

Principles and technique of the electro-  
physiological analysis of colour reception  
with the aid of microelectrodes

BY

RAGNAR GRANIT AND GUNNAR SVAETICHIN

Separat ur *Upsala Läkareförenings förhandlingar*.  
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ALMQVIST & WIKSELLS BOKTRYCKERI-A.-B.

From the Physiology Institute, Helsingfors.

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According to measurements of the electrical retinal reaction of the frog's eye in response to stimulation with an equal energy spectrum, the cones have their maximum around 0.550—0.560  $\mu$  (GRANIT & WREDE, 1937), the rods between 0.500—0.510  $\mu$  (GRANIT & MUNSTERHJELM, 1937, but see also CHAFFEE & HAMPSON, 1924, GRANIT, 1937). From the point of view of possible mechanisms of colour reception it is necessary to realize that the rod-maximum, due to visual purple, in the first instance must be regarded as an experimental complication tending to interfere with the isolation of cone properties. Dark-adaptation has to be avoided with the aid of definite criteria for its inception (see *e. g.* GRANIT & WREDE, 1937).

The reason for this is partly that the cone spectrum is known to mediate all the complex colour perceptions of the human eye, and partly that, with no sensations to guide us, we may mistake a rod for a greensensitive cone. Also mixtures of rod and cone sensitivities may be mistaken for special types of cones. If the capacity to discriminate colours is due to different receptors being sensitive to different regions of the spectrum, then the experiment wanted is one which tests this supposition and proves it to be true or false for *cone* vision alone. It is a superfluous task to show that, in general, some retinal areas or nerve fibres are more sensitive to green, others to red. What is wanted is an experiment that is strictly quantitative and for which energy conditions of the stimulus as well as state of adaptation are carefully controlled.

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The general facts are quite well known. In the papers by GRANIT and WREDE (1937) and WRIGHT and GRANIT (1938) electroretinograms are shown illustrating that *e.g.* a green-sensitive off-effect may go with a red-sensitive *b*-wave, that it is necessary to separate a special blue- or violet-sensitive part of the cone curve from the rest of it, and that the red end of the cone curve is selectively depressed by red-adaptation. Also HARTLINE (1937) has found that different fibres in the optic nerve are selectively sensitive to different parts of the spectrum. All these facts mean that the electrophysiological aspects of colour vision are interesting only inasmuch as they are put on a strictly quantitative basis and dark-adaptation as well as other sources of error are excluded. In the experiments reviewed in the summary by WRIGHT and GRANIT (1938) dark-adaptation was controlled.

The work is full of pitfalls, and, having now devoted some time to it, we have decided to publish a separate brief account of our technique together with a discussion of the sources of error in this kind of experimentation. We shall also describe some of our results, enough to show that, even when all possible precautions including the avoidance of rods are observed, single or a limited number of fibres in the optic nerve need not have the spectral distribution of sensitivity characterizing a larger assembly of elements. The main results were preliminarily reported by one of us (R.G.) at a lecture in Uppsala on April 19th 1939 and are here offered as a homage to the host at that occasion, a man who himself has enriched the field of colour vision with important contributions.

### The problem.

The aim of this work has been to measure for cone vision in the frog's eye the amount of energy necessary to elicit a barely perceptible minimum of impulses in different regions of the spectrum. To this end a microelectrode was used for restricting the area to be recorded from, and a microilluminator was added to the equipment to facilitate localization of the response. HARTLINE's (1938) beautiful technique for isolating single fibres is an ideal one but too laborious for use in work which requires such a great number of observations as this does. Assume, for instance, that some fibres are exceedingly thin or that their spectral properties are represented by very small populations. Then a very great number of experiments is required before the existence of such fibres can be established with any degree of certainty. There is no short-cut to knowledge in this question. The work to be per-

formed probably will last for years. A technique by which »attempts to obtain single fibres are successful in only a very small percentage of trials» (HARTLINE, 1938) is not very suitable for the purpose aimed at. Therefore it was felt necessary to develop microelectrodes and use them for collecting sensitivity curves in the simplest possible manner, that is by listening to the impulses in a loudspeaker and reducing energy of illumination in different parts of the spectrum till, for a given location of the microelectrode, the discharge just became subthreshold.

### Technique and procedure.

#### *General description.*

An important feature of HARTLINE's (1938) technique was preserved, that is, to isolate the fibres where they spread fan-like over the inside of the retina, rather than in the optic nerve. The opened eye of a completely light-adapted frog is put under a binocular microscope (ZEISS's model) and the microelectrode is applied with the aid of a micromanipulator vertically against the surface of the retina. The sort of picture obtained upon illumination of the eye is shown in fig. 1. The whole eye was illuminated with wave-length 0.600  $\mu$ . As can be seen the number of active fibres is very limited, and further restriction is then obtained by diminishing the illuminated area and the energy of the spectrum. The latter task is carried out with a graded neutral wedge, pushed into the beam of light till nothing whatever is seen on the screen of the cathode ray or heard in the loudspeaker. The wedge reading for this moment is recorded. The principle therefore, as has been stated, is to measure energy at the threshold for different wave-lengths.

Now one might think that much the easier way would be to photograph the discharge for an equal energy spectrum and afterwards evaluate frequency of discharge as a function of wave-length. This method would do for a *non*-restricted average response, provided that one knew the frequency as a function of energy for a number of selected wave-lengths. But considering how many different types of fibres there are (HARTLINE, 1938), such a procedure would be of very limited use in an analysis of single or a restricted number of fibres. If the individual frequency-energy curves differ in rate of rise or start from different absolute thresholds, then in an ideal case broad or narrow distribution curves would be obtained dependent upon the function »frequency of discharge-energy». With less luck curves of any kind might be had. The result would be a »pseudo-solution» of the problem of

colour reception, a number of uninterpretable curves with maxima in different parts of the spectrum.

It is clear that the criticism here levelled against the rather elaborate procedure of using an equal energy spectrum with still greater justification can be directed against the very simple experiment of testing different spots on the retina for spectral sensitivity with a number of colour filters.

#### Technical details.

1. *Microelectrodes*. These consisted of silver pins stuck into a glass capillary and drawn out in a flame to a thin point. A number of such microelectrodes, cut to a suitable diameter, were

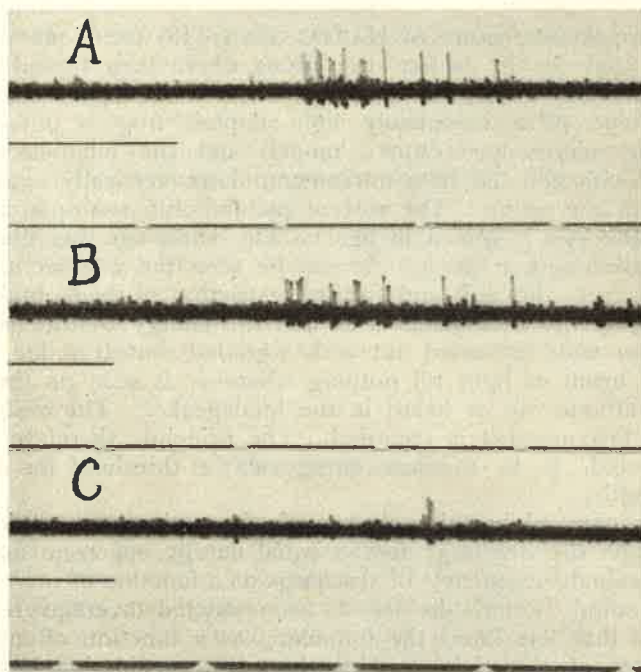


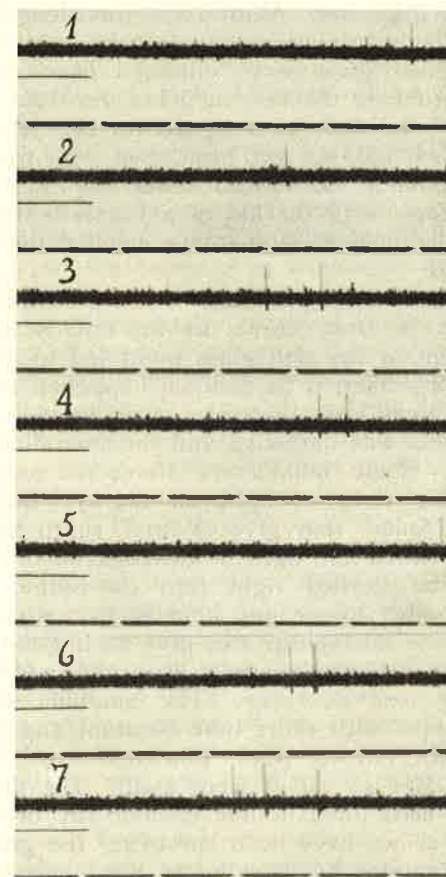
Fig. 1. Illustrates the degree of localization obtained with microelectrode alone without microilluminator as well as an experiment in which energy at the threshold was measured. Illumination on film and time in  $\frac{1}{5}$  sec. below oscillograph line.

A—C: off-response to wave-length  $0.600 \mu$  at three energy levels: A: 125, B: 66, C: 7 units of energy (E). Note decrease of frequency and number of fibres, and increase of latent period from A to C.

1—7: values near or just at threshold for different wave-lengths in an experiment with an off-element of maximal sensitivity (S) in  $0.600 \mu$  for which  $E = 2.5$  and  $S =$

tested with the aid of a preparation and the best ones selected. As indifferent electrode served silver-silverchloride leads to a cotton pad behind the excised, opened frog's eye.

2. *Microilluminators*. These were made by drawing a glass rod into a fine point, bending it to a suitable arc, and silvering it.



396. The records 1 and 5 are just *at* threshold for  $0.650 \mu$  and  $0.500 \mu$ , when sometimes no response, sometimes one impulse after a very long latency was obtained.

Wave-lengths and energies for the different responses are: 1,  $0.650 \mu$ ,  $E = 54$ ; 2,  $0.600 \mu$ ,  $E = 2.5$ ; 3,  $0.560 \mu$ ,  $E = 3.4$ ; 4,  $0.500 \mu$ ,  $E = 15$ ; 5,  $0.500 \mu$ ,  $E = 13$ ; 6,  $0.450 \mu$ ,  $E = 68$ . The last record 7 shows response to  $0.450 \mu$  later in the experiment when dark-adaptation has increased sensitivity of the responding element to short wave-lengths. E is here only 8.1 and the threshold is at E 5.4. Before dark-adaptation the relative sensitivities (S %) were:  $0.650 \mu$  4.7,  $0.600 \mu$  100,  $0.560 \mu$  74,  $0.500 \mu$  20,  $0.450 \mu$  3.8.

The silver was covered with fixative. Light from the monochromator was focussed on the thick end of the rod and reached the point by internal reflexion from the silver mirror. Tests were made with silvered rods of different types of glass using our photocell-galvanometer system to find out whether the light transmitted after internal reflexion differed in energy distribution from the light focussed on to the glass rod. Within the wave-lengths used in this work the microilluminators were found to be reliable. Later rods of specially purified glass were obtained from ZEISS. In these preliminary experiments the new model of the WRIGHT colorimeter (WRIGHT, 1939) was used, as designed for this laboratory by Mr G. C. NEWTON. It has not yet been used with frog's eyes.

The microilluminator was moved under the ZEISS binocular preparation microscope with the aid of a JANSE-PETERFI micromanipulator. A similar micromanipulator controlled the movements of the microelectrode.

During the preliminary operations with the microilluminator the opened eye was lit from above by the microscope lamp. The slightest movement of the thin glass point led to an off-discharge, heard in the loudspeaker, if its shadow happened to pass a sensitive area. Such areas were therefore easily located. This having been done the field was darkened and the microilluminator pushed near the retina. Some millimeters above the retina it throws a diffusely spreading cone of light on the eye, but as soon as a place has been found that gives a brief, sharp rattling noise in the loudspeaker when the light is switched on or off, the microilluminator can be pushed right into the retina, and then it is generally not possible to see any kind of halo whatsoever around it. Quite often the microelectrodes give such good definition that microillumination contributes relatively little to the end result (cf. fig. 1).

3. *Amplifying and recording.* The amplifier was of the condenser-coupled type with short time constant and balanced input stage (see *e.g.* MATTHEWS, 1938, TÖNNIES, 1938). For the initial stages MARCONI MH 40 valves were used. The final stage could alternatively be used for a double cathode ray or a loudspeaker. So far, as long as we have been surveying the ground, we have made most observations by listening to the loudspeaker. In some cases our small observation cathode ray with a sweep-circuit has been run alongside the preparation. Speed is essential since the opened eyes dark-adapt in about 20 min. (see GRANIT & WREDE, 1937) and then interference from the rods makes the whole experiment unreliable as an analysis of cone vision.

4. *Monochromator.* This was the Tutton instrument used as described by GRANIT and MUNSTERHJELM (1937). The light source was a straight filament quartz lamp driven from our high capacity

Nife cells at the amperage corresponding to the mean colour temperature of  $2800^{\circ}$  K for which it was calibrated at the Philips Co. testing department. Slit-width was constant throughout the experiment. Purity for colorimetric purposes with the slit-widths used should be reasonably good down to about  $0.450 \mu$ .

5. *Energy control.* For this purpose was available a Moll vacuum thermopile, a Siemens so-called supergalvanometer with special amplifying autocollimation optics for reading off the deflexions of the mirror. With these instruments the wedge readings corresponding to a constant deflexion of 3.9 mm of the mirror galvanometer were measured for every  $0.010 \mu$  between  $0.450$  and  $0.700 \mu$ . Thus were obtained the wedge readings for an equal energy spectrum. The gradient density of the neutral tint wedge for the same wave-lengths was measured with a sensitive potassium photocell. Knowing the gradient density per millimeter of the wedge as well as the readings in millimeter for equal energy, it was an easy matter to calculate the actual energy at any wave-length for any experimentally obtained wedge reading. For extreme reductions of intensity an extra filter of known transmission in the spectrum could be put in. The density of the accessory filter was of the order of 1.25, the gradient density/millimeter of the wedge of the order of 0.017, both figures, of course, functions of wave-length. These figures are here given merely to indicate order of magnitude of the factors used in the calculations.

6. *Preparation.* The frogs (*R. esculenta*) were taken directly from the basin and put into our standard light-adaptation apparatus (20 000 m.c.), described in detail in ZEWI's (1939) work from this laboratory on the regeneration of visual purple. From this work we know that light-adaptation in our apparatus is practically complete in 20 min. The eyes were then excised, opened, and generally a segment was cut off as this seemed to delay dark-adaptation apart from making the retina more accessible. All operations and adjustments under the microscope were carried out under a bright lamp and the eye was illuminated under the microscope by its lamp till the final arrangements were completed. The eye in the microscope was kept under a blackened metal cover with holes for the micromanipulator and the microscope objective.

## Results.

### *Treatment of the data.*

For a given combination of microilluminator and microelectrode the relative energies at the threshold ( $E$ ) for a number of wave-lengths was determined as quickly as possible with exposures at intervals of half a minute. Little energy is needed in a spectral

region of high sensitivity and much energy to compensate for low sensitivity at some other wave-length. Considering this, the results are best given in terms of the inverse value of  $E$ , or relative sensitivity

$$S = 1/E \times 1000.$$

$S$  having been computed it was generally found convenient for graphical comparison to present the results in terms of per cent of the maximal value for  $S$ . For equal energy measurements  $E = 100$  was the unit of our scale, to which all wave-lengths were adjusted.

#### *Dark-adaptation as a source of error.*

A given locus on the retina with a sharply defined discharge is not immediately maximally sensitive. Its sensitivity in the dark increases rapidly to a semi-stationary level, so that the initial values generally have to be discarded. Then follows a period of stationary or slowly increasing sensitivity lasting for some 15 min. during which the experiment has to be run (cf. GRANIT & WREDE, 1937). After that follows dark-adaptation of the rods, easily recognized by the extremely fast and regular rise of sensitivity to short wave-lengths. During the semi-stationary period it is necessary, as a check on the conditions, to repeat observations with one or two wave-lengths falling within the spectrum common to rods and cones. A slow rise in sensitivity of the control may be accepted, and intervening observations can then be corrected by interpolating between the controls, but a greater rise nearly always means onset of dark-adaptation and hence cessation of the experiment. A test with wave-length  $0.500 \mu$  immediately gives the information wanted for deciding for or against onset of dark-adaptation.

Do rods and cones go to the same fibres in the optic nerve? If they do, as is definitely indicated by histological work (see *e. g.* POLYAK, 1936), then all conclusions as to colour reception from experiments of this character are invalid unless at the same time it is shown that dark-adaptation or the absorption spectrum of visual purple has been excluded. The physiological evidence definitely favours convergence. Thus illumination with any wave-length causes diminution of a successive electrical retinal response to any other wave-length (see WRIGHT & GRANIT, 1938), and, independently of state of adaptation, the retinal response to the red end of the spectrum diminishes during dark-adaptation (THERMAN, 1939) etc. For a discussion of the facts showing rods and cones to compete about »right of way» the reader is referred to two reviews (GRANIT, 1938, WRIGHT & GRANIT, 1938).

Our experiments unanimously show that ultimately dark-adaptation nearly always takes place. Now we have not tried to find out *in casu* whether at the threshold one or several fibres are present. Most likely we always get the most sensitive fibre. HARTLINE's technique would seem to be the best one for this purpose, since, with a microelectrode on the retina, there is always the risk of a change from one fibre to another. But as all evidence supports the conclusion that rods and cones compete about the same fibre we are compelled to act as if this were so until proofs to the contrary become available. Actually we have some cases in which dark-adaptation of a certain retinal locus has not taken place. This may mean that there are isolated fibres which always are connected to cones, but it is just as likely that the permanent cone character of such fibres is due to physiological causes of which many could be suggested. In the experiment of fig. 1 with a single element, the absorption spectrum, as shown by the records, shifts, from  $0.600-0.580 \mu$  in the beginning, to short wave-lengths at the end of the experiment. This is a good instance of a single fibre showing a Purkinje shift, and we have many such experiments.

#### *Electrode on a great number of fibres.*

After cutting the microelectrode till its cross section reaches about a millimeter, illumination of a very large area of the retina (excluding microelectrode) would be expected to give an idea about the average spectral distribution of sensitivity of a cone population. We have 61 such observations obtained with 13 frogs from experiments in which only the initial values immediately after light-adaptation have been used. The distribution of sensitivity obtained from averaging these observations is shown in fig. 2. Sensitivity is given in per cent of the maximum placed in  $0.560 \mu$ . The index in all these experiments has been the moment of disappearance of the *initial outburst* of impulses in response to illumination. The off-effect has been neglected. The curve shown in broken lines is from the work of GRANIT and WREDE (1937) and illustrates size of the *b*-wave of the retinal electrical response to stimulation with an equal energy spectrum in an otherwise similar experiment. In our experiment it is important to observe that the microelectrode should be in the shadow. Illumination of it may give a photo-electrical effect which the ear may mistake for impulses.

The two curves of fig. 2 should be related by an equation expressing size of potential as a function of energy absorbed. To judge by the work of CHAFFEE and HAMPSON (1924) size of *b*-wave for all wave-lengths (dark-adapted eye) is proportional to the logarithm of the intensity, provided that the latter be sufficiently high so that actually cones are excited. With an equal energy

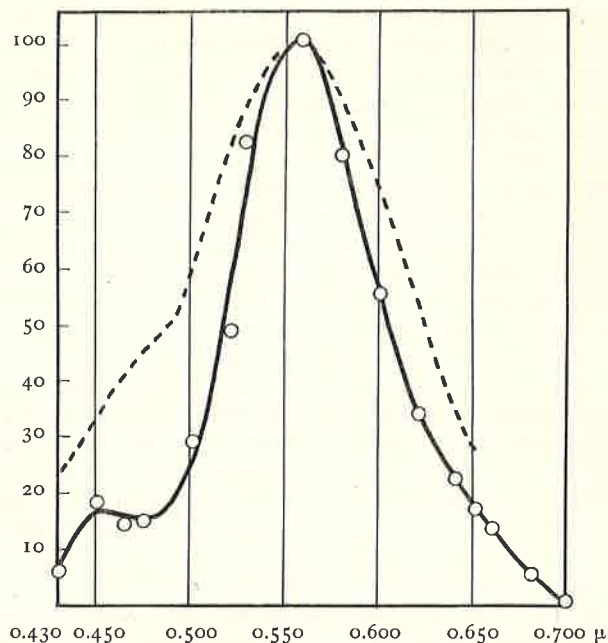


Fig. 2. Curve drawn through points marking observations shows inverse value of relative energy at the threshold for a large number of fibres and for a large area illuminated with the wave-lengths marked on the abscissae. The curve drawn in broken lines illustrates size of potential of the response to illumination with an equal energy spectrum, according to GRANIT and WREDE (1937).

spectrum energy of illumination is constant but it is here assumed to be absorbed in proportion to the sensitivity  $S$  of the retinal elements concerned. Therefore the two curves should be related by CHAFFEE and HAMPSON's (1924) equation, suitably transformed for this case,

$$P = k \log S + k_1,$$

in which  $P$  is size of potential ( $b$ -wave) in response to stimulation with an equal energy spectrum,  $S$  is sensitivity, obtained by us directly in terms of »sensitivity at the threshold» with a spectrum of known energy distribution, and  $k$  and  $k_1$  are constants.

Actually this equation perfectly describes the relation between the curves of fig. 2 within the wave-lengths 0.650—0.510  $\mu$ . Outside 0.650  $\mu$  no values for size of potential were available. But for the short wave-lengths 0.500—0.450  $\mu$  the values for size of potential ( $P$ ) are relatively too large to fit the curve for the equation, as derived from values for the region 0.650—0.510  $\mu$ . This

can also be seen by direct inspection of the curves of fig. 2. This means that the »blue» elements are not identical with the »red» ones. Both curves show a hump in the blue end which also indicates that elements with a different absorption spectrum from the ones giving the otherwise symmetrical curve are activated in the short wave-lengths. (For a discussion of this question see GRANIT & WREDE, 1937.)

#### *Restriction of active units.*

As soon as the active units are sufficiently reduced by micro-electrode and microilluminator it is found that immediately after light-adaptation most units only discharge with an off-effect. According to HARTLINE (1938) some fibres should discharge at »on» (onset of illumination), others at »off», other again at both »on» and »off». Our experience is that in the well light-adapted eye »on»-units are very rare, »on-off»-units reasonably common, and pure »off»-units the very great majority of fibres. At this stage of our work we have but rarely cared for checking up on the number of active units under the electrodes, and so are not prepared to attach very great importance to this finding. But from the practical point of view it means that we have either listened to »on» or »off» when both have been present or to pure off-effects alone. Generally time does not permit one to run parallel observations on the thresholds of both »on» and »off»-elements since, as a rule, the latter have a very much lower threshold than the former. Before the vanishing point of both »on» and »off» has been determined for a number of wave-lengths the eye may have dark-adapted and the whole experiment come to a premature end.

The accuracy with which the threshold can be determined acoustically is very much determined by the nature of the discharge. We have in the first instance aimed at finding places which answer to »on» or »off» with a brief, sharply defined rattle — the typical discharge immediately after light-adaptation — and the energy at the moment of cessation of this noise may be located to the millimeter on the wedge (gradient density per millimeter discussed above). As it is important to get several observations before dark-adaptation sets in we have used 5 mm steps on the wedge and placed the threshold in the middle between the last point where it was heard and not heard. This is always possible with a light-adapted eye even when the discharge is not of the sharply set-off type. Naturally one has to be careful in the long run not to limit ones observations to one type of discharge only, as this may impose undesirable restrictions on the experiment.

The onset of dark-adaptation is quite often headed by the be-

ginning of a disturbing spontaneous discharge that can be made to disappear by renewed light-adaptation. Both »on» and »off» become drawn out and less distinct in the dark-adapted eye. The threshold is not as precise as in the light-adapted condition and the increase in sensitivity is thousandfold counted from the semi-stationary stage.

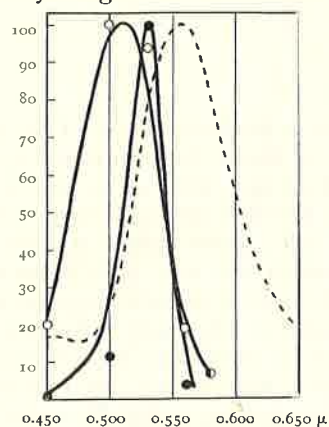


Fig. 3.

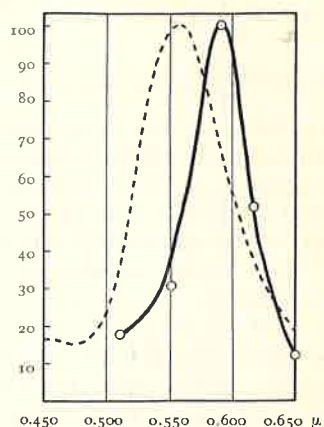


Fig. 4.

Fig. 3—10. In all these figures the curve drawn in broken lines is the one drawn in full in fig. 2. The curves drawn through the observations illustrate distribution of inverse value of relative energy in different parts of the spectrum, as obtained from experiments in which both microelectrode and microillumination has been used. See description in text.

#### Quantitative observations with restricted units.

In the curves, to follow, the average distribution of sensitivity of the cone population (from fig. 2) is put in for comparison in every instant. These curves, selected from 54 experiments, refer to experiments where we feel convinced that dark-adaptation has been recognized, or avoided with every possible care.

1. *A mixed case.* Fig. 3 shows an experiment in which we succeeded in following »on» and »off» simultaneously. The on- and off-effects did not belong to the same fibre to judge from their different spectral distribution and the fact that  $S_{\max}$  for »off» equals 2 120 and for »on» only 234.  $S_{\max}$  for »off» is at 0.500  $\mu$  which in conjunction with the high sensitivity probably means that some dark-adaptation has taken place. As has been found by GRANIT and WREDE (1937) the off-effect commonly shows signs of dark-adaptation before the initial *b*-wave. The interesting fact, shown by fig. 3, is that the curve for the on-discharge is very steep and narrow with a maximum around 0.530  $\mu$  and that it

does not coincide with the other two curves. The differences between the simultaneously recorded on- and off-effects are particularly striking.

2. *Maximum in the long wave-lengths.* Fig. 4 represents a very sharply defined off-effect with  $S_{\max} = 32$  somewhere between 0.590 and 0.615  $\mu$ . The intermediate region was not covered. The

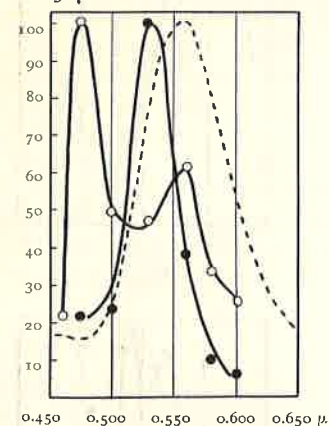


Fig. 5.

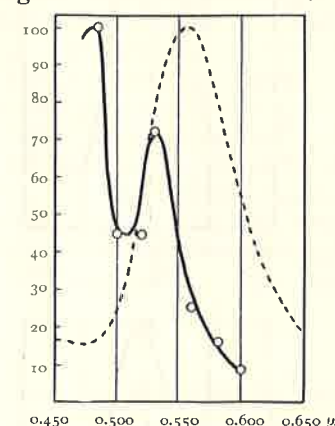


Fig. 6.

narrow sensitivity band again is striking, as well as the fact that in 0.550  $\mu$ , which for an average cone population is very nearly maximal, the sensitivity is as low as 30 % of the maximum. It is worth nothing that a relative maximal sensitivity  $S = 32$  is a fairly low value. The single element of fig. 1 is of this type.

3. *Maximum in the short wave-lengths.* In both fig. 5 and fig. 6 there are curves with two maxima, probably indicating the presence of two elements rather than of one element with a complex curve. For these curves the maxima in the short wave-lengths are outside 0.500  $\mu$ , the maximum of the rod curve. These maxima would have to be located between 0.450—0.470  $\mu$ . In fig. 5 the second hump in the curve is at about 0.560  $\mu$ , but, on the assumption of two elements, the broad and low curve may well have its maximum further out in the long wave-lengths. In fig. 6 the second lower maximum is at 0.530  $\mu$ . In fig. 5 the narrow curve, which also has its maximum in 0.530  $\mu$ , refers to the on-outburst whereas the curve with two maxima refers to the *simultaneously* recorded off-effect. In fig. 5  $S_{\max} = 406$  for »off» and 28 for »on», in fig. 6  $S_{\max} = 1 190$ . Despite the high sensitivity in the latter case there were no signs of dark-adaptation.

4. *A pure »on»-element.* Fig. 7 shows 5 points obtained in an experiment where the microelectrode picked up a pure »on»-dis-

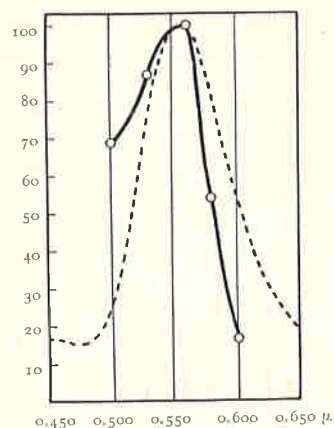


Fig. 7.

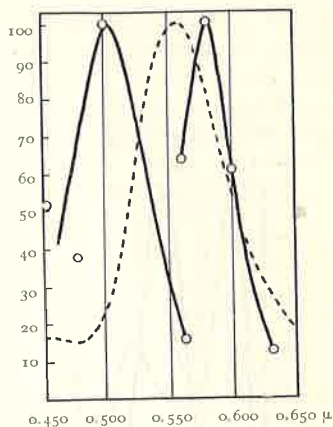


Fig. 8.

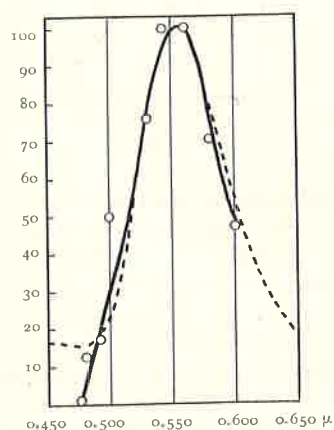


Fig. 9.

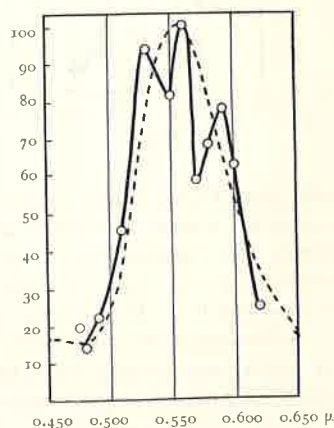


Fig. 10.

charge. The maximum rose a little too fast from  $S = 10$  to  $S = 60$  to satisfy our criteria for avoidance of dark-adaptation, so that it is possible that the curve gradually widens towards the short wave-lengths on account of some dark-adaptation taking place. Nevertheless, as  $0.600 \mu$  refers to a very early observation (nr. 2), the sensitivity to red is exceptionally low in this case. The discharge had a diffuse character.

5. *Early and late off-effect.* In the experiment shown in fig. 8 we obtained the points on the narrow curve with maximum in  $0.580 \mu$  in the beginning of the experiment. Sensitivity was then 27. Somewhat later, when dark-adaptation had taken place, the

sensitivity rose to 65 and the maximum shifted to the region of  $0.500 \mu$ . The early off-effect has the distribution of sensitivity recorded in fig. 1 for a single element. In both cases dark-adaptation moved the maximum to the short wave-lengths.

6. *Average curve without hump in the blue.* In fig. 9 is shown a case in which the readings fell on the average curve. It is interesting because of the fact that the hump in the blue end is absent.

7. *Three maxima.* Finally fig. 10 illustrates an experiment in which the readings were obtained with a very high degree of precision so that the presence of three maxima could not be put down to chance variations. The maxima are in  $0.530$ ,  $0.560$  and  $0.580 \mu$ . That several elements took part in the reaction could be determined by listening to the discharge. In the red end it had the character of a couple of sharp shots, in the blue end it was a diffuse noise. This indicates that in the red there were a couple of large impulses, and in the blue several small ones.  $S_{\max}$  was 26.

#### Sources of error.

Before drawing any conclusions from the experimental material presented we have to consider the effect of the three most important sources of error on the curves presented.

1. The number of elements contributing to the discharge recorded may be too large. The net result of this effect would be a tendency of the curves to gather around the average curve, that is to become relatively too broad.

2. Dark-adaptation may take place and this change would also lead to an expansion of the curves but in this case towards the short wave-lengths with a maximum in  $0.500 \mu$ .

3. Some elements may suddenly cease to discharge. In this case it is difficult to forecast what will happen, but on the whole it would be expected to favour precise definition. Our general experience is that when a given place under the microelectrode ceases to discharge, death is very quick in coming on, and with it absolute silence.

4. The most important source of error would seem to be the »switch-board effect» (GRANIT & MUNSTERHJELM, 1937). In these experiments it has turned up as sudden inexplicable changes of threshold indicating a rotation of activity among the retinal elements, seen also with single elements. Such phenomena could explain, for instance, curves of the type shown in fig. 10. They may well play an important rôle in colour reception. But the fact that many curves have been so narrow will be difficult to explain except by assuming the existence of several absorption bands of the kind shown for a single element in fig. 1.

### Conclusion.

From the point of view of colour reception our main question is: what is the spectral distribution of sensitivity of the elements of the cone population giving the average curve of fig. 2? We repeat that the final answer to this question only can be given on the basis of a very large experimental material. Our experiments show that quite often very narrow bands of sensitivity are obtained, which may occupy maxima at different levels of sensitivity between 0.450—0.600  $\mu$ . Specimens of such curves are given in figs. 4—10. They show that the average curve of fig. 2 is a compound curve. The elements that have contributed to it can hardly be less than three in number, a »blue» (maximum around 0.460—0.480  $\mu$ ), a »green» (around 0.500—0.530  $\mu$ ), and a »red» or »yellow» (0.580—0.600  $\mu$ ) one, but it is quite probable that the number of elementary colour-receivers is still greater. Nevertheless THOMAS YOUNG seems to have been right in assuming a minimal number of three elementary colour receivers. Among the curves obtained experimentally many are too narrow to be explained on the basis of sources of error.

### Summary.

In this paper is described the technique and the sources of error in picking up impulses with microelectrodes from single or a limited number of elements in the frog's retina in response to stimulation with spectral light of known energy distribution.

The experiments have been carried out with the light-adapted eye, and it is shown that from the point of view of an analysis of colour reception dark-adaptation is the most serious source of error encountered.

Sensitivity curves have been determined by listening to the discharge in the optic nerve and measuring the amount of energy necessary for the threshold.

In fig. 2 is shown the average sensitivity distribution of a cone population in the frog's eye.

Figs. 1 and 4—10 show that restriction of the retinal area, recorded from, leads to the average curve being split up into elementary curves, comprising relatively narrow bands in the spectrum.

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