Focus of the PhD-Project: (Prof. Dr. Dieter Bruns)

The function of vesicular SNARE proteins in Ca^{2+}-dependent vesicle fusion studied in autaptic cultures of hippocampal neurons

The Ca^{2+}-dependent exocytosis of neurotransmitter-containing vesicles is the elementary signal of inter-neuronal communication. Precision, speed and modulation of this process are characterizing features for discrete signalling and for the plasticity of neuronal information processing. Recent studies hypothesize that the SNARE proteins Syntaxin 1, SNAP-25 and Synaptobrevin are essential for Ca^{2+}-dependent exocytosis of secretory organelles. Unclear remained however, how these proteins function in exocytosis and to what extent their structural components (aminoterminus, SNARE-domain und transmembrane region) control different properties of the exocytotic mechanism.

Therefore this PhD project focuses on a structure-function analysis of the vesicular SNARE protein synaptobrevin II. Autaptic cultures of hippocampal neurons provide an ideal model system to study stimulus-secretion coupling, the pool of release competent vesicles as well as the time course of quantal signalling using a single-electrode approach. In the last years we analysed v-SNARE action in the exocytosis of chromaffin cells with membrane capacitance measurements and carbon fiber amperometry by studying the functional impact of various v-SNARE deficiencies (Synaptobrevin II ko, Cellubrevin ko and a new double-null mutant, sybII/ceb dko). These experiments revealed that v-SNAREs control distinct properties of the exocytotic mechanism, the recruitment of vesicles to the pool of release-competent vesicles ('priming') and the expansion of exocytotic fusion pores indicating their action throughout exocytosis from priming to fusion (Borisovska et al., 2005).

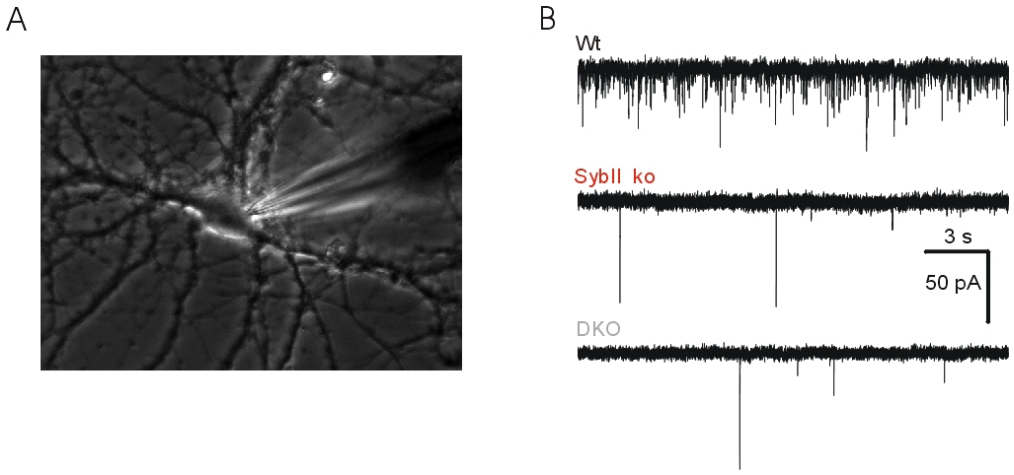

Figure 1: Quantal signaling in hippocampal neurons in culture. Synaptic signals recorded in the neuronal network (A) of sybII-ko or in dko cells are nearly eliminated compared with controls (B)

Synaptobrevin II-deficient neurons exhibit a nearly complete loss of evoked transmitter release and a strong reduction of spontaneous exocytosis (Figure 1). By expressing mutated variants of synaptobrevin in sybII-deficient neurons this project will clarify how structural components of the v-SNARE protein determine central properties of neuronal exocytosis. Using a gain-of-function approach, it is expected that the project will shed new light on the mode of v-SNARE action in rapid Ca^{2+}-triggered neurotransmitter release.

Methods:

Patch clamp techniques, high resolution membrane capacitance measurements, carbon fiber amperometry, electrical measurements of exocytotic fusion pores, UV-Flash photolysis of caged compounds, rapid ratiometric calcium imaging, trans gene technology (genetically-deficient mouse models), electron microscopy and biochemical techniques (e.g. protein-protein-interaction *in vitro*), gene transfer with viruses (e.g. Semliki-Forest virus).

Selected Publications

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Schütz, D., Lang, T., Zilly, F., Jahn, R., and Bruns, D. (2005). A dual function for munc-18 in exocytosis. *Eur J Neurosci.* **21**, 2419-32.

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Kesavan, J., Borisovska, M. and Bruns, D., 2007. v-SNARE actions during Ca^{2+}-triggered exocytosis. *Cell*, **131**, 351-363.

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Collaborators

Thomas Südhof, University of Texas Southwestern, Howard Hughes Medical Institute, Dallas, Texas 75235

Reinhard Jahn, Max-Planck-Institut für biophysikalische Chemie, Am Fassberg, 37077 Göttingen, Germany

Jacob Soerenson, Max-Planck-Institut für biophysikalische Chemie, Am Fassberg, 37077 Göttingen, Germany

Alain Marty, Laboratoire de Physiologie Cérébrale, Université Paris 5/CNRS, 45 Rue des Saints Pères, 75006 Paris, France

Thierry Galli, Membrane Traffic in Neuronal and Epithelial Morphogenesis, Institut National de la Santé et de la Recherche Médicale Avenir Team, Institut Jacques Monod, Unité Mixte de Recherche 7592, Centre National de la Recherche Scientifique, Universités Paris 6 et 7, 75005 Paris, France

Dieter Langosch, Lehrstuhl für Chemie der Biopolymere, TU München-Weihenstephan, 85354 Freising, Germany

Nikolai. S. Veselovsky, National Academy of Sciences of Ukraine, Bogomoletz Institute of Physiology, Department of Neuronal Networks, Bogomoletz str. 4, Kiev, 01024, Ukraine
Erica Jaffe, Instituto Venez. De Investigaciones Cientificas, Caracas, Venezuela
Tao Xu, Institute of Biophysics, Chinese Academy of Sciences
15 Datun Road, Chaoyang District, 100101 Beijing, China

On Campus collaborations
Jens Rettig and **Ulf Matti**, Physiologisches Institut, Universität des Saarlandes, 66424 Homburg, Germany
Veit Flockerzi, Institut für Pharmakologie und Toxikologie, Universität des Saarlandes, 66424 Homburg, Germany
Marc Freichel, Institut für Pharmakologie und Toxikologie, Universität des Saarlandes, 66424 Homburg, Germany