Summary

Learning and memory are fundamental processes for animal survival. Memory stabilization is mediated by long-lasting modifications of synapse strength and structure. This is known as synaptic plasticity and may occur at either side (pre- or post-) of the synapse. The main objective of this thesis was to examine the effects of altered hippocampal neurotransmission on molecular mechanisms related to memory. With respect to presynaptic plasticity this was investigated using inbred and mutant mice that expressed a gain-of-function in the $Ca_V2.1$ calcium channel. In addition, the effect of reducing postsynaptic plasticity on gene transcription during memory reconsolidation was investigated. Reconsolidation is a re-stabilization process after memory recall, which updates the strength and content of memories. It can be expected that expanding the knowledge on the molecular mechanisms of memory reconsolidation may provide therapeutic entry points for the treatment of anxiety-related disorders. The studies conducted in this thesis further our understanding of the impact of genetically or pharmacologically changed neurotransmission on memory.

Chapter 2 provides a comprehensive analysis of synaptic differences in the cognitively distinct C57BL/6J (C57) and DBA/2J (DBA) inbred strains, with DBA mice performing less than C57 mice. To understand why both strains differ in cognitive functions we investigated the synaptic hippocampal protein content, ultrastructural morphology and synaptic functioning. Hippocampi of DBA mice showed lower expression of presynaptic proteins involved in exocytosis (including essential regulatory proteins such as Rab3A/C, Syntaxin1B and Munc18-1) and calcium-sensing protein RASAL1. Moreover, ultrastructural analysis of CA1 synapses revealed a significant reduction of the synaptic vesicle pool in DBA compared with C57. At the functional level, reduced paired-pulse facilitation and enhanced short-term depression indicated altered transmitter release and/or refilling mechanisms of glutamatergic synapses of DBA mice. Taken together, our data suggest that mice of the DBA strain show a compromised presynaptic phenotype that affects the dynamic properties of neurotransmitter release and plasticity. In combination with previously documented postsynaptic deficiencies, this phenotype may underlie the compromised performance in hippocampal-dependent memory tasks in DBA mice.

In **chapters 3-5** the effects of increased glutamatergic neurotransmission on aspects of hippocampal learning and memory were explored. To this end we investigated mice with different familial hemiplegic migraine type 1 (FHM1) knock-in missense mutations (R192Q and S218L) in the *CACNA1A* gene. FHM1 is a monogenic form of migraine with aura, in which transient hemiplegia is present during the aura phase of the attacks. Previous research had shown that FHM1 mutations affect the function of voltage-gated $Ca_V2.1$ calcium channels, leading to increased (cortical) excitatory neurotransmission. The severity of the symptoms, both in patients and mice, differs between the two FHM1 mutations: the R192Q mutation causes 'pure' hemiplegic migraine, whereas the S218L mutation causes a complex phenotype of hemiplegic migraine, ataxia, seizures, and (sometimes fatal) delayed brain edema after only a mild head trauma.

In **chapter 3** we aimed to explain cognitive and memory difficulties observed in familial hemiplegic migraine (FHM) patients by comparing hippocampal memory, neurotransmission and plasticity in the CA1 area between FHM1 and wild-type mice. Hippocampal excitatory neurotransmission (field potentials, stimulus-response relation) and LTP induction and maintenance were enhanced in R192Q mice, whereas LTD was not affected by the mutation. Surprisingly, hippocampal learning and memory were significantly impaired in these mice. In conclusion, our data suggest that abnormally enhanced plasticity can be as unfavorable to efficient learning as reduced plasticity. This provides clues to how enhanced neuronal excitability not only confers susceptibility for migraine attacks but also may influence cognitive function.

Chapter 4 further examines the magnitude of the cognitive and behavioral deficits in FHM1 mice. Behavioral phenotypes were determined using both automated home-cage phenotyping and conventional learning/memory and anxiety-related tests. Both FHM1 strains showed impaired hippocampus-dependent memory in contextual fear conditioning. Homozygous S218L mutant mice exhibited a more severe impairment and additional cognitive and behavioral symptoms compared with heterozygotes of the S218L strain and homozygotes of the R192Q strain. These results are in line with the magnitude of the gain-of-function effects and the clinical phenotypes observed in patients with these mutations.

In **chapter 5** the underlying transcriptional mechanisms of hippocampal dysfunction in FHM1 mutants were analyzed. Gene expression in hippocampi of FHM1 mutant mice was determined using deep serial analysis of gene expression by next-generation sequencing (deepSAGE-seq). Although we detected a set of differentially expressed genes, partly attributable to remnant 129/Ola background

in the mutant mice, changes in expression level could not be validated using qPCR in the same set of samples. Our results indicate that at a cellular level only minor changes in transcription may occur.

In **chapter 6** we investigated mechanisms activated during hippocampal memory reconsolidation, under basal conditions or when initial plasticity in neurotransmission was disrupted. Recently retrieved memories become labile and require a reconsolidation process to re-stabilize and persist further. This is governed by several molecular processes to alter synaptic strength. One of the primary steps engaged in this process is endocytosis of GluA2-containing AMPA receptors to temporarily decrease synaptic strength to allow modification by gene (and protein) expression. Using cap-analysis of gene expression and next generation sequencing (CAGE-seq), we studied hippocampal transcriptome changes early (at 30 minutes) and late (6 hours) after memory retrieval in two sets of experiments. First, we compared reconsolidation of aversive with neutral memory. Early aversive memory reconsolidation induced transcription factor activity and protein phosphatase and ubiquitin ligase binding activity. At a late phase of aversive memory reconsolidation, adaptations in synaptic strength were observed, such as regulation of transporter channels and glutamate receptors. In the second experiment, we observed fewer expression changes during aversive memory reconsolidation when AMPA receptor endocytosis was inhibited during retrieval, compared with normal retrieval. Early during reconsolidation, inhibition of AMPA receptor trafficking induced the expression of microRNA 207 (*Mir207*). In conclusion, we found that retrieval and subsequent reconsolidation of aversive memory, in comparison with neutral memory, engaged specific transcriptional programs. This is the first study investigating the temporal transcriptome profile associated with memory reconsolidation.

Chapter 7 provides a general discussion of the implications of the main findings in the above experimental chapters and suggestions for future strategies in the study of these topics. With the studies of inbred and mutant mouse strains described in this thesis, an effect was shown of alterations in molecular function that affect the degree of neurotransmission, on associated learning and memory. A changed basal activity or level of proteins that affect neurotransmitter release, either positively or negatively, was associated with a negative effect on cognitive functioning. These findings validate the importance of presynaptic molecular machinery in synaptic plasticity important for memory formation.