Published in final edited form as:

Dev Neurobiol. 2011 December ; 71(12): 1286–1296. doi:10.1002/dneu.20894.

Visual Circuit Assembly in *Drosophila*

Krishna V. Melnattur¹ and Chi-Hon Lee^{1,*}

¹Section on Neuronal Connectivity, Laboratory of Gene Regulation and Development, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD 20892, USA

Abstract

Both insect and vertebrate visual circuits are organized into orderly arrays of columnar and layered synaptic units that correspond to the array of photoreceptors in the eye. Recent genetic studies in *Drosophila* have yielded insights into the molecular and cellular mechanisms that pattern the layers and columns and establish specific connections within the synaptic units. A sequence of inductive events and complex cellular interactions coordinates the assembly of visual circuits. Photoreceptor-derived ligands, such as hedgehog and Jelly-Belly, induce target development and expression of specific adhesion molecules, which in turn serve as guidance cues for photoreceptor axons. Afferents are directed to specific layers by adhesive afferent-target interactions mediated by leucine-rich repeat proteins and cadherins, which are restricted spatially and/or modulated dynamically. Afferents are restricted to their topographically appropriate columns by repulsive interactions between afferents and by autocrine Activin signalling. Finally, Dscam-mediated repulsive interactions between target neuron dendrites ensure appropriate combinations of post-synaptic elements at synapses. Essentially all of these *Drosophila* molecules have vertebrate homologs, some of which are known to carry out analogous functions. Thus, the studies of *Drosophila* visual circuit development would shed light on neural circuit assembly in general.

Introduction

The fly visual system is comprised of the compound eye and four optic ganglia, viz. the lamina, the medulla, the lobula and the lobula plate (Figure 1A, Meinterzhagen and Hanson, 1993). The visual world is perceived by the eye as patterns of activation of photoreceptor neurons in the ~800 unit eyes, or ommatidia which are organised in a crystalline array. Each ommatidium has eight photoreceptor neurons, or retinula (R) cells, divided into three classes based on their relative position and opsin gene expression. The six “outer” photoreceptors (R1–R6) express the Rh1-opsin, respond to a broad spectrum of light and are thought to be functionally analogous to vertebrate rod cells in that they together represent an achromatic channel and are responsive to low-intensity light. The two “inner” photoreceptors R7 and R8 respond to a more restricted range of wavelengths, and mediate chromatic discrimination

*To whom correspondence should be addressed: Chi-Hon Lee, M.D., Ph.D., Section on Neuronal Connectivity, Laboratory of Gene Regulation and Development, Eunice Kennedy Shriver National Institute of Child Health and Human Development, National Institutes of Health, Building 18T, Room 106, Bethesda, MD 20892, USA, Tel: 301-435-1940, Fax: 301-496-4491, leechih@mail.nih.gov.

and are thus thought to be analogous to vertebrate cone cells. They can be further subdivided based on their opsin gene expression pattern – R7 photoreceptors express either the Rh3 or Rh4 opsin which are both maximally responsive to ultraviolet light; R8 photoreceptors in contrast express either the Rh5 or Rh6 opsin, which are maximally sensitive to blue or green light, respectively. From a functional point of view, the first level of connectivity is chiefly concerned with directing the outputs of photoreceptors of different spectral sensitivities to appropriate target neurons in the lamina and medulla while preserving their retinal spatial relationships, a process called retinotopy.

R cell axons from a single ommatidium are initially bundled into a single fascicle, and project in a retinotopic fashion towards the first optic ganglion, the lamina. R1–R6 axons terminate in the lamina, defasciculate and make stereotyped projections to connect with specific lamina neurons organised in cylindrical units called cartridges (Trujillo-Cenoz, 1965; Braitenburg, 1967). R7/R8 axons in contrast, project together through the lamina and terminate in the second optic ganglion, the medulla. The medulla neuropil is organised into layers and columns. Each medulla column receives direct input from one R7/R8 pair and indirect input (via the lamina neurons) from the R1–R6 cells that “see” the same point in space. The medulla column thus represents the basic functional unit of the fly visual system. R7, R8 and the lamina neuron axons terminate in different layers suggesting that layers are differentially responsive to visual stimuli, a notion further supported by an activity-labelling study (Bausenwein and Fischbach, 1992). Neuroscientists since Cajal have noted the striking similarities between the overall organisation and morphology of the fly peripheral visual system and the vertebrate retina, and drawn analogies between the fly lamina/medulla and the vertebrate inner/outer plexiform layers in particular. (Cajal and Sanchez, 1915; Mienertzhagen, 1993).

Visual information carried by R7, R8 and lamina neurons is processed by some 50 types of medulla neurons (Fischbach and Dietrich, 1989). Recent EM and fluorescence microscopy studies have begun to establish the synaptic circuitry of the medulla (Gao et al., 2008; Morante and Desplan, 2008; Takemura and Meinertzhagen, 2008;). There are thought to be thirty columnar neuronal classes which are present in most if not every column. The intrinsic types interconnect layers of the medulla column, while the transmedullary types connect, in a topographic fashion, specific medulla layers to the third and fourth optic ganglia – the lobula and lobula plate. Specific visual projection neurons are thought to transfer visual information from the lobula to optic glomeruli in the ventral protocerebrum and from the lobula plate to the ventral ganglion. These feed-forward visual circuits, outlined in Figure 1B, represent only the information transfer from eye to the brain while the feedback circuits are left out for clarity.

Because of the abundant genetic tools that have resulted from the past few decades of work on eye patterning, much of the work on fly visual circuit development has focused on the genes that control the projection patterns of R cell axons to the lamina and the medulla. Classical developmental analyses at the light and electron microscopic levels have revealed complex cellular interactions that occur sequentially to establish photoreceptor projection patterns. Large genetic screens based on either histology or visual-driven behaviours have provided molecular handles for dissecting these cellular events (Garrity et al., 1996;

Newsome et al., 2000; Clandinin et al., 2001; Lee et al., 2001; Berger et al., 2008). Mosaic techniques that allow the generation of single-cell mutant clones in R cell or target neurons have greatly facilitated the dissection of several key biochemical pathways involved in these processes. This work on development of photoreceptor projections forms a framework to use to analyse development of the visual circuit.

In this review we will focus on the development of synaptic connections between photoreceptors and their target neurons in the lamina and medulla. Broadly speaking, synaptic target selection by afferent axons can be thought to take place in three stages – navigation to the right target ganglion (in this case lamina vs. medulla), selection of the appropriate target region within the ganglion and finally synapsing on the right target neurons within the region. We will dwell briefly on the first phase and describe the second and third stages in more detail, highlighting the insights into cellular and molecular mechanisms that have come from analyses of mutants that disrupt the patterns of specific connectivity seen in these ganglia. These analyses have revealed the importance of repulsive afferent-afferent and target dendrodendritic interactions, as well as permissive and instructive afferent-target interactions in shaping the patterns of innervation of afferent axons and target dendrites in the lamina and medulla.

Synaptic target selection in the lamina

Within each ommatidium, the R8 cell differentiates first and projects its axon through the lamina to the medulla. The R1–R6 cells are next to differentiate, their axons bundle together and extend along the R8 axon but terminate in the lamina plexus between rows of glia. The R7 cells are the last to differentiate and their axons, like those of R8, project through the lamina to the medulla to terminate in a different medulla layer from the R8 axons. The termination of the R1–R6 cells in the lamina has been shown to be unrelated to the timing of axonal outgrowth from the retina, but instead to result from interactions between afferent axons and lamina glia which provide an as-yet unidentified stop signal (Poeck et al., 2001; Suh et al 2002; Chotard et al. 2005).

Lamina development is directly coupled to the innervation of retinal axons. The arriving R1–R6 axons secrete hedgehog protein, and the EGF ligand spitz which induce neurogenesis and differentiation in the lamina ensuring that precisely the correct complement of lamina neurons is generated per ommatidium (Huang and Kunes 1996, 1998; Huang et al., 1998). Differentiated lamina neurons correctly associate with the R1–R6 axons to form cartridges via two matching nephrin/NEPH1 family cell-adhesion receptors: Hibiris expressed on the lamina neurons and Roughest on retinal axons (Umetsu et al., 2006, Sugie et al., 2010). Thus afferent derived signals ensure that there are matching numbers of lamina cartridges and ommatidia, that each cartridge has the right number and kinds of target neurons and that the appropriate adhesion receptor is expressed in the lamina neurons to match retinal afferents'. But how do afferent axons select retinotopically appropriate cartridges?

Neural Superposition

As a consequence of the curvature of the eye and the angular position of photoreceptors within an ommatidium, R1–R6 cells from 6 different ommatidia arranged in a specific

relative position “see” the same point in visual space (i.e. an R1 cell from one ommatidium, R2 from the next one and so on), and therefore have to project to the same lamina cartridge, one that is different from the R1–R6 bundle’s ‘ommatidial’ cartridge. This phenomenon is termed neural superposition (Figure 2A). How is this achieved? Elegant work (Clandinin and Zipursky 2000) involving genetic ablations of R cells and transformation of the orientations of R cells within the ommatidium highlighted the importance of afferent-afferent interactions between R cells, and ommatidial orientation in pattern generation.

Insights into the potential molecular mechanisms mediating the interactions among R1–R6 growth cones came from a forward genetic screen based on R1–R6-dependent optomotor behaviour (Clandinin et al., 2001; Lee et al., 2001;). This genetic screen for motion blind mutants identified the protocadherin Flamingo (Fmi) as important for elaboration of the specific R1–R6 pattern (Lee et al., 2003). R1–R6 axons mutant for Flamingo form lamina cartridges containing variable number of terminals (3–15) (Lee et al., 2003). Flamingo function appears to be required at the stage when individual axons separate out from ommatidial bundle to target the appropriate cartridge. Clonal analysis has shown that its function is required in R cell axons to influence neighbouring R cell afferents, likely through repulsive homophilic interactions (Chen and Clandinin, 2008).

The same behavioural screen also identified mutations in the homophilic adhesive receptor N-cadherin (CadN), the receptor tyrosine phosphatase LAR and its cytoplasmic partner Liprin- α . The identification of these molecules and their functional studies highlight the importance of the interactions between R1–R6 growth cones and their target lamina neurons. CadN mutant R1–R6 photoreceptors do not segregate from the ommatidial bundle to innervate lamina cartridges (Lee et al., 2001). CadN is broadly expressed on both afferents and targets. Clonal analyses further revealed that CadN is required in both afferent axons and target lamina neurons (Prakash et al., 2005), so it is thought to act as a permissive agent mediating afferent-target interactions. The phosphatase LAR and the scaffolding protein Liprin- α share a similar mutant phenotype with CadN but are required only in the photoreceptor afferents (Clandinin et al., 2001; Choe et al., 2006). Genetic and biochemical studies further suggest that LAR interacts with CadN to mediate this afferent-target interaction process (Prakash et al., 2009). Thus permissive afferent-target and repulsive afferent-afferent interactions are important in choice of spatially appropriate cartridge, but what governs formation of specific synapses within a cartridge?

Synaptic connections in the lamina cartridge

The synaptic circuit in the lamina has been determined by serial EM reconstruction *in toto* and the majority of pre- and post-synaptic elements from over ten different neural types have been identified (Meinertzhagen and O. Neil, 1991). However, functional and developmental studies have not yet fully explored these complex connections and previous studies have instead focused on the tetrad synapses (Hiesinger et al., 2006; Rister et al., 2007; Katsov and Clandinin 2008; Joesch et al., 2010). Every R1–R6 terminal establishes approximately fifty presynaptic sites with four different postsynaptic elements, which include an invariant pair of L1 and L2 and two variable elements out of the lamina neuron L3, amacrine cells and/or glia. Analysis of a large collection of novel and known mis-sorting

mutants that resulted in a variable number of R1–R6 axons (3–12) innervating in a given cartridge revealed that surprisingly in all these mutants photoreceptor axons formed a fixed number of synapses per R1–R6 terminal irrespective of cartridge composition, strongly suggesting that synapse frequency is presynaptically determined (Hiesinger et al., 2006). How then, are L1 and L2 post-synaptic elements invariably part of every tetrad synapse?

Early EM work on the timing of lamina neuron dendritic outgrowth hinted at the possibility of interactions between dendrites regulating this process (Frohlich and Meinertzhagen, 1983; Figure 2A). Thus, at 50% pupal development dendrites of L1 and L2 were seen to extend to and mingle extensively over the photoreceptor terminal with numerous L1/L1 and L2/L2 pairs. By 70% pupal development the L1 and L2 dendrites assumed an alternating sequence with selective regression of dendrites lacking the correct neighbour (Meinertzhagen et al. 2000). Recent work suggests that the homophilic repulsive receptors Dscam1 and Dscam2 act redundantly to mediate this process of synaptic exclusion. Thus, in lamina cartridges doubly mutant for Dscam1 and Dscam2, L1 and L2 postsynaptic elements were found in essentially random combinations at tetrad synapses with many L1/L1 and L2/L2 pairs, a phenotype that was more severe than that observed for the Dscam1 or Dscam2 single mutant (Millard et al., 2010).

Synaptic target selection in the medulla

The medulla neuropil is patterned into layers and columns: the afferents, including R7, R8 and lamina axons, innervate specific medulla layers and their axonal terminals are restricted to their topographically appropriate columns (or axonal tiling). Layer-specific targeting segregates specific types of information to appropriate layers while axonal tiling preserves the spatial information from retina to the medulla target columns. In contrast to the lamina, the complexity of the medulla neuropil has meant that progress towards delineating circuitry of the medulla has been slower. Recent immunocytochemical and EM studies (Gao et al., 2008; Morante and Desplan, 2008; Takemura et al., 2008) have begun to unravel the pattern of specific connections in the distal medulla (layers M1–6), and have led to the partial circuit outlined in Fig. 1B.

A few organizational features have begun to emerge. First, most afferents form large presynaptic termini in one or two specific layers within which their postsynaptic partners elaborate dendritic arbors. However, synaptic contacts, in dyad or triad configurations, are often concentrated in but not strictly confined to specific medulla layers. For example, in R7s, over 70% of the presynaptic sites are located at their terminal boutons and stem but in R8s, the presynaptic sites are distributed along the axons in the medulla neuropil. Second, the motion pathway mediated by lamina neurons L1 and L2 is largely segregated from the color pathway, which is mediated by chromatic R7 and R8 as well as achromatic L3. Distinct sets of columnar neurons relay motion or color inputs to different layers in the lobula. Third, besides the aforementioned synaptic connections, which are largely confined to one or several medulla columns, wide-field neurons interconnect neighbouring medulla columns. For example, the amacrine neuron Dm8 pools inputs from approximately sixteen R7s and relays these signals to transmedulla neurons.

How are such elaborate connections established in the medulla? Studies in the past decade suggest that the medulla circuits are assembled in sequential and discernable stages. The medulla neuropil is dynamically patterned into layers and columns, and at each stage afferents are guided to specific medulla layers and restricted to their topographically appropriate columns. Developmental analysis of R cell projections into the medulla supports the view that layer specific projections proceed in distinct stages during which afferent axons respond to specific cues in target layers (Figure 2B; Ting et al., 2005). In late third-instar larvae and early pupae R8 and R7 axons target sequentially to temporary layers of medulla followed by the lamina neurons L1-L5 which intercalate between the R8 and R7 temporary layers. At the mid pupal stage, R8 and R7 axons begin extending out to their final recipient layers – R8 axons to the R7 temporary layer, which becomes M3, the final R8 recipient layer and R7 axons to layer M6, the final R7 recipient layer. Ablation studies suggest interactions between classes of afferents are not important for layer-specific targeting, which is presumably mediated by afferent-target interactions. Thus afferent growth cones terminate in specific layers at specific developmental stages, presumably by recognising target-derived cues.

Step-by-step assembly could conceivably limit the number of potential synaptic partners the afferents encounter, thereby gradually reducing the complexity of synaptic pairing. In addition, it might reflect the development of afferents and targets induced by their interactions. While the medulla is partially developed at the time of photoreceptor innervation, R-cell afferents provide Jelly-Belly-mediated anterograde signals to the medulla neurons (Bazigou et al., 2007). Removing the ligand Jelly-Belly in the R-cells or its receptor Alk tyrosine kinase in the medulla resulted in disrupted expression of specific cell-adhesion molecules (such as Flamingo and Roughest/IrreC) in the medulla neurons and the subsequent mistargeting of R8 axons.

R8 targeting

R8 axons are the first afferents to arrive at the developing optic lobe and they establish a gross retinotopic map at least in part through a DWnt4-dependent mechanism during the larval stage (Sato et al., 2006). R8 axons terminate temporarily at a superficial layer of the medulla. Targeting to the temporary layer and forming a regular array of R8 growth cones requires two transmembrane receptors, Flamingo and Golden Goal (gogo), as well as the actin-cytoskeletal regulator Hu-li tai shao (Hts) (Ohler et al., 2011; Tomasi et al., 2008; Senti et al., 2003; Lee et al., 2003). Gogo and Flamingo are dynamically expressed on both R8 and R7 axons; however, they are functionally required only in R8s at this stage. Phenotypic analyses suggest that they mediate repulsive axon-axon interactions among R8s as well as R8-target interactions at the temporary layer. Gogo physically interacts with Huli tai shao (Hts) and likely regulates the axonal cytoskeleton through Hts. Interestingly, over-expressing Gogo caused R8 axons to be stalled at the temporary layer, suggesting that down-regulation of Gogo activity is required for R8s to migrate to their final target layer.

During the mid-pupal stage, the R8 growth cones regain motility and extend to their final target layer. This process depends on the instructive cues mediated by the leucine rich repeat (LRR) protein Capricious (Shinza-Kameda et al., 2006). Capricious is expressed on R8

axons and the R8-recipient layer but not on R7 axons or in the R7-recipient layer. Mutant R8 axons lacking Capricious mistargeted to the R7 recipient layer and crossed over into neighbouring columns. Conversely ectopic expression of Capricious in R7 axons resulted in their mistargeting to the R8 layer. Capricious is thought to exert its effects via homophilic afferent-target interactions. Further support for the instructive role of Capricious comes from recent work on the R7-specific transcription factor NF-YC. R7 cells mutant for NF-YC mistargeted to the R8 layer, and ectopically expressed Capricious. This activation of Capricious in the NF-YC mutant was shown to result from a de-repression of the R8 specific transcription factor Senseless in R7 cells (Morey et al., 2008). Senseless has previously been shown to regulate expression of the R8 specific opsins – Rh5 and Rh6. The same transcriptional program thus regulates expression of sensory receptors and targeting molecules.

Intriguingly, in contrast to R7 cells (see below), the processes of layer specific targeting and tiling to a single column have not been genetically separable in R8. Thus, all of the mutations identified so far that disrupt R8 layer specific targeting also appear to result in R8 terminal tiling defects suggesting that the two processes are intrinsically linked in R8 axons. Alternatively, the molecules that regulate layer specific targeting of R8 axons could also independently function to tile R8 terminals.

R7 targeting

A behavioural screen for R7-driven behaviours identified mutations in CadN that caused R7 axons to mistarget to the R8-recipient layer (Lee et al., 2001). Subsequent developmental analyses have demonstrated that CadN is required on both R7 axons and medulla target neurons at the early stage of targeting for R7 axons to reach their temporary layer and at the later stage for growth cone extension to the final recipient layer (Ting et al., 2005; Nern et al., 2005). CadN thus mediates a permissive afferent-target interaction for layer-specific R7 targeting. In vertebrates, N-cadherin plays an important role in layer-specific targeting of retinal ganglion neurons in the chicken tectum (Inoue and Sanes, 1997) and in lamination in the zebra fish retina (Masai et al., 2003). In flies, the CadN-mediated interaction appears to be solely adhesive in nature since the cytoplasmic region of CadN, hence its signalling activity, is not required in R7s (Yonekura et al., 2006).

Mutations in the receptor tyrosine phosphatase LAR also resulted in R7 axon mistargeting to the R8 recipient layer. However, LAR function appears to be required exclusively in the afferent growth cones at the second stage of R7 targeting (Clandinin et al., 2001; Maurel-Zafran et al., 2001; Ting et al., 2005). It is unclear though if LAR function is required for R7 axons to extend from their temporary layer, or to maintain R7 termination in the recipient layer – the mutant phenotype is consistent with both possibilities. Subsequent genetic and biochemical studies indicated that LAR's phosphatase activity is largely dispensable in R7s. Instead, LAR functions through the scaffolding protein Liprin- α and the GEF Trio to modulate the actin cytoskeleton (Hofmeyer et al., 2006; Hofmeyer and Treisman, 2009). In addition, a recent study has identified two related fly liprins, Liprin β and γ that have distinct functions in R7 targeting: Liprin- β seems to act in parallel to Liprin- α to prevent R7 axons from overshooting their target M6 layer while Liprin- γ in contrast, competes with Liprin- α

for binding to LAR, possibly negatively regulating LAR activity (Astigarraga et al., 2010). In other systems including worms, vertebrates and the *Drosophila* neuromuscular junction, LAR and Liprin- α have been implicated in the establishment of active zone during the early stage of synaptogenesis (reviewed in Stryker and Johnson, 2007). While it remains poorly characterized, R7 synaptogenesis might involve LAR and Liprin- α at the second stage of R7 target selection.

Many of the molecules that regulate layer specific targeting of the R7 and R8 axons are widely expressed, yet appear to have specific roles in targeting. How might this be effected? One possibility is that these cell adhesion molecules are dynamically expressed on afferent growth cones and target dendrites in such a manner as to enable formation of stable contacts only at particular developmental times. Support for this temporal regulation hypothesis comes from work on the transcription factor Sequoia (Petrovich and Hummel, 2008). Sequoia is required for both R7 and R8 targeting: R7 mutant axons stalled at the R8-recipient layer, and R8 mutant axons stalled at their temporary layer. Sequoia protein levels are dynamically regulated in R7 and R8 cells—with Sequoia protein levels shown to be rising sequentially in neighbouring R8 and R7 cells, coincident with their axonal projections into the medulla. When the Sequoia expression level was constitutively maintained at a high level in R8 cells, R8 axons projected beyond the M3 recipient layer to terminate in the M6 layer, a switch that was dependent on CadN function. Significantly however, CadN overexpression in R8 cells did not affect their layer-specific projections suggesting that Sequoia has other targets in addition to CadN.

Early work suggested the importance of afferent-afferent repulsive interactions in tiling of R7 axons to specific columns (Ashley and Katz, 1994). Recent work has begun to delineate the molecular mechanisms of tiling and revealed a somewhat surprising role for autocrine signalling (Ting et al., 2007). R7 axons were shown to secrete the TGF- β ligand Activin which binds to its receptor Baboon, resulting in the phosphorylation of the transcriptional factor dSmad2. dSmad2 complexes with Importin- α 3, and the complex is then transported from R7 growth cones into the nucleus by the Dynein-Dynactin motor. Disruptions in different components of this pathway caused R7 axons to wander aberrantly into neighbouring columns, suggesting that the autocrine Activin signalling reduces the motility of R7 growth cones. The downstream targets of dSmad2 that block R7 growth motility, however, have not been identified. Significantly the penetrance of mutant phenotypes was enhanced by ablating neighbouring R7 axons suggesting that R7 axons deficient for Activin signalling remained sensitive to repulsion from neighbouring axons. Recent work suggests that the Ig superfamily protein Turtle might at least in part, mediate this repulsion (Ferguson et al., 2009).

Lamina neuron targeting

The tools to specifically label lamina neuron afferents have only recently been generated – studies of the mechanisms of lamina neuron targeting are therefore still in their infancy. Recent work has implicated CadN in layer specific termination of different lamina neuron classes (Nern et al., 2008), and Dscam in tiling of one class of lamina neurons (Millard et al., 2007).

CadN requirement in lamina neuron targeting was shown to be cell-type but not layer specific. Thus, while CadN function was required for correct layer-specific termination of L1, L3 and L4 axon terminals, and for extension of the interstitial branches of L5, it appeared to be dispensable for L2 targeting. Furthermore, CadN appears to act at different developmental stages in different classes of lamina neurons. Thus, while L3 axons require CadN function in the first quarter of pupal development to terminate in appropriate layers, L5 axons only require CadN in the last quarter of pupal development. Finally, while CadN is widely expressed in the medulla, CadN expression on one lamina neuron growth cone (L2) in one layer (M2) seemed to regulate the targeting of the process of another class of neurons (L5) to that layer (Nern et al., 2008). Taken together, these observations suggest that dynamic regulation of CadN expression is key to the different functions of this widely expressed molecule.

A careful developmental analysis of the projections of L1 neurons into the medulla suggested that contact mediated repulsion might mediate tiling of L1 axons to single columns, and implicated Dscam2 as the effector of this process. Thus, wild-type L1 axonal processes appeared to overlap with processes from neighbouring columns at mid-pupal stages and only retract into single columns by about 70% pupal development. Dscam2 mutant L1 axonal arbours in contrast failed to retract resulting in late pupal L1 axonal arbours that spanned several columns (Millard et al., 2007). However, Dscam2 function was not required for the tiling of lamina neuron L2 or for the photoreceptors R7 and R8. Thus, while Dscams are attractive candidates to mediate contact-based repulsion, a Dscam based code is unlikely to generally mediate tiling of afferent axons in the medulla.

Conclusions and future directions

Over the last ten years, drosophilists have used eye specific mosaics to genetically dissect the process of visual circuit assembly. What insights into visual circuit assembly have we gained from this approach and what do the coming years have in store?

Perhaps naively, one might expect afferent-target interactions to be largely mediated by molecules of restricted expression patterns that tag in-growing afferent and their specific target dendrites with unique identities. A few of the molecules identified in these mutational genetic screens (notably, Capricious) do appear to conform to this prototype in that their expression is restricted to afferents and their target layers, and they function in a classically instructive fashion. But, this appears to be more the exception than the rule -many of the molecules (notably CadN) identified through the genetic approaches appear to be broadly expressed in many classes of afferents and target layers and act in a permissive fashion to mediate targeting of different afferents at different stages. Dynamic regulation of expression level or activity in different cells might provide additional specificity. This notion is supported by the observations that Sequoia dynamically regulates CadN expression in R7 and R8 cells (Petrovich and Hummel, 2008) and that LAR modulates CadN activity in R1–R6 cells (Prakash et al., 2009).

That most of the molecules identified in these genetic screens have turned out to be broadly expressed permissive molecules rather than specifically expressed instructive molecules

might reflect as much on the nature of the screens as on the nature of the system. Thus, if afferents utilised multiple instructive molecules in a redundant fashion mutations in any one of them might not significantly disrupt targeting. Indeed, recent work has revealed significant redundancy in wiring of the visual system. Thus, the related Ig superfamily proteins Dscam1 and 2 function redundantly in the lamina neurons L1 and L2 to mediate homotypic repulsion that ensures that each photoreceptor tetrad synapse in the lamina cartridge includes a single post-synaptic element from each of L1 and L2 (Millard et al., 2010). However, redundancy is not restricted to members of a family of related proteins. Thus, Activin-mediated autocrine signalling in individual R7 axons within a column appears to act partially redundantly with contact-mediated repulsion between neighbouring columns to tile R7 axons in the medulla. Furthermore, as recent careful work on the Liprin scaffolding proteins has demonstrated, growth cone motility is regulated by a balance of the activities of different molecules that can interact but also have independent functions (Astigarraga et al., 2010; Prakash et al., 2009). Clearly, understanding how the activities of the different molecules identified in the genetic screens are integrated into regulatory networks that direct growth cone behaviour is likely to be a focus of attention in the coming years. As these molecules and likely their mechanisms of action are evolutionarily conserved, these endeavours would be greatly assisted by studying the candidate molecules identified by *in vitro* studies in vertebrates.

While there has been some progress in delineating the mechanisms of target selection in the lamina, our understanding of the cellular events underlying specific wiring in the medulla is still rather afferent-centric. Thus, as little is as yet known about the development of medulla neurons the layer specific targeting of afferents has been used as a surrogate for synaptic target specificity. Recent ultrastructural studies revealed that this approximation is poor at least for certain medulla afferents such as R8 and L3. As tools to specifically label developing medulla neurons and their synapses become available, we anticipate getting a clearer picture of how specific synaptic connections are established in the medulla.

Acknowledgments

We would like to apologize to our colleagues whose works were not discussed here because of space limitation. We thank Dr. Chun-Yuan Ting for help with figures. K.V.M. and C.H.L. are supported by the Intramural Research Program of Eunice Kennedy Shriver National Institute of Child Health and Human Development (HD008748).

References

- Ashley JA, Katz FN. Competition and position-dependent targeting in the development of the *Drosophila* R7 visual projections. *Development*. 1994; 120:1537–1547. [PubMed: 8050361]
- Astigarraga S, Hofmeyer K, Farajian R, Treisman JE. Three *Drosophila* liprins interact to control synapse formation. *J Neurosci*. 2010; 30:15358–15368. [PubMed: 21084592]
- Bausenwein B, Fischbach KF. Activity labeling patterns in the medulla of *Drosophila melanogaster* caused by motion stimuli. *Cell Tissue Res*. 1992; 270:25–35. [PubMed: 1423522]
- Bazigou E, Apitz H, Johansson J, Loren CE, Hirst EMA, Chen PL, Palmer RH, Salecker I. Anterograde Jelly belly and Alk Receptor Tyrosine Kinase Signaling Mediates Retinal Axon Targeting in *Drosophila*. *Cell*. 2007; 128:961–975. [PubMed: 17350579]
- Braitenberg V. Patterns of projection in the visual system of the fly. I. Retina-lamina projections. *Exp Brain Res*. 1967; 3:271–298. [PubMed: 6030825]

- Berger J, Senti KA, Senti G, Newsome TP, Ösling B, Dickson BJ, Suzuki T. Systematic identification of genes that regulate neuronal wiring in the *Drosophila* visual system. *PLoS Genetics*. 2008;4.
- Cajal SR, Sanchez D. Contribucion al conocimiento de los centros nerviosos de los insectos. *Trab Lab Invest Biol*. 1915; 13:1–167.
- Chen PL, Clandinin TR. The Cadherin Flamingo Mediates Level-Dependent Interactions that Guide Photoreceptor Target Choice in *Drosophila*. *Neuron*. 2008; 58:26–33. [PubMed: 18400160]
- Choe KM, Prakash S, Bright A, Clandinin TR. Liprin- α is required for photoreceptor target selection in *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America*. 2006; 103:11601–11606. [PubMed: 16864799]
- Chotard C, Leung W, Salecker I. glial cells missing and gcm2 cell autonomously regulate both glial and neuronal development in the visual system of *Drosophila*. *Neuron*. 2005; 48:237–251. [PubMed: 16242405]
- Clandinin TR, Lee CH, Herman T, Lee RC, Yang AY, Ovasapyan S, Zipursky SL. *Drosophila* LAR regulates R1–R6 and R7 target specificity in the visual system. *Neuron*. 2001; 32:237–248. [PubMed: 11683994]
- Clandinin TR, Zipursky SL. Afferent growth cone interactions control synaptic specificity in the *Drosophila* visual system. *Neuron*. 2000; 28:427–436. [PubMed: 11144353]
- Ferguson K, Long H, Cameron S, Chang WT, Rao Y. The conserved Ig superfamily member turtle mediates axonal tiling in *Drosophila*. *Journal of Neuroscience*. 2009; 29:14151–14159. [PubMed: 19906964]
- Fischbach KF, Ditttrich APM. The optic lobe of *Drosophila melanogaster*. I. A Golgi analysis of wild-type structure. *Cell and Tissue Research*. 1989; 258:441–475.
- Frohlich A, Meinertzhagen IA. Quantitative features of synapse formation in the fly's visual system. I. The presynaptic photoreceptor terminal. *J Neurosci*. 1983; 3:2336–2349. [PubMed: 6631484]
- Garrity PA, Rao Y, Salecker I, McGlade J, Pawson T, Zipursky SL. *Drosophila* photoreceptor axon guidance and targeting requires the dreadlocks SH2/SH3 adapter protein. *C ell*. 1996; 85:639–650.
- Gao S, Takemura Sy, Ting CY, Huang S, Lu Z, Luan H, Rister J, Thum AS, Yang M, Hong ST, Wang JW, Odenwald WF, White BH, Meinertzhagen IA, Lee CH. The Neural Substrate of Spectral Preference in *Drosophila*. *Neuron*. 2008; 60:328–342. [PubMed: 18957224]
- Hiesinger PR, Zhai RG, Zhou Y, Koh TW, Mehta SQ, Schulze KL, Cao Y, Verstreken P, Clandinin TR, Fischbach KF, Meinertzhagen IA, Bellen HJ. Activity-Independent Prespecification of Synaptic Partners in the Visual Map of *Drosophila*. *Current Biology*. 2006; 16:1835–1843. [PubMed: 16979562]
- Hofmeyer K, Maurel-Zaffran C, Sink H, Treisman JE. Liprin- α has LAR-independent functions in R7 photoreceptor axon targeting. *Proceedings of the National Academy of Sciences of the United States of America*. 2006; 103:11595–11600. [PubMed: 16864797]
- Hofmeyer K, Treisman JE. The receptor protein tyrosine phosphatase LAR promotes R7 photoreceptor axon targeting by a phosphatase-independent signaling mechanism. *Proceedings of the National Academy of Sciences of the United States of America*. 2009; 106:19399–19404. [PubMed: 19889974]
- Huang Z, Kunes S. Hedgehog, transmitted along retinal axons, triggers neurogenesis in the developing visual centers of the *Drosophila* brain. *C ell*. 1996; 86:411–422.
- Huang Z, Kunes S. Signals transmitted along retinal axons in *Drosophila*: Hedgehog signal reception and the cell circuitry of lamina cartridge assembly. *Development*. 1998; 125:3753–3764. [PubMed: 9729484]
- Huang Z, Shilo BZ, Kunes S. A retinal axon fascicle uses Spitz, an EGF receptor ligand, to construct a synaptic cartridge in the brain of *Drosophila*. *Cell*. 1998; 95:693–703. [PubMed: 9845371]
- Inoue A, Sanes JR. Lamina-specific connectivity in the brain: regulation by N-cadherin, neurotrophins, and glycoconjugates. *Science*. 1997; 276:1428–1431. [PubMed: 9162013]
- Joesch M, Schnell B, Raghu SV, Reiff DF, Borst A. ON and OFF pathways in *Drosophila* motion vision. *Nature*. 468:300–304. [PubMed: 21068841]
- Lee CH, Herman T, Clandinin TR, Lee R, Zipursky SL. N-cadherin regulates target specificity in the *Drosophila* visual system. *Neuron*. 2001; 30:437–450. [PubMed: 11395005]

- Lee RC, Clandinin TR, Lee CH, Chen PL, Meinertzhagen IA, Zipursky SL. The protocadherin Flamingo is required for axon target selection in the *Drosophila* visual system. *Nature Neuroscience*. 2003; 6:557–563.
- Masai I, Lele Z, Yamaguchi M, Komori A, Nakata A, Nishiwaki Y, Wada H, Tanaka H, Nojima Y, Hammerschmidt M, Wilson SW, Okamoto H. N-cadherin mediates retinal lamination, maintenance of forebrain compartments and patterning of retinal neurites. *Development*. 2003; 130:2479–2494. [PubMed: 12702661]
- Maurel-Zaffran C, Suzuki T, Gahmon G, Treisman JE, Dickson BJ. Cell-autonomous and -nonautonomous functions of LAR in R7 photoreceptor axon targeting. *Neuron*. 2001; 32:225–235. [PubMed: 11683993]
- Meinertzhagen IA, O'Neil SD. Synaptic organization of columnar elements in the lamina of the wild type in *Drosophila melanogaster*. *Journal of Comparative Neurology*. 1991; 305:232–263. [PubMed: 1902848]
- Meinertzhagen IA. The synaptic populations of the fly's optic neuropil and their dynamic regulation: parallels with the vertebrate retina. *Progress in Retinal Research*. 1993; 12:13–39.
- Meinertzhagen, IA.; Hanson, TE. The development of the optic lobe. In: Bate, M.; Martinez Arias, A., editors. *The development of Drosophila melanogaster*. Plainview, NY: Cold Spring Harbor Laboratory Press; 1993. p. 1363-1491.
- Meinertzhagen IA, Piper ST, Sun XJ, Frohlich A. Neurite morphogenesis of identified visual interneurons and its relationship to photoreceptor synaptogenesis in the flies, *Musca domestica* and *Drosophila melanogaster*. *Eur J Neurosci*. 2000; 12:1342–1356. [PubMed: 10762363]
- Millard SS, Flanagan JJ, Pappu KS, Wu W, Zipursky SL. Dscam2 mediates axonal tiling in the *Drosophila* visual system. *Nature*. 2007; 447:720–724. [PubMed: 17554308]
- Millard SS, Lu Z, Zipursky SL, Meinertzhagen IA. *Drosophila* dscam proteins regulate postsynaptic specificity at multiple-contact synapses. *Neuron*. 2010; 67:761–768. [PubMed: 20826308]
- Morante J, Desplan C. The Color-Vision Circuit in the Medulla of *Drosophila*. *Current Biology*. 2008; 18:553–565. [PubMed: 18403201]
- Morey M, Yee SK, Herman T, Nern A, Blanco E, Zipursky SL. Coordinate control of synaptic-layer specificity and rhodopsins in photoreceptor neurons. *Nature*. 2008; 456:795–799. [PubMed: 18978774]
- Nern A, Nguyen LVT, Herman T, Prakash S, Clandinin TR, Zipursky SL. An isoform-specific allele of *Drosophila* N-cadherin disrupts a late step of R7 targeting. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102:12944–12949. [PubMed: 16123134]
- Nern A, Zhu Y, Zipursky SL. Local N-Cadherin Interactions Mediate Distinct Steps in the Targeting of Lamina Neurons. *Neuron*. 2008; 58:34–41. [PubMed: 18400161]
- Newsome TP, Asling B, Dickson BJ. Analysis of *Drosophila* photoreceptor axon guidance in eye-specific mosaics. *Development*. 2000; 127:851–860. [PubMed: 10648243]
- Ohler S, Hakeda-Suzuki S, Suzuki T. Hts, the *Drosophila* homologue of adducin, physically interacts with the transmembrane receptor golden goal to guide photoreceptor axons. *Dev Dyn*. 240:135–148. [PubMed: 21128303]
- Petrovic M, Hummel T. Temporal identity in axonal target layer recognition. *Nature*. 2008; 456:800–803. [PubMed: 18978776]
- Poeck B, Fischer S, Gunning D, Zipursky SL, Salecker I. Glial cells mediate target layer selection of retinal axons in the developing visual system of *Drosophila*. *Neuron*. 2001; 29:99–113. [PubMed: 11182084]
- Prakash S, Caldwell JC, Eberl DF, Clandinin TR. *Drosophila* N-cadherin mediates an attractive interaction between photoreceptor axons and their targets. *Nature Neuroscience*. 2005; 8:443–450.
- Prakash S, McLendon HM, Dubreuil CI, Ghose A, Hwa J, Dennehy KA, Tomalty KMH, Clark KL, Van Vactor D, Clandinin TR. Complex interactions amongst N-cadherin, DLAR, and Liprin-α regulate *Drosophila* photoreceptor axon targeting. *Developmental Biology*. 2009; 336:10–19. [PubMed: 19766621]

- Rister J, Pauls D, Schnell B, Ting CY, Lee CH, Sinakevitch I, Morante J, Strausfeld NJ, Ito K, Heisenberg M. Dissection of the Peripheral Motion Channel in the Visual System of *Drosophila melanogaster*. *Neuron*. 2007; 56:155–170. [PubMed: 17920022]
- Sato M, Umetsu D, Murakami S, Yasugi T, Tabata T. DWnt4 regulates the dorsoventral specificity of retinal projections in the *Drosophila melanogaster* visual system. *Nature Neuroscience*. 2006; 9:67–75.
- Senti KA, Usui T, Boucke K, Greber U, Uemura T, Dickson BJ. Flamingo regulates R8 axon-axon and axon-target interactions in the *Drosophila* visual system. *Current Biology*. 2003; 13:828–832. [PubMed: 12747830]
- Shinza-Kameda M, Takasu E, Sakurai K, Hayashi S, Nose A. Regulation of layer-specific targeting by reciprocal expression of a cell adhesion molecule, Capricious. *Neuron*. 2006; 49:205–213. [PubMed: 16423695]
- Stryker E, Johnson KG. LAR, liprin alpha and the regulation of active zone morphogenesis. *J Cell Sci*. 2007; 120:3723–3728. [PubMed: 17959628]
- Sugie A, Umetsu D, Yasugi T, Fischbach KF, Tabata T. Recognition of pre- and postsynaptic neurons via nephrin/NEPH1 homologs is a basis for the formation of the *Drosophila* retinotopic map. *Development*. 2010; 137:3303–3313. [PubMed: 20724453]
- Suh GSB, Poeck B, Chouard T, Oron E, Segal D, Chamovitz DA, Zipursky SL. *Drosophila* JAB1/CSN5 acts in photoreceptor cells to induce glial cells. *Neuron*. 2002; 33:35–46. [PubMed: 11779478]
- Takemura SY, Lu Z, Meinertzhagen IA. Synaptic circuits of the *Drosophila* optic lobe: The input terminals to the medulla. *Journal of Comparative Neurology*. 2008; 509:493–513. [PubMed: 18537121]
- Ting CY, Herman T, Yonekura S, Gao S, Wang J, Serpe M, O'Connor MB, Zipursky SL, Lee CH. Tiling of R7 Axons in the *Drosophila* Visual System Is Mediated Both by Transduction of an Activin Signal to the Nucleus and by Mutual Repulsion. *Neuron*. 2007; 56:793–806. [PubMed: 18054857]
- Ting CY, Yonekura S, Chung P, Hsu SN, Robertson HM, Chiba A, Lee CH. *Drosophila* N-cadherin functions in the first stage of the two-stage layer-selection process of R7 photoreceptor afferents. *Development*. 2005; 132:953–963. [PubMed: 15673571]
- Tomasi T, Hakeda-Suzuki S, Ohler S, Schleiffer A, Suzuki T. The Transmembrane Protein Golden Goal Regulates R8 Photoreceptor Axon-Axon and Axon-Target Interactions. *Neuron*. 2008; 57:691–704. [PubMed: 18341990]
- Trujillo-Cenoz O. Some aspects of the structural organization of the intermediate retina of dipterans. *J Ultrastruct Res*. 1965; 13:1–33. [PubMed: 5840545]
- Umetsu D, Murakami S, Sato M, Tabata T. The highly ordered assembly of retinal axons and their synaptic partners is regulated by Hedgehog/single-minded in the *Drosophila* visual system. *Development*. 2006; 133:791–800. [PubMed: 16439478]
- Yonekura S, Ting CY, Neves G, Hung K, Hsu SN, Chiba A, Chess A, Lee CH. The variable transmembrane domain of *Drosophila* N-cadherin regulates adhesive activity. *Mol Cell Biol*. 2006; 26:6598–6608. [PubMed: 16914742]

Figure 1.

Synaptic circuitry of the adult fly visual system. (A) Schematic of feed-forward circuits from the eye to the brain. The visual system comprises the retina (compound eye) and four optic ganglia viz. the lamina, medulla, lobula and lobula plate. The 'outer' R cells R1–R6 (blue) project axons from the retina to the lamina where they connect with lamina neurons in lamina cartridges. The 'inner' R cells R7 (yellow) and R8 (red) project through the lamina to terminate in the M6 and M3 layers of the medulla, respectively. The lamina neurons L1–L5 (green) terminate in layers M1–M5. There are thought to be ~50 classes of medulla neurons, three of which are shown. Distal medulla amacrine cells (Dm neurons, orange) arborise extensively in specific distal medulla layers. Transmedullary (Tm) neurons (purple) connect specific layers of the medulla with specific layers of the lobula. TmY neurons (pink), in contrast connect the medulla with the lobula and lobula plate. Visual projection neurons are thought to connect the lobula with 'optic glomeruli' in the ventro-lateral protocerebrum. One lobula columnar (LC) neuron is shown in brown. (B) Motion and colour circuits in the lamina and medulla. The motion pathways are mediated by the lamina neurons L1 and L2, which relay achromatic R1–R6 input to a set of medulla neurons (striped circles) distinct from the direct targets of R7 and R8. L1 and L2 are not only electrically coupled via gap

junctions (double blue line) in the lamina, but are also interconnected indirectly through centrifugal neurons C2 and C3 in the medulla. In the colour pathway, R7 and R8 provide chromatic inputs for the columnar neurons Tm5 and Tm9, respectively. These neurons also receive an indirect achromatic input via L3. The wide-field amacrine neuron Dm8 pools inputs from approximately sixteen R7s and relays these signals to transmedulla neurons.

Figure 2.

Sequential development of specific connectivity in the lamina and medulla. (A) Neural superposition and tetrad synapse assembly in the lamina. The panels from the 3rd instar larval stage through to 50hrs after puparium formation (APF) depict R1–R6 axon extension to the lamina and targeting to spatially appropriate targets, whereas the panels from 50hrs APF to 70hrs APF are expanded views of a single R cell axon terminal highlighting the process of synaptogenesis. From the 3rd instar larval stage to about 30hrs APF, R cell axons from the retina extend towards the lamina. R1–R6 cell axons (blue) terminate at the lamina plexus. By this stage R8 axons (red) have extended beyond the lamina to terminate in the medulla. At around 40hrs APF, R1–R6 growth cones defasciculate and extend in a stereotypic pattern towards their targets. At this stage, R7 (yellow) and lamina neuron (green) axons have followed R8 axons to terminate in the medulla. At about 50hrs APF, R1–R6 growth cones elongate to form mature terminals and lamina neuron neurites start to grow out along the R cell surface towards nascent pre-synaptic sites. Right, an expanded view of a single R cell axon terminal where L1 (dark green) and L2 (pale green) neurites randomly explore the surface of the terminal (blue) to contact nascent presynaptic sites (pale yellow), forming numerous L1/L1 and L2/L2 pairs. Between 50hrsAPF and 70hrs APF, L1/L1 and

L2/L2 partnerships are excluded from the same presynaptic site. Neurites denied post-synaptic access retract, resulting in the invariant alternation of L1 and L2 neurites a long the length of the R cell terminal. By about 70hrs APF, each nascent presynaptic site accumulates two other postsynaptic elements and matures to form a stable tetrad synapse (bright yellow). (B) Layer specific targeting of afferent axons in the medulla. The R8 (red), R7 (yellow) photoreceptor and L1–L5 lamina neurons (LNs, green) axons project to specific layers in the medulla in two distinct stages. Left, between the 3rd instar larval stage and 30hrs APF, the R8, R7 and lamina neurons project sequentially to distinct medulla layers. R8 differentiates first and extends its axon past the lamina to terminate in the superficial medulla (R8 temporary layer). The later differentiating R7 axons fasciculate with the pioneer R8 axons but terminate in a deeper layer in the medulla (R7 temporary layer) just below the R8 temporary layer. The lamina neurons differentiate last, and their axons terminate between the R8 and R7 temporary layers increasing the separation between them. Middle, at around 50hrs APF the R8 and R7 growth cones regain motility and project to their final recipient layers (M3 and M6 respectively). Right, between 70hrs APF and eclosion, the terminals of R8, R7 and L1–L5 lamina neurons are restricted to their characteristic adult-specific medulla layers, as indicated.