NEUROSCIENCE

Brain function marries anatomy

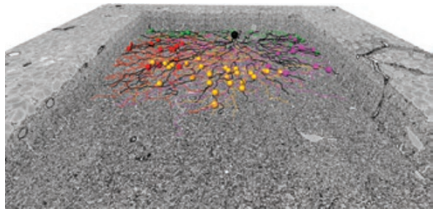
Researchers have taken first steps toward functional connectomics. By combining large-scale serial electron microscopy and functional imaging data, the structure of neural networks can be related to their function.

To undertake the formidable task of understanding the brain, its billions of interconnected neurons have to be carefully traced into a comprehensive diagram. But resolving the fine anatomy of these connections is only part of the story. Understanding the network will also require information about each neuron's function in the circuit. The study of a neuron's function in relation to its connections has been called 'functional connectomics' and requires the right blend of *in vivo* physiology and network anatomy methods—and a lot of hard work.

Two-photon calcium imaging can be used to characterize the functional properties (firing patterns) of neurons in the living rodent brain. Thereafter, one can use high-resolution imaging methods such as serial electron microscopy to reconstruct and trace the local network surrounding these neurons. Two groups have now independently published the fruits of such efforts and have taken the first steps in relating the observed wiring and functional patterns to rules of connectivity.

Clay Reid and colleagues at Harvard University were interested in studying neural circuits responsible for coding visual processing. They first analyzed the activity of a cohort of excitatory cells in the mouse primary visual cortex in response to visual stimuli of different orientations using *in vivo* functional imaging. Immediately after, they extracted the exact volume of tissue and analyzed its fine anatomy using serial section transmission electron microscopy (Bock *et al.*, 2011).

The researchers cut 40–50-nanometer thin sections from a block of brain tissue using a diamond knife and collected each of the sections by hand. They imaged and reconstructed over 1,000 sections, a feat that was made possible by building a microscopy system that enabled them to take millions of pictures over several months.



A large, three-dimensional volume from mouse retina imaged using serial block-face electron microscopy. Image courtesy of K. Briggman.

Out of the thousands of cell bodies contained in this volume of tissue, they focused on ten pyramidal neurons that they had previously functionally characterized. After manually tracing their hundreds of connections to other neurons, they examined the functional logic of the network. "We found one main result," explains Reid. "For the most part, inhibitory cells receive inputs from nearby excitatory cells without regard for the detailed physiological properties of those excitatory cells." This finding is consistent with previous hypotheses that inhibition does not have an active role in computing qualities such as orientation in this circuit.

On the other side of the Atlantic, the group of Winfried Denk at the Max Planck Institute, Heidelberg was using similar techniques to unveil the wiring specificities of neuronal circuits present in the mouse retina. The group was interested in ganglion cells that compute the direction of motion of an object in the visual field (Briggman *et al.*, 2011). These ganglion cells will fire when an object moves in their 'preferred' direction and will not when it moves in the opposite 'null' direction.

Kevin Briggman and Moritz Helmstaedter, postdocs in Denk's laboratory, first used two-photon calcium imaging to characterize the activity patterns of a group of ganglion cells in response to moving-bar stimuli projected onto retina isolated from mice. Then they imaged the tissue using serial block face electron microscopy—a method Denk and colleagues developed in 2004 in which the imaging and cutting is done in an automated fashion. "The technique was born out of the necessity to automate the sectioning, and by doing this, it allowed us to reduce the section thickness to 25 nanometers, which was critical to follow even the thinnest axons and dendrites," explains Briggman.

The group then used the reconstructed images to manually trace the skeletons of several ganglion cells that had been functionally characterized and identified a large fraction of the synaptic contacts these cells have with inhibitory amacrine cells. They found that the ganglion cells received asymmetric inhibitory inputs that were oriented along the null direction. It is this wiring asymmetry that confers direction selectivity upon the ganglion cells.

Although this was an incredible technical achievement, the two teams imaged only a relatively small volume of the brain (less than 1% of a cubic millimeter). To tackle larger volumes, the speed of both image acquisition and analysis will need to be improved. The bottleneck is mainly the analysis. "It's somewhat frustrating that we have automated data acquisition but still rely on manual annotation," says Briggman. "An important next step that we and our collaborators are working on is how to involve computers in the analysis." In the future, researchers hope to use computer algorithms that can aid human tracing efforts or do all the segmentation automatically.

Although these goals are still a ways off, Reid is optimistic: "I certainly hope that by the time we have our next dataset there will be something we can use that can be faster than 100% manual segmentation. I am sure it will happen."

Erika Pastrana

RESEARCH PAPERS

Bock, D.D. *et al.* Network anatomy and *in vivo* physiology of visual cortical neurons. *Nature* **471**, 177–182 (2011).

Briggman, K.L. *et al.* Wiring specificity in the direction-selectivity circuit of the retina. *Nature* **471**, 183–188 (2011).