Research Foci of DFG Research Unit FOR 2419

Dynamic changes in synaptic strength, termed synaptic plasticity, is a cellular mechanism for the dynamic adaptation of neuronal networks that is widely recognized to underlie cognitive functions such as learning and memory. In seven projects, FOR 2419 scientists investigate different aspects of activity-dependent mechanisms with respect to structural and functional synaptic plasticity at the molecular and cellular level. To achieve a maximum horizontal cohesion, all projects focus on a single synapse type, the glutamatergic synapse in the mammalian central nervous system. State-of-the-art optogenetic techniques are applied to bridge the molecular level of synaptic research with systemic analyses of neuronal networks that regulate cognition and behavior in intact animals.

Most of the molecular components at synapses are highly dynamic due to active transport and diffusion. Moreover, they undergo rapid turnover. Central questions of the FOR 2419 research unit are: i.) which mechanisms guarantee stability in neuronal connectivity in such a dynamic system? ii.) how do these mechanisms encode memory, especially when very stable and very dynamic synapses co-exist on individual neurons? To pursue these questions, activity-dependent subcellular mechanisms that stabilize single and cooperating glutamatergic synapses will be investigated.

Recent methodological progress opens up new approaches for longstanding questions by combining cell biological, electrophysiological and optogenetic methods with state-of-the-art microscopic techniques, such as high-pressure freezing for electron microscopy, dSTORM, FRAP, confocal spinning disc microscopy, two-photon microscopy and TIRF. The FOR 2419 focuses on the following aspects of synaptic plasticity:

a) Specificity of activity-dependent cytoskeletal transport of synaptic components to and from the postsynaptic compartment

b) Role of the endoplasmatic reticulum for the structural stability and long-term synaptic plasticity in postsynaptic spines

c) Changes in pre- and postsynaptic fine-structure in long-term potentiation

d) Effect of specific activity patterns on the lifetime and strength of synapses as well as the connectivity of neuronal networks

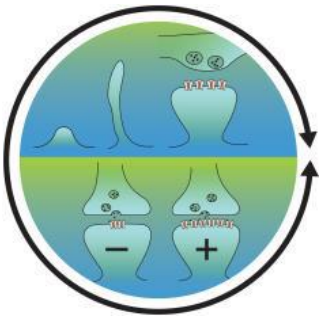
The rapid turnover of neurotransmitter receptors, elements of the cytoskeleton as well as of postsynaptic signaling and scaffolding molecules is part of a complex dynamic equilibrium of delivery, removal and recycling of synaptic components in synaptic plasticity. According to the *synaptic tagging* hypothesis, synapses, which were previously active, are preferentially supplied with plasticity relevant product (PRPs), as compared to naïve synapses. Therefore, a major cell biological approach within the FOR 2419 focusses on the trafficking of PRPs to excitatory spine synapses via microtubules and actin filaments. In general, the subcellular transport is regulated by the composition of motor-cargo complexes through microtubule- and actin-binding proteins, as well as by posttranslational modifications (PTMs) of tubulin. The FOR 2419 projects analyze transport regulation on all these levels: Marina Mikhaylova's group investigates the dynamics of motor proteins of the kinesin, dynein and myosin families using superresolution time-lapse microscopy (dSTORM, STED) (Kevenaar et al., 2016). Moreover, Froylan Calderon de Anda's and Wolfgang Wagner's groups analyze synaptic motor proteins of the myosin family whose functions have not yet been studied so far (Kneussel and Wagner, 2013). The role of calcium signaling for trafficking is important in this context, just as the interaction of myosins with the kinase TAO2. In humans, the gene encoding TAO2 is located on chromosome 16p11.2, a region that carries substantial susceptibility to autism spectrum disorders (ASD) and schizophrenia. Therefore, the question how TAO2 may be causative of ASD is studied in TAO2 mutant mice (de Anda et al., 2012). In newly generated tubulin knock-in mouse models, Matthias Kneussel's group explores tubulin-PTMs that contribute to the activity-dependent modification of tubulin. PTM patterns, the "tubulin code", are thought to provide selected specificity at tubulin tracks (Janke and Kneussel, 2010). In all above-mentioned projects, mouse models are used in addition to neuronal cell and slice cultures. They should help to analyze cellular components, the plasticity of neuronal networks and learning and memory.

A further focus of FOR 2419 scientists is the endoplasmatic reticulum (ER) that can be actively transported into dendritic spines by the motor protein myosin (Wagner et al., 2011). The function of spine ER is currently unknown. Thomas Oertner's group found that a certain form of long-term depression (LTD) of synaptic

transmission is only identified in ER-positive spines (Holbro et al., 2009). Using optogenetic stimulation and two-photon calcium imaging, his group investigates the question whether the transient invasion of ER into individual spine synapses mediates a kind of metaplasticity. In addition, Michael Frotscher's group analyses the protein synaptopodin that stabilizes ER structures in spines with state-of-the-art electron microscopic (EM) techniques (Studer et al., 2014). Moreover, EM immunogold studies on high-pressure frozen material are supplemented with two-photon microscopy, to monitor the time course of activity-induced structural changes at identified synapses and to record calcium transients in postsynaptic spines.

Beyond the molecular and cell biological studies of the FOR 2419, Christine Gee and Simon Wiegert's project investigates the effect of specific activity patterns on the lifetime and strength of synapses as well as the connectivity of neuronal networks in organotypic slice cultures (Wiegert and Oertner, 2013).

The aim of the FOR 2419 is to elucidate the mechanisms of synaptic plasticity and network connectivity for a better understanding of the nervous system in health and disease.



Synaptic plasticity, the capability of synapses to change their structure including formation and elimination of synapses as well as their functional strength, may be induced e.g. by long-term potentiation (LTP) that is a cellular model for learning and memory. Structural modifications (upper picture) such as the formation and maturation of dendritic spines contribute to new synaptic connections. An example for functional modifications (lower picture) is the increase in postsynaptic neurotransmitter receptors at synapses that leads to an enhancement of synaptic transmission (+).

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