

QUANTITATIVE ULTRASTRUCTURAL ANALYSIS THREE-DIMENSIONAL  
RECONSTRUCTIONS OF SYNAPTIC STRUCTURES OF THE BRAIN

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An investigation was made at electron microscope level (serial ultrathin sections) of changes in the 3-dimensional (3D) morphology of dendritic spines and synapses in dentate gyrus and CA3 hippocampal areas in rats and ground squirrels. For comparative ultrastructural analyses of synapses we used different functional states of rat brain: 1) spatial learning in water maze; 2) 2) stress-restraint; 3) subcutaneous injection of peptide FGL that is mimetic of receptor site of fibroblast growth factor (FGL) which improve memory. Besides we investigated synapses in alternative functional states of Yakutian ground squirrels when brain temperature drops to 0°C during hibernation. For qualitative estimation: volume; surface area of dendritic spines and distances between synapses we used commercial software 3D-Studio-max and software developed by Drs. K.Harris and J. Fiala (<http://synapse-web.org/tools/index.stm>): Align and Trace. All 3D-reconstructed: postsynaptic densities; dendritic segments and dendritic spines; axonal segments and presynaptic boutons; mitochondria; smooth endoplasmic reticulum; Golgi stacks; multivesicular bodies etc. were represented as triangular surfaces.

Our analyses included: 1) scanning of serial images as \*.bmp files; 2) alignment of neighboring images in “Align” software 3) preparing of contours (by hand) of each studied structure and generation of 3D- reconstructions with triangular surfaces as \*.wrl/vmrl files; 4) quantitative estimation (volume, surface area, mass centers, distances between mass centers) using special software; 5) preparation of 3D images using 3D-Studio-max и Adobe Photoshop.

We demonstrate 3D movie to show all steps of analyses synaptic structures. We discuss new paradigms of synapse organization.

1. Popov VI, Medvedev NI, Kraev IV, Gabbott PL, Davies HA, Lynch M, Cowley TR, Berezin V, Bock E, Stewart MG. A cell adhesion molecule mimetic, FGL peptide, induces alterations in synapse and dendritic spine structure in the dentate gyrus of aged rats: a three-dimensional ultrastructural study. European Journal of Neuroscience, 27(2), pp.301-314, 2008

2. Popov VI, Medvedev NI, Patrushev IV, Ignat'ev DA, Morenkov ED, Stewart MG. Reversible reduction in dendritic spines in CA1 of rat and ground squirrel subjected to hypothermia-normothermia in vivo: A three-dimensional electron microscope study. *Neuroscience*. 149(3), pp.549-560, 2007
3. Popov VI, Deev AA, Klimenko OA, Kraev IV, Kuz'minykh SB, Medvedev NI, Patrushev IV, Popov RV, Rogachevskii VV, Khutsian SS, Stewart MG, Fesenko EE. [Three-dimensional reconstruction of synapse and dendritic spines in the hippocampus of rats and ground squirrels: new paradigms of the structure and function of a synapse]. *Zh Vyssh Nerv Deiat Im I P Pavlova*, 54(1):120-129, 2004