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"Red" and "Green" Receptors in the Retina of Tropidonotus.

By

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Received 3 October 1942.

In our survey of colour receptors in the retina, carried out with micro-electrodes stuck into the opened eye, frogs, rats, pigeons, guinea pigs, the Greek tortoise and a number of fishes have been studied (GRANIT and SVAETICHIN, 1939, GRANIT 1941—42). In this paper the work is extended to a snake (*Tropidonotus natrix*) which is of interest because of its pure cone retina (see *e. g.* WALLS, 1934).

The technique for energy control of the spectrum and application of the micro-electrode has been described in the earlier papers of this series (cf. in particular GRANIT and SVAETICHIN, 1939, and GRANIT 1941, a) and need not be redescribed. The snakes were anaesthetized with urethane (20 %) and the micro-electrode inserted with micromanipulator into the opened eye under suitable magnification of the visual field. Successful experiments were carried out with 11 eyes from which 18 series were obtained, a series referring to a given location of the micro-electrode and comprising a large enough number of observations for establishing the spectral locus of its sensitivity curve. The number of values (threshold determinations) obtained in such a series may vary from 5 to 50, each figure being fixed upon after several positive and negative readings on both sides of the threshold. The values are averaged and plotted in per cent of the inverse value of the energy necessary for a threshold response in each wave-length tested.

General Observations.

Spikes from single elements or from elements synchronized to act as singles are sometimes obtained, though not as often and easily as in mammals. Relatively restricted discharges are the rule.

The slow respiration may cause variations in the level of sensitivity which have to be followed by continuously calibrating with the wavelength chosen as control — nearly always the maximum.

In animals with rods and some type of visual purple nearly all receptors later enter upon a secondary phase of dark adaptation pushing the region of maximal sensitivity towards the spectral locus of the top of the absorption curve for visual purple. This always means a shift of the sensitivity towards the short wavelengths. It also means that the spectral properties of elements located by the microelectrode have to be analysed before dark-adaptation sets in. In the cone-retina of the snake this complication is absent and there is no need for light-adapting the animals. To be true, the green region of the spectrum has sometimes become more prominent later in an experiment when the animal has been lying in the dark box and its eye only illuminated by threshold stimuli. But this need not mean more than that the "green" elements are more sensitive to the light adaptation necessary for inserting the micro-electrode, and the change is not of the order of magnitude characterizing dark-adaptation in eyes containing visual purple. The pure cone eye of the Greek tortoise behaved in a similar fashion (GRANIT, 1941 b).

In the retina of the snake low intensities merely elicit responses *to* illumination, no off-discharges. To stronger stimuli, however, most elements react with both on- and off-responses. Pure off-elements have not been found, which, of course, need but mean that they are rare.

The bulb is very soft and small in this animal and consequently easily damaged when opened. Urethane has to be given cautiously in repeated doses of 0.2 cc to ensure optimal conditions. Neither the isolated eye nor the eye of a snake, which does not breathe after urethane, responds to light. Several animals were lost owing to the difficulties mentioned.

The Colour Receptors.

Elsewhere I have pointed out (GRANIT, 1941, e, f) that the animals with good colour vision possess one broad dominator band and some narrow modulator bands in other regions of the spectrum. The dominator is assumed to represent "brightness" or "white" modulated to "colour" by the modulators. The spectral properties of the visual receptors of *Tropidonotus* conform to this scheme.

The broad dominator band is plotted in fig. 1 from 5 series, one of them referring to an isolated spike. The maximum of the dominator is in 0.560 μ and to all appearance the curve is identical with the dominator found in, for instance, frogs (GRANIT, 1941, f) and cats (GRANIT, unpublished) and with the luminosity curve of the photopic human eye. In the pigeon the dominator appeared shifted somewhat to the right, probably owing to selective absorption by the coloured oil globules (GRANIT, 1942, b).

Fig. 2 shows 7 series with maximum in 0.600 μ. All except 2 had a secondary hump in the green though the level of this hump

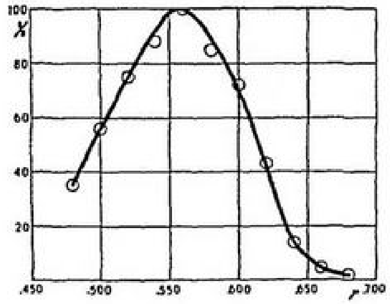

Fig. 1. Average spectral distribution of sensitivity of 150 values from 5 series with "dominator" elements. Spectrum of equal quantum intensity.

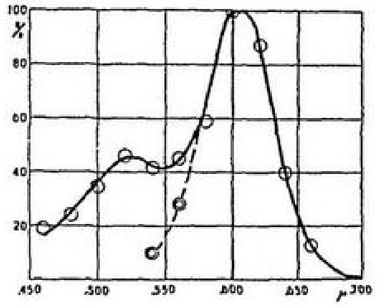

Fig. 2. Average spectral distribution of sensitivity of 124 values from 7 series with well marked "red" modulator and moderate or small secondary hump in the green. Dotted line refers to 2 cases in which hump in the green absent or small. Spectrum of equal quantum intensity.

varied from case to case. In the two remaining series the curve passed down towards 0.540 μ, as shown by the broken line of the figure, and the threshold of these two elements was so high as to exclude continuation of the experiment farther towards the short wave-lengths. Thus we do not know whether or not a small hump in the green would have appeared in these cases too. However it is clear that the "red" element of fig. 2 with maximum around 0.600 μ is of the narrow modulator type.

Fig. 3 illustrates 2 series in which the "green" element, coupled to the "red" one, is still more prominent than in fig. 2. It is conceivable that, by combining in a certain proportion, the "red" and "green" modulators may add up to the distribution curve for the dominator. A step in this direction is indicated by the series illustrated by the open circles in fig. 4. The curve drawn through these observations, (as many as 37) comes very near the dominator. The red is emphasized by a hump around 0.600 μ. This hump is still more marked in the second curve of the same figure, illustrating another series based on 19 observations, most of which fell around the maxima. The values for 0.520 and 0.540 μ are single values only, taken at the end of the experiment, and may therefore be too low.

A process of fusion of the two modulators into a dominator would certainly provide an attractive explanation of the facts that the dominator is broad and symmetrically placed with respect to the two narrow modulators and that the experiments prove them to be combinable in different proportions. It

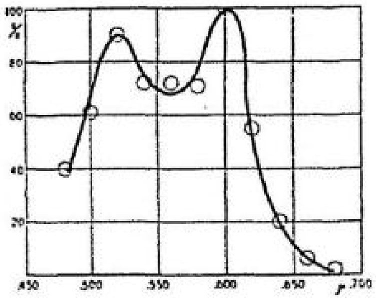

Fig. 3. As fig. 2, but with large rise in the green. Averages of 73 values from 2 series.

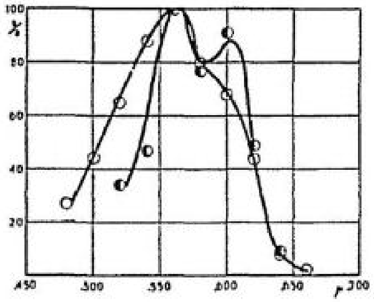

Fig. 4. As fig. 1, but dominator with hump in the red. Averages of 56 values from 2 series.

would also explain that in the animals taken in the spring the dominator was rare and the "red" modulator common whereas in those taken in the autumn the dominator was common and the modulator rare. The "green" modulator was never obtained in the isolated state but that it also is narrow is clearly indicated by the results. Isolated elements were found with all types of curves.

Knowing from the work of POLYAK (1936) that both rods and cones and (or) several cones converge towards the same ganglion cell and that the micro-electrode picks up the discharge from this layer, we can understand results such as those of figs 1—4 by glancing at the diagram of fig. 5. The convergence of "red" and "green" elements towards the same final common path is there illustrated schematically. The different proportions of "red: green" may be thought to represent the experimentally determined curves of figs. 1—3.

If there be a "blue" modulator in the eye of the snake it must be represented by relatively few receptors or by very insensitive ones. It has not been seen in these experiments.

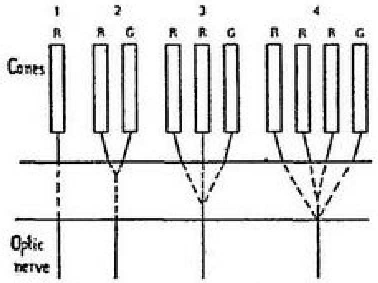

Fig. 5. Diagram illustrating convergence towards optic nerve of "red" and "green" cones in different proportions suggesting an explanation of the curves of figs. 1—4 in terms of two elementary modulators. R = "red" cone, G = "green" cone. 1, "red" modulator alone as in fig. 2 dotted curve; 2, "red" and "green" combining to dominator of fig. 1; 3, two "red" and one "green" modulator combine to give curve of fig. 3; 4, three "red" and one "green" modulator combine to give curve drawn in full of fig. 2.

The "red" modulator at 0.600 μ seems to be very constant in different animals. The curve of fig. 2 could just as well have been taken from a frog or a rat. The "green" modulators vary in location from 0.500—0.530 μ in different animals. On account of the visual purple, which in animals with mixed retinae is a source of complication in the green region of the spectrum, modulators in this region may often be very difficult to measure with precision. Again, the steepness of the "red" modulator towards the left side of the spectrum may be exaggerated by the filtering effect of haemoglobin (GRANIT, 1941, c) around 0.520—0.560 μ. The eyes of snakes like those of rats sometimes tend to bleed a little when opened.

The eye of *Tropidonotus* has been used by v. STUDNITZ (1940) for extraction of his so-called "Zapfensubstanz". In his experiments extracts were made on whole bulbs(!) and extinction analyzed with the "Stufenphotometer" of PULFRICH. A very broad composite curve was obtained with maxima — according to v. STUDNITZ — in 0.468, 0.555 and 0.655 μ. They seem to bear no clear relation to the data obtained with the far more accurate electrophysiological method which, in addition, registers what actually is being led up to the brain.

Summary.

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

distribution of sensitivity to monochromatic light of more or less restricted discharges in this cone eye.

There is a broad dominator band of sensitivity with maximum in 0.560 μ, similar to the dominator found in the frog's eye.

There are further two narrow modulator bands of sensitivity with maxima in respectively 0.600 and 0.520 μ.

The "red" modulator is sometimes obtained in the practically isolated state but generally the "green" modulator appears coupled to it so that a secondary hump of variable size is found with maximum in 0.520 μ.

The author is indebted to the Rockefeller Foundation for a grant to this laboratory.

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