this factor affects the visibility curves in my experiments depends upon the unknown concentration in the living retina but also upon its manner of distribution. If the outer limbs of the rods could be treated as a model absorption trough, then a decrease in the concentration, owing to light-adaptation, would lead to a decrease in the width of the curves. But LYTHGOE (BAYLISS, LYTHGOE and TANSLEY, 1936, LYTHGOE, 1940) and GRANIT (GRANIT ET AL., 1938, 1939) have given reasons for believing that the formation of a surface film of visual purple is an essential factor in the sensitization of the eye during dark-adaptation. An over-simplified physical scheme may therefore not be applicable to this case.

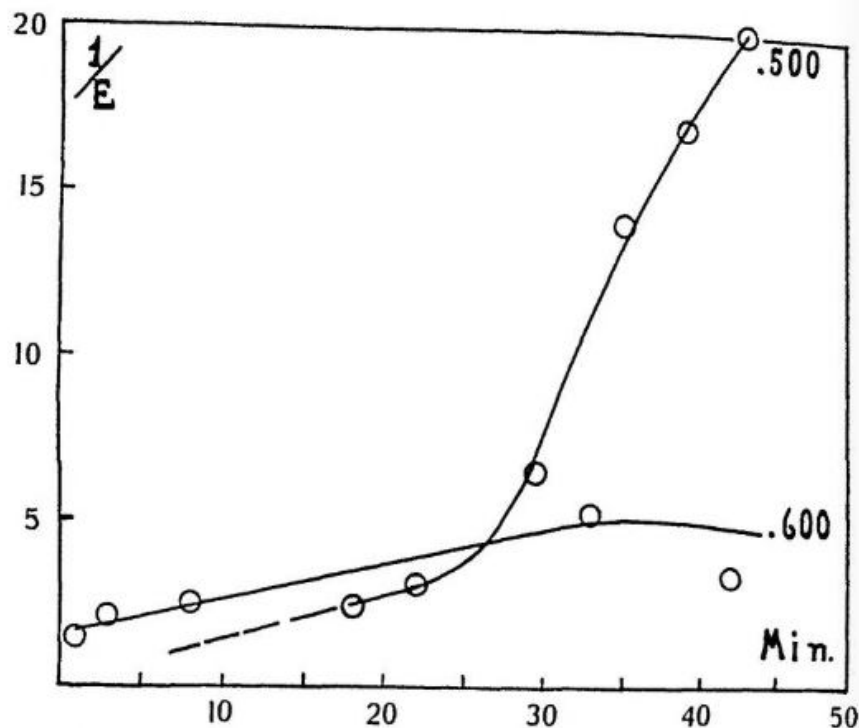


Fig. 9. Shows recovery of sensitivity as in fig. 6 for the wave-lengths 0.500 and 0.600 μ in the experiment analyzed in fig. 8 with regard to the distribution of sensitivity.

But the experiments themselves have disclosed a fact which in this connexion would seem to be of significance. This is the two-step rise of the recovery curves for wave-length 0.500 μ . Also with the less discriminative retinal response an initial slow rise is followed by a later fast increase in the sensitivity of the eye (WREDE, 1937; RIGGS, 1937; GRANIT ET AL. 1938, 1939), demonstrated with frogs. The initial slow phase in the regeneration at wave-length 0.500 μ represents the time taken up by the so-called "intermediate process" of GRANIT ET AL. We suggested that during this time some chemical organization of the visual purple molecule in relation to the cell took place, as during this early phase neither the sensitivity nor other properties of the eye corresponded to what is meant by a state of dark-adaptation. The late fast phase represented dark-adaptation proper which made the visual purple "active" physiologically. LYTGOE (1940) in discussing the work of GRANIT ET AL. (1938, 1939)

suggested that the visual purple, which was inactive from the point of view of dark-adaptation proper, nevertheless had some function to carry out, probably in connexion with photopic vision. We can see now that the "green" substance which either is visual purple itself or a modification of it, really does take part in what is known as "cone vision", but correctly should be termed daylight or photopic vision. This result could only be obtained with the discriminative micro-electrode technique used above.

It is regrettable that the as such highly convenient plot of log threshold against time for the process of dark-adaptation should have come to acquire some theoretical significance (HECHT, 1923) on account of the superficial likeness of this curve to the bimolecular isotherm supposed to represent visual purple regeneration. From the theoretical point of view much the better plot is the one used above, inverse value of energy necessary for the threshold, against time, which also enables comparison with the absorption curve for visual purple. The simple, less sensitive, but very useful method of measuring size of the electroretinogram during dark-adaptation in combination with simultaneous measurements of the concentration of visual purple in similarly treated control eyes (GRANIT ET AL., 1938, 1939) showed that the size of the electroretinogram bore no simple relation to the amount of visual purple. And yet the electroretinogram must measure sensitivity to light even though the relation is not one of direct proportionality (GRANIT, 1938). Moderate light-adaptation greatly diminished the size of the electroretinogram without reducing the concentration of visual purple of a well dark-adapted eye. On the other hand, the light-adapted eye did not become sensitive in proportion to the rise of the concentration of visual purple during dark-adaptation. Not until the concentration had reached a relatively high value did a fast rise of sensitivity (dark-adaptation proper) set in. But before that something took place in the eye. And it was this period that we ascribed to the time needed for an "intermediate process" preceding the sensitization of the retina. That the chemistry of regeneration of the visual purple molecule, and perhaps the molecule itself, is different during this phase is suggested by the slow rise of the recovery curve.

Some Difficulties for the Duplicity Theory.

In fig. 10 I have illustrated the probable place of the micro-electrode, including the assumption that the few cones are of the kind that share the final common paths with the rods (POLYAK, 1936). Many facts indicate that the micro-electrode picks up the discharge from the final common path (GRANIT, 1941), among them also the high degree of isolation that sometimes is obtained with it (see the illustrations of typical responses).

It is clear that the well-known assumptions of the duplicity theory that the rods cease to function in daylight vision, and that they are colour blind, will find it difficult to survive these experiments. One could perhaps ascribe the "red" substance which, after all, also may be a modification of the visual purple molecule, to the few cones present in the rat's retina. But only a desire to save a dogma could lead to the hypothesis that the "green" substance of the light-adapted rat's eye is a cone substance. Every-

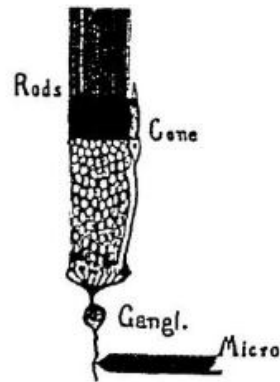


Fig. 10. Schematic drawing (lacking bipolars and amacrine) illustrating the probable locus of the micro-electrode with regard to a receptive unit consisting of several rods and a single cone connected to the same final common path. The cone is a little too large. The diameter of the very slender and elongated rods is 0.75μ (SCHULTZE, 1866).

thing points to the fact that visual purple, probably in a slightly modified state, is active also in the light-adapted state and in the same elements.

Most curious is the fact that the experiments show the same active unit to give the visibility curve with the two maxima in red and green. Before definitely accepting this fact one would like to be able to support it with a greater number of experiments, also with other animals. It is, of course, of prime importance from the theoretical point of view. But even if further experiments should show that the "red" and "green" substances were coupled to different neurites with very similar spikes, there is no escape from the fact that they must be closely connected in order to account for their always being together.

Colour Vision in Rats.

Do the rats see colours? Experiments by WALTON (1933) suggest that they can learn to discriminate the red and green parts of the spectrum from each other. It would seem difficult to accept other conclusions drawn from his experiments as filters were used and only the red end was relatively pure. Seeing how narrow the sensitivity bands may be one must emphasize this point of view, especially with regard to further experimentation with training methods. The ideal procedure would be to locate the sensitivity bands of different animals with the micro-electrode technique and then try to find out whether the animal utilizes the particular peripheral mechanism of discrimination found. From the point of view of the properties of the latter, however, the second task is of no immediate interest.

The mechanism of colour reception of the rat's retina is probably of a very elementary type. Thus, for instance, in the frog's retina the region of low sensitivity between red and green is filled out and possesses a maximum in 0.560μ , covering the region where the rat has its minimum. In fact, the frog's photopic spectrum is almost identical with the human one, but frogs may have a more prominent "blue" receptor (GRANIT and SVAETICHIN, 1939). Despite this, the properties of the peripheral mechanism of the rat's eye in many ways throw more light upon the nature of the peripheral mechanism in relation to vision. But some caution is suggested by the circumstance that the micro-electrode technique is selective in the sense that it chooses the most common case, even when one is careful not to restrict one's observations to isolated elements. If there are other substances or combinations in the rat's eye they must be very rare or insensitive in comparison with the one described above, but one cannot now assert that the rat only possesses the red-green mechanism described in this paper.

Summary.

Micro-electrode, amplifier and cathode ray oscillograph have been used for the recording of "spikes" from restricted units in the retina of anaesthetized albino rats in response to monochromatic light of known energy content.

A description of the types of discharge seen in isolated elements is given on p. 95.

The main problem has been to study the distribution of sensitivity to monochromatic light of more or less restricted units.

In the dark-adapted state their visibility curves often correspond very well with LYTHGOE'S absorption curve for visual purple. But some of them have an additional lower maximum around 0.600μ .

In such cases good light-adaptation suppresses the green part of the spectrum relatively more and there may remain above the threshold of the monochromator only the red-sensitive part, which in the isolated state still has its maximum around 0.600μ , descends steeply towards the yellow and less steeply towards the longer wave-lengths (figg. 6—8).

The "red" and the "green" element recover from light-adaptation along slowly rising curves, the "green" one probably somewhat faster so that at a certain stage of light-adaptation both are present giving a characteristic visibility curve with two maxima around respectively 0.500 and 0.600μ (fig. 8). But after a time, depending upon the previous period of light-adaptation, the "green" element begins to recover from light-adaptation at a very much faster rate than previously (dark-adaptation proper).

In the initial stages of light-adaptation the "green" substance often has a narrower visibility curve than is suggested by the properties of visual purple. This in connexion with its slow initial rate of regeneration has led to the suggestion that it is not visual purple itself but a slightly modified visual purple molecule. On the other hand, a haemoglobin-oxyhaemoglobinfilter, due to blood in the bulb, can be shown to affect the curve for the "green" substance in such a manner as to make it narrower.

The "red" and "green" elements are always coupled, and, though not conclusive on this point, the evidence at present suggests that the two elements are connected to the same fibre in the optic nerve.

It is suggested that the red-green system of the rat's retina represents the prototype of such systems in mammalian retinae.

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