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Methods to Visualize Structural and Functional Changes at Synapses

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Editorial

At excitatory synapses in the mammalian brain, presynaptically-released glutamate activates receptors embedded in the postsynaptic membrane[1,2]. The strength of these synaptic connections undergoes experience-dependent plasticity, a property that allows for diverse forms of learning and memory[3,4,5,6,7,8,9]. Pathological changes to the numbers of synapses and their signaling properties leads to the altered circuit activity and information processing displayed in numerous neurological and psychiatric disorders[10,11,12]. As such, there is a critical need to understand the underlying mechanisms that govern neural connectivity and the strength of synaptic connections.

Excitatory synapses form on dendritic spines, tiny postsynaptic protrusions containing scaffold proteins, receptors, signaling factors, cytoskeletal proteins, and trafficking organelles[1,2,13,14,15,16]. These micron-sized signaling compartments allow for control of synaptic strength on a synapse-by-synapse basis[13,16]. Dendritic spine morphology varies along the length of the neuronal dendrites, with spine shapes typically described as thin, filopodial, stubby, mushroom, or branched[13]. Importantly, spine shape is correlated with synaptic function and stability. For example, larger spines have increased numbers of receptors and greater synaptic strength[16,17,18]. Unlike thin spines, larger spines can persist for months *in vivo*, a property that may allow for stable synaptic connections and long-term memory storage[19,20].

Spine morphology and density along a dendrite are commonly tracked to probe the degree of neural connectivity and the properties of excitatory synapses within a particular brain region, in both normal and disease states[13]. A variety of methods have been developed to visualize spine morphology in different tissue preparations and model systems using standard light and fluorescence microscopy, as well as super-resolution and electron microscopy (EM)[21]. These powerful imaging techniques have provided tremendous insight into our current understanding of brain organization, function, and disease. This method collection includes important imaging techniques to monitor the morphological complexity of dendrites and spines, as well as optogenetic tools to activate and measure neural activity in dendrites and single spines.

Pekarek et al.[22] present a relatively rapid, high-throughput protocol to image and quantify the structure of neuronal dendrites deep within intact nervous tissue. The authors perform Clear Lipid-exchanged Acrylamide-hybridized Rigid Imaging/immunostaining/*in situ* hybridization-compatible Tissue-hYdrogel (CLARITY), a tissue clearing technique that removes lipids from the brain tissue and helps minimize light scattering during subsequent imaging[23]. After clearing, Z-stacks of neurons expressing fluorescent proteins were collected by confocal or two-photon microscopy. Image analysis and automated quantification allowed for precise measurements of spine density and shape, as well as dendritic tree complexity. This protocol allows for simultaneous imaging of structures ranging from single spines to dendritic trees and removes the time consuming and potentially damaging step of thin-section cutting. This technique will be highly useful to researchers looking to quantify changes in spine morphology and dendritic complexity in intact brain tissues following the introduction or removal of a gene(s) of interest.

Developed over 100 years ago[24], Golgi staining remains a powerful tool to visualize nervous system organization and neuronal morphology. In this method, sparse populations of neurons are stained by microcrystalization of silver chromate, allowing for visualization of axons, dendritic trees, and spines[25]. Frankfurt and Bowman[26] detail a rapid Golgi impregnation protocol to visualize neurons and dendritic spines in the rat hippocampus and medial prefrontal cortex. The authors demonstrate how to prepare, stain, and image tissue for subsequent light microscopy, as well as quantify spine density along dendritic trees. The protocol described here yields consistent, reliable impregnation of neurons, and reduces exposure to toxic chemicals.

While light and fluorescence microscopy are well suited for imaging dendrites and spines, EM allows for nanometer resolution visualization of pre- and postsynaptic

structures[21]. EM approaches are typically used to measure the ultrastructural properties of synapses, including the dimensions of the postsynaptic density, as well as the numbers and size of synaptic vesicles[21]. In this collection, Śliwińska et al.[27] provide a protocol to acquire three-dimensional (3D) EM images by Serial Block-face scanning Electron Microscopy (SBEM) in hippocampal mouse tissue[28,29]. Specifically, they describe how to prepare samples, embed tissue in resin, mount and trim samples, perform SBEM, and analyze images to generate 3D reconstructions. This protocol generates high-contrast images with well-defined membranes and can be adapted for a variety of tissue types.

During development of the nervous system, neurons undergo dramatic morphological changes as synaptic connections are formed and refined by neural activity[30,31]. To track neuronal maturation in the developing cerebellum, Chan et al.[32] provide a protocol for *in vivo* postnatal electroporation of developing granule neurons. Here, researchers injected and electroporated DNA into the hindbrain of postnatal mouse pups to transfect a sparse population of neurons with fluorescent proteins. High-resolution confocal microscopy and 3D imaging allowed researchers to characterize the morphology of dendrites and dendritic claws over several stages of postnatal development. Importantly, this approach could allow for the insertion or removal of genes that may impact granule cell maturation and function and will be of high interest to researchers studying cerebellar development and granule neuron maturation.

Finally, the protocols by Cuentas-Condori and Miller[33] employ super-resolution imaging[34], optogenetics[35], and live-cell imaging of calcium dynamics[36] to study the activity of GABAergic neurons in the nematode, *C. elegans*. Using a confocal microscope equipped for super-resolution imaging[37], the authors express fluorescent proteins in dendrites to measure the frequency of different spine shapes. Furthermore, the authors assess neural function by expression of an optogenetic protein presynaptically and a calcium indicator postsynaptically. Following light activation of terminals, calcium transients were observed within individual dendritic spines. These protocols, along with recent findings[38], highlight the exciting use of *C. elegans* as a powerful model system to track spine morphology and synaptic activity.

Taken together, the protocols described in this methods collection will add to the already rich toolbox available to visualize neural structure and function[21]. The implementation of these methods in future studies will likely provide additional insights into the processes that drive learning-related synaptic plasticity and the cellular mechanisms that underlie disease states.

Disclosures

The authors have nothing to disclose.

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