Imaging approaches to characterize Ca^{2+}-dependent vesicle trafficking steps in ribbon synapses of the retina (Prof. Frank Schmitz/ Dr. Karin Schwarz)

Ribbon synapses are high performance synapses in the retina and inner ear with a particularly high vesicle turnover. Ribbon synapses can release large numbers of synaptic vesicles within a very short timescale and maintain fast vesicle exocytosis for long periods of time. The synaptic ribbon is a presynaptic specialization in the active zone of ribbon synapses that speeds synaptic vesicle trafficking and provides the ribbon synapse with a large pool of release ready vesicles. Synaptic ribbons define hot spots of exocytosis, endocytosis and vesicle trafficking in this synapse. How these functions are mediated and which proteins are involved is only poorly understood.
Our lab identified proteins that are present at these hot spots (e.g. RIBEYE, Munc119, GCAP2, CSP and others) and our aim is to characterize the function of these proteins. In the present project, vesicle turnover at ribbon synapses will be analyzed with a variety of molecular, genetic and imaging approaches using wildtype and genetically altered mice.
We will use Ca^{2+}-imaging techniques to analyze the importance of synaptic ribbons and synaptic ribbon-associated proteins for the regulation of intracellular Ca^{2+}-levels and Ca^{2+}-dependent signalling cascades. The physiological importance of the ribbon-linked protein network for synaptic vesicle trafficking will be determined with various physiological approaches (imaging with FM1-43, exocytosis reporters, time lapse microscopy) and imaging techniques, e.g. two-photon-microscopy. These techniques will be combined with biochemical and molecular approaches (Yeast-Two-hybrid- and related techniques), biochemical techniques (immunoprecipitations, „pulldown“ analyses) as well as morphological assays. Bipolar and photoreceptor cells from the mouse retina as well as organotypic retina cultures will be used as model systems for the physiological analyses. We expect from these analyses insights on how the ribbon works at a molecular level and how synaptic ribbon proteins are involved in vision. These analyses will also help to better understand diseases of the visual system because mutations of ribbon synaptic proteins are often linked to disturbances in vision.

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