Fachbereich Biologie (10)

JOHANNES GUTENBERG UNIVERSITÄT MAINZ

AG Functional Neurobiology (AG Heine)
Institute for Developmental and Neurobiology (IDN)

June 2023

Bachelor project

Immunostaining of hippocampal neurons to reveal changes in active zone composition after VGCC/scaffolds genetic and acute manipulations

The structural organization of the presynaptic terminal is of fundamental importance for the neurotransmitter release. As a basis of neuronal communication this process represents the major determinant of high cognitive functions such as memory and behavior. In the presynaptic active zone, voltage-gated calcium channels' (VGCC) proximity to docked synaptic vesicles is essential for fast neurotransmitter release. Such proximity is ensured by several scaffolding proteins (such as RIM, RBP2, Munc-13, Bassoon and Piccolo), collectively known as cytomatrix of the active zone. Notably, the scaffold protein Bassoon was shown to specifically bring Cav2.1 channels close to synaptic vesicles, through an indirect interaction mediated by a PXXP/SH3 binding domain. Interestingly, deletion of this binding domain results in decreased channel abundance and impaired vesicle release. However, the mechanisms by which individual VGCC/scaffold interactions affect the structural architecture of the active zone is still elusive. Therefore, we aim to study how genetic or acute manipulations of the tight VGCC/scaffold binding structurally affects other components of the presynaptic release machinery. To do so, in cultured hippocampal neurons we will disrupt VGCC/scaffold interactions in a short time scale by delivering cell-penetrating peptides that mimic known binding sites between VGCC and scaffold proteins. In parallel, we will up- or downregulate Bassoon to disturb VGCC/scaffold interactions in a prolonged time scale.

In this project, we will investigate structural changes of active zone composition following acute VGCC/scaffold interference. Furthermore, we will study release machinery's architecture following overexpression and shRNA-mediated downregulation of the scaffolding protein Bassoon.

While carrying out this project, you will learn how to genetically manipulate protein expression, label synaptic proteins through immunocytochemistry staining of hippocampal neurons and how to visualize fluorescently labelled proteins using confocal microcopy in primary hippocampal neurons. Moreover, you will be introduced to image analysis software ImageJ and Icy for quantitative evaluations of protein abundance and localization within synapses.

If you are interested, please contact Michela Borghi (mborghi@uni-mainz.de).

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