THE CONTROL OF PHOTO-PIGMENTARY 
RESPONSES IN EYELESS CATFISH 

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I. INTRODUCTION 

Many colour-changing animals have been found to show pigmentary responses to variations in light intensity even after removal of the eyes. Responses of this type have been observed in certain lacertilians: Phrynosoma (Redfield, 1918), Anolis (Hadley, 1929, 1931), Chameleo (Zoond & Eyre, 1934), and teleosts: Phoxinus (von Frisch, 1911b, 1912), Amiurus (Abramowitz, 1936), Lepadogaster and other shallow water species (Wykes, 1937). A similar response occurs also in the lamprey (Young, 1935b). In all these species the melanophores contract in darkness and expand in bright light. 

The physiological control of this type of response is still incompletely known. Zoond & Eyre (1934) from their work on chameleons concluded that the response resulted from a spinal reflex with an autonomic motor component. Hadley's experiments on Anolis (1931) and Parker's on Phrynosoma (1938) suggest that the melanophores respond directly to light as independent effectors. Von Frisch demonstrated that the response is nervously controlled in Phoxinus, the pineal body being the chief photoreceptor, though the response is not completely prevented by its removal. His results are confirmed by those of Scharrer (1928) who succeeded in establishing conditioned reflexes to light in eyeless Phoxinus. Photoreceptors were found to be localized in the diencephalon, the skin being insensitive to light. In the ammocoete Young (1935b) concluded that the paling which occurs when the animals pass from light to darkness is probably due to "the inhibition of posterior pituitary secretion by nervous impulses set up by the change of illumination of the pineal complex". 

The following experiments were undertaken in order to discover how far the nervous control of the response in teleosts resembles that of the chameleons. 

II. METHODS 

The pigmentary responses of Amiurus nebulosus and Fundulus heteroclitus have already been studied in considerable detail. These species were therefore tested by preliminary experiment to find which would be most suitable for the present investigation.
In each case a dozen eyeless fishes were used. Six were left for 24 hr. in a tank in the darkroom, while six remained for 30 min. in a white bowl above which a 100 W. lamp was hung at a distance of 50 cm. The time allowed under each set of conditions was shown by previous experiments to be sufficient to elicit a maximum pigmentary response and was insufficient for a change of more than 2° C. in the water temperature. At the end of this period the fishes were fixed in 40% formaldehyde. The diameters of fifty melanophores were then measured, using an eyepiece micrometer and a magnification of 252 diameters. Microscopic examination of the state of the melanophores before and after fixation showed that the fixative had no detectable effect on the condition of these cells. Similar methods have been used in the study of colour response by Brown (1936) and Wykes (1937). In Fundulus the melanophores in the large scales overlying the pineal organ were used, while in Amiurus, for reasons which will appear later, the melanophores of the pelvic fin were measured. Thus the diameters of 300 melanophores were measured in each case and their mean value calculated. In eyeless Fundulus it was found that the melanophores reached a mean diameter of 101-8 ± 1-32μ under the 100 W. light, contracting to 40-8 ± 0-48μ in darkness, while the melanophores of eyeless Amiurus ranged from a diameter of 114-6 ± 1-45μ in the light to 49-8 ± 1-0μ in darkness. The range is thus very similar in the two species. The response is not, however, so spectacular as these figures might suggest and indeed seems to have been completely overlooked by Bray (1918) who states that blinded catfishes “remain dark under all light conditions”. The change may best be appreciated by observing the colour of the paired fins as they lie against a white background. After a period of darkness these fins appear completely transparent, becoming gradually dark again under the influence of light.

Amiurus was chosen as the better species for further investigation since its greater size facilitates all operative procedures.

In all the experiments to be reviewed, the mean melanophore diameter, obtained by the method already described, was used as a quantitative basis for the estimation of colour response. This method is particularly valid in the case of Amiurus where melanophores are the only type of pigment cells which occur. The experiments were carried out in a photographic darkroom at a room temperature of 20 ± 2° C. Running water was not employed since, during the winter months, the temperature drops to about 6° C. At this temperature the colour responses of Amiurus are almost completely in abeyance and the melanophores remain expanded under all conditions both of background and illumination. The fishes varied from 19 to 14 cm. in length, having a mean length of 17-2 cm. In all cases the eyes were removed so that responses to background colour were eliminated.

III. EXPERIMENTS ON NERVOUS CONTROL

In order to assess quantitatively the effect of nerve section on the photic response, the six spinal nerves innervating the left pelvic fin were sectioned in a group of twelve eyeless fishes. Since the nerve supply of the right fin remained normal it served as a control. Six of these fishes were left in the darkroom tank and six were
placed under the 100 W. light. After suitable periods the two groups were fixed and the mean melanophore diameter of the right and left pelvic fins was measured. The normal fins, as already described, showed a marked melanophore response, the diameter reaching $114.6\mu$ in the lighted specimens and $49.8\mu$ in those from the darkroom. The melanophores of the denervated fins remained expanded under both conditions, those in the light having a diameter of $127.6\pm1.9\mu$ while that of those in the darkroom was $119.4\pm1.1\mu$. It is difficult to ascribe this persistent melanophore expansion to an injury discharge in the severed fibres since it lasts unchanged for several days. It seems rather that the melanophores of the denervated region are cut off from some nervous activity which, under suitable conditions, leads to melanophore contraction in the normally innervated skin.

From this experiment it appeared that the path of this activity lay along the spinal nerves. A further series was, however, necessary in order to discover whether it consisted of simple spinal reflexes or whether the brain also was involved. In a group of six eyeless fishes the cord and sympathetic chains were sectioned at the level of the 8th vertebra, so separating the reflex activity of the cord from that involving the brain. The melanophores are probably influenced by motor nerves of the autonomic system which have been found in *Phoxinus* (von Frisch, 1911a; Wernœ, 1926) to leave the cord at the 15th vertebra. The section of the cord was therefore made above this level.

The operated animals, after being allowed a day or two for recovery, were placed in the darkroom tank for 24 hr. and then fixed. The mean diameter of the pelvic fin melanophores was measured and found to be $110.2\mu$, indicating that the melanophore contraction had been to a large extent inhibited in the posterior region. Thus, if the paling reaction is nervous in origin, it must depend, in this species at least, upon impulses passing through the brain. The fact that it does not occur to any appreciable extent in a denervated region suggests that independent effector action plays little or no part in the response. This conclusion was supported by a series of experiments on excised skin, the melanophores of which remained expanded under all light conditions, though contracting rapidly in 1:1000 adrenaline chloride solution. Moreover, no local response to light could be obtained in the normal fish. To test this possibility a fish was confined in a glass tube through which water passed. A narrow beam of light was then focused on the skin by means of a lens. This experiment, with various modifications, was tried on several individuals but with entirely negative results.

In the case of background responses denervated areas respond in accordance with, though much more slowly than, the rest of the body. These slow parallel responses were never noted in the reactions to light intensity. This observation was first made by Abramowitz (1936) and has been further confirmed in my own work.

The fact that the melanophores are subject to nervous control may be further demonstrated by electrical stimulation of the cord. In the fishes used for this experiment the melanophores were already expanded and the nerves to the pelvic fin cut. The fishes were then decapitated and eviscerated in order to eliminate circulatory changes. In the stimulating circuit condenser discharges through a thermionic
pentode were varied in strength and frequency by means of variable resistances. A cathode ray oscillograph was used for calibration. With the electrodes placed in the vertebral canal and a discharge voltage to which the tongue was barely sensitive, stimulation at frequencies as low as 10 per sec. resulted in melanophore contraction in all innervated regions, the pelvic fin alone remaining dark. This response was reversible and could be repeated. It took several minutes to develop fully and persisted without change when the stimulation was retained for much longer periods. The threshold frequency varied in different fishes and would rise during the course of the experiment due perhaps to the fatigue of the system or to the inadequate oxygenation of the tissues involved. The relation between response and frequency is indicated in the following protocol.

<table>
<thead>
<tr>
<th>No. of fish</th>
<th>Frequency</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>B105</td>
<td>2 per sec.</td>
<td>No macroscopic effect after 10 min.</td>
</tr>
<tr>
<td>B106</td>
<td>4</td>
<td>No macroscopic effect after 10 min.</td>
</tr>
<tr>
<td>B109</td>
<td>10</td>
<td>Macroscopic paling in 5 min.</td>
</tr>
<tr>
<td>B100</td>
<td>50</td>
<td>Macroscopic paling in 2 min.</td>
</tr>
<tr>
<td></td>
<td>Stimulation stopped</td>
<td>Fish darkened again within 10 min.</td>
</tr>
<tr>
<td></td>
<td>Stimulation repeated</td>
<td>Macroscopic paling in 2 min.</td>
</tr>
</tbody>
</table>

From these results it appears that a state of melanophore contraction could be maintained by an exceedingly low tonic discharge.

Evidence concerning the sensory origin of nervous control is inconclusive. The work of von Frisch and Scharrer on Phoxinus suggested that the pineal might be an active photoreceptor. In six eyeless Amiurus, therefore, the brain cavity was opened dorsally and the pineal and part of the roof of the diencephalon destroyed with a hot needle. The flap of bone was then returned into place and the skin fastened back with suture thread. The response to light was found to persist, however, after this operation. No post-mortem sections of the brain were made so that it is not possible to say how much of the diencephalon was destroyed. The results obtained on Phoxinus suggest that the tissue of the whole of that region of the brain may be sensitive to light. Nevertheless it is, perhaps, significant that in Phoxinus and Lampetra and to a lesser extent in Fundulus, the skull over the diencephalon is thin and the skin only slightly pigmented. In Amiurus, however, the dorsal roof of the skull is thick and the skin above it very deeply pigmented so that the brain tissues seem to be well protected from the influence of light.

IV. EXPERIMENTAL HYPOPHYSECTOMY

Abramowitz (1936) showed that the photic response continues in Amiurus after hypophysectomy. If eyeless fishes, newly hypophysectomized, are tested in the darkroom and bright light they give pigmentary responses similar to those of the normal fish under corresponding conditions. It appears from this that the response is in no way connected with a variable secretion of pituitary hormones. It is known, nevertheless, that extracts of catfish pituitary, or obstetrical pituitrin induce melanophore expansion when injected into light-coloured catfish (Parker, 1934). A
pituitary hormone is known to be the cause of normal melanophore expansion in cyclostomes, elasmobranchs and amphibians. It is possible, therefore, that there is a fairly constant concentration of such a hormone in the blood of Amiurus and that the supply of the hormone to the melanophores is varied by an alteration in the calibre of the skin capillaries which are under nervous control. Section of the vaso-motor fibres, then, would lead to a relaxation of the capillaries and a consequent increase in the supply of the expanding hormone. This possibility was emphasized by the fact, pointed out to me by Dr Osborne, that hypophysectomized catfish do, in the course of a few days, show a definite melanophore contraction.

A number of hypophysectomized fishes were therefore kept alive for several days. It was found that the degree of melanophore contraction in bright light diminished rather rapidly during the first 5 days after hypophysectomy but appeared then to reach a steady state which was maintained so that a distinct response might still be obtained after 8 days. Six such fishes, kept for 8 days after hypophysectomy gave a mean melanophore diameter of 71.8 μ under the 100 W. light as against the 114.6 μ obtained under similar conditions in the normal fish. The darkness-contraction, however, remained unchanged, the melanophores reaching a mean diameter of 50.8 μ as against 49.8 μ in the normal fish. Such a contraction could, as before, be prevented by denervation. The mean diameter of the melanophores in a denervated fin was found to be 77.2 μ. It has been suggested that this gradual paling might be attributed to the sickness of post-operative animals. The fishes nevertheless seemed thoroughly healthy and the only casualties were due to infection with the water mould Saprolegnia, to which, under laboratory conditions, the fishes are very susceptible. Moreover, none of the pinealectomized fishes showed such a paling.

This restriction of the photic response after hypophysectomy is almost certainly due, then, to the disappearance of the pituitary hormone, infundin, from the blood. The fact that the response is consistently maintained under such conditions, except in denervated areas, indicates that it is primarily under nervous control and that the presence of the pituitary hormone is of secondary importance. The latter acts as an augmenter rather than as the initiator of the pigmentary response.

V. DISCUSSION

The significance of the pituitary hormones in colour-changing teleosts has been discussed by various authors. The summary of this work, given by Kleinholz (1935), shows that the results obtained from injection of various pituitary extracts and tests with isolated scales have been inconclusive and in certain cases contradictory.

The above experiments on blinded Amiurus have shown that in the absence of the pituitary the photic response is considerably restricted. The fact that the response remains, precludes the possibility, however, that it is under direct pituitary control. The most probable supposition is that under normal conditions the pituitary hormone in the blood augments the melanophore expansion which occurs in bright light and leads to a similar expansion under all light conditions once the nervous control is removed. It is unable, however, to prevent the melanophore contraction.
resulting from nervous stimulation either in darkness or by electrical means. Sand (1935) has suggested that the stimulus of light inhibits a tonic motor discharge which, in the darkness, produces melanophore contraction. The fact that such contraction can be maintained by an extremely low frequency of stimulation emphasizes this possibility.

The photosensory receptors involved have not been isolated. Certainly the pineal does not function alone in this respect. The possibility that other parts of the wall of the diencephalon are photosensitive remains. Nervous tissue appears to be more generally sensitive to light than has previously been realized. Young (1935a) showed that in the lamprey the spinal cord, when scraped free of pigment dorsally, became immediately sensitive to light. Rodewald (1935), while investigating the effect of light on the pituitary of *Rana temporaria*, found that in eyeless frogs the stump of the optic nerve functions as a receptor for light. But it is extremely doubtful whether in *Amiurus* light could penetrate through the skull and heavily pigmented skin which overlie the diencephalon. In lacertilians also there appear to be differences in the methods of non-visual photoreception. Clausen & Mofshin (1936) made use of the varied oxygen consumption of *Anolis carolinensis* in light and darkness as an index of photoreception. In tests in which the eyes, skin and pineal were incapacitated respectively, analysis showed that the eyes and skin were almost equally important as light receptors while the pineal was also functional in this respect though in a lesser degree. Parker (1938), however, found no evidence of light sensitivity in the pineal of *Phrynosoma*, though it is a well-developed organ in this species. It is not known whether the pineal is a functional photoreceptor in the chameleon. Zoond & Bockenham (1935) were, however, able to show that photoreception is a function of the general skin surface in this species. This may also be true in *Amiurus*. This conclusion was previously reached by van Heusen (1917), who used the swimming movements of blinded catfishes as indicative of photoreception, so extending the previous work of Eigenmann (1899) on *Amblyopsis*.

In chameleons the photic response appears, on the basis of the experiments performed by Zoond & Eyre (1934), to be controlled by spinal reflexes, though it is noteworthy that Parker (1938) has adduced these results as evidence in favour of independent effector activity. In *Amiurus*, on the other hand, the response is under a nervous control which is centralized in the brain. This difference is reflected in the pigmentary behaviour of the two types, for the fish always changes colour as a whole and the localized responses to local stimuli which occur in the chameleon cannot be obtained. The fact that the photic response in *Phoxinus* can be used as a basis for the building up of conditioned reflexes, suggests that, in this species also, control is centralized in the brain.

The possibility of a pituitary control of colour has not yet been fully investigated in the chameleon, though it has been recently found to occur in other lacertilians (Hadley, 1931; Kleinholz, 1935; Parker, 1938). These workers have recorded that various species of lizard become permanently pale after hypophysectomy but will still darken after injection of pituitary extract. It is not clear, however, what part the pituitary secretion plays in the normal colour changes of these animals. In
Amiurus the nervous control supplies what may be regarded as a switch mechanism by which the melanophore contraction is turned on or off. The pituitary hormone on the other hand controls the full range of the response and is probably responsible for the slowness with which the reaction reaches its equilibrium condition.

VI. SUMMARY

1. In common with certain other teleosts and lacertilians, eyeless Amiurus nebulosus and Fundulus heteroclitus show a pigmentary response to changes in intensity of illumination. The melanophores contract in the darkness and expand in bright light. The control of this photic response was investigated in Amiurus.

2. The contraction in darkness was not obtained in areas denervated by section of spinal nerves nor in the posterior part of the body after section of the cord. The response is therefore under the control of nervous reflexes passing through the brain. A similar melanophore contraction can be obtained by electrical stimulation of the cord at extremely low frequencies.

3. The response remained in pinealectomized animals. Photoreceptors may possibly be located in the skin or the wall of the diencephalon may be sensitive to light.

4. After hypophysectomy the response continues but the degree of melanophore expansion in bright light is diminished. The expanding hormone of the pituitary is therefore important in that it augments a melanophore response which is under nervous control.

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REFERENCES


