NEUROSCIENCE

Unraveling synapse diversity

Array tomography opens the door to the large-scale exploration of molecular diversity of individual brain synapses.

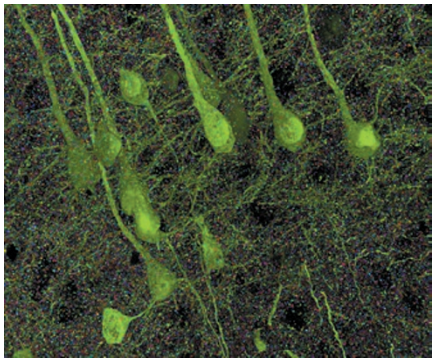
Neurons connect to each other through small cellular structures called synapses. Synapses are essential to neuronal function because they are responsible for passing the electrical or chemical signals from cell to cell. Thus, they contain extensive arrays of molecular machinery that serve to link the two cellular membranes together and carry out the signaling process.

A single neuron can make tens of thousands of synaptic contacts with other neurons, and in one cubic millimeter of brain tissue, more than a billion synapses can be found. Traditionally, synapses have been classified based solely on neurotransmitter identity, but it has now become increasingly clear that these groupings fall short of disentangling the vast molecular heterogeneity of these important cellular structures. The systematic analysis of this heterogeneity has remained elusive owing to the scarcity of methods to discriminate, detect and analyze a high number of individual synapses *in situ*.

Several years ago Stephen Smith and co-inventor Kristina Micheva of Stanford University developed a technology called array tomography, which involves serially cutting nanometer-thin sections of brain tissue and then analyzing them using light or electron microscopy. This type of approach can help resolve the fine details of many synapses jammed together across large fields of view and entire neuronal circuits.

Among the advances of array tomography is that several rounds of antibody staining can be performed on the same section, allowing immunofluorescence detection of up to 17 different markers, as has been shown to date. Array tomography also allows quantitative studies to be performed with high reliability because both staining and imaging are independent of depth within a tissue sample. Finally, this method can be scaled up using automated imaging instrumentation.

In a recent study, Smith, Micheva and their colleagues applied array tomography to directly explore the molecular diversity of brain connections. They focused on the discrimination and analysis of glutamatergic and GABAergic synapses in the mouse cortex, trying to identify every single synapse contained in a volume of tissue as unambiguously as possible. Among their achievements was the discovery of a robust marker that can identify the vast majority of cortical synapses, synapsin I, and the development of a conjoint of markers that can help identify glutamatergic and GABAergic synapses with more confidence. These findings open the door to the refinement of molecular subtypes in these broad synapse categories.



Array tomographic image of neurons and synapses in the mouse cortex. Dendrites, branches and axons are shown in green, and individual synapses are the multicolored dots. Image courtesy of Stephen Smith.

Smith is particularly excited about using the technology to analyze synaptic molecules that relate to synaptic function and plasticity as well as developmental adhesion and guidance molecules often found at the synapse. "Pretty soon we'll be able to look at single synapses and get a strong sense about their functional status (by looking at molecules such as AMPA receptors) and at the same time make good assumptions about the reconstruction of the circuit by guessing who the post- and presynaptic neurons are based on adhesion and guidance molecules at the synapse," he explains.

As a physiologist turned to neuroanatomist in the 21st century, Smith thinks that bringing together technologies such as this one with the use of genetically targetable fluorescent probes and optogenetic tools will soon enable a new golden age of neurophysiology.

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RESEARCH PAPERS

Micheva, K.D. *et al.* Single-synapse analysis of a diverse synapse population: proteomic imaging methods and markers. *Neuron* **68**, 639–653 (2010).