**Editor's Summary**

The function of any neural circuit, such as the retina in the eye, is directly related to its connectivity, as determined by the functions of different classes of neurons and their choice of partners. Those attributes are governed by the genetic makeup and molecular interactions of each neuron, but it has been difficult to demonstrate a continuous link from the genetic level to that of cellular structure and circuit function. In-Jung Kim et al. have developed a novel multidisciplinary approach to the problem and apply it to retinal ganglion cells. They have discovered a new type of retinal neuron, identified by a genetic marker, with a unique cell shape, and a unique function. All cells of this type ‘point’ in a single direction. Remarkably, these cells respond best to objects moving in the direction predicted biophysically by their structure: upwards. The finding raises an intriguing question: why has the mouse invested so heavily in sensitivity to upward motion?

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**Undergraduate Journal Club**


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**LETTERS**

Molecular identification of a retinal cell type that responds to upward motion

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The retina contains complex circuits of neurons that control visual behavior. Various theories propose that cell type (RGC), cell number, and cell-to-cell connections among RGCs are part of the network that controls visual behavior. Using a genetic labeling method, we have identified a new class of RGC. These cells have a unique cell shape and are selectively activated by upward motion. The findings suggest that the genetic make-up of neurons can be decoded to predict their function in circuits controlling visual behavior.

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**Eye and Retina**

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**Fig. 1:** A drawing of a section through the human eye with a schematic enlargement of the retina.
The retina has over 50 known cell types belonging to the 5 traditional classes (receptors, horizontal, bipolar, amacrine, ganglion) - mostly from rabbit.

Generalized diagram of the retina circuitry shows both vertical and horizontal connections.
This study reports on a new type of retinal ganglion cell, identified by (1) molecular marker (junctional adhesion molecule, JAM-B) and (2) by asymmetrical dendrites, by its connectivity in the inner plexiform layer, and (4) by its directional sensitivity to moving light.

JAM-B (junctional adhesion molecule B) is expressed in retinal ganglion cells, labeled here with YFP genetic construct. Flattened whole mount.

**Cellular Adhesion Molecules in Retinal Ganglion Cells**

Previous work from Yamagata and Sanes demonstrated that cellular adhesion molecules in the Immunoglobin super family (IgSF) guide synaptic contacts within a specific lamina of the retina.


A Survey of 200 IgSF genes in mouse retina revealed one gene, JAM-B is expressed in just a few Retinal ganglion cells (Junctional Adhesion Molecules – B).

In-situ hybridization of Post Embryonic day 7 (P7) retina to probes for JAM-B (in red) and a probe for all Retinal Ganglion Cell-specific gene, THY1, (in Green)

Right Whole mount shows JAM-B positive RGCs in whole mount.

JAM-B positive RGCs are “dispersed” in retina

JAM-B is involved in tight junction assembly in developing blood cells

An ecologist would call this spatial distribution “dispersed” (maximal distance between cells) – (territorial birds, trees competing for limited resources)

clumped  |  random  |  dispersed

clumped  |  random  |  dispersed

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**Genetic Construct Used to make retinal ganglion cells fluorescent**

Transgenic system for marking RGCs with YFP. CreER, tamoxifen-activated Cre; open triangles, loxP sites.

Transgenic system for marking RGCs with YFP. CreER, tamoxifen-activated Cre; open triangles, loxP sites.
Cre-recombinase is fused with mutated estrogen receptor making a tamoxifen-dependent Cre – that can be turned on simply by injecting tamoxifen.

**Materials and Methods**

Construction of Plasmid and Generation of Transgenic Mice. 500 ng Cre-ER is propagated by drawing the 349 bp XbaI fragment from Cre-ER App in the XbaI site of the expression vector CMV3. Cre-ER was inserted in the XbaI site of the expression vector CMV3. SV40 early promoter was excised from the CMV3 vector by digestion with ClaI and SpeI. Cre-ER (SV40) was cloned into the expression vector CMV3, generating the expression vector CMV3 Cre-ER. Cre-ER is expressed in the brain regions of mice that have been treated with tamoxifen. The expression of Cre-ER is regulated by the expression of the CMV3 vector.

**Figure 1.**

**A**

- SV40 early promoter
- Cre-ER Mutant
- poly A

**B**

- Cre-ER
- JAM-B
- Cre-ER
- bacterial artificial chromosome

**C**

**D**

**E**

**F**

**G**

**H**

**I**

**J**

**K**

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**M**

**N**

**O**

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**V**

**W**

**X**

**Y**

**Z**

**Supplementary Information**

In the mammalian retina, ten parallel, stacked representations are maintained by ten populations of amacrine cells, which are functionally distinct from one another. These amacrine cells are characterized by their synaptic inputs and outputs, which they receive and send. The afferent inputs to these amacrine cells are mainly from photoreceptors, horizontal cells, and other amacrine cells. The efferent outputs of these amacrine cells are to ganglion cells, which are the output neurons of the retina. These ganglion cells are divided into several subtypes, each with a different role in processing visual information. The functional diversity of amacrine cells is related to their anatomical position, as they are distributed throughout the retina, from the inner plexiform layer (IPL) to the ganglion cell layer (GCL). Amacrine cells are essential for the normal functioning of the retina, as they modulate the responsiveness of ganglion cells to light and color, and they play a crucial role in the visual processing of the brain.
Asymmetry of dendrites

Central retina asymmetric
Ventral retina symmetric, also dorsal margins

Arrow: Asymmetric dendrites
Blue star: Symmetric dendrites
Optic nerve: White star

Physiology of J-RGC

Illuminate with blue or UV light. Guide electrode toward cell body, whole cell patch. Stimulate with white light.

J-RGC are off cells

Non-YFP positive cells are equally on cells and off cells

Response to spot size

Slanted trajectory: response to a bright spot moving downward produces a delayed excitation, and then a surround excitation with less delay. At the appropriate speed these two events occur simultaneously.

Spatio-temporal receptive field measured by reverse correlation with flickering bars.
Directional preference for flickering bars averaged for bars positioned at 45 deg intervals around circle centered on receptive field.

Cells with symmetric dendrites were not directionally selective.

Requirements for a motion detector:
1.) Two spatially separated inputs.
2) Temporal asymmetry in processing two inputs.
3) Nonlinear interaction between two inputs.

General Motion Detection Schemes

Two targets for J-RGC’s

One outstanding question is why the mouse has invested so heavily in sensitivity to upward motion. We have been no more successful than Dräger and Hubel in guessing what that significance might be. By mating JAM-B–CreER mice to other transgenics bearing appropriate Cre-activated channels or toxins, we may be able to inactivate this pathway and thereby directly test its function.
One possible mechanism is suggested by the finding that inhibitory synapses on some RGCs are concentrated at distal dendrites. Distal inhibition on the asymmetric dendrites of J-RGCs could account for their asymmetrically displaced surround.