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# Building a projection map for photoreceptor neurons in the *Drosophila* optic lobes

Javier Morante, Claude Desplan*

*Department of Biology, New York University, 1009 Silver Building, 100 Washington Square East, New York, NY 10003, USA*

**Abstract**

The sensory tasks performed by the eye are diverse and complex. In *Drosophila*, the eye performs motion detection for navigation as well as detection of the quality of light (color and polarized light). Both types of inputs are processed separately, as different photoreceptors are specialized in these tasks and contact different target cell layers in the optic lobe. However, their respective outputs are likely to be integrated in higher brain centers. Here, we discuss the cell diversity and potential role of the several ganglia that form the fly optic lobe. We also discuss the power of modern genetic tools to provide the potential to trace the visual neural networks.


*Keywords:* Lamina; Medulla; Optic lobe; Photoreceptor; Retina

## 1. Modular organization of the visual system: multiple processing steps

The *Drosophila* compound eye is formed by approximately 800 independent eyes, called ommatidia, each disposed in a hexagonal array. An adult ommatidium contains 8 photoreceptors cells (PRs) and 11 accessory cells. PRs can be classified in two subtypes: six outer PRs (R1–6) surrounding two inner PRs (R7 and R8). R1–6 contain the wide spectrum photopigment rhodopsin 1 (Rh1) and are important for motion detection (Fig. 1A) [1]. They are highly sensitive and bear long and wide rhabdomeres (the dramatic apical microvillar extensions that contain the Rhs). They can be considered homologous to the vertebrate rods. R7 and R8 have smaller rhabdomeres and express different Rhs. This, along with their topographic organization, with R7 placed on top of R8 along the distal-proximal axis of the retina, strongly suggest that they are involved in color vision, thus resembling the vertebrate cones. The ommatidia can be divided into three subtypes based on the Rh content of their inner PRs: the pale subtype (30%) contains the UV-sensitive Rh3 in R7 and the blue-sensitive Rh5 in R8, while the yellow subtype contains the UV-Rh4 in R7 and the green-Rh6 in R8 (Fig. 1B). A third kind of ommatidia is specialized in detecting the vector of polarized light whereby the orthogonal arrangement of the R7 and R8 microvilli serves as a polarizing filter. They are found in the dorsal rim area and contain the UV-Rh3 in both R7 and R8, thus allowing the comparison of polarization of the light rather than any particular wavelength (Fig. 1C).

The spatial distribution and the characteristic expression of *rhodopsin* genes in outer and inner PRs reflects the different functions of R1–6 versus R7 and R8. This means that the information transmitted by the outer and inner PRs must be processed in two different ways, and indeed, the outer and inner PRs project to two different targets in the optic lobe: R1–6 project to the first optic ganglion called the lamina, while processing of information coming from R7 and R8 for color discrimination starts in the projections of these cells to two distinct layers of the second optic ganglion; the medulla. R7 terminates in the deeper ‘M6’ layer while R8 synapses in the superficial ‘M3’ layer (Fig. 2) [2].

During the last decade, extensive progress has been made in understanding several aspects of eye development, in particular the developmental mechanisms leading to the formation of PRs. This led to the identification of the conserved cascade of ‘eye determination genes’ such as Pax6. Then the movement of the morphogenetic furrow, which results from the specific activation of the Hedgehog, Decapentaplegic, Notch and Wingless pathways leads to the recruitment of evenly spaced R8 PRs [3,4]. Through sequential activation of the EGF receptor and Notch pathway, R8 recruits all other PRs while further signaling along the equatorial-to-polar axis leads to the establishment of planar cell polarity that allows the appropriate projection pattern in the lamina [5]; finally, recent studies have described the genes involved in

* Corresponding author. Tel.: +1-212-998-8218; fax: +1-212-995-4710.
*E-mail address:* claude.desplan@nyu.edu (C. Desplan).

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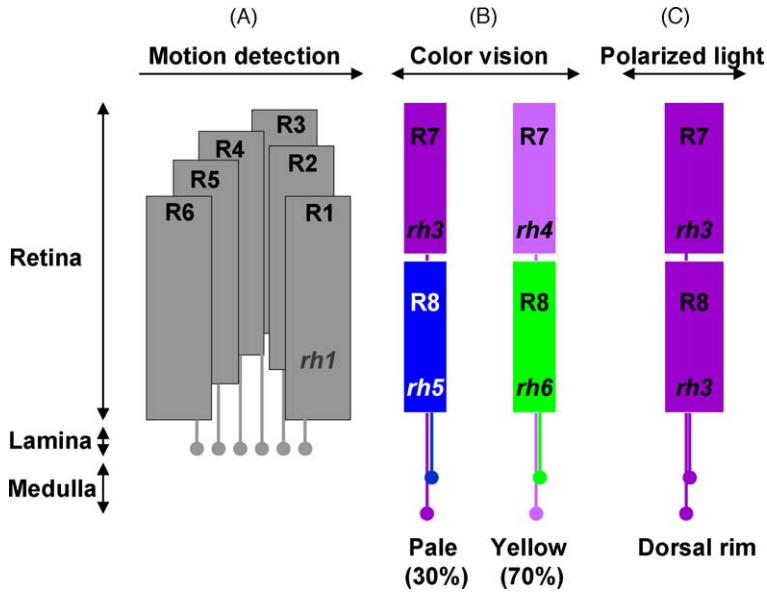


Fig. 1. Photoreceptor subtypes in the *Drosophila* eye. (A) Schematic diagram showing the distribution of outer PRs (R1–6) in a single ommatidium. R1–6, containing a broad spectrum rhodopsin (Rh1), have long and broad rhabdomeres and project to the lamina. Three subtypes of ommatidia can be defined by their inner PRs (R7 and R8): the pale subtype (30%) contains Rh3 (UV) in R7 and Rh5 (blue) in R8, while the yellow subtype (70%) contains Rh4 (UV) in R7 and Rh6 (green) in R8 (B). They are involved in color vision. A special third kind of inner PRs located in the dorsal rim area has large rhabdomeres that contain Rh3 in both R7 and R8 and are involved in the detection of the vector of polarized light (C). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the differentiation of the various classes of PRs and the expression of specific Rhs [6–8].

While less understood, the genetic control of axonal connection to either the lamina or medulla has also been partially elucidated through sophisticated genetic screens [9,10]. These screens involved generating homozygous mutant eyes for a given gene in an otherwise normal animal by targeting mitotic recombination selectively to the eye pri-

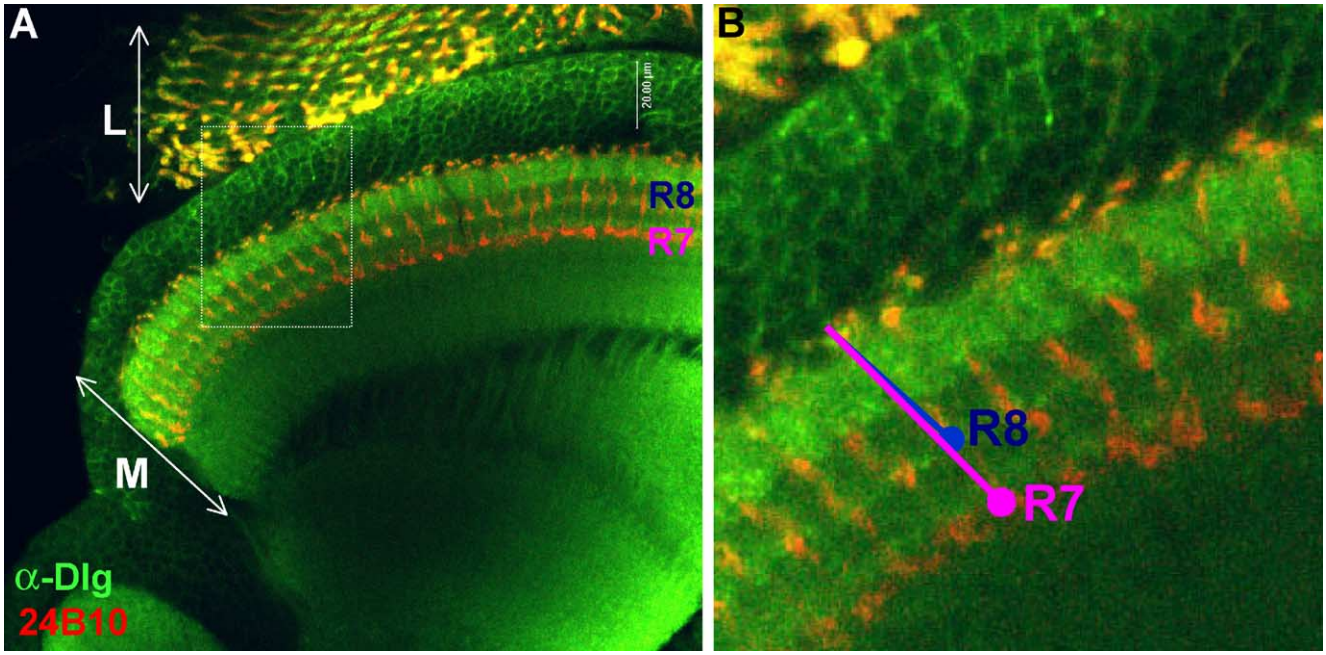


Fig. 2. Distribution of R7 and R8 axonal projections and synaptic contacts in the medulla. (A) R7 and R8 projections revealed by the 24B10 antibody contact two different layers in the medulla. The synaptic marker disc large (Dlg) reveals the complexity in the number of synaptic contacts that R7 and R8 make in the medulla. (B) High magnification of R7 and R8 axonal termini in the medulla. Although both axons fasciculate at the entry of the medulla, R8 terminates in the 'M3' layer (blue line) and R7 projects deeper, to the 'M6' layer (purple line). L: lamina; M: medulla. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

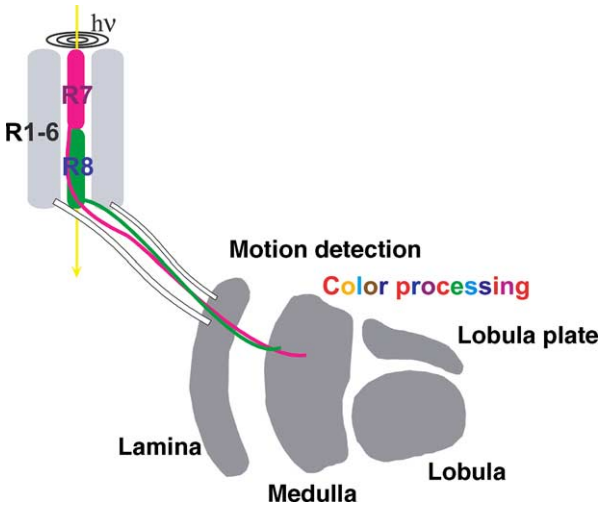


Fig. 3. Differential targets and functions of outer vs. inner PRs. R1–6 project to the lamina, the first step in processing images. However, R7 and R8 bypass the lamina and project to the medulla, the first step in the color vision pathway. The lobula and the lobula plate form the lobula complex. They are the optic neuropils involved in higher processing of both color vision and motion-detection pathways.

mordium [9,11]. Most of these screens are then performed in the context of a behavioral screen to test for incorrect information processing. For instance loss of the optomotor response, which measures motion detection, tests for defects in outer PR processing, whereas loss of UV-light attraction identifies inner PR processing defects. Several of the genes identified in these screens (e.g. *N-cadherin*, *lar*, and *flamingo*) led to focusing attention onto specific process of axon targeting [10,12,13].

However, in spite of this work, remarkably very little is known about the molecular control of specification of the cells that are the target for PRs in the optic lobes. This is particularly true for the medulla where processing of color vision occurs: although many cell types have been identified by Golgi impregnation [2,14], their functional meaning has remained elusive.

### *1.1. The optic lobes*

The fly optic lobes are formed by several structures that represent different levels of processing: the lamina, the medulla, and the lobula complex, formed by the lobula and the lobula plate (Fig. 3).

## 2. The first optic neuropil: the lamina

The lamina is the best characterized optic neuropil and exhibits a remarkably common organization in columns even in distant species of insects [15]. There are an estimated 6000 cells in the lamina of *Drosophila* [16]. R1–6 synapse to the lamina cartridges, the functional units of the lamina which are composed of ∼13 cells: the processes of five monopolar cells (L1–5), one or two amacrine cells, as well as three medulla neurons (C2, C3, and T1) and three glial cells. Inside each cartridge, the predominant synapses for the PRs are the tetrad synapses formed by R1–6 that contact L1–2 monopolar and amacrine cells [17].

Induction of lamina cartridge development occurs during the third larval stage. At this stage, the outer PR neurons are in proximity to but not directly connected to their targets: the six outer PRs from one ommatidium fasciculate and their growth cones stop between two glial layers in the lamina plexus in response to a glial signal [18,19]. In fact, the particular cartridge that was induced by an axon bundle will not be innervated by any of the axons that induced it, but instead will be innervated later in the pupae by six outer PRs originating in six neighboring ommatidia that see the same point in space. Fig. 4 (generously provided by Nicolas Franceschini) clearly shows how the bundle of six PR axons coming from one ommatidium de-fasciculates and targets six different cartridges to generate the neural superposition pattern [20–22]. This complex configuration enhances the signal-to-noise ratio of the response to a signal in the visual field by increasing resolution and photon detection [13,20,23,24]. The interaction among PR growth cones at the time of innervation is extremely important for target selection by R1–6. Mutations that delete specific PRs or disrupt retinal organization indicate that R3 and R4 are required for the appropriate target selection of the remaining outer PR axons, while R1 and R6 are required for the precise projection of R2 and R5 [23]. One of the molecules involved in this process is Flamingo, a cadherin-related cell surface protein. It is required in the outer PR growth cones during mid-pupation [13], at the time when the growth cones make their choice of projection to the neural cartridges. *Flamingo* mutants show cartridges with a variable number of R1–6 axon projections (from 3 to 15), although the tetrad synapses appear to form correctly in the new target [13].

R1–6 not only innervate the cartridges but also control both their neurogenesis [25–28] and gliogenesis [29]. Similarly, in the mouse olfactory system, olfactory sensory neurons send pioneer axons to trigger the neurogenesis of the olfactory bulb [30]. In the fly visual system, Hedgehog, and the EGF receptor ligand Spitz play a critical role in this process [27,28]. Hedgehog is initially expressed in the developing PRs and then is transmitted along the PR axons, acting in two steps. First, it induces entry of the lamina precursor cells into their last cell division but is not directly involved in their final differentiation [27]. Retinal axons deprived of Hedgehog fail to induce lamina neurogenesis, similar to mutants lacking PRs (e.g. *eyes absent* or *sine oculis*) [25]. Exogenous expression of Hedgehog in somatic clones in the optic lobes of these mutant PRs rescues the initiation of cell division, but fails to induce the expression of differentiation markers in lamina cells [27], which requires additional cues. This is achieved as a second function of Hedgehog, which is to induce the onset of the EGF receptor expression in lamina precursor cells. Indeed, expression of a dominant negative

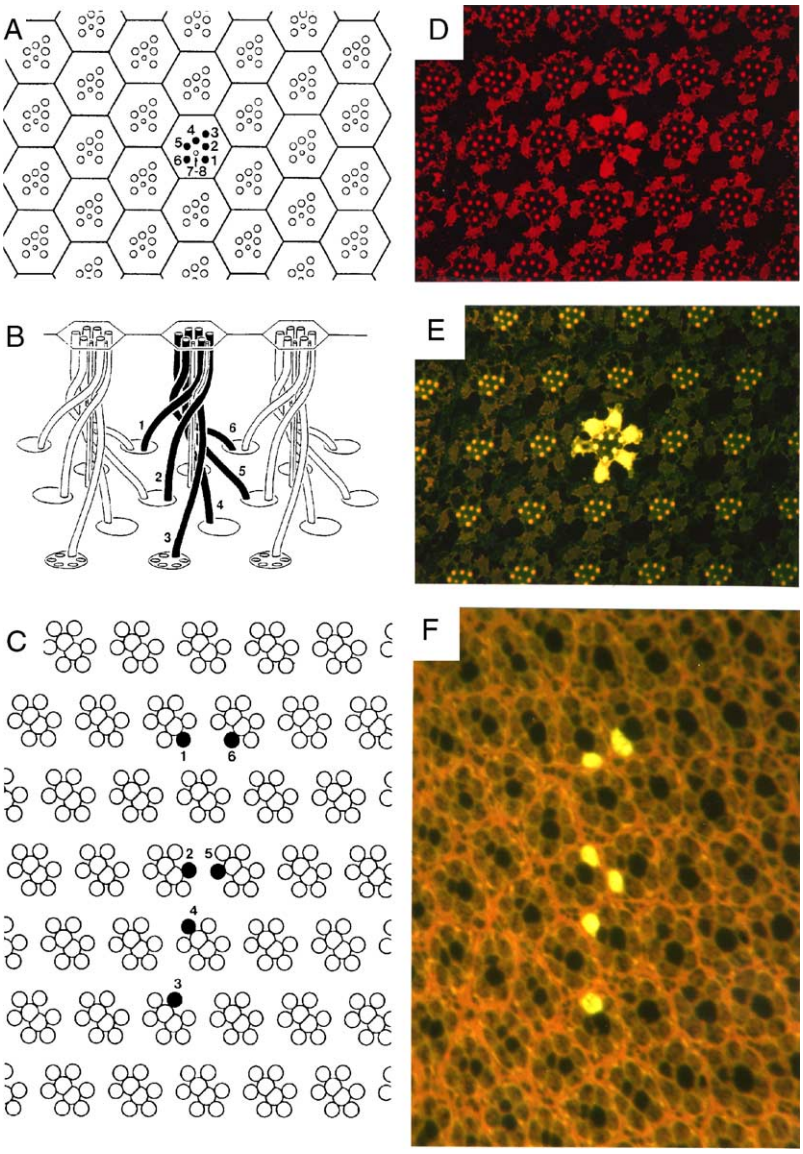


Fig. 4. Dye-induced photopermeabilization allows neuronal tracing of photoreceptor neurons in the housefly *Musca domestica*. The red fluorescing dye, SR101, applied to the extracellular space enters the photoreceptors that are induced by irradiating a single facet for 30 min with green light. (A) Schematic drawing of the fly retina showing the regular pattern of PR in each ommatidium. (B) The bundle of six axons coming from a given ommatidium defsciculate and contact six different lamina cartridges in the lamina. (C) Projection pattern of R1–6 (darkly labeled) stemming from a single ommatidium. (D) Red fluorescence of SR101 in the retina and (E) fluorescence of the dye LY that has permeated into the six peripheral receptors cells within the treated facet. (F) Photomicrograph of a cross-section through the lamina showing the yellow fluorescence of LY in the six terminals from those six receptors labeled in panel (E). This image was generously provided by Nicolas Franceschini and more details can be found in Ref. [52].

form of the EGF receptor using the flp-out technique (see below) in the late third instar stage prevents the expression of differentiation markers in the lamina cartridges without affecting the expression of early markers such as Dac [28]. Finally, Spitz, an EGFR ligand, also transmitted by PR axons, acts on the cartridges neurons, suggesting a role in their final differentiation [28]. Spitz mutant retinal axons induce the Hedgehog-dependent events during lamina development but fail to induce neuronal differentiation of the cartridges. This phenotype can be rescued with specific expression of Spitz in the eye. Thus, Hedgehog and the EGF-like ligand Spitz are transmitted along PR axons as anterograde signals that act in two steps, first controlling the proliferation and second the differentiation of the lamina cartridges.

Thus, the lamina cartridges represent the first step in visual processing as they are the direct targets for the outer PRs and are presumably involved in the initiation of the motion-detection pathway.

## 3. The second optic neuropil: the medulla

The medulla is the target area for the inner PRs, the cells thought to be involved in color vision in the fly. Color vi-

sion is the perception of different wavelengths of light. One prerequisite to discriminate colors is to be able to compare the inputs of at least two PRs focusing on the same point in space that are sensitive to different wavelengths of light. *Drosophila* contains the machinery to perform color vision, as R7 and R8 look in the same direction and contain photopigments sensitive to different wavelengths. Therefore, it is likely that the fly color vision system relies on comparing the inputs of R7 and R8. Their projections bypass the lamina cartridges and innervate the medulla neuropil, fasciculating together at the entry of the medulla and projecting in two separate layers, R8 in the M3 layer and R7 deeper in the M6 layer (Fig. 2). To understand how color vision is processed, it will be necessary to identify the target neurons of R7 and R8 and to study the connections established between the cells of the medulla. This work started almost 100 years ago with the pioneering works of Cajal and Sanchez in 1915 [14]. Since then, much effort, in particular by Fischbach and Dittrich, has identified the many cell types in the medulla [2]. However, no direct connections between R7 and R8 projection neurons have been revealed, making it essential to revisit the problem with the modern tools of fly genetics to understand how color vision is processed.

The medulla is the most complex optic neuropil with regard to the number of cell types. Formed by approximately 40,000 cells [16], it is divided by the serpentine layer (defined by the axons of tangential neurons) into a distal or outer medulla, and a proximal or inner medulla [2]. Classical studies using Golgi impregnations have revealed the multistratification of the medulla, with 10 layers [2]. In larger flies such as *Musca*, detailed studies have estimated to 120 units the number of cells participating in a single medulla column [31]. The same complexity is also found in *Drosophila* [2] (J.M. and C.D., unpublished observations). Although some medulla cells maintain the retinotopic map, making synapses in columns (the visual-sampling unit of the retina) [32], other cells are able to make synapses in several columns (J.M. and C.D., unpublished observations). The medulla is involved in processing the inputs of R7 and R8, likely to mediate color vision. It also receives inputs from the outer PR primary target neurons coming from the lamina [2], suggesting an additional role as an intermediate target in the motion-detection pathway before a higher processing occurs in the lobula plate.

Transmedullary (Tm) cells represent one of the best characterized cells in the medulla both in terms of morphology and physiology. Distributed as retinotopic palisades in the medulla cortex, they possess multiple lateral ramifications contacting several medulla layers and a main ramification projecting into the lobula. A special Tm cell type (TmY) exhibits an additional bifurcation in its axons, which also project to the lobula plate. Approximately 40 morphological species of Tm cells have been recognized [2,33]. In *Calliphora*, Douglass and Strausfeld [34] have recently performed physiological recordings in several kinds of medulla cells. They showed that Tm cells respond to a local flicker stimulus but not to motion direction or orientation, ruling out the involvement of Tm and TmY cells in motion-detection processing [34] and raising the possibility that Tm cells are involved in other visual tasks such as color vision processing. Indeed, Tm cells contact specific subsets of inner PRs (J.M. and C.D., unpublished observations).

In conclusion, the medulla represents an intricate of cells with an unknown function and axonal projections coming directly from inner PRs and lamina cells as an intermediate target suggesting a dual role in the color vision and motion-detection pathway.

## 4. The lobula complex: lobula and lobula plate

The last step in the visual pathway, at least in the optic lobe, is the lobula complex formed by the lobula plate and the lobula. This system has been studied in detail in large flies such as *Musca domestica* [35] and recently also in *Drosophila* [36]. The best characterized neurons among the ∼15,000 neurons in the lobula plate [16] are the giant neurons that are responsible for motion detection. Classical studies using Golgi impregnations have divided the giant neurons in two groups: the horizontal and the vertical system. A fixed number of 3 cells comprises the horizontal system in large flies (as well as in *Drosophila*), while the number in the vertical system is more variable, from 5 to 7 in *Drosophila* [37] or 9 to 11 in large flies. Recently, Luo and coworkers have identified a gene specifically expressed in the *Drosophila* giant neurons as an enhancer trap line (Gal4-3A), allowing them to use the MARCM system (see below) to trace their dendritic arborizations and study their lineage [36]. The dendrites of the vertical and horizontal systems ramify broadly in the lobula plate with a high degree of overlap. Their axons project medially around the periesophageal region where they synapse with fibers coming from the ocelli (another adult visual system involved in the detection of the horizon during flight) and cervical connective fibers [33,36].

Despite the well-defined broad dendritic arborizations of the giant neurons in the lobula plate, there is little information on the pre- or post-synaptic cells they contact. The input neurons to the lobula plate appear to maintain a retinotopic organization, but the output neurons do not [38]. This is to be expected as a very small number of large neurons (fed by the target neurons of outer PRs) are involved in the motion-detection pathway while other parts of the lobula are implicated in other pathways such as color vision (fed by the projections of the inner PR targets). One of the most striking characteristics of the lobula plate cells is their extensive dendritic arborizations in the optic lobe, occupying the main part of the medulla neuropil, confirming their role in visual processing.

## 5. New tools to delineate the visual circuitry

One of the major difficulties to describe the visual circuitry is the lack of effective tools to understand such a complex neural network. The pioneering work of Cajal and Sanchez [14] as well as most recent studies performed in the 1980s [2,39], have relied on Golgi impregnations to randomly label cells in the optic lobe. These have established a well characterized map of the lamina [40] but are less clear and incredibly more complicated for the medulla and lobula plate [2]. Recently, modern approaches have relied on genetic rather than histologic tools to reveal sensory circuits.

Neurotoxins represent a potential method to map neural circuitries [41]. Some bacterial neurotoxins such as tetanus toxin can move through synapses, although preliminary tests have revealed that they are inefficient to transfer synapses in flies, worms, or plants (P. Brulet, personal communication).

Plant lectins have also been used as trans-synaptic tracers in neuroanatomical studies for mapping central neural pathways. They bind specific moieties attached to glycoproteins and glycolipids in the plasma membrane and are taken up by endocytosis, thus passing through synapses and being transported in dendrites and axons in both directions. Wheat germ agglutinin (WGA) has been extensively used to trace circuits [42]. The mouse olfactory pathway has been traced by expressing WGA in all olfactory sensory neurons [43,44] or in subsets expressing a given odorant receptor [45]. WGA was detected not only in the second order neurons of the olfactory bulb, the mitral cells, but also in the third order neurons located in the olfactory cortex, revealing efficient transynaptic transport that helped elucidating the stereotyped map in this cortical area [45]. Transmission of WGA from outer PRs to target neurons has also been demonstrated [44]. For instance, after labeling the outer PRs, the lectin was detected immunohistochemically or by electronic microscope in lamina cell bodies and their axons terminals projecting to the medulla, suggesting that WGA can cross multiple synapses [44,46]. Despite these results, the transfer of WGA is extremely limited and requires very strong expression in the upstream cell (J.M. and C.D., unpublished data). To overcome this limitation, improved versions of this technique are being developed, such as coupling the lectin to GFP to facilitate its in vivo visualization.

Finally, Lee and Luo have designed a new approach to trace single neurons using a genetic mosaic system called MARCM (mosaic analysis with a repressible cell marker) [47]. This is a dual system relying on two yeast transcriptional regulators: Gal4 and Gal80. Gal4 binds to its target site, UAS, to activate transcription, while Gal80 blocks Gal4 function, resulting in repression. In MARCM, Gal80 is expressed broadly (e.g. using the tubulin promoter) but the generation of a FRT-based mitotic recombination leads to the loss of the Gal80 repressor in few cells, and thus, to expression of the reporter (GFP). This way, a mosaic of GFP-expressing cells (including neurons in the brain) can be randomly generated. By inducing mitotic recombination events early or late, one can generate large clones, or single labeled neurons [47,48]. This MARCM method relies on mitotically active cells, making the method particularly useful for marking clones in the developing nervous system. A second system to trace single neurons in the CNS involves the flp-out system [49,50]. In this system, a reporter gene is separated from its promoter by a cassette flanked by FRT sites that can be removed by activation of the flip recombinase (itself driven by the heat shock promoter). This system is particularly appropriate to mark post-mitotic cells as there is no need for a mitotic event. By heat shocking at different times, single isolated post-mitotic cells, or clones of cells can be marked. Both methods have been used successfully for studying the representation of the glomerular olfactory map in the *Drosophila* brain [48,50] or to determine the development of the *Drosophila* mushroom bodies and describe the dendritic patterns of its neurons [51]. We have utilized both systems successfully, labeling cells in the optic lobe previously described trough the Golgi impregnation, demonstrating that these techniques will be applicable for studying neural circuitries in the fly visual system.

## 6. Future directions

In order to understand motion detection and color vision, it is critical to identify the circuitry that starts with the contacts between PRs and their targets in the optic lobe and finishes in higher brain centers. However, it is now most critical, to first define the neural substrates that participate in the visual pathway and second, to identify the genes expressed in these cells. Once these objectives are achieved, it will be relatively easy to manipulate the system and to define unequivocally the functions and the contribution of the neural cells in the processing of the color vision and motion detection.

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