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## Neuroscience: Retinal Projectome Reveals Organizing Principles of the Visual System

A new study using zebrafish genetics and whole-brain imaging has identified more than 50 retinal ganglion cell morphologies and produced the first comprehensive map of connectivity between retina and its target visual centers.

Keisuke Yonehara  
and Botond Roska\*

When a predator fish attacks a prey fish, different features of the predator's image and motion, such as its boundaries, color, approach and lateral motion, are extracted separately by different types of ganglion cells, the output neurons in the prey's retina. Each ganglion cell type consists of a mosaic of ganglion cells covering the retinal surface. The extracted features are sent in parallel to distinct visual centers by ganglion cell axons. The brain of the prey interprets the visual scene by integrating messages from the different ganglion cell types and then plans and executes a motor output that provides a potential escape from the predator. Similarly, the predator uses its own set of ganglion cell types and extracted features to track and catch its prey.

General consensus among researchers has been that the vertebrate retina has about 20 distinct types of retinal ganglion cells and, therefore, they extract 20 different features from the visual scene. In this issue of *Current Biology*,

Robles *et al.* [1] present the first complete connectivity map between the retina and central brain regions of zebrafish. When both dendritic morphology and central projections are taken into account, the data suggest that more than 50 ganglion cell morphologies exist. This new result further emphasizes the large number of parallel computations that are performed at the front end of the visual system.

In attempts to identify retinal ganglion cell morphologies, researchers in the field have relied mainly on three different experimental approaches. The first approach is random sparse labeling of ganglion cells using fluorescent or other dyes, and reconstruction of dendritic morphology [2–4]. The second is serial electron microscopy to reconstruct the fine structure of neurons [5]. One limitation of these two approaches is that they cannot look at axonal projections. The third approach is genetic labeling of specific cell types [6–12], which allows researchers to relate dendritic morphology, axonal projection and physiology of identified ganglion cell types. The number of available markers is far from complete,

however, and we still lack systematic approaches for identifying such markers. To date, all existing classifications have been based on dendritic and somatic morphology.

Robles *et al.* [1] mapped the connectivity between the retina and the central projection targets and classified ganglion cells based on the combination of dendritic morphology and axonal projection patterns. Taking advantage of the advanced genetics available in zebrafish as well as the fact that the larvae are transparent, they were able to image the entire retinal projection pathway using confocal microscopy. Ganglion cells were labeled sparsely, less than 1% at a time, which allowed the characterization of dendritic morphology and axonal projection patterns. Their work provides at least three key insights into the organizing principles of the vertebrate visual system.

The first insight concerns the structural diversity of ganglion cells. Robles *et al.* [1] identified 20 stereotyped axonal projection classes based on the 18 projection sites they found (Figure 1). The projection sites consist of nine sublaminal divisions within the tectum (homologous to the mammalian superior colliculus) and nine extratectal arborizing fields. Combining the projection patterns with distinct dendritic morphologies, more than 50 ganglion cell morphologies were identified. Importantly, the authors found that ganglion cells with the same dendritic morphology could be further categorized into multiple structural types based on the axon projection pattern. This echoes a

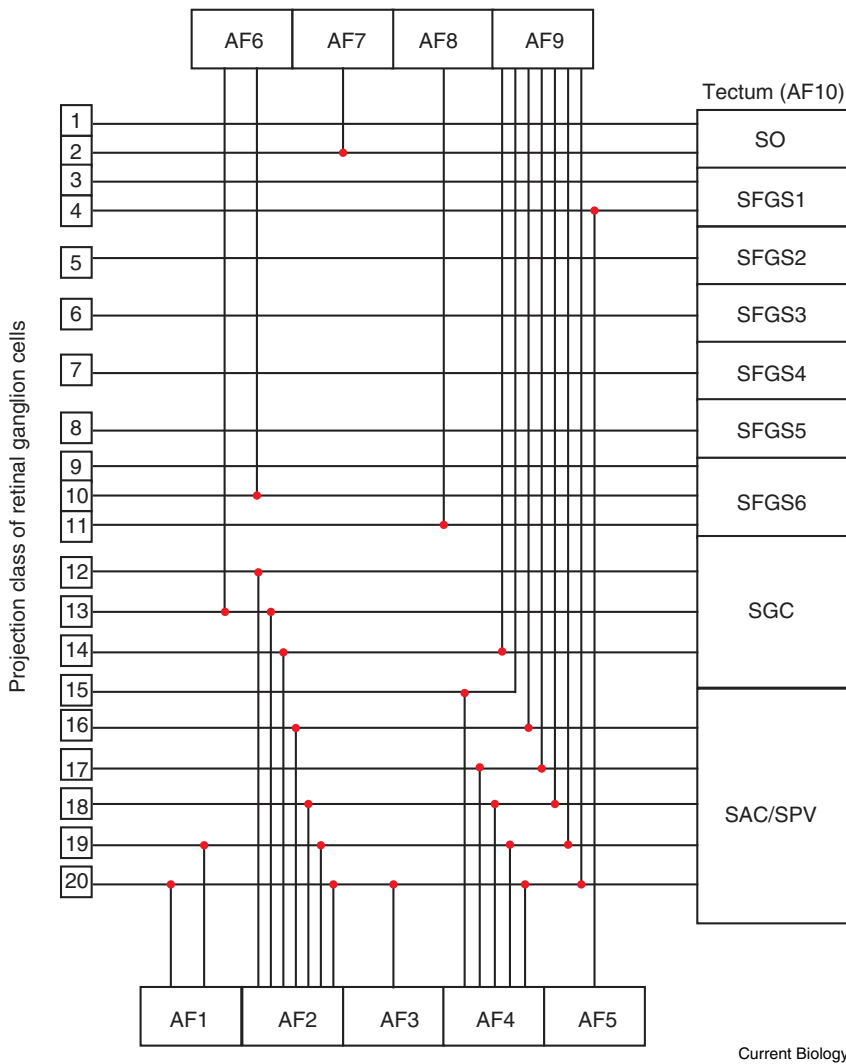


Figure 1. Wiring diagram of axons from 20 distinct projection classes of ganglion cells to 18 distinct innervation sites in higher centers.

Retinal ganglion cells of zebrafish larvae were classified into 20 distinct projection classes based on which of the innervation sites they project onto. The innervation sites consist of nine distinct tectal sublaminæ and nine extratectal arborizing fields (AFs). Red points indicate branching points of axons. Individual projection classes could be further categorized into distinct structural types based on dendritic morphology.

finding in the mouse visual system: Hong *et al.* [13] showed that dendritic morphology does not predict the layer of the superior colliculus in which ganglion cell axons terminate. It remains to be determined whether ganglion cells with the same dendritic morphology but different axon projection patterns have different response properties, or whether they carry the same signals to different regions.

The second insight is about the significant divergence and convergence of ganglion cell pathways. Robles *et al.* [1] found that

many ganglion cells send axon collaterals to different combinations of tectal and extratectal sites. In turn, most of the 18 innervation sites received retinal inputs from a combination of ganglion cells with different dendritic morphology. Therefore, the general trend is that a ganglion cell mosaic sends its extracted feature to many target sites, and a target site receives a combination of different features. The extensive divergence and convergence of visual pathways may represent a key feature of the visual system.

The third insight is about projection patterns of retinal mosaics. A conventional view is that a brain region that receives input from the retina has access to every spatial location within the retina. However, Robles *et al.* [1] showed that some retinal projection sites receive input from only a part of the retinal surface. There are at least two ways to explain this topographic bias. Either the old idea that each dendritic mosaic covers the entire retinal surface is incorrect, or ganglion cells within the same dendritic mosaic may have heterogeneous axon projection patterns. For example, nasal and temporal ganglion cells may project to different target sites. The data presented in this paper favor the second explanation. The physiological significance of such a biased projection remains unclear but the authors propose ecological reasons, arguing that visual information from different retinal regions could be used for different biological purposes, for example, ventral retina may specialize in detecting predators from above [14,15].

Such a beautiful and comprehensive description of the projectome of retinal ganglion cells in zebrafish not only provides a complete picture of connectivity between selected brain regions, but also helps us to focus our attention on important challenges for understanding the visual system. If there are many retinal axon projection sites, and most of them combine visual features from a number of retinal mosaics, it is likely that the next set of visual centers also receives combinatorial projections from various sites. Why is vision based on such a feature-combinatorial system and how do the different centers respond during a visual behavior? One would predict that the zebrafish, which is genetically accessible and in which the activity of every cell in the brain can be recorded [16] is an ideal model system to provide an answer to these questions.

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## Planar Cell Polarity: The Importance of Getting It Backwards

The core and Fat-Dachsous signaling systems locally align planar cell polarities in *Drosophila* epithelia. Three recent papers address how coupling between these systems can be altered and reversed by the products of the gene *prickle*.

Seth S. Blair

The accurate polarization of cells along the plane of an epithelium can orient molecules and structures within single cells, regulate the direction of cell and tissue rearrangements, and bias differentiation choices. Look at the hairs on your arm. Think of your inner ear. While mechanisms for this planar cell polarity (PCP) can differ, two molecular systems involved in PCP are apparently shared from flies to vertebrates: the ‘core’ polarity system, and the Fat (Ft)–Dachsous (Ds) system. Three recent papers, including one published in this issue of *Current Biology*, now present interesting new details about how to strengthen, weaken, and in particular reverse the coupling between these systems in *Drosophila*, due to two different products of a single gene [1–3].

As in many fields of biology, PCP has moved from elegant, singular theories to the reality of multiple parallel mechanisms that intersect on several levels [4,5]. This can make things a bit hard on the casual — or even professional — fan of PCP. Complexity has a way of rendering Occam’s razor

a bit duller and less reliable. One distrusts the simplest explanation (once bitten, twice shy) but, having admitted that there are several reasonable ways to get the same result, the search for a powerful experimental test becomes more difficult. Many find themselves, like the cells, repeating and reinterpreting the work of their neighbors, albeit with twists, some subtle, some profound. What has improved, however, is our ability to look in detail at the cell-by-cell planar polarization of the proteins most intimately involved in the process, rather than the final outcome. This nicely narrows interpretations, and has confirmed and extended some old ideas in lovely detail.

Protein polarizations are important because they are not just an outcome but — in tissues like the *Drosophila* wing, abdomen and eye — a cause of PCP. Some of the polarized proteins are also signals that can direct polarization in adjacent cells, which in turn propagate that local alignment to their neighbors. Add amplification and feedback, and any slight tendency towards polarization turns into a robust, self-reinforcing property

of repeated, interlocked polarities across a field of cells.

Having multiple local alignment systems likely adds another level of robustness [4,5]. In the core system, signaling between cells is carried by the Wnt receptor Frizzled (Fz), the multipass transmembrane protein Van Gogh/Strabismus (Vang/Stbm) and the homophilic cadherin Flamingo/Starry night (Fmi/Stan), which is modulated and localized by the cytoplasmic proteins Disheveled (Dsh), Diego and Prickle (Pk). Fz, Dsh and Diego concentrate on one face of a cell and Vang/Stbm and Pk concentrate on the opposite; Fmi/Stan co-concentrates with both (Figure 1A). In the Ft–Ds system, signaling is carried by heterophilic binding between the Ft and Ds protocadherins, with Ds and the myosin Dachs concentrated more reliably on one face, and Ft weakly concentrated on the opposite (Figure 1A).

How — and how well — are these two systems integrated in *Drosophila*? It depends a bit on the type of polarity, which includes biased cell divisions, hair polarities, and polarized fate choices. Ft and Ds protein polarization appears largely unaffected by the core polarity system (although I will discuss an intriguing new exception below), and there are polarities where the Ft–Ds system seems to work largely alone. Each system can independently influence the polarity of abdominal hairs when the other system has been disrupted [4,6]. Nonetheless, in the wing and eye, core proteins polarize