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Wiring visual circuits, one eye at a time

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Abstract

A study uses light-gated ion channels to control the activity of neurons in each eye, thereby revealing how the timing of neuronal firing dictates whether visual circuits segregate from one another or stay mixed.

The most famous circuits in the brain are the ones representing the eyes. Every neuroscience student is taught Sperry's classic eye-rotation experiments that showed how neighboring cells in the retina project to neighboring portions of their targets, and their chemical affinity for one another¹. Every card-carrying neuroscientist is also expected to know the work of Hubel and Wiesel that showed that axons representing the two eyes are segregated into non-overlapping domains in the dorsal lateral geniculate nucleus (dLGN) and visual cortex². Given the prominence of these findings, it is not surprising that ever since Sperry, Hubel and Wiesel shared the Nobel Prize 30 years ago, retinotopic and eye-specific maps have been premier models for exploring how the brain wires up during development.

Three sets of findings in this field have been replicated many times and therefore stand as undisputed facts. First, the output neurons of the eyes, called retinal ganglion cells (RGCs), connect diffusely with their targets and then refine their axons into retinotopic and eye-specific connections. Second, both retino-topic and eye-specific connections undergo refinement before visual experience. Third, RGCs spontaneously fire action potentials during the period of retinotopic and eye-specific mapping, and drugs or genetic mutations that alter those firing patterns tend to perturb RGC axon refinement³. Those three sets of facts aside, there is surprisingly little consensus about the activity-dependent mechanisms that instruct retinotopic and, especially, eye-specific mapping. It is still unclear, for instance, whether or not cortical ocular dominance columns develop by pruning and refinement, and whether RGC activity influences that process^{3–6}. And although it is generally accepted that both guidance molecules and spontaneous activity are important for retinotopic and eye-specific targeting³, which aspects of that activity are essential remains hotly contested—to the point where some have argued that activity plays merely a permissive, secondary role to guidance molecules^{3,4,6}.

Fortunately, the crucial experiments needed to resolve these issues and drive the field forward have become clear. To understand whether and how spontaneous retinal activity instructs the layout of visual maps, it is necessary to systematically control patterns of activity in each eye and then carefully examine how those activity patterns influence the development of RGC connections in the brain. In this issue of *Nature Neuroscience*, Zhang

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et al. describe those long-awaited experiments⁷ and, in doing so, provide several insights into how the relative timing of action potentials influences visual circuit wiring.

The authors used a mouse line expressing Channelrhodopsin-2 (ChR2) in RGCs⁸ and flashed blue light into the eyes of the mice to stimulate RGC firing (Fig. 1). The authors confirmed that blue light flashes induced RGC spiking by recording RGC responses *in vitro*. They also beautifully confirmed that the flash-induced action potentials were communicated from RGCs to postsynaptic target neurons, by monitoring calcium transients of neurons in the superior colliculus *in vivo*.

Once they had control over neural activity in the developing retina, Zhang *et al.*⁷ were positioned to carry out some key experiments to test the long-standing notion that ‘neurons that fire together, wire together’. First, they alternated the timing of RGC firing in the left and the right eyes and evaluated how this manipulation modulated the pattern of RGC connections in the superior colliculus. Their first set of experiments used mice at postnatal day 9, an age when eye-specific segregation is mostly completed in mice⁹. However, synchronous stimulation of the eyes for 12 hours a day caused RGC axons to revert to an intermingled state. In contrast, asynchronous stimulation had no effect; the axons remained separated in the superior colliculus. Those results suggest that shortly after eye-specific sorting occurs, RGC axons are still malleable, but only in one direction. This phenomenon seems to have a critical period, because when the same experiments were done starting at postnatal day 14, no rewiring was observed.

The authors knew they were onto something interesting, but they wanted to pinpoint the exact parameters needed to maintain ocular segregation. Therefore, they stimulated the eyes of some mice synchronously (0 millisecond offset), and stimulated the eyes of other mice asynchronously. Among the asynchronous group, different cohorts of mice received stimulation with temporal offsets ranging from 20 to 200 milliseconds. The critical time window turned out to be ~100 milliseconds: if the offset was less than 100 milliseconds, RGC axons desegregated, whereas if the offset was longer than 100 milliseconds, RGC axons did not alter their connections. These experiments are reminiscent of earlier studies that used electrical stimulation of the optic nerves to modulate ocular dominance column plasticity in cats¹⁰. The new study from Zhang *et al.*⁷ is unprecedented, however, because it defines the exact timing requirements for maintaining eye-specific segregation.

What are the activity-based rules for driving the initial segregation of eye-specific connections in the brain? In the mouse, axons from the two eyes sort out largely during the first postnatal week⁹, so Zhang *et al.*⁷ injected the eyes of newborn wild-type mice with a virus encoding ChR2. Four days later, those mice expressed ChR2 in a subset of RGCs, rendering them vulnerable to blue light-induced activation. The authors then asked how synchronous versus asynchronous stimulation affects early RGC axon refinement. The results were clear: pulsing both eyes at the same time disrupted the emergence of eye-specific connections in the brain, whereas pulsing the eyes asynchronously actually enhanced it (Fig. 1). Collectively, these data support the model of activity-dependent segregation put forth by Shatz and co-workers¹¹. Indeed, the results of Zhang *et al.*⁷ stand as clear evidence for ‘fire together, wire together’ in the context of early visual circuit wiring.

Most previous studies that focused on eye-specific segregation did so in the dLGN^{3,9}. Accordingly, the authors also tested whether the activity-based timing rules they saw in the superior colliculus also apply for RGC inputs to the dLGN. Indeed, the same rules applied⁷.

At some level, this is surprising given that RGC inputs to the superior colliculus mature earlier than RGC inputs to the dLGN¹². These results suggest that the temporal constraints

on activity-dependent refinement may apply broadly across different visual targets, and perhaps even to ocular dominance columns in cortex.

Another important question that Zhang *et al.*⁷ addressed is whether the activity-based timing rules they discovered could be used to fix miswired visual connections. To address this, the authors turned to the $\beta 2$ nicotinic acetylcholine receptor knockout (*Chrn2*^{-/-}) mouse^{3,12-14}. The patterns of spontaneous RGC firing in *Chrn2*^{-/-} mice remain contested^{6,13,14}, but everyone agrees that *Chrn2*^{-/-} mice have blurred retinotopy and abnormal eye-specific segregation in the superior colliculus and dLGN^{3,13}. By crossing the ChR2 mice to *Chrn2*^{-/-} mice, Zhang *et al.*⁷ discovered that early asynchronous activation of the two eyes greatly improved ocular segregation in the brains of *ChR2;Chrn2*^{-/-} mice. Thus, by identifying the key parameters for normal activity-dependent refinement, Zhang *et al.*⁷ also tapped into regimes that can rewire and rescue visual circuits.

The results of Zhang *et al.*⁷ lend new understanding in the field of visual circuit development. They also open up several new avenues for research. For example, now that the timing rules for eye-specific segregation are known, one can quantitatively address which features of naturally occurring spontaneous activity, such as those characteristic of retinal waves^{3,13,14}, are important for visual mapping. Using the ChR2-based approach in conjunction with genetic tools to alter synaptic transmission¹⁵, one could also test how the activity of target neurons contributes to the plasticity rules that drive axon segregation. In the meantime, the results of Zhang *et al.*⁷ indicate that the field of visual circuit development first defined by Sperry, Hubel and Wiesel more than four decades ago is headed into a new era in which the essential features of activity-dependent wiring will be understood.

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Figure 1. The timing of optogenetic stimulation instructs the architecture of visual circuits (a) Schematic of the visual pathway from the eyes to the superior colliculus and the experimental procedure used to independently control neural activity in the two eyes. Each eye projects heavily to the contralateral superior colliculus and provides some input to the ipsilateral superior colliculus. Normally, axons from the two eyes minimally overlap (yellow) after eye opening. Lightbulbs 1 and 2 represent the stimulation apparatus used to evoke activity of retinal output neurons (RGCs) in the left eye (red; 1) or in the right eye (green; 2) of mice that harbor Channelrhodopsin-2 (ChR2) in RGCs. (b) Synchronous stimulation of the two eyes before or just after the normal period of eye-specific segregation causes increased overlap of contralateral and ipsilateral RGC projections to the superior colliculus. (c) Asynchronous stimulation of the two eyes causes the degree of overlap to decrease. Similar results are observed in other visual targets, and the timing of stimulation both during the experiment and across development is important for plastic changes in RGC projections.