CHAPTER 8

Optic Lobe Development

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Abstract

The optic lobes comprise approximately half of the fly's brain. In four major synaptic ganglia, or neuropils, the visual input from the compound eyes is received and processed for higher order visual functions like motion detection and color vision. A common characteristic of vertebrate and invertebrate visual systems is the point-to-point mapping of the visual world to synaptic layers in the brain, referred to as visuotopy. Vision requires the parallel extraction of numerous parameters in a visuotopic manner. Consequently, the optic neuropils are arranged in columns and perpendicularly oriented synaptic layers that allow for the selective establishment of synapses between columnar neurons. How this exquisite synaptic specificity is established during approximately 100 hours of brain development is still poorly understood. However, the optic lobe contains one of the best characterized brain structures in any organism—both anatomically and developmentally. Moreover, numerous molecules and their function illuminate some of the basic mechanisms involved in brain wiring. The emerging picture is that the development of the visual system of *Drosophila* is (epi-)genetically hard-wired; it supplies the emerging fly with vision without requiring neuronal activity for fine tuning of neuronal connectivity. Elucidating the genetic and cellular principles by which gene activity directs the assembly of the optic lobe is therefore a fascinating task and the focus of this chapter.

Introduction

Several comprehensive works cover the description of early events during optic lobe development in *Drosophila*,[1-3] whereas most recent reviews focus on the molecules and mechanisms during the establishment of synaptic connectivity in the visual system.[4-6] The present chapter focuses on optic lobe development from the viewpoint of neurogenetics: How can a surprisingly low number of genes encode the wiring of a complicated brain structure? An answer must encompass all levels of the developmental program, from cellular differentation and movement to the molecules and mechanisms that provide meaningful synapse formation signals. In particular, we will focus on the events and mechanisms that lead to the recognition of synaptic partners. What is the mechanism of such recognition events? What are the molecular players at the level of the cell surface during recognition events and what are the mechanisms for their precise, dynamically regulated expression pattern? And, finally, how plastic is this program, i.e., to what extent is the final synaptic wiring pattern determined by the genetic program?

Recognition of different cell types is not confined to the nervous system and is a general requirement in the development of multicellular organisms. Without cell recognition, recruitment of cells into developing tissues would be impossible. Recognition between different cell types is especially demanding in the nervous system where neurons have to synapse with specific partners, often thousands of cell body diameters apart. Due to their regular, columnar and layered organization visual

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systems are well suited to investigate the genetic determination and developmental rules that underlie the establishment of neuronal connectivity. The repetitive organization of about 750 visual units or columns on each side of the fly's head allow the detection of minor disturbances. The visual system of *Drosophila* has the further advantage that an exceptionally powerful toolbox can be applied to genetically dissect the developmental programs.

The Adult Visual System Is Organized into Parallel Visuotoptic Functional Pathways

The adult optic lobes of coleoptera, lepidoptera and diptera[7,8] are subdivided into four neuropils, the lamina, medulla, lobula and lobula plate (Figs. 1A, 2A). Photoreceptor projections from the eye directly innervate the first two neuropils, lamina and medulla. In *Drosophila*, each single eye, or ommatidium, of the compound eye contains eight different photoreceptor cell types. Their light-sensing protrusion, the rhabdomeres, receive light along seven different optical axes underneath a single lens. The outer 6 rhabdomeres are formed by retinula cells R1-6; the inner rhabdomere comprises distally R7 and proximally R8. In all ommatidia, except those of the dorsal rim, the inner rhabdomeres are much thinner than the outer ones. Functionally, the outer photoreceptors are responsible for spatial vision, whereas the inner photoreceptors convey color vision.

Three types of ommatidia can be distinguished[9] according to the rhodopsin (Rh) content of the inner retinula cells R7 and R8: 30% of ommatidia are of the pale subtype, where R7 contains the UV-sensitive Rh3 and R8 the blue-sensitive Rh5, while the remaining 70% are of the yellow subtype and contain UV-sensitive Rh4 in R7 and green-sensitive Rh6 in R8. Both types are randomly distributed due to the stochastic expression pattern of the transcription factor and Dioxin receptor homolog *spineless* in R7 cells. The expression of Spineless in R7 cells specifies it as a Rh4 cell. R7 then dictates the fate of the R8 cell to also assume the yellow subtype. In the absence of *spineless* or in *spineless* mutants, all R7 and most R8 cells adopt the pale (Rh5) fate, whereas overexpression of *spineless* is sufficient to induce the yellow R7 fate.[10] The molecular mechanism that determines the stochastic expression of Spineless as well as the functional significance of the random pale/yellow ommatidia distribution are currently unknown.

In addition to these two major ommatidial types there is a dorsal rim area of the compound eyes[9,11] which is specialized for the detection of polarized light. Here R7 and R8 rhabdomeres have larger diameters and both express the UV-sensitive Rh3. As the microvilli of both cell types are perpendicularly oriented with respect to each other, this allows the evaluation of the vector of

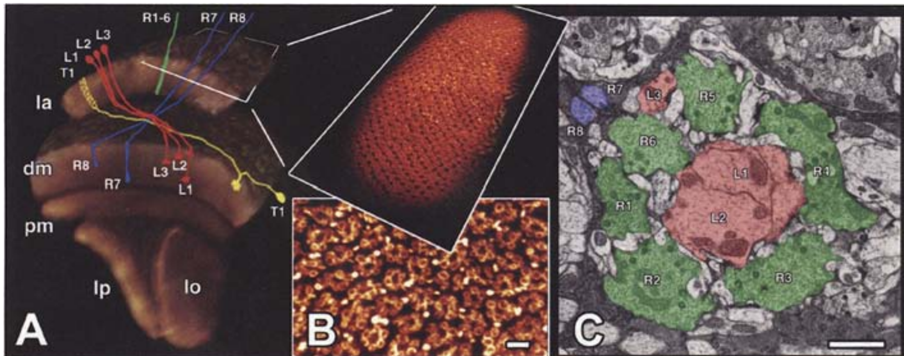

Figure 1. The Drosophila optic lobe. A) Volume rendered optic lobe neuropils based on synaptic staining (n-Syb). Selected characterized cell types are depicted based on Golgi studies.[18] la, lamina; dm, distal medulla; pm, proximal medulla; lp, lobula plate; lo, lobula. B) The primary visual map. Lamina cross-sections of confocal images based on a photoreceptor-specific antibody staining. Scale bar 5μm. C) EM micrograph of a single unit (cartridge) of the visual map in the lamina. Color code as in A. Scale bar 1μm.

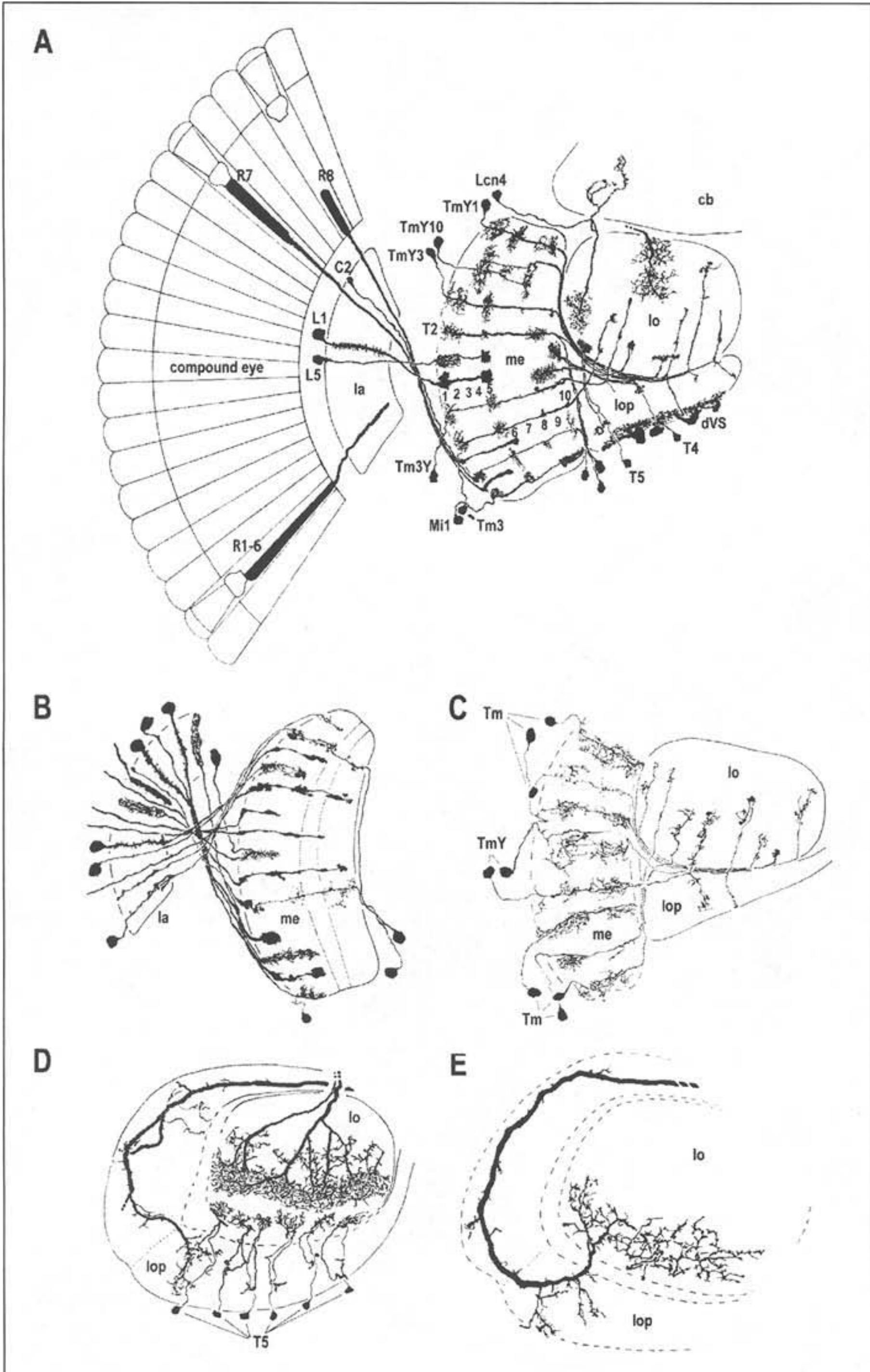

Figure 2, legend viewed on following page.

Figure 2, viewed on previous page. Golgi Gestalten of neurons in wild type and mutant optic lobes. A) Composite scheme of the left compound eye and optic lobe with camera lucida drawings of Golgi impregnated neurons of wild type flies selected to illustrate the layering of the medulla neuropil, e.g., L1, L5, Mi1, Tm3, Tm3Y, T2, TmY3, TmY1, but not TmY10 are potential interactors in the distal medulla as their arborizations overlap in layers M1 and M5 (see numbers without prefix). Original camera lucida drawings taken from Fischbach and Dittrich (1989).[18] B, C) Camera lucida drawings of columnar neurons in the optic lobe of the *small optic lobes*^{KS58} mutant display a partial loss of stratification (modified from[126]). D, E) Camera lucida drawings of some examples of neuronal cell types surviving congenital sensory deprivation in completely eyeless *sine oculis*^{2} flies. Sprouting of medulla tangentials into the lobula complex can be seen (modified from[91]). la, lamina; me, medulla; lo, lobula; lop, lobula plate; cb, central brain. Naked numbers 1-10 depict medulla layers M1-M10. dVS, dendrites of giant vertical neurons of the lobula plate; all others labels are names of neuronal cell types following the nomenclature of Fischbach and Dittrich (1989).[18]

light polarization rather than wavelength. The homeodomain transcription factor homothorax is both necessary and sufficient for R7/R8 to adopt the polarization-sensitive dorsal rim fate instead of the color-sensitive default state. Homothorax increases rhabdomere size and uncouples R7-R8 communication to allow both cells to express the same opsin rather than different ones as required for color vision. Homothorax expression is induced by the dorsally expressed genes of the *iroquois* complex and the *wingless (wg)* pathway.[12]

The outer photoreceptors responsible for spatial vision terminate in the first optic ganglion, the lamina, whereas the inner photoreceptors responsible for color vision project through the lamina into the second and major optic neuropil, the medulla (Figs. 1,2). It has to be expected that the different types of R7 and R8 retinula cells described above project to specialized target neurons in the optic lobe. In fact, in the locust, the neuronal pathways of the dorsal rim region could be traced via neurons in the dorsal rim of the medulla to the lower unit of the anterior optic tubercle.[13] It is noteworthy, that the decision about the type of opsin occurs in the midpupal stage, after the axons have found their way into the brain and during the period of synapse specification and formation. It is not known whether the opsin decision also influences target choices in the maturing neuropil of *Drosophila*.

The axons of the eight retinula cells per ommatidium project to the adult brain following the neural superposition rule[14-16] which secures that axons from retinula cells obtaining information from the same point in space project into the same cartridge of the lamina or column of the medulla. In larval development, the R1-6 axons of a single ommatidium form a common fascicle with their leading R8 axon and follow it through the larval optic stalk into the larval lamina plexus in a retinotopic fashion. They distribute themselves to six different, neighbouring lamina cartridges and establish a visuotopically correct map only later.[2,17] The R8 and the following R7 axons directly project into the medulla in a correct retino- and visuotopic manner, as discussed in detail in section 4 of this chapter. The six outer R-cell terminals of a single ommatidium are presynaptic to the dendrites of lamina monopolar neurons L1, L2 and L3 in six different lamina cartridges, while the L-cell dendrites receive input from R-cells coming from six different ommatidia. The axons of the lamina monopolar cells L1-5 (only the first three are postsynaptic to R1-6) of a single cartridge project via the first optic chiasm into specific layers of isotopic medulla columns (Figs. 2,3). While a single lamina cartridge receives input from retinula cells with identical optical axes of six neighbouring ommatidia, a medulla column samples such information from 7 ommatidia, transmitted via 5 different direct neuronal channels (L1, L2, L3, R7 and R8).

In summary, while R7 and R8 directly form retinotopic projections in the medulla, R1-R6 undergo axon terminal resorting according to the principle of neural superposition to match the orientation of the optical axes of the adult rhabdomeres (visuotopy). The visuotopic organization is a general feature of all image processing visual systems in invertebrates as well as in vertebrates.

Most of the visual interneurons of *Drosophila* have been described in Golgi studies.[18] They can be classified into many columnar and fewer tangential types, the axons of which are oriented perpendicular to each other. By mere evaluation of the structural features (Fig. 2A) it has been

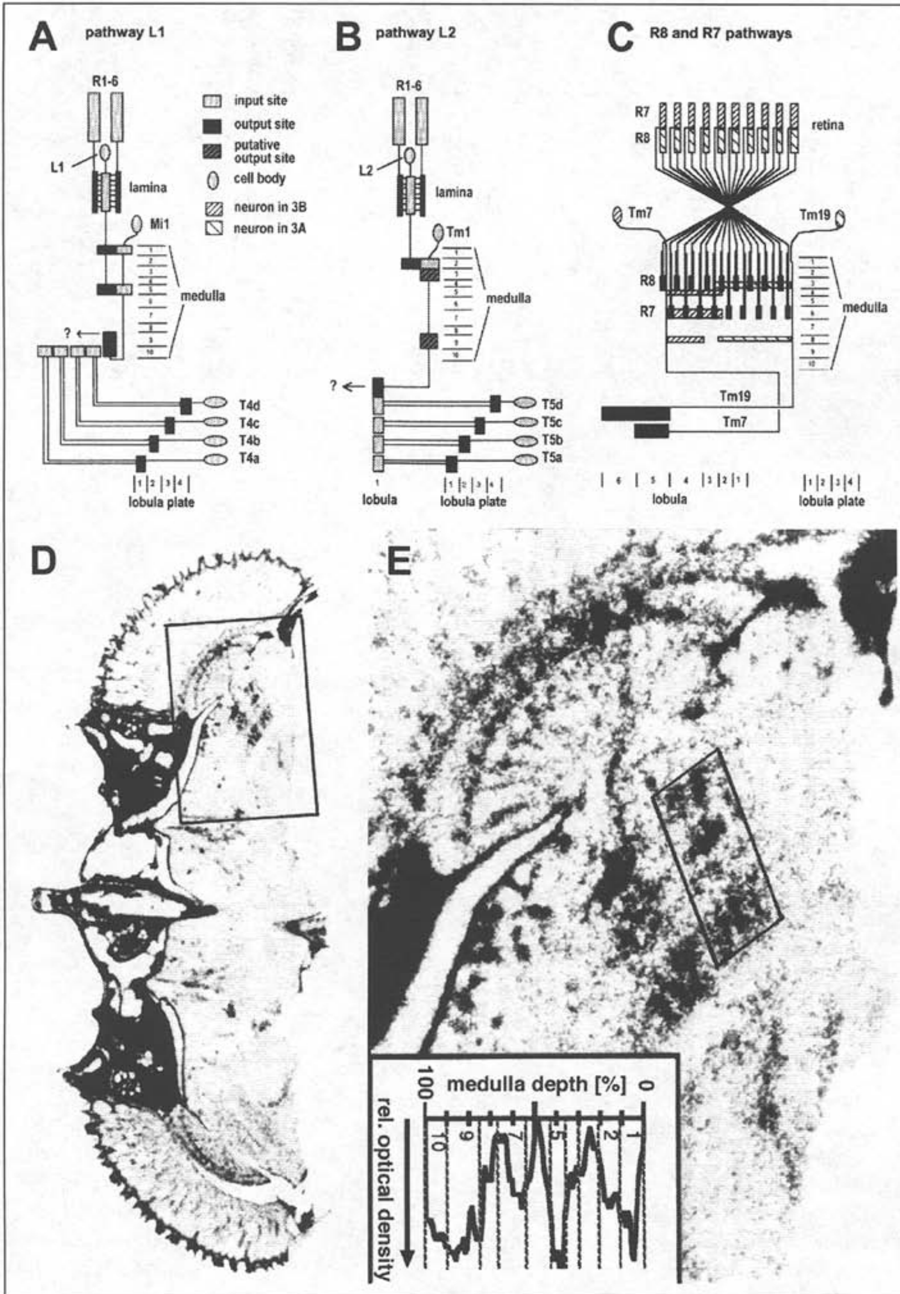

Figure 3. Visuotopically organized pathways in the optic lobe. A-C) Peripheral separation of visuotopically organized functional pathways requires the organization of the optic lobe in columns and layers. Three functional pathways in the optic lobe are shown which are inferred from the relationship of layered arborizations of all known cellular Golgi profiles. Legend continued on following page.

Figure 3, viewed on previous page. For simplicity, at the level of the medulla only typical neuronal types are shown. The L1 and L2 pathways are fed by R1-6 and function in spatial vision, the R8 and R7 pathways in color vision. D) depicts an ^{3}H-2-deoxyglucose autoradiogram of a horizontal brain section after unilateral 120 min stimulation in two 15 x 15 degree sectors of the right visual field. The right optic lobe autoradiogram is enlarged in E. The anterior visual field window (posterior medulla sector) was stimulated by upward motion, the posterior visual field window by horizontal progressive motion of the same spatial wavelength (using a sinusoidally modulated gray scale). In both cases visuotopically situated columnar neurons of the L1 and L2 pathway layers (A,B) have taken up radioactive deoxyglucose. The density profile of the medulla sector stimulated by upward motion is shown in the inset. It is obvious that the R7/R8 pathway layers M3 and M6 are silent under these conditions (modified from Fischbach et al 1992[22]).

claimed that several, visuotopically organized, parallel visual pathways co-exist[19] (Fig. 3A-C). In combination with 2-deoxyglucose studies[20] a clear structural separation between the pathways for motion detection and colour vision could be demonstrated[18-22] (see Fig. 3D,E).

This neuronal organization of the visual system of *Drosophila* contrasts sharply with the olfactory system, where olfactory receptor cells with the same chemosensory specificity converge in so called glomeruli of the antennal lobe onto single large interneurons (relay neurons) that project to the mushroom bodies and the lateral protocerebrum[23,24] (see Chapters by R. Stocker and by V. Rodrigues and T. Hummel). However, it has recently been pointed out that the output level of the visual system is also comparable to the olfactory system, as visuotopically organized lobula output neurons of the same type converge in so-called optic glomeruli, where they synapse onto large projection neurons[25,26] (Fig. 4). It is therefore tempting to suggest that the visuotopic, parallel pathway organization is an evolutionary added feature of the visual system.

What is known about the cellular and molecular mechanisms that enable the visuotopic and pathway-specific wiring in the optic lobe? We will first review data related to the dependence of visual neuropil development on retinal innervation and will consider some of the functions of known cellular and molecular factors involved in axonal pathfinding, target recognition and synaptogenesis.

Lamina Development

Retinal Innervation: Axon Outgrowth and Interdependence with Optic Lobe Development

Axon outgrowth from the retina occurs in a developmental wave following the wave of cellular differentiation in the eye disc. The first (pioneer) axons grow out from R8, followed by R2&R5, R3&R4, then R1&R6 and R7 follow last.[27] The retinal axons project through the tubular optic stalk that consists of a monolayer of surface glia and forms before axon ingrowth under the control the focal adhesion kinase Fak56D.[28] The larval photoreceptor organ, the Bolwig's organ, is dispensable for adult wild-type photoreceptor axons to project normally and is thus not an essential pioneer of axonal navigation to the lamina. Bolwig's organ later transforms into the four photoreceptors of an extra-retinal posterior "eyelet", the so-called "Hofbauer-Buchner eyelet",[29] which is involved in the generation of circadian rhythm.[30] The best characterized signal transduction pathway required for photoreceptor growth cone guidance includes the Insulin receptor on the cell surface[31] and intracellularly *dreadlocks* (*dock*, a SH2/SH3 adaptor protein), *pak* (p21 activated protein kinase), *trio* (a Rho family guanine exchange factor that activates Rac), *misshapen* (a Ste20-like serine/threonine kinase) and *bifocal* (a putative cytoskeletal regulator).[32-37] These molecular components have been proposed to constitute a signal transduction cascade from the cell surface to the actin cytoskeleton. Targeting choices of the different photoreceptor subtypes and the upstream guidance receptors are described in more detail below.

While maintainance of the fly's retina requires that retinal axons connect to the optic lobe,[25] it is well established that retinae develop quite normally in ectopic positions without connections to the brain, either achieved by transplantation[38] or by ectopic expression of eyeless.[39] Also the

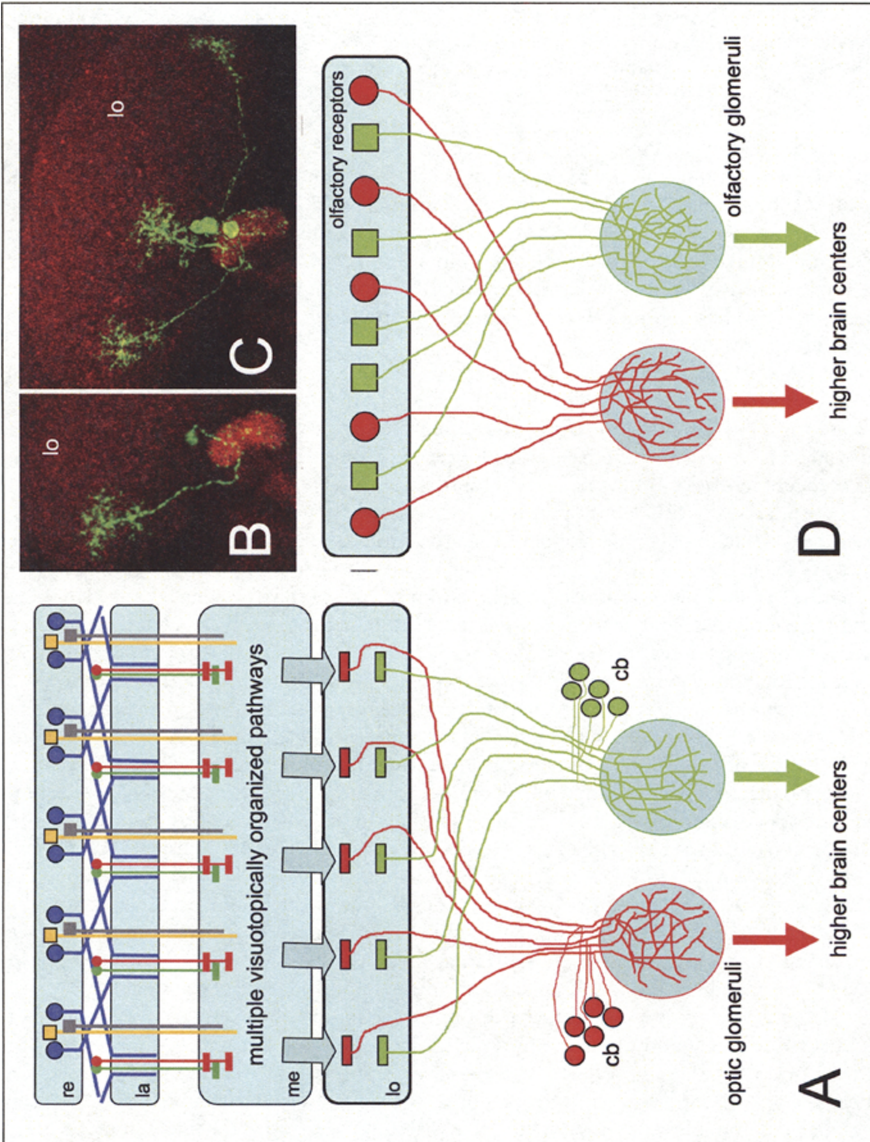

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Figure 4, viewed on previous page. Comparing wiring principles of the olfactory and the visual system. A) Schematic view of the visual system. Visuotopy is maintained up to the lobula complex (lobula plate has been omitted for simplicity). Different sets of lobula columnar neurons project to specific optic glomeruli, where they terminate in a nonvisuotopic manner. B,C) GFP marked neurons resulting from MARCM using the *irreC/rst*-specific Gal4 driver NP2044. Background staining with an IrreC/Rst-specific antibody. B) The terminals of a single LC12 neuron branch throughout its glomerulus. C) A clone of three such LC12 neurons subserving different parts of the visual field are shown. D) Schematic view of the organization of the olfactory system. Here all olfactory receptor cells of the same kind directly project to the same glomerulus. re, retina; la, lamina; me, medulla; lo, lobula; cb, cell bodies.

unconnected phenotype of the *disconnected* mutant, in which the retinula cell axons of the compound eye do not connect to the brain, demonstrates that retina development, which proceeds normally, is autonomous.[40] This does not hold for the optic lobe, the development of which strongly depends on retinal innervation (Fig. 2D,E). It was already demonstrated by Power in 1943 and confirmed by Hinke in 1961 that optic lobe volume strongly correlates with the facet number of the compound eye.[41,42] In his volumetric studies Power found that eyeless flies do not develop a lamina at all and have a drastically reduced medulla and lobula complex (about 80% and 60% reduction respectively).

Optic lobe interneurons are the progeny of two groups of progenitor cells, arranged in the outer and inner optic anlagen. The lamina (together with the distal part of the medulla, see below) arise from the outer optic anlage.[43] The strong correlation of lamina size with the number of ommatidia is the direct consequence of an inductive influence of ingrowing retinula (R) cell axons on neurogenesis of lamina neurons[44,45] and lamina glia.[46] Photoreceptor innervation thus triggers the final cell-cycle of lamina precursor cells. Hedgehog, that is released from R-cell axons, induces the generation of lamina monopolar neurons from lamina precursor cells which—in the absence of Hedgehog—are arrested in the G1 phase.[47-49] Hedgehog transport in photoreceptors has recently been shown to depend on the competition between targeting signals of the Hedgehog N- and C-termini. After Hedgehog cleavage, the N-terminal domain is targeted to the retina, while the C-terminal domain is responsible for Hedgehog transport along the axon.[50] Together with Hedgehog, the epidermal growth factor receptor (EGFR) ligand Spitz is transported down the photoreceptor axons. The postsynaptic precursor cells express EGFR and are thus initiated to assemble the postsynaptic cell complement for the lamina cartridge.[47] By the concerted action of Hedgehog and Spitz, the number of presynaptic neurons determines the size of the postsynaptic neuronal population. The five lamina cell types L1-L5 are thereby specified. As young retinal ommatidia are added anteriorly, this also implies that the lamina grows from posterior to anterior. Lamina precursor cells as well as glia cells require the transcription factor Glia cells missing (*gcm*) and Glia cells missing 2 (*gcm2*), that were previously thought to be exclusively required for glial cell fate determination.[51] Of further importance on the side of the lamina precursor cells is the gene product of *dally*. In *dally* loss-of-function mutants the lamina precursors do not perform the second division that is triggered by ingrowing retinal fibres.[52] Dally is a heparan sulfate proteoglycan attached to the membrane via a GPI-anchor and able to modulate Hedgehog signaling.[53]

The dependence of lamina differentiation upon the ingrowth of retinal fibres provides a straight-forward programming of retinotopic projections along the anterior-posterior axis. As a wave of differentiation (visible as the so-called morphogenetic furrow) sweeps along the eye-imaginal disc from posterior to anterior during the late larval and early pupal stage, the new ommatidial axon bundles leave the eye imaginal disc anteriorly and accordingly induce lamina development also at its anterior margin.[27,54] Maturation of the eye imaginal disc and the lamina therefore occurs in parallel from posterior to anterior. Apoptosis of excess cells concludes the wave of development in the lamina. In vertebrates, Eph receptor tyrosine kinases have critical roles in retinotopic map formation. *Drosophila* contains only one Eph gene, which has indeed been implicated in the targeting of retinotopic projections, although the precise cellular requirement and mechanism are less clear.[55]

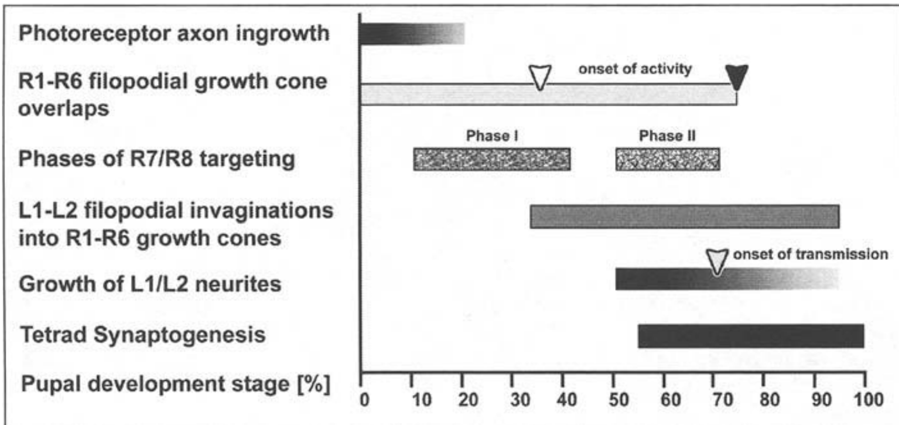

Figure 5. Timeline of morphogenetic events during pupal optic lobe wiring. Depicted is the temporal succession of different phases of photoreceptor and lamina monopolar cell (L1/L2) growth, incorporating data from different fly species. Innervation of the anterior lamina by photoreceptors axons is complete by 20% of pupal development. Transient filopodial-growth cone invaginations and overlaps amongst R1-R6 growth cones can be observed up to 75% of pupal development. The arrowheads show the approximate onset of R1-R6 responses recorded using sharp electrodes in the blowfly *Calliphora* (open arrowhead)[127] or whole-cell recordings (filled arrowhead) from dissociated ommatidia in *Drosophila*.[128] In the first half of pupal development R1-R6 terminals are resorted according to the neural superposition rule.[2,17] Two phases of R7/R8 target layer selection have been distinguished in the medulla.[98] Growth of L1/L2 neurites and filopodial growth cone invaginations from at least one L1/L2 axon into R1-R6 growth cones can be observed through most of the second half of pupation. The grey arrowhead indicates the approximate onset of synaptic transmission to L1/L2 based on *Calliphora* data.[127] Synaptogenesis takes place in the second half of pupal development and culminates in the formation of tetrads which unite elements of four different cell types at a single synapse.[2] Modified from I. A. Meinertzhagen et al (2000).[129]

No such helpful temporal gradient does exist when the establishment of retinotopy along the dorso-ventral axes is considered. How is it secured that dorsal retinula axons project into the dorsal lamina and ventral retinula axons project into the ventral lamina? By the use of eye mutants with reduced facet number, it was demonstrated that navigation of ommatidial bundles is independent of each other: Single bundles navigate more or less correctly in the absence of neighbouring one. Genetically wild type axons are even able to innervate their correct brain region, when surrounding fibres are misprojecting due to the glass genotype.[56] Which cues are these axons using for their navigation?

DWnt4, a *Drosophila* member of the Wnt family of secreted glycoproteins, is specifically expressed in the ventral half of the developing lamina in the third instar larval stage.[57] In the absence of DWnt4, ventral retinal axons misproject to the dorsal lamina and can be redirected towards an ectopic source of DWnt4. Wnt glycoproteins are known to activate via Frizzled (Fz) receptors canonical (ß-catenin dependent) as well as noncanonical (ß-catenin independent) signaling pathways. Ventral retinula cells missing the Dfrizzled2 (Dfz2) receptor or the directly interacting Dishevelled protein often misroute their axons dorsally and it could be shown that interference with noncanonical but not with canonical signaling affects axon targeting along the dorso-ventral axis. These results suggest that secreted DWnt4 from the ventral lamina acts as an attractant for retinal axons that express Dfz2. In dorsal retinula cells the expression of the genes of the *iroquois* complex seem to attenuate the competence of Dfz2 to respond to DWnt4.[57]

Stop and Go at The Marginal Glia

In the larva, the lamina neuropil (called lamina plexus at this stage) contains the R1-6 terminals and is sandwiched between layers of glial cells. Distally of the R1-6 terminals, the epithelial glial cells are situated and proximally the lamina marginal glial cells. They separate the R1-6 terminals from the layer of medulla glia. Several lines of evidence suggest that the lamina marginal glial cells represent an intermediate target for R1-6 growth cones and cause them to stop at this point. In nonstop mutants[58,59] glial cell development is disrupted and the axons of R1-6 do not terminate in the lamina, but project down into the medulla. Nonstop is a ubiquitin-specific protease that is required in glia cells. Similarly, the absence of marginal glia in clones mutant for *Medea*, which codes for a DPP signal transducer, results in R1-6 axon projection defects.[60]

Contacting glial cells as intermediate targets may be the price retinula cells have to pay for regulating the neurogenesis of their postsynaptic partners. These still have to differentiate and it is not before the second half of pupal development that synapses are being formed[2,61,62] (Fig. 5).

Neither the molecular nature of the stop signal emitted by marginal glial cells nor the receptor in R-cells are currently known. However, it was shown that the absence of the receptor tyrosine phosphatase PTP69D in photoreceptors sometimes leads to their projection into the medulla.[63] As PTP69D is also required for the correct targeting of R7 to layer M6 of the medulla (in its absence R7 terminates in M3 like R8) it has been suggested that PTP69D plays a permissive role in R1-6 and R7 axonal targeting by helping to defasciculate from the leading R8 axon.[64]

After having stopped at the marginal glia, R1-6 growth cones are hanging around for quite a while. Apparently the reception of nitric oxide (NO), which is produced by lamina cells, is required for these growth cones not to project further down into the medulla.[65] Furthermore, Brakeless, a nuclear protein is needed in retinula cells to stop their axons at the marginal glia.[66,67] Interestingly, Brakeless acts as a transcriptional repressor of the *runt* pair rule gene, which encodes the Runt transcription factor required for R7 and R8 axonal projections into the medulla. If repression of *runt* by Brakeless is abolished in R2 and R5 cells only, this is sufficient to induce the projection of all six outer R-cells into the medulla.[68] This fact clearly indicates the existence of interactions between the R-cell terminals in larval development. As R2 and R5 are determined directly after the R8-cell, their axons are the first to follow the R8 axon. When the first three axons of an ommatidial bundle project into the medulla, the trailing axons might be forced to follow due to fasciculative forces. During the pupal stage, afferent-afferent interactions also seem to play an important role in the sprouting of the outer R-cell terminals to their correct visuotopic cartridges.[2,17,69]

Neural Superposition: Correcting the Initial Retinotopic Projections in the Lamina

Initially, in the larvae, all outer R-cell axons from a single ommatidium form a single fascicle. They terminate together, sandwiched between the epithelial and marginal glia in the lamina plexus, retaining their spatial relationship in the ommatidium. A column of 5 lamina monopolar neurons is induced by the incoming photoreceptors distally. Lamina monopolar axons fasciculate with the R7/8 axons of the corresponding ommatidium and project towards the medulla. Due to the axonal ingrowth from new ommatidia and the corresponding recruitment of lamina neurons and glia along the posterior—anterior axes, a precise retinotopic map is established. However, the retinotopic map is of little use for R1-R6 in the lamina, as the R1-R6 from a single ommatidium look at different points in visual space. In order to obtain a visuotopic map from here, R-cell axons have to be resorted so that axons coming from retinula cells looking at the same point in space in the adult are united in a single cartridge. This process takes place in the first half of pupal development[2,17] (see Figs. 5,6). It is interesting to note that the extensive resorting and hence rewiring of photoreceptor terminals in the lamina is a peculiarity solely made necessary by the fact that *Drosophila* ommatidia, like all Diptera, contain a split or open rhabdom system; the rhabdomeres receive light from different points in space under the same lens. A single secreted protein, Spacemaker, is necessary and sufficient for the formation of an open system.[70]

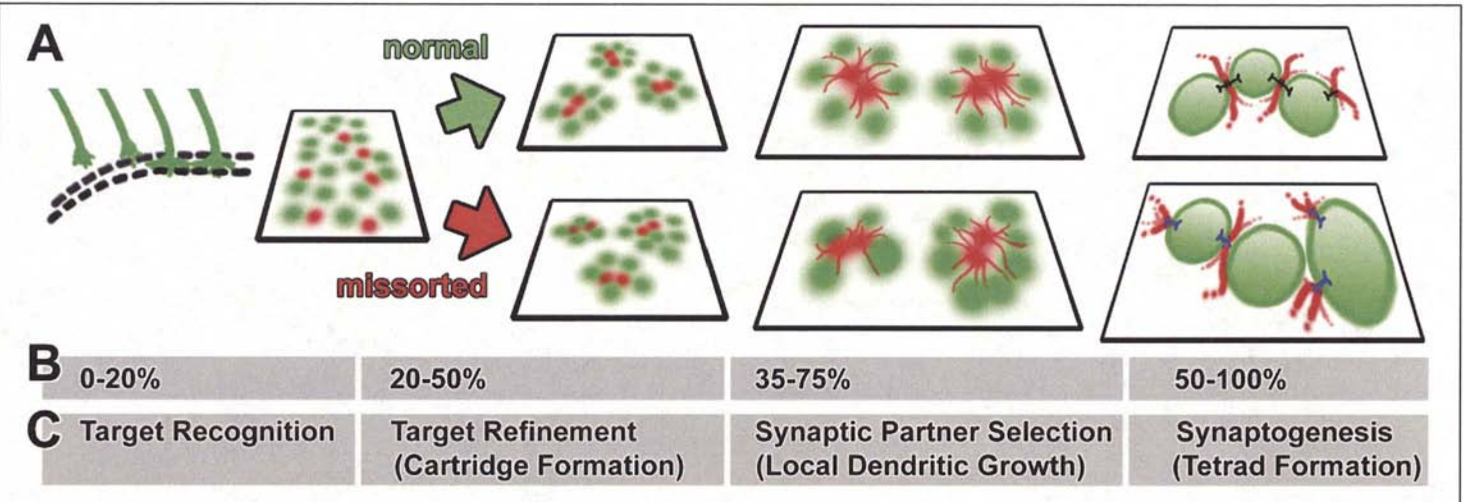

Figure 6. The Development of the Drosophila Visual Map. Time series of developmental steps normally leading to the formation of visuotopically correct synapses. A) R1-R6 growth cones initially stop at the marginal glia. Sorting of photoreceptor terminals into cartridges that map neighboring points in space occurs during the first half of pupal development. The second half is characterized by synapse formation between synaptic partners that were prespecified during cartridge formation. A normal number of synapses forms in photoreceptor terminals independent of synaptic partner accuracy. Green, photoreceptor terminals; red, postsynaptic lamina monopolar cells. B) Time scale. C) Naming of developmental steps. Adapted from Hiesinger et al 2006[71].

By visualizing projections from single ommatidia labeled with DiI and by deleting subsets of retinula cells, it was demonstrated that interactions among the R-cell population itself regulate cartridge selection.[17] First it was shown that remaining R-cell terminals in mutants for *phyllopod* (R1, R6 and R7 are transformed into cone cells), *lozenge^{sprite}* (transforms R3 and R4 into R7 cells) and *seven-up* (transforms R3 and R4 and in addition R1 and R6 into R7) are still able to defasciculate and to initiate their search for a lamina target. Therefore, this basic behavior seems to be independent of other R-terminals in the bundle. However, when R1 and R6 were absent, the final projections of the remaining R3 and R4 terminals were invariably correct, while those of R2 and R5 sometimes showed defects. R1 and R6 are therefore not required for the correct projections of R3 and R4, but do influence R2 and R5 targeting. In *lozenge^{sprite}*, absence of R3 and R4 leads to highly aberrant targeting of the remaining R-cell axons (R1, R2, R5, R6). In the *seven-up* mutant, where in addition R1 and R6 are missing, the remaining R2 and R5 are always making targeting errors.[17] In conclusion these results indicate a specific interaction between R-cell axons with regard to their final projections in the lamina.

Mutations in many genes have been identified in large screens using the eyFLP method[64] that affect this photoreceptor terminal resorting and thus lead to cartridges with too few or too many R-cell terminals.[71] The list contains several guidance receptors and cell adhesion molecules, including DLar (a receptor tyrosine phosphatase), DN-Cadherin (a classical cadherin) and Flamingo (a protocadherin).[72-74] For N-Cadherin mediation of attractive interaction between photoreceptor axons during visual map formation has been demonstrated.[75] All of these are also required for the targeting of R7/R8 in the medulla, as discussed in more detail below.

Guidance cues like the above-mentioned cell adhesion molecules must be accurately spatiotemporally regulated and localized in order to provide meaningful synapse formation signals. Vesicle trafficking has been implicated in the localization of cell adhesion molecules in photoreceptors mutant for neuronal synaptobrevin, which encodes a vesicle protein critically required for vesicle fusion.[76] More recently, loss of a vesicle-associated protein, the exocyst component Sec15, has been shown to cause specific cartridge sorting and R7/R8 projection defects (Fig. 7). Importantly, photoreceptors mutant for *sec15* display mislocalization phenotypes for a specific subset of guidance molecules, including DLar.[77] Which intracellular compartments are responsible for the dynamic and precise trafficking and localization of guidance receptors is unknown.

Synapse Formation in the Lamina Is Activity-Independent and Synapse Number Is Presynaptically Determined

In vertebrates the refinement of retinotopic maps in the visual system is strongly affected by electric neuronal activity and by competition between presynaptic terminals.[78,79] Although visual deprivation in early adulthood does reduce synapse number in the visual system of *Drosophila*,[80] neuronal activity is not required for synaptic partner selection, synapse formation or refinement of synapse numbers in pupal photoreceptors. The emerging fly is thus provided with a prespecified, functional visual system that has been built by activity-independent mechanisms.[71] The argument is based on the evaluation of the brain structure of mutants with defects in the generation of electrical potentials (*norpA^{P24}*: phospholipase C, required for phototransduction[81] and *trp^{343}*; *trpl^{302}*: Ca^{2+} channels required for evoked and spontaneous electrical potentials), or with defects in the conduction of electrical potentials (*para^{ts1}*: sodium channel), or with defects in the release of neurotransmitter (*hdcjk910*, a histidine decarboxylase[82,83]) and *synaptotagmin* (a Ca^{2+}-sensor required for neurotransmitter release[84]). Importantly, in spite of the absence of spontaneous or evoked electrical activity, cartridge sorting according to the principle of neural superposition as well as the formation of the correct number of synapses in each cartridge are normal.[71] Per R-cell terminal about 50 evenly spaced synapses are formed.[85,86]

Synapse number is not only independent of electrical activity, but also independent from hypo- or hyperinnervation of a single cartridge by R-cell terminals (Fig. 6). Synapse constancy per R-cell terminal was first suggested for house flies[87] and recently shown for *Drosophila*.[71] In a collection of cartridge missorting mutants, terminals in aberrant cartridges nevertheless form

a normal number of synapses with the postsynaptic L1-3 neurons. The number of synapses per R-cell terminal does not correlate with the number of terminals per cartridge, which shows that there is no competition for limited postsynaptic contact provided by L1-3. The presynaptic R-cell terminal is exerting control.[71] Nothing is known about the mechanism that restricts the number of synapses at the presynaptic site.

Medulla and Lobula Complex Development

As compared to the lamina, the neuropils of the medulla and lobula complex are structurally much more complex and house many more neuronal types.[18] Columnar organization is retained and there is a one to one correspondence between lamina cartridges and medulla columns, in spite of the fact that the connecting fibres cross in the outer (first) optic chiasm in the horizontal plane. These fine-grained, isotopic point-to-point connections are also retained between the medulla and the lobula complex through the axon bundles in the inner optic chiasm. However, at the level of the lobula output neurons, the number of repetitive elements is reduced.[26,88,89] While the lamina neuropil is only weakly stratified (e.g., the L4 collaterals are restricted to the proximal lamina layer), stratification of the medulla, lobula and and lobula plate is pronounced (Fig. 2A). Based on profiles of Golgi impregnated neurons, the medulla has been divided into ten different layers (M1-M10), the lobula into six layers (Lo1-Lo6) and the lobula plate into four layers (Lop1-Lop4).[18] Layers M1-M6 constitute the distal medulla and layers M8-10 the proximal medulla. In structural brain mutants like *small optic lobes (sol)*[90] the layering of the neuropil can be severely disturbed (Fig. 2B,C). Both parts of the medulla are separated by the serpentine layer M7, which houses large tangential axons and dendrites of medulla columnar neurons projecting to or from the Cucatti bundle. Columnar neurons of the distal medulla, like lamina monopolar cells, are derived from the outer optic anlage, while columnar neurons of the proximal medulla and the lobula complex derive from the inner optic anlage.[2,43]

In contrast to the lamina, that is completely dependent on retinal innervation, medulla and lobula complex rudiments do exist in completely eyeless flies.[41,91] (Fig. 2D,E). These rudiments are not exclusively built by descendants of the inner optic anlage; they still contain columnar neurons derived from the outer optic anlage[91] and cell loss seems mainly be due to degeneration of differentiated neurons rather than to a lack of proliferation of neuronal precursors, as massive axonal degeneration has been decribed at the level of the inner optic chiam in eyeless *sine oculis* pupae.[92] This indicates that the final division of the precursors of these neurons does not depend on induction by innervation of R7/R8 or of lamina monopolar axons.

It is also very telling that the neuropil rudiments of medulla, lobula and lobula plate are still isotopically connected by columnar neurons in such completely eyeless flies[91] (Fig. 2D). Visuotopy in the wild type optic lobe is therefore not completely induced by the ordered ingrowth of retinula cells. Also layering, at least at the level of the lobula, is partially retained. However, a reliable feature of the optic lobe rudiments of completely eyeless flies is the fusion of the posterior medulla neuropil with the lobula plate. This fusion seems to result from the sprouting of medulla tangentials into the lobula plate[91] (Fig. 2D,E). The relative independence of the deeper layers of optic lobe neuropils from eye development may reflect their intensive invasion by neurons that house their cell bodies in regions of the central brain.[26]

The Importance of Compartment Boundaries

Glial septa define neuronal compartments in the developing central brain as well as in the optic lobe.[93] One such border separates the outer optic anlage and its descendants from the inner optic anlage and its offspring. During development lamina cells are in very close proximity to cells of the lobula cortex. These cell populations never intermingle in wild type flies. The Robo/Slit receptor/ligand system was recently shown to be of importance for the maintenance of the separation of these cell populations. Slit is secreted by lamina glia and repels Robo-positive neurons of the lobula complex.[94] The *egghead (egh)* gene is also involved in the establishment of this compartment border.[95] In the absence of *egh*, some R1-R6 axons project abnormally to the medulla. This

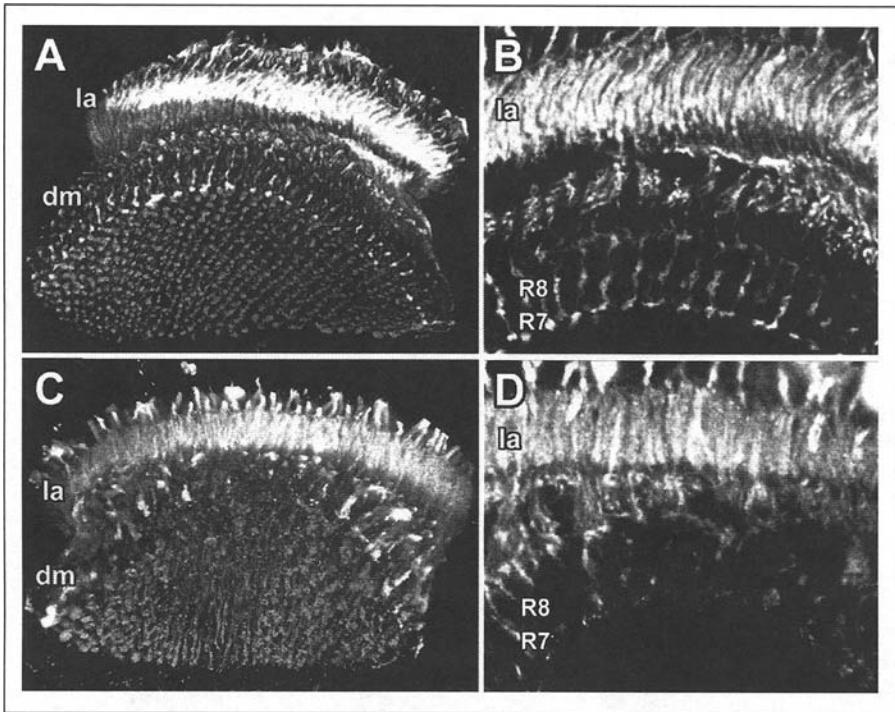

Figure 7. Normal and aberrant photoreceptor projections in the optic lobe. A, C) 3D reconstructions from confocal stacks of photoreceptor projections in newly eclosed flies, viewed from inside the brain. B, D) Projections views of respective brains at higher magnification. A, B) Wild type R1-R6 projections form a dense synaptic layer in the lamina (la). R7/R8 project through the outer chiasm and terminate in separate layers in the medulla (dm, distal medulla). C, D) Mutants defective for correct synaptic partner selection (shown here are photoreceptors mutant for *sec15*) are characterized by a loss of the precise and regular projection pattern in both neuropils (adapted from Mehta et al 2005[77]).

is not due to a loss of *egh* function in the eye or in the neurons and glia of the lamina. Instead, clonal analysis and cell-specific rescue experiments showed that *egh* is required in cells of the lobula complex primordium, which abuts the lamina and medulla in the developing larval brain. In the absence of *egh*, sheath-like glial processes at the boundary region delimiting lamina glia and lobula cortex are in disorder and inappropriate invasion of lobula cortex cells across this boundary region disrupts the pattern of lamina marginal glia which normally provides the stop signal for R1-6 axons.[95] *egghead* encodes a beta4-mannosyltransferase[96] which is involved in Glycosphingolipid biosynthesis. Glycosphingolipids have been implicated in EGFR signaling in *Drosophila*.[97]

Selecting the Correct Medulla Target Layer

In the medulla the visual information channels fed by R1-6 are relayed via lamina neuron processes to higher order interneurons. In addition, photoreceptors R7/8 terminate and form synapses exclusively in the medulla (Fig. 7A), where therefore the color vision circuit is predicted to reside[9,19] (Fig. 3). Layering of the medulla reflects the requirement for the establishment of visuotopically organized synapses between these different sets of columnar neurons. In the adult optic lobe the five lamina monopolar neurons and R7 and R8 terminate in different layers of the distal medulla.[18] This enables them to relay on characteristic sets of higher order columnar neurons which project to the lobula complex, most importantly onto transmedulla cells (Tm) projecting to the lobula and

transmedulla Y cells (TmY), the axons of which branch in the inner optic chiasm and terminate in lobula and lobula plate (Fig. 2). By inspection of R8 and R7 targeting it was shown that the adult situation is established in an at least two-staged layer-selection process[98] (Fig. 5). During early pupal development the newest leading R8 axon terminates superficially in the distal medulla neuropile and is overtaken by the following R7 axon that temporarily occupies the immediate adjacent deeper layer. These temporary layers of R8 and R7 are more and more pushed apart by the growth cones of the five lamina monopolar cells, which follow and elaborate their arborizations in the space between the R-cell terminals during the first 40% of pupal development. The lamina gradient of maturation from posterior (oldest) to anterior (youngest) is thus reflected as a spatial gradient of the thickness of the medulla in the horizontal plane during early pupal development. At about 50% of pupal development all R7 and R8 growth cones simultaneously become mobile again and target to their final layers M6 and M3.[6,98] The nature of the global trigger of this event is still unknown.

In *sevenless* mutants lacking R7, the axons of R8 and lamina monopolar neurons behave normally during targeting stage I (Fig. 5). The same is true for R8 and R7 terminals in the absence of lamina monopolar neurons. Therefore R8, R7 and L1-L5 axons target independently to their temporary terminal layers at the first layer-selection stage.[98] This layer selection therefore does not seem to depend on interactions between the afferents, but rather on interactions with cells in the target area.

Some factors have been identified that are required for target layer selection. One interesting example is the homophilic cell adhesion protein Capricious (CAPS) with leucine rich repeats, which is present only in R8 and in medulla cells, but not in other retinula cells and not in the lamina.[99] In the medulla neuropil of the third larval instar CAPS is uniformly expressed, but is restricted to specific layers during pupal development sparing the final R7 recipient layer. In flies mutant for *caps*, R-cell terminals in the medulla do not form a regular array and many R8-cell terminals seem to invade neighbouring columns. If CAPS is misexpressed in R7 cells, the first stage of R7 target layer selection is only mildly affected, but the growth cones remain in the final R8 recipient layer. This is evidence that CAPS plays an instructive role in the targeting of R8 terminals.[99]

Other factors required for target layer selection of retinula cells are more widely expressed in the target region and may play a permissive role, e.g., N-cadherin.[6,72] Homophilic cell adhesion mediated by the extracellular domain rather than signaling is important, because the cytoplasmic domain is dispensable not only for N-cadherin mediated cell adhesion in S2 cells but also for targeting of R7 growth cones. However, the cytoplasmic domain is required for normal R7 growth cone morphology.[100] In the lamina, N-cadherin seems to function in a very similar way in the targeting of R1-6 axons as it is expressed and required in the R-cells as well as in the lamina monopolar neurons.[75]

As N-cadherin is not exclusively expressed in specific subsets of neurons in the respective target areas, it is worth mentioning that N-cadherin exists in 12 splice isoforms. In fact, it could be shown that the isoform specific *N-cad (18Astop)* allele selectively affects the second stage of R7 target selection.[101] This allele eliminates the six isoforms containing alternative exon 18A. N-cadherin isoforms containing exon 18B are sufficient for the first stage of R7 targeting to its temporary layer, while the 18A isoforms are preferentially expressed in R7 during the second half of pupal development and are necessary for R7 to terminate in the appropriate synaptic layer M6 of the medulla.[101] However, it is very unlikely that the N-cadherin isoforms constitute something like a combinatorial code for the selective recognition of synaptic partners, as expression of any isoform is able to rescue the function of the other and the various isoforms mediate promiscuous heterophilic interactions with each other.[98,101] The function of the structural variations in the isoforms is thus still unknown. It is conceivable that they affect interactions with other proteins rather than homophilic adhesiveness. Therefore N-cadherin can be considered as a homophilic cell adhesion protein providing permissive stabilizing interactions in target selection.

Mutant alleles of the receptor tyrosine phosphatase LAR and its downstream interactor, the scaffolding protein Liprin-α, produce N-cadherin mutant-like targeting defects of R-axons.[74,102-104] Both proteins are expressed like N-cadherin in all R-cells and in neurons of the target areas and

their involvement in the regulation of N-cadherin has been shown.[105] However, the requirement of Liprin-α and LAR for R-cell targeting is exclusively on the presynaptic site.[103,104] This implies that N-cadherin regulation is different on the dendritic and on the axonal site. Two heparan sulfate proteoglycans have been identified as ligands for LAR: Dally-like and Syndecan. Both have been implicated in LAR-dependent axon guidance: Syndecan as a promotor and Dally-like as an inhibitor of LAR signaling.[106-108]

The G-protein coupled, 7-pass transmembrane receptor Flamingo is an atypical cadherin, which has recently been shown to regulate synaptogenesis at the neuromuscular junction. In addition, Flamingo is required to prevent axonal and synaptic degeneration in *Drosophila*.[109] Its involvement in optic lobe development is also well established.[73,110,111] Mutations in the *flamingo (fmi)* gene have been discovered in screens for abnormal R-cell connectivity[111] and for defects in visual behaviour.[73] While Flamingo is required for the sorting of R1-6 terminals to their correct lamina cartridges, it has at least two important functions during R8 axon targeting as well: it facilitates competitive interactions between adjacent R8 axons to ensure their correct spacing[73,111] and it promotes the formation of stable connections between R8 axons and their target cells in the medulla.[110,111] The tiling function of Flamingo is not restricted to axonal projections. In other systems, it has been shown to function in the shaping of dendritic fields as well[112] and it was recently shown that ingrowing R8 axons induce layer-specific expression of Flamingo in the medulla via Jelly belly (Jeb) signaling.[110] Its receptor, the anaplastic lymphoma kinase (Alk), is expressed and required in target neurons in the optic lobe. Jeb is generated by photoreceptor axons and controls target selection of R1-R6 axons in the lamina and R8 axons in the medulla. Loss of Jeb/Alk function affects medulla layer-specific expression not only of Flamingo, but also of two cell-adhesion molecules of the immunoglobulin superfamily, Roughest/IrreC and Kirre/Dumbfounded.[110] These closely related single pass transmembrane proteins are known from their function in muscle fusion,[113,114] eye development[27,115] and optic chiasm formation.[116-118] Loss of Roughest/IrreC leads to misrouting via the inner optic chiasm of posterior R8/R7 and lamina monopolar axons to their visuotopic target area.[116] The axonal bundles in the first optic chiasm which connect single lamina cartridges with isotopic medulla columns tend to fasciculate in loss of function mutants,[118] which copies the loss of *flamingo* phenotype in the first chiasm,[73] indicating that the Roughest/IrreC protein helps to keep columnar fibre bundles apart from each other.

Columnar Tiling

While the stratification of columnar neurons reflects their cell type specific connectivity, it is the lateral extent of the arborizations that determines the visuotopic precision of the adult neurons and affects the size and position of their visual fields. It was shown in a classical paper that competition between R7 terminals occurs to a limited degree in the target region.[119] In the third instar, R7 axons transiently display overlapping halos of filopodia, but in genetic mosaics vacant sites are only invaded by neighbouring R7 terminal extensions, if extra R7 axons due to the *more inner photoreceptors* mutation are available in the juxtaposed medulla columns.[119]

The appropriation of territory by neuronal arborizations has at least two aspects. First, the processes of the same neuron have to recognize and arrange themselves. Dendritic as well as axonal arborizations should more or less evenly cover their appropriate target space. Second, neurons of the same type should respect each others territory. The second process is known as "tiling", but the first process is related. It has to be assumed that in both processes recognition of "self" or of "same kind" has to be followed by repulsion. Interestingly homophilic receptors of the conserved family of the Down syndrome cell adhesion molecules (DSCAMs), members of the immunoglobulin superfamily, have been found to function in both aspects of neuronal tiling.[120-122] There are four Dscam genes in the *Drosophila* genome, called Dscam and Dscam2-4. Dscam is special in that it displays an extraordinary molecular diversity. Due to four casettes of alternative spliced exons it can generate 38016 different proteins.[123] Most interestingly, isoform-specific homophilic adhesion seems to induce repulsion in dendrites and thus helps to avoid selfcrossing and contributes to an even coverage of the dendritic field in all four classes of dendrite arborization neurons, a group of sensory neurons with a stereotyped dendritic branching pattern.[120,122] For Dscam2 two isoforms

(Dscam2A and Dscam2B) have been described. They are able to mediate isoform-specific homophilic adhesion in S2-cells and do not bind to other Dscam family members. Dscam2 plays a role in tiling among L1 terminals (Fig. 2A) within the distal medulla. Dscam2 homophilic interactions mediate repulsion between L1 axonal terminals in neighbouring columns. Loss of Dscam2 function leads to an overlap of the L1 terminals.[121]

The repulsive effect of proteins that are able to mediate homophilic adhesion in cell culture experiments demonstrates the importance of signaling for the understanding of cellular responses in vivo. Due to the high number of different types of columnar neurons in the medulla of *Drosophila*[18] it is likely that still other receptors will be described that function in tiling of columnar cell types.

Connecting Optic Lobes with and across the Central Brain

Neuronal connections between the optic lobe and central brain have recently been systematically mapped in considerable detail.[26] Comparably little is known about the development of these projections. Through the study of the transcription factor Atonal, which is originally known to be required for the specification of the R8 ommatidial founder photoreceptor, a dorsal cluster of optic lobe neurons was discovered that connects both optic lobes across the central brain during larval development.[124] The dorsal cluster neurons project contralaterally towards the lobula complex where they fan out over the lobula complex and inner chiasm and additionally form a precise number of projections towards the medulla. This reproducibly accurate projection pattern has been employed to identify an integrative signaling network encompassing the Jun N-terminal kinase, the GTPase Rac, the secreted morphogen Wnt, its receptor Frizzled, the FGF Branchless and the FGF receptor. Importantly, this network regulates the extension and retraction of axonal branches, but not axon guidance, indicating that these processes are regulated independently.[125] Finally, the dorsal cluster neuron projections have also been shown to form independent of neuronal activity, further supporting the notion that wiring of the optic lobes, from cellular differentiation down to the specification of synapses, follow a genetic program.[71,125]

Concluding Remarks

While many steps in optic lobe development are still not yet understood, it is clear that a combination of timing of neuronal and glial cell fate specification, axonal outgrowth, of inductive events and of specific recognition processes between "self" and "not self" direct the wiring of the neural machinery of the optic lobe. It is therefore a genetically encoded developmental program that ensures all aspects of vision required for the survival of the newly emerging fly. Adult optic lobe development is optimized for speed and precision. However, the adult optic lobe also displays a certain degree of plasticity. Deprivation of visual input after the optic lobe is formed can lead to a reduction in synapse numbers in the lamina during a critical time window in early adulthood.[80] However, such plasticity is apparently not required to wire a functional optic lobe. It is therefore an important realization that a brain structure like the *Drosophila* optic lobe is as much the product of a genetically encoded developmental program as the eye or a wing. Given the rich genetic tool box available and the wealth of knowledge about *Drosophila* development, the optic lobe is a wonderful model system to decipher this developmental program and attain knowledge about the extend to which a brain structure can be "genetically encoded".

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