Building connections

Studies in the developing retina provide insights into neural circuit assembly

By Arjun Krishnaswamy

Much of a child’s first year is spent asleep, punctuated by the occasional feeding, fit, or flatulence. The serenity is only skin deep, as a flurry of action goes on beneath. The brain is building itself at a frantic pace during this year, forming millions of synapses per second. Remarkably, speed does not incur a cost in precision—children reach their first birthday with specific, largely mature patterns of connectivity already formed, ready to become terrible at 2. How the brain manages this remarkable feat is not altogether clear.

We do know part of the story. It begins with the generation of neurons, which migrate to appropriate locations and extend axons long distances to target areas (1). The puzzle is what happens next, when axons and dendrites form their remarkably specific patterns of connectivity. Forming synapses is an astonishingly promiscuous process. Decades of study indicate that neurons readily synapse with each other if they share matched transmitters and receptors, as well as generic organizing molecules. Yet, axons in fact synapse with only small subsets of potential targets and often only on specific portions of a target—for example, a distal dendrite, a soma, or an axon hillock (1, 2).

How do these selective connections arise? One idea finds its roots in the classical work by Langley and Sperry: Selectivity between potential synaptic partners arises because they have a specific chemical affinity for one another (3–5). Endowing neurons with this affinity might be the role for the large families of recognition molecules that take up ~3% of the genome (1). There is ample evidence that such proteins regulate cell-cell contact but little evidence that their combinatorial expression patterns are related to specific connectivity. Could such expression patterns encode some or all of the nervous system’s blueprint?

Retinal circuit assembly

Model for selective synapse formation and circuit assembly in the retina. Progressively narrowing the options available to developing neurons leads to specific connections among interneurons and retinal ganglion cells. Modified from (8).

**Visualizing the wiring of the retina**

Conceptually, deciphering the code requires us to draw links among genes, circuitry, and function. Technically, this requires a list of the neural components to be wired, tools to mark and manipulate them, and methods to map circuitry. For much of the central nervous system, this is a tall order, but the retina is a notable exception.

The retina is a sophisticated neural computer whose parallel circuits preprocess the visual scene to highlight salient features before this information is transmitted to the brain (6). Each of the 30 or more retinal circuits begins with photoreceptors, which work as photon detectors. Each ends with a retinal ganglion cell (RGC), which acts as a feature detector that conveys visual information to the brain. There are ~30 types of RGCs, each uniquely attuned to features such as motion, color, contrast, and so on. In between are 70 or more types of interneurons. RGCs are endowed with their preference for different features via the synapses that they receive from specific subsets of interneurons, called amacrine cells (ACs) and bipolar cells (BCs) (6, 7). How this precision is established and its contribution to retinal circuitry are unclear.

Initially, methods to map retinal circuitry were lacking. An ever-growing compendium of mouse lines that let one mark and manipulate many of the ~100 cell types in the retina, however, made this problem tractable.

We designed and built a custom two-photon microscope–physiology rig to provide spatiotemporally restricted optogenetic excitation to hundreds of genetically defined interneurons in the mouse retina while recording intracellularly from defined RGC subtypes. Armed with this approach, we could map functional connectivity among the retinal types and study how these maps arise.

**Cadherins direct layer-specific innervation**

Using the technique described above, we first discovered a pair of related BCs that provide visual input to directionally selective RGCs (DSRGCs) (8). Anatomically, these BC axons target layers occupied by DSRGC dendrites, which suggests that BCs recognize these locations in the retinal neuropil. Using genetic screens, we discovered that a pair of cadherins were expressed by these BCs and later determined that these cadherins position the BC axons in layers containing DSRGCs dendrites. This organization is critical for DSRGC visual responses—perturbing it by deleting cadherins disconnects BC input and effectively renders DSRGCs blind (8). Conceptually, choosing a layer mitigates the wiring chal-
A “SIDEKICK” ENHANCES PAIRING WITH POTENTIAL SYNAPTIC PARTNERS

Layering alone cannot explain the circuitry of the retina. Simply put, there are ~30 kinds of retinal circuits but only 5 to 10 layers in which to contain their wiring—how do neurons find each other within this tangled thicket? We reasoned that such selectivity should result in enriched connectivity between a subset of colaminar neurons and set out to determine whether such patterns existed by functionally mapping more than a dozen colaminar interneuron-RGC pairs. We discovered that ACs defined by expression of the vesicular glutamate transporter 3 (VG3-ACs) connect well to one type of RGC, W3B RGCs, but poorly to other RGCs in the same layer (9). This selectivity is remarkable because VG3-W3B connections are interdigitated with those of several others.

Such complexity poses a serious wiring challenge for these two neurons and suggests the presence of organizers within layers that operate at an incredibly fine scale. Parallel experiments confirmed this suspicion—VG3 and W3B connect strongly because they both express a homophilic immunoglobulin superfamily (IgSF) adhesion molecule called Sidekick 2 (9). Connections of VG3 and W3B with equally proximate partners that are Sidekick-negative are substantially weaker. This indicates that recognition biases local connectivity in favor of particular pairings.

A HIERARCHY OF WIRING CHOICES BUILDS RETINAL CIRCUITS

This work leads to a speculative model for retinal circuit assembly that revolves around progressively narrowing the options available to developing neurons (see the figure) (8, 9). This model is attractive because it unburdens the genome of having to provide unique recognition for every synapse. For example, the division of labor with cadherins for layers and IgSFs for targets might allow the same IgSFs to be used in several layers at once. Reality might be even simpler, since ~10 other kinds of wiring choices have been documented, each enacted by largely distinct genetic programs (I). Combinations of such genetic programs might direct neurons through a hierarchical sequence of choices that result in particular connectivity patterns.

Do neurons use all of these strategies? Or only a subset? How are they combined? Could the path of a given neuron influence the wiring of another? And perhaps most important, how do activity and experience act on this initial scaffold to refine patterns of connectivity? Understanding these systems-level interactions among circuit patterning rules is critical for developmental neurobiology’s ultimate goal—to know how to build a brain. This goal, if realized, might offer major clues to brain function and new avenues of diagnosis and treatment of dysfunctions caused by mental illness.

REFERENCES

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