Application Note

Symmetric Synapse

related instrument Leica EM ICE

Medical Research

Natural Resources

Industrial Manufacturing
Symmetric Synapse
Clathrin coated endocytosis pit in the postsynaptic dendrite

PROCEDURE
WT hippocampal neurons were plated at a density of 80,000 cells/cm² on 6 mm sapphire disks for 14 days. Sample were frozen using a high-pressure freezer (EM ICE Leica) under a pressure of 2100 bar by mounting it into a sandwich support with extracellular solution containing 15% of Ficoll 400, to assess ice crystal damage. The Cryo-fixation was achieved within milliseconds allowing simultaneous immobilization of all macromolecular components. After freezing, sample was transferred into cryovials containing 1% glutaraldehyde, 1% osmium tetroxide, 1% milliQ water in anhydrous acetone and processed in an automated freeze-substitution device (AFS2, Leica). Samples were gradually warmed from -90°C to -50°C (increment of 8°C/h); from -50°C to -20°C (increment of 6°C/h) At -20°C samples were kept for 12h and finally warmed to 20°C (increment of 5°C/h). After the freeze-substitution step sample was contrasted with 0.1% uranyl acetate for 1 h, and embedded in EPON. Infiltration with EPON resin was performed at room temperature by following steps: infiltrated with 30% EPON/Acetone for 3 h, followed by 70% EPON/Acetone for 3 h, and overnight incubation in 90% EPON at 4°C. Finally, sample was placed into the capsules filled with pure EPON and further polymerized for 48h at 60°C. Approximately 200um square areas containing cells were randomly selected, sectioned at 40nm thickness using a Leica Ultracut (UCT), and collected on Formvar-coated (0.5%) 200-mesh copper grids. Those sections were further examined on Zeiss 900 or FEI Tecnai G20 electron microscope operated at 120 keV (FEI). Electron micrographs (2048x2048 pixels) were collected in Veleta camera (FEI, 2048x2048 pixels) at 135,000x magnification (pixel size 0.375nm). The sections were contrasted with 2% uranyl acetate in milliQ water for 3 min, followed by washing in water, air drying and incubation with lead citrate (0.15 m lead citrate, 0.12 m sodium citrate in CO2-free dH2O) for 15s.

Courtesy:
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RESULTS

Zooming into a Symmetric Synapse

Symmetric synaps (arrow heads) formed by axon terminal containing pleomorphic synaptic vesicles and the postsynaptic dendrite. In the postsynaptic dendrite marked with arrows is a clathrin coated endocytosis pit. Bar is 200nm.