Posttranslational regulation of synaptic strength

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2012

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Englisch summary

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Moving, sensing, thinking, learning and of course reading this thesis are just a few examples of the endless list of actions that are controlled by the brain. Mankind has been puzzled by its function for many centuries. The first descriptions in the field of neuroscience appeared several hundred years B.C. Technological advances such as the invention of the microscope and new surgical techniques allowed the transition from mere macroscopic, descriptive anatomy to microscopic and functional analysis of the healthy and diseased brain. A major breakthrough occurred in the late 1800s when Santiago Ramón y Cajal for the first time visualized and described the nerve cell (*neuron*) as the structural and functional unit of the brain.

The human brain consists of approximately 100 billion (10^{11}) neurons. A typical nerve cells has many processes, multiple dendrites and one axon. In the brain, *axons* and *dendrites* contact each other by forming about 100 trillion synapses (10^{14}), making it an extremely powerful information processing system. A single neuron receives information from many others, integrates this information and transmits the result via its axon to the dendrites of other neurons in the brain. In most cases, axon and dendrite are not directly connected, but are separated by a synaptic cleft. To bridge this gap, most neurotransmission is of chemical nature. Signaling molecules, so-called *neurotransmitters*, are packaged into synaptic vesicles in the presynaptic terminal (Figure 1.1). Vesicles then dock at the plasma membrane at the *active zone* (AZ) and get 'primed' for fusion. To send information from one cell to another, an action potential (AP; a transient change in membrane potential) propagates to the presynaptic terminals of the sending cell, depolarizes the presynaptic membrane and opens voltage-gated calcium channels, leading to an influx of calcium (Ca^{2+}) ions into the terminal. Ca^{2+} is sensed by proteins, mainly synaptotagmins, of the presynaptic release machinery and triggers fusion (also called *exocytosis*) of synaptic vesicles with the plasma membrane. As a consequence, neurotransmitter is released from the synaptic vesicle into the synaptic cleft and diffuses to the postsynaptic cell. Here, the neurotransmitter binds to receptors, which either causes changes in membrane potential

or activates intracellular signaling cascades. (Figure 1.2). Many different proteins are needed to control the different steps of this synaptic vesicle cycle to ensure fast and precise release of neurotransmitters. In addition, a neuron can modify the strength of synaptic transmission in response to changes in the environment. This adaptation is for instance important during learning and is generally referred to as *plasticity*.

My PhD project aimed to contribute to the understanding of the molecular mechanisms of synaptic plasticity. To this end, I mainly studied the function of two proteins: liprin-α2 en Munc18-1. Furthermore, I investigated how the function of these proteins modified in response to changes in network activity. During this adaptation small chemical groups are added or removed from the proteins, a process called *posttranslational modification*.

Chapter 2 investigates the role of the active zone proteins liprin-α1 and liprin- α2 in synaptic transmission in mature synapses. We show that liprin- α2 but not liprin- α1 is important for proper spontaneous and evoked release. Loss of liprin- α2 leads to mislocalization of several other presynaptic proteins, showing that liprin-α2 is important for clustering presynaptic proteins and maintenance of the active zone. We show that liprin-α2 protein levels and turnover are regulated in an activity- and proteasome-dependent manner. Hence, liprin-α2 plays an important and upstream role in the modulation of synaptic strength.

Chapter 3 en 4 study the modifcation of the protein Munc18-1 by *phosphorylation* (i.e. addition of a phosphate group) by different *kinases* (i.e. proteins that add phosphate groups).
Chapter 3 describes a novel inhibitory ERK-dependent signaling pathway that controls synaptic transmission via phosphorylation of the presynaptic protein Munc18-1. We identify Munc18-1 as a substrate for ERK *in vitro* upon increased network activity and *in vivo* after footshock-induced stress. Phosphorylation recruits a synaptic E3 ligase subunit and triggers ubiquitination and subsequent proteasomal degradation of Munc18-1 and decreased vesicle release. Preventing phosphorylation leads to increased synaptic efficacy and blocks the inhibitory effect of retrograde signaling via the CB1 receptor. Thus, activity-dependent ERK phosphorylation of Munc18-1 homeostatically controls neuronal activity.

Chapter 4 studies the effect of Cdk5 phosphorylation of Munc18-1. In neurons, Cdk5 activation results in reallocation of synaptic strength, including weakening of some connections while strengthening others (Mitra et al., 2011). We show that Cdk5 phosphorylation of the presynaptic protein Munc18-1 plays an important role in the regulation of synaptic strength. In the absence of phosphorylation, neurons show reduced spontaneous and evoked release, RRP size and release probability. Hence, Cdk5 phosphorylation of Munc18-1 does not underlie Cdk5-dependent silencing of synapses, but may play a role in neuronal silencing induced increase of presynaptic strength.

Chapter 5 describes a novel software routine (SynD) for automatic analysis of neuronal morphology. The program allows extraction of morphological and intensity data for soma, neurites and synapses from immunofluorescent images and minimizes analysis time and observer bias.

Chapter 6 summarizes the main findings and attempts to generate a model on how synaptic strength is modulated in the brain by combinatorial action of posttranslational modifications. In particular, I will focus on the regulation of Liprin-α2 and Munc18-1 and the possible implications for human health. The results in this thesis show that one protein is influenced by many others and that already small changes, such as the addition of a single phosphate group, can have strong effects on the function of a protein. Small changes in the structure of a protein, for instance due to point mutations, or in signaling cascades can therefore have a great impact on the performance of the human brain. Further research is now investigating the described pathways in the context of Alzheimer's disease, anxiety disorders and epilepsy.