NEUROSCIENCE

Mapmaking with barcoded neurons

In the mouse brain, researchers use sequencing to map long-range neuronal projections…a lot of projections.

When Tony Zador took a position at Cold Spring Harbor Laboratory 17 years ago, he wanted to explore the neural circuits that underlie cognitive tasks, and he set about establishing rodent models for decision making. By 2009, there were tools for testing whether a circuit gave rise to a behavior; projections could be traced and specific neurons could be stimulated or inhibited. But each test could take longer than a year and end in a negative result. "We really needed a way to screen out the vast majority of potential circuit hypotheses for what might underlie a particular behavior on the basis of the anatomy," says Zador.

Around the same time, Zador learned about Brainbow, a method for uniquely labeling individual neurons with colors from a large palette of fluorescent protein combinations. He soon realized that replacing color labels with sequence tags would bring the advantages of a sequencing readout: ease and throughput.

A successful tagging strategy was developed by graduate student Ian Peikon, who engineered synaptic proteins to tow barcodes to pre- and postsynaptic terminals. The proteins included an RNA-binding domain that recognized hairpin sequences next to each barcode. Although more work was needed before these tools could be used to trace neural connections, graduate student Justus Kebschull noticed that they could be used to map long-range axonal projections, and thus multiplexed analysis of projections by sequencing, or MAPseq, was born.

MAPseq requires that a viral library be injected in specific spots in the brain. Each viral genome encodes a GFP transcript with a unique barcode, along with the synaptic protein. Sequencing barcode RNA from brain sections provides a quantitative readout for axonal density in each section, making it possible to map thousands of neurons. By contrast, traditional tracing methods can be highly precise but also painstaking and very slow, even when automated.

As their first case study, the researchers chose the locus coeruleus, a brainstem region responsible for generating the noradrenaline signal that snaps the brain to attention. It was believed to broadly innervate the cerebral cortex, but there were conflicting ideas about whether the neuronal mapping is haphazard or structured. "We were interested in the possibility that signals could be more precise, more selective than if every neuron projected everywhere," says Zador. Indeed, MAPseq detected preferred cortical targets for individual neurons, a phenomenon that was not observed before.

MAPseq uses Sindbis virus, which can infect single cells in any brain region in organisms from arthropods to mammals, including primates. In principle, the method could be adapted to target specific neuronal subsets through restriction of expression with a cell-type-specific recombinase, but this would require a different type of virus.

The scientists have ramped up MAPseq to produce an all-to-all projection pattern of an entire mouse brain in about a week for a few thousand dollars, Zador says. To improve spatial resolution at target sites, they now use laser-capture microdissection to retrieve RNA from portions of sections that have been registered against an anatomical atlas. Fluorescent *in situ* sequencing has also been successful in providing precise spatial information, though the method is currently too slow to work at the whole-brain scale. Yet Zador is not hung up on spatial resolution. His background in theoretical neuroscience informs his view that what matters is the connectivity diagram, rather than the precise spatial position of every neuron.

His team is pushing forward with the original goal of identifying circuits. They have fused pre- and postsynaptic proteins to extracellular domains that can form covalent bonds in synapses that they cohabit, and then carried out sequencing of paired barcodes in individual droplets or by physical linking after immunoprecipitation. Even bigger plans are afoot for attributing function to circuits. "My dream," Zador says, "is to measure neural activity using two-photon microscopy in a behaving animal, then look at that circuit using MAPseq and *in situ* sequencing and figure out how all those neurons are connected to each other and where they go."

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RESEARCH PAPERS

Kebschull, J.M. *et al.* High-throughput mapping of single-neuron projections by sequencing of barcoded RNA. *Neuron* **91**, 975–987 (2016).