Chapter 1

The anatomical organization of the compound eye's visual system

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Introduction

The visual system of the fly's compound eye is noted both for its modular composition and for crystalline regularity. In the compound eye, each module or ommatidium has a fixed complement of 26 cells that includes eight uniquely identified photoreceptor neurons (Ready et al., 1976). An outer ring of six cells, R1–R6, surrounds two central cells R7 and R8 in each ommatidium. Backed by extensive genetic analysis of their development and function, R1–R6 constitute by far the best-understood sensory neurons in any invertebrate visual system, and among the best-known neurons of any nervous system. During its development from the eye imaginal disk, the pattern of ommatidia in the compound eye is impressed upon neurogenesis in the primordia of the underlying optic lobe (Meinertzhagen and Hanson, 1993) and, as a result, the optic lobe neuropiles are likewise modular in their composition, comprising a clear array of cartridges in the first neuropile, the lamina (Braitenberg, 1967) and a less obvious array of columns in the second neuropile, the medulla (Campos-Ortega and Strausfeld, 1972; Strausfeld and Campos-Ortega, 1972). The lamina and medulla are some of the most orderly and well-characterized neuropiles of the entire fly's brain, and models for the brains of all animal species, invertebrate or otherwise.

Often overlooked or simply not acknowledged, most essential details of the neuroanatomy of the optic lobe were established not in *Drosophila*, but in larger fly species – mostly the housefly *Musca domestica*, before observations on *Drosophila* became ascendant. Anatomical studies on the optic lobe in *Drosophila* are, in fact, undergoing an intense renaissance at the time of writing this review, yielding to new genetic and imaging technologies that support a sense of promise that many long-outstanding questions will soon be resolved. Particular issues include the number of individual cell types, their synaptic circuits, and neurotransmitter systems, and whether each type is discrete, distinguishable from all other types. The groundwork for these questions in *Drosophila* was laid by a commendably accurate report of the cell types derived from Golgi impregnation (Fischbach and Dittrich, 1989), which is still current. Anticipating the topic of this chapter, Meinertzhagen and Hanson (1993) provide summary diagrams of the adult optic lobe that occasional readers have found useful.

The compound eye

The compound eyes are the most obvious of the fly's seven visual systems (Hofbauer and Buchner, 1989), and their regular array of corneal lenslets has been a favorite object for microscopists since the time of Hooke (1665). Each corneal facet is a regular hexagon with two horizontal sides, that forms part of an array with horizontal z rows aligned parallel to an equator and two oblique rows (x,y). At its greatest vertical height, each eye contains about 30 such rows, divided equally between dorsal and ventral ommatidia on either side of the equator, with a similar number of x and y rows (Ready et al., 1976). The hexagonal shape of the ommatidium, and the ommatidial lattice that results, is refined during development, when excess pigment and bristle cells are removed (Cagan and Ready, 1989). It is therefore the loss of these cells that confers the regularity of the ommatidial photoreceptor array that is critical for the eyes' isotropic spatial resolution.

The ommatidium and its pattern of axonal projection

The organization and structure of photoreceptor neurons (Ready et al., 1976), and their rhodopsin expression patterns (e.g., Mikeladze-Dvali et al., 2005) in *Drosophila* have all been extensively reviewed elsewhere. Each photoreceptor neuron has a single light-absorbing rhabdomere that is separate from that of its neighbors, with the two rhabdomeres of R7 and R8 situated axially in tandem, that of R7 sitting on top of R8 (Ready et al., 1976). Starting with R8, R1–R6 assemble in a developmental sequence as three pairs of neurons, in which R2 and R5 are recruited first, followed by R3/R4 and then by R1/R6; R7 is added last (Tomlinson and Ready, 1987). Thus, R1–R6, which in many ways are matched in their anatomy and function, with each expressing a single rhodopsin Rh1 (O'Tousa et al., 1985), are not in fact a single class but are actually paired,

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as in other insect ommatidia. When viewed in cross-section, the six outer rhabdomeres of R1–R6 form an asymmetrical trapezoidal pattern that is reflected at an equator, a line of mirror-image pattern symmetry between ommatidia in the dorsal and ventral regions of the eye field (Dietrich, 1909). A consequence of that pattern is that the optical axis of each photoreceptor diverges slightly from those of other photoreceptors in the same ommatidium, each photoreceptor as a result viewing a slightly different point in visual space (Kirschfeld, 1967; Franceschini and Kirschfeld, 1971). Congruence between the angle of their divergence and the angular divergence between the optical axes of neighbouring ommatidia, imparted by the curvature of the retina, means that the axis of each R1–R6 photoreceptor exactly aligns with another R1–R6 photoreceptor sitting behind a neighboring facet, so that one photoreceptor each beneath seven such facets then view the same point in visual space. The axons of R1–R6 innervate the lamina, and while these enter the lamina as ommatidial bundles, the individual axons of each single bundle diverge at the distal face of the lamina neuropile. During that divergence, the axons of exactly those photoreceptors that view the same point in space then converge upon a single cartridge in the lamina (Braitenberg, 1967; Trujillo-Cenóz, 1965), in a pattern of so-called neural superposition (Kirschfeld, 1967). The axon sorting zone distal to the lamina is a complex layer of interweaving, a miracle of morphogenesis within which axon trajectories are established with great accuracy (Horridge and Meinertzhagen, 1970). Dorsal and ventral ommatidia have mirror symmetrical patterns of interweaving, and because more axons extend in a direction towards the equator than away from it, a zone of hyperinnervation is formed by cartridge rows on either side of the equator with a reciprocal zone of hypoinnervation at the lamina's rim (Meinertzhagen, 1972; Fröhlich and Meinertzhagen, 1987). These details were all firmly established from studies on large fly species, chiefly on *Musca* and the blowfly *Calliphora*.

The three main systems of photoreceptor input to the visual system are thus R1–R6, R7, and R8. R1–R6 provide input to motion-sensing pathways (Heisenberg and Buchner, 1977; Joesch et al., 2011; Rister et al., 2007), while R7 and R8 provide independent spectral inputs to the medulla (e.g., Heisenberg and Buchner, 1977; Gao et al., 2008). R7 and R8 with respective peak sensitivities in the UV (R7) and blue (R8) each express one of two rhodopsins (Morante and Desplan, 2004). Each cell type thereby comprises in turn two subtypes, and all four subtypes have distinct spectral sensitivities (Hardie and Kirschfeld, 1983). Pairs of R7 and R8 cells coordinately express a particular rhodopsin to construct one of two types of ommatidial rhodopsin partnerships, pale or yellow (Franceschini et al., 1981; Mazzoni et al., 2008). The R1–R6 and R7/R8 systems, previously considered independent (Yamaguchi et al., 2008), have recently been shown to converge, R7 and R8 also contributing to the motion pathway (Wardill et al., 2012). A proposed structural basis for that convergence is provided by gap junctions that form in a shallow zone of the lamina within which the axons of R7 and R8 make glancing contact with that of R6, and less frequently with R1 (Shaw et al., 1989). The opportunity for that contact arises, in turn, from the sorting zone of photoreceptor axons that enables neural superposition, which requires the axon of R6 to pass between those of R7 and R8 to reach its correct cartridge.

The terminals of R1–R6

The synaptic terminals of R1–R6 in the lamina are God's gift to neuroanatomy. They are aligned like drinking straws, so that a single section samples many profiles, thus allowing rigorous quantification of synaptic organelles. Exploiting these features, and the opportunity to generate whole-eye mosaics of mutant neural genes that would be lethal elsewhere in the nervous system (Stowers and Schwarz, 1999; Newsome et al., 2000) the synaptic terminals of R1–R6 have provided a test bed for the diagnosis of mutant synaptic gene action. Selected examples include genes that regulate: mitochondrial transport (Stowers et al., 2003); vesicle endocytosis (Fabian-Fine et al., 2003; Dickman et al., 2005); or the role of activity on axon sorting and photoreceptor synapses (Hiesinger et al., 2006). R1–R6 terminals form tetrad synapses that release histamine (Hardie, 1989; Sarthy, 1991). Capitate projections are synaptic organelles formed where neighboring epithelial glia (below) invaginate into a R1–R6 terminal to form a stalked organelle with a spherical ~200-nm head, single or multiple, borne on a ~80 nm diameter stalk (Stark and Carlson, 1986). The base of the stalk is a site of endocytotic membrane retrieval (Fabian-Fine et al., 2003), and the head a postulated site of histamine recycling (Fabian-Fine et al., 2003) expressing the AMPylation protein Fic (Rahman et al., 2012), the whole functioning as a proposed integrated recycling organelle.

The optic lobe

The fly's visual world maps upon four separate, successive neuropiles of the optic lobe, which sits beneath the compound eye. These comprise (Fig. 1.1): first, the distal curved lamina; beneath it the large, concentric second neuropile, the medulla; and, beneath this, two face-to-face neuropiles, the lobula and posterior lobula plate, that lie orthogonal to the medulla's internal face (Strausfeld, 1976). These neuropiles are all modular, with an array of columns – called cartridges in the lamina – that exactly matches that of the overlying ommatidia (Braitenberg, 1967). Each ommatidium projects as an axon bundle, which then undergoes the pattern of divergence required for neural superposition, as described above. Each cartridge projects, in turn, as a bundle of 11 axons that connects it to the medulla by way of the external chiasma (Strausfeld, 1971a; Meinertzhagen, 1976). Each horizontal row of cartridge axon bundles inverts its anteroposterior sequence as a coherent sheet, each sheet folding over on itself in parallel with its neighbors, either by a clockwise twist in the dorsal half of the right eye, above the equator, or by a counterclockwise twist in the ventral half, to map onto a

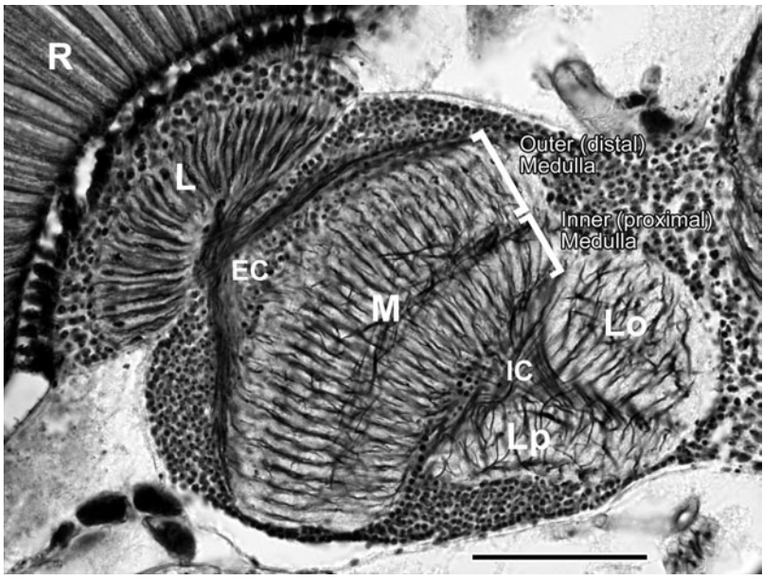

Fig. 1.1. The *Drosophila* visual system in horizontal section, showing rows of cartridges parallel to the equator in the lamina (L) connecting with rows of columns in the medulla (M) via the external chiasma (EC). Outer (distal strata M1–M6) and inner (proximal strata M7–M10) halves of the medulla are separated by a middle stratum connecting to Cuccatti's bundle, that contains many of the medulla's tangential neurons. Axons extend between the medulla's proximal face and the lobula and lobula plate neuropiles via the inner chiasm. R, retina; IC, internal chiasma; Lo, lobula; Lp, lobula plate. Scale bar: 50 μm. (Image of Bodian preparation; reproduced from Takemura et al., 2008.)

horizontal row of medulla columns (Braitenberg, 1970). A corresponding inner chiasma with a more complex composition connects the medulla with the neuropiles of the lobula complex (see below).

These tracts are constituted by axons of columnar relay neurons, having their axon running the length of a column. Accounts especially by Strausfeld and others using Golgi impregnation and other classical light microscopic methods, established a library of cell types in different fly species (e.g., Strausfeld, 1979, 1971b, 1976; Strausfeld and Lee, 1992). Major studies on *Drosophila* came only after these earlier accounts and, at least initially, were mainly confirmatory.

Definition of morphological cell types

The landmark Golgi study of Fischbach and Dittrich (1989) provided what is still the most comprehensive single published account of cell types in *Drosophila*, assigning neurons to classes based on the direction of axon outgrowth – whether at right angles to the neuropile, as for columnar neurons, so as to project a retinotopic map onto the lamina, medulla, and lobula – or across the neuropile, as for tangential neurons. Further distinctions among these are based on the extent and stratum of each cell's dendrite arborizations (Figs. 1.2, 1.3). The third class of intrinsic neurons are distinct from both columnar and tangential neurons insofar as they arborize only in a single neuropile, and are thus the substrate for local circuit interactions. While having much to commend it, a parallel nomenclature of "columnar neurons," which contact photoreceptors from a single ommatidium only, and "non-columnar neurons" that integrate information from broader receptor fields (Morante and Desplan, 2008), will not be adopted in this account.

From the evidence of Golgi impregnation alone, the optic lobe in *Drosophila* has a total of 113 morphological cell types, although even this large number appears to be a considerable underestimate, perhaps by about a third (Drs. A. Nern and G.M. Rubin, personal communication). Of these, the lamina has 12 types of neurons (Tuthill et al., 2013; Fig. 1.2), while the medulla has a reported minimum of 59, thus at least half the optic lobe's total. The cells are arranged in columns, one per ommatidium, and strata, ten in the medulla, six identified in the lobula and four in the lobula plate (Fischbach and Dittrich, 1989). The numbers of strata are thus in some proportion to the numbers of types of co-stratifying neurons they segregate (see below).

In addition to cells that relay within or between the optic neuropiles, visual projection neurons connect the optic lobe with the central brain. Among the 44 types identified in a screen of *Gal4* lines, 24 are associated with the lobula, of which 14 arborize specifically in the lobula and the remaining 10 contribute not only to the lobula, but also the medulla and/or lobula plate (Otsuna and Ito, 2006).

Some particular types of neurons and their numbers of subtypes

Including the lamina cells identified below, which all appear to be definitively identified, Golgi impregnation also reveals the main classes of columnar relay neurons (Fischbach and Dittrich, 1989). For the medulla, these are: the single class of five lamina L-cell types, which terminate in the distal medulla (Fig. 1.2); transmedulla cells (Tm, 30 including all reported subtypes – see Fig. 1.3), which penetrate the medulla and terminate in the lobula; and similar to these, TmY cells (14 including all subtypes), which have an axon that splits to terminate in both the lobula and lobula plate. Medulla intrinsic cells (Mi,

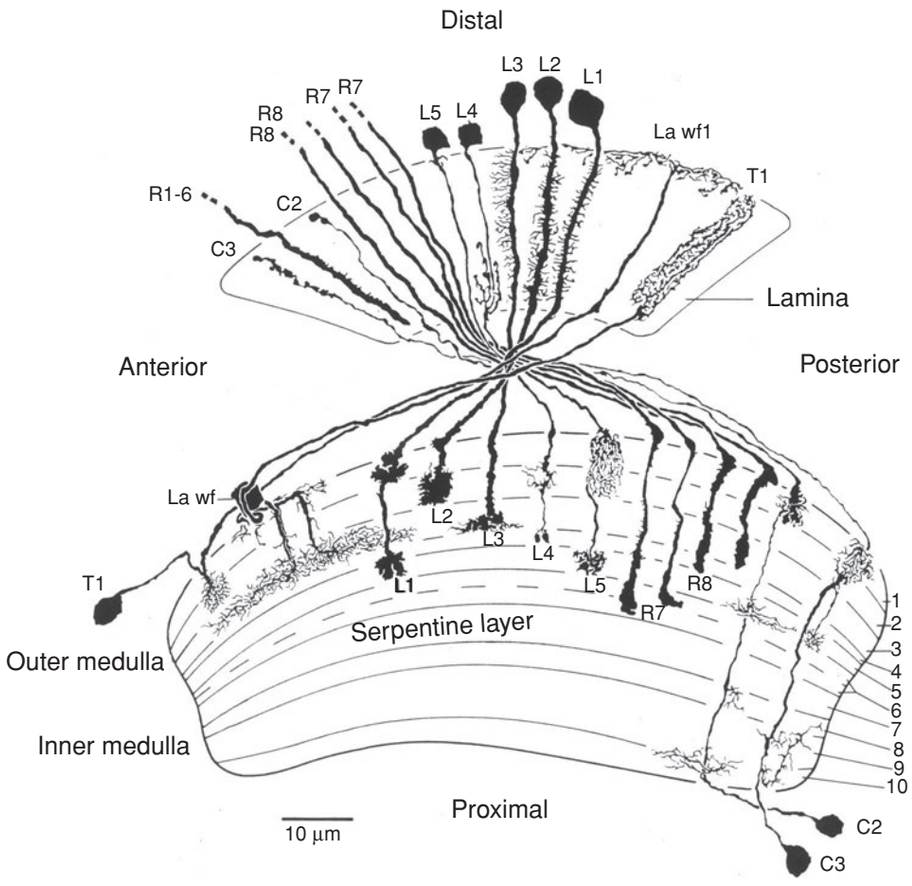

Fig. 1.2. The lamina's cell types impregnated by the Golgi method, shown in the same plane as in Fig. 1.1. Photoreceptor neurons R1–R6 innervate the lamina; lamina columnar cells L1–L5 relay to the medulla; photoreceptor neurons R7 and R8 innervate the distal medulla; T1 and a lamina wide-field cell (La wf1), both with somata in the medulla cortex, and C2 and C3, with somata between the posterior edge of the medulla and the lobula plate cortex, all innervate the lamina from a centrifugal direction. Lamina tangential and intrinsic (amacrine) neurons are omitted. (Reproduced from Meinertzhagen and Hanson, 1993, after Fischbach and Dittrich, 1989.)

12 reported subtypes) do not project to the lobula, but instead connect distal with proximal medulla strata, between strata 1 and 6 and strata 8 and 10, typically with dendritic arbor(s) in the former and a terminal in the latter and so relaying signals centripetally. T1 and two C cells (C2 and C3) are additional classes that project centrifugally from the medulla to the lamina (Fig. 1.2). The lobula and lobula plate neuropiles have four additional classes of columnar neuron, three with cell bodies in the lobula plate cortex: Tlp, Y and T cells. Translobula plate neurons (Tlp, seven reported subtypes) connect different layers of the lobula plate with lobula stratum Lo4. Y cells (five subtypes) have an axon that penetrates the lobula plate to bifurcate in the inner chiasma and project to both the lobula and proximal medulla, although no clear morphological distinction between dendrites and terminals is obvious. T cells (11 reported subtypes) also have their cell body in the lobula plate cortex, but form two major types depending on whether they arborize in the medulla or not. T2 and T3, for example, do, and, like the medulla centrifugal neurons, C2 and C3, with cell bodies nearby, both arborize in the proximal medulla (like Y cells), with T2 also arborizing in the distal medulla; in addition, both T2 and T3 project to the lobula. Similar to these, T4 also has an axon that divides in the inner chiasma, doubles back, and then innervates the lobula. T5 does the same but does not arborize in the medulla. T4 and T5 are numerous, with apparently up to four representatives per column (see below). In addition to these medulla neurons, lobula columnar neurons (Lcn, six reported Golgi subtypes) have cell bodies outside the optic lobe, arborizing in the deep lobula and projecting to the central brain. They form one of the many classes of visual projection neuron that project between optic lobe and brain, of which *Gal4* lines identify 14 more associated with the lobula (Otsuna and Ito, 2006), see above. Further illustrated details of cell types in *Drosophila* and associated nomenclatural issues are to be found in both these publications (Fischbach and Dittrich, 1989; Otsuna and Ito, 2006).

In addition to these columnar neurons, tangential neurons have an axon that spreads across the visual field, in many cases with an exuberant arbor, ten reported subtypes in the medulla, six in the lobula, and two in the lobula plate. Those of the lobula plate (Fischbach and Dittrich, 1989), the lobula plate giant tangential cells (LGTCs), have received particular attention because they signal wide-field information on motion, either horizontal (HS cells) or vertical (VS cells). Finally, additional cell types include the optic lobe intrinsic, or amacrine, neurons, those of the lamina (Lai), the medulla's distal (Dm, nine subtypes) or proximal (Pm, three subtypes) strata, or the lobula (Li, two subtypes).

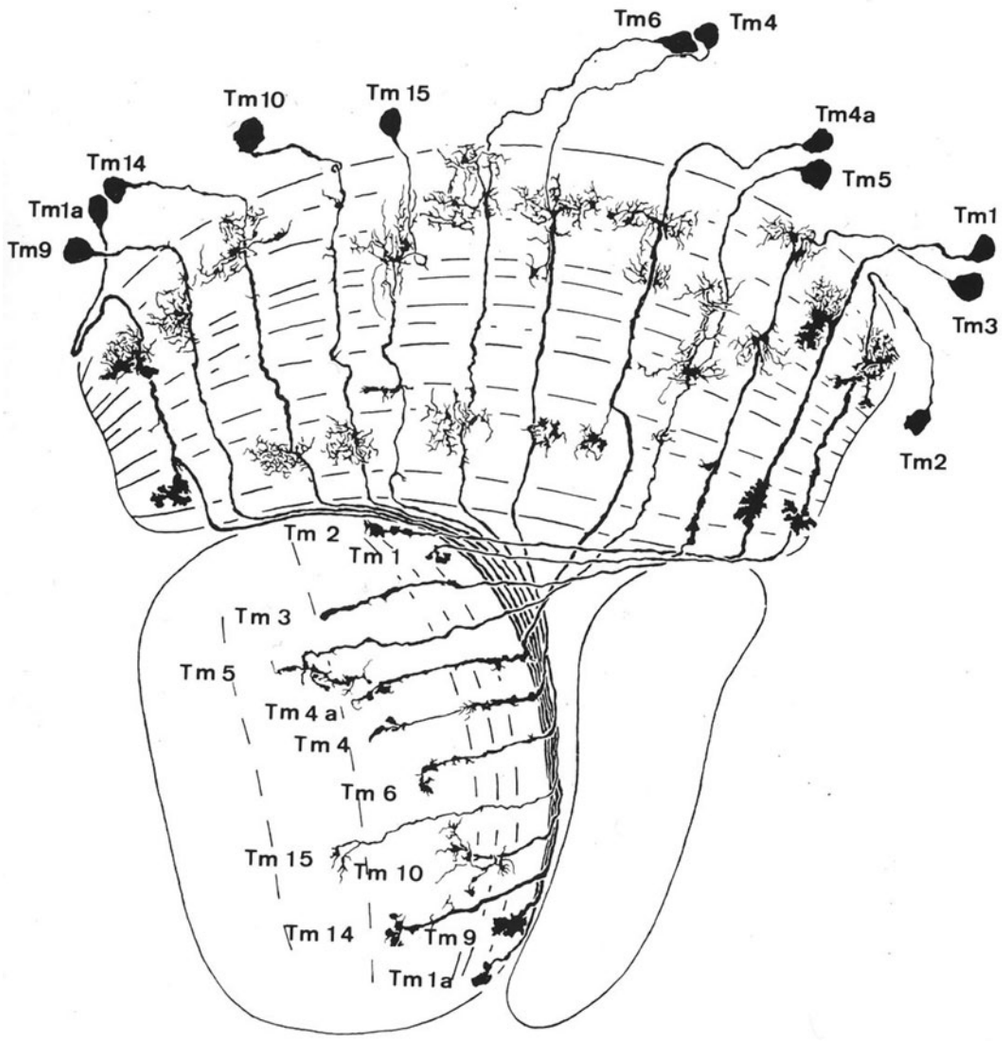

Fig. 1.3. Selected transmedulla (Tm) cells with somata in the medulla cortex, having axons that penetrate the medulla and terminate in the lobula. Tm1 and Tm2 are L2's chief targets (Takemura et al., 2011). Shown with the same orientation as in Fig. 1.2. (Reproduced from Meinertzhagen and Hanson, 1993, after Fischbach and Dittrich, 1989.)

In most cases these neurons have exquisite morphological phenotypes, and the careful assignment of a cell to a particular class has relied on accurate human observation and judgment that is particularly critical in the relay pathways for the many types of columnar neuron that connect successive strata and neuropiles. These judgments support an elaborate taxonomy based on several features: the direction of the axon, the site of its termination, the stratum of arborizations (from the ten in the medulla), and the width of the arbor (whether confined to a single column or extending across multiple columns). In parallel, screens of two major *Gal4* driver collections (Jenett et al., 2012; Hayashi et al., 2002) reveal the 12 cell types of the lamina (Tuthill et al., 2013), and many other classes of neuron, especially for the medulla (Drs. A. Nern and G.M. Rubin, personal communication) and lobula (Otsuna and Ito, 2006), some not previously reported from Golgi impregnation. In a more limited way, a *Gal4* line for the histamine channel protein gene *ort* (Gengs et al., 2002) expresses in neurons that are candidate targets for photoreceptor histamine release. It identifies L1–L3 (Rister et al., 2007) and several medulla cells, including a medulla amacrine cell Dm8 and four transmedulla cells, Tm2, Tm5, Tm9, and Tm20 (Gao et al., 2008).

Insofar as the taxonomy of cell types is based on human judgments, it is to some extent subjective. The close agreement between the forms of these cells seen from Golgi impregnation (Fischbach and Dittrich, 1989) and those seen in single *ort*-expressing neurons (Gao et al., 2008) rather gratifyingly implies that the human arbitration of different cell classes actually mirrors developmental decisions made by the fly. The latter must ultimately reflect the genetic steps that specify each neuron type. For example, brain-specific homeobox protein is expressed in lamina cells L4 and L5 and in medulla cell Mi1, and is required to specify the fate of all three (Hasegawa et al., 2013).

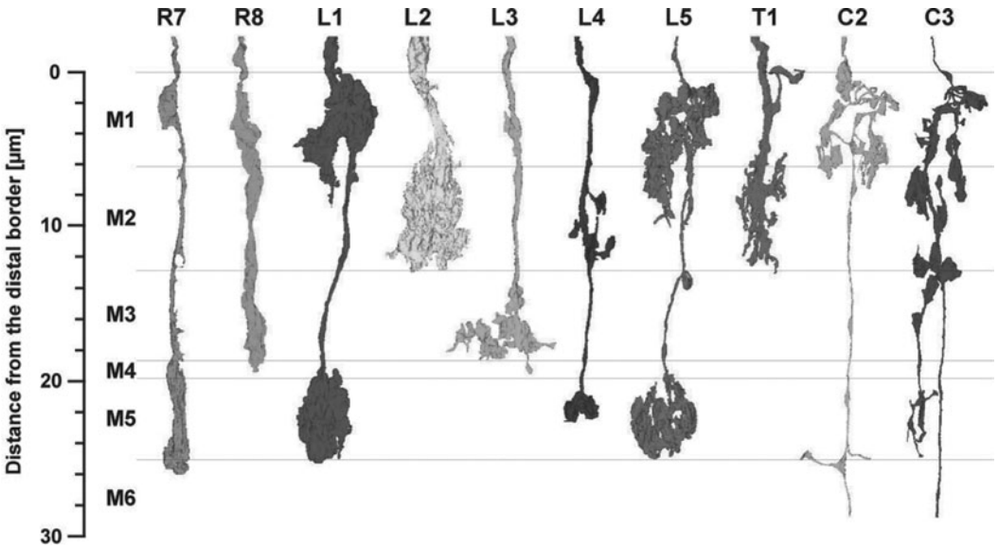

Fig. 1.4. Terminals of ten neurons reconstructed from serial-section EM with input terminals in strata M1–M6 of the distal medulla. The neurons are: R7 and R8, L1–L5, C2 and C3, and T1. Viewed from anterior looking posterior, in the plane of the chiasma. (Reproduced from Takemura et al., 2008.) A black and white version of this figure will appear in some formats. For the colour version, please refer to the plate section.

In addition to congruence between Golgi and genetic evidence, some neurons – such as L2 and Tm2 (Meinertzhagen et al., 2009) – have also been studied from serial-section EM, from which technically demanding three-dimensional reconstructions reveal yet a third means to view the same cells (Fig. 1.4), one that exerts no bias upon the choice of particular cells, but in which it may not be possible to reconstruct all tiny neurites.

With this spirit of conquest over some of the technically difficult approaches, and a groundswell of opinion to support the view that each type is discrete, morphologically determinate, and discriminable from all other types, it is still difficult to assess the exact extent of variation among the arbors of the same cell type, and to assert the absence of yet more subtle subtypes. Thus Tm5 identified from Golgi impregnation is now seen from inspection of repeated examples in a reporter line to comprise three subtypes, each with a minute difference in its arborization (Gao et al., 2008). We may anticipate other such subtleties, although close inspection of 379 cells reconstructed from serial-EM, most as multiple representatives of 56 classes of medulla neurons (Takemura et al., 2013), does not reveal widespread cases.

Finally, the neurotransmitter phenotype of the optic lobe's cell types contributes another layer of evidence, although this is often conflicting. Inconsistencies, especially between transmitter immunolabelings and genetic reporter lines are even obvious in the simple lamina (e.g., Kolodziejczyk et al., 2008). These become more obvious in the deeper neuropiles, among the cells identified by reporter lines for acetylcholine (Cha-positive: Raghu et al., 2011), glutamate (dvGlut-positive: Raghu and Borst, 2011), and GABA (dVGAT-positive: Raghu et al., 2013). Used to drive green fluorescent protein (GFP) these lines provide clear evidence of cell morphology, sometimes identifying hitherto unreported cell types, but sometimes supporting neurotransmitter phenotypes that are at variance with other evidence. To give but one example, L4 is ChAT-immunoreactive (Kolodziejczyk et al., 2008) and expresses *Cha* transcripts (Takemura et al., 2011), both implying its cholinergic nature, but expresses with a *dVGAT-Gal4* reporter, consistent with a GABA phenotype (Raghu et al., 2013).

The lamina: A tiny constituency of identified neurons

The lamina's distinctive array of cartridges, one per ommatidium (Braitenberg, 1967) – thus numbering more than 750 (Ready et al., 1976), is a particular feature of this neuropile in flies. All the optic neuropiles are, in fact, modular but the appearance of that modularity in the lamina of flies arises from the principle of neural superposition, because each cartridge is surrounded by the terminals of R1–R6 that converge upon it from neighboring ommatidia, and because these are wrapped in turn by isolating glia. More than this, each cartridge has an identical cellular composition. Present in every cartridge are five lamina monopolar cells L1–L5, two medulla centrifugal cells C2 and C3, and a third medulla cell T1. T1 is a mystery: morphologically it appears to be centrifugal but in *Drosophila* it lacks presynaptic sites in either lamina or medulla (Takemura et al., 2008). C2 and C3 have cell bodies that arise from deep in the optic lobe, in the cortex of the lobula plate. They have a GABA phenotype (Kolodziejczyk et al., 2008) and thus qualify as a substrate for inhibitory centrifugal feedback between medulla and lamina.

To these five are added contributions from four other less well-characterized cell types that are infraperiodic, having fewer cells than there are cartridges. (a) Two are wide-field neurons (Lawf1, Lawf2) having processes that spread into neighboring cartridges. Lawf2 is labelled by a *Gal4* line for the transcription factor Homothorax *hth-Gal4* (Hasegawa et al., 2011) and has recently been independently confirmed

(Tuthill et al., 2013). Lawf1 and Lawf2 arborize in different medulla strata, Lawf1 in M1 and M4, Lawf2 in M1 and M8–M10 (Hasegawa et al., 2011). Both were considered tangential cells (Kolodziejczyk et al., 2008) although the direction of their axons, orthogonal to the face of the medulla, is in fact columnar. Lawf1 is probably a cell that expresses GFP driven by a *Gal4* line for choline acetyltransferase (*Cha-Gal4*) and was redesignated *Cha*-Tan, while Lawf2 expresses a *Gal4* for the ionotropic GABAA receptor subunit RDL, *rdl-Gal4*, and was redesignated *rdl*-Tan (Kolodziejczyk et al., 2008). The complete forms of these cells have now been confirmed as Lawf1 and Lawf2 (Tuthill et al., 2013), and partial EM reconstructions and their synapses reported (Rivera-Alba et al., 2011). There are many such cells, but not one each per cartridge. (b) A third cell type is the highly synaptic lamina amacrine (Lai) neuron. These have cell bodies beneath the lamina with ascending axons that spread synaptic processes into a number of cartridges, those of a single cartridge probably deriving from a single Lai cell. The amacrine processes partner the basket arborizations from T1 cells, both cells contributing one of a pair of neurites that lies between neighboring R1–R6 terminals. (c) Except for Lawf2, all the above cells were reported from Golgi impregnation (Fischbach and Dittrich, 1989), along with a fourth, final cell type. (d) The latter is a lamina tangential neuron partly reported by Fischbach and Dittrich (1989) as Lat, now reported to correspond to about four cells per optic lobe (Tuthill et al., 2013) that innervate a distal plexus of the lamina. These cells arborize in the anterior, so-called accessory medulla involved in circadian regulation (Helfrich-Förster et al., 2007). A second contender for the Lat cell arises from a pair of somata in the posterior protocerebrum with bilateral axons that bypass the medulla of both sides, traverse the chiasma and posterior margin of the lamina, to give rise to upwardly directed varicose neurites that penetrate the lamina cortex. These cells are called LBO5HT in large fly species and are 5-HT immunoreactive (Nässel, 1991). They lack synaptic release sites and are thought to be sources of 5-HT acting as a neuromodulator, for example mediating circadian changes in the visual system (Pyza and Meinertzhagen, 1996). Resolving the candidacy of these two cells must await further evidence.

The lamina's synapses

The cartridge is like a wooden interlocking burr puzzle with tightly packed space-filling cells. These are predominantly cylindrical in shape, and their mutual packing is mostly the problem of how to fit all cell profiles optimally into the cartridge cross-section. This fit reflects a compromise between two complex demands: first, wiring economy – to minimize the distance between connections; and second, volume exclusion – the displacement of large neurites from regions that are rich in synaptic connections (Rivera-Alba et al., 2011).

A complete matrix of synaptic connections between the cells in a single wild-type cartridge has been reported (Meinertzhagen and O'Neil, 1991), as have estimates of pathway strength derived from the numbers of such connections (Meinertzhagen and Sorra, 2001), reports that have recently been amplified (Rivera-Alba et al., 2011). Thus, taking 20 synapses as the threshold, the strongest pathways are from R1–R6 to L1–L3 and amacrine Lai cell processes, each R1–R6 terminal forming about such 50 input synapses. Each synapse is a tetrad that releases histamine; the terminals of R7 and R8 in the medulla also contain histamine (Pollack and Hofbauer, 1991). Other strong pathways include the synaptic connections from amacrine cell neurites, which feed back to R1–R6 or provide input to L3, T1 or epithelial glia (see below), for which the neurotransmitter may be glutamate (Sinakevitch and Strausfeld, 2004; Kolodziejczyk et al., 2008). The amacrine feedback synapses onto T1 beg to be better characterized. These occur at so-called gnarl contacts, where a thin sheet from surrounding epithelial glia is interposed so as to occlude a direct contact between the amacrine and T1 cells. The same is variably true for feedback synapses to R1–R6, where a thin sheet of epithelial glia intrudes at some but not all sites of amacrine synaptic contact, possibly nullifying the presence of a synapse between these two neurons (Meinertzhagen and O'Neil, 1991). Taking a lower threshold of eight synapses brings in additional pathways from amacrine to L2, as well as inputs to L2 from C2 and C3, and the collaterals of L4 that invade from the two anterior neighbouring cartridges (Meinertzhagen and Sorra, 2001). L5 lacks clear or significant synaptic engagements in the lamina.

The medulla, a plenitude of cell types

As summarized above, the medulla has an entire army of morphological cell types, at least half of all those reported for the optic lobe (Fischbach and Dittrich, 1989). Most are columnar, and of the medulla's 59 or so cell types reported from Golgi impregnation, possibly 30 are in turn Tm cells. The dendrites of these neurons can be restricted to a single column, or spread widely. Thus, dendrites of the same cell class can either extend outside column borders, often intermingling with those of others of the same cell type, so as to shingle the retinal field, or abut the neighboring column borders so as to tile the field. A number of genes are now identified that mediate the tiling of neurite arbors, for example through homophilic interactions between immunoglobulin family members Turtle, that mediate repulsion between R7 terminals (Ferguson et al., 2009), and Dscam2, for the terminals of L1 (Millard et al., 2007). In addition to columnar neurons, tangential neurons are fewer in number (ten reported for the medulla, but likely a considerable underestimate), and spread laterally, usually within just a single stratum, and often across the entire medulla field.

As first realized long ago for *Musca* (Campos-Ortega and Strausfeld, 1972), the medulla's array of columns is home to two patterns of columnar cell types. From counts of both the cells in the medulla cortex and the number of columns these populate, it is clear that on average only about 35 of the >60 cell types occupy each medulla column (calculated in Meinertzhagen and

Sorra, 2001), with a corresponding number of 13.5 for lobula columns, which contain 26 reported cell types. Clearly therefore, not all cell types have an axon in all columns. Some – such as Tm1 and Tm2 (Takemura et al., 2011) – are in all, while many others are not. Campos-Ortega and Strausfeld (1972) refer to the former as synperiodic (1 cell : 1 column). and perhaps only 25 types, including input terminals, are synperiodic, present as one cell in every column (Drs. A. Nern and G.M. Rubin, personal communication; Takemura et al., 2013) and the axons of all other types scattered less frequently. Two classes, Tm3 and Tm4 – along with T4 and T5, are ultraperiodic, having multiple representatives in each column; together with the 25 cells above, these are all considered to be modular, because they are found in each and every column (Takemura et al., 2013). Essentially nothing is known about how other medulla cell types might populate the array of columns, however. They include those that arborize within one column but are infraperiodic, having fewer cells than medulla columns (1 cell : *n* columns). Many may have arborizations in every column and thus can be predicted to pool information from multiple columns. Defining their spacing relative to synperiodic cells depends on identifying the position of the axon relative to the borders of neighboring columns, but in neither case are these well defined. Moreover, the lateral spread of dendrites may ensure an even representation in neighboring columns, for example by tiling the medulla's array of columns (Millard et al., 2007; Ferguson et al., 2009), without close reference to the position of the axon that generates the dendrites. In practice, it may therefore be difficult to distinguish between infraperiodic cells and those that are aperiodic, lacking a fixed distribution among columns. In addition to columnar cells, each column contains the neurites of tangential and local amacrine-like cells with wide-field arborizations not easily reconstructed by means of EM (Takemura et al., 2013).

Given the variable composition of infra- and aperiodic cell types, relative to the defined contributions from modular neurons, there can be no clear unit structure of the medulla neuropile. Unlike the lamina, this is anyway unlikely to contain a fixed blend of cell types, and insofar as the distribution patterns of medulla cells may be random, there may be no minimal structural unit, or medullon (Campos-Ortega and Strausfeld, 1972), containing all representative cell types. Two types of column may correspond to the pale and yellow subtypes of R7 and R8 pairs in the ommatidia, and the pattern of these across the eye is random (Bell et al., 2007). Some cell types may be very few in number, too, which will hinder the final search for their connections, while six other types seen from EM reconstructions (Takemura et al., 2013) are simply not reported from Golgi impregnation.

Single-cell clones from *Gal4* lines that report the expression of different transcription factors have already been used to identify a large number of medulla cell types and their likely contribution to spectral pathways (Morante and Desplan, 2008). Further analyses from reporter lines can be relied upon to confirm and add many other details.

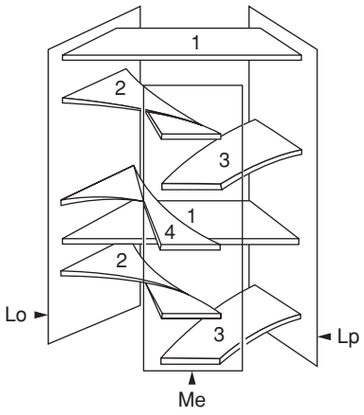

Fig. 1.5. Sheets of axons in the internal chiasma of *Musca*, with alternating direct and twisted strata between medulla (Me), lobula (Lo), and lobula plate (Lp) neuropiles. (Reproduced from Meinertzhagen and Hanson, 1993.)

Finally, the medulla's busy marketplace of neurites is, like any social network, highly stratified. Each stratum can be viewed as delimiting the network's combinatorial complexity, the range and number of contacts formed between synaptic partners, and thus as a corollary of packing so many different cell types into a single neuropile. Inputs arriving from the lamina establish the six strata of the distal medulla by terminating at specific strata, which they accomplish in a sequence of steps during which afferent input axons respond to specific cues in target layers (Ting et al., 2005). First, in the late third-instar larva and early pupa, axons from R7 and R8 grow to temporary layers in the medulla, R8 arriving before R7 and terminating more superficially. The axons of L1–L5 then follow, insinuating themselves between the temporary layers formed by R7 and R8. In the mid pupa, R8 axons then extend down to the R7 temporary layer, to form their final recipient stratum, M3. R7 axons then descend yet deeper to their final recipient stratum, M6. Interactions between classes of afferent axons are not needed for each class to locate its specific stratum, which it does instead presumably through afferent–target interactions (Ting et al., 2005). These steps require the actions of a range of identified cell adhesion molecules, as recently reviewed (Schwabe and Clandinin, 2012).

Going down: The neuropiles of the lobula complex

The lobula complex comprises two neuropiles, the lobula and its thinner, flatter, posterior partner, the lobula plate (Strausfeld, 1976). At the proximal surface of the medulla, the axonal composition of column bundles is not clear and awaits resolution. The axons that connect the medulla with lobula and lobula plate neuropiles through the internal chiasma are, like those of the external chiasma, are also arranged as a succession of coherent, horizontal sheets of axon bundles. The arrangement of these is much more complex than in the external chiasma, however. In *Musca* (Braitenberg, 1970), each layer of axons in the inner chiasma is reported to contain four sheets (Fig. 1.5): (1) an unfolded sheet between lobula and lobula plate; (2) a folded sheet generating the inverted projection of a row of medulla

columns upon a row of lobula columns, with a counterclockwise twist; (3) an unfolded sheet of a row of medulla columns upon a row of lobula plate columns; and (4) a folded sheet generating the inverted projection of a row of medulla columns upon a row of lobula columns, like (2) but with a clockwise rotation (Braitenberg, 1970). In the *Musca* lobula some large terminals form a hexagonal array that occupies every second column in every second row, i.e., one in six columns. The regularity of this array suggests that some infraperiodic cells at least must have a fixed distribution. These inputs have yet to be identified in *Drosophila*, however, although the lobula receives columnar input predominantly from medulla Tm and TmY cells.

The medulla interneurons of the lamina's two major cell types, L1 and L2, overlap the arbors of bushy T cells that have cell bodies in the lobula plate cortex (Strausfeld, 1984), of which T4 and T5 in large fly species both have up to four cells per column (Strausfeld and Lee, 1991) and *Drosophila* has four subtypes, a–d, overall (Fischbach and Dittrich, 1989). Each subtype segregates into one of the four strata of the lobula plate, a specific stratum for each subtype (Fischbach and Dittrich, 1989). These strata also segregate the dendrites of HS and VS cells, to which the terminals of T cells provide proposed anatomical synaptic input, albeit identified only for T4 input to an HS cell (Strausfeld and Lee, 1991; Takemura et al., 2013). Information on the lobula plate's HS and VS cells, either HS cells that signal horizontal motion (Hengstenberg et al., 1982) during rotation about the fly's vertical axis, or VS cells that signal rotation around vertical axes within the fly's equatorial plane (Krapp and Hengstenberg, 1997), is mostly derived from studies on large fly species, although recordings have been reported from dye-filled *Drosophila* HS cells (Schnell et al., 2010), and T cell inputs to giant LPTCs of the lobula plate have also recently been shown to be functional in *Drosophila*. Thus genetic interruption of that input by T4/T5-Gal 4 driven expression of two effector lines, UAS-*shi* or UAS-*Kir2.1*, procures conditional blockade of motion-sensitive responses in the LPTCs, but not responses to flicker (Schnell et al., 2012). Dendrites of both T4 and T5 cells express both Rdl-type GABA receptors, and are thus presumed to receive inhibitory input (Raghu et al., 2007), as well as Dα7-type nicotinic cholinoceptor subunits specifically on higher-order dendritic branches (Raghu et al., 2009). These expression patterns suggest that directional selectivity of the LPTCs is achieved by dendritic integration among excitatory cholinergic inputs and inhibitory GABA-ergic inputs from local motion detectors having opposite preferred directions.

In *Drosophila* three HS and six VS cells are reported (Scott et al., 2002; Rajashekhar and Shamprasad, 2004). In addition, three classes of neuron on each side of the brain that express the transcription factor *Odd-skipped* project into the lobula plate as tangential neurons; one has a contralateral and two have both ipsi- and contralateral projections (Levy and Larsen, 2013).

The lobula plate's four strata are thus defined in *Drosophila* by two criteria: first, the presence of dendrites from the HS and VS cells; and second, the segregation of terminals from T4 and T5's four subtypes, a, b, c, and d (Fischbach and Dittrich, 1989; albeit subtype T4b is missing from their account). To these two criteria should be added a third, the stimulus-specific uptake of ^{3}H-2-deoxyglucose (2-DOG) when the fly is exposed to large-field gratings moving in a preferred direction and with a specific orientation (Buchner and Buchner, 1984; Buchner et al., 1984; Bausenwein and Fischbach, 1992). In sequence, the four strata are: An inner stratum Lopl (or HS layer), next to the inner chiasma, containing most of the dendrites of the HS cells and the terminals of T4a and T5a, which 2-DOG labels by front-to-back motion; next, stratum Lop2, which contains the terminals of T5b and probably T4b and which 2-DOG labels by back-to-front motion; next, stratum Lop3, which contains the terminals of T4c and T5c and which 2-DOG labels by upward motion; and last, the most posterior stratum Lop4 (or VS layer), which contains most dendrites of the VS neurons and the terminals of T4d and T5d, and which 2-DOG labels by downward motion. The lobula plate's outputs from these tangential cells relay information about directional motion in anti-parallel preferred directions to descending pathways which then project to the circuits of the thoracic nervous system that mediate flight, as identified in large fly species (e.g., Strausfeld, 1989; Strausfeld and Lee, 1991).

The axons of columnar neurons in the lobula segregate and project next to a group of discrete optic glomeruli in the lateral protocerebrum (Otsuna and Ito, 2006; Strausfeld and Okamura, 2007). These have been compared with those of the olfactory system (Mu et al., 2012). Eleven glomeruli in the posterior ventral, and seven in the posterior region of the lateral procerebrum each receive exclusive and often monolithic input from a single class of lobula columnar neuron (Lcn), while the optic tubercle is an additional glomerulus that receives non-Lcn input as well (K. Shinomiya, personal communication). Fourteen types of visual projection neuron have been identified extending between the lobula and protocerebrum (Otsuna and Ito, 2006) and although little is known about their function, the lobula as a whole is predicted to be involved in detecting object features (Douglass and Strausfeld, 2003a) but also exhibits motion sensing elements (Douglass and Strausfeld, 2003b). Two such neurons are tangential cells, LT10 and LT11, which a recent report implicates in the detection of second-order motion (Zhang et al., 2013).

The optic lobe's synaptic circuits

Since the time of Ramón y Cajal (Cajal and Sánchez, 1915), synaptic circuits in the optic lobe have been constructed from contacts between neurons, terminal to dendrite, with the specificity of those contacts dictated by the co-stratification of both. Such constructions rely upon three basic assumptions: the correct identification of the axon's terminal and dendrites for each optic lobe neuron; the assignment of an exclusively presynaptic role to the former, and a postsynaptic role to the latter; and the assignment of neither role to the axon itself. While true in general, each assumption is often violated (Takemura et al., 2008), and sites of synaptic contact can, in fact, only be confirmed

at present from electron microscopy (EM). More than this, EM studies often reveal synapses between unexpected synaptic partners, and thus reveal the importance of local circuit as well as relay neurons. Moreover, EM accounts alone reveal the numbers of synaptic contacts, and thus the likely pathway strength, between partner neurons. The existence and strength of connections predicted by terminal-to-dendrite overlaps, and those seen in EM reconstructions, have received recent quantitative comparison in a column of the medulla, where their correlation is seen in fact to be highly variable (Takemura et al., 2013).

Violated though these assumptions may be, it seems most likely that strata are one way to limit synaptic interactions to those between neurons that co-arborize in the same stratum. Using this line of reasoning, Bausenwein et al. (1992) superimposed the density profiles of Golgi impregnated columnar cell types to analyze the connectivity between the medulla strata. This approach assumes that the density of arborizations reflects accurately the density of synaptic contacts, as opposed to their dispersion, but clearly identified at least three main visual pathways.

Pathway 1 has input pathways in strata M1 and M5 and connects stratum M10 to the lobula plate, with its HS and VS LPTCs (Borst et al., 2010). Pathway 2 has input in stratum M2 and connects stratum M9 to superficial layers in the lobula, which in turn connect to the lobula plate. These pathways are proposed to receive input from R1–R6, either via L1 (terminating in M1 and M5) or L2 (terminating in M2), and their neurons have narrow-field dendritic arbors. The pathways were originally suggested to play a major role in motion detection, a conclusion supported by the stimulus-specific 2-DOG labeled bands seen after wide-field visual stimulation (Bausenwein and Fischbach, 1992). That suggestion was later validated by genetic dissection approaches for L1 (pathway 1) and L2 (pathway 2), that suggested, in turn, that these lamina neurons provide inputs to two motion-sensing channels (Rister et al., 2007).

Pathway 3 has input in M8 either from stratum M3 (pathway 3a) or from M4 and M6 (pathway 3b), layers that get their major input from L3 and R8 or L4 and R7, respectively. This pathway then connects M8 to deep layers of the lobula. Some neurons of pathway 3 have wide-field dendrites that must pool inputs over multiple columns that have been suggested to be involved in computing form and spectral information. One such pathway for the latter comes from pooled R7 inputs to an amacrine neuron, Dm8, and subserves UV phototaxis (Gao et al., 2008).

Overall, we see that divergence at the first synapse, the R1–R6 tetrads (Meinertzhagen and O'Neil, 1991), establishes input to pathways 1, 2, and 3a, whereas R8 and R7 are thought to provide input to pathways 3a and 3b, respectively. The synaptic contacts observed from serial-section EM largely bear out these suggestions but add a multitude of new details.

The motivation of motion

Interest in the organization of insect visual systems rests in large measure on a cornerstone computational model of motion detection, the Reichardt elementary motion detector (EMD). This computes correlations between input signals that are separated in time and space to predict motion-sensing outputs (for review see Borst and Egelhaaf, 1989; Borst et al., 2010). The attraction of the EMD detector lies both in its computational simplicity and in its robustness. No less, for decades it has offered vision scientists a simple solution to a compelling problem in neurobiology. But knowledge of the EMD's biological implementation as actual connections between specific neurons has always remained tantalisingly incomplete. Certain cell types have been implicated from terminal-to-dendrite overlap criteria and electrophysiological recordings, notably in the medulla (for review, see Douglass and Strausfeld, 2003a), but only recent EM evidence of the actual connections made by identified neurons reveals those anatomically qualified to act as circuits underlying this detector (Takemura et al., 2013).

Past accounts from all fly species have given particular attention to pathways 1 and 2, above, for L1 and L2. Following earlier suggestions both are now known to provide the substrate for motion sensing. Thus, interrupting synaptic function in L1 and L2 together suppresses optomotor (Rister et al., 2007; Clark et al., 2011) and electrophysiological (Joesch et al., 2010) responses to wide-field motion stimuli. By virtue of its proposed electrical coupling to the other by means of gap junctions, either neuron alone may, however, produce a wild-type motion response (Joesch et al., 2010). Differential effects have been reported after separately inactivating either cell, leaving the other intact. Thus, separately L1 may signal posterior-to-anterior motion across the retina and L2 anterior-to-posterior motion (Rister et al., 2007), or light and dark moving edges (Clark et al., 2011) respectively; or a yet wider range of even more subtle behavioral deficits that reveal the roles of these two cells in basic motion detection (Tuthill et al., 2013).

L1's and L2's pathways in the medulla, and the cells that constitute these, are now known. For the L2 pathway the chief targets are Tm1 and Tm2, representing a binary split that generates two parallel pathways (Takemura et al., 2011), rather as upstream L1 and L2 receive matched inputs from R1–R6 tetrads in the lamina (Meinertzhagen and Sorra, 2001). Compared with the input to L1/L2 pairs at lamina tetrads, however, these inputs are not matched exactly and only two thirds of L2's synapses provide input to both Tm1 and Tm2 (Takemura et al., 2013). L2 also provides input to Tm4 from the same column and the Tm4 cells of neighbouring columns. For the L1 pathway, each L1 terminal has two major targets: Mi1, which receives input almost exclusively within a single column, and a group of Tm3 cells which, like L2's Tm4 targets, have dendrites spreading in from neighbouring columns. Together these two cell types contribute 85% of the identified inputs to T4 and are therefore T4's sole major pathways from L1 (Takemura et al., 2013).

What of the medulla inputs from three other L-cell types? L5, long considered a synaptic orphan (Takemura et al., 2008), forms only a few casual synapses in the lamina, but is highly synaptic in the medulla, where it receives massive input from