Cell Reports Methods

CellPress
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Preview

An optical approach for mapping functional connectivity at single-cell resolution in brain circuits

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https://doi.org/10.1016/j.crmeth.2022.100272

In the current issue of *Cell Reports Methods*, Spampinato et al. demonstrate a multiplexed system combining holographic photo-stimulation and functional imaging that may offer a generalizable approach for revealing how signals interact in complex neural circuits.

A major goal in neuroscience is to determine the connectivity of neurons within circuits. This goal is motivated by the notion that knowing the connectivity will enable understanding of how neural circuits process signals and produce behaviors. Attaining this goal is made difficult by the fact that most neural circuits are composed of dozens, if not hundreds, of distinct cell types, and each type typically exhibits specific and intricate connectivity with other types. Indeed, connectomics—a set of techniques that elucidates the anatomical connectivity between thousands of neurons at the resolution of individual synapses—has produced novel insights into the cell types and connections within neural circuits (Bae et al., 2018). However, like any approach, it has limitations. Most notably, connectomics doesn't directly reveal functional information about the strength with which a signal in one neuron influences the activity of a post-synaptic neuron or, perhaps more importantly, how that influence depends on the concurrent activity of other neurons in the circuit.

A new study by Spampinato et al. (2022) introduces a powerful synthesis of approaches that may begin to elucidate the functional connectivity in many brain circuits. They devise a framework combining several established optical methods to manipulate and measure the activity of individual neurons: optogenetics, holography, and calcium-dependent fluorescence imaging. They use the retina as a testbed neural circuit for developing and validating these methods because the retina is one of the best understood brain circuits, with robustly identifiable cell types that are genetically accessible (Masland, 2012). Overall, their approach has the potential to efficiently map how diverse spatial and temporal patterns of activity in a circuit influence subsequent patterns of activity, thereby mapping connectivity functionally.

In more detail, Spampinato et al. (2022) begin by virally expressing an optogenetic construct, CoChR (Klapoetke et al., 2014), in a specific type of retinal bipolar cell called rod bipolar cells (Figure 1). These cells connect to rod, but not cone, photoreceptors; they are specialized to transmit rod signals in the darkest of conditions (e.g., a starlit night) to other neurons. These signals eventually converge onto retinal ganglion cells (RGCs), the axons of which form the optic nerve, and provide the sole source of visual information to the brain. Once the rod bipolar cells express CoChR, they become light sensitive and can be stimulated with arbitrary spatiotemporal patterns of light. To stimulate individual or combinations of rod bipolar cells, the authors used a method, called multiplexed temporally focused light shaping, developed previously by this group in the lab of Dr. Valentina Emiliani (Hernandez et al., 2016). The method allows temporally focused two-photon activation of multiple cells expressing optogenetic constructs across a volume of tissue, with a spatial resolution matching the size of individual neurons. Because bipolar cells generally signal using graded changes in their membrane potential, instead of action potentials, the authors also show that this holographic photostimulation technique can produce graded changes in the activation of bipolar cells.

Using holographic techniques to manipulate neural circuit activity has been achieved by several labs previously. However, the consequences of these manipulations have been assayed primarily by changes in animal behavior (Carrillo-Reid et al., 2019). While certainly useful and interesting, behavior-based approaches do not reveal the functional connectivity among neurons. To meet this challenge, Spampinato et al. (2022) express a calcium-dependent fluorescence indicator (GCaMP6s) (Chen et al., 2013) in RGCs while also expressing CoChR in rod bipolar cells. Changes in intracellular calcium reflect RGC excitability driven by pre-synaptic rod bipolar cells. Thus, the authors are able to measure changes in RGC activity using two-photon calcium imaging (Denk et al., 1990) in response to optogenetic activation of individual and small collections of rod bipolar cells (Figure 1). From these measurements, the authors begin to deduce the properties of functional connectivity between rod bipolar cells and different RGC types, and in particular the degree of spatial convergence in this system. This is reminiscent of previous studies that stimulated individual cone photoreceptors with different patterns of light while using multi-electrode arrays to measure the resulting activity among RGCs, thereby mapping the functional connectivity and convergence from cones to diverse RGC types (Field et al., 2010).

The approaches utilized in this study have some limitations. While calcium imaging of neural activity is common and useful, it is a somewhat indirect measure of spiking activity, and in general, it lacks

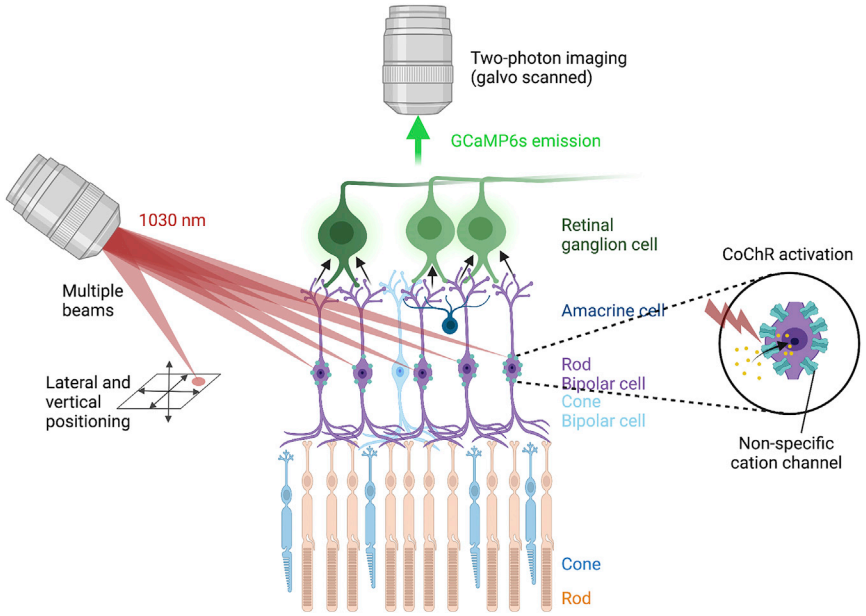

Figure 1. Holographic photostimulation and GaCAMP imaging in *ex vivo* retina

Spampinato et al. (2022) designed a system combining two-photon guided holographic photostimulation and calcium fluorescence imaging to study functional connectivity in *ex vivo* retina. Rod bipolar cells and retinal ganglion cells were virally targeted using specific promoters to express optogenetic constructs of CoChR and GCaMP6s, respectively. Two-photon holographic stimulation (1,030 nm) was used to activate CoChR in individual rod bipolar cells causing them to depolarize (left inset), and a two-photon galvo-based scanning system was used to image the calcium (GCaMP6s) responses of downstream retinal ganglion cells (right inset). The authors report that this approach provides the flexibility to target cells at different locations and depth for photostimulation and measure post-synaptic neural responses, thus enabling mapping the functional connectivity in neural circuits.

the temporal resolution needed for tracking the precise spike times generated in neural circuits like the retina (Berry et al., 1997). Additionally, while the spatial precision of holographic two-photon stimulation is impressive, maintaining the precision and uniformity of stimulation across a set of densely packed neurons can be challenging. Advances in photonics and genetically engineered fluorescent calcium and voltage indicators (Dana et al., 2019), however, are likely to relax these limitations in the near future.

The long-term potential of this approach and these technologies is quite exciting. The use of two-photon optogenetic excitation concurrent with two-photon imaging of neural activity allows for interrogating neural connectivity in neural circuits in both *ex vivo* and *in vivo* experiments. The ability to manipulate the activity of one or a small number of neurons by spatiotemporally focused light allows for (but does not guarantee) production of patterns of activity that are more likely to replicate patterns occurring naturally, thereby yielding more nuanced and potentially more accurate conclusions about how different kinds of neurons influence and communicate with one another to shape perception and behavior. A fundamental understanding of the information processing in the brain requires more than just elucidating the anatomical connections or passively observing patterns of neural activity while animals behave. Ultimately, it requires causal manipulations and measurements that are tuned to the scale and specificity of information processing. We know that one critical scale is that of individual neurons and their interactions with other neurons, a.k.a. functional connectivity. Of course, other scales also matter such as individual synapses, signal integration in dendritic arbors, as well as interactions between entire brain regions. But to the extent that spikes are the currency of rapid information exchange in the brain, and circuits define the nature of this processing, the approach illustrated in this article holds tremendous promise for understanding brain function.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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