The many roles of starburst amacrine cells

Richard H. Masland
Howard Hughes Medical Institute, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA

Starburst amacrine cells release two classical neurotransmitters, ACh and GABA. In a tour de force of paired-cell recording, Zheng et al. now show that the starburst cells are mutually excitatory during early development but mutually inhibitory in adult animals. The change occurs by remodeling of both the cholinergic and the GABAergic synapses between starburst cells. The finding gives a precise mechanistic basis for the developmental waves of activity in the retina.

Introduction
The rod amacrine excepted, starburst amacrine cells are the most numerous amacrine cells of the mammalian retina. They are found in species from turtles to macaques. Because they are both numerous and widely overlapping, they occupy a substantial fraction of all available volume of the inner synaptic layer of the retina. In the rabbit, where they have been most intensively studied, starburst amacrine cells deploy a total of six meters of dendrite per millimeter of retinal surface, for a total of more than two kilometers of starburst dendrite in the retina as a whole [1]. Only one other known retinal neuron is within an order of magnitude of this amount. A cell using so much precious 'wire' must do something important, and an incisive new paper by Jimmy Zhou and his colleagues [2] now shows that it does not one but several important things, changing its role dramatically during the course of development.

Synapses that change during development
In early postnatal days, the developing retina is repeatedly swept by waves of electrophysiological activity. These waves create correlated firing in the retinal ganglion cells, and the correlated activity is thought to instruct the topographical ordering of the central visual system [3]. The starburst cells are unusual in that they synthesize and release two classical neurotransmitters, ACh and GABA, and pharmacological evidence had suggested that the early developmental waves are mediated by both, because GABA can act in an excitatory way during early life [4,5]. How are the waves propagated? Anatomists have reported synapses between starburst cells [6,7], but their existence has always been somewhat controversial. The technical breakthrough was reliable recording from pairs of starburst cells, which allowed Zheng et al. to observe directly the starburst-to-starburst synapses. It was found that the cells communicate directly with each other, without intervention of any other retinal cells. Excitation of this sort is entirely adequate to explain the propagation of early developmental waves.

A reason to doubt starburst-to-starburst synapses was that they would appear to create a positive feedback, in which excitation produces even more excitation, inevitably leading to epilepsy-like instability. It turns out that this does not happen because the starburst cell synapses are remodeled before the circuitry and excitability of the cells reaches the adult level. Nicotinic ACh receptor (nAChR)-mediated excitation between starburst cells drops to an almost undetectable level (cholinergic synapses from starburst to ganglion cells remain). At the same time, the GABAergic synapses among starburst cells switch from being excitatory to inhibitory. The cells are no longer at risk of overstimulation, because excitatory interactions between starburst cells have been reduced at one of their sources and reversed at the other.

Starburst function in the adult retina
Zheng et al. convincingly resolve a long-standing ambiguity about starburst function – the mechanism of release of the two neurotransmitters. It was shown long ago that both neurotransmitters are released from the cells, but the mechanism of release has always been problematic [8], with some suggestion that part of the GABA release is transporter-mediated. In the new experiments, pairs of cells were studied in a medium in which Ca\(^{2+}\) entry was blocked by a high concentration of Cd\(^{2+}\). Under normal ionic conditions, both ACh and GABA were released in a quantal fashion. In the high-Cd\(^{2+}\) medium, synaptic potentials mediated by both neurotransmitters were entirely eliminated. These findings are hallmarks of standard vesicular release. This was confirmed by an elegant experiment in which caged Ca\(^{2+}\) was injected into one of the cells via the recording pipette. When Ca\(^{2+}\) was subsequently uncaged by a brief pulse of UV light, the injected cell released both cholinergic and GABAergic vesicles, as evidenced by quantal excitatory and inhibitory responses from the second starburst cell.

What do these results say about the role of the starburst cells in adult retinas? A strong possibility is that they account for the unusually strong lateral inhibition observed for starburst responses to light [9,10]. Another is that they might participate in more complex circuits. It has now been shown that sectors of the starburst dendritic arbor (Figure 1) are capable of independent activity [9–12] and that each sector reports on only a particular direction of a moving stimulus [13]. If starburst cells obey a wiring postulate requiring that sectors having the same directional orientation all...
synapse on a single retinal ganglion cell (and not on others), then the directional preference of these starburst sectors will be passed on to the ganglion cell, which will itself become directionally selective [14,15]. Because the strongest determinant of direction selectivity is GABA-mediated inhibition, removal of the uncertainty about the mechanism of GABA release brings the mechanism of direction selectivity one step closer to a definitive solution.

Concluding remarks

A couple of points remain to be understood. Are ACh and GABA packaged and secreted together, or at different sites? Zheng et al. intriguingly find differences in the Ca²⁺ sensitivity of ACh and GABA release, which would suggest release from different vesicular pools. And what is the role of cholinergic synapses in the adult animal? They are known to excite the directionally selective ganglion cells, but this appears to occur in a non-directional way. Do they have a modulatory function, entirely independent of directional selectivity? Or do they participate in more complex circuitry, perhaps involving local microcircuits presynaptic to the direction-selective ganglion cell [16–18]? Paired-cell recordings between starburst cells and other types of retinal neurons might also help to answer these questions.

References