S-POTENTIALS FROM LUMINOSITY UNITS IN THE RETINA OF FISH (CYPRINIDAE)

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SUMMARY

1. S-potentials were recorded in fish from units which never responded by depolarization. These hyperpolarizing units are the L-units of Svaetichin & MacNichol (1958).

2. Figure 5 shows some sets of action spectra from a single unit. For each curve the criterion of action was hyperpolarization to a fixed level, by lights of various wave-lengths. When these lights fell upon zero background (circles) the curves show that two kinds of cone contribute to the action spectrum, one with the 620 nm pigment of Marks and one with the 680 nm pigment of Naka & Rushton (1966a).

3. When the lights fell upon (i) a fixed green background (triangles, Fig. 5), or (ii) a fixed red one (squares), the action spectra changed in a way that indicated greater prominence of (i) the 680 nm system (ii) the 540 nm green system that was not conspicuous without adaptation to red.

4. These observations (on the tench *Tinca*) are contrary to the conclusions of Svaetichin & McNichol (on Gerridae) that the action spectrum is unaltered in shape by adaptation to coloured lights. The contribution of the green cones, for example, was actually absolutely greater under deep red adaptation.

5. It is concluded that L-units receive signals from 680, 620, 540 nm and possibly also the blue cones, that the quantum catch in all these contribute to the hyperpolarization produced, but their interaction is more complicated than the simple addition of independent cone effects.

INTRODUCTION

In their pioneer description of various types of S-potential, Svaetichin & MacNichol (1958, see also MacNichol & Svaetichin 1958) drew a major distinction between the colour units that responded by depolarization to light of long wave-length, and the Luminosity units that always responded by hyperpolarization, no matter what the wave-length. From their work it looked as though L-units were organized more simply than C-units, not only because they had only one direction of response, but because the

spectral sensitivity, as shown by records of potential *versus* wave-length was not changed differently by adapting to lights of different wave-lengths. The results were as though all the cone pigments were mixed together in the class of cones that served L-units, and indeed they suggested that this was the case.

We have no experience with cones of the Gerridae that they studied. Those who have examined single cones of the Cyprinidae by micro-spectrophotometry (Marks 1963, 1965; Liebmann & Entine, 1964) have not reported mixed pigments. We have repeated some of Svaetichin & Mac-Nichol's observations on the L-units of Cyprinidae, and find the S-potentials less simple than with C-units and their spectral sensitivity by no means unchanged by adaptation to coloured lights. Instead of L-units being the easiest to analyse we have found them the hardest, and have not succeeded in understanding properly the underlying organization.

Note. References Naka & Rushton (1966*a*, *b*) will be quoted for short as (NR*a*), (NR*b*).

METHODS

The fish, usually tench (*Tinca*), were kept and prepared as described previously (NR*a*). The retina was left in the optic cup mounted in a dark, cool, moist chamber with apertures for the light stimulus and for the micro-electrode. This was a potassium-citrate filled micropipette. The optical arrangements were as shown in Fig. 1 of (NR*a*), with the shutter usually placed in the spectral beam 1 only, so that a steady adapting background could be added through beam 2.

RESULTS

Figure 1 shows a typical set of records obtained on a stationary time base so that each response is represented by a vertical line whose length is proportional to the amount of hyperpolarization. The X-shift was coupled to the wedge W_1 (Fig. 1, NR*a*) by means of the potentiometer P so that the displacement to the right is proportional to log I, where I is flash intensity (see scale at bottom of Fig. 1). Each frame in Fig. 1 (*a*) shows response with a light stimulus of a different wave-length (as indicated) and within each frame the effect of increasing the intensity of that light is seen. In Fig. 1 (*b*) the same thing is recorded except that now there is a steady background of strong deep red light upon which the stimulating flash falls. As would be expected with L-units all records still show hyperpolarization, but con-trary to the findings of Svaetichin & MacNichol (1958) the deep red background appears to depress excitability to deep red flashes (720 nm) more than to green ones (520 nm).

Figure 1 (*a*) shows only a few of the records taken without background in this series. Figure 2 shows nearly the whole set, with only the extremity of each potential excursion plotted as a point. For the set at each wave-length the lateral displacement of the points in Fig. 2 (as in Fig. 1) represents log I, but each set has been laterally displaced a known arbitrary amount

for clearness of presentation. It is plain that the curves of Fig. 2 are not of constant shape and no fixed template could describe them all. Before trying to analyse this situation, it may help to consider a preliminary and unusual example of great simplicity.
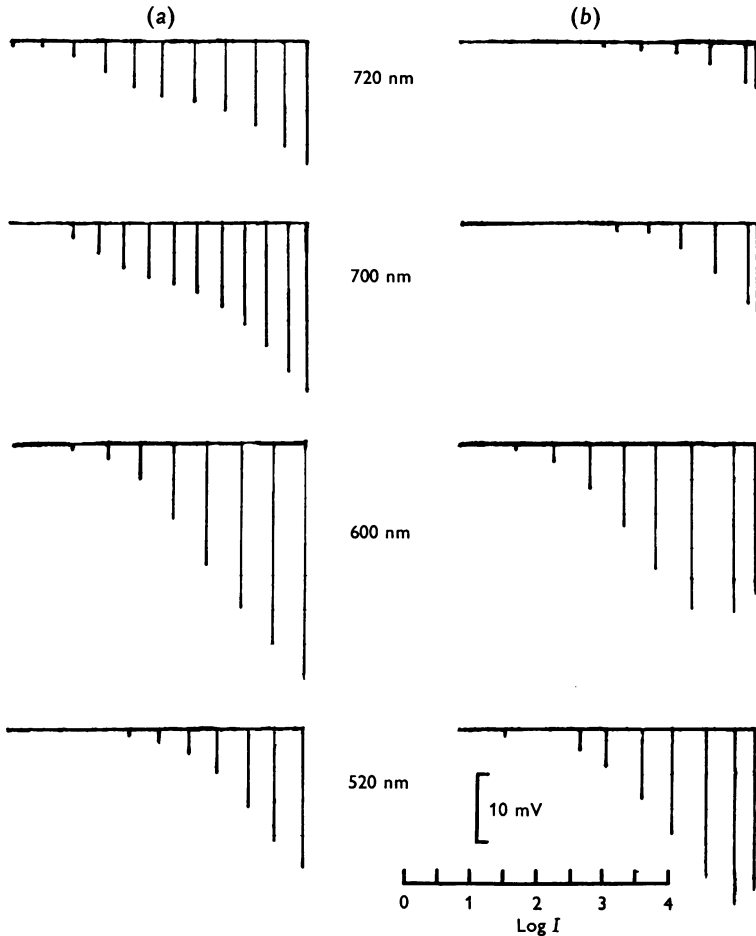

Fig. 1. S-potentials from a L-unit on stationary time base. Wave-length of light flash in each row as indicated; log intensity proportional to lateral displacement (see scale below). Flash applied (*a*) to dark retina (*b*) to retina illuminated by steady deep red light.

Figure 3 shows sets of results plotted as in Fig. 2. All these curves have the same shape, which is a tanh curve, and the curve drawn by a suitable template fits them all (or nearly so). Knowing for each wave-length the relative log quantum energy that corresponds to the position of the template, we may plot the log quantum spectral sensitivity curve (Fig. 4

open circles). It corresponds to the green cones and has a maximum at about 550 nm. This exceptional unit therefore appears to be controlled only by green cones whose action is to hyperpolarize. We have classed the unit as L since it is never depolarized, but clearly a R/G colour-unit with inactive red cones would behave similarly. R/G units do behave like this under strong deep red adaptation, but no such adaptation was applied to the unit of Fig. 3.
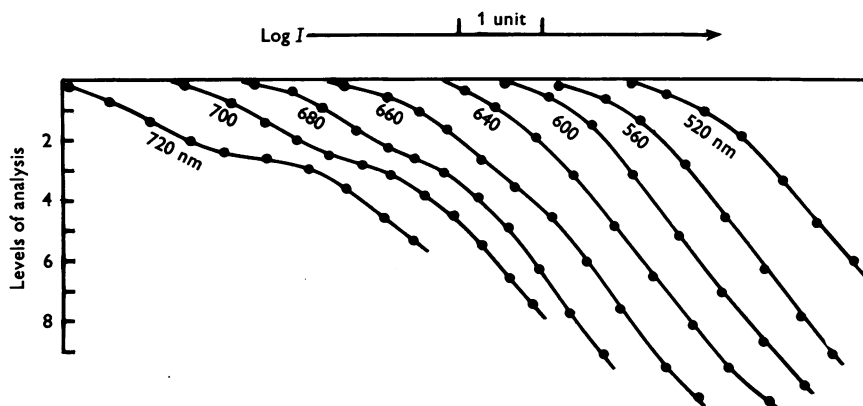

Fig. 2. The lower extremities of the potentials of Fig. 1(a) are represented by the filled circles on the sets 720, 700, 600 and 520 nm. Each set is displaced horizontally a known arbitrary amount for clearness, and other sets are included in Fig. 2 that are omitted from Fig. 1(a). Scale on the left gives nine arbitrary levels of hyperpolarization each of which may be chosen as criterion of action for an action spectrum. The log quantum energy corresponding to the intersection of level n with each curve gives action spectrum n (circles) in Fig. 5.
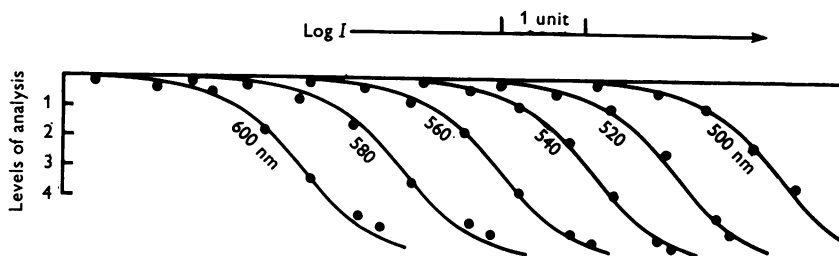

Fig. 3. L-potentials plotted as in Fig. 2. The curve drawn through the points is a fixed tanh-shaped template.

On the left of Fig. 3 is a scale of four levels. If at each level a horizontal line was drawn across the figure it would cut all the curves at distances measurable on the log I scale. Knowing the quantum energy of the spectrometer and the arbitrary displacement of the set of points for each wavelength, we may calculate the light energy at each point of intersection. The results are plotted in Fig. 4 as filled circles, curve 2, for instance,

being the intersections of horizontal level 2. The computed log energies for each wave-length are plotted downwards (scale on right) to give a log action spectrum (scale upwards on left). The criterion of action for curve 2 is hyperpolarization just down to level 2 in Fig. 3.
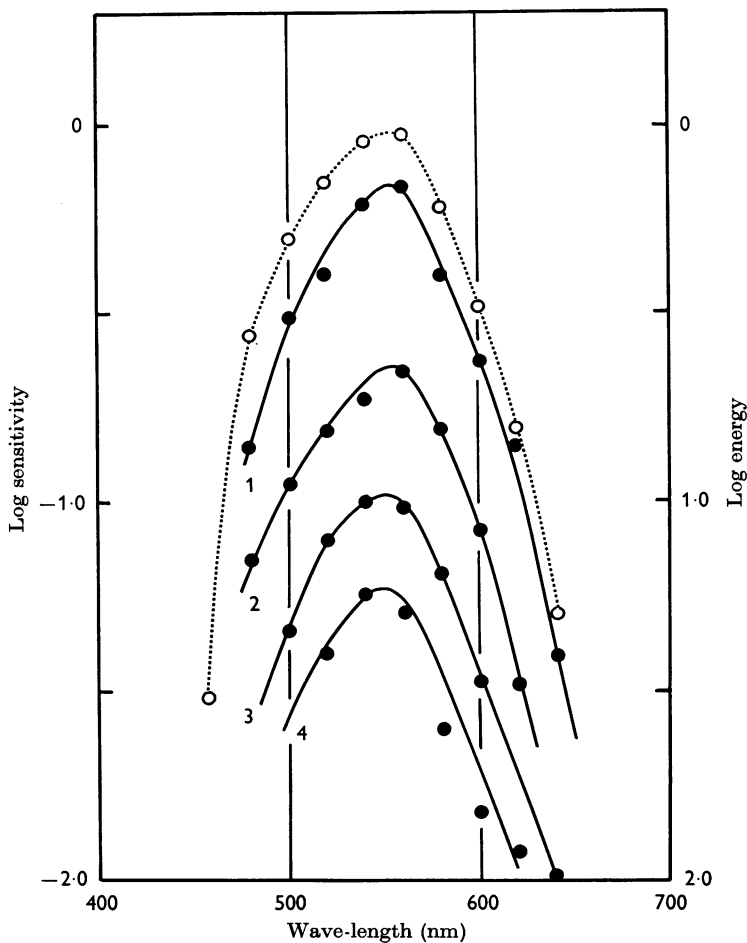

Fig. 4. Action spectra from Fig. 3. Open circles from shift of template, filled circles curve *n* when criterion of action is hyperpolarization to level *n* in Fig. 3.

This is the method that will be used to display the complex results of Fig. 2; in Fig. 3 the answer is obvious. If all the curves lie on one template, the shape of the action spectrum will remain unchanged whatever be the level chosen for the horizontal—as the filled circles of Fig. 4 show.

Since it needs more energy to hyperpolarize to a deeper level (Fig. 3), the action spectra for deep levels appear low in Fig. 4. Now we turn back to Fig. 2 and by drawing horizontals through the family of curves at various

levels we plot the action spectra for various fixed amounts of hyper-polarization. The whole experiment was repeated (on the same unit) with a steady background of deep red light, and again with a steady background of blue-green light (peak transmission about 500 nm). All these results are plotted in Fig. 5.
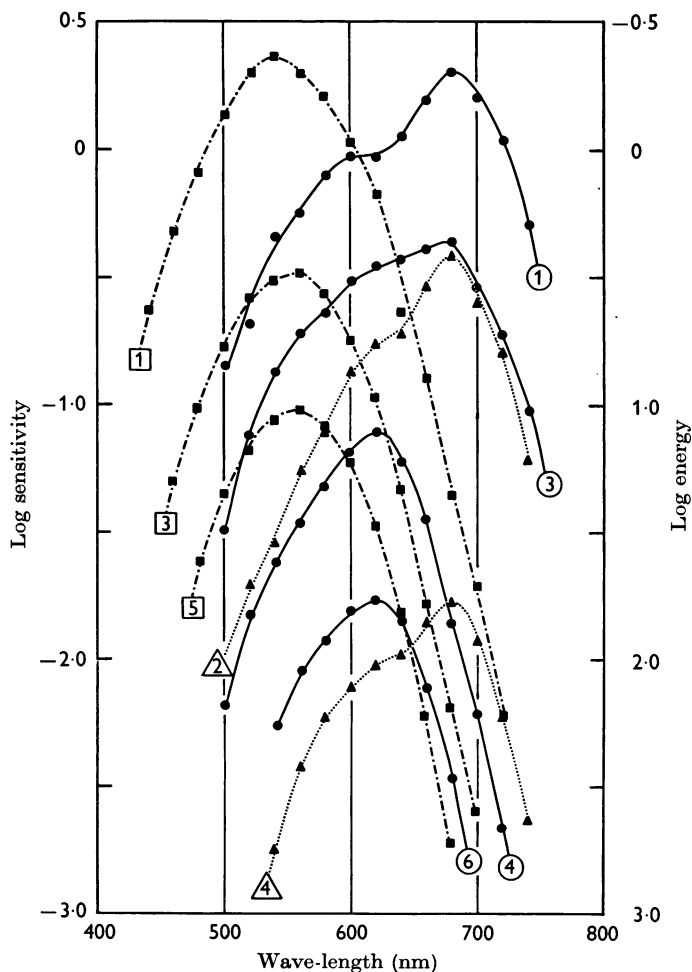

Fig. 5. Action spectra from the L-unit of Fig. 2. Numbers at end of each curve give the level of hyperpolarization (Fig. 2) used as criterion of action. Circles when flashes fell upon dark retina, triangles when flashes fell upon steady green adapting light, squares when adapting light was red.

The action spectra joined by the continuous lines through circles in Fig. 5 were obtained with zero background. The numbers refer to the level (Fig. 2) of hyperpolarization used as criterion of 'action', levels (1), (3), (4) and (6) being plotted. In (1) there is a clear peak at 680 nm that

corresponds to the deep-red cones which supply the R/G units. And there appears to be a second peak at 620 nm where Marks (1963, 1965) and Liebmann & Entine (1964) found the absorption maximum in some of the single cones they studied. As we go from level (1) through (3), (4) to (6), the 680 nm peak becomes inconspicuous and we are left with what looks like the action spectrum of 620 nm alone.

The dotted curves through triangles show at two levels of hyper-polarization (2) and (4) the effect of a fixed green steady background (500 nm) upon the action spectrum. It is now very similar to (1) of the former series, and hardly changes its shape with the level of hyperpolariza-tion chosen as criterion of action.
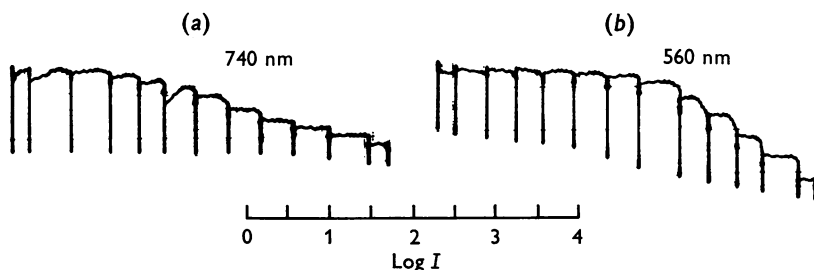

Fig. 6. Here the flash was always a deep red light of *fixed* intensity and the steady *background* was altered for each exposure as shown by the lateral displacement in conjunction with the scale of log *I*. In (*a*) the background was 740 nm, in (*b*) 560 nm. It is seen that moderate green adaptation, though not changing the resting potential, increases the response to a fixed red flash.

The interrupted curves through squares show at three levels of hyper-polarization (1), (3) and (5) the effect of a fixed deep red steady back-ground upon the action spectrum, and results are very surprising. Not only do the green cones (540 nm) now appear clearly and alone, but they are far more sensitive *absolutely* than they were when there was no back-ground at all (circles curve 1). What might have been expected is that the deep red background by depressing the sensitivity of the red cones (620 nm) and the deep-red cones (680 nm) would lower the level of the continuous curves so far that the curves of green cones (540 nm) would stand revealed at their own (undepressed) level which before was covered. But there seems no escape from the remarkable fact that the deep red steady back-ground makes green cones appear at least 10 times as sensitive as they were with zero background! A similar enhancement of green by a red background is to be seen in Fig. 1.

Not only can a red background enhance the L-response from green cones, but a green background can enhance the response from deep red cones as Fig. 6 shows. In both (*a*) and (*b*) the flash was a deep red light of

fixed intensity. The background in (a) was a light of 740 nm, in (b) of 560 nm, and in both figures the displacement to the right was proportional to log I of *the steady background* that was increased by step between each flash. In both cases the increase in steady light produced an increase in the steady hyperpolarization of the unit. But whereas in (a) this was associated with a response to the flash that progressively diminished, in (b) a less simple pattern of response was found. Moderate green backgrounds hardly increased the steady hyperpolarization of the unit, but they increased conspicuously the size of the response to the deep red flash.

Analytical discussion

The treatment of this paper has been rather qualitative and the evidence of the curves in Fig. 5 is convincing more to intuition than to intellect. Nevertheless, the results will yield a little to analysis. In Fig. 2 from which the continuous curves through circles were obtained, the curve at 720 nm has the shape of a tanh curve which saturates at amplitude level 3 and at higher values of log I somehow hyperpolarizes further. The second stage in hyperpolarization appears to be the entry of a second mechanism that is far more prominent at 600 nm. From Fig. 5 a reasonable interpretation is that at the peak of curve 1 (circles) the L-unit is hyperpolarized by the deep-red cones (680 nm), but at lower sensitivity levels the red cones (620 nm) add to the hyperpolarization produced.

In Fig. 7 some of the curves of Fig. 2 have been replotted to examine that interpretation. The filled circles are simply replots of the points of Fig. 2 for each wave-length. The continuous curve drawn through the circles of the 740 nm curve (that was not included in Fig. 2) is the tanh curve that describes the S-potential as a function of light energy for a single mechanism,

$$V = U.I/(I+I_{\frac{1}{2}}),\qquad(1)$$

where V is the recorded potential height, U its maximum value, I the energy of the light stimulus and $I_{\frac{1}{2}}$ the value of I that makes $V = \frac{1}{2}U$.

The same curve has been drawn through the left portions of all the curves in Fig. 7 up to that of wave-length 640 nm. This fixed template of standard tanh shape fits well the low luminance portions of all these curves and is what would be expected if in this region only one pigment was effective. By measuring at each wave-length the relative quantum energy necessary to displace the tanh template to a fixed position, we obtain the action spectrum of this pigment. It is plotted in Fig. 8 as curve A, and clearly corresponds to the deep red cones (680 nm).

If now, returning to Fig. 2, we examine the curves from 520 nm to 640 nm they all fit reasonably a single template of much greater amplitude than that for 720 nm. Its action spectrum is plotted as open circles in

Fig. 8 and corresponds to the 620 nm red pigment. The open circles in
Fig. 7 show what remains when the 680 nm template is subtracted from
the recorded filled circles. The dotted curve that has been drawn through
them is the '620 nm template' seen alone through the filled circles of
640 nm. We thus have a simple result. At wave-lengths below 650 nm the
single '620 nm template' fits the results; at wave-lengths above 650 nm the
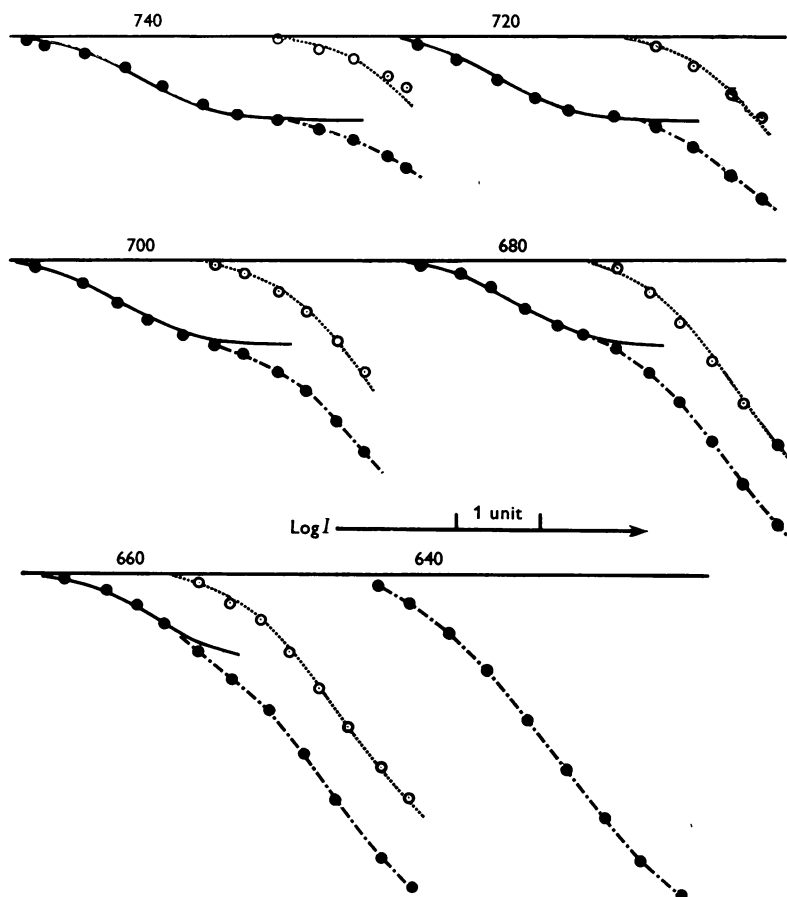effects of 620 and 680 nm add.

Fig. 7. Filled circles, replot from Fig. 2. Continuous line is the tanh template for
680 nm pigment. Open circles show the result of subtracting the 680 nm template
from filled circles. Dotted curve through open circles is the curve of 640 nm.

But though simple to say this is not so simple to understand. For below
650 nm there seems no good reason why the contribution from the deep
red pigment should be left out; and above 650 nm, though the '620 nm
template' fits the open circles of Fig. 7 well enough, the action spectrum

that results (open circles Fig. 8) falls precipitously along the dotted line instead of following the more plausible curve of triangle that is a replot of the continuous curve 4, Fig. 5. It is as though curves *A* and *B*, Fig. 8, ran true only when well apart, and became depressed as they approached and could not meet each other.
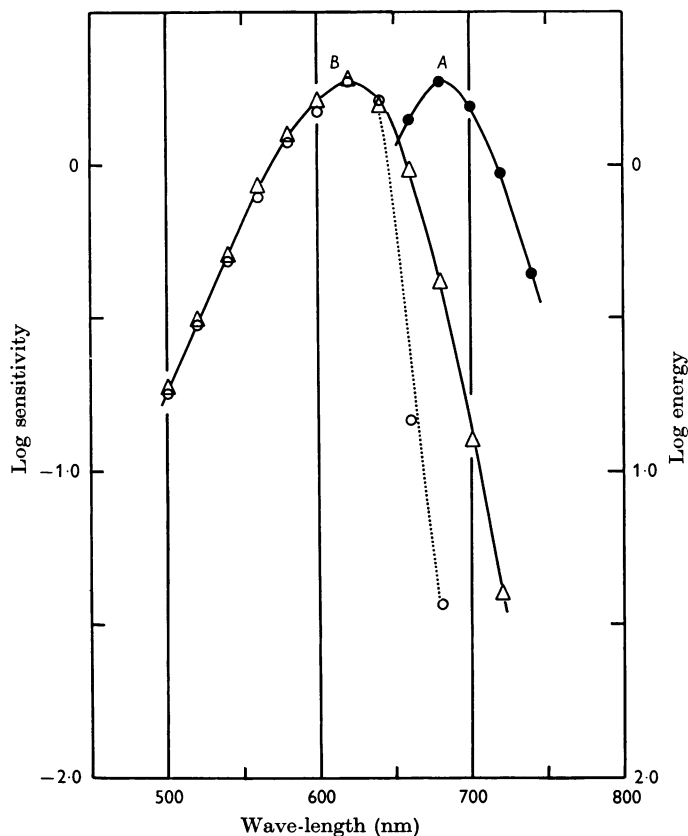

Fig. 8. Filled circles, action spectrum from continuous line template of Fig. 7. Open circles (dotted curve) action spectrum from dotted template of Fig. 7; open circles at shorter wave-lengths, action spectrum of same template from Fig. 2. Triangles, replot from Fig. 5, circles, curve 4.

The beginnings of an explanation can be given along the lines of the model proposed in the previous paper (NR*b*) to describe quantitatively the relation between S-potential and quantum catch in the red and green pigments that influence the *R/G* unit.

Figure 9 shows the modified model more appropriate to the L-unit. The difference is that both the 680 nm battery and the 620 nm battery hyper-polarize, and there is no isolating resistance *r* to stop the 680 nm system *A* from shunting the 620 nm system *B*.

The exact quantitative results of this system are tedious to work out, but one can see at once the essential features. In the far red, the only active system will be A; B will have zero conductance and the 680 nm tanh template should fit the results of Fig. 7 and give the 680 nm action potential as found. In the same way below 600 nm the conductance of system A will be nearly zero and so the results will fit the '620 nm template' giving the 620 nm action potential as found. Since the maximum amplitude of depolarization by 620 nm is much greater than by 680 nm, the resistance R_B must be much smaller than R_A. Thus so long as the switches of B are more strongly stimulated by light than those of A, the current from A will be mainly shunted by B and the recorded potential V will not be very different whether the A switches are on or off. This is why we can neglect the contribution of the 680 nm system at wave-lengths below 650 nm.
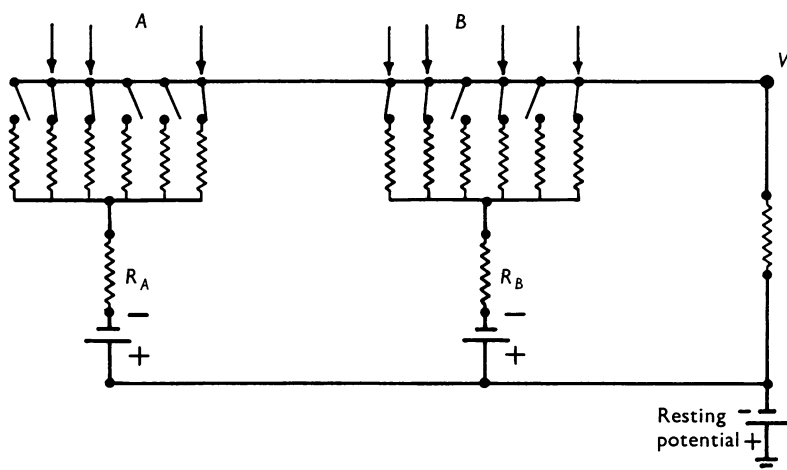

Fig. 9. Membrane model for consideration in analysis.

At wave-lengths just above 650 nm on the other hand the conductance of systems A and B, Fig. 9, become comparable and each shunts the other. Thus the two contributions to V are less than would be expected if we assume simple additivity with no shunting. The dotted template of Fig. 7 is drawn on the assumption of simple additivity and the low V values that should have been attributed to shunting were interpreted as a low quantum catch. That is why the dotted action spectrum in Fig. 8 fell so sharply as soon as curve B approached the region where A is excited sufficiently to act as a good shunt.

Though this explanation may well be starting on the right lines, unfortunately it does not carry us far. The '620 nm template' does not have the expected tanh shape proper to a single pigment—a curve that should run

nearly linear for about 1·5 log I units. The '620 nm template' is linear for about 2·5 units, and thus appears to be compounded of more than one pigment. Since we know from Fig. 5 that the green 540 nm pigment can contribute, it should clearly find a place in the model. As seen in Fig. 7, record 660, where the end of the '680 nm template' overlaps with the start of the '620 nm template', the result gives the appearance of an extension of the linear range. Thus the '620 nm template' itself may be due to the summed contribution of some or all of the 680, 620 and 540 nm pigments. Since we know the action spectra of the three pigments we know the relative lateral shifts of their tanh curves for each wave-length. But we do not know the amplitudes of the 620 and 540 nm curves, nor the extent to which simple additivity is modified by shunting, so it is not worth while computing a plausible arrangement. More important, any such scheme will fail altogether to account for some features that arise with a steady background.

For a single pigment system, if a flash of intensity I_1 is added to a fixed steady background light I_0, the S-potential V_1 (measured from the steady value V_0 due to the background I_0) is found to exhibit the usual tanh relation to log I_1. This is what must follow if the flash I_1 simply adds to I_0 and gives a total potential $(V_1 + V_0)$ as though $(I_1 + I_0)$ was a single flash.

For with zero background the general relation is

$$V/U = I/(I + I_{\frac{1}{2}}).\tag{1}$$

With background I_0

$$(V_1 + V_0)/U = (I_1 + I_0)/(I_1 + I_0 + I_{\frac{1}{2}}).$$

With I_1 zero

$$V_0/U = I_0/(I_0 + I_{\frac{1}{2}}).$$

Subtracting

$$V_1/U = \frac{I_1}{I_1 + I_0 + I_{\frac{1}{2}}} \cdot \frac{I_{\frac{1}{2}}}{I_0 + I_{\frac{1}{2}}}.\tag{2}$$

Relation (2) is the same as (1) with change of scales; $I_{\frac{1}{2}}$ is increased by I_0 and U by the factor $I_{\frac{1}{2}}/(I_0 + I_{\frac{1}{2}})$.

Nothing of this kind, however, can explain the way that the action of green cones is *enhanced* in sensitivity by a steady red background (Fig. 5 squares) and red cones by steady green backgrounds Fig. 6(b). Such enhancement was found at the ganglion cell level in the goldfish (a species closely related to the tench we studied) by Wagner, MacNichol & Wolbasht (1960, Fig. 9) who describe it in terms of the balance of excitation and inhibition—an organization which is possibly controlled by S-potentials.

This red/green opposition in L-units recalls the opposition in R/G-units and suggests the possibility that L-units receive directly or indirectly some

output from the R/G unit. It must be remarked, however, that L-units are situated nearer to the cones than are the colour units, thus R/G would have to feed back at least to the level of L to produce this effect. It is conceivable, however, that all S-potentials are concerned with feed-back, and exert their influence upon the cones themselves. For example, the colour units might feed back to the twin cones that Marks (1965) finds are usually red/green and occasionally green/blue (just as we find the C-units are usually R/G and occasionally G/B). There might result a differential change in the scaling of the light-to-signal transduction (e.g. greater sensitivity for red, less for green, in green adaptation) which would affect signals from these cones in whatever paths they followed.

We cannot speak of this, however, for we do not understand the part played by S-potentials in retinal organization.

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