Summary

The role of TrkB/BDNF-signaling in the developing and immature brain is multifaceted. However, it has been difficult to study the effects of BDNF in the intact adult brain, without influencing the development of the brain. It is not clear if discrete regions of the brain and specific cell types have different sensitivities to TrkB signaling in the mature brain. This thesis aims at elucidating the role of TrkB signaling in adult cortical plasticity to answer some of the following questions.

What are the structural and functional effects of inhibiting TrkB signaling in the adult cortex? Are these effects different in a similar cell type from another brain area? Can the pre- and post-synaptic effects of TrkB signaling be discerned? Does TrkB signaling act as a competitive substrate for cortical plasticity? What are the mechanisms that bring about these effects?

Due to the lack of appropriate tools to address these long-standing questions, a new approach had to be developed to counteract the problems. **Chapter 2** deals with the development of transgenic mice that express a membrane targeted enhanced green fluorescent protein (EGFP-F). The transgene expression starts at 6 weeks in individual excitatory neurons of the neocortex and hippocampus in a Golgi-staining like fashion. The EGFP-F expression levels are strong enough to perform acute and chronic in vivo imaging of layer II/III pyramidal neurons of the adult visual cortex from two different transgenic lines. Multiple transgenes can be expressed in the same individual neurons to facilitate morphological and functional analyses on the effects of different proteins**.**

In **Chapter 3,** viral-mediated Cre expression is used to increase the flexibility of this Cre-lox recombination approach to regulate gene expression in a temporal and spatial fashion. We use two different viral

systems, Adeno-associated virus (AAV) and Lentiviruses to deliver Cre stereotactically and drive the expression of β-galactosidase in R26R Cre-reporter mice. The recombination occurs in the first week of transduction and the expression pattern is similar in both viruses. There is stable transduction without any observable toxic effects.

We take this a step forward in **Chapter 4** by driving mosaic expression of a Cre-dependent functional protein, the dominant negative form of the TrkB receptor, TrkB.T1, fused to EGFP (TrkB.T1-EGFP). This allows us to visualize the individual neurons in which TrkB signaling is inhibited. TrkB.T1-EGFP expressing neurons from layer II/III of the primary visual cortex (V1) show fewer mature spines but have more of immature spine forms and filopodia when compared to EGFP-F expressing neurons. The amplitude and frequency of miniature excitatory postsynaptic currents (mEPSCs) are also reduced in these neurons. The reduction in the head size of the mature spines indicates that postsynaptic TrkB signaling is essential for spine maintenance in the adult V1. However, such morphological and electrophysiological changes are not observed in the adult hippocampal CA1. This suggests that different mechanisms govern synaptic maintenance in distinct brain areas.

In **Chapter 5,** we express TrkB.T1-EGFP in all pyramidal neurons of V1 by using a broad expressing Cre mouse. In comparison to the broad expression EGFP-F mouse, TrkB.T1-EGFP expressing neurons do not show a difference in the spine density and spine type. There is no change in the amplitude and frequency of mEPSCs. The spine head size is also similar, suggesting that inhibition of TrkB signaling in a large population of pyramidal neurons does not affect spine maintenance and other excitatory

properties. However, the inhibitory input to these neurons is reduced, as characterized by fewer Parvalbumin expressing puncta. The size and intensity of these puncta and the frequency of miniature inhibitory postsynaptic currents (mIPSCs) are also lower.

We hypothesize that synaptic maintenance by postsynaptic TrkB signaling is a competitive process that could possibly be controlled by the inhibitory system. The population activity seems to be a crucial determinant to trigger inhibition. If TrkB signaling is reduced in a single neuron, excitation is diminished in only this cell and does not affect the network activity. On the other hand, when TrkB signaling is disrupted in a majority of neurons in V1, the population activity also reduces to initiate a negative feedback of the inhibitory input.

In conclusion -

- The generated transgenic mice can drive mosaic expression of transgenes in the adult forebrain without affecting the development or the environment of these neurons.
- Multiple Cre-dependent transgenes can be expressed in the same individual neurons of the adult cortex to facilitate gene interaction studies.
- Postsynaptic TrkB signaling regulates synaptic maintenance in a bidirectional manner in the adult visual cortex, but not in the hippocampal CA1.
- Synaptic maintenance by TrkB signaling is a competitive process regulated by the inhibitory input which, in turn, is triggered by population activity.

Identifying the role of pre and postsynaptic effects of TrkB in the thalamocortical synapses and studying dynamic changes during synapse formation and maintenance by in vivo imaging would be useful complementary studies.