

## THE PHOTOPOSITIVE\* RESPONSE OF THE FROG (*RANA PIPIENS*) UNDER PHOTOPIC AND SCOTOPIC CONDITIONS

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Under suitable experimental conditions (Boycott, Mrosovsky & Muntz, 1964) frogs jump towards the light (Pearse, 1910; Muntz, 1962*b*). This response, which is released most readily by blue light, appears to be a function of the retinal projection to the diencephalon (Muntz, 1962*a, b*). The responsiveness to blue is very marked, and is largely independent of the intensity and saturation of the stimulating lights. It was also possible to show that inhibition was involved, since adding green light to blue reduced its effectiveness (Muntz, 1962*b*). As a result of further experiments (Muntz, 1963*a, b*), it was suggested that the response was due to a synergistic action of the green rods and the cones, opposed by an inhibitory action from the red rods. This hypothesis is described in more detail in the Discussion.

If the inhibitory effect is due to the red rods, dark-adaptation should have a very marked effect on the spectral response curve, since it will accentuate the contribution of the inhibitory receptor to the behaviour. This should result in a decreased responsiveness of the animals to light, and also in an accentuation of the effectiveness of blue light relative to other colours. Boycott *et al.* (1964) reported differences in the behaviour of dark-adapted and light-adapted frogs to light. The data in that experiment were, however, insufficient to test the predictions made above.

### METHODS

*Subjects* were thirty *Rana pipiens* adults, with a body length between 2 and 3 in., obtained from a dealer. One subject died during the experiment, so reported results are based on 29 subjects. They were kept in individual aquaria, 25 × 25 cm., and 20 cm. deep, containing water and a brick on which they could sit. During the experiment they were fed occasionally on mealworms.

*Apparatus.* The apparatus is shown in Fig. 1. It was constructed of Plexiglass, either painted matt black or transparent as shown, 16 cm. deep, and containing approximately 2 cm. of water. A trial was started by placing an animal at *S*, and the animal responded by moving towards the transparent panel *P*, made of transparent Plexi-

\* It is not clear whether the response of the frog to light should be called positive phototropism, or positive phototaxis (see Boycott *et al.* 1964). The term photopositive will be used here in order to avoid this problem.

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glass covered with tracing paper. One half of this panel was illuminated, the other half remained dark.

If the subject did not respond within 10 sec., it was shocked by two electrodes which lined the two lateral walls of the apparatus. The shock, which was turned on automatically by a 10 sec. timer, consisted of pulses of 20 V. d.c. at a rate of 18 per min. The transparent panel *P* prevented animals from escaping too rapidly, since preliminary experiments suggested that they chose much less reliably under these conditions, and the narrow starting chamber ensured that the animals reached the choice area without any large bias towards one side or the other.

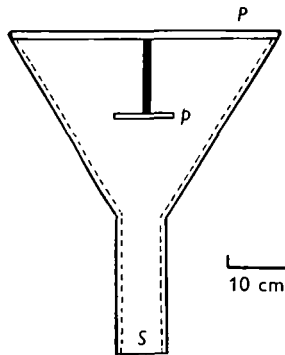


Fig. 1. Plan of apparatus. *S*, starting point; *P* and *p* transparent panels. The position of the electrodes is shown by the dashed lines.

*Stimuli.* The colour of the light was controlled by six interference filters, and the intensity by neutral density filters. The light source was a 500 W. projector bulb. The relative energy of the different colours, when at full intensity, was calculated from the maker's spectrophotometric transmission curves, assuming that the source had a colour temperature of 3098° K. The absolute energy of light reaching the apparatus, when no filters were in the beam, was determined using a S.E.I. photometer, and the maximum energy available through each filter was then calculated in W./cm.<sup>2</sup> from the maker's spectrophotometric curves. For this calculation it was assumed that at 550 m $\mu$  one lumen is produced by 0.0016 W.

These procedures are subject to various inaccuracies. In particular, they rely on the estimate of the colour temperature of the projector bulb. This is, however, probably not critical, since an error of as much as 375° K in the estimated colour temperature would cause an error of only 0.14 log units in the relative intensities at 450 and 650 m $\mu$  which is small in relation to the 0.8 log unit steps in intensity used in the experiment. Furthermore, errors in calibration will not affect differences in responding to the same colours under the two conditions of adaptation.

*Experimental procedure.* The animals were tested after either being dark-adapted for 1 hr. (scotopic conditions), or after light-adaptation (photopic conditions). Animals were dark-adapted by being placed, still in their home aquaria, in a thick cardboard box covered with black paper in a dark room. Light-adaptation was accomplished by leaving the animals in their usual living conditions, where they were illuminated by a double row of 40 W. fluorescent lamps, 5 ft. above the aquaria.

Half the animals were tested for 2 days, as described below, under scotopic conditions, and then, 7 days later, for 2 days under photopic conditions. The other half of the animals were tested under photopic conditions first, and scotopic conditions second.

Table 1. *Characteristics of the filters used in the present experiment*

Dominant wavelength (m $\mu$ )	462	494	528	585	615	670
Half-maximum bandwidth (m $\mu$ )	8	11	8	9	9	10
Maximum energy obtainable ( $\mu\text{w./cm.}^2$ )	10.7	20.4	23.8	35.4	40.6	67.2
Correction factor necessary for equal quantum spectrum (log units)	-0.20	-0.58	-0.65	-0.82	-0.88	-1.10
Correction factors used in experiment	-0.2	-0.6	-0.6	-0.8	-0.8	-1.0

Irrespective of the conditions of adaptation, the animals were tested by exactly the same procedure. The six colours used were equated to give an equal energy quantum spectrum to the nearest 0.2 log units. The actual corrections used are shown in the bottom row of Table (1). On the first day of testing each subject received eighteen tests: one test on each of the six colours at the greatest intensity available, followed by one test on each colour with the intensity reduced by 0.8 log units, followed by a third test on each colour reduced in intensity by a further 0.8 log units. This procedure was followed by further tests on the second day, reducing the intensity of the light by two more steps of 0.8 log units. The animals received eleven tests in all on the second day as the 670 m $\mu$  filter was not used at the lowest intensity. Each animal thus received a total of fifty-eight tests, twenty-nine under photopic conditions, and twenty-nine under scotopic conditions.

Each combination of colour and intensity was seen once only by each animal under a given adaptation procedure. For half the animals this combination appeared on the left-hand side of the apparatus, and for the other half of the animals on the right-hand side of the apparatus. The animals were tested in groups of ten, five scotopic and five photopic, in rotation. To complete a test on a given colour and intensity for all ten animals took approximately  $\frac{1}{2}$  hr., so that the state of adaptation of the animals cannot have been seriously affected by the tests.

#### RESULTS

Every frog received one test on each of the fifty-eight conditions of intensity, colour, and adaptation procedure used. A Cochran Q-test was applied to the results, which yielded a value of Q of 152.9, with 57 degrees of freedom. This is significant at better than the 0.1 % level, so it may be concluded that the probability of choosing the light window was not the same under all the different conditions.

The animals were tested in a forced-choice situation, so it would be expected that the illuminated window would be chosen by 50 % of the frogs, whether or not the

illumination was above threshold. The data were corrected for this effect according to the formula

$$P_{\text{corr.}} = \frac{P_{\text{obs.}} - 0.5}{1 - 0.5},$$

where  $P_{\text{corr.}}$  is the corrected probability of detection, and  $P_{\text{obs.}}$  is the observed probability of choosing the illuminated window (see Blackwell, 1953). With this correction a value of one shows perfect detection, a value of zero a chance score. The corrected data, for the different combinations of colour and intensity used, are shown in Fig. 2.

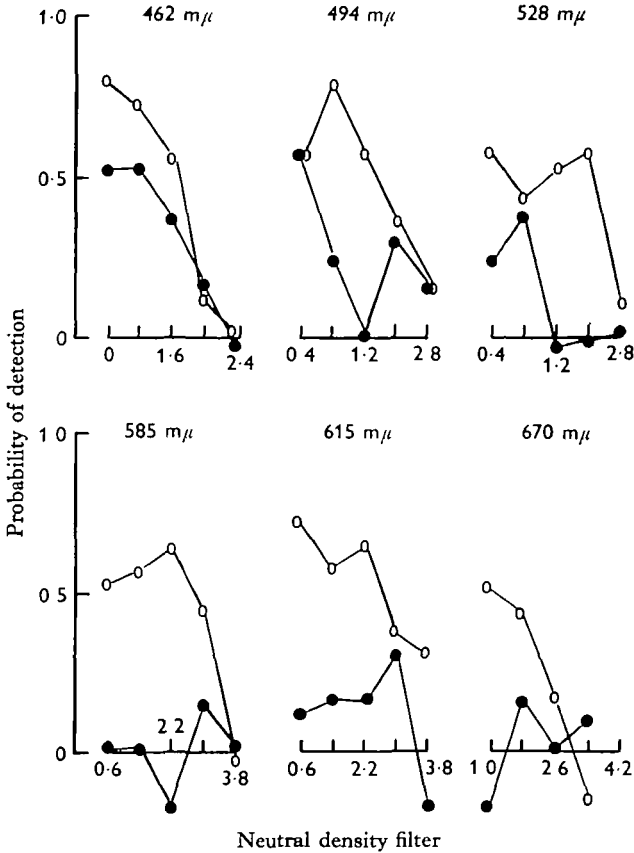


Fig. 2. Probability of detection versus intensity for the different colour used. The open circles show the results when the animals were light-adapted, the filled circles results when they were dark-adapted.

It can be seen from the figure that, under photopic conditions, the photopositive behaviour occurred for all colours tested. This was not true for the scotopic conditions, however, where the photopositive behaviour was only shown to the two colours of shortest wavelength; with longer wavelengths scores were only slightly above chance, even when high illuminations were used. The figure also shows that the photopositive behaviour was much less marked when the animals were dark-adapted. Only five of the twenty-nine animals made more choices of the lighted window when dark-adapted than when light-adapted, and a sign test applied to the results was significant at better

than the 0.1% level of confidence ( $r = 5$ ,  $N = 27$ ,  $P = 0.0015$ ). One animal chose the darkened window more frequently than the illuminated window under both conditions of adaptation; during the first day of both photopic and scotopic testing it made only two out of eighteen of its responses to the illuminated window. The reason for this difference is not known.

Dark-adaptation thus reduced the strength of the photopositive response. This was true for all the colours tested, but from Fig. 2 it is apparent that the effect was strongest for the greens and yellows (528–615  $m\mu$ ).

The dark-adapted animals were much more strongly photopositive to blue than to the other colours, the preference for the lighted window being only just above chance for the longer wavelengths. Under photopic conditions, however, the preference for blue was less marked, and in fact was only apparent with the higher intensities. This is shown in Fig. 3 where the responses to the two highest intensities used have been

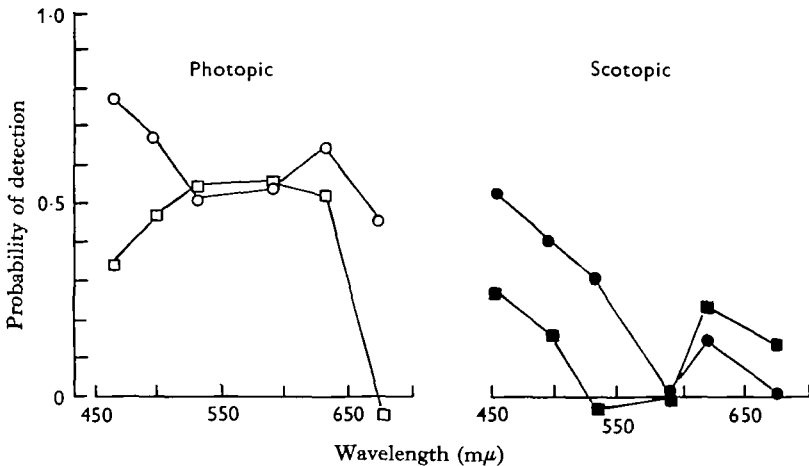


Fig. 3. Effect of intensity on spectral response curves. The left-hand figure shows the results when the animals were light-adapted, the right-hand figure results when they were dark-adapted. Circles, number of responses to the two highest intensities used; squares, number of responses with the next highest intensities used.

pooled, as have also the responses to the next two highest intensities. The figure shows that under photopic conditions the animals were more responsive to blue and yellow than to green when the intensity of the stimulating lights was high, and the curve is similar to the spectral response curves published previously for *R. temporaria* in Muntz (1962*b*). With the two lower intensities, however, the curve has a different form; the ends of the spectrum are no longer more effective than the greens, and a broad smooth curve results. A sign test shows that increasing the intensity of the lights caused a significant increase in the number of frogs responding positively to the two blue lights ( $r = 4$ ,  $N = 20$ ,  $P = 0.006$ ) and to the two red lights ( $r = 3$ ,  $N = 20$ ,  $P = 0.001$ ), but there was no significant effect of intensity on the number of responses to the two greens ( $r = 9$ ,  $N = 19$ ,  $P = 0.5$ ). When tested under scotopic conditions this interaction between intensity and colour was not apparent, the animals being most responsive to blue irrespective of intensity.

Fig. 3 shows spectral response curves, in which the frequency with which the illuminated window was chosen is plotted against wavelength for an equal quantum spectrum. Conventionally, animals' responses to colour are presented as spectral sensitivity curves, in which the energy necessary for some criterion response is plotted against wavelength. The present data cannot be presented in that way, since for many colours the frequency with which the illuminated window was chosen never differed appreciably from that expected on chance. Furthermore, when more than one retinal receptor is involved, and there is an interaction between wavelength and intensity, the form of the spectral sensitivity curve depends on the criterion chosen, and there is no longer any obvious advantage of spectral sensitivity curves over spectral response curves.

#### DISCUSSION

Under photopic conditions, with low illuminations, the spectral response curve is maximal in the green, and falls off in the blue and red. With high intensities the blue sensitivity becomes apparent, and the relative effectiveness of the yellows and red also increase, so that a bimodal curve with a minimum in the green, such as has been described on previous occasions, results. This interaction between the spectral response curve and intensity has not been reported previously, because previous experiments were all carried out with bright lights of equal energy, which were compared by the frogs in pairs.

The two photopic curves of Fig. 3 are very similar to two curves reported by Silver (1963) for *Xenopus laevis*, using changes in the colour of the skin as the response. Thus Silver found that when the animals were on a white background, and had to respond by becoming lighter, a smooth curve with a maximum in the green was obtained. When, however, the animals were on a black background, and were required to respond by becoming darker, a bimodal curve with maxima in the blue and yellow, and a minimum in the green, was obtained. Silver's experimental techniques differed markedly from those used in the present experiment. Thus she used changes in skin colour as the response, as against choices of an illuminated window. However, Denton & Pirenne (1954) found no difference between spectral sensitivity obtained using changes in the colour of the skin as the response, and that obtained using phototactic behaviour. Unpublished results on *Xenopus* obtained by the writer, using the technique described in Muntz (1963*b*) for the tadpole, also revealed typical bimodal curves in the phototactic situation. Silver's results also differ in that they are spectral sensitivity curves, showing the intensity of light necessary for a constant response, whereas the present results are spectral response curves, showing the number of responses for a given intensity of light. Spectral response curves will differ from spectral sensitivity curves in their shape, depending on the relationship that holds between intensity and responsiveness. They will not, however, differ in the position of the maxima and minima, and so may be compared on this basis.

The photopic results obtained in the present experiment thus suggest that Silver's two curves may be two examples of a family of curves obtainable under different conditions, and not represent two basically different mechanisms. Silver's results show that the darkening reaction, which reveals the characteristic bimodal curve with the green minimum, required higher intensities of light to reach threshold in the greens.

Similarly, under photopic conditions, the bimodal curve of *Rana pipiens* requires higher intensities of light for its development.

The failure of the frogs to respond preferentially to blue under photopic conditions with low illuminations probably has little effect on their behaviour in their normal environment. This condition will only occur when the animals are light-adapted, during the daytime, and there will be many brightly illuminated areas under such conditions for the animals to respond to. This is particularly true since the behaviour is photopositive, so that the animals will be responding to the brightest lights available. Low illuminations will occur at night, but then the animals will be dark-adapted and the sensitivity to blue will be maintained.

The present experiment was designed to test the hypothesis put forward in Muntz (1963 *b*). In this hypothesis it was assumed that the spectral characteristics of the frog's photopositive behaviour are due to a positive effect from the green rods and cones acting synergistically; the greater this positive effect, the more likely the frogs are to show photopositive behaviour. This positive effect was assumed to be divided by a negative effect from the red rods. The result of this division was held to account for the form of the spectral response curve. In general terms the hypothesis was confirmed: the responsiveness to blue was accentuated by dark-adaptation, and the animals also became less strongly photopositive. Both these effects are predicted, since dark-adaptation accentuates the contribution of the inhibitory receptor.

It is less easy, however, to put the hypothesis into a quantitative form, since there are a large number of unknown parameters involved. Thus we do not know the relative contributions of the three receptors to the behaviour, nor by what rules their effects should be combined. Nor do we know what relationship to expect between the responsiveness of the animals in the apparatus used, and the output of the retinal mechanism. We do, however, have good data on the spectral characteristics of the different retinal elements (Donner & Reuter, 1962; Granit, 1942). We also know that under scotopic conditions the red rods will be more sensitive than the cones, while under photopic conditions the reverse will apply, and it is very probable that the green rods will be intermediate between the rods and the cones under both sets of conditions (Rushton, 1959; Donner & Reuter, 1962). It should therefore be possible to make plausible assumptions for the factors which are unknown, and derive spectral response curves for photopic conditions. Following this, by changing the sensitivities of the three receptors appropriately, but leaving all the other assumptions unchanged, it should be possible to obtain scotopic curves agreeing with the experiment data.

This has been done in Fig. 4, making the following five assumptions:

- (i) the contribution of a receptor to the animal's behaviour is proportional to the logarithm of the intensity of the stimulating light,  $I$ .
- (ii) the contributions of the two positive receptors (the green rods and the cones) are multiplied together, and then divided by the contribution of the inhibitory receptors (the red rods).
- (iii) the three receptors produce a 'dark light' (Barlow, 1956) when there is no illumination. For simplicity it is assumed that this dark light has a value of one in each case.
- (iv) the relative sensitivities of the three receptors under photopic and scotopic conditions are as shown in Fig. 5.

(v) the number of green rods and red rods contributing to the behaviour are equal, and the number of cones contributing is twice as large.

On the basis of these assumptions the contribution  $C$  of a receptor to the animal's behaviour is given by

$$C = m (\log I - \theta) + I,$$

where  $\theta$  is the threshold of the receptor under the conditions being considered, and is obtained from Fig. 5. It is also assumed that where  $\theta$  is greater than  $\log I$ ,  $C$  is equal to one: that is, the bracket cannot have a negative value, but equals zero when the receptor is below threshold. The number  $I$  is the dark light (assumption (iii)) and  $m$  is put equal to one for the green and red rods, and to two for the cones (assumption (v)). Having calculated the contributions of the three receptors, the animal's responsiveness may be calculated from the rule given under assumption (ii).

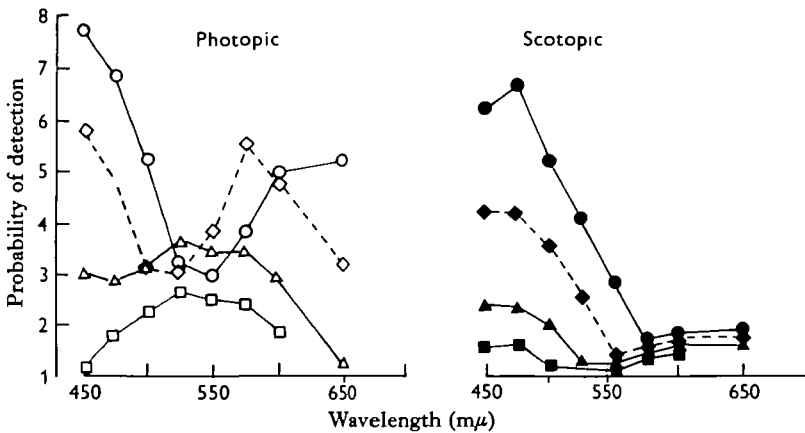


Fig. 4. Theoretical spectral response curves. The left-hand figure shows photopic curves, the right-hand figure shows scotopic curves. The squares, diamonds, triangles, and circles show the effect of increasing the intensity in 0.4 log. unit steps.

The results of these calculations, for selected wavelengths and intensities, are shown in Fig. 4. A responsiveness value of one defines the absolute threshold, since this value is obtained with the dark light alone. Although there are some differences, Fig. 4 agrees very well with the experimental results. Thus in both cases there is an interaction between the spectral response curves and intensity under photopic conditions, with the bimodal spectral response curve only appearing at higher intensities. Fig. 3 shows spectral response curves differing in intensity by 1.6 log. units, and the theoretical curves show that an intensity change of this order could account for the differences. In both the experimental and theoretical curves, the effect of intensity has little effect on the responsiveness to the green, and in fact makes it slightly smaller in each case. Both the theoretical and experimental results show much less interaction between intensity and the spectral response curve under scotopic than photopic conditions, and in both cases under scotopic conditions the blue responsiveness is more marked, the minimum is displaced further towards the long wavelengths, and the secondary rise in the yellow is smaller. The theoretical results also agree with the experimental findings in showing that the animals will be less responsive under scotopic conditions, although the retinal receptors are more sensitive.



The experimental results can thus be explained in full with the five assumptions listed on p. 107. These assumptions appear reasonable, and most of them are probably not critical, and could be altered substantially without greatly affecting the form of the results. Assumption (iii) is necessary because of assumption (ii), otherwise under those conditions where the positive effect alone is released, the stimulating light being below the inhibitory receptor's threshold, the responsiveness of the animal would reach

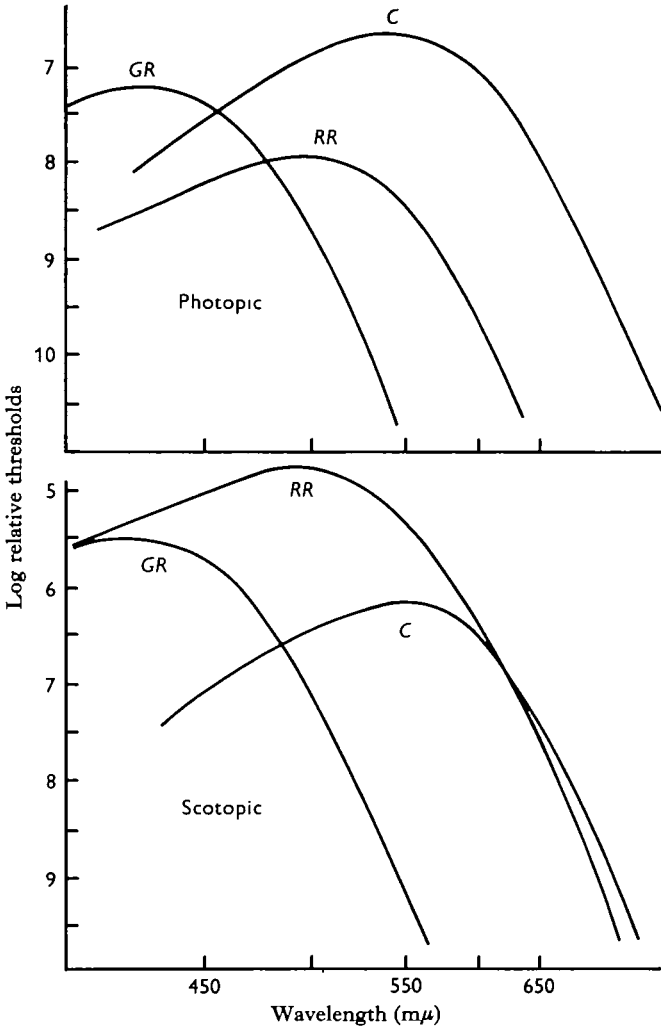


Fig. 5. Assumed relative sensitivities of the three receptors under photopic and scotopic conditions. GR, green rods; RR, red rods; C, cones.

infinity. A 'dark light' has been proposed on several occasions (Barlow, 1956; Rushton, 1961). In the present calculations its value has been arbitrarily set at one for all three receptors; although it would probably be more realistic to assume different dark lights for the different receptors, in the absence of any detailed information on this, the simplest assumption is probably the best.

In assumption (iv) the relative sensitivities chosen are arbitrary, within the restrictions given on p. 107. The increase in rod sensitivity of 2.8 log. units, and in cone sensitivity of 2 log. units, on dark-adaptation, seem reasonable, and the crossing point for the rod and cone curves under scotopic conditions (610 m $\mu$ ) is the same as that selected by Willmer (1964). The shape of the green and red rod curves was derived from Dartnall's nomogram, and the shape of the cone curve from Granit's photopic dominator.

In the model presented here the contributions of the three receptors are assumed to combine multiplicatively. This assumption was made to explain the finding that adding the green light to blue reduces its effectiveness (Muntz, 1962*b*), which cannot be explained if a simple additive relationship is proposed. Physiological data suggest, however, that addition and subtraction may be more common than multiplication and division within the nervous system, as for example occurs in the interaction of excitation and inhibition in the *Limulus* eye (Hartline & Ratliff, 1958). A multiplicative relationship can, however, be expressed as an additive relationship if a logarithmic transformation is used, and the same results can then be obtained (Hailman, 1964).

Various complicating effects may well be involved in the behaviour. For example, no consideration has been given to latency effects; if the red rods have a greater latency of response than the green rods and cones (which is very probable), stimulation of the eye could release a positive effect which is subsequently terminated by a slower but greater negative effect. This would give results in some respects similar to division. However, the form of the model presented here appears to be simpler, and can account for the facts. Another complication is that the frog has other photoreceptors apart from the eye, in particular the pineal eye (Dodt & Heerd, 1962), and the skin (Pearse, 1910). It is possible that these receptors are also affecting the behaviour.

#### SUMMARY

1. It has been suggested that the spectral characteristics of the frog's photopositive response are determined by a synergistic action of the green rods and the cones, opposed by an inhibitory effect from the red rods. If this is the case, dark-adaptation should markedly affect the form of the spectral response curves, and also make the animals less strongly photopositive, since it will accentuate the contribution of the inhibitory receptor.

2. These predictions were tested with *Rana pipiens*, and both were confirmed. By making appropriate assumptions it was possible to generate theoretical curves from the hypothesis, which agree in detail with the experimental results.

#### REFERENCES

- BARLOW, H. B. (1956). Retinal noise and absolute threshold. *J. opt. Soc. Amer.* **46**, 634-9.  
 BLACKWELL, H. R. (1953). Psychophysical threshold: experimental studies of methods of measurement. *Engineering Research Bulletin* 36, Ann Arbor: Univ. of Michigan.  
 BOYCOTT, B. B., MROSOVSKY, N. & MUNTZ, W. R. A. (1964). Black and white preferences in the frog, *Rana temporaria*, and other Anura. *J. Exp. Biol.* **41**, 865-77.  
 DENTON, E. J. & PIRENNE, M. H. (1954). The visual sensitivity of the toad, *Xenopus laevis*. *J. Physiol.* **125**, 181-207.  
 DODT, E. & HEERD, E. (1962). Mode of action of pineal fibres in frogs. *J. Neurophysiol.* **25**, 405-29.  
 DONNER, K. O. & REUTER, T. (1962). The spectral sensitivity and photopigment of the green rods in the frog's retina. *Vision Res.* **2**, 357-72.

- GRANIT, R. (1942). Colour receptors of the frog's retina. *Acta physiol. Scand.* **3**, 137-51.
- HAILMAN, J. P. (1964). Coding of the colour preference of the gull chick. *Nature, Lond.*, **204**, 710.
- HARTLINE, H. K. & RATLIFF, F. (1958). Spatial summation of inhibitory influences in the eye of *Limulus*, and the mutual interaction of receptor units. *J. Gen. Physiol.* **14**, 1049-66.
- MUNTZ, W. R. A. (1962*a*). Microelectrode recordings from the diencephalon of the frog (*Rana pipiens*) and a blue-sensitive system. *Neurophysiol.* **25**, 699-711.
- MUNTZ, W. R. A. (1962*b*). Effectiveness of different colours of light in releasing the positive phototactic behaviour of frogs, and a possible function of the retinal projection to the diencephalon. *J. Neurophysiol.* **25**, 712-20.
- MUNTZ, W. R. A. (1963*a*). Phototaxis and green rods in Urodeles. *Nature, Lond.*, **199**, 620.
- MUNTZ, W. R. A. (1963*b*). The development of phototaxis in the frog (*Rana temporaria*). *J. Exp. Biol.* **40**, 371-9.
- PEARSE, A. S. (1910). The reactions of amphibians to light. *Proc. Amer. Acad. Arts Sci.* **45**, 159-208.
- RUSHTON, W. A. H. (1959). Excitation pools in the frog's retina. *J. Physiol.* **149**, 327-45.
- RUSHTON, W. A. H. (1961). Peripheral coding in the nervous system. In *Sensory Communication*, ed. Rosenblith, W. A., New York: Wiley.
- SILVER, P. H. (1963). Two spectral sensitivity curves of *Xenopus laevis* obtained by using the melanophore response to light on white and black background. *J. Physiol.* **169**, 1-9.
- WILLMER, E. N. (1961). Human colour vision and the perception of blue. *J. Theoret. Biol.* **2**, 141-79.