Retinal development: **On the crest of an exciting wave**

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Propagated waves of excitation in developing neural tissues may be a critical feature of maturation. Recent findings shed new light on the mechanisms underlying these waves.

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Waves of electrical excitation can course spontaneously across brain tissues. These more-or-less periodic and coordinated patterns of activity can be observed in the cortex, hippocampus, spinal cord and retina; they are alluring, but their functional roles remain elusive. Neurobiologists have focussed on the coordinated activity of ganglion cells in developing retinae, for good reasons. The vertebrate retina is a fairly well understood, laminated planar tissue from which can be recorded the activity of single cells as well as groups of cells. There is compelling evidence that neuronal activity is required for normal adult patterns of connectivity in higher visual centers [1]. In the retina, characteristic patterns of spontaneous, periodic activity occur only during developmental stages when the ganglion cells are establishing connections within their primary target, the lateral geniculate nucleus (LGN). The retina would thus seem an ideal system for investigating the mechanisms and functions of spontaneous propagated electrical activity, and this optimism is borne out by a number of recent findings.

In all vertebrates, the retina is structured as a transparent, 200–300 micron-thick laminar sheet (Fig. 1). The light-capturing rods and cones lay along the distal edge of the retina, at the back of the eye. Layers of interconnecting second- and third-order neurons conduct light-evoked signals from rods and cones to the ganglion cells, which line the proximal edge of the retina, nearest the front of the eye. Ganglion cells send their axons from the eye to the higher visual centers. The activity of individual ganglion cells — in the form of action potentials — can be recorded with microelectrodes. The activity of large groups of ganglion cells can be visualized with either a planar array of multiple electrodes that presses against the retinal surface adjacent to the ganglion cell somata, or by video imaging of intracellular calcium concentrations.

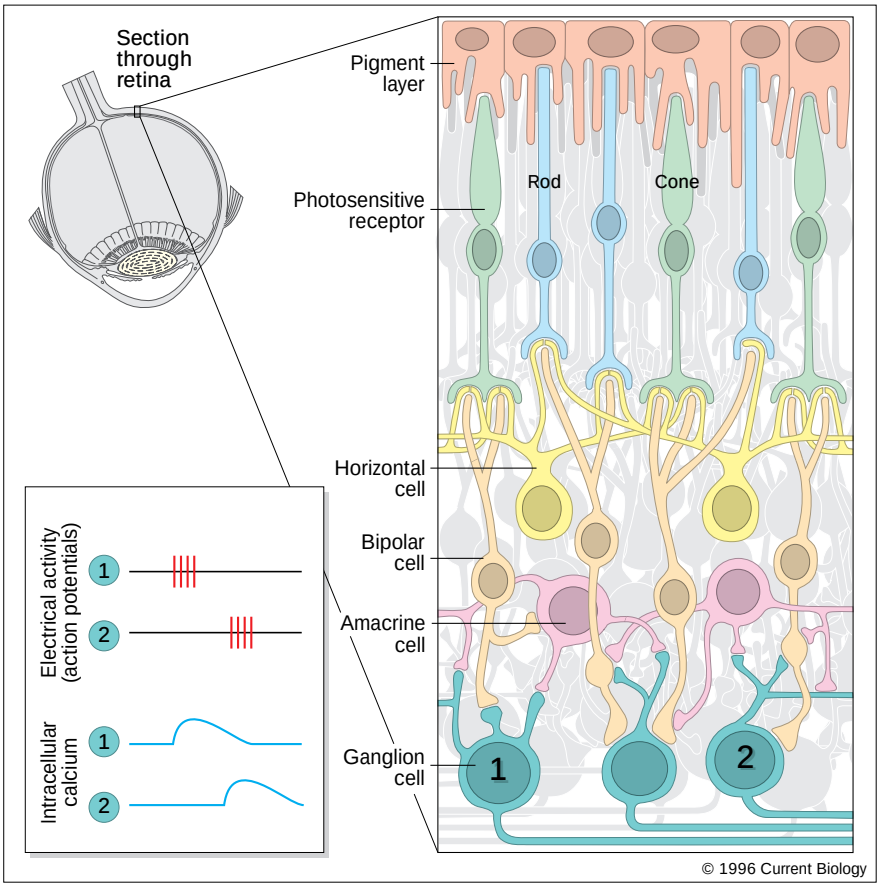
Individual ganglion cells in the developing mammalian retina are known suddenly and randomly to fire a burst of action potentials and then remain silent or refractory for a period of several tens of seconds; such bursts of activity can progress wave-like from one ganglion cell to the next. The first sign of this was detected when ganglion cells in one-day-old rabbits were found to discharge spike activity in short bursts [2]. More recently, in the embryonic rat retina, the bursting patterns of many neighbouring ganglion cells were found to be temporally correlated, suggesting that the activity patterns are coordinated or even synchronized [3]. The use of a planar electrode array subsequently revealed that, in the neonatal ferret, the bursts of activity move from one cell to the next and propagate across the retina as a wave [4]. Each ganglion cell bursts for 4–10 seconds and a wave of bursts propagates across the retina at speeds up to 200 microns per second [4,5].

Until recently, missing from the scene has been even the semblance of an explicit model for propagated waves in the retina. Burgi and Grzywacz [6] have begun to fill this void. They developed a computerized model which simulates the propagated waves of spike discharges that they measured in the turtle retina. In the model, depolarization of one or a few ganglion cells elevates extracellular potassium and releases glutamate. Diffusion of potassium and synaptic excitation of neighboring cells mediates the spread of excitation across the retina. The model correctly simulates the requirement for glutamate-activated receptors and actions potentials for wave propagation, the refractory period between bursts and the velocity of wave propagation (~30 microns per second). Future work will require refining the model to account for the faster velocities (150–300 microns per second), and the absence of a need for glutamate-gated channels, that characterize wave propagation in mammalian retinae.

Previous studies showed that not all cell types in any one area of the retina participate in the activity bursts or propagated waves [4,5]. It remained a mystery how selective a burst or wave was with respect to cell type or spatial location. Recently, Wong and Oakley [7] have found that ON and OFF type β-type ganglion cells — activated and depressed, respectively, when light falls on their receptive field centres — burst nearly synchronously in retinae from nine-day-old animals, but by day seventeen their activity was almost uncorrelated. A particularly striking observation was that the ON cells burst at a much lower rate than the OFF cells. These results provide strong evidence for the existence of sub-networks of synchronously bursting cells in the neonatal mammalian retina, and argue against the notion that the propagated waves reflect diffusion of an excitatory molecule, such as extracellular potassium, that would be expected indiscriminately to depolarize most cells as it passed laterally across the retina.

Figure 1

A cross section through the retina, showing the various cell types. The inset at the bottom left illustrates the bursts of electrical activity and changes in intracellular calcium level that occur as an activity waves passes successively through ganglion cells 1 and 2.



From previous work, we knew that the acetylcholine receptor antagonist curare suppresses the periodic bursts of spikes in the neonatal rabbit retina [2]. Other reports have also implicated acetylcholine in retinal wave generation, but until recently it was not known how acetylcholine and its receptors fitted into any scheme for burst initiation and/or wave propagation. Feller *et al.* [8] simultaneously recorded calcium waves across the retina and monitored synaptic currents in individual ganglion cells under voltage clamp. Their findings showed that spontaneous, compound synaptic currents occurred concomitantly with passage of the propagated wave past a ganglion cell. These synaptic currents resulted from the activation of nicotinic acetylcholine receptors: curare blocked both the transient calcium increases and the synaptic currents. We can now conclude that activation of nicotinic acetylcholine receptors on ganglion cells themselves plays an integral role in the initiation and propagation of the calcium waves.

We know that, once a burst occurs in one or a few neighboring ganglion cells, in order for a wave to propagate some signal has to radiate from the region encompassing these few ganglion cells to excite more distant neighbors. Glutamate is not the excitatory neurotransmitter, because glutamate receptor antagonists do not block waves [5]. Although potassium may the diffusible excitatory molecule for wave propagation in turtle retinae, its diffusional rate is likely to be too slow for mammalian waves. Acetylcholine is the best candidate for being the excitatory molecule that mediates wave propagation. As amacrine cells — the neurons presynaptic to the ganglion cells — are the only retinal cells to synthesize acetylcholine, they must be involved in lateral transmission of the excitation. The exact role of the amacrine cells is still unclear, and more research will be required to elucidate the connections. We can, however, rule out two of the older ideas that have been postulated to account for waves.

One of those older ideas was that gap junction coupling is required for wave propagation. In developing neonatal cortex, propagating calcium waves most probably require gap junction coupling between neurons [9]. Gap junctions are present in the developing mammalian retina, and it was suggested that they might play a pivotal role in propagated bursts of spiking activity or calcium waves. This idea is probably erroneous. Penn *et al.* [10] reported that individual β-type ganglion cells, known to participate in bursts and wave propagation, are not coupled *via* gap junctions to

any other cells. Furthermore, although the neonatal form of bursting and wave propagation disappears by postnatal day twenty one, the extent of dye-coupling between γ- and α-type ganglion cells and other neurons, including amacrine cells, continues to increase well beyond day twenty-one, clearly at odds with a gap junction-based model for wave propagation.

The other old idea was that retinal waves are a form of 'spreading depression'. During episodes of spreading depression, retinal and other neural tissues become transiently hyperexcited and then depressed [11]. In amphibians and birds, spreading depression propagates across the retina as a milky white wave that travels at around 10–20 microns per second. The propagation of action potentials and calcium waves in neonatal retinas might just be an expression of spreading depression. Gouras [12] showed nearly forty years ago that a burst of action potentials occurs when spreading depression passes a ganglion cell. Mori *et al.* [11] demonstrated that spreading depression was stimulated by brief puffs onto the retina of potassium, aspartate or glutamate, or by touching the retina. Spreading depression was not blocked by tetrodotoxin, an antagonist of the voltage-sensitive sodium channels, however it was associated with elevated level of extracellular potassium.

Several characteristics of propagated spike and calcium waves in neonatal mammalian retinae make it unlikely that they are a form of spreading depression. The propagation speed is faster for retinal activity waves (150–300 microns per second) than for spreading depression (10–20 microns per second). Unlike spreading depression, the propagation of calcium waves is blocked by tetrodotoxin. Furthermore, calcium waves cannot be initiated by poking the retina (M. Feller, personal communication). Finally, although a brief puff of potassium can initiate both spreading depression and a calcium wave, neither glutamate nor aspartate can initiate a wave in the neonatal mammalian retina.

It has been argued that the coordinated firing of groups of neurons aids in the establishment of connection patterns in target tissues [1]. The notion is summed by the phrase 'neurons that fire together wire together'. That Wong *et al.* [7] found different patterns of coordinated activity in ON and OFF ganglion cells adds credence to the idea, because separate layers of ON and OFF cells are found in the LGN of some mammalian species. The difference in spontaneous activities of the ON and OFF cells arose at the time (postnatal day seventeen) when the ON/OFF layering is being established in the LGN.

But as appealing as the idea that activity waves help sculpt retinal–LGN connections may be, it would be prudent to consider also the possibility that they play a role in the development and maturation of the retina itself. Sernagor and Grzywacz [13,14] have suggested that bursts of action potentials in young turtle retina may help sculpt the receptive-field properties ganglion cells. Furthermore, Gu and Spitzer [15] have shown that, in developing spinal cord neurons, calcium transients play roles in neurotransmitter expression, ion channel maturation and neurite extension. Similar functional roles might exist for activity bursts and waves in the developing retina. We are clearly inching closer to the time when spontaneous bursting and wave propagation are understood well enough to at least allow their experimental manipulation by pharmacological intervention. Then we can begin directly testing the functional roles for the spontaneous activity.

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