The dynamic membrane traffic at the neuronal synapse

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The membrane and protein recycling mechanisms at the synapse form synaptic vesicles through endocytosis, allowing neuronal cells to sustain high rates of activity. Animal models of defective endocytosis accumulate recycling intermediates and endosomes at their synapses and present neurodegeneration and/or early lethality. Endosomes are molecular sorting stations for vesicular traffic, but little is known about synaptic endosomes and their roles. The proposed studies aim to understand membrane traffic at the synapse with focus on endosomes, and will examine mice that lack essential endocytic genes using a combination of super-resolution imaging and electron microscopy. We will also apply a genomics approach to these animal models to identify signaling pathways that originate from abnormal synapses and lead to neurodegeneration.

The conserved membrane and protein recycling mechanisms at the synapse, which result in the formation of homogeneously-sized synaptic vesicles (SVs) through a process of endocytosis, allow neuronal cells to sustain high rates of activity. The high speed of SV recycling and the small size of synapses have traditionally hampered the studies of synaptic organelles. Recently, mouse models of defective endocytosis were generated whose synapses accumulate recycling intermediates that are stable for minutes to hours, as opposed to milliseconds to seconds in unaltered synapses. Moreover, current advances in super-resolution fluorescence microscopy make it possible to inspect synaptic organelles at molecular level. Taken together, the mammalian models that accumulate recycling intermediates at the synapse and super-resolution microscopy provide a powerful way to address some long-standing questions in the field of synaptic physiology.

Our research proposal focuses on endosomes, probably the most transient, most heterogeneous and certainly the least understood synaptic structures. They are central to membrane trafficking at the synapse, and control sorting of SV proteins, recycling of SVs and degradation of synaptic membrane proteins and lipids. We consider that endosomes at the synapse are not just independent entities, but they form a tightly coordinated network with SVs and other cellular organelles, and imbalances in such network directly result in altered SV recycling and in perturbations of the neuronal signaling landscape that leads to neurodegeneration.

We have recently characterized animals with impaired function of the endocytic adaptor endophilin that, in addition to defective SV recycling (accumulation of clathrin-coated vesicles and reduction in the number of SVs), also show neurodegeneration and a prominent accumulation of endosomes at the synapses.

Independent studies on mice without synaptojanin 1 (a lipid phosphatase) and dynamin (a GTPase), two major endophilin interactors, demonstrated that endosomal compartments are also up-regulated in the absence of these key endocytic proteins. The use of three animal models with the similar phenotypes ensures that we are not looking at a gene-specific change, but rather at a defective endocytic process. We are addressing two main questions:

UNRAVELING SYNAPTIC VESICLE FORMATION FROM ENDOSOMAL COMPARTMENTS

Besides clathrin-mediated endocytosis, which is considered a major pathway of SV recycling under physiological conditions, there are additional ways to generate SVs, but their mechanisms are not well understood. It has been previously suggested that endosomal intermediates may undergo fragmentation, or may bud vesicles (either in a coat-independent or coat-dependent manner). We are now using advanced imaging (including super-resolution microscopy – Figure 1), electron microscopy (EM) and EM tomography at the synapses accumulate recycling/endosomal intermediates due to impaired endocytosis in order to distinguish between these possibilities. Using the aforementioned animal models, but also RNA interference and pharmacological agents, we are further investigating whether endosomal compartments function independently of each other, or they build a coordinated network, possibly also with SVs and other cellular organelles.

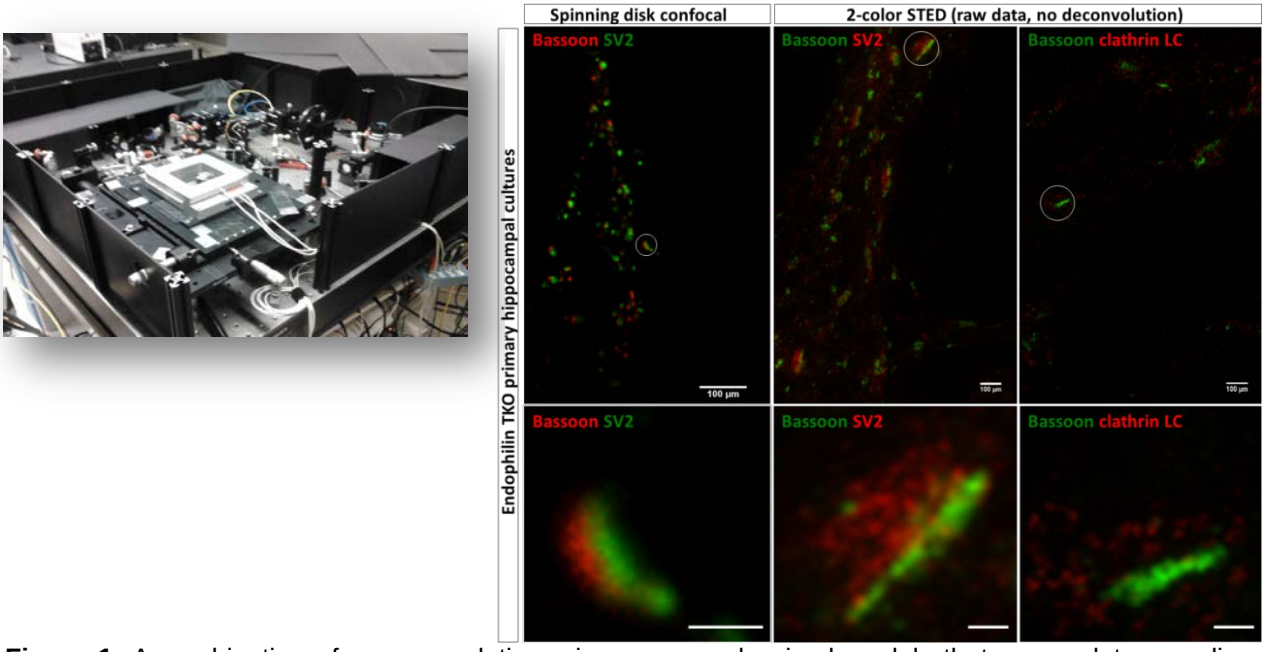

Figure 1. A combination of super-resolution microscopy and animal models that accumulate recycling intermediates at the synapse provide a powerful way to address some long-standing questions in the field of synaptic physiology. Left: Custom-made two-color STED microscope (photo courtesy of Fabian Göttfert and Stefan Hell). Right: Two-color super-resolution images of synapses without endophilin immunostained for Bassoon protein (as marker for an active zone), synaptic vesicle protein 2 (SV2, as marker for SVs) and clathrin light chain protein (clathrin LC, as marker of clathrin-coated vesicles). The labelling of various endosomal markers at the mutant synapses is in progress.

ALTERED SYNAPTIC MEMBRANE TRAFFICKING AND NEURODEGENERATION

Neurodegenerative diseases arise, at least in part, by imbalances in synaptic function and communication between neurons. Animals with defective endocytosis display prominent signs of neurodegeneration, and several key endocytic proteins have been linked to neurodegenerative diseases (e.g. Parkinson's disease). We are presently exploring how the perturbed endocytic processes at the synapse change the neuronal signaling landscape and lead to neurodegeneration. To this end, we are applying next-generation sequencing and a multi-dimensional genomic analysis to the mouse models of impaired endocytosis, namely the mice without endophilin and synaptojanin-1 proteins, in order to identify signaling pathways that originate from altered membrane trafficking at the synapse and lead to neurodegeneration. The preliminary leads from genomics and pathway analysis will be validated by quantitative proteomics. Further characterization of implicated proteins will be performed by biochemical, physiological and imaging experiments, depending on the leads to be followed.

Altogether, we expect that the proposed experiments will deepen our knowledge of the dynamic membrane trafficking at the synapse and provide details on the molecular mechanisms of endocytic processes in the nerve cells as well as reveal their relevance for the nervous system physiology. Given the central role of these processes for the nervous system function, our research proposal will likely lead to the better understanding of neurodegenerative diseases.