NEUROSCIENCE

Connectomics at the single-cell level

Trans-synaptic tracing that starts from a single neuron provides a detailed view of that neuron's inputs.

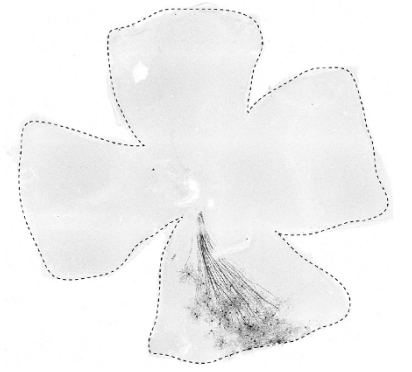
Trans-synaptic retrograde tracing in bulk has been highly successful in revealing the connectome in the mouse brain. However, bulk labeling of neurons does not provide a detailed view of the connections individual neurons make. For example, with this technology, it is difficult to distinguish whether individual neurons in a given brain region receive inputs from multiple cell types or even multiple brain regions, or whether different neurons in the region of interest are targeted by different cell types or input from more than one brain region.

Botond Roska and his team at the Friedrich Miescher Institute for Biomedical Research in Basel, Switzerland, are working to solve this dilemma in the visual system, where ganglion cells in the retina convey sensory information to the lateral geniculate nucleus (LGN) and then the cortex. The circuitry in the retina is fairly well understood, but this is not the case for the LGN, which was thought to be a simple relay structure. In light of sparse, yet contradictory, electrophysiological evidence, Roska and his team set out to address this conundrum. "It is very clear that you can only ask [this question] at the single-cell level," says Roska. "Once you don't know a brain region, the only thing that makes sense is either to record all of them or one by one."

The researchers developed a method to visualize the retinal ganglion cells that projected to single LGN neurons, following the one-by-one strategy Roska proposed. It took more than two years for Roska's co-worker Santiago Rompani to work out the technical details. The researchers inject low concentrations of AAV (adeno-associated virus) encoding Cre–GFP into area V1 in the visual cortex. The virus is taken up by projection neurons and transported back to their soma, producing a fluorescent signal in the nuclei of sparsely distributed neurons in the LGN. The researchers then electroporate various plasmids into a single GFP-labeled cell. This is the most difficult part of the approach, as the LGN is "in the middle of the brain," Roska says. The electroporated plasmids prepare the targeted cell for infection with an mCherry-encoding rabies virus tracer that then trans-synaptically infects the retinal ganglion cells that connect to the LGN neuron. Spread of the rabies virus is restricted by the monosynaptic nature of the engineered virus, which limits infection and mCherry expression to the ganglion cells directly connected to the targeted LGN cell.

To determine the different classes of ganglion cells that connect to individual LGN neurons, the researchers imaged the labeled ganglion cells in retinal explants. However, the cells had to be accurately traced for determination of the ganglion cell class. To do this, Roska and his team tried automated tracing approaches. "But it's not bulletproof, and in many of these tracings the problem is that you get one mistake and the whole thing is totally wrong," says Roska. He therefore used the services of a company that does tracing for electron microscopy, and "we just taught them how to do tracing…for confocal," says Roska. His co-worker Fiona Müllner spearheaded the subsequent statistical analysis of the data.

The team found three modes that described how retinal ganglion cells connect with LGN neurons. First, they observed a simple relay mode, as previously postulated, although "with a little bit of a twist," according to Roska.



A cluster of ganglion cells in the retina that connect to a single LGN neuron. Reprinted from Rompani *et al.* (2017) with permission from Elsevier.

In this mode, several ganglion cells of the same class and an outlier ganglion cell type (although with shared features) connected to a single LGN neuron. Second, they found LGN cells that received inputs from clusters of different ganglion cell types. And finally, they observed LGN neurons that received binocular input. "It just blew our mind that…we started to see binocular cells," says Roska.

At this stage, the approach is fairly tedious, but Roska and his team are working to modify the technology in order to achieve higher throughput. They are also planning to use their technology in other organisms. "The next one will be the marmoset," says Roska. He is interested in understanding the binocular LGN cells better, and thinks that correlating their presence with features of the visual system in various animals could be informative.

Nina Vogt

RESEARCH PAPERS
Rompani, S.B. *et al.* Different modes of visual integration in the lateral geniculate nucleus revealed by single-cell-initiated transsynaptic tracing. *Neuron* **93**, 767–776 (2017).