New microscope decodes complex eye circuitry (w/ Video)

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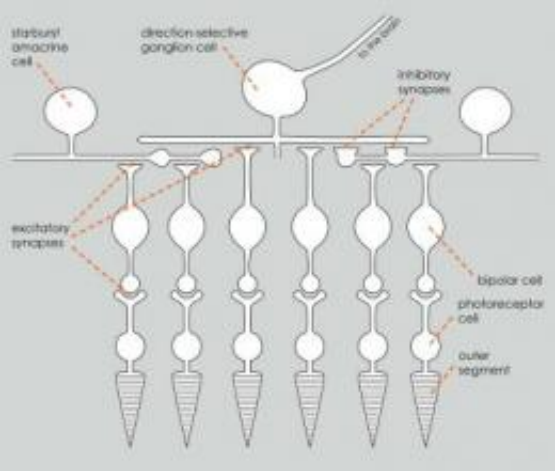


In the outer segments of the photo receptor cells the optic signals are transduced into electrical signals. Excitatory and inhibitory synapses convey these signals to the ganglion cells. Credit: www.somedonkey.com

The sensory cells in the retina of the mammalian eye convert light stimuli into electrical signals and transmit them via downstream interneurons to the retinal ganglion cells which, in turn, forward them to the brain. The interneurons are connected to each other in such a way that the individual ganglion cells receive visual information from a circular area of the visual field known as the receptive field. Some ganglion cells are only activated, for example, when light falls on the centre of their receptive fields and the edge remains dark (ON cells). The opposite is the case for other ganglion cells (OFF cells). And there

are also ganglion cells that are activated by light that sweeps across their receptive fields in a particular direction; motion in the opposite (null-) direction inhibits activation.

Starburst amacrine cells, which modulate the activity of the ganglion cells through inhibitory synaptic connections, play an important role in this direction selectivity. The same research group at the Max Planck Institute in Heidelberg demonstrated a number of years ago that starburst amacrine cells are activated by moving stimuli. Each branch in the circular dendrite tree reacts preferentially to stimuli that move away from the cell body; movements in the opposite direction, towards the cell body, inhibit its activity. In the central area around the cell body dendrites function only as receivers of synaptic signals, while the dendrites on the periphery act as transmitters as well – and, therefore, double as axons. Whether these dendrites cause the direction selectivity in the ganglion cells or whether the ganglion cells "compute" it using other signals was unclear up to now.

Max Planck researchers Kevin Briggman, Moritz Helmstaedter and Winfried Denk have now discovered that, although the cells themselves are symmetrical, the synapses between retinal ganglion cells and starburst amacrine cells are distributed asymmetrically: seen from the ganglion cell, the starburst cell dendrites connected with it run in the direction opposite to the preferred direction of motion. "Ganglion cells prefer amacrine-cell dendrites that run along the null-direction," says Winfried Denk.

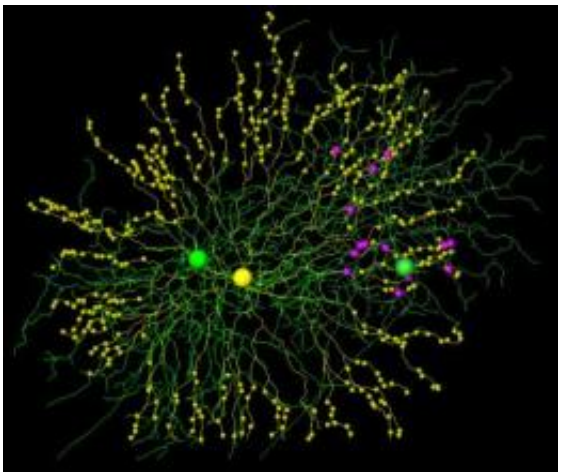
According to previous studies by Winfried Denk and his research group, the electrical characteristics of the dendrites, which emerge starlike from the cell bodies of amacrine cells, play a crucial role here. The further they are located from the centre of the cell toward the edge, the easier they are to excite; therefore, stimuli are transmitted preferentially in this direction. This mechanism does not require but is helped by inhibitory

influences between neighbouring amacrine cells, known as lateral inhibition. "A ganglion cell can thus differentiate between movements from different directions simply by making connections with certain starburst amacrine cell dendrites - namely those that prevent activation of the ganglion cell in null-direction through their inhibitory synapses. These are precisely the amacrine cell dendrites that run along this direction," explains Winfried Denk.

Functional and structural analysis

This discovery was made possible by combining two different microscopy methods. The scientists succeeded, first, in determining the preferred motion direction of the ganglion cells using a two-photon fluorescence microscope. A calcium-sensitive fluorescent dye indicated in response to which stimuli calcium flows into the cells - a process that signals electrical activity in cells.

They then measured the exact trajectory of all of the dendrites of these ganglion cells and those of connected amacrine cells with the help of a new electron microscopy method known as serial block face electron microscopy. This process enabled them to produce a volumetric image by repeatedly scanning the surface of a tissue sample using the electron beam of a scanning electron microscope. A thin "slice" is shaved off the sample surface after each scan is complete, using an extremely sharp diamond knife. These slices are thinner than 25 nanometers, just about one thousandth of the thickness of a human hair.



Cells and synapses reconstructed from serial block face electron microscopy data. A single starburst amacrine cell (yellow, note synaptic varicosities) and two direction-selective ganglion cells (green). Even though there is substantial dendritic overlap with both cells, all connections (magenta) go to the right ganglion cell. Credit: Kevin Briggman

The high three-dimensional resolution of this method enabled the scientists to trace the fine, densely packed branched dendrites of retinal neurons and clearly identify the synapses between them. The complete automation of the imaging process enables them to record data sets with thousands and even tens of thousands of sections "while on holiday or attending a conference," says Winfried Denk. "For the first time, minute cell structures can now be viewed at a high resolution in larger chunks of tissue. This procedure will also play an indispensable role in the clarification of the circuit patterns of all regions of the nervous system in the future."

More information: Kevin L Briggman, Moritz Helmstaedter, Winfried Denk, Wiring specificity in the direction-selectivity circuit of the retina, *Nature*, March 10, 2011