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Title: Effect of extracellular space width on geometric tortuosity in 3D reconstruction of neuropil

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Authors: ***J. P. KINNEY**[1,2], J. SPACEK[3], T. M. BARTOL[2], C. BAJAJ[4], K. HARRIS[4], T. J. SEJNOWSKI[2,5,6];
[1]Bioengineering, UCSD, San Diego, CA; [2]Salk Inst. for Biol. Study, La Jolla, CA; [3]Charles Univ., Hradec Kralove, Czech Republic; [4]Univ. of Texas at Austin, Austin, TX; [5]Univ. of California San Diego, San Diego, CA; [6]Howard Hughes Med. Inst., San Diego, CA

At hippocampal CA1 synapses vesicularly-released glutamate is not confined to the synaptic cleft, but instead, spills out into the extracellular space (ECS). How far the neurotransmitter diffuses from its point of release is determined by the activity of glutamate transporters which remove glutamate from the ECS and the tortuosity of the surrounding neuropil which describes how the rate of diffusion of neurotransmitter in the neuropil is diminished compared to free diffusion. The total tortuosity is defined as the product of a viscous component and a geometric component. The focus of this work is on the geometric component of tortuosity which captures the effect of longer diffusion paths in the neuropil and will be measured in the absence of glutamate transporters. Here we characterize the effect of extracellular width on geometric tortuosity in a 3D reconstruction of 180 cubic microns of rat hippocampal neuropil in CA1 stratum radiatum. Perfusion-fixed tissue was stained and cut in 50 nm thick sections before being photographed with transmission electron microscopy through serial sections. The membranes of every dendritic, axonal, and glial process in each section were manually traced from the micrographs at a resolution of 2.3 nm per pixel using RECONSTRUCT (http://synapses.bu.edu). The set of contours from each process were used to reconstruct the plasma membrane. The 3D computer model of neuropil consists of water-tight, triangulated surfaces representing the outer surface of the lipid bilayer of each process in the neuropil. We manipulate the location of the surface on the nanometer scale such that the width of the extracellular space is a specified distance and is uniform throughout the reconstruction. The ECS width is then a variable parameter and will be swept over the range 1 nm to 80 nm. For each configuration of the model the geometric tortuosity is measured by simulating neurotransmitter diffusion in MCell.

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