

THE DEVELOPMENT OF PHOTOTAXIS IN THE FROG (*RANA TEMPORARIA*)

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Many experiments have shown that when a frog is disturbed it escapes towards the light, and that blue light is more effective than any other colour in guiding this response (see Pearse, 1910, for a review). A previously reported experiment (Muntz, 1962*b*) has shown that the greater effectiveness of blue light is not simply due to the animals' possessing a greater sensitivity to light in this part of the spectrum, but involves colour vision; the animals will respond preferentially to blue irrespective of the intensity or saturation of the stimulating lights. They will, for example, choose a blue illuminated window in preference to a window illuminated by an additive mixture of the same blue light together with a green light, in spite of the fact that the latter window contains as much blue as the former, as well as the green.

It seems probable that this response is mediated by the retinal projection to the diencephalon, for microelectrode recordings have shown that the fibres projecting to this area without exception respond more strongly to blue light than to light of any other colour (Muntz, 1962*a*). The response of these optic nerve fibres is again independent of the saturation and intensity of the stimulating light, in the same way as the phototactic behaviour of the intact animal. It therefore seems that the analysis of colour which is shown by frogs in the phototactic situation must be occurring in the retina itself. The following experiment attempts to throw light on the retinal receptors involved by correlating the development of the behaviour with the development of the retinal elements.

METHODS

The experiments were done on tadpoles of *Rana temporaria*, collected from a pond in the Oxford University Parks, and kept in aquaria at room temperature illuminated by a north-facing window. The water was replaced by fresh pond water every week, and the tadpoles obtained their food from the weed included with the pond water. Adult frogs for test no. VI were obtained from a dealer.

Phototactic behaviour was tested in the Y-maze shown in Fig. 1. The maze was constructed out of black Perspex, except for the windows W_1 and W_2 , which were of transparent Perspex covered with tracing paper. The maze was filled with water to a depth of 2 in. Each window was illuminated by a 500 W. projector at a distance of 21 in. Different colours of light were obtained by using six Wratten filters having an average half-maximum bandwidth of 30 $m\mu$. Details of these filters, and of the methods used in calibration, are given in Muntz (1962*a*). The filters were calibrated

to give equal energy, and calibrations were accurate to within 5%. The illumination at W_1 and W_2 was $21 \mu\text{W./cm}^2$. In the present experiment the same filters were used throughout, so that inaccuracies in calibration cannot have affected the changes in behaviour which were observed.

The six filters used could be arranged in fifteen pairs. Each filter was, however, also paired with darkness, so that there were twenty-one ways of illuminating the windows W_1 and W_2 . On any test eight animals were shown each of these twenty-one possibilities, four animals with W_1 illuminated by one member of each pair, and four with

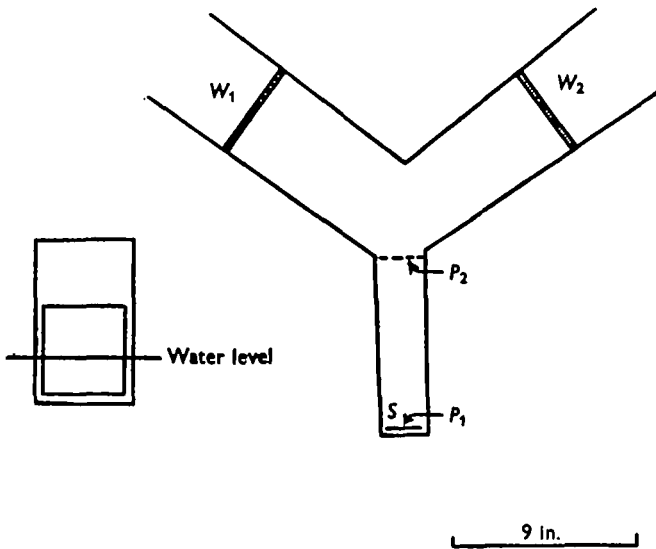


Fig. 1. Plan view of apparatus. Inset: end view of one of the illuminated windows.

Table 1

Test no.	Approx. stage according to Saxén (1954)	Mean length* (mm.)	Appearance	Age after arrival in days
I	6	16	Hind-limb buds visible	0
II	6-7	20	Hind-limb buds length of anus, toes visible as bulges	8
III	7	20.6	Hind limbs with joints and toes clearly visible	20
IV	7	21.5	Hind limbs between $\frac{1}{2}$ and $\frac{1}{3}$ length of tail, no sign of fore limbs	32
V	9-10	16	Fore limbs emerged, sitting posture	46
VI	Adult†	40	—	—

* Calculated from animals fixed in Bouin's solution.

† Adults obtained from a dealer.

W_1 illuminated by the other member of the pair. Animals were taken one at a time from the aquarium in a small net and given a trial in the maze, after which they were placed in another aquarium. For a trial the animal was placed in the starting arm of the maze (S). The partition P_1 was then slowly moved up to the position P_2 , forcing the

animal to make a choice. Sometimes the animal could be seen swimming into one of the arms of the maze, but more often it was invisible against the black of the maze and could only be found by turning on the general room illumination after the trial. When all the animals had undergone one trial, the procedure was repeated, each animal undergoing a second trial and being returned to the original aquarium. This was repeated until all twenty-one pairs of stimuli had been seen by eight animals, so that there were 168 trials in all, which were completed in one day. In the early stages of the experiment about sixty animals were used, so that each animal underwent an

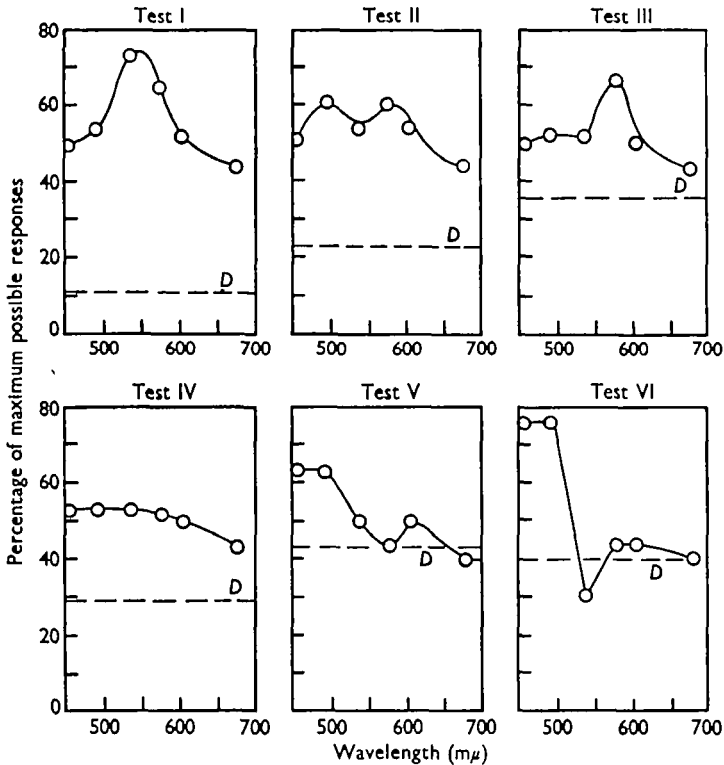


Fig. 2. Choices of different colours during the six tests. The line labelled *D* shows the number of occasions on which darkness was chosen.

average of 2.8 (168 divided by 60) trials before the testing schedule was completed. The number of animals decreased during the experiment to about twenty, at which stage each animal received an average of 8.4 trials during a testing day.

The tadpoles were tested at intervals between 8 and 14 days. The stages of development reached on the different tests are shown above (Table 1). From Saxén's (1954) data we can estimate that the animals were about 14 days old on entering the laboratory.

After each day's testing eight animals were fixed in Bouin's solution. The lengths given in Table 1 were calculated from these animals. Subsequently the heads of these animals were sectioned, bleached and stained, and the stage of retinal development was determined. Two stains were used, both of which gave good results: haematoxylin and eosin, and Masson's ponceau-acid fuchsin-light green stain.

RESULTS

Behavioural results are shown in Figs. 2 and 3. In Fig. 2 the number of times each colour was chosen, expressed as a percentage of the maximum number of times it might have been chosen, is shown for the different tests. Kendall's coefficient of agreement u (Kendall, 1948) was in every case significant at better than the 1% level of confidence. The horizontal line labelled D in each case shows the percentage of occasions on which darkness was chosen. It can be seen that initially the animals were

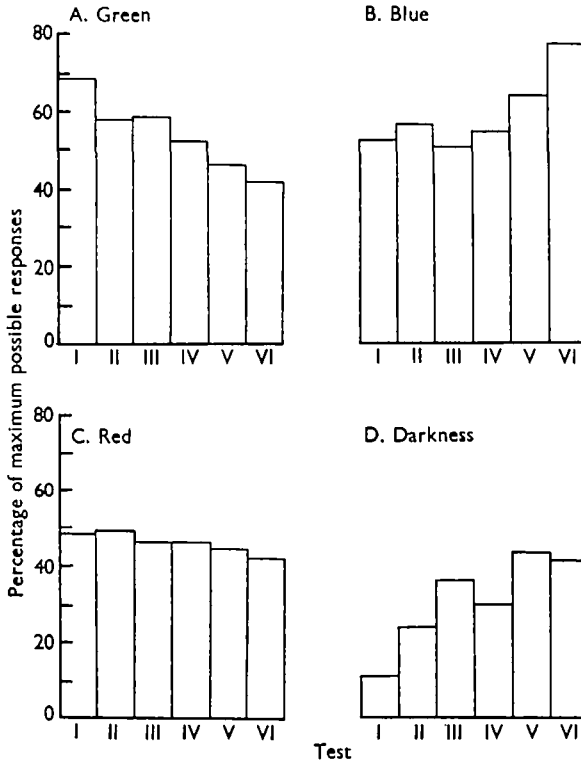


Fig. 3. Change in percentage responses to green, blue, red and darkness. A. Responses to two green lights used (λ_{\max} . 530 and 570 $m\mu$). B. Responses to two blue lights used (λ_{\max} . 460 and 495 $m\mu$). C. Responses to two red lights used (λ_{\max} . 608 and 675 $m\mu$). D. Responses to darkness.

positively phototactic, with green light (approx. 550 $m\mu$) being chosen more often than any other colour. Gradually the number of responses to green light decreases, while the number of choices of darkness and of blue light increases, until in the adult (test no. VI) blue light is chosen very much more often than either darkness or any other colour. The adults in fact showed little phototactic behaviour to any colour of light except blue: darkness was chosen almost equally often as any light with a wavelength longer than about 510 $m\mu$. The results of test no. VI agree well with results previously reported (Muntz, 1962*b*).

These changes are readily visible in Fig. 3, in which the average responsiveness to the two blue colours used (λ_{\max} . 460 and 495 $m\mu$), the two greens (λ_{\max} . 530 and

570 m μ), the two reds (λ_{max} . 608 and 675 m μ), and to darkness are shown for the different stages.

Fig. 4 shows the appearance of the visual cells at the time of tests I, IV and VI. Even in the youngest tadpoles used the rods and cones can be clearly distinguished. It can be seen that the cones change little over the experiment, but that there is a considerable development of the rods, and in particular of the outer segment. There was also an increase in the density of receptors. Only rods and cones are shown; it was not possible to distinguish the green rods with any certainty except in adults. The histological pictures are from light-adapted animals, and agree well with those published by Saxén (1954).

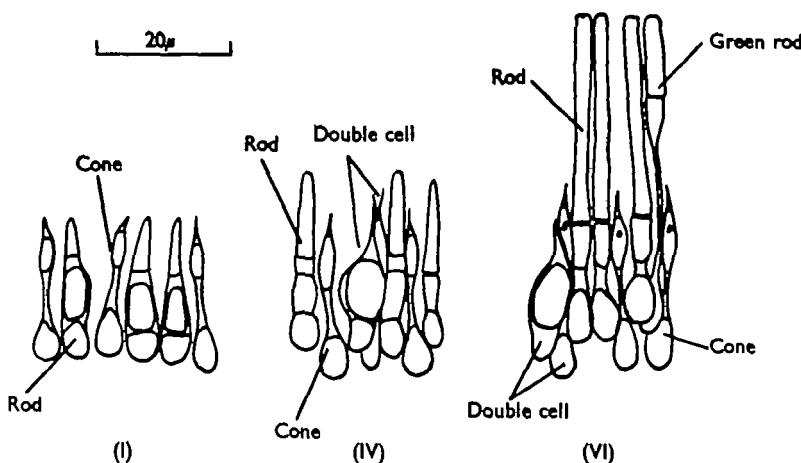


Fig. 4. Appearance of visual cells at time of tests I, IV and VI

DISCUSSION

The fact that, in adults, the responsiveness to blue light is decreased by adding a green light to it (Muntz, 1962*b*) suggests very strongly that in phototaxis an inhibitory effect is involved as well as an excitatory one. This is borne out by the present experiment; the gradual rise in the percentage of choices of darkness with age is difficult to explain except by the gradual maturation of some inhibitory mechanism. In the present experiment green light was chosen slightly less often than darkness by the adults, but the effect was small and may have been due to chance; in previous experiments frogs were found to respond positively even to green light (Muntz, 1962*b*). Thus adult frogs appear to be positively phototactic throughout the visible spectrum, strongly so to blue light, and to a small degree to light of other colours. We must therefore assume that the inhibitory effect is smaller than the excitatory effect throughout the spectrum. In the green, however, the positive effect outweighs the negative effect by much less than it does in the blue, and slightly less than in the orange or yellow.

The colour which is least effective for positive phototaxis is at about 510 m μ , so that the negative effect might be expected to be maximal at about this point. This suggests that the negative effect is due to the red rods, which are maximally effective

at 502 m μ . The time over which the inhibitory effect increases supports the idea that the red rod underlies the inhibitory effect. Thus Birukow (1949), using the optomotor response, found that tadpoles initially have photopic vision alone, and that scotopic vision only starts at 14 days (approximately Saxén's stage 6, or the age of the tadpoles during test I of this experiment) and develops slowly. Majima (1925) also found that tadpoles initially have photopic vision alone, scotopic vision developing considerably later. Saxén found that cone movements start at stage 2 or 3, but that rod movements start only at stage 7, and concludes, on the basis of this and Birukow and Majima's results, that cone function starts earlier than rod function, which only starts at stage 7 and gradually increases until metamorphosis. In the present experiment test I reveals a smooth curve maximal in the green. This curve appears to be a photopic sensitivity curve and so can be attributed to the cones,* and there are very few choices of darkness at this point. However, from test II (Saxén's stage 6-7) onwards the responses to darkness increase steadily. This is exactly what would be expected if the red rods underlie the inhibitory effect.

If we accept that the red rod is the cause of the inhibitory effect, we can deduce roughly the form of the excitatory effect. It must be maximal in the blue, and extend over the whole spectrum so that at any point it is greater than the inhibitory effect. Fig. 5 shows a possible arrangement; the inhibitory curve is the absorption spectrum of visual purple, and the excitatory curve has been constructed so that when it is divided by the inhibitory curve the resultant is as shown in Fig. 5 B. The exact relation between the excitatory and inhibitory effects is not critical for the general form of the curves.

It is fairly clear that the excitatory effect is mediated, at any rate in part, by the green rods. These rods have been shown to absorb maximally in the blue (Denton & Wyllie, 1955), and Dartnall (1957) has extracted a pigment, maximal at 430-440 m μ , which is probably the green rod pigment. Further, *Salamandra*, which lacks green rods, is negatively phototactic and insensitive to blue light, whereas *Triturus*, in which green rods are present (and also various anurans which have green rods) has a positive phototactic response curve similar to that of the frog (Muntz, in preparation). However, it is unlikely that only the green rods are involved in the positive effect, for Denton & Wyllie (1955) and Dartnall (1957) agree that they are insensitive to green light, whereas the positive effect which is postulated here must extend over the whole spectrum. Furthermore, no visual pigment has been extracted with an absorption spectrum as broad as that which would be required for the positive effect postulated here. One possibility is that the positive effect is mediated by a synergistic action of the cones and the green rods, which, acting together, could give a spectral response curve as broad as is necessary (Fig. 6). Donner & Rushton (1959) have shown that in certain circumstances the cones and the green rods can act together in a synergistic

* Wald (1945-6) has shown that *Rana catesbiana*, the bull-frog, has visual pigments based on vitamin A₂ before metamorphosis, and only gets the typical frog pigments, based on vitamin A₁ when metamorphosis is complete. This would make the curve obtained in test I incompatible with cone function, for it does not agree with the maximum of photopic sensitivity expected for a system based on vitamin A₂, which would be at about 610 m μ . However, Wald's finding does not apply to all anurans, and Collins, Love & Morton (1953) have shown that in *R. temporaria* and *R. esculenta* rhodopsin with λ_{max} 502 m μ is the only visual pigment in both tadpoles and adults. In these species, therefore, the photopic sensitivity of the tadpole would be expected to be maximal at about 560 m μ .

Day, and Walls (1942) maintains that, in view of the position of the nucleus, the green rod is more cone-like than rod-like. In this respect it is worth noting that the positive phototaxis of young tadpoles is probably mediated by the cones alone, and there is no evidence to suggest that the neural connexions serving this are subsequently broken.

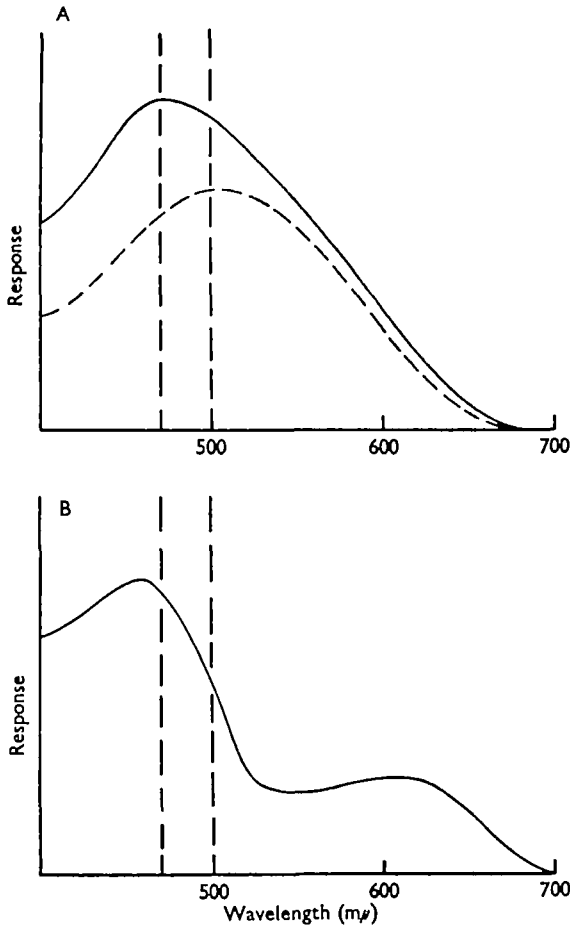


Fig. 5. A possible mechanism underlying phototropism in Amphibia. (A) Response curves of positive (continuous line) and negative (dashed line) effects. (B) Percentage by which positive effect exceeds negative effect at different wavelengths.

The present experiment rests on the assumption that the analysis of colour being studied occurs in the retina itself. This assumption relies on the evidence showing that the microelectrode recordings of Muntz (1962 *a*) were made from the terminal arbores of the optic nerve fibres projecting to the diencephalon, and not from the thalamic neurones themselves or their axons. Apart from the evidence given in that paper, two further reports have since been read which describe experimental results from the isolated retina identical to those obtained from the diencephalon, and which thus confirm this assumption. Thus Liberman (1957), recording single units in isolated retinal preparations, showed that, in response to diffuse illumination, *on*-fibres give a prolonged response to blue light, but only a short burst of impulses to red light,

irrespective of the intensity of the stimulating light. This behaviour, which is identical to that found for the *on*-fibres in the diencephalon, did not occur with *on-off*- or *off*-fibres. Orlov (1961) has also presented results from isolated retinas which agree well with the responses found in the diencephalon. Recording massed responses from the optic nerve in light-adapted retinæ, he found prolonged oscillations in response to blue light, but only a rapidly terminating response on stimulation with red or green light. These oscillations occurred when the stimulating light was of shorter wave-

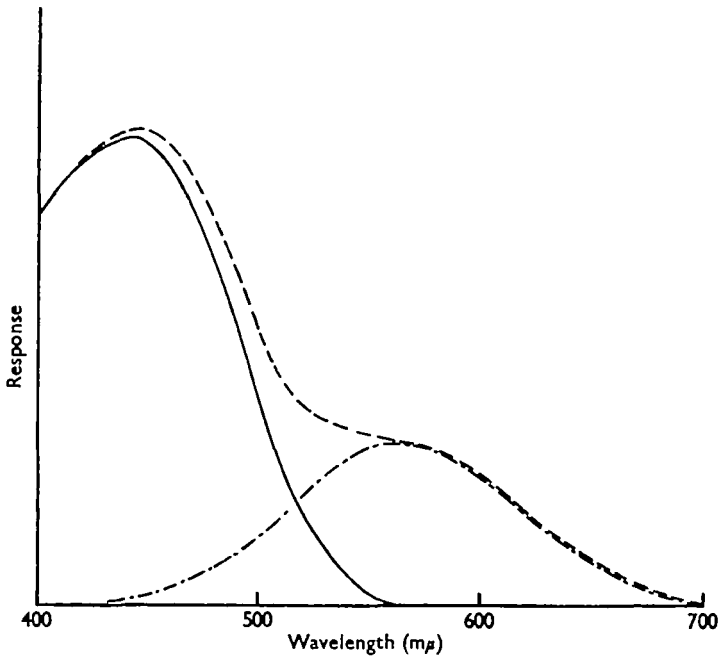


Fig. 6. Suggested compound form of positive effect. Full line, Dartnall's blue pigment. Dashed line with dots, cone-response curve. Dashed line, resultant positive effect.

length than $480\text{ m}\mu$, but not when the wavelength was longer than $520\text{ m}\mu$, with a transitional zone between, which agrees precisely with the spectral response curves recorded in the diencephalon. With large electrodes oscillations were also recorded from the diencephalon, which had the same spectral response curves as the individual units.

Thus, although the evidence is not conclusive, the following hypothesis can explain the facts relating to phototaxis in frogs in a simple way. In young tadpoles the behaviour is entirely governed by the cones, which have an excitatory effect. Later the red rods, which have an inhibitory effect, and the green rods, which (like the cones) have an excitatory effect, gradually develop, so that in the adult the green rods and cones are acting synergistically in an excitatory way, but are opposed by the inhibitory effect of the red rods. A possible function of such a mechanism has been suggested in Muntz (1962*b*).

SUMMARY

1. The development of phototaxis in the frog was studied by testing the behaviour of tadpoles at different stages of their development. The development of the retina at each stage was checked from histological preparations.

2. Young tadpoles responded most strongly to green light (approx. 550 m μ). Gradually the number of responses to green light decreased, while more responses were made to blue light, and also to darkness. Adult frogs respond most strongly to blue light; light of wavelengths longer than about 510 m μ is responded to only slightly more often than darkness.

3. It was concluded that the phototactic behaviour of frogs is probably mediated by a synergistic action of the green rods and the cones, which have an excitatory effect, and which are opposed by an inhibitory effect of the red rods.

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