

Chapter 1

General Introduction

Cognitive function is critical to the survival of many animal species, for instance, enabling adequate responses to external stimuli and adapting to changes in the environment. In particular, learning and memory are vital to an organism's ability to use previous information in avoiding acute danger and in navigating known environments to find nutrition. At the basis of learning and memory lies molecular and cellular plasticity in various neuronal circuits and brain structures 1. This plasticity involves molecular, structural and functional adaptation of synaptic efficacy 2. Fine-tuning of synaptic strength is critical to acquire, maintain and modify memories. If this plasticity process becomes altered at some level, either by genetic and/or environmental factors, it may affect processes involved in learning and memory.

In this thesis I discuss the effects of genetic variation and mutations on memory and their underlying molecular mechanisms in the hippocampus, a brain structure important for memory formation and retrieval. In addition, I studied the molecular events linked to retrieval and updating of an existing memory with new information. Part one of this introduction highlights relevant scientific background, i.e., the role of the hippocampus, and the cellular and molecular plasticity mechanisms involved in learning and memory. Part two covers the experimental designs, including the latest “-omics” techniques that have been applied in the experimental chapters to study models of (aberrant) hippocampal plasticity.

Figure 1: Involvement of the hippocampus in different types of memory.

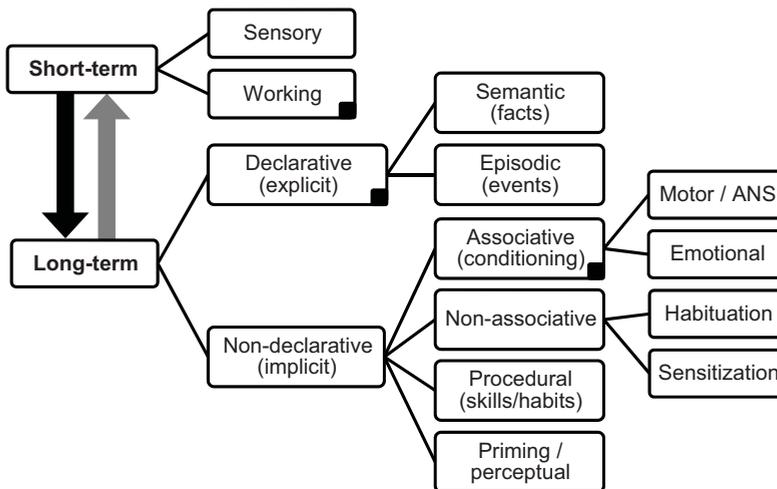
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Distinctions between memory types are usually made based on the interval during which information is retained (i.e., short- or long-term memory) and the content of the memory. This can be further divided in categories of explicit (factual) and implicit memory (e.g., subconscious learnt procedures such as riding a bike). Different sets of brain regions interact for a certain type of memory, often involving both neocortical and other (striatum, amygdala, cerebellum) areas. The hippocampus has a critical role in declarative memory, but is also involved in several other types of memory (indicated by black squares). When information is retrieved from long-term memory, it may enter the working/short-term memory to update it with new information (grey arrow). Figure adapted from reference ¹⁴².

I. Scientific background

Types of learning and memory mediated by the hippocampus

Learning and memory are often linked to the ability to consciously remember facts and events, also known as declarative memory. It can be subdivided into semantic (facts) and episodic (events). The first deals with categories of facts and attributes (e.g., “the Eiffel tower is in Paris”), whereas episodic memory involves associations to personal events (e.g., “I went to a concert in Paris, next to the Eiffel tower”). Other implicit procedures, such as skills and simple associative learning (classical conditioning), are grouped under non-declarative memory (Figure 1). As each type of memory depends on unique interactions between brain regions, dysfunction or damage to a specific area may cause specific problems with memory. A well-known example of this is the case of patient H.M. who developed a specific type of amnesia after bilateral medial temporal lobectomy³. Although unable to form new long-term declarative memories, he could still learn skills (procedural memory). His case, together with similar patient reports and primate studies, helped to identify the importance of the hippocampal system in declarative memory functioning^{4,5}. Evidence for additional functions in learning and memory soon emerged from rodent studies, indicating a role for the hippocampus in spatial navigation⁶ and emotional (contextual fear) memory^{7,8}.



Involvement in such diverse memory processes (declarative, spatial and contextual) may be attributable to the complex functional organization of the hippocampus. Regions with distinct patterns of gene expression and diverse anatomical connections to other brain centers exist (reviewed in ⁹. For instance, dorsal-ventral areas differentially contribute to particular types of memory. The ventral hippocampus has higher connectivity to areas that mediate emotional responses, such as the amygdala, and has been implicated in anxiety-related behaviors ¹⁰. The dorsal hippocampus is predominantly involved in associative memory processes that involve spatial and temporal cues. Impairments in spatial memory occur with dorsal hippocampal lesions, but not with similar lesions in the ventral area ¹¹. With respect to emotional memory, the dorsal hippocampus is important for contextual representations, whereas the ventral area may modulate behaviors related to motivation and anxiety ^{12,13}.

Storage and modulation of memory: consolidation and reconsolidation

Memories are not static. Within the hippocampus and other brain regions, memories are continuously being formed, lost or maintained and modified to integrate new information ¹⁴. Temporal profiles of memory formation are usually studied using learning paradigms based on single (brief) trials in rodents. Such tasks are useful to probe the timing of cellular and molecular events, or the functional consequences of perturbing these events during memory formation. One of the paradigms often applied to study hippocampal memory is contextual fear conditioning (CFC). I will highlight the processing of memory on the basis of CFC in the hippocampus (Figure 2), aspects of which can be generalized –to certain extent– to other brain areas and types of memory.

Learning starts with the acquisition of new information, which is temporarily stored in short-term memories (STM). Some of these subsequently undergo *consolidation*, i.e., stabilization of STM into long-term memory (LTM) ¹⁵. Consolidation in the hippocampus lasts up to 6 hours, requiring local cellular ^{16,17} and molecular ^{18–23} changes to occur before the memory becomes insensitive to disruption. System's consolidation entails the gradual transfer of LTM content to additional brain areas (mainly cortical) that leads to formation of a “remote” memory, which is to a large extent independent of the hippocampus, persisting for weeks or up to a year in rodents ^{14,24,25}. Once consolidated, a LTM is not fixed. Re-encounter with the CFC context induces retrieval, i.e., the LTM is re-called and

manifests itself as a behavioral and autonomic fear response. Retrieval for a short period of time re-activates brain areas where the LTM was encoded, causing protein degradation and destabilization of the memory^{26,27}. *Reconsolidation* is required to maintain or modify the strength of the memory and assimilate new experiences. Similar to consolidation, this process requires molecular changes to re-stabilize the LTM²⁸⁻³⁰.

Gaining insight of memory consolidation and reconsolidation processes is relevant to the study of neurobiological disorders causing cognitive dysfunction. Furthermore, understanding reconsolidation is important in finding effective pharmacological treatment strategies to adapt aberrant associative memories in anxiety disorders, such as post-traumatic stress disorder^{31,32}.

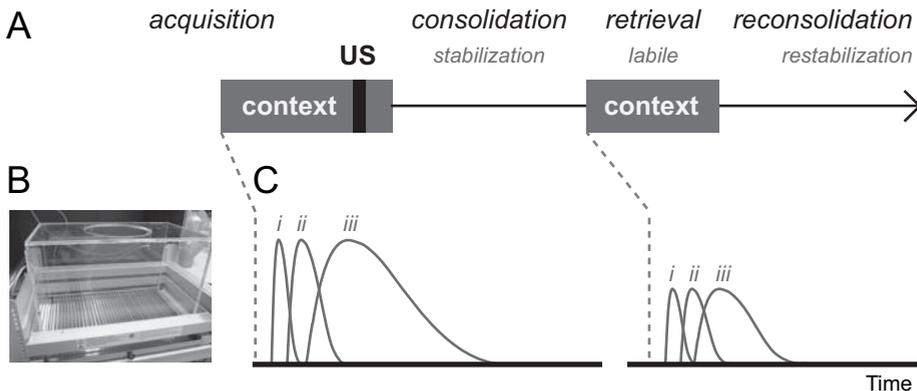


Figure 2: Contextual fear conditioning paradigms. **A:** In rodents, contextual fear memory is acquired by allowing the animal to explore a new context (**B**: cage with a grid floor) for a few minutes, the conditioned stimulus (CS). The exploration phase is followed by administration of an aversive unconditioned stimulus to the animal (US; mild foot shock). Hereafter the memory consolidates, i.e., it stabilizes in molecular terms (see C) over the course of a few hours-days. Upon return to the context after 24 hours, retrieval of the memory occurs. This renders the memory labile, allowing modification of its strength and/or content. Reconsolidation is needed to re-stabilize the (updated) memory in order to persist. **C:** Both consolidation and reconsolidation require glutamate receptor activation, which subsequently activates kinase pathways and transcription factors (*i*). These acute responses are followed by receptor trafficking at the synapse and a peak of immediate-early response genes (*ii*), and later by changes in protein expression (*iii*). The latter category involves proteins that modify synaptic strength by either changing the physiological strength (receptor and auxiliary subunits), mobility of membrane proteins, or synapse structure. The difference in consolidation and reconsolidation lies in the pathways involved; interfering with distinct transcription factors and IEGs (black text in C) will lead to impairments in one process but not the other. For example, partial knockdown of the *Egr1* gene impairs reconsolidation, whereas *C/EBP β* , *NGFI-B* and *BDNF* are specifically required for consolidation^{47,64-66}.

Synaptic mechanisms associated with memory

The cellular element essential for neuronal function is the synapse, a specialized structure where pre- and postsynaptic parts of two neurons communicate via electrical or chemical (neurotransmitter) signals with either excitatory or inhibitory effects. Chemical synapses are made up of axon terminals at the presynaptic compartment, where neurotransmitters are stored in synaptic vesicles. These may be released into the extracellular space between the neurons (synaptic cleft) by activity-dependent exocytosis. The resulting increase of neurotransmitter in the synaptic cleft activates ligand-binding receptors on the postsynaptic membrane of the receiving neuron. Excitatory neurotransmission constitutes the brain's primary mode of signaling; up to 90% of synapses may release the excitatory neurotransmitter glutamate^{33,34}. Signaling through glutamate and its receptors plays a central role in memory formation³⁵, although inhibitory neurotransmission through the neurotransmitter gamma-aminobutyric acid (GABA), and neuromodulation by neurotransmitters, such as serotonin, norepinephrine, glucocorticoids and endocannabinoids, also provide important molecular mechanisms for formation and modulation of memory^{35,36}. Essential for memory processing are adaptations in the strength of synaptic transmission (synaptic plasticity), i.e., physiological, molecular and cellular changes that are described in the following paragraphs.

Long-term memory and LTP

In 1894, the scientist Ramón y Cajal postulated that plasticity in the number and strength of neural connections constitutes the physical basis of learning³⁷. Adding to this theory, Hebb proposed that synapses undergo long-term potentiation (LTP): activity-dependent modifications to strengthen the connection³⁸. Later work by Bliss supported this theory and found that LTP causes a persistent increase in strength that may last as long as a memory^{39,40}. LTP has since been the principal model for research of cellular and molecular events underlying learning and memory^{2,41,42}. The opposite process, long-term depression (LTD) may also be involved in learning, especially in the cerebellum where it constitutes the main form of plasticity^{40,43}. However, LTP has been studied more extensively for its link to hippocampal (fear) memory consolidation. Although a causal link between LTP (as an experimentally induced phenomenon) and memory formation *in vivo* has been difficult to demonstrate, a number of observations support its involvement in

fear memory. Rats learning an inhibitory avoidance task show an LTP-like enhancement of excitatory transmission in the hippocampus that occludes subsequent induction of LTP⁴⁴. Inversely, inducing LTP may block fear memory⁴⁵. Further properties of LTP emulate the neuropsychological phases in memory formation. LTP has an early (induction) and late (maintenance) phase, with similarities to respectively short- and long-term memory. At the basis of LTP is modification of synaptic strength by molecular mechanisms, i.e., epigenetic modification, gene transcription and protein adaptations (translation, degradation, modification and localization)⁴⁶. Induction of LTP relies on adaptations in readily available proteins, whereas persistence of LTP, like memory consolidation, depends on temporal profiles of gene expression and protein synthesis⁴⁷⁻⁴⁹.

Molecular mechanisms of LTP induction

Induction of synaptic plasticity and LTP are activity-dependent processes and thus intertwined with (glutamatergic) synaptic transmission. At the presynaptic compartment, exocytosis and subsequent endocytosis to re-assemble vesicles is governed by an extensive network of interacting proteins⁵⁰. Exocytosis occurs at the active zone at the synaptic terminal, where synaptic vesicles dock in the vicinity of high-voltage-gated calcium channels (hVGCC). Docking and localization near hVGCCs is mediated by respectively SNARE and Rab3-RIM protein complexes. Fusion to the presynaptic membrane is calcium-dependent triggered by axonal membrane depolarization, which opens hVGCCs. Calcium influx activates calcium-sensing proteins, inducing conformational changes in the SNARE-synaptic membrane protein complex. This causes fusion of the vesicle with the presynaptic membrane, releasing the neurotransmitter from the synaptic vesicles. Importantly, presynaptic calcium sensors and hVGCCs also contribute to short-term plasticity, facilitating neurotransmitter release^{51,52}.

After release into the synaptic cleft, glutamate binds to two types of receptors at the postsynaptic membrane; metabotropic (mGluRs) and ionotropic (kainate, N-methyl-D-aspartate: NMDA, α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid: AMPA) receptors. The mGluRs are G-protein coupled receptors, which upon activation affect intracellular signaling cascades, mostly leading to indirect modulation of ionotropic receptor activity. AMPA- and NMDA- type glutamate receptors are ligand-gated cation (K^+ , Na^+ and Ca^{2+}) channels, which upon activation by glutamate induce an excitatory postsynaptic current. Of these,

NMDA receptors are critically involved in LTP induction, but require a slight membrane depolarization by the faster AMPA receptors to facilitate their activation⁵³. As a result of their activation, a postsynaptic current may become an action potential, but more importantly, the resulting calcium influx triggers intracellular signaling that activates protein kinases. Specifically, the activation of calcium/calmodulin dependent kinase II (CamKII) and calcium/phospholipid protein kinase C (PKC) is important in the early RNA and protein-synthesis independent phase of LTP. Phosphorylation of their targets, AMPA receptors, leads to enhanced function and membrane insertion, resulting in synaptic potentiation⁵⁴. Notably, mice lacking isoforms of these kinases show normal NMDA-mediated neurotransmission but have impairments in LTP induction, spatial memory and fear conditioning⁵⁵⁻⁵⁹.

Molecular mechanisms supporting LTP and memory consolidation

In order to sustain LTP and consolidate a memory, a number of further changes to the synapse are required, i.e., phosphorylation of proteins by activated kinases leading to i) induction of gene expression and protein synthesis, ii) receptor trafficking, and iii) synapse structural reorganization (Figure 2).

i) Induction of RNA and protein synthesis. CaMKII, PKC and other kinases (protein kinase A - PKA and mitogen-activated protein kinase - MAPK) are necessary for maintenance of LTP and memory consolidation through activation of transcriptional programs⁶⁰⁻⁶². These kinases phosphorylate transcription factors, inducing expression of plasticity related genes, coding for proteins required in adapting and maintaining synaptic strength⁶³. An essential transcription factor is the cAMP response element-binding protein (CREB), which among other transcription factors, regulates expression of immediate-early response genes (IEGs) important in memory consolidation⁴⁷. These include genes for transcription factors Ccaat-enhancer-binding protein (*C/EBPβ*), Early growth-response 1 (*Egr1/zif268*), *c-Fos*, *c-Jun* and neuromodulatory factors, such as nerve growth factor IB (*NGFI-B*) and brain-derived neurotrophic factor (*BDNF*)^{47,64-66}. Phased transcription in the hippocampus, peaking directly after acquisition and 3-6 h later, is important for memory consolidation²⁰. In addition to transcription, translation (protein synthesis) at the cell body and more importantly, at or near synapses (in dendrites), where plasticity was induced is also needed for consolidation^{63,67,68}.

- ii) Receptor trafficking. As a direct result of phosphorylation by kinases, the conductance, number and type of postsynaptic AMPA receptors is increased ⁵⁴. The primary response to LTP induction is a rapid increase in membrane receptors containing the calcium-permeable GluA1 subunit ⁶⁹. These are gradually replaced by calcium-impermeable GluA2-containing AMPA receptors, resulting in a lasting increase in synaptic strength ⁷⁰. The importance of GluA2 receptors is illustrated by conditional (CA1 area) knock-out mice. These mice initially revealed enhanced LTP (mediated by the calcium-permeable GluA2-lacking AMPARs). Yet, they are impaired in NMDA receptor-dependent learning, as revealed by deficient contextual fear and spatial learning and memory ⁷¹. Nevertheless, the effects of GluA2 insertion on long-term memory maintenance may differ per type of memory and brain area. In the hippocampus, disruption of GluA2 expression at the membrane, a day after learning a task, affects spatial but not fear conditioning memory ^{72,73}.
- iii) Structural changes. To maintain a consolidated memory over longer periods of time, structural changes occur in neurons. The number of synaptic contacts (dendritic spines) may increase to re-wire the local network on a neuron ^{74,75}. At single synapses involved in the memory, scaling supported by actin cytoskeletal rearrangements modifies the synaptic strength ^{76,77}.

The contribution to memory by molecular mechanisms in the postsynaptic compartment, as described above, has historically received a great deal of attention. Less research has been conducted on presynaptic forms of plasticity, likely because presynaptic LTP requires specific stimulation protocols, initially producing controversial results ^{2,78}. However, several groups have shown induction of presynaptic LTP by a protocol (theta-burst stimulation) that simulates naturally occurring hippocampal activity ⁷⁹⁻⁸¹. Presynaptic plasticity has been demonstrated in a number of hippocampal areas. Like its postsynaptic component, mossy fiber-CA3 synapses of the hippocampus undergo presynaptic LTP mediated by kinases, specifically PKA activation ⁸². In addition, it has been suggested that retrograde signaling may increase release efficiency, contributing to LTP at CA3-CA1 synapses of the hippocampus ^{78,82}. Furthermore, the active zone of the presynaptic compartment, may also contribute to structural plasticity through size and efficacy scaling of synaptic contacts ^{83,84}.

Similarities and differences in memory consolidation and reconsolidation

After acquisition or retrieval of a memory (re-)stabilization is needed by respectively consolidation or reconsolidation, to maintain or modify its strength and content⁸⁵. Both processes rely on transcription and protein synthesis; as with consolidation administration of selective inhibitors can disrupt memory reconsolidation^{28,30,86,87}. Although these findings suggest overlap with molecular mechanisms involved in consolidation and reconsolidation, they are thought to entail distinct cellular processes⁸⁸⁻⁹⁰. Within the hippocampus, transcription factors may be differentially engaged by re-consolidation⁹⁰. Subsequent gene expression is limited to a select subset of genes involved in consolidation⁹¹⁻⁹³. Pronounced differences are however observed with regard to AMPA receptor trafficking^{29,30}. Reconsolidation of contextual fear memory depends on a biphasic wave of AMPA receptor surface expression, characterized by initial endocytosis but followed by a wave of receptor re-insertion²⁹. Blocking this wave by inhibiting endocytosis of GluA2-containing receptors, disrupts the modification of memory strength that occurs with re-consolidation, leading to increased fear²⁹.

II. Experimental design

Learning and memory research has focused mainly on postsynaptic synaptic plasticity mechanisms underlying memory consolidation. However, the short- and long-term synaptic plasticity mechanisms important for memory require both pre- and postsynaptic modifications⁸². The main objective of this thesis was to examine the effect of genetically or molecularly altered neurotransmission on molecular mechanisms related to memory in the hippocampus. First, the association of presynaptic molecular and cellular mechanisms is investigated in two strains of mice known to differ in learning and memory performance. Next, the effects of increased neurotransmission by gain-of-function mutations in the presynaptic Ca_v2.1 voltage-gated calcium channel were examined on various aspects of learning and memory. Finally, to extend our perspective on molecular mechanisms of reconsolidation, transcriptional changes during reconsolidation associated with to normal and disrupted AMPA receptor mediated neurotransmission are discussed. In the following section, the models and novel techniques will be described that were used to investigate these topics.

Models for hippocampal memory and altered synaptic plasticity

Mouse strains show performance differences in hippocampal memory tasks

In the study of hippocampal learning and memory, rodents (especially mice) are frequently used due to the availability of transgenic strategies and the array of tasks that they can learn relatively fast⁹⁴. Individual variation in learning can be mimicked by genetically diverse mouse strains. The standard C57BL/6J (C57) strain performs quite well in hippocampus-dependent cognitive tasks, whereas this may be impaired in other strains, such as CBA/J and DBA/2J (DBA)^{95,96}. Studies into associated molecular mechanisms have mainly focused on postsynaptic plasticity. Differences in level and activity of some of the previously described important protein kinases (PKC, PKA and MAPK) negatively affect spatial and fear memory formation in DBA mice but may be reversed by pharmacological treatment⁹⁷⁻¹⁰⁰. However, a reduced capacity for paired-pulse facilitation (PPF) has also been observed⁹⁵, which suggests the presence of changes in presynaptic dynamics and short-term plasticity that may further contribute to the physiological and behavioral phenotype in DBA mice.

FHM1 Cav2.1 channel mutations cause neuronal hyperexcitability

Neuronal membrane depolarization triggers opening of voltage-gated calcium channels, allowing an influx of calcium, one of the principal intracellular signaling molecules. This influx activates calcium-dependent enzymes and sensors, and, depending on the type of channel, leads to changes in gene expression and facilitates neurotransmitter release¹⁰¹⁻¹⁰³. The class of voltage-gated calcium channels consists of several types of channels, which differ in their mode of activation (high- or low-voltage-activated), biophysical properties and pharmacological response to toxin-derived blockers¹⁰⁴. Voltage-activated channels consist of a pore-forming $\alpha 1$ -subunit and auxiliary subunits (β , $\alpha 2\delta$, and γ). Ten different genes encode $\alpha 1$ -subunits, of which the Cav2 family encodes those most important for neurotransmitter release, i.e., the high-voltage activated Cav2.1 (P/Q-type) and Cav2.2 (N-type) channels. In the cortex, release of the excitatory neurotransmitter glutamate depends predominantly on Cav2.1 channels¹⁰⁵. Although both channel types contribute to transmitter release at hippocampal synapses, Cav2.1 channels may have a more dominant effect on release kinetics¹⁰⁶. The relative contribution of Cav2.1 channels to spike-evoked presynaptic Ca^{2+} influx is likely involved: a higher number of ions have been estimated to enter

through a single $\text{Ca}_v2.1$ channel in comparison with $\text{Ca}_v2.2$ ¹⁰⁷. In addition to its role in neurotransmitter release, $\text{Ca}_v2.1$ channels are important for regulating the size of the presynaptic active zone ⁸³ and expression of proteins involved in the release machinery ¹⁰⁸.

Clinical and experimental findings in the field of migraine research indicate that increased excitatory neurotransmission is a hallmark feature of this disease ¹⁰⁹. In a particular subset of patients with familial hemiplegic migraine (-type 1: FHM1), increased glutamatergic transmission is caused by gain-of-function mutations in the *CACNA1A* gene. Introduction of missense mutations R192Q and S218L in two transgenic FHM1 knock-in mouse models, generated by a gene targeting approach, revealed that $\text{Ca}_v2.1$ channel dynamics are altered with increasing Ca^{2+} influx and release of glutamate ¹¹⁰⁻¹¹³. Short-term forms of plasticity may also be affected by FHM1 mutations ¹¹⁴. In the R192Q mouse model, the relatively mild form of FHM1 associated with this mutation does not induce an evident migraine-like phenotype, although signs of spontaneous head pain were identified ^{115,116}. Mice that are transgenic for the S218L mutation, show a severe phenotype resembling that of FHM1 S218L patients that show, in addition to hemiplegic migraine, symptoms of cerebellar ataxia, epilepsy and, sometimes fatal brain edema after a mild head trauma ^{111,117,118}. Although $\text{Ca}_v2.1$ channels are ubiquitously expressed throughout the brain, regional differences in abundance exist. High protein expression has been found in the hippocampus ¹¹⁰. Furthermore, mRNA *in situ* hybridization indicates predominant expression in the CA3 region of the hippocampus ¹¹⁹. Taking into consideration the functional importance $\text{Ca}_v2.1$ channels for synaptic plasticity, cognitive and behavioral phenotypes related to hippocampal dysfunction in FHM1 mice are expected.

High-throughput -omics techniques used in the chapters

For several experiments, “next-generation” high-throughput techniques have been applied to dissect the hippocampal proteome and transcriptome. Hypothesis generating -omics techniques are a suitable means to generate new insights into downstream effects of mutations or molecular substrates of learning and memory. The proteome was studied (**Chapter 2**) using mass spectrometry-based quantification. Transcriptome differences (**Chapters 5 and 6**) were determined using tag-based next-generation sequencing methods. Furthermore, cognitive and behavioral analysis (**Chapters 3 and 4**) using conventional testing combined with

automated high-throughput phenotyping (*phenomics*) was applied to determine the impact of Cav2.1 mutations.

Mass spectrometry-based quantification of the proteome

Techniques based on mass spectrometry (MS) provide a powerful entry to identify and quantify a large number of proteins in a single experiment. Different strategies can be employed, i.e., by quantification of labeled peptides or of previously specified peptides in a label-free manner. The first is used in the popular iTRAQ (isobaric tags for relative and absolute quantification) technique that allows for simultaneous quantification of peptides originating from multiple samples, each labelled with a tag to assign the proteins to a sample. Disadvantages of this method are that it underestimates changes in protein abundance and is less suited for complex experimental designs involving many samples^{120,121}. These shortcomings in full proteome investigations are better covered in a recently developed label-free workflow, i.e., sequential window acquisition of all theoretical fragment ion mass spectra (SWATH)^{122,123}.

Transcriptome quantification with tag-based next-generation sequencing

In the past decade, the development of next-generation sequencing (NGS) has advanced the methods to determine the full range of genomic transcription. In contrast to microarray methods, examining the transcriptome using NGS is not biased by predefined probes. Furthermore, NGS methods provide absolute quantification data as expression is measured in counts rather than in arbitrary units. Thus, the detection range for transcript expression is theoretically only limited by the sequencing depth¹²⁴. Indeed, initial RNA-sequencing studies validating the method, have found that differential expression can be reliably measured and exceeds the sensitivity of microarrays^{125,126}. Tag-based NGS methods allow for a reduced sequencing depth without loss of information on expression levels¹²⁷, by limiting the reads to either the 5'- or 3'-end of transcripts (Figure 3). Depending on the method of choice, information on differential transcript expression is also preserved, e.g., when alternative transcription start or end locations are used by a single gene.

With capped analysis of gene expression by next-generation sequencing (CAGE-seq), the 5' cap is used to select mRNAs for sequencing ¹²⁸. The resulting data on transcription start sites (TSS) for each gene can be used to search for proximal promoters to establish the type of transcription factors initiating gene expression, e.g., in response to a stimulus ¹²⁹. The other technique, deep serial analysis of gene expression by next-generation sequencing (deepSAGE) selects poly-adenylated RNAs and sequences from the 3' most CATG site ¹²⁷. Although the tag-based methods have not been directly compared, they are likely equally suited to investigate differential gene expression.

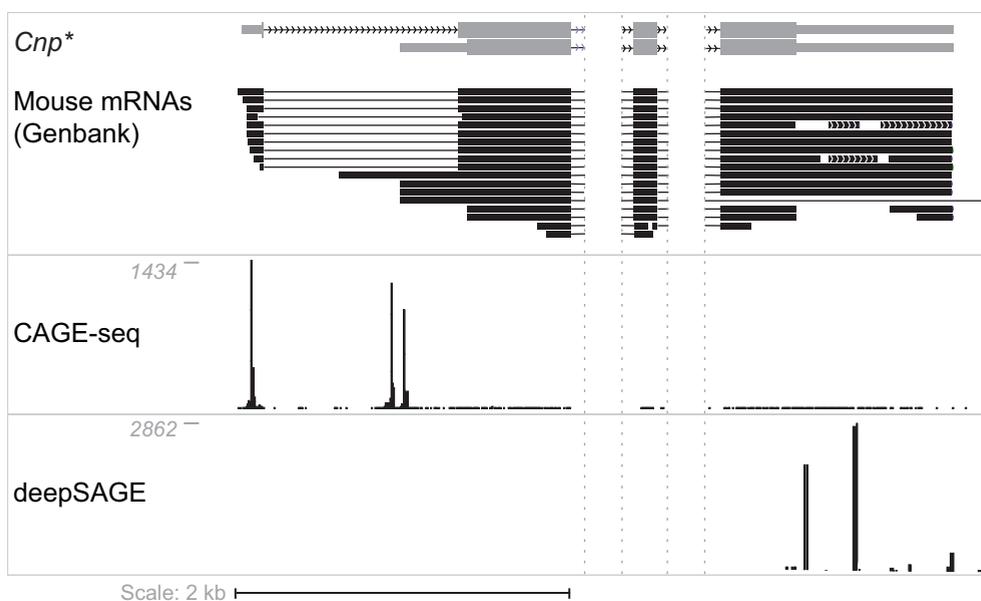


Figure 3: Tag-based sequencing methods. A screenshot of the UCSC mouse genome browser (mm10), showing the transcript variants (black; mRNAs from GenBank) of the *Cnp* gene (grey), which is abundantly expressed in the hippocampus. Introns have been shortened for illustration purposes (dashed boundaries). RNA-sequencing data is displayed in the lower half of the image. The data-tracks contain the number of tags (peak height, maximum indicated by numbers in grey) present in experimental data for two independent hippocampal samples. The first sample was measured using CAGE-seq. The three tag peaks indicate different transcription start site (TSS) positions for mRNA. Some low-level “noise” is also detected throughout the length of exons. The second sample was measured with deepSAGE and shows peaks at 3'-CATG sites most proximal to the poly-A tail of the transcripts.

Automated home-cage phenotyping of behavior and cognition

Conventional behavioral testing has provided many paradigms to study behavior, learning and memory. Nevertheless, these paradigms are often labor-intensive and their results may be confounded by laboratory (protocol) differences¹³⁰, and stress induced by animal handling¹³¹⁻¹³³. High-throughput automated home-cage phenotyping reduces human interference with testing and further improves standardization of test environments and protocols¹³⁴. Furthermore, behavior can be continuously monitored over longer periods of time (days to weeks) to provide information on natural behaviors^{135,136} in addition to tasks for learning and executive function^{137,138}. Combined automated home-cage and conventional testing allows for a comprehensive evaluation of complex behavioral phenotypes, e.g., induced by drug treatments¹³⁹ or genetic disorders^{140,141}.

Outline of the thesis

The experimental chapters in this thesis discuss the cellular and molecular mechanisms at the basis of learning and memory in the hippocampus. Topics of particular interest are the contribution of (presynaptic) proteins involved in excitatory neurotransmission. This was studied in different mouse strains and models with gain-of-function mutations in the presynaptic Cav2.1 voltage-gated calcium channel. Furthermore, molecular adaptations during memory reconsolidation were investigated.

In **Chapter 2**, the inbred strains C57 and DBA were compared with respect to the effect of genetic diversity on molecular composition, structure and function of hippocampal synapses. Proteomic analysis revealed a reduction of several proteins involved in DBA mice compared with C57. Further evidence for a presynaptic phenotype was found using ultrastructural microscopy and physiological experiments. In brief, our data suggest that in addition to well-described strain-specific post-synaptic differences, the change in dynamic properties of presynaptic transmitter release may underlie compromised synaptic processing related to cognitive impairments in DBA mice.

In Chapters 3 to 5 the behavioral, functional and/or molecular effects of increased activity-dependent glutamate release were investigated using two FHM1 mouse models with gain-of-function missense mutations R192Q or S218L in the Cav2.1 channel ^{110,111}.

In **chapter 3** we investigated the impact of the R192Q mutation on hippocampal physiology and cognitive functioning. In addition to changed dynamic properties of the calcium channel, LTP was increased by two-fold in mutant mice, whereas LTD was unaffected. Unexpectedly, both spatial and contextual fear memory were significantly impaired in mutant mice. The results suggest that abnormally enhanced hippocampal neuroplasticity may disturb memory formation.

In **chapter 4** we elaborated on the behavioral and cognitive effects of both the R192Q and S218L mutations. The presence of a cognitive and behavioral difference between R192Q and S218L fits with the notion that patients and mice with the S218L mutation have more pronounced clinical and neurobiological symptoms.

In **Chapter 5** we examined whether differences at the transcriptional level in the hippocampus of the *Cacna1a* mutants may be involved in the memory formation deficit in these mice. We applied deepSAGE and detected a small set of genes with differential expression. However, the initial results could not be validated by quantitative PCR. Although minor transcriptional changes might occur in hippocampus of *Cacna1a* mutants, the sensitivity for detection may differ per method.

In **Chapter 6**, the temporal profile of transcriptional changes underlying reconsolidation of memory is discussed. Early- and late-phase hippocampal gene expression changes after retrieval of contextual fear memory were determined using CAGE-seq. In addition, the effect on gene expression when blocking a key process in reconsolidation, i.e., AMPA receptor endocytosis, was examined. Retrieval of aversive memory was found to engage specific transcriptional programs during early and late reconsolidation.

Chapters 1 and 7 contain the Introduction and Discussion sections that put the experiments performed in this thesis in the appropriate context.

