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ELECTRICAL ACTIVITY IN THE OPTIC TECTUM AND COLOUR CHANGE IN THE MINNOW (*PHOXINUS PHOXINUS* L.)*

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INTRODUCTION

The relationship between the electroencephalogram recorded from the optic tectum and light has been recorded in two species of fish. Enger (1957) recorded electroencephalograms from free-swimming codfish, *Gadus callarias*, and Schadé & Weiler (1959) from the goldfish, *Carassius auratus*. No work has been done on the relationship between the electroencephalogram and the colour of the skin. Gentle (1971 *a*, *b*) has shown that the optic tectum is necessary for colour change to occur, and the experiments to be described were designed to investigate the relationship between the electroencephalogram and the colour of the minnow.

MATERIALS AND METHODS

The fish used were 6 cm long adults collected from the River Chess outside Rickmansworth. They were kept in large sinks in the laboratory. To estimate the colour of the fish they were compared with a series of nine standard grey tints derived from the Ostwald White-Grey-Black series (Healey, 1967). Light grey being 0 and very dark grey 8, and for the sake of convenience the numbers are referred to as the Derived Ostwald Index (D.O.I.).

All operations were performed using a solution of 0·008% MS.222 (Tricaine methane-sulphonate, Sandoz Ltd.). The electrical activity of the tectum was recorded by means of bipolar tungsten electrodes 1 cm long and with a tip diameter of 25 μm. They were insulated except for the tip with Araldite PZ820 resin and had a resistance of 3·6 kΩ. The leads from the electrodes were connected to a Tektronix 122 pre-amplifier and displayed on a Tektronix 502 A oscilloscope and photographed. The electrodes were positioned using a Leitz micromanipulator and the fish was held by two pairs of clamps. The respiratory water was fed into the mouth directly and was collected in a trough placed under the fish.

The recordings from the fish, which were undergoing colour change as a result of background reversal, were taken with implanted electrodes. The electrodes were placed on the tectum and fixed onto the skull with denture repair cement. After the cement had dried the animals were transferred to the apparatus shown in Fig. 1,

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which enabled the background to be changed without disturbing the fish. The apparatus consisted of a rectangular box made of white Perspex. The fish was held by means of a tube carrying respiratory water into the mouth and two V-shaped pieces of clear Perspex (*A*). The holding device was fixed onto another piece of clear Perspex (*C*) which was raised from the floor of the box by ledges on three sides. Attached to the free edge of *C* was an upright plate of white Perspex. '*B*' was a piece of black Perspex which could be slid between *C* and the bottom of the box *E* to present the fish with a black background. If *B* was slid out the fish was presented with the white background produced by *E*. Two plates of black Perspex (*D*) were made to form the two sides of a box and when they were put together they formed the walls. When *D* was removed the sides of the box formed the white walls.

On completion of the experiment the fish were decapitated and the position of the electrodes was verified histologically.

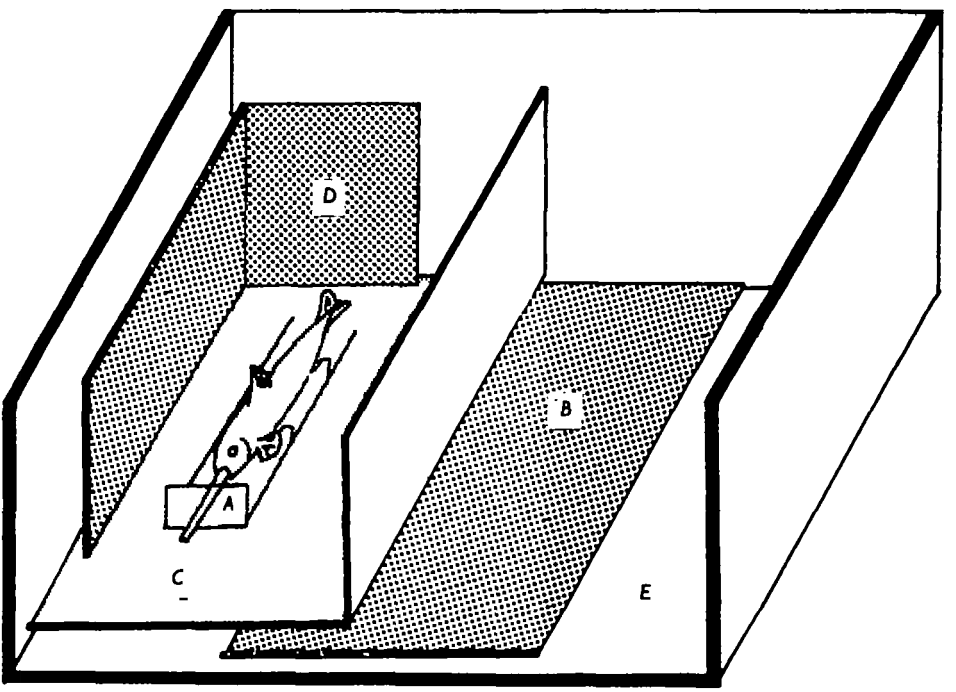

Fig. 1. Diagram of the background-reversal apparatus used to record E.E.G.s of the minnow with electrodes implanted in the tectum. *A*, *C*, clear Perspex; *D*, *B*, black Perspex; *E* and the sides of the box, white Perspex.

RESULTS

The superficial E.E.G. *in normal fish*

Recordings were taken from each of eight unanaesthetized fish, held lightly in two clamps. The fish were in dim light and the extraneous noise was reduced to that of the electronic apparatus used. The electrodes were lowered onto the exposed tectum so that they just penetrated the surface, the dorsal skull bones having been previously removed under anaesthetic. The positions of the electrodes and the recordings obtained are shown in Fig. 2.

There appeared to be two basic rhythms, a slow one of 6–14 Hz and a faster one of 18–24 Hz. The amplitude of the slower being 20–112 μV and the faster 6–18 μV. Slight differences in frequency were also noted. Position *A* showed a variation in low frequency (L.F.) of 6–12 Hz with a mean of 7·5; *B* with a variation of 8–14 Hz and a mean of 10·5; *F* a variation of 4–14 Hz and a mean of 9·6 and *G*, *H* and *I* a variation of 8–11 Hz and a mean of 8·9. The high frequency (H.F.) showed the same variation of 18–24 Hz throughout the tectum.

In position *C* a marked periodicity of high and low activity was found. The L.F. and the H.F. were both present but there were periods in the record where the H.F. was apparently absent and only the L.F. was seen.

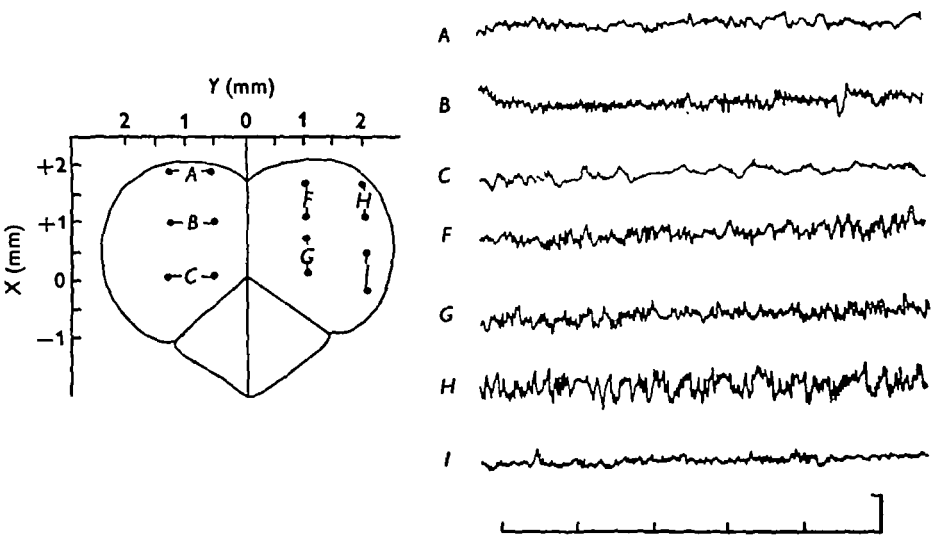

Fig. 2. The positions of the electrodes and the surface E.E.G.s recorded in normal fish. The horizontal line is marked in sec and the (vertical) amplitude calibration is 100 μV.

The E.E.G. *following anaesthesia*

The MS.222 solution used to give deep anaesthesia greatly reduced the amplitude of the E.E.G. In all the six fish tested there was only a small amount of very low-level activity which could not be discerned above the general noise level of the electrodes. During recording the fish maintained a permanently dark tint.

The E.E.G. *in darkness*

Four fish were used and they were clamped throughout the experiment. Darkness was simulated by covering their eyes with a light-proof shield which was sufficiently extensive to cover not only the eye but most of the side of the head. The results from a single fish are shown in Fig. 3 and the recording position was *G* (Fig. 2). The first record was taken in the light; the eye was then covered and records were taken immediately after covering and 15 and 30 min after covering. The eye covers were then removed and recordings were taken.

The initial effect of covering the eyes was that the amplitude of the E.E.G. increased and the increase persisted for 15 min. The amplitude then dropped after 30 min to a very low level, and finally when the covers were removed the amplitude increased again.

The fish were fully white at the beginning of the test when the eyes were not covered, but at the end of the 30 min covering period the fish assumed an intermediate shade of about D.O.I. value of 4. All the fish used gave the same result.

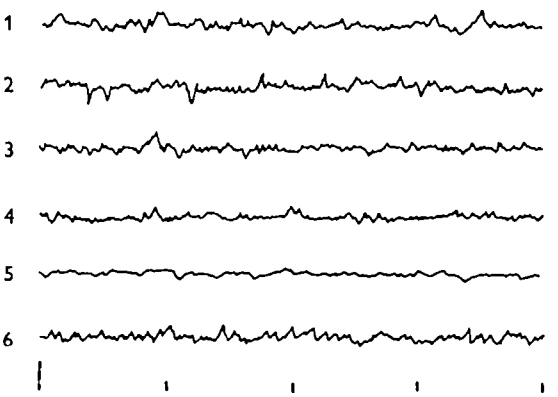

Fig. 3. The E.E.G. pattern in light and in darkness. Time marks in seconds and amplitude 100 μV. 1, In light. 2, Transferred to darkness. 3, Darkness 5 min. 4, Darkness 15 min. 5, Darkness 30 min. 6, Returned to the light.

The E.E.G. *and blinding*

As in the previous experiment the fish were clamped throughout. Recordings were taken from one fish before and after blinding. Blinding was accomplished by the bilateral section of the optic tracts under anaesthetic, with the E.E.G. being recorded 10 min and 30 min after recovery. The E.E.G. patterns were recorded from fish which had been blinded for intervals of 5 h, 2 day and 5 day, and for each record a separate fish was used because it was not possible to record from the same fish on more than one occasion. A group of eight fish had the E.E.G. recorded after having been blinded for four months. All the records are shown in Fig. 4.

The amplitude was greatly affected by blinding, and a comparison of the 10- and 30 min records with those of the normal fish showed that there was almost no activity present. The H.F. and the L.F. could only just be seen and it was not possible to compare their frequencies. A partial increase in amplitude was seen in the 5 h fish but this did not increase any further in the 24 h animal. Both the 2 day and the 5-day fish showed increases in amplitude. All records were taken from position *G* (Fig. 2). All the fish were fully dark except the 5-day fish which had begun the post-blinding paling (Gentle, 1971*a*) and had reached a value of D.O.I. 6·5.

The recordings from fish blinded for four months were taken to see whether the E.E.G. pattern had been regained fully and whether any differences were present between the dark and light fish at this time. The amplitude was found to be lower in the blind fish than in normal fish but the results from the blinded fish were not sufficiently numerous to allow an accurate assessment of the pattern. There did,

However, appear to be a very slight increase in amplitude in the records from the fish which had lightened to a D.O.I. value of 3. The fish which remained dark (D.O.I. 7·5) tended to have E.E.G. records of rather smaller amplitude.

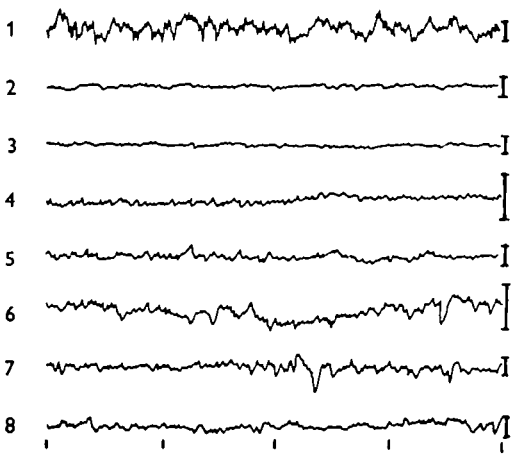

Fig. 4

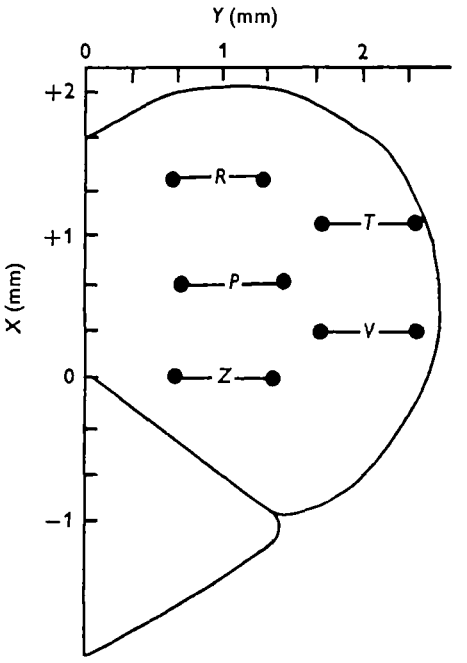

Fig. 5

Fig. 4. The E.E.G. pattern in the minnow before and after blinding. Time marks in seconds and amplitude 50 μV. 1, Normal fish. 2, Blind 10 min. 3, Blind 30 min. 4, Blind 24 h. 5, Blind 2 days. 6, Blind 5 days. 7, Blind 4 months D.O.I. 7. 8, Blind 4 months D.O.I. 3.

Fig. 5. The position of the electrodes used to record the E.E.G.s during background reversal.

The E.E.G. *following background reversal*

(*a*) *Method*

Recordings were taken from five regions of the optic tectum, labelled *R*, *P*, *Z*, *T* and *V* in Fig. 5. Region *Z* is the region that when destroyed in blind fish causes them to turn pale (Gentle, 1971*a*). Five depths in each region were used, these being (using the terminology of Leghissa, 1955): (1) stratum plexiforme et fibrosum externum, (2) stratum plexiforme internum, (3) stratum griseum internum, (4) stratum fibrosum profundum, (5) stratum griseum periventriculare.

The electrodes were lowered into the tectum and cemented to the remaining skull bones. When the cement was dry, and before the fish was allowed to recover from the anaesthetic, it was injected with 0·166 mg/100 g body weight of turbocurarine chloride (Burroughs Wellcome). The turbocurarine chloride prevented the fish from swimming away and also stopped the strong opercular beat from masking the record. The fish was allowed to recover from the anaesthetic on the black background. A record of the tectal activity was taken on the black background and it was then changed to white. Recordings were taken immediately after the change to white and

then at intervals of 5, 10 and 20 min. The background was then returned to the black and the same recordings were taken as on the white. The background was then changed to the white again and the initial recordings repeated.

Fifteen seconds of recordings were taken at each time, and an analysis of 10 sec of this was carried out. The analysis consisted of selecting certain amplitude limits and counting the number of frequencies present.

(*b*) *Results*

The amplitude variation from fish to fish was considerable, and this prevented an accurate analysis of amplitude; the results are confined to the analysis of the frequency.

Depth 1: *stratum plexiforme et fibrosum externum.* The records from this depth had the same pattern as the superficial recordings, showing the L.F. of 6–13 Hz and the H.F. of 18–24 Hz. The frequencies did not differ from the black to the white and the pattern was seen in all the recording sites (Fig. 6).

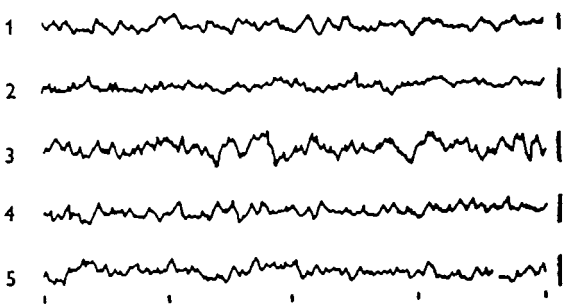

Fig. 6. The E.E.G. patterns at depths, 1, 2 and 3 in all positions and at depth 5 position *Z* during background reversal. Time marks in seconds and amplitude 25 μV. 1, Depth 1. 2, Depth 2. 3, Depth 3. 4, Depth 5 on white background. 5, Depth 5 on black background.

Depth 2: *stratum plexiforme internum.* In the recordings from this depth there was an increase in the amount of H.F. activity so that the variation was from 20–30 Hz and the mean values were all at least 4 Hz more than at depth 1 (Fig. 6).

Depth 3: *stratum griseum internum.* There was an increase in the H.F. activity in this region to give mean values of 40 Hz. The L.F. showed a slight reduction in frequency to give values of 4–5 Hz (Fig. 6). No change in pattern was seen on either background.

Depth 4: *stratum fibrosum profundum.* At this depth there was a reduction in the H.F. activity to give values of 11–30 Hz. All the regions showed the same pattern, and at this depth there were changes observed when the background was changed. When the fish was transferred to the black background from the white, a difference of approximately 10 Hz was observed in the H.F. rhythm. For example in position *P* it increased from 17 Hz on the white to 28 Hz on the black. This increase in the frequency of the H.F. rhythm persisted throughout the 20 min period on the black background. The results from position *Z* and *P* are shown in Table 1 and E.E.G. pattern *Z* in Fig. 7.

The L.F. activity did not differ from that at the other depths. There was a marked

cyclic activity in position *Z*. The amplitude of the E.E.G. was suddenly reduced to almost nil for a period of 2 sec followed by a gradual return to normal (Fig. 7).

Depth 5: *stratum griseum periventriculare.* The L.F. did not change with the background and showed a 6–10 Hz pattern. The H.F. showed an increase in frequency when the fish was on the black background similar to that seen at depth 4, with the increase in the region of 10 Hz (Fig. 6). The recordings in all the regions showed the same pattern.

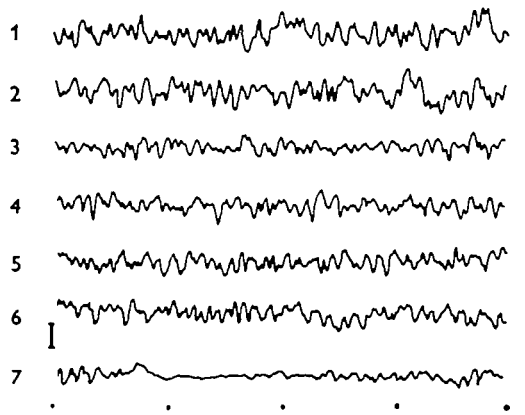

Fig. 7. The E.E.G. pattern at depth 4 position *Z* during background reversal. Time marks in seconds and amplitude 100 μV. 1, White zero time. 2, White 5 min. 3, White 20 min. 4, Black zero time. 5, Black 5 min. 6, Black 20 min. 7, Cyclic activity.

Table 1. *The high-frequency activity (Hz) in the stratum fibrosum profundum on black and white backgrounds*

Background	Time (min)	Region *Z*		Region *P*	
		Variation	Mean	Variation	Mean
White	0	11–15	12	15–25	21
	5	12–16	12·5	15–25	16
	20	12–16	14·9	13–21	17·5
Black	0	17–23	19·9	25–31	28
	5	20–26	22·2	20–30	25
	20	19–25	22·3	23–30	27

DISCUSSION

The superficial E.E.G. pattern in the minnow agreed well with that of the codfish (Enger, 1957) and the goldfish (Schadé & Weiler, 1959). The two rhythms in the minnow are 6–14 and 18–24 Hz, in the codfish 8–13 and 14–32 Hz and in the goldfish 7–14 and 18–24 Hz.

Schadé & Weiler (1959) reported that after cutting the optic nerve on one side the spontaneous electrical activity of the contralateral half of the optic tectum is reduced. In the minnow the initial effect of bilateral blinding was to reduce the level of the E.E.G. amplitude throughout the whole of the tectum. The amplitude of the E.E.G. was almost nil after the first 30 min following blinding but it increased after 5 h and this increase continued for several months. In no case did the normal E.E.G. reappear in blinded fish.

In the normal goldfish the amplitude of the spontaneous activity is maintained and even increased in the dark (Schadé & Weiler, 1959). In the minnow, the change from the light to darkness resulted in an increase in activity possibly corresponding to the 'OFF' discharge of the retina. This high activity was maintained for 15 min or more and may be due to the spontaneous discharge of the retina reported by Granit (1955) in the frog. The activity of the minnow tectum decreased after 30 min in the dark. Adrian & Mathews (1927, 1928*a*, *b*) found a similar decrease in the spontaneous activity of the optic tract of the conger eel, *Conger vulgaris*. They found that the impulses in the optic tract increased rapidly in frequency when the light was turned off and then the rate declined at first rapidly and then more slowly until finally the nerve lost all activity. Finally in the minnow, when the light was turned on the E.E.G. pattern showed a burst of activity possibly corresponding to the 'ON' responses of the retina.

At depths 1, 2 and 3 no difference was found when the background was changed. This can be explained by the fact that this region corresponds to the ventral retinal projection (Gentle, 1968) and background reversal would not effect the illumination of the ventral retina. These layers therefore seem to be both functionally and correspondingly anatomically distinct, namely, they receive the fibres from the optic tract and interpret the visual input. At depths 4 and 5 the H.F. increases by approximately 10 Hz on the black background. Depth 4 is the main efferent fibre layer of the tectum and depth 5 is a layer of small neurones which give rise to the tectal efferent fibres.

To conclude on the E.E.G. and colour in the minnow two points are important. First, in all conditions where the superficial E.E.G. was very reduced the fish darkened; e.g. anaesthetized, blinded, after death and in darkness. Secondly, in the two deepest layers of the tectum there is an increase in the H.F. activity when the fish is placed on a black background and it is in this region where the fibres pass out of the optic tectum to the paling centre in the anterior medulla.

SUMMARY

1. The electrical activity of the optic tectum was recorded from the minnow under various conditions to investigate its relationship to colour change.

2. The superficial E.E.G. was found to consist of two rhythms a 6–14 Hz (20–112 V) and a 18–24 Hz (6–18 μV).

3. When the fish were deeply anaesthetized the E.E.G. was reduced virtually to nothing.

4. Almost no activity was present in the optic tectum 30 min after bilateral blinding. There was an increase in activity after 5 h and this continued for 5 or more days but never returned to normal.

5. In darkness the activity of the superficial E.E.G. first increased and then decreased, and when the eyes were re-exposed to light the activity increased again.

6. The E.E.G. patterns were recorded and analysed from various depths and positions in the optic tectum during background reversal. In the stratum plexiforme et fibrosum externum, plexiforme internum and griseum internum no changes were observed. In the stratum fibrosum profundum and griseum periventriculare an

increase in the high-frequency activity of approximately 10 Hz was observed on a black background.

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REFERENCES

ADRIAN, E. D. & MATHEWS, R. (1927). The action of light on the eye. I. The discharge of impulses in the optic nerve and its relation to electric changes in the retina. *J. Physiol., Lond.* **63**, 378–414.

ADRIAN, E. D. & MATHEWS, R. (1928*a*). The action of light on the eye. II. The processes involved in retinal excitation. *J. Physiol., Lond.* **64**, 279–301.

ADRIAN, E. D. & MATHEWS, R. (1928*b*). The action of light on the eye. III. The interaction of retinal neurons. *J. Physiol., Lond.* **65**, 273–98.

ENGER, P. S. (1957). The electroencephalograms of the codfish (*Gadus callarias*). Spontaneous electrical activity and reactions to photic and acoustic stimulation. *Acta. physiol. scand.* **39**, 37–71.

GENTLE, M. J. (1968). Ph.D. thesis, University of London.

GENTLE, M. J. (1971*a*). The central nervous control of colour change in the minnow (*Phoxinus phoxinus* L.). I. Blinding and the effects of tectal removal on normal and blind fish. *J. exp. Biol.* **54**, 83–91.

GENTLE, M. J. (1971*b*). The central nervous control of colour change in the minnow (*Phoxinus phoxinus* L.). II. Tectal ablations in normal fish. *J. exp. Biol.* **54**, 93–102.

HEALEY, E. G. (1967). Experimental evidence of regeneration of nerve fibres controlling colour changes after anterior spinal section in the minnow (*Phoxinus phoxinus* L.). *Proc. Roy. Soc.* B **168**, 57–81.

LEGHISSA, S. (1955). La struttura microscopia e la citoarchitettonica del tetto ottico des pesci teleostei. *Z. Anat. Entwgesch.* **118**, 427–63.

SCHADÉ, J. P. & WEILER, I. J. (1959). Electroencephalographic patterns of the goldfish (*Carassius auratus*). *J. exp. Biol.* **36**, 435–52.