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Dendritic spine morphology analysis

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We use this protocol and it's working

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Abstract

Dendritic spines are key sites of excitatory synaptic signaling, and their morphology reflects changes in connectivity and plasticity. This protocol outlines imaging and quantitative analysis of spine features, including density, length, head diameter, and subtype classification, enabling detection of structural alterations across experimental conditions.

Materials

- Fatal Plus (Vortech Pharmaceuticals, Catalog #0298-9373-68)
- Paraformaldehyde (PFA; Sigma Aldrich, Catalog #P6148)
- Glutaraldehyde (Fisher Scientific, Catalog #BP2547)
- Peristaltic pump (Cole Parmer)
- Leica vibratome (VT1000S) (settings noted: speed 70, frequency 7)
- 0.1 M PB (phosphate buffer)
- 48-well plate
- Sodium azide (Fisher, Catalog #BP9221)
- Nikon Eclipse FN1 upright microscope with 10× objective and 40× water objective
- Air table
- Tissue chamber: 50 × 75 mm plastic base with a 60 × 10 mm petri dish epoxied to the base
- Platinum wire and alligator clip (for ground connection)
- Electric current source (negative terminal connection to micropipette)
- Glass micropipettes
- Lucifer yellow dye, 8% (ThermoFisher, Catalog #L453)
- Micropipettes (A-M Systems, Catalog #603500)
- Manual micromanipulator (secured with magnets; provides ~45° injection angle)
- Small petri dish containing 1× PBS and DAPI
- Dental wax
- Filter paper
- Glass slides with two 125 µm spacers (Electron Microscopy Sciences, Catalog #70327-20S)
- Kimwipe
- Vectashield (Vector Labs, Catalog #H1000)
- Coverslips (Warner, Catalog #64-0716)
- Nail polish (for sealing)
- General lab supplies (e.g., instruments to pull micropipettes, mounting supplies)
- Nikon Ti2 C2 confocal microscope (Nikon, Tokyo, Japan)
- Plan Apo 60×/1.40 NA oil-immersion objective
- Plan Fluor 10×/0.75 NA air objective
- Nikon Elements 4.20.02 image capture software
- Fiji (ImageJ) image processing software
- Huygens Deconvolution System (16.05, Scientific Volume Imaging, the Netherlands)
- Neurolucida 360 (2.70.1, MBF Biosciences, Williston, Vermont)
- Neurolucida Explorer (2.70.1, MBF Biosciences, Williston, Vermont)
- Microsoft Excel (Redmond, WA)
- Image file format: .tif

Perfusions and brain tissue processing

- 1 Anesthetize mice with Fatal Plus and transcardially perfuse with cold 1% paraformaldehyde (PFA) for 1 min, followed immediately by 4% PFA with 0.125% glutaraldehyde for 10 min using a peristaltic pump for consistent administration.
- 2 Immediately extract each brain and drop-fix in 4% PFA with 0.125% glutaraldehyde for 8–12 h at 4 °C.
- 3 After fixation, coronally slice brains into 250 µm thick sections using a Leica vibratome (VT1000S) set to speed 70 and frequency 7, slicing in 0.1 M phosphate buffer (PB).
- 4 Store slices at 4 °C, one slice per well in a 48-well plate, submerged in 0.1 M PB containing 0.1% sodium azide.

Iontophoretic microinjections

- 5 Set up the Nikon Eclipse FN1 upright microscope (10× and 40× water objectives) on an air table and prepare the tissue chamber (50 × 75 mm plastic base with a 60 × 10 mm petri dish epoxied to the base). Attach a platinum wire and connect the ground to the bath with an alligator clip.
- 6 Fill a glass micropipette with ~2 µL of 8% Lucifer yellow dye and connect the negative terminal of the current source to the micropipette. Use freshly pulled micropipettes with highly tapered tips.
- 7 Secure the manual micromanipulator on the air table with magnets to provide an approximately 45° injection angle.
- 8 Place brain slices in a small petri dish containing 1× PBS and DAPI for 5 min at room temperature.
- 9 After DAPI incubation, place slices on dental wax and adhere the tissue to a piece of filter paper; transfer the filter paper to the tissue chamber filled with 1× PBS and weight down for stability.
- 10 Use the 10× objective to visualize and advance the micropipette tip in X, Y, and Z until the tip is a few micrometers above the tissue, then switch to the 40× objective to advance the tip into layer 2/3 of ventral CA1.
- 11 Once the microelectrode contacts a neuron, apply 2 nA of negative current for 5 min to fill the neuron with Lucifer yellow. After 5 min, turn off the current and remove the micropipette.

- 12 If the neuron does not fully fill with dye after penetration, remove the electrode and exclude that neuron from analysis. Inject multiple neurons per hemisphere per animal as needed.
- 13 After injection, move the filter paper with the tissue back into the chamber containing 1× PBS. Carefully lift the tissue off the paper and place it on a glass slide with two 125 µm spacers. Remove excess PBS with a Kimwipe and air-dry the tissue for 1 min.
- 14 Add one drop of Vectashield directly to the slice, place the coverslip, seal with nail polish, and store injected tissue at 4 °C in the dark.

Confocal microscopy

- 15 For high-resolution dendrite imaging, use a Nikon Ti2 C2 confocal microscope with a Plan Apo 60×/1.40 NA oil-immersion objective to acquire 3D z-stacks of secondary dendrites that meet selection criteria (within 80 µm working distance, relatively parallel to section surface, no overlap with other branches, located 40–120 µm from the soma).
- 16 Acquire z-stacks with Nikon Elements using a step size of 0.1 µm, image size 1024 × 512 px, zoom 4.8×, line averaging 4×, and acquisition rate of 1 frame/s.
- 17 For lower-magnification slice imaging use the Plan Fluor 10×/0.75 NA air objective and acquire stitched images by automatically stitching multiple adjacent 10× images with 25% blending overlap (each frame 1024 × 1024 px, acquisition rate 1 frame/s); subsequently acquire 10× stitched images with the same parameters.
- 18 Perform post-image processing in Fiji (ImageJ); each image file is two-channel — use Split Channels to separate channels prior to further analysis.

Dendritic spine morphometry analysis

- 19 Deconvolve confocal z-stacks using Huygens Deconvolution System (16.05, Scientific Volume Imaging). Use the following settings: deconvolution algorithm: GMLE; maximum iterations: 10; signal to noise ratio: 15; quality: 0.003. Save deconvolved images in .tif format.
- 20 Import deconvolved image stacks into Neurolucida 360 (2.70.1) for dendritic spine analysis and use the semi-automatic directional kernel algorithm to trace the dendrite. Exclude the outer 5 µm of each dendrite from the trace.
- 21 Examine all assigned trace points to ensure they match the dendrite diameter and position in X, Y, and Z planes; adjust points as necessary.

- 22 Perform automatic dendritic spine reconstruction using the voxel-clustering algorithm with these parameters: outer range = 5 μm ; minimum height = 0.3 μm ; detector sensitivity = 80%; minimum count = 8 voxels.
- 23 Manually review the semi-automatically identified spines to ensure accuracy; add missing spines by increasing detector sensitivity if needed, and use the merge and slice tools to correct any morphology or backbone point errors.
- 24 Allow Neurolucida 360 to automatically classify each dendritic spine as a dendritic filopodium, thin spine, stubby spine, or mushroom spine based on constant parameters.
- 25 Export three-dimensional dendrite reconstructions to Neurolucida Explorer (2.70.1) for branched structure analysis to obtain measurements such as dendrite length; number of spines; spine length; counts of thin, stubby, mushroom spines, and filopodia; spine head diameter; and spine neck diameter. Export the analysis output to Microsoft Excel for further processing.
- 26 Calculate spine density as the number of spines per 10 μm of dendrite length. For each mouse, average the values for all dendrites corresponding to that mouse. To analyze apical and basal dendrites separately, average values for either apical or basal dendrites per mouse. Include a mouse in statistical analyses only if a minimum of 100 μm of dendrite length has been reconstructed and analyzed.

Protocol references

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