Odonatologica 16(1): 25-35 *March 1, 1987*

POSTEMBRYONIC DEVELOPMENT OF THE OPTIC LOBE IN *LESTES EURINUS* SAY: MORPHOLOGY (ZYGOPTERA: LESTIDAE)

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Received January 28, 1986 / Revised and Accepted April 3, 1986

The postembryonic development of the visual apparatus was traced from early larval instars to the adult stage. During the larval life, the optic lobe shows minor changes in its structure. It consists of 3 neuropiles interconnected through 2 chiasmata. The development of the optic lobe is the result of the mitotic activity of the outer and inner optic anlages. This activity decreases during the last instar larva and eventually stops. The anlages have completely disappeared in the adult stage.

INTRODUCTION

LAVOIE et al. (1981) and LAVOIE-DORNIK et al. (1981) have shown that there was in the literature an impressive number of investigations on arthropod vision, and pointed out the importance of such research in insects in general (MEINERZTHAGEN, 1973, 1975, 1976, 1977) and particularly in such groups as Diptera (FRÖHLICH & MEINERTZHAGEN, 1982; HAUSEN et al., 1980; HORRIDGE & MEINERTZHAGEN, 1970; NICOL & MEINERTZHAGEN, 1982a, 1982b), Orthoptera (KIEN, 1974a, 1974b) and Hymenoptera (RIBI, 1975a, 1975b, 1976, 1977, 1979). They also emphasized that the work in this field in Odonata was not as significant as in other groups in spite of an increasing interest in recent years, most of which has been involved with Anisoptera (VIALLANES, 1884; ZAWARZIN, 1914; RICHARD & GAUDIN, 1959; MOUZE, 1972, 1974a, 1974b, 1975, 1978a, 1978b, 1979, 1980; MEINERTZHAGEN et al., 1980; ARMET-KIBEL et al., 1977; MEINERTZHAGEN & ARMET-KIBEL, 1982). In Zygoptera, even though some authors have studied the ultrastructure of the compound eye (NINOMIYA et al., 1969) or its histology and morphometry

(LAVOIE et al., 1978a, 1978b, 1984), there are so far no detailed studies on the histology and morphology of the optic lobe.

The objective of this study is to describe the morphology of the optic lobe in *Lestes eurinus* from early larval instars to the adult stage.

MATERIAL AND METHODS

Specimens came from laboratory rearings (PELLERIN & PILON, 1978) where larvae were obtained from eggs and reared individually under constant photoperiod and temperature. There were 15 larval instars.

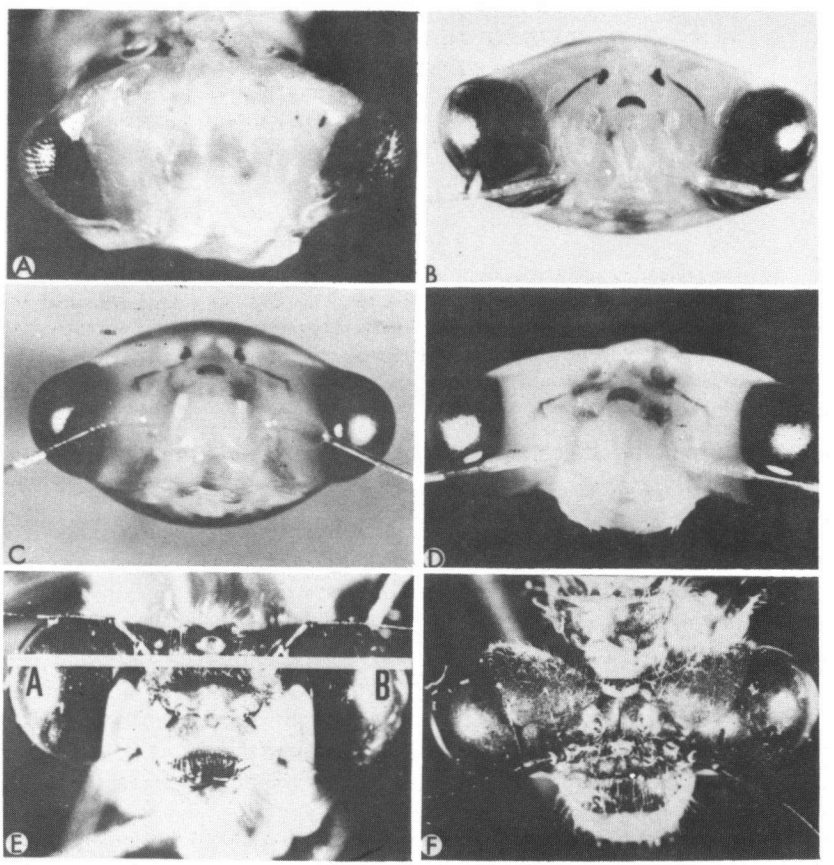

Fig. 1. Evolution in the shape of the head and in the position of the compound eye during post-embryonic development in *Lestes eurinus:* (A) 6th instar; — (B) 9th instar; — (C) 11th instar; — (D) 14th instar; — (E) adult, frontal view; — (F) adult, dorsal view. — [AB: plane for histological cuts (parallel to AB in adults, perpendicular to AB in larvae); larval labial mask removed].

Larvae were beheaded in a Susa Heidenhain solution (GABE, 1968) and left in this fixative for 24 hours. After dehydration, heads were stored for more than one month in normal butylic alcohol to soften the cuticle. They were then impregnated under vacuum conditions and imbedded in Paraplast plus at 60° C. Depending of the developmental stage they were then cut into 4 to 15 μm serial sections. In the case of larval heads, sections were made along a transversal plane while, in the case of adult heads, they were made along a frontal plane (Fig. 1E). The reason for this is that larvae have a prognathous head, while in the adult stage the head is of the orthognathous type, therefore the two planes have to be used to follow the change of orientation of the visual apparatus in metamorphosis. Sections were stained with Mallory blue before examination, using a light microscope Leitz laborlux 12; the photomicrographs were obtained with a light microscope Axiomat.

In the case of third instar larvae, due to their small size, fixative techniques for electron microscopy had to be applied before examination with a light microscope. In this case, larvae were beheaded in a 3% solution of glutaraldehyde (0.12 M) and left in this fixative for one hour at 25° C. After being rinsed in a phosphate-buffered solution (0.4 M; pH 7.4) three times for 10 minutes, heads were postfixed in 1% osmium (0.12 M ; 7% dextrose) for two hours and then rinsed in distilled water. After dehydration, tissues were impregnated (SPURR, 1969) and imbedded in Spurr, sections (0.3-1.0 μm) were stained with toluidine blue.

Scanning-electron microscope preparations were obtained using a standard method (HAYAT, 1978). Preparations were then gold coated and examined under a JEOL JSM-35 scanning-microscope.

OBSERVATIONS AND DISCUSSION

The visual apparatus of *L. eurinus* is included in a head capsule, flattened dorso-ventrally (Fig. 2B). This characteristic shape of the head is more pronounced as development towards the adult stage is progressing. In early instar the compound eyes look like small dark buds on each side of the head. As development is proceeding, the number and the size of ommatidia increase, so that each compound eye in the adult stage is a voluminous structure (Fig. 1E, F), though the zygopteran eyes never meet medially, as is the case in some Anisoptera, and never reach the size of anisopteran eyes.

STRUCTURE OF THE OPTIC LOBE

The optic lobe is a relatively constant morphological structure in Odonata. It is formed by a complex of nervous structures situated between the compound eyes and the brain (Fig. 2).

From the eye towards the centre of the head, the optic lobe is made up of a certain number of structures. First, there are the post-retinal fibres produced by new ommatidia. These fibres form bundles growing in a centripetal direction, innervating the first optic ganglion (lamina), and transmit visual information to this ganglion. In growing these bundles are going through an extracellular space, the size of which varies according to the developmental stage. This space is more important in early larval instars. Before reaching the second optic ganglion (medulla), these nervous fibres, showing different path patterns, are responsible

for the formation of the external chiasma. From the medulla, a third series of fibres with crossed axons forms the internal chiasma and grows towards the third optic ganglion (lobula). From this last synaptic zone, a fourth series of fibres (optic nerve) emerge to connect with the brain protocerebron.

In Anisoptera as well as in Zygoptera there are always three visual neuropiles:

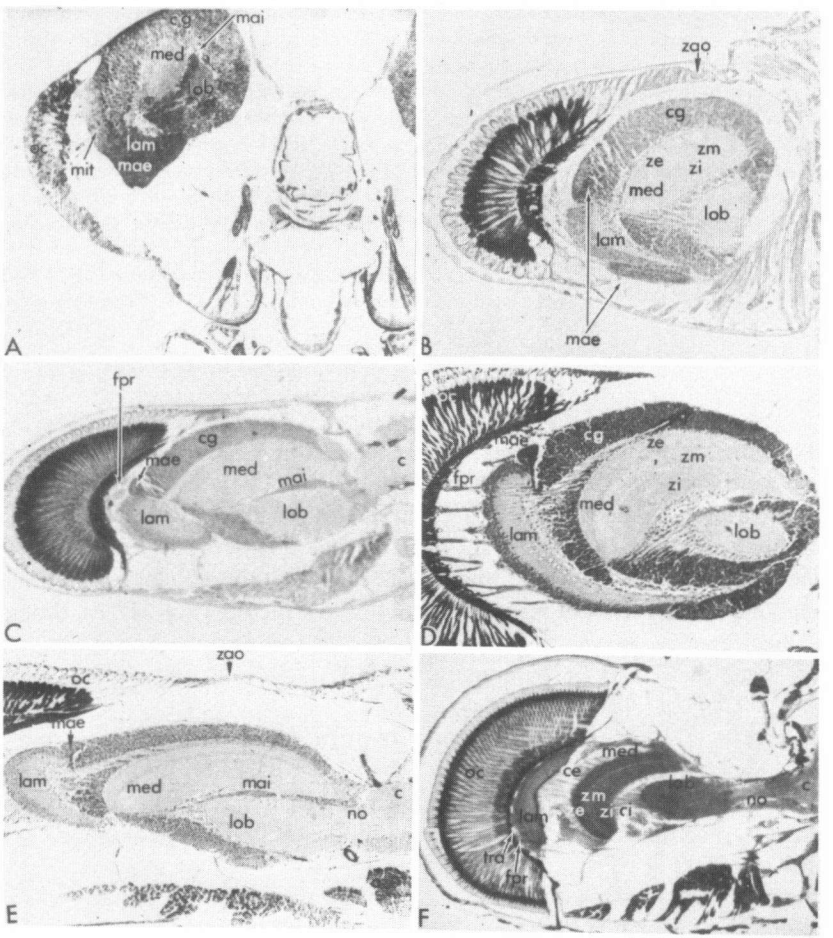

Fig. 2. Evolution of the structure of the visual apparatus during postembryonic development in *Lestes eurinus:* (A) 3rd instar (× 34); — (B) 6th instar (× 30); — (C) 10th instar (× 20); — (D) 14th instar (× 18); — (E) 15 th instar (× 18); — (F) adult (×54); — [C: brain; — ce: external chiasma; — ci: internal chiasma; — cg: ganglion cells; — fpr: post-retinal fibres; — lam: lamina; — lob: lobula; — mae: outer optic anlage; — mai: inner optic anlage; — med: medulla; — mit: mitosis; — no: optic nerve; — oc: compound eye; — tra: trachea; — zao: ocular growth zone; — ze: external zone; — zi: internal zone; — zm: median zone].

lamina, medulla and lobula, which are of about the same shape and in the same position in all species (LAVOIE et al., 1978a, 1978b,; MOUZE, 1972).

In early instars, the three neuropiles are already formed although it is difficult to differentiate the ganglion cells and which neuropile they belong to (Fig. 2a). In these instars the optic lobe is a compact mass, leaning against the head dorsal epidermis. It appears as if only one bundle of post-retinal fibres connects a compound eye to the optic lobe. In the sixth larval instar, the whole of the optic lobe is somewhat spherical in shape and a clockwise movement of the whole apparatus has already begun (Fig. 2B).

Whereas the general shape of the optic lobe is somewhat spherical in early instars, it tends to become more and more flattened as larval development proceeds towards metamorphosis. During this period, the optic lobe is horizontally stretched between the eye and the protocerebron, thus causing its dorso-ventral flattening (Fig. 2E). It is during the metamorphosis that the greater changes take place because of the change from prognathous to orthognathous head orientation.

STRUCTURE OF THE OPTIC GANGLIONS

Lamina

The lamina is the first neurophile on the path of the post-retinal fibres (Figs 2-3) to the first synaptic zone (RIBI, 1975a, 1975b, 1976, 1977, 1979). It is the most ventral of the neuropiles. Its somewhat oval shape in early instars is different from the characteristic blade shape in the more mature larvae. The lamina is bounded laterally by the outer optic anlage. At the sixth instar the structure of the lamina is typical and consists of three different zones (Fig. 2B). The external zone receives the fibres from the compound eye. Their number increases as new ommatidia are being added to the eye. This zone contains the neuron bodies, with which neurommatidia or ganglion cells are in juxtaposition. These neurommatidia are the ones receiving the visual information from the eye, since the nervous fibres from each ommatidium are connected with these receptive cells. The median zone is of about the same thickness as the external one, but is fibrous in nature and forms the core of the lamina. It is delicately striated, the striae being axially orientated. The internal zone is a thin fibrous layer, with some sparsely spread nuclei.

As larval development proceeds towards metamorphosis, the lamina progressively moves away from the optic lobe towards the compound eye, thus reducing more and more the extracellular space. The lamina flattens, but increases in volume. The median zone is becoming more important in size than the external zone. The lamina is almost parallel to the eye in the 11th instar.

In the last two instars, the lamina occupies an intermediate dorso-ventral

position between the eye and the protocerebron and looks like being suspended from the outer optic anlage (Fig. 2D, E). The lamina continues its displacement towards the eye and adopts the eye curvature. Because of this, the post-retinal fibres get more and more contracted. Due to the greater number of ommatidia there are a greater number of bundles innervating this neuropile.

During metamorphosis the lamina approaches the compound eye so that in the adult stage it is intimately leaning against it (RIBI, 1975a, 1975b, 1976, 1977, 1979; MEINERTZHAGEN, 1975). Contrary to what has been reported for Anisoptera (MOUZE, 1972, fig. 2F), the structure of the lamina in Zygoptera is not modified in an important way. There are only some minor changes in shape and in position but it is always composed of three zones. In the internal zone the nuclei have disappeared but the fibrous nature still exists.

Medulla

The medulla in the early instars is oval in shape and situated on the dorsal side of the optic lobe; in more mature instars it takes on a more flattened shape (Fig. 2A). In the last instar the medulla is dorso-ventrally flattened and also adopts the curvature of the eye (Fig. 2E). During metamorphosis the medulla is situated nearer to the eye than previously because it still follows the displacement of the lamina.

The medulla is more voluminous than the lamina and covered with a thick layer of ganglion cells. The concave side faces the brain and the convex side the lamina. Although this ganglion is formed of many finely striated layers, three concentric zones are readily distinguished (Fig. 2D): an external zone receiving the nervous fibres from the external chiasma; a median thin zone formed of two alignments of small nuclei; and an internal zone from which nervous fibres innervate the lobula through the internal chiasma.

There are two types of connections between the lamina and the medulla. The first type is axonal in nature and consists of bundles of nervous fibres which form the external chiasma. The second type is through the outer optic anlage which consists of an aggregation of neuroblasts. This anlage connects the lamina and the medulla through its internal and external margins. It is a very active centre of cell proliferation, insuring the continuous growth of these two ganglions throughout the larval life.

Lobula

In early instars this third neuropile looks like a small drop suspended from the medulla (Fig. 2A). It is the ganglion closest to the brain and its neuropilar content appears homogeneous. At this stage the lobula is of greater size than the lamina but it is smaller than the medulla. Here also it is difficult to ascertain to which

neuropile the ganglion cells belong, as the whole optic lobe appears like a compact mass. In late instars, the oval shape is retained, although somewhat flattened (Fig. 2D). This neuropile also retains its original position in relation to the other two neuropiles. This third optic ganglion does not undergo as much transformation as the other two neuropiles. In fact, it is the only one to retain its original position in relation to the brain.

BULLOCK & HORRIDGE (1965) pointed out that the lobula could consist of four different zones on account of the four different types of axons found in this neuropile. This study shows that the lobula may be divided in three easily recognizable zones, like the other two neuropiles, which are of the same composition except for a smaller number of ganglion cells.

The nervous connection between the lobula and the medulla is axonal in nature. The crossed axons of the nervous fibres form the internal chiasma (Fig. 3). Some nervous fibres emerge from the lobula to form the optic nerve which innervates the protocerebron (Fig. 2E).

The outer optic anlage

In early instars the outer optic anlage is quite voluminous and occupies a greater surface than in late instars when compared to the other components of the optic lobe (Fig. 2A). It does not have the characteristic shape it will take in late instars but simply consists of a mass of mitotic cells, sign of great mitotic activity. It holds a dorso-lateral position in the optic lobe, quite far from the ocular growth zone.

In more mature larvae, this anlage presents its characteristic shape with well defined external and internal margins which are asymmetrical (Figs 2D, 3A). The external margin contributes cells to the lamina, the thicker internal margin to the medulla. The latter cells are richer in chromatin than the former ones. This type of growth of the lamina and the medulla is at the origin of the formation of the external chiasma. As the lamina grows in a ventral direction, the medulla in a dorsal direction (MOUZE, 1972, 1980), the nervous fibres have to cross each other.

In the last instar the anlage is situated dorsal to the lamina so that the displacement of the latter towards the eye brings it closer to the eye growth zone (Fig. 2E). It is in this final instar that the mitotic activity decreases until eventually it ceases completely. This cessation of activity brings the complete disappearance of this anlage in the adult stage (Fig. 2F).

The inner optic anlage

The inner optic anlage in early instars (Fig. 2A) is dorso-ventrally flattened between the medulla and the lobula. At this stage its volume in relation to the

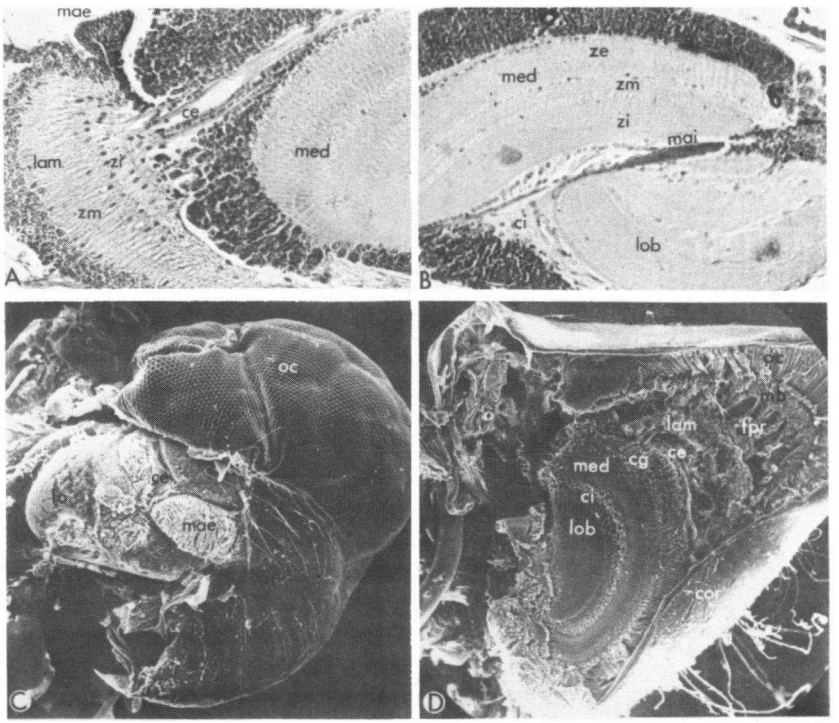

Fig. 3. Structure in a 14th instar larva of the outer optic anlage (A: × 45) and of the inner optic anlage (B: × 37), and a scanning electron microscope aspect of the visual apparatus of a 15th instar (C: × 37; D: × 33). — [ce: external chiasma; — cg: ganglion cells; — ci: internal chiasma; — cor: cornea; — fpr: post-retinal fibres; — lam: lamina; — lob: lobula; — lo: optic lobe; — mae: outer optic anlage; — mai: inner optic anlage; — mb: basal membrane; — med: medulla; — oc: compound eye; — ze: external zone; — zi: internal zone; — zm: median zone].

other structures of the optic lobe is important, although it is less voluminous than the outer optic anlage. During the larval life its development is similar to the latter. Being positioned between the medulla and the lobula, it has to follow the displacement of the optic lobe as a whole. That is why it becomes more and more flattened dorso-ventrally (Fig. 2E). As is the case with the outer optic anlage, its mitotic activity, decreasing to a standstill during the last larval instar, results in its complete disparition in the adult stage (Fig. 2F).

CONCLUSION

The optic ganglions formed during the embryonic development rapidly develop during larval life because of the mitotic activities of the outer and inner

optic anlages. These anlages are active throughout larval life but this activity decreases in the last instar larva to a complete standstill. In the adult stage, these anlages completely disappear.

During post-embryonic development the optic lobe is subjected to modifications in shape, position and structure. For example, its shape and position between the compound eye and the brain vary from one larval stage to the other. The extracellular space between the post-retinal zone and the first neuropile is very important in early instars. But during the larval development the lamina tends to be closer to the photoreceptors of the eye, therefore this extracellular space looses its importance and shrinks, while the post-retinal fibers are growing shorter.

During the imaginal metamorphosis the change of orientation of the head from prognathous to orthognathous brings about modifications in the visual apparatus.

ACKNOWLEDGEMENTS

This research has been supported by the National Research Council in Natural Sciences and Engineering of Canada and by the "Cooperation Franco-Québécoise (programme intégré 01-02-40-70)".

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