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SYSTEMS NEUROSCIENCE

# Diversity in sight

**A systematic analysis of bipolar cells, which act as a central signalling conduit in the retina, reveals that the neurons' diverse responses to light are generated largely by feedback from neighbouring amacrine cells.**

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The world that you see is not the world that exists — it has been heavily retouched by your retina. The modified image uses less computational power than the raw form because, before being sent to the brain, it is packaged into more than 30 representations that emphasize specific features of the visual scene. The content of these messages is partially understood, but much less is known about the neural machinery that creates them. In a paper online in *Nature*, Franke *et al.*[1] report methods and findings that shine a light on the heart of this retinal machinery.

The vertebrate retina contains five main classes of neuron[2]. First, photoreceptor cells detect light and send a synaptic output that connects to bipolar cells. Horizontal cells in the outer retina can modify this signal, but bipolar cells are the backbone of the system, sending outputs to ganglion and amacrine cells in the inner retina. Amacrine cells receive inputs not only from bipolar cells but also from other amacrine cells, and both bipolar and amacrine cells have synapses with retinal ganglion cells. These are the final output neurons of the retina — their bundled projections make up the optic nerve that leads to the brain.

But each of these five classes also has subtypes. As a result, the retina contains more than 60 types of neuron, each performing a different task. This circuitry extracts which features of the incoming information can be efficiently transmitted to the brain. For example, many retinal ganglion cells are sensitive to change — they respond to the turning on or off of a light, but are relatively quiescent when light levels are steady. Others are sensitive only to moving objects, and even to objects moving in a particular, pre-specified direction[3].

The conventional way to study the neurons involved in creating these diverse messages is to make electrical recordings from individual cells, but this strategy cannot measure enough cells to accurately reflect the diversity of neuronal interactions. Franke and colleagues therefore developed a technique to directly analyse the synaptic outputs of many of the bipolar cells in a mouse retina simultaneously by recording the activity of a protein that fluoresces in response to glutamate (the neurotransmitter molecule released by bipolar cells).

The termini of a bipolar cell, called varicosities, are not a one-way street — in addition to releasing glutamate[4,5], they receive inputs back from amacrine cells. Thus, each varicosity integrates different kinds of information. By recording only the cells' outputs, the authors could look directly at the main driver of the final visual response to light.

The researchers recorded galaxies of fluorescent spots, marking glutamate-release sites, in isolated retinas that had been mounted under a microscope and exposed to light. They used statistical techniques to isolate the spots that corresponded to individual synaptic varicosities and to clusters of varicosities belonging to a single type of bipolar cell. Importantly, they verified that these spots represented bipolar-cell termini by filling individual bipolar cells in the same retinas with a second fluorescent dye that enabled direct observation of both the varicosities and the processes that connect them to the main body of the bipolar cell.

Franke *et al.* applied a battery of different light stimuli to the retina, designed to reveal the distinguishing features of different types of bipolar cell. Their first major finding was that interactions with amacrine cells increase the diversity of the different bipolar cells' outputs to ganglion cells — they 'decorrelate' the response of one bipolar cell type from the responses of others. When the authors stimulated the retina with spots of light small enough to hit one bipolar cell, responses were relatively uniform from cell to cell. But when they used a wider spot that stimulated not only a bipolar cell but also many of its neighbouring amacrine cells, the responses of different bipolar cell types were distinct.

Amacrine cells come in two broad groups: small-field cells, which communicate locally, and wide-field cells, which can project across the retinal surface[6]. The small-field cells use glycine as a neurotransmitter, whereas the wide-field cells use GABA molecules. Franke and colleagues' second major finding was

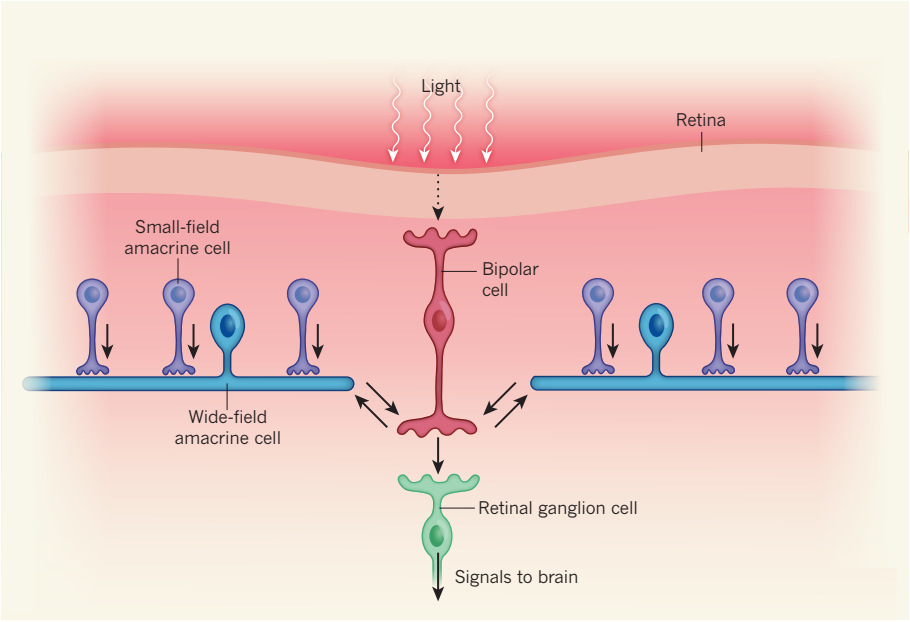


**Figure 1 | Generating diversity in bipolar cells.** Bipolar cells are the backbone of the neuronal circuitry in the vertebrate retina, receiving information (indicated by dotted arrow) from light-sensing photoreceptor cells (not shown) and sending signals (black arrows) through synaptic outputs to amacrine and retinal ganglion cells. Bipolar cells also receive signals from amacrine cells. There are two types of amacrine cell, small- and wide-field cells. Franke *et al.*[1] report that wide-field amacrine cells send signals that modulate the activity of bipolar cells, and are in turn modulated by small-field cells. There are many types of small-field amacrine cell, leading to diverse bipolar-cell responses to light that feed to the brain via retinal ganglion cells.

that small- and wide-field cells have different roles in mediating bipolar-cell responses. By pharmacologically manipulating these neurotransmitters, the authors showed that the major driver of bipolar-cell behavioural diversity is the GABA-releasing wide-field cells, but they are in turn controlled by the glycine-releasing small-field cells (Fig. 1). Moreover, different bipolar-cell types receive inputs from different sets of GABA-releasing cells, which in turn receive input from different sets of small-field cells. Thus, the diversity of bipolar-cell outputs comes from circuits of amacrine cells, not individual amacrine-cell types, as was often imagined.

Finally, Franke *et al.* began the process of refining the definitions of bipolar-cell types. For example, they show that all 'OFF' bipolar-cell types (those whose activity is inhibited by light) make brief, paradoxical 'ON' responses at times when they should be inhibited. These are not trivial differences — they reflect meaningful diversity in signals sent by the bipolar cell to the ganglion cell.

A major advantage of the new methods is that they allow huge samples of cells to be studied side-by-side, and the detailed study of individual subtypes to become practical. A next step will be to understand in more detail the responses of the different types of bipolar cell to the changing scenes observed by freely roaming mice. This will not be easy.

The challenge is how to choose test stimuli, and how to interpret the bipolar-cell responses to them. It is unlikely that naturalistic responses can be achieved using spots of light, striped patterns or even the 'chirp' stimuli used by Franke and colleagues, which change in intensity or flicker rate over time. It is widely thought that visual scenes similar to those that would be encountered in the wild would be appropriate. But there is no agreement on how to meaningfully analyse responses to such stimuli. Where in a natural scene does the stimulus to an individual retinal cell start, and where does it end? For once, it seems that our methods have outrun our ideas for how to use them. Think hard, people. ■

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1. Franke, K. *et al. Nature* http://dx.doi.org/10.1038/nature21394 (2017).
2. Masland, R. H. *Neuron* **76,** 266–280 (2012).
3. Vaney, D. I., Sivyer, B. & Taylor, W. R. *Nature Rev. Neurosci.* **13,** 194–208 (2012).
4. Borghuis, B. G., Marvin, J. S., Looger, L. L. & Demb, J. B. *J. Neurosci.* **33,** 10972–10985 (2013).
5. Marvin, J. S. *et al. Nature Methods* **10,** 162–170 (2013).
6. Masland, R. H. *Vis. Neurosci.* **29,** 3–9 (2012).