Pre-existing neuronal pathways in the developing optic lobes of Drosophila

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Summary

We have identified a set of larval neurones in the developing adult optic lobes of Drosophila by selectively labelling cells that have undergone only a few mitoses. A cluster of three cells is located in each of the optic lobes near the insertion site of the optic stalk. Their axons fasciculate with fibres of the larval optic nerve, the Bolwig’s nerve, and then form part of the posterior optic tract. These cells are likely to be first order interneurones of the larval visual system. Unlike the Bolwig’s nerve, they persist into the adult stage. The possibility of a pioneering function of the larval visual system during formation of the adult optic lobe neuropil is discussed.

Key words: Drosophila, selective labelling, pioneer neurones, optic lobes.

Introduction

The larval and imaginal stages of a holometabolous insect are essentially different with respect to morphology and behaviour. The larval structures are created during embryogenesis whereas the adult structures are formed during postembryonic development. The transformation of the larva into the adult also requires a complex remodelling of the nervous system. The adult nervous system is generated by addition of new neurones as well as reorganization of some larval elements and the degeneration of others (Heywood, 1965; Nordlander & Edwards, 1968; White & Kankel, 1978; Technau & Heisenberg, 1982; Fischbach & Technau, 1984; Booker & Truman, 1987; Truman & Bate, 1988). Addition of new neurones in the central nervous system (CNS) occurs during larval life by the proliferation of a stereotyped array of stem cells (‘neuroblasts’; Booker & Truman, 1987; Truman & Bate, 1988). Differentiation of these neurones is initiated in the larva and continues into the pupal stage. The newly growing nerve fibres produced by these neurones have to establish specific synaptic contacts to provide adult functions. Since the adult axons are integrated into a pre-existing larval neuronal network, it is likely that they use this larval neuronal scaffold as a matrix for orientation. Larval neurones would then function as pioneers for imaginal neurones analogous to the embryonic pioneers which establish the first axon pathways during embryogenesis and which seem to be an important source of guidance for subsequent larval fibers (Bate, 1976, 1978; Bentley & Keshishian, 1982; Taghert et al, 1982; Bastiani et al, 1985).

In adult Drosophila the optic lobes constitute a major part of the brain. Proper development of the optic lobes requires the ordered ingrowth of the retinal input fibres from the eye imaginal discs (Meinertzhagen, 1973; Meyerowitz & Kankel, 1978; Fischbach, 1983; Fischbach & Technau, 1984). A larval nerve, called Bolwig’s nerve, which originates from a cluster of 12 larval photoreceptor cells located near the mouth hooks (Bolwig, 1946; Melamed & Trujillo-Cenoz, 1975; Zipursky et al, 1984; Steller et al, 1987) provides an essential bridge between the eye disc and the optic lobe, and mediates the normal ingrowth of these fibres (Steller et al, 1987). On the other hand, the optic lobe rudiments of eyeless sine oculis (so) mutants still clearly show a columnar and stratified organization revealing organizing properties independent from the eye disc (Fischbach, 1983). It therefore seems likely that larval visual interneurones might be essential for these residual, organizing properties of the adult optic lobes.

In the following, we describe a set of three early differentiated larval neurones which occupy a central position in the developing adult optic lobes of the Drosophila brain.

Materials and methods

Stocks
Oregon R wildtype flies of Drosophila melanogaster were used.

Labelling embryonic cells with HRP
Embryos at the syncytial blastoderm stage were globally
labelled with HRP as described previously (Technau, 1986; Tix et al. 1989). During the first and early second larval stages, the HRP is evenly distributed at a high concentration throughout all parts of the CNS, and thus reflects the fact that mitotic activity of neuroblasts is low during embryogenesis (Hartenstein & Campos-Ortega, 1985; Technau, 1987) and early larval stages (Truman & Bate, 1988). During the third larval stage massive proliferation of adult precursor cells and a
considerable increase in the volume of these cells leads to an almost complete dilution of the marker in certain areas of the thoracic neuromeres and the brain. The labelled individuals were raised until they reached a given stage of postembryonic development, varying between the second larval instar and 6-day-old flies.

Histology
Larvae, pupae or adult flies were dissected in 0.1 M-phosphate buffer (PB, pH 7.2) and the CNS and (in the larval and early pupal stages) the attached eye/antennal discs were removed. During the lateral extension of the growing optic lobes in third instar larval brains, a represents an oblique frontal, C an oblique horizontal and E a frontal plane of view. B is a close-up of the left optic lobe in A and D of the right optic lobe in C. H is an oblique frontal section through one brain hemisphere. The broken line in H marks the border between the optic lobe and the central brain region. Cells within the dotted lines in H belong to the outer (OA) and inner optic anlagen (IA). The cell bodies of the optic lobe pioneers (OLP) at this stage are located near the insertion site of the optic stalk (OS; D,E,F,H) surrounded by the outer optic anlage (OA; H). They are either arranged as a cluster (F) or two of them are immediate neighbours and the third is shifted a small distance apart (G). Their unramified axons fasciculate and run straight through the optic lobe (OL) towards the central brain (CB; A,B,D,E). The larval optic nerve, the Bolwig’s nerve (BN), enters the optic lobe through the optic stalk, projects towards the cell bodies of the OLPs (B,C,E,G) and fasciculates with their axons (C–E). Occasionally, a few glial cells (G; D) become labelled in the lamina region. Arrowheads in D mark the BN/OLP pathway. Arrowheads in G point to Bolwig’s nerve fibres approaching the OLP tract. Arrows in upper right corner of A,C and H indicate the orientation of the specimen: d, dorsal; l, lateral; p, posterior; AD, antennal disc; BN, Bolwig’s nerve; CB, central brain; ED, eye disc; G, glial cell; IA, inner optic anlage; La, lamina; Lo, lobula complex; Me, medulla; OA, outer optic anlage; OL, optic lobe; OLP, optic lobe pioneers; OS, optic stalk. (G,H): composite photographs. Bars (A,C) 50 μm; (B,D,F–H) 20 μm.

Results
In order to identify larval neurones that might serve a pioneering function in the developing adult optic lobes we applied a simple method consisting of the selective labelling of cells that have undergone only a few mitoses (Tix et al. 1989). HRP injected into early embryos becomes almost completely diluted during postembryonic development in massively proliferating adult tissues, whereas larval cells, which have undergone a low number of mitoses, remain intensively labelled.

In the optic lobes, the label is completely diluted in all cells except three which remain intensely stained (Fig. 1). The three cells in question are well-differentiated neurones, i.e. they have already extended axons. During the lateral extension of the growing optic lobes in third instar larvae, their cell bodies are carried away from the central brain and their axons elongate. Their cell bodies now occupy a position in the most distal region of the optic lobe near the insertion site of the optic stalk (Fig. 1D,E,F,H). They are larger than the surrounding cell bodies of the optic ganglia. Their unbranched axons are fasciculated and project straight through the optic lobe towards the central brain (Fig. 1A,B,D,E). Since these cells are the first differentiated cells in the optic lobe primordium, we will refer to them as the optic lobe pioneers (OLPs).

The larval visual nerve, the Bolwig’s nerve, is also intensively labelled (Fig. 1B–E) and can be traced in its entire length from the mouth hooks via the antennal

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**Fig. 1.** Whole-mount preparations (A–D,F,G), camera-lucida drawing (E) and semithin section (H) showing the location and projection pattern of HRP-labelled OLPs in third instar larval brains. A represents an oblique frontal, C an oblique horizontal and E a frontal plane of view. B is a close-up of the left optic lobe in A and D of the right optic lobe in C. H is an oblique frontal section through one brain hemisphere. The broken line in H marks the border between the optic lobe and the central brain region. Cells within the dotted lines in H belong to the outer (OA) and inner optic anlagen (IA). The cell bodies of the optic lobe pioneers (OLP) at this stage are located near the insertion site of the optic stalk (OS; D,E,F,H) surrounded by the outer optic anlage (OA; H). They are either arranged as a cluster (F) or two of them are immediate neighbours and the third is shifted a small distance apart (G). Their unramified axons fasciculate and run straight through the optic lobe (OL) towards the central brain (CB; A,B,D,E). The larval optic nerve, the Bolwig’s nerve (BN), enters the optic lobe through the optic stalk, projects towards the cell bodies of the OLPs (B,C,E,G) and fasciculates with their axons (C–E). Occasionally, a few glial cells (G; D) become labelled in the lamina region. Arrowheads in D mark the BN/OLP pathway. Arrowheads in G point to Bolwig’s nerve fibres approaching the OLP tract. Arrows in upper right corner of A,C and H indicate the orientation of the specimen: d, dorsal; l, lateral; p, posterior; AD, antennal disc; BN, Bolwig’s nerve; CB, central brain; ED, eye disc; G, glial cell; IA, inner optic anlage; La, lamina; Lo, lobula complex; Me, medulla; OA, outer optic anlage; OL, optic lobe; OLP, optic lobe pioneers; OS, optic stalk. (G,H): composite photographs. Bars (A,C) 50 μm; (B,D,F–H) 20 μm.

**Generation of three-dimensional model of pupal optic lobe**
Optical sectioning of fixed whole-mount preparation was carried out on an Olympus inverted microscope equipped with Nomarski optics, using a 20×, 0.8 N.A. oil immersion objective lens (Olympus). The specimen was illuminated with a 12 V, 50 W halogen lamp. The images were acquired by a Peltier-cooled CCD camera (Photometrics Ltd, Tuscan AR) equipped with a 1340×1037 pixel CCD chip (Kodak-Videk). The entire process of focusing the objective, opening and closing shutters, and storing the digital data was controlled by a Microvax II workstation (Digital Equipment Corporation) coupled to a 32 Mbyte Mercury Zip 3232+ array processor (Mercury Computer Systems Inc., Lowell MA) and a Parallax model 1280 display system (Parallax Graphics Inc., Santa Clara CA). The data were stored on a large format, 2 Gbyte, optical disc (Emulex Corp./Optimen 1000, Costa Mesa CA). Optical sections were obtained by stepping the focus through the sample at 2.5 μm intervals.

The bulk of the data processing and computer model building were performed on a Vax 8650 computer system (Digital Equipment Corp.) with attached Parallax display stations. All of the software used is also compatible with the Microvax II system.

The tracings shown were generated interactively with the computer graphics display system, by entering selected data with an on-screen cursor. The modelling software package, written in Fortran, allows the viewing of consecutive images so that axon paths could be traced over many focal planes (Chen & Agard, in preparation). The images shown here were photographed using a Dunn digital camera system (Log E-Dunn) with Ektachrome 200 slide film.

**Histology**
Larvae, pupae or adult flies were dissected in 0.1 M-phosphate buffer (PB, pH 7.2) and the CNS and (in the larval and early pupal stages) the attached eye/antennal discs were removed. Following fixation, DAB reaction and dehydration, the preparations were either embedded as whole mounts on slides or cut into semithin sections (1.5 μm) which were counterstained with a solution of 0.05% methylene blue, 0.01% toluidine blue and 0.05% borax at 60°C.

**Immunohistochemistry**
MAb 22C10 has been isolated during a search for MAb against the Drosophila nervous system (Fujita et al. 1982) and has been kindly provided to us by A. Ferrus. CNS and eye/antennal discs were removed in PBS and fixed for 1 h in 4% paraformaldehyde (in PBS). The antibody staining was performed according to the protocol of Jan & Jan (1982). The stained preparations were dehydrated in an ethanoll series and embedded in Epon as whole mounts.

**Illustrations**
Labelled cells in the whole-mount preparations were photographed using Nomarski optics or drawn by use of a camera lucida device (Zeiss).
Fig. 2. HRP-labelled OLPs in whole-mount preparations of brains from early wildtype pupae (ca. 10 h (C), 28 h (B) and 30 h (A) after puparium formation, apf). (A) Frontal view of total pupal brain. As a result of progressive proliferation and differentiation of the surrounding adult optic lobe cells the cell bodies of the OLPs have become shifted apart from the insertion site of the optic stalk. Note that the central brain region still carries a large number of labelled cells. (B) Horizontal view at a medial focal plane of the same optic lobe shown as a 3D model in Fig. 3. The white arrow points to the site where OLPs and centrifugal medulla tangential neurones (CF) meet to form part of the posterior optic tract. For further explanations see Fig. 3. In A the Bolwig’s nerve (BN) is already almost completely degenerated. In B a few Bolwig’s nerve fibres are still visible. In C the proximal part of the Bolwig’s nerve is still present, whereas the distal part, spanning from the mouth hooks to the eye–antennal disc, is already in an advanced stage of degeneration. The large HRP-containing cell associated with the Bolwig’s nerve on the surface of the eye disc probably represents a glial cell (G) phagocytosing degenerating material. Arrows at the bottom of A–C indicate the orientation of the objects: a, anterior; d, dorsal; l, lateral. BN, Bolwig’s nerve; CB, central brain; CF, centrifugal fibres; ED, eye disc; G, presumably glial cell; La, lamina-region; Me, medulla; MC, medulla cortex; LC, lobula complex cortex; Lo, lobula; LP, lobula plate; OL, optic lobes; OLP, optic lobe pioneers; X2, second optic chiasm. (A–C); composite photographs. Bar, (A) 50 μm; (B,C) 30 μm.

and eye imaginal discs through the optic stalk into the optic lobe. Here most of the Bolwig’s nerve fibres project medially, pass the cell bodies of the OLPs and fasciculate with their axons (Fig. 1B–E). Our preparations did not allow us to precisely localize the termination sites of the Bolwig’s nerve fibres. Most of them seem to terminate at the level of the presumptive medulla, but a few of them may accompany the OLP axons over a longer distance towards the central brain. The larval photoreceptor cells and the OLPs appear to be born at the same time. Thus, assuming that they extend growth cones at the same time, the axons of the Bolwig’s nerve are likely to reach the optic lobes after the OLP axons have pioneered a pathway through the optic lobe.

The number and location of the OLPs is rather constant. In 57 of 62 third instar larval optic lobes there were three OLPs. In three cases we found four and in two cases only two of these cells. In the five exceptional cases the contralateral optic lobe carried three OLPs.
Fig. 3. Three-dimensional computer model showing the projection patterns of HRP-labelled neurones in the left-side optic lobe of an early wildtype pupa (28 h apf; compare medial focal plane of the same optic lobe in Fig. 2B). In stereo-couples A and B the view is along the dorsoventral axis, in C along the posterior–anterior axis and in D along the distal–proximal axis. In all pictures, the surface of the optic lobe is shown in dark blue (the lines follow the periphery of the optic lobe and the border between the optic lobe and the central brain region on consecutive optical sections), the OLPs are red, the remnants of the degenerating Bolwig's nerve are green and centrifugal fibres are yellow. A (same view as B) also shows the outlines of the optic lobe neuropils (light blue). The OLP fibres (red) tangentially run along the prospective distal medulla surface \((Me)\) from its prospective posterior to its prospective anterior margin and then enter the pathway of the posterior optic tract \((PO)\). The cell bodies of the OLPs occupy a position in the outer optic chiasm between the developing lamina \((La)\) and medulla neuropils. An additional set of HRP-labelled fibres (yellow), not previously detectable in the larva, now centrifugally invades the medulla, lobula \((Lo)\) and lobula plate \((LP)\). Several of them project towards the anterior margin of the medulla by using the pathway of the posterior optic tract, as do the OLPs. The orientation of the object is indicated by the coordinates at the bottom of each picture: (,), anterior; X, dorsal; O, proximal. \(Me\), medulla; \(La\), lamina; \(Lo\), lobula; \(LP\), lobula plate; \(PO\), posterior optic tract.
Early neuronal pathways in Drosophila optic lobes

Fig. 4. Optic lobe pioneers in wildtype adult CNS. (A) Frontal view of an optic lobe of a 6-day-old adult fly which received HRP at the syncytial blastoderm stage. (B) Whole-mount preparation of a wildtype adult brain stained with MAb 22C10. In the optic lobes (OL) the antibody binds to the OLP fibres (arrows) and retinula cell axons whereas most of the optic lobe neuropil becomes only weakly labelled. Arrowheads point to the region where the OLP fibres bend to follow the posterior optic tract. Arrows at the bottom of A, B indicate the orientation of the objects: d, dorsal; l, lateral. CB, central brain; OL, optic lobe; OLP, optic lobe pioneers (axon fascicle); SG, suboesophageal ganglia. (A): composite photograph. Bar, (A) 20 μm; (B) 100 μm.

Fig. 5. Whole-mount preparations of wildtype third instar larval brains and eye–antennal discs stained with MAb 22C10. The antibody intensively stains the Bolwig’s nerve (BN) and retinula cells demonstrating the course of their axons (Zipursky et al. 1984). The Bolwig’s nerve can be traced from its origin near the mouth hooks via the antennal (AD) and eye disc (ED) from where it runs through the optic stalk (OS) together with the adult photoreceptor cell axons (A). Arriving in the optic lobe the retinula cell axons fan out whereas Bolwig’s nerve fibres fasciculate with the OLP tract to project centrally into the optic lobe (OLP/BN). The cell bodies of the OLPs are not detected by the antibody (B). As opposed to the Bolwig’s nerve which degenerates in the early pupa the OLPs survive metamorphosis (see Fig. 4). AD, antennal disc; BN, Bolwig’s nerve; ED, eye disc; OL, optic lobe; OLP/BN, optic lobe pioneers/Bolwig’s nerve fascicle; OS, optic stalk. (B): composite photograph. Bar, (A) 50 μm; (B) 20 μm.

Either all cell bodies of the OLPs are arranged as a cluster (in 65% of the cases; Fig. 1F) or two of the cells are immediately adjacent to each other whereas the third (or fourth) cell body is separated by a few cell diameters (in 35% of the cases; Fig. 1G).

Differentiation of the adult optic lobe neurones begins in the third instar larva and is most evident during early pupal stages when the neuropil of the three optic ganglia (lamina, medulla and lobula-complex) becomes clearly distinguishable (Meinertzhagen, 1973). In the process of differentiation, during the early pupal stage, the brain hemispheres elongate more and more in a lateral direction, changing from a circular into a more oval shape (Fig. 2A). The optic stalk broadens and gets shorter and the retina becomes attached to the optic lobe. As a result of proliferation and differentiation of the surrounding imaginal cells, the cell bodies of the three OLPs are now occupying a position in the outer optic chiasm between the developing lamina and medulla neuropil (Figs 2B; 3A).
The OLP axons are oriented along what will become the distal surface of the medullar neuropil (Figs 2B; 3), after morphogenetic movements rotate it later in development. Thus, the OLPs project tangentially on top of the medulla from its less developed posterior to its more developed anterior edge. In the early pupa, the prospective posterior edge of the medulla is in the distal (lateral) region and the prospective anterior edge is in the proximal region of the optic lobe. Later it is rotated about 90° to occupy its final position. Thus, the wave of differentiation which sweeps over the eye imaginal discs (Ready et al. 1976; Campos-Ortega & Hofbauer, 1977; Tomlinson & Ready, 1987) and concomitantly over the optic lobes (Meinertzhagen, 1973; White & Kankel, 1978) in the medulla region is directed parallel to the OLPs' pathway. The axons of the adult large field medulla tangential neurones (MT-cells; Fischbach & Dittrich, 1988) leave the medulla neuropil at its anterior edge and then enter the posterior optic tract (Cuccati-bundle) together with the OLPs. We do not know where the OLP axons terminate, since we have not been able to trace their pathway through the central brain region where it becomes obscured by a large number of HRP-labelled cells.

In the early pupa, HRP-labelled centrifugal fibres appear in the optic lobes in addition to the OLPs (Figs 2B; 3). A fraction of these fibres clearly belong to the MT-cells since they approach the anterior margin of the medulla via the posterior optic tract and invade the median medulla neuropil. As opposed to the OLPs, these HRP-labelled centrifugal fibres and additional ones projecting into the lobula complex are not yet detectable in the optic lobes of late larvae. Thus, they do not grow into the optic lobe before the early pupal stage when differentiation of the optic lobe neuropil is in a more advanced stage. As deduced from their intensive HRP-staining they must have passed through a small number of mitoses.

About 10 h after puparium formation (apf) the first signs of degeneration appear in Bolwig's nerve and on the eye disc a few large non-neuronal cells associated with the Bolwig's nerve become labelled with HRP (Fig. 2C). Since they do not manifest the HRP before this stage, these cells may be phagocytizing degenerating material of the Bolwig's nerve and thereby accumulating HRP by an indirect route. Degeneration of the nerve proceeds in an anterograde direction until about 30 h apf, when the nerve has completely vanished (Fig. 2A). In contrast to the Bolwig's nerve, the OLPs persist through metamorphosis and are still found in 6-day-old adult flies (older flies were not inspected). Their number, location and axonal projection pattern correspond to that of the preceding stages (Fig. 4A,B). These observations are substantiated by antibody staining. MAb 22C10 (Fujita et al. 1982) labels the Bolwig's nerve in its entire length as well as the newly growing retinula cell axons following the Bolwig's nerve through the optic stalk into the optic lobe where they radiate in a fan-shaped manner (Zipursky et al. 1984; Fig. 5A). Whereas the cell bodies of the OLPs are not detected by the antibody, the fascicle formed by the OLPs and the Bolwig's nerve is weakly stained (Fig. 5B). Following degeneration of the Bolwig's nerve, the OLP fibres remain labelled (Fig. 4B).

Discussion

Visual behaviour of the Drosophila larva is very primitive when compared to the highly developed visual performance of the adult fly. Accordingly, the larval visual system consists of only a few elements. Each of the larval visual organs comprises 12 photoreceptor cells which arise during embryogenesis and establish contacts with the brain hemispheres (Steller et al. 1987). The target cells of the larval photoreceptor cells in the brain were previously unknown. We have now identified a set of three neurones (OLPs) in the developing optic lobes which for the following reasons seem to represent target cells of the Bolwig's nerve. (1) Fibres of the Bolwig's nerve project directly towards the OLPs cell bodies and fasciculate with their axons. (2) Among the cells of the optic lobes the OLPs are the only ones derived from a small number of cell divisions, suggesting that they do not stem from the optic anlagen which give rise to the adult optic lobe neurones. (3) They are already in a fully differentiated state before there is any detectable fibre growth in the remainder of the optic lobes. It therefore seems that, like the larval photoreceptor cells, the OLPs differentiate in the embryo and that they are the only larval cells in the prospective optic lobes. (4) In third instar larvae, at least some of the Bolwig's nerve fibres terminate in the region of the optic lobe some distance apart from the central brain region. Therefore, interneurones have to connect Bolwig's nerve to higher order visual centres in the brain. The OLPs are the best candidates for these primary interneurones.

Although we were not able to precisely localize the sites at which the Bolwig's nerve fibres terminate, our material suggests that there exists heterogeneity among the fibres in this respect. It appears that a subpopulation of the Bolwig's nerve fibres is pioneered by the OLP tract over a longer distance towards the central brain in order to make contact with a more proximal set of interneurones. Different termination sites of the larval receptor cell fibres would correspond to the situation in the adult where short (R1–6) and long (R7/8) retinula cell fibres terminate at different levels. Indeed, recent evidence for the expression of different opsin genes in the larval photoreceptor suggests that it may be made up of distinct cell types, as is the compound eye (Pollock & Benzer, 1988). Provided that short Bolwig's nerve fibres form synapses with the OLPs, the OLPs would be homologous with lamina cells, whereas more proximal postsynaptic elements of longer Bolwig's nerve fibres would correspond to medulla neurones. This hypothesis will be addressed in future studies at the ultrastructural level.

The elements of the larval visual system, the Bolwig's nerve and the OLPs, provide the core around which the formation of the adult visual system takes place during.
postembryonic development. A pioneer function of the Bolwig's nerve involving the guidance of the retinula axons from the eye disc to the optic lobe has been previously suggested (Meinertzhagen, 1973; Melamed & Trujillo-Cenoz, 1975). Recent, more direct evidence based on mutant analysis supports this hypothesis and indicates that the Bolwig's nerve does pioneer the optic stalk route that retinula cell axons follow to the optic lobe (Steller et al. 1987).

Normal organization of the optic lobes depends critically on proper innervation by the retinula cells. Absence of retinal innervation causes massive degeneration of optic lobe neurones (Power, 1943; Meinertzhagen, 1973; Meyerowitz & Kankel, 1978; Fischbach & Technau, 1984). However, the analysis of eye mutants suggests that there are additional organizing properties in the optic lobes that are independent of retinal innervation. Thus, among the neurones surviving congenital sensory deprivation in eyeless sine oculus (so) flies, there are tangential as well as columnar neurones that retain an isotopic and stratified organization in the optic lobe rudiments (Fischbach, 1983). In disconnected (disco) mutant flies, although photoreceptor cells are present, they remain disconnected from the brain due to misrouting of the Bolwig's nerve. In these flies, the optic lobe rudiments are significantly smaller than in so flies (Steller et al. 1987). Since the Bolwig's nerve is present in so but not in disco flies, Steller et al. proposed that Bolwig's nerve itself contributes to the normal development of the optic lobes. Alternatively, or in addition, this role may be attributed to the OLPs, because in disco mutant brains labelling with HRP is negative for the OLPs (Fischbach, Boschert, Tix & Technau, in preparation). Meinertzhagen (1973) found that the first axon pathways in the optic lobes are laid down along the 'ocellar' bundle (which corresponds to the Bolwig's nerve) and argued that this bundle is critical in the establishment of the optic neuropils. In this paper, we show that this bundle does not consist only of Bolwig's nerve. It is actually formed by the axons of the OLPs and by Bolwig's nerve fibres fasciculating with the OLPs. We suggest, therefore, that the OLPs are of initial importance for the selforganizing properties of the optic lobe neuropil.

A pioneering function of the OLPs during optic lobe development is further corroborated by the following arguments. (1) They are the first cells to differentiate in the prospective optic lobe region. (2) They maintain a core-position, with their large cell bodies facing the insertion site of the optic stalk and their axons running centrally through the optic lobe to the central brain. (3) The OLPs' pathway runs tangentially to the prospective medulla neuropil. Large field medulla tangential neurones together with the OLPs run along the posterior optic tract which is one of the most prominent connections to the central brain. In the early pupal stage, fibres of medulla tangential neurones centrifugally grow along this tract towards the medulla. Therefore, the presumed pioneering function of the OLP-tract could be primarily involved in the organization of the tangential elements of the optic lobes. This assumption is supported by the fact that tangential neurones are less affected by the absence of retinal innervation than columnar neurones (Fischbach, 1983). (4) Whereas ingrowth of retinula cell fibres into the optic lobe is almost complete by the end of the third larval instar, the main bulk of the optic lobe neuropil is formed during the early- and mid-pupal stages. Correspondingly, the Bolwig's nerve degenerates in the early pupa whereas the OLPs persist throughout metamorphosis. (5) Inspection of various structural brain mutants shows a correlation between the absence or misrouting of the OLPs and defects in the optic lobes (Fischbach et al. in preparation).

The development of the visual system reflects the main strategies that lead to the metamorphosis of the nervous system: addition of new elements as in the eye discs and the optic lobe anlagen; degeneration of some larval elements, as is the case of Bolwig's nerve and integration into the adult network and possibly reorganization of other larval elements as exemplified by the OLPs. Thus, the OLPs, like other persisting CNS neurones may execute four successive functions. (1) They pioneer the route of larval receptor cell axons within the optic lobe. (2) As first order interneurones they process visual informations in the larva. (3) In the late larval and in the pupal stage, they serve as pioneer fibres for the formation of the adult optic lobe neuropil. (4) Becoming integrated into this neuropil, they could participate in processing visual information in the adult.

We thank Michael Bate, Michael Caudy and Karl-Friedrich Fischbach for helpful discussions and carefully reviewing the text, José Campos-Ortega for working facilities and comments on the manuscript, Eva Varus for expert technical assistance and Alberto Ferrus for providing MAb 22C10. We would also like to thank David Agard and John Sedat in whose laboratories the three-dimensional models were generated and Hans Chen for help in software development. This work was supported by a grant from the Deutsche Forschungsgemeinschaft to G.M.T.

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(Accepted 11 January 1989)