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Methods to Visualize Structural and Functional Changes at Synapses

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Methods to Visualize Structural and Functional Changes at Synapses

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ARTICLES DISCUSSED:

1. Pekarek, B. T., Hunt, P. J., Belfort, B. D. W., Liu, G., Arenkiel, B. R. Imaging and quantification of intact neuronal dendrites via CLARITY tissue clearing. *Journal of Visualized Experiments: JoVE*. (170), e62532 (2021).
2. Frankfurt, M., Bowman, R. Rapid Golgi stain for dendritic spine visualization in hippocampus and prefrontal cortex. *Journal of Visualized Experiments: JoVE*. (178), e63404 (2021).
3. Śliwińska, M. A., Cały, A., Szymański, J., Radwańska, K. Serial Block-Face Scanning Electron Microscopy (SBEM) for the study of dendritic spines. *Journal of Visualized Experiments: JoVE*. (176), e62712 (2021).
4. Chan, U., Gautam, D., West, A. E. Utilizing in vivo postnatal electroporation to study cerebellar granule neuron morphology and synapse development. *Journal of Visualized Experiments: JoVE*. (172), e62568 (2021).
5. Cuentas-Condori, A., Miller III, D. M. Imaging dendritic spines in *Caenorhabditis elegans*. *Journal of Visualized Experiments: JoVE*. (175), e62676 (2021).

EDITORIAL TEXT:

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^{3–9}. 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^{10–12}. 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 tiny postsynaptic protrusions on dendritic spines that contain scaffold proteins, receptors, signaling factors, cytoskeletal proteins, and trafficking organelles^{1,2,13–16}. 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 branched¹³. Importantly, spine shape is correlated with synaptic function and stability. For example, larger spines have increased number of receptors and greater synaptic strength^{16–18}.

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 states¹³. 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/*i*n situ hybridization-compatible Tissue-hydrogel (CLARITY), a tissue clearing technique that removes lipids from the brain tissue and helps minimize light scattering during subsequent imaging²³. 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 ago²⁴, Golgi staining remains a powerful tool to visualize nervous system organization and neuronal morphology. In this method, sparse populations of neurons are stained by microcrystallization of silver chromate, allowing for visualization of axons, dendritic trees, and spines²⁵. Frankfurt and Bowman²⁶ 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 structures²¹. 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 vesicles²¹. 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 Miller³³ employ super-resolution imaging³⁴, optogenetics³⁵, and live-cell imaging of calcium dynamics³⁶ to study the activity of GABAergic neurons in the nematode, *C. elegans*. Using a confocal microscope equipped for super-resolution imaging³⁷, 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 findings³⁸, 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 function²¹. 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.

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The authors have nothing to disclose.

REFERENCES:

1. Okabe, S. Molecular anatomy of the postsynaptic density. *Molecular and Cellular Neurosciences*. **34** (4), 503–518 (2007).
2. Sheng, M., Hoogenraad, C. C. The postsynaptic architecture of excitatory synapses: a more quantitative view. *Annual Review of Biochemistry*. **76**, 823–847 (2007).
3. Citri, A., Malenka, R. C. Synaptic plasticity: multiple forms, functions, and mechanisms. *Neuropsychopharmacology*. **33** (1), 18–41 (2008).

- 133 4. Derkach, V. A. et al. Regulatory mechanisms of AMPA receptors in synaptic plasticity.
134 *Nature Reviews. Neuroscience*. **8** (2), 101–113 (2007).
- 135 5. Herring, B. E., Nicoll, R. A. Long-term potentiation: From CaMKII to AMPA receptor
136 trafficking. *Annu Rev Physiol*. **78**, 351–365 (2016).
- 137 6. Newpher, T. M., Ehlers, M. D. Glutamate receptor dynamics in dendritic microdomains.
138 *Neuron*. **58** (4), 472–497 (2008).
- 139 7. Shepherd, J. D., Huganir, R. L. The cell biology of synaptic plasticity: AMPA receptor
140 trafficking. *Annual Review of Cell and Developmental Biology*. **23**, 613–643 (2007).
- 141 8. Malenka, R. C., Bear, M. F. LTP and LTD: an embarrassment of riches. *Neuron*. **44** (1), 5–
142 21 (2004).
- 143 9. Nicoll, R. A. A Brief History of Long-Term Potentiation. *Neuron*. **93** (2), 281–290 (2017).
- 144 10. Blanpied, T. A., Ehlers, M. D. Microanatomy of dendritic spines: emerging principles of
145 synaptic pathology in psychiatric and neurological disease. *Biological Psychiatry*. **55** (12), 1121–
146 1127 (2004).
- 147 11. Penzes, P. et al. Dendritic spine pathology in neuropsychiatric disorders. *Nature*
148 *Neuroscience*. **14** (3), 285–293 (2011).
- 149 12. Newpher, T. M. et al. Regulation of spine structural plasticity by Arc/Arg3.1. *Seminars in*
150 *Cell & Developmental Biology*. **77**, 25–32 (2018).
- 151 13. Bourne, J. N., Harris K. M. Balancing structure and function at hippocampal dendritic
152 spines. *Annual Review of Neuroscience*. **31**, 47–67 (2008).
- 153 14. Hotulainen, P., Hoogenraad, C. C. Actin in dendritic spines: connecting dynamics to
154 function. *The Journal of Cell Biology*. **189** (4), 619–629 (2010).
- 155 15. Kennedy, M. J., Ehlers, M. D. Organelles and trafficking machinery for postsynaptic
156 plasticity. *Annual Review of Neuroscience*. **29**, 325–362 (2006).
- 157 16. Nishiyama, J., Yasuda, R. Biochemical Computation for Spine Structural Plasticity.
158 *Neuron*. **87** (1), 63–75 (2015).
- 159 17. Holtmaat, A. J. et al. Transient and persistent dendritic spines in the neocortex in vivo.
160 *Neuron*. **45** (2), 279–291 (2005).
- 161 18. Matsuzaki, M. et al. Structural basis of long-term potentiation in single dendritic spines.
162 *Nature*. **429** (6993), 761–766 (2004).
- 163 19. Kasai, H. et al. Structure-stability-function relationships of dendritic spines. *Trends in*
164 *Neurosciences*. **26** (7), 360–368 (2003).
- 165 20. Hayashi-Takagi, A. et al. Labelling and optical erasure of synaptic memory traces in the
166 motor cortex. *Nature*. **525** (7569), 333–338 (2015).
- 167 21. Arenkiel, B. R., Ehlers, M. D. Molecular genetics and imaging technologies for circuit-
168 based neuroanatomy. *Nature*. **461** (7266), 900–907 (2009).
- 169 22. Pekarek, B. T. et al. Imaging and quantification of intact neuronal dendrites via CLARITY
170 tissue clearing. *Journal of Visualized Experiments: JoVE*. (170) (2021).
- 171 23. Chung, K. et al. Structural and molecular interrogation of intact biological systems.
172 *Nature*. **497** (7449), 332–337 (2013).
- 173 24. Pannese, E. The Golgi stain: invention, diffusion and impact on neurosciences. *Journal of*
174 *the History of the Neurosciences*. **8** (2), 132–140 (1999).
- 175 25. Gabbott, P. L., Somogyi, J. The 'single' section Golgi-impregnation procedure:
176 methodological description. *Journal of Neuroscience Methods*. **11** (4), 221–230 (1984).

26. Frankfurt, M., Bowman, R. Rapid Golgi stain for dendritic spine visualization in hippocampus and prefrontal cortex. *Journal of Visualized Experiments: JoVE*. **178** (2021).
27. Sliwinska, M. A. et al. Serial Block-Face Scanning Electron Microscopy (SBEM) for the Study of Dendritic Spines. *Journal of Visualized Experiments: JoVE*. (176) (2021).
28. Wanner, A. A., Kirschmann, M. A., Genoud, C. Challenges of microtome-based serial block-face scanning electron microscopy in neuroscience. *Journal of Microscopy*. **259** (2), 137–142 (2015).
29. Smith, D., Starborg, T. Serial block face scanning electron microscopy in cell biology: Applications and technology. *Tissue & Cell*. **57**, 111–122 (2019).
30. Reichardt, L. F. Neurotrophin-regulated signalling pathways. *Philosophical Transactions of the Royal Society of London. Series B. Biological Sciences*. **361** (1473), 1545–1564 (2006).
31. Kolodkin, A. L., Tessier-Lavigne, M. Mechanisms and molecules of neuronal wiring: a primer. *Cold Spring Harbor Perspectives in Biology*. **3** (6) (2011).
32. Chan, U., Gautam, D., West, A. E. Utilizing in vivo postnatal electroporation to study cerebellar granule neuron morphology and synapse development. *Journal of Visualized Experiments: JoVE*. (172) (2021).
33. Cuentas-Condori, A., Miller 3rd, D. M. Imaging dendritic spines in *Caenorhabditis elegans*. *Journal of Visualized Experiments: JoVE*. (175) (2021).
34. Jacquemet, G. et al. The cell biologist's guide to super-resolution microscopy. *Journal of Cell Science*. **133** (11) (2020).
35. Rajasethupathy, P., Ferenczi, E., Deisseroth, K. Targeting Neural Circuits. *Cell*. **165** (3), 524–534 (2016).
36. Lin, M. Z., Schnitzer, M. J. Genetically encoded indicators of neuronal activity. *Nature Neuroscience*. **19** (9), 1142–1153 (2016).
37. Huff, J. The Airyscan detector from ZEISS: confocal imaging with improved signal-to-noise ratio and super-resolution. *Nature Methods*. **12**, i–ii (2015).
38. Cuentas-Condori, A. et al. *C. elegans* neurons have functional dendritic spines. *eLife*. **8** (2019).