

FUNCTIONAL DEVELOPMENT OF VISUAL PATHWAYS IN LARVAL SARDINES AND ANCHOVIES¹

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INTRODUCTION

One of the principal purposes of studying the nervous system is to correlate the observed gross and microscopic structure with the dynamic activity of the animal, that is, its behavior patterns. Coordinated swimming movements, feeding, and schooling can be observed in larval fish as soon as the neural pathways, sensory and motor, are sufficiently developed. On the other hand, mating and spawning behavior, which frequently involve extensive migratory movements, require sexual maturity and stimulation from endocrine glands.

This paper describes centers and fiber tracts in the central nervous system of sardine and anchovy which are involved in relaying visual information. Evidence is presented concerning the time during larval development when these morphological features make their first appearance.

Virtually nothing is known about the developing nervous system in sardine and anchovy larvae, and no detailed study of the optic system of these fish exists. Therefore, extensive larval and some adult material of the two species was examined for the present paper.

The species used were *Sardinops caerulea* (Girard) and *Engraulis mordax* (Girard). Isolated brains of adult specimens, fixed in ten percent neutralized formalin, were cut at 10 micra in transverse and sagittal serial sections; all sections were mounted and stained by the Klüver-Barrera (1953) or by the Ziesmer-Bodian (1952) method. Different methods of silver impregnation were tried on larval stages. Best results were obtained with the Ranson-Pyridine (1911) and the Bodian (Ziesmer, 1952) methods.

THE VISUAL SYSTEM IN THE ADULT

In its external form the central nervous system appears typically teleostean, as seen in Figures 1 and 2. That vision is the dominant sense becomes evident when one compares the relatively large optic lobes with the small forebrain and the small cerebellum. The eyes are so large that their median curvatures are adjacent to each other; they have displaced the small forebrain dorso-caudally.

Chiasma opticum and corpus geniculatum laterale

A complete decussation of the optic nerves occurs at the optic chiasma in all fish. The optic nerve displays considerable lamination in sardine and anchovy.

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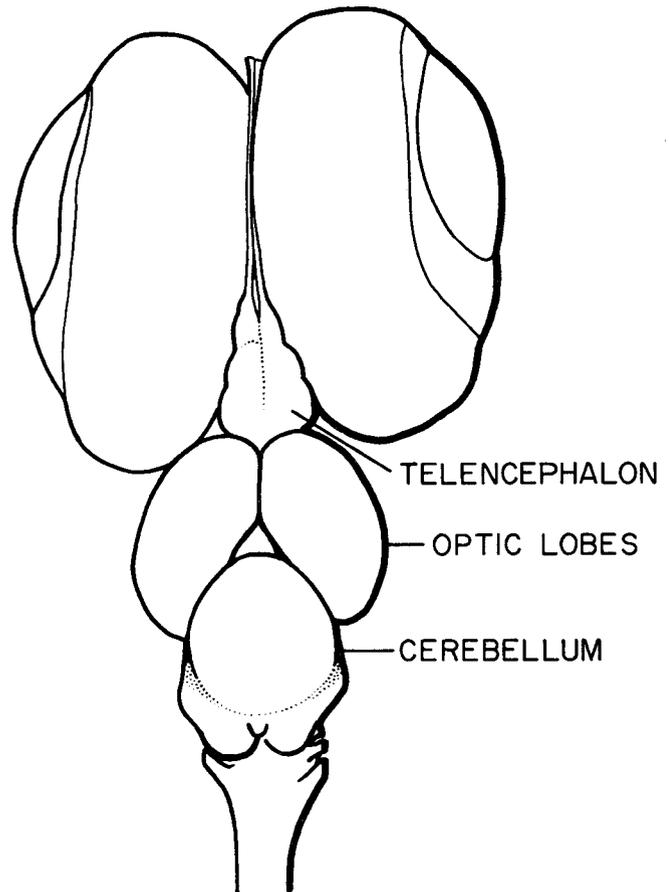


FIGURE 1. Dorsal view of eyes and brain of an adult anchovy.

This pleated condition is common in teleosts, but only in a few species are these bundles known to interdigitate with those of the nerve from the other side. An interdigitating chiasma is common in the herring family as first described for *Clupea harengus* by Weber (1827) where one nerve simply perforates the other. Solger (1877) reported for *Engraulis encrasi-cholus* that four bundles from the right are penetrated by three bundles from the left. An interdigitating chiasma, however, is not confined to the herring family. Gross (1903) noted that in *Abramis* each nerve splits into a large ventral and a small dorsal bundle which interlace with the bundles of the opposite nerve. Lissner (1923) showed that in *Rhodeus amarus* two bundles from the right eye separate three bundles from the left eye. Meader (1934) found similar conditions in *Osmerus* and *Campostoma*. Accord-

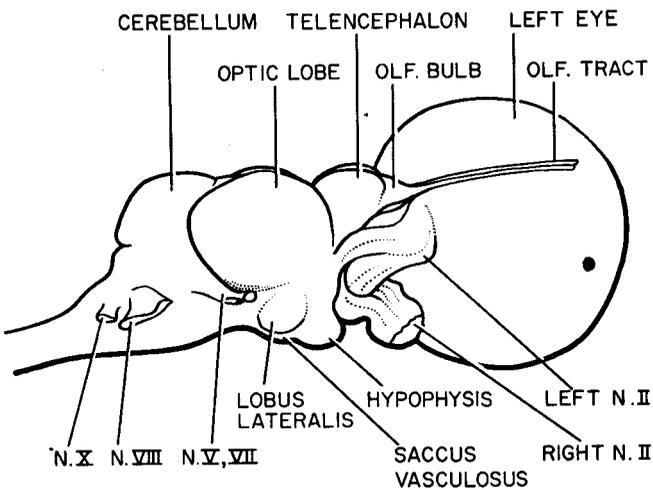


FIGURE 2. Lateral view of the brain of an adult anchovy. The right eye has been removed.

ing to Ströer (1936) in *Salmo salar* each optic nerve consists of two bundles which interdigitate in the chiasma, and which apparently do not re-unite but form the dorsal and ventral optic tracts. The largest number of optic nerve fiber bundles which interdigitate seems to occur in squirrel fish where Meader (1934) described from six to nine mutually perforating bundles in *Holocentrus*.

Each optic nerve of *Engraulis mordax* consists of six or seven laminae. In the chiasma these individual strands do not all separate and interweave with those from the other side. Three bundles often penetrate four of the other nerve (Fig. 3), or three are separated by two of the nerve from the other eye.

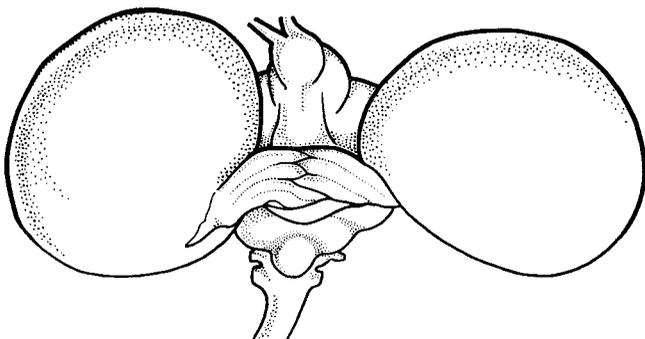


FIGURE 3. Frontal view of eyes and brain of an adult anchovy, showing the optic chiasma. Three bundles of the optic nerve from the right eye penetrate four bundles of the left nerve.

After the complete decussation in the interdigitating chiasma, the individual laminae of either side unite again where the optic tracts penetrate a thin bony capsule before entering the diencephalon. At this level we can see that the optic tract separates into two main portions, the tractus opticus dorsalis (medialis) and the tractus opticus ventralis (lateralis) (Fig. 4). The dorsal optic tract invades the fronto-dorsal portion of the optic tectum, whereas the ventral tract continues farther caudad and branches into

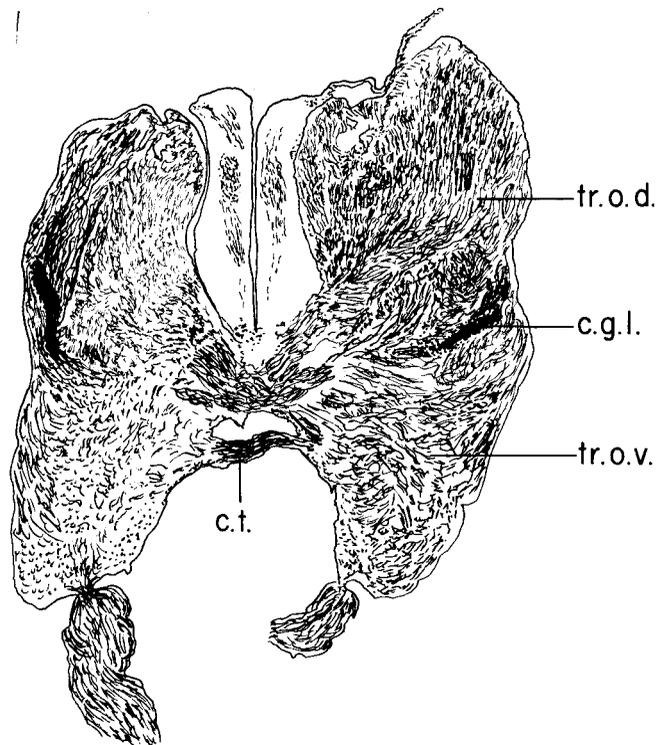


FIGURE 4. Transverse section through the brain of an adult anchovy at the level of the lateral geniculate nucleus (Bodian stain—30x). Tr. o. d.: dorsal optic tract; tr. o. v.: ventral optic tract; c. g. l.: lateral geniculate nucleus; c. t.: transverse commissure. The last bundle of the ventral optic tract passes through a thin bony lamella into the cranial cavity (constrictions).

the latero-caudal parts of the tectum. A small bundle branches off the dorsal tract medially and continues caudad where it joins the commissura minor. This is the fasciculus medialis nervi optici. (Bellonci, 1888; Kappers, 1906). The most lateral fibers form the fasciculus geniculatus tracti optici (Meador, 1934) and go to the corpus geniculatum laterale. Some apparently pass around this nucleus but many seem to terminate there, or form collaterals (Fig. 4). The geniculate fascicle is not clearly separated from the main dorsal tract, but its course can easily be observed for a certain distance by following this tract distally from its endings in the geniculate body.

The corpus geniculatum laterale is one of the most conspicuous optic centers and can be found throughout the vertebrate series. In mammals it becomes the relay station for visual impulses to the cortical representation area, and the tectal connection diminishes. In fish this center does not show the highly laminated structure that it does in some specialized mammals, although it can be folded in some (Franz, 1912). Franz could not identify the lateral geniculate body in *Ameiurus* and certain other bottom dwelling fish, but it is present in all teleosts with well developed vision.

In *Sardinops* and *Engraulis* the lateral geniculate extends very far rostrad, where all optic nerve fibers are still tightly grouped together, and it can be seen in the lateral wall of the dorsal optic tract as a thin lamella. A little farther caudad, its long axis which

was almost vertical changes to a dorsolateral-ventromedial direction (Fig. 4). The caudal portion of the lateral geniculate body is almost horizontally oriented. This nucleus consists of a very dense net of fibers intermingled with a few cells and enveloped by a layer of small cells. In sections, one can see that fibers of the geniculate fascicle stream predominantly into the ventral portion of the lateral geniculate body.

Tectum opticum and torus longitudinalis

In all fish which are equipped with good vision the optic tectum is a dominant part of the brain. As the name designates, the optic tectum covers the basal parts of the midbrain like a roof and receives visual information. Phylogenetically, it maintains this highly developed state through birds. In mammals, however, the tectum becomes reduced in relative size because of the greatly enlarged telencephalon; but, as superior colliculus, it probably retains the former reflex functions. Recapitulating phylogeny, the midbrain roof is found as a dominating part of the brain in the embryonic development of mammals. In teleost fish the optic tectum shows a greater differentiation in its fiber architecture than does any other part of the central nervous system.

The optic tectum has been studied extensively and can be divided into various layers. Ramon (1899) described ten strata, and, more recently, Leghissa (1955) distinguished seven layers which can be recognized in all teleosts. Beginning from the optic ventricle towards the surface, they are: 1) stratum griseum periventriculare, 2) stratum fibrosum profundum, 3) stratum griseum internum, 4) stratum plexiforme internum, 5) stratum griseum externum, 6) stratum

plexiforme et fibrosum externum, 7) stratum fibrosum marginale (Fig. 5).

Afferent fibers from retinal ganglion cells spread over the surface of the contralateral tectum. The course of these myelinated axons can be followed in the outer layers, the separation of which is not very distinct in sardine and anchovy. According to Leghissa (1955) retinal fibers of the dorsal optic tract are mixed with olfactory fibers of the strio-tectal tract in these layers. Optic terminal fibers curve sharply towards the inside where they synapse, mostly in the plexiform and fibrous layer, with dendrites of deeper situated neurons. Some fiber strands traverse the entire tectum obliquely from the superficial layer to the deep fiber layer. Leghissa (1955) claims that these fibers are efferent from the tectum and are axons from marginal neurons in the external plexiform and fiber layer. These fiber bundles are especially numerous in the more caudal portion of the tectum (Fig. 6). They join other fiber bundles in the deep fibrous layer, most of which course medially and cross over to the contralateral tectum as the fasciculus commissuralis intertectalis, or they have synapses in the contralateral torus longitudinalis.

The torus longitudinalis is a paired median structure which connects the tectal halves in the midline. Present in most teleosts, although rather small in some like anchovy and sardine (Fig. 5), the torus longitudinalis begins rostrally at the commissura posterior and becomes a paired structure caudally. At its anterior pole the torus can be seen to incorporate the fibers from the pars dorsalis of the posterior commissure (Fig. 7). Kudo's (1923) claim that axons of granule cells of the torus enter superficial tectal lay-

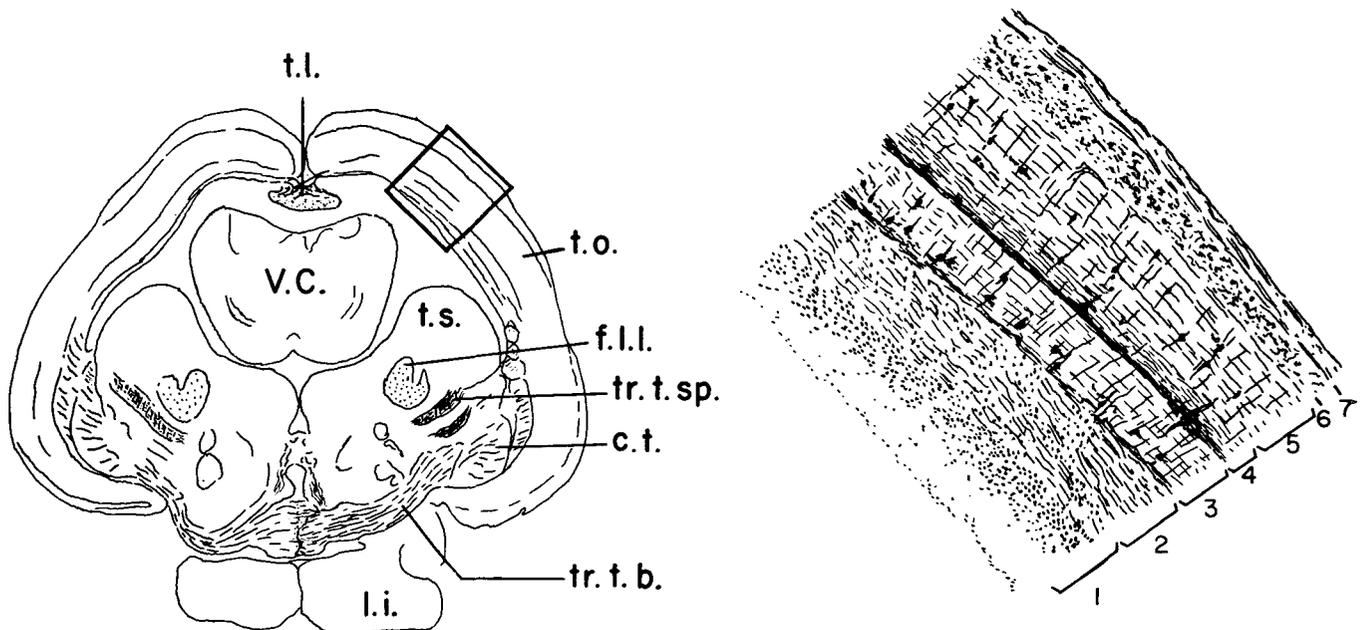


FIGURE 5. Transverse section through the midbrain of an adult anchovy, diagrammatically on the left (30x). Higher magnification of part of the optic tectum on the right (Bodian stain—100x). t.l.: torus longitudinalis; t.o.: optic tectum; t.s.: torus semicircularis; v.c.: valvula cerebelli; l.i.: inferior lobe of thalamus; f.l.l.: fasciculus longitudinalis lateralis (lateral lemniscus); tr. t. sp.: tecto-spinal tract; tr. t. b.: tecto-bulbar tract; c.t.: transverse commissure. Numbers 1 to 7 indicate the seven strata of the optic tectum.

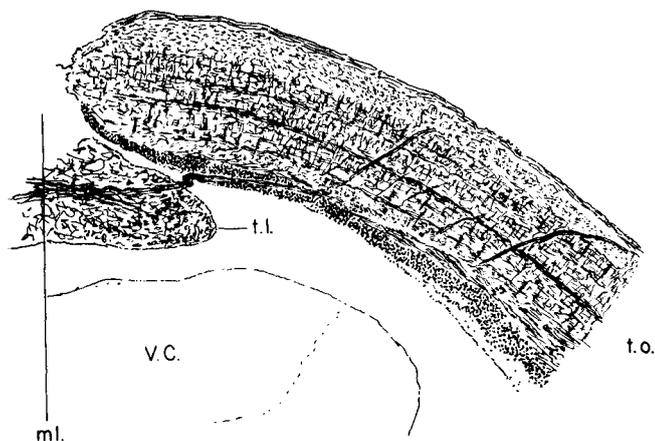


FIGURE 6. Transverse section through the optic tectum 550 micra farther caudad than Figure 5. (Bodian stain—50x). ml.: midline; other abbreviations see legend to Figure 5. Oblique bundles of fibers from layers 6 or 7 can be seen streaming into the efferent fiber layer.

ers, was disputed by Leghissa (1955). P. Ramon actually showed that axons of neurons in the torus enter the stratum opticum of the ipsilateral tectum (Ramon, 1899; Fig. 4). Our material does not show such connection. Axons of torus neurons form fiber bundles which stream rostrally and seem to leave the torus with the pars dorsalis of the posterior commissure. The connections of this dorsal part of the posterior commissure had been the cause of dispute (Kappers, Huber, Crosby, 1936, page 919). Leghissa (1955) describes part of the dorsal portion entering the tractus mesencephalo-cerebellaris anterior. The torus longitudinalis is a major visual center, interrelating visual

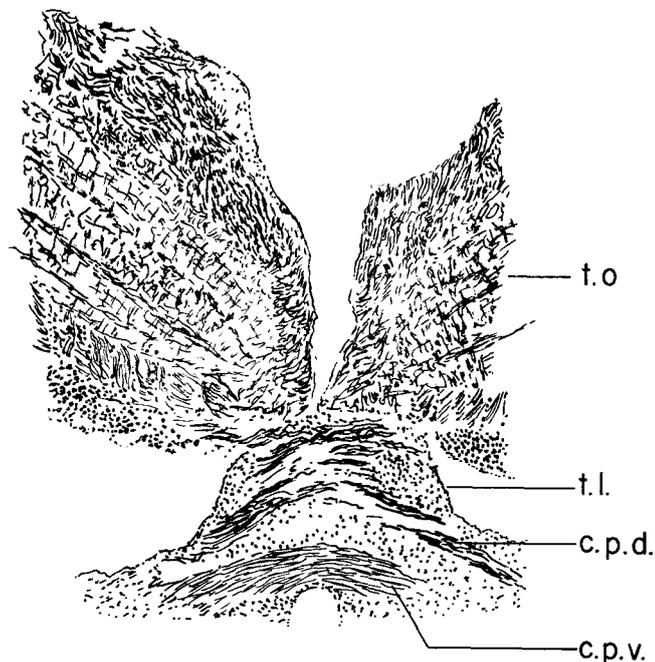


FIGURE 7. Transverse section through the anterior portion of the optic tectum (Bodian stain—100x), t.o.: optic tectum; t.l.: torus longitudinalis; c. p. d.: dorsal part of posterior commissure; c. p. v.: ventral part of posterior commissure.

information between the two lateral optic tecta, and may also play a role in correlating visual with gravistatic information, as suggested by the connection with the cerebellum.

There is extensive differentiation of efferent tracts. The tractus tectobulbaris is a large bundle which crosses as the commissura ansulata before proceeding to the medulla (Fig. 5). Another is the tractus tecto-spinalis which leads to the spinal cord. The commissura transversa receives tectal fibers, some of which connect to the ganglion isthmi.

Other nuclei concerned with vision

In addition to the geniculate body, the tectum opticum and the torus longitudinalis, there are several smaller nuclei situated in the basal mesencephalon and the thalamus. Almost all of these are concerned with relaying visual information because they have connections with the above three main centers and also with each other. Unfortunately, the nomenclature of these nuclei is in a disorderly state. Many apparently homologous structures were given different names by different authors and often the same name was applied to different structures (Kappers, Huber, and Crosby, 1936, page 925). We wish to mention only a few which are easily identified in our sections.

The corpus glomerulosum pars anterior (Franz, 1912; nucleus anterior thalami of earlier authors) lies caudal and slightly dorsal to the lateral geniculate (Fig. 8). It is connected with the latter and also with the tectum. A pars rotunda of this usually elongated corpus glomerulosum complex is absent in sardine and anchovy and apparently is missing in all physostomes which have been examined.



FIGURE 8. Transverse section through the right ventral midbrain and thalamus (Bodian stain—50x). t.o.: optic tectum; n.i.: nucleus intermedius; n.a.: nucleus anterior (corpus glomerulosum pars anterior); tr. o. v.: ventral optic tract.

Another optic relay station in close proximity to the geniculate and pars anterior of the glomerulosum complex is the nucleus intermedius (Goldstein, 1905; Brickner, 1929) which receives a loose bundle from the optic tract and also connects to the tectum and the geniculate (Fig. 8).

DEVELOPMENT OF VISUAL STRUCTURES IN THE LARVA

An attempt was made to determine on which day after hatching visual structures make their first appearance. This was not easy because of the very small size of the larvae and because of the difficulty in staining the first fine nerve fibers. The larvae were hatched and reared at 18° C. All yolk sac larvae were *Sardinops*, the later stages *Sardinops* as well as *Engraulis*.

On the first day, the day of hatching, neurons are not yet differentiated; migration of neuroblasts in the mesencephalon has not begun. The lateral optic vesicles have formed a double layer and show the first signs of lens formation in their lateral walls. The cells in the lens placode are arranged spherically (Fig. 9). There is no differentiation of cells in the inner retinal layer of this secondary optic vesicle.

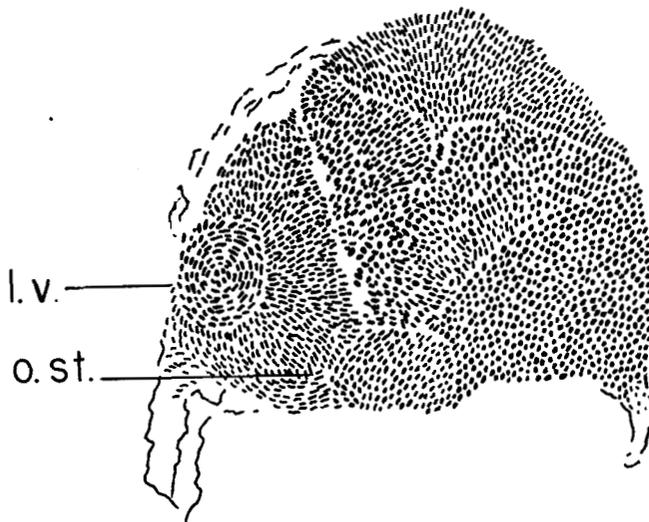


FIGURE 9. Cross section through the brain of a larval sardine on the day of hatching (Bodian stain—200x). l.v.: lens vesicle; o.st.: optic stalk.

On the second day (average length 4.93 mm) the eye is very advanced; pigment is present, visual cells are developed and the stratification of the retina into different layers is progressing (Fig. 10). At this stage no optic nerve fibers can be seen. The dorsal mid-brain shows a layer of nerve fiber endings; however, these may not be visual but somatic afferent. According to Leghissa (1955) the latter reach the tectum before retinal fibers. The gut of the two day old is still closed and yolk is present.

At three days of age (average length 5.27 mm) the cells of the bacillary layer in the retina have a greenish outer segment as in the eye of the adult fish. This probably indicates the presence of visual pigment. Optic nerve fibers leaving the eye and decussating with fibers from the other eye can be observed (Fig. 11). These visual fibers can be followed into the tectum where the fibrous layer now appears greatly enlarged. The foregut is opening in the three day old larva although the yolk sac has not been completely absorbed.

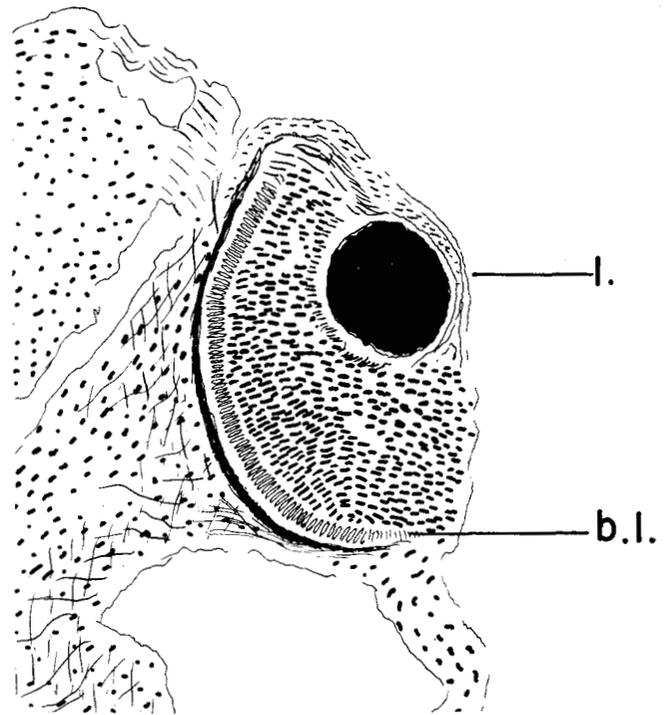


FIGURE 10. Cross section through the eye of a two-day old sardine (Bodian stain—300x). lens (l) and bacillary layer (b. l.) are well developed.

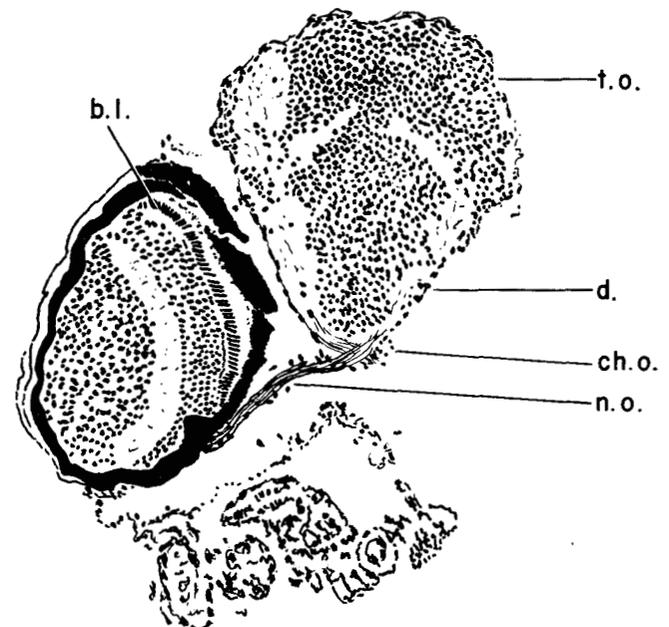


FIGURE 11. Three-day old sardine. Cross section through eye, thalamus and developing midbrain (Bodian stain—200x). b.l.: bacillary layer; n.o.: optic nerve; ch. o.: optic chiasma; d.: diencephalon; t.o.: optic tectum.

In the five day old larva (average length 5.56 mm), optic nerve fibers are well myelinated and appear yellowish-brown in Ranson-stained material (Fig. 12). In this section we can detect the first indication of an interdigitating chiasma. A small ventral bundle

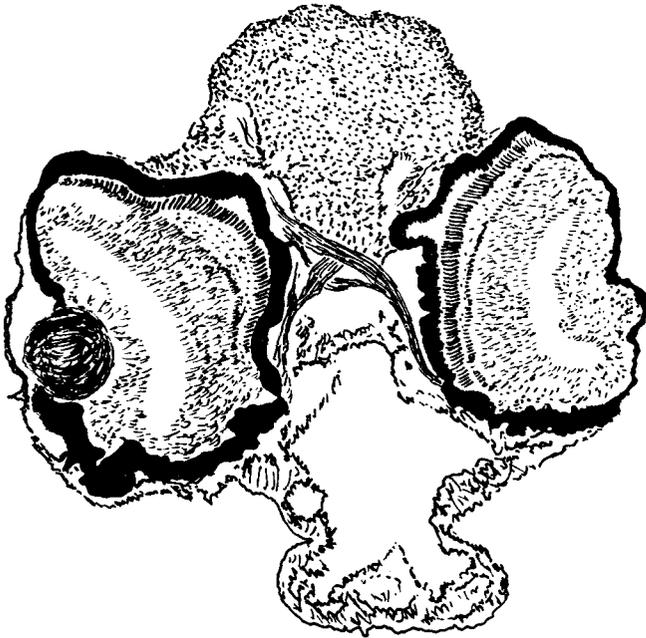


FIGURE 12. Five-day old sardine. Cross section through the head at the level of the optic chiasma (Ranson stain—200x). The optic nerve from the left seems to perforate the nerve from the right.

branches off the main part of the nerve and runs ventral to the nerve from the left eye. Retinal fibers can be easily followed in their course through the lateral portion of the diencephalon into the optic tectum.

The later development and differentiation of the visual system proceeds at a slower pace. The number of optic nerve fibers increases; the superficial part of the optic tectum becomes thicker as the different layers develop, whereas the underlying matrix of undifferentiated nerve cells decreases in size. This cellular layer is mostly concentrated dorsomedially where the torus longitudinalis will form. The torus longitudinalis appears in its final form relatively late, although fibers from the efferent fiber stratum connect the two tectal halves at an early stage. One of the earliest visual centers to develop is the lateral geniculate nucleus which can be detected in the five day old larva. However, the dense net of unmyelinated fibers inside this nucleus can be seen in later stages only.

PHYSIOLOGICAL CONSIDERATIONS

Nerve fibers from retinal ganglion cells connect to the optic tectum in three day old larval sardines reared at 18° C. At this stage the young larva appears to begin feeding on its own; the yolk is almost absorbed and the foregut is opening. The eyes and connecting neural pathways are slowly assuming their important role in providing necessary information about the surrounding environment. An important question is how well the young larva is able to see with a visual system at such an early developmental state. There are about 40 individual fibers in the optic tract of the five day old larva and these fibers

terminate in a relatively small dorsolateral area of the contralateral tectum. It is known from experimental histological studies on the visual system of fish (Lubsen, 1921; Akert, 1949) that there is a topographic projection of the retina onto the optic tectum. In this work small lesions are made in different parts of the retina. The axons of destroyed ganglion cells degenerate, as do their myelin sheaths, and the degenerating myelin impregnates better with osmium salts than does normal myelin. The course of degenerating nerve fibers can subsequently be followed to an approximate location on the optic tectum. The details and the precision of this orderly projection have been worked out for several fresh water fish (Schwassmann and Kruger, 1965). Objects in the dorsal field of view which stimulate the ventral retina are projected to the medial portion of the contralateral tectum. The anterior visual field is represented rostrally on the tectum, whereas the posterior field can be detected in the caudal portion and the ventral field projects to the lateroventral portion. Receptive fields of individual ganglion cells were found to be very small (many less than four degrees) corresponding to an area of about one third of a millimeter in diameter on the retina of a 10 mm eye.

Obviously, true shape perception and good visual acuity depend on a precisely defined topographical projection of a great number of retinal axons onto the optic tectum. These requirements are certainly not fulfilled in the larva of only five days. The young sardine at the beginning of the feeding stage might be capable of a very coarse type of movement perception. Food particles might be detected by a partial shadowing effect on the eye, and their direction could be determined by alternate and simultaneous stimulation of both eyes.

Some indirect evidence that the precise retino-tectal projection is functional only at a later stage after feeding has already begun is found in Székely's studies with *Triturus* larvae (Székely, 1954). He exchanged different halves of the embryonic retinae resulting in a characteristic reversal of the corresponding visual fields which he noted by observing the feeding behavior. However, this reversal occurred only after the larva had been feeding normally for one week.

Only behavioral studies with young sardine larvae can provide information about the role of the early visual system in the detection and capture of food. The fact that the visual pathways and centers are operational toward the end of the yolk sac stage means that vision begins to play an important role in the life of the growing larva.

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