

Study of the membrane organization of OLN-93 oligodendroglial cells

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Oligodendrocytes (OLGs) are the myelin-producing cells of the central nervous system. Abnormalities in myelin development or destruction of its structure have severe pathological consequences, causing diseases such as multiple sclerosis (1). Based on detergent extractions and co-localization studies, several myelin proteins have been identified in cholesterol/sphingolipid-rich membrane microdomains, so-called membrane rafts (2-8). Within the framework of multiple sclerosis research, the final aim of this project is to study the effects of cytokines and cholesterol-lowering drugs on the membrane organization of OLGs. Our current research efforts focus on the lateral diffusion of raft and non-raft components. Therefore, OLN-93 oligodendroglial cells were labeled with the raft marker Bodipy FL-C5 sphingomyelin or with the non-raft marker DiD. Cells were grown in 8-well glass-bottom LabTek chambers. Confocal laser scanning microscope (CLSM) imaging and fluorescence correlation spectroscopy (FCS) were carried out on these cells at ~24°C with a Zeiss LSM 510 META CLSM and a Zeiss Confocor 2 setup. Data were collected at the bottom membrane (i.e. close to the glass cover slip) of the cells. The measurement conditions were: 488 nm Ar ion laser at 0.1% maximum power (~5 µW at the sample position) and 633 nm red HeNe laser at 0.1% maximum power for Bodipy FL-C5 sphingomyelin and DiD respectively, and a 40x, NA 1.2 water immersion objective. Free and anomalous diffusion models were used to analyze the data with Zeiss Confocor, Globals (LFD, UIUC) and Origin (Prof. Dr. M. Hof and A. Benda) software packages. The effects of cholesterol depletion (methyl-β-cyclodextrin), disruption of the actin cytoskeleton (latrunculin A) and disruption of microtubules (colchicin) on the diffusion of Bodipy FL-C5 sphingomyelin and DiD will be discussed as well as the influence of bleaching and cellular motion.

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