Sleep and memory
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CHAPTER 1

General introduction
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1. SLEEP AND SLEEP LOSS

Sleep is an active, repetitive and reversible behavior that is proposed to serve several different functions. Many of the hypotheses on the function of sleep focus on several aspects of homeostasis, such as energy balance, thermoregulation, tissue restoration, detoxification and immune function (Adam and Oswald, 1977; Walker and Berger, 1980; McGinty and Szymusiak, 1990; Benington and Heller, 1995; Berger and Phillips, 1995; Inoué et al., 1995; Maquet, 1995; Krueger et al., 2003). One of the most exciting hypotheses is that sleep makes an important contribution to brain plasticity and learning and memory processes (Graves et al., 2001; Maquet, 2001; Ellenbogen et al., 2006; Walker and Stickgold, 2006; Diekelmann et al., 2009).

Given the potential importance of sleep in human physiology and health, insufficient sleep may have serious consequences. This is a pressing issue since recurrent sleep loss is a serious problem that affects many people at every level of our society (Bonnet and Arand, 1995; Hublin et al., 2001; Rajaratnam and Arendt, 2001). Some reports have suggested that our sleep time has decreased with 20% over the last century (Ferrara and De Gennaro, 2001). Importantly, not only adults experience sleep loss. The incidence of sleep loss and excessive daytime sleepiness is becoming a great issue in children and adolescents as well (Meijer et al., 2000; Frederiksen et al., 2004; Teixeira et al., 2004; Van den Bulck, 2004; Thomson and Christakis, 2005; Gibson et al., 2006).

There are several factors in modern society that may explain this decrease in sleep time. First of all, the use of artificial lightning, which has severely changed the traditional timing of human activities (Dinges, 1995). In addition, it often occurs that we sacrifice some sleep time to cope with our many social demands and daily interests, which are amongst others influenced by the unlimited access to television and internet (Vioque et al., 2000; Ohida et al., 2001; Van den Bulck, 2004; Thompson and Christakis, 2005). Another factor is work related. A 24-hour economy requires shift work, which leads to sleep loss in a large part of our modern society (Rajaratnam and Arendt, 2001). The sleep of a night shift worker is on average reduced by 2-4 hours and shift work is often associated with severe sleepiness (Akerstedt, 2003).

Acute sleep loss is associated with a decline in vigilance and attention (Corsi-Cabrera et al., 1996; McCarthy and Waters, 1997; Lim and Dinges, 2008; Tucker et al., 2009) which results in an increased risk for human errors and accidents (Webb, 1995; Leger, 1995). Whereas one may initially recover from the acute effects after subsequent sleep, frequent or chronic loss of sleep may induce neurobiological changes that accumulate over time, which may result in serious health complaints. Short and disturbed sleep have been identified as risk factors for various disorders, including cardiovascular diseases (Schwarz et al., 1999; Ayas et al., 2003; Gangwisch et al., 2006) and psychiatric disorders (Ford et al., 1989; Chang et al., 1997; Riemann and Voderholzer, 2003).

2. SLEEP AND MEMORY

The focus of this thesis will be on the consequences of sleep loss for memory processing and its possible underlying mechanisms. The notion that sleep is important for memory has been around for
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a very long time. The first experimental data supporting this came from a study by Jenkins and Dallenbach in 1924. They showed that memory retention was better in the morning after a night of sleep than in the evening after an equivalent amount of time awake. Since these findings could have been related to time of day rather than the occurrence of sleep, later studies directly compared periods of normal sleep with sleep deprivation (SD). Many of these studies have shown that SD negatively affects memory processes (Stickgold et al., 2000; Drummond and Brown, 2001; Kim et al., 2001; Graves et al., 2003; Curcio et al., 2006; Ferrara et al., 2006; Gais et al., 2006; Yoo et al., 2007; Alvarenga et al., 2008; Walker, 2008; Mograss et al., 2009; Vecsey et al., 2009; Yoo et al., 2009), which has contributed to the hypothesis that sleep has an important function in memory processing and the underlying molecular mechanisms of brain plasticity (Graves et al., 2001; Maquet, 2001; Ellenbogen et al., 2006; Walker and Stickgold, 2006; Diekelmann et al., 2009). Also, the observation of changes in sleep parameters after learning (Smith et al., 1980; Smith and Rose, 1997; Stickgold et al., 2000; Walker et al., 2002), as well as the study of cellular activity during the different sleep states after a learning task (Kudrimoti et al., 1999; Hasselmo, 2008), have contributed to this hypothesis. However, a major problem in this area of research is that the terms sleep and memory both refer to complex phenomena, neither one of which can be treated as a single event. Therefore it is important to clarify these terms and their possible relationships.

Generally speaking sleep can be divided into two main states, rapid eye movement (REM) sleep (also known as paradoxical sleep) and Non-REM (NREM) sleep. These two states alternate through the night in humans in a 90-minute cycle. NREM sleep is often further subdivided into stage 1-4, which correspond to increasingly deeper stages of sleep. Stage 3 and 4 together are called ‘slow wave sleep’ (SWS), based on the general occurrence of slow, low-frequency (0.5-4 Hz), cortical oscillations in the electroencephalogram (EEG). Although the NREM/REM cycle length remains largely stable across the night, the ratio of NREM to REM within each 90-minute cycle changes. Early in the night NREM dominates and REM sleep prevails in the latter half of the night. In humans, NREM sleep occupies approximately 80% and REM sleep around 20% of the total sleep time. NREM and REM sleep radically differ from each other in electrophysiological characteristics and neurochemical regulatory mechanisms, and perhaps even in function as well (Hobson & Pace-Schott, 2002). Therefore, sleep cannot be considered a homogeneous state.

Just as sleep, also memory cannot be treated as a single state. Memory can be divided into at least two distinct forms according to its temporal properties: short term memory (STM), which involves temporal storage of information and lasts no longer then a few hours, and long term memory (LTM), which lasts from several hours to days or even longer (McGaugh, 1966; Davis and Squire, 1984). Furthermore, it is known that multiple memory systems store different classes of memory in different brain regions. A popular classification for memories is based on the distinction between declarative and nondeclarative memories (Squire, 1992; Squire and Zola, 1996). Declarative memory is considered as consciously accessible memories of facts (semantic) and events (episodic) (i.e., knowing “what”). This type of memory is crucially dependent on structures of the medial temporal lobe, including the hippocampus (Eichenbaum, 2000). The nondeclarative category includes procedural memory, such as the learning of skills (i.e., knowing “how”). Procedural memory is in part
dependent on the striatum, which is situated in the forebrain (Kreitzer and Malenka, 2008). Furthermore, memories can be explicitly or implicitly learned, which means that they can be consciously acquired or acquired without conscious knowledge. In general, declarative material is explicitly learned and procedural material is often implicitly learned (Squire, 1986; Squire and Zola-Morgan, 1988).

Memory processing encompasses several stages, including acquisition, consolidation and retrieval that occur over time. Acquisition is the uptake of new information during learning and the encoding of this information into a memory trace that is initially still unstable. Subsequently, this memory representation may go through a stage of consolidation during which it becomes more stable and often resistant to interference (Davis and Squire, 1984; McGaugh, 2000). Retrieval refers to the process of recalling a stored memory. It is believed to destabilize the memory, making it labile and susceptible to either reconsolidation or to degradation when the stored information is no longer valid. Reconsolidation is the process of restabilizing the labile memory trace (Nader, 2003). When a destabilized memory is not reconsolidated, it can degrade relatively quickly, although it is unclear whether the memory trace is actually weakened or only made inaccessible to recall processes.

3. SLEEP DEPRIVATION AND MEMORY STAGES

Various behavioral studies have described the negative consequences of inadequate sleep prior to learning on memory encoding. Several human studies exposed subjects to SD prior to training in a learning task and showed that declarative memory encoding was significantly disrupted. These effects were found for the encoding of recognition and temporal memory (Morris et al., 1960; Harrison & Horne, 2000; Yoo et al., 2007), verbal memory (Drummond et al., 2000; Drummond & Brown, 2001) and emotional memory (Walker and Stickgold, 2006). Also animal studies have shown that pre-training SD impairs memory encoding of numerous declarative learning tasks, especially of hippocampus-dependent learning tasks. Learning of hippocampus-independent tasks was more resistant to prior SD (McDermott et al., 2003; Guan et al., 2004; Ruskin et al., 2004).

In addition to the impact of prior SD on memory encoding a substantial body of work also demonstrates the impact of SD following learning on memory consolidation. Several human studies show that SD following training affects the consolidation of memory in various procedural tasks, such as memory for a visual discrimination task (Karni et al., 1994, Stickgold et al., 2000). Also declarative memory for spatial information and recognition memory is impaired by SD (Ferrara et al., 2006; Mograss et al., 2009). In human research the use of partial or selective post-training SD are common. The first half of nocturnal human sleep is dominated by SWS whereas REM sleep dominates during the second half. Wakefulness during the first half of the night prevented an improvement in a declarative paired-associate list task, whereas wakefulness during the second half of the night prevented an improvement in a procedural mirror tracing task (Plihal and Born, 1997). These results have been taken to suggest that SWS in humans is important for declarative memory and REM sleep for procedural memory. However, most data on the effects of SD on declarative memory are derived
from animal studies. For example, it is shown that SD following training negatively affects recognition (Palchykova et al., 2006), contextual fear (Graves et al., 2003; Vecsey et al., 2009) and spatial memory (Smith and Rose, 1996, 1997).

It is suggested from human literature that there is a critical time window following training which is sensitive to sleep loss. For example, it has been shown that SD during the first night following task acquisition reduces performance upon testing a week later, whereas SD during the following nights had no effect (Smith, 1993; Smith and Whittaker, 1987). Animal studies support such a critical time window and show an even more specified time window. SD during the first 5 to 6 hours of the first resting phase following training impairs memory, whereas delayed SD for 5 to 6 hours with an onset 5 to 6 hours following training has no effect (Graves et al., 2003; Palchykova et al., 2006; Vecsey et al., 2009).

4. SLEEP DEPRIVATION AND HIPPOCAMPUS-DEPENDENT MEMORY

It is shown that SD prior to learning as well as after learning negatively affects declarative memory processes. As mentioned previously, one of the key brain structures involved in declarative memories is the hippocampus, which is located in the medial temporal lobe (Eichenbaum, 2000) and appears to be particularly sensitive to SD (Smith and Rose, 1996, 1997; Graves et al., 2003; Ferrara et al., 2006, 2008; Vecsey et al., 2009).

The hippocampus can be divided in several major subregions: the dentate gyrus (DG), Cornu Ammonis 3 (CA3), Cornu Ammonis 1 (CA1) and the subiculum; see Fig. 1 (Amaral and Witter, 1989; Granger et al., 1996). The granule cells in the DG receive most input from the entorhinal cortex via the perforant path. Subsequently, these cells project to the pyramidal cells in the CA3 area via the mossy fibers. In addition, the CA3 region also receives direct input via the perforant path. It is suggested that these two major afferent inputs of the CA3 may contribute differentially to encoding (DG-originated mossy fibers input) and retrieval of spatial memory (perforant path input) (Lee and Kesner, 2004). The CA1 area receives input from projections of the CA3 pyramidal cells via the Schäffer collaterals as well as from the entorhinal cortex via the perforant path (Nakashiba et al., 2008). Finally the pyramidal cells of the CA1 area project back to the entorhinal cortex, either directly or via the subiculum, and project to the main limbic circuit, including the mammillary bodies, anterior thalamic nuclei and the cingulate limbic cortex (Vinogradova, 2001).

The hippocampus is known for its rapid formation of spatial representations of the environment (i.e., spatial maps). In addition, it can rapidly develop associations between a specific context and the event that occurred within that context, for example an association between an aversive stimulus such as a foot shock and the spatial environment in which it occurred (i.e., contextual fear conditioning) or an association between spatial cues and the location of an invisible platform (i.e., spatial learning in the Morris water maze). Lesion studies have clearly demonstrated the critical involvement of the hippocampus in such tasks as spatial learning in the Morris water maze (Schenk and Morris, 1985) or contextual fear conditioning (Phillips and LeDoux, 1992; Chen et al.,
Both tasks also have a hippocampus-independent version. For example, in case of fear conditioning this task is called cued fear conditioning and instead of the requirement for an association between a specific context and a shock, this task depends on the association between a neutral conditioned stimulus, such as a light or a tone cue, with an aversive shock stimulus (Phillips and LeDoux, 1992).

Various studies in rodents have shown that SD selectively impairs hippocampus-dependent memory processing. For example, it is shown that SD prior to training impairs behavioral performance in a hippocampus-dependent, contextual fear conditioning task, but not in a hippocampus-independent cued fear conditioning task (McDermott et al., 2003; Ruskin et al., 2004). Also by using the water maze it is shown that SD prior to training affects specifically hippocampus-dependent spatial memory, but not hippocampus-independent non-spatial memory (Guan et al., 2004; Yang et al., 2008). Also human studies show effects of SD on memory encoding for learning tasks that involve the hippocampus. A single night of SD produces a significant deficit in hippocampal activity during episodic memory encoding, resulting in worse subsequent retention (Yoo et al., 2007). Also mild sleep disruption that suppressed slow wave activity (SWA) and induced shallow sleep without reducing total sleep time is sufficient to affect subsequent successful encoding-related hippocampal activation and memory performance (Van der Werf et al., 2009).

Figure 1. The hippocampus is a key brain structure involved in declarative memories. (A) A schematic representation of the position of the hippocampus within a rat brain. (B) A schematic picture of the major subregions of the hippocampus: dentate gyrus (DG), cornu ammonis 3 (CA3); cornu ammonis 1 (CA1) and the subiculum (Sub).
Next to the effects of SD prior to learning on memory encoding also the effects of post-training SD on hippocampus-dependent memory consolidation have been clearly demonstrated. In rodent studies it is shown that SD selectively impairs memory consolidation for hippocampus-dependent contextual fear conditioning (Graves et al., 2003; Vescey et al., 2009) and for hippocampus-dependent spatial information in a Morris water maze task (Smith and Rose, 1996, 1997). Human studies confirm that SD after learning affects hippocampus-dependent spatial memory consolidation. For example, it is shown that SD prevents the increase in performance in a spatial learning task that is seen after a night of sleep (Ferrara et al., 2006, 2008). The effects of SD on the consolidation of new memories are under extensive investigation. In most human and animal studies subjects are immediately sleep deprived following a learning task that was performed near or in the resting phase. However, in real life, subjects do not only learn right before they go to sleep, but often learn during their active phase. For this reason, it is important to examine the effects of SD when learning occurs at the beginning of the active phase, which is what we did in one of the studies described in this thesis.

In addition to its role in the formation of new memories, the hippocampus is also thought to be important for adaptation of existing memories that require updating because of changes in former associations. Such adaptation of memories and learned behaviors is an important aspect of successfully coping with changes that frequently occur in our surroundings, e.g., in the case of moving to a new home, school, or job. In the so called match-mismatch process the hippocampus compares actual sensory information from the environment with previously stored representations (Gothard et al., 1996; Knight, 1996). To investigate the effects of experimental manipulations, such as SD, on this process, subjects are confronted with changes in a familiar context, for example in case of rodents the relocation of a hidden platform in the Morris water maze or the relocation of a food reward in a maze. While the effect of SD on the formation of new memories has been topic of numerous studies, the effects of SD on the adaptation of existing memories have so far received scarce attention. For this reason we examined in one of the chapters of this thesis not only the effects of SD on memory formation but also on memory adaptation.

5. HIPPOCAMPAL SYNAPTIC PLASTICITY

Clearly, SD affects hippocampal functioning. Recently, scientists started to determine the underlying cellular mechanisms of this hippocampal deficit. Although behavioral and electrophysiological studies in humans show the importance of sleep and the detrimental effects of SD on memory processes, animal experiments are essential to determine the underlying neuronal and molecular mechanisms.

At a cellular level, changes in synaptic strength and membrane excitability are thought to be critical for the formation of memories (Bliss and Collingridge, 1993). This mechanism is based on the lasting modulation of the synaptic contact between two neurons (Hebb, 1949; Viana di Prisco, 1984; Siegelbaum and Kandel, 1991; Bailey et al., 2000). Long term potentiation (LTP) and long term depression (LTD) are widely accepted cellular models for synaptic plasticity underlying certain types
of learning and memory (Malenka and Bear, 2004). In LTP, the strength of a synapse is increased following high frequency stimulation (HFS). As a result the postsynaptic neuron can be more easily excited. Conversely in LTD, the strength of a synapse is decreased following low frequency stimulation (LFS). As a consequence the neuron will be less excitable. The molecular mechanisms of LTP and LTD have been extensively characterized, especially in the hippocampus (Malenka and Nicoll, 1999). The most prominent-or at least the most commonly studied- forms of LTP in the hippocampus require the activation of postsynaptic N-methyl-D-aspartate (NMDA) type and α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA) type of glutamate receptors (Collingridge et al., 1983; Dudek and Bear, 1992; Bear and Abraham, 1996).

NMDA receptors consist of two families of homologous subunits, the NR1 and NR2 subunits (Meguro et al., 1992; Kutsuwada et al., 1992), and are assembled from one NR1 and one or more NR2 subunits (Hollmann and Heinemann, 1994; Sheng et al., 1994). Several isoforms of the NR2 subunit exist (NR2A-D) that control the properties of the NMDA receptor channel, including the threshold for the removal of the voltage-dependent Mg\(^{2+}\) block from the channel (Cull-Candy et al., 2001). AMPA receptors are composed of four different subunits (GluR1-4) that can assemble in different combinations (Hollmann and Heinemann, 1994). The GluR1 subunit is predominantly expressed in the forebrain, including the hippocampus (Squire, 1992) and is regulated by protein phosphorylation on its intracellular carboxy-terminal domain (Roche et al., 1996).

When the postsynaptic membrane is sufficiently depolarized during NMDA receptor-induced LTP, this relieves the voltage-dependent Mg\(^{2+}\) block from the NMDA channel (Mayer et al., 1984). Now the channel can be activated by glutamate resulting in a postsynaptic Ca\(^{2+}\) influx through these channels which is associated with the activation of a number of kinases, among others cAMP dependent protein kinase A (PKA), which leads to the phosphorylation of AMPA receptors at the serine (S) sites of the GluR1 subunit. Phosphorylation of S831 increases the single channel conductance (Benke et al., 1998; Derkach et al., 1999) and phosphorylation of S845 increases the mean open probability and the number of GluR1 containing AMPA receptors into the synapse (Esteban et al., 2003).

Over the last decade it has been demonstrated in various studies that total SD as well as selective REM SD and sleep interruption impair LTP induction in the hippocampus in vivo as well as ex vivo (Campbell et al., 2002; Davis et al 2003; McDermott et al., 2003; Romcy-Pereiro & Pavlides, 2004; Kim et al., 2005; Kopp et al., 2006; Tartar et al., 2006; Ravassard et al., 2009; Vecsey et al., 2009). Furthermore, it is shown that SD after LTP induction impairs LTP maintenance (Ishikawa et al., 2006).

Recent studies have sought to determine the synaptic mechanisms of the SD-induced inhibition of LTP. Most of these studies focused on long periods of SD. It is for example shown that 72 hours of SD led to a reduction in NMDA receptor function in the hippocampus, most likely because of altered NMDA receptor surface expression (McDermott et al., 2006). But also total SD for as little as 4 hours altered NMDA receptor function (Kopp et al., 2006). The latter study shows an alteration in the molecular composition of synaptically activated NMDA receptors. Yet, few studies have been performed on AMPA receptor function; although there is some indication that at least long term REM
SD affects AMPA receptor expression. Seventy-five hours of REM SD not only decreased protein levels of the NR1 subunit of the NMDA receptor, but also protein levels of the GluR1 subunit of the AMPA receptor (Ravassard et al., 2009). Since few studies examined AMPA receptor functioning following relatively short periods of SD, which are more common in everyday life, we focused on this topic in one of the chapters of this thesis (see Fig. 2).

6. CREB, PROTEIN SYNTHESIS AND MEMORY STORAGE

Various studies have shown that protein synthesis is required to maintain LTP and to form LTM (Davis and Squire, 1984; Frey et al., 1993; Huang et al., 1994; Nguyen and Kandel, 1996). It is clearly demonstrated that inhibition of protein synthesis around the time of learning blocks LTM (Abel et al., 1997; Bourjouladze et al., 1998; Schafe et al., 1999).

One significant element in the regulation of neuronal gene expression and protein synthesis is 3',5'-cyclic adenosine monophosphate (cAMP) response element binding protein (CREB). This transcription factor binds as a dimer to cAMP-response element (CRE) sites, a promoter region of several genes, and thereby promotes the synthesis of a wide variety of proteins involved in LTM (Lanahan and Worley, 1998; Lonze and Ginty, 2002). The importance of CREB, in the maintenance of LTP and in the formation of LTM has been clearly shown in several studies (Frank and Greenberg, 1994; Yin and Tully, 1996; Abel and Kandel, 1998; Silva et al., 1998). Furthermore, this protein is extensively studied in hippocampus-dependent tasks.

Several intracellular signaling cascades are involved in CREB-regulated gene transcription, including the ones that involve adenylyl cyclase (AC), cAMP (Dash et al., 1991), Ca^{2+} (Dash et al., 1991; Sheng et al., 1991) and mitogen activated protein kinase (MAPK) (Xing et al., 1996). CREB can be activated and phosphorylated via several kinases, including protein kinase A (PKA), protein kinase C (PKC), calmodulin kinase (CaMK) IV (Brindle and Montminy, 1992; Sassone-Corsi, 1995) and MAPK (Martin et al., 1997; Michael et al., 1998). Each of these kinases phosphorylates CREB at the S133 site (Gonzalez and Montminy, 1989; Gonzalez et al., 1989). Phosphorylation of S133 recruits the CREB binding protein (CBP), to the CREB/CRE complex and promotes transcription of downstream genes such as the immediate early genes *c-fos* (Sheng et al., 1990) and *zif268* (Bozon et al., 2003), whose products, in turn, induce the transcription of late downstream genes, and activate direct ‘effector’ proteins, such as structural proteins, signaling enzymes or growth factors, that are essential for LTM (Lanahan and Worley, 1998; Lonze and Ginty, 2002).

Treatments that suppress CREB impair hippocampus-dependent memory for contextual fear conditioning and spatial water maze learning (Bourjouladze et al., 1994; Guzowski and McGaugh, 1997). Conversely, treatments that induce CREB expression enhance the formation of hippocampus-dependent memory (Josselyn et al., 2001; Chen et al., 2003; Brightwell et al., 2007). Furthermore, it is shown that hippocampus-dependent spatial learning increases CREB phosphorylation (Mizuno et al., 2002; Colombo et al., 2003). Training in a spatial task is associated with progressively increasing levels of phosphorylated CREB (pCREB). pCREB expression in response to training is low on the first
day but is increased on subsequent training days. These temporary increases in pCREB continue to show up after late training sessions, when animals have already mastered the task (Mizuno et al., 2002; Porte et al., 2008).

Since it is demonstrated that CREB is critically involved in LTM formation in several hippocampus-dependent tasks that are known to be affected by SD, it is an ideal candidate to study when examining the effects of SD on memory formation at a molecular level (see Fig. 2). In addition, the time-window during which CREB phosphorylation is increased following training makes it particularly interesting to study in relation to SD. It is shown that pCREB expression is up-regulated for discrete periods of time during the first 6 hours following training for hippocampus-dependent contextual fear conditioning and passive avoidance learning (Bernabeu et al., 1997; Stanciu et al., 2001). Furthermore, it is shown that inhibition of protein synthesis and protein kinase A (PKA), a kinase that phosphorylates CREB, within a similar time range impair the formation of contextual fear conditioning (Bernabeu et al., 1997; Bourtchouladze et al., 1998; Wallenstein et al., 2002). Similar to inhibition of PKA and protein synthesis, post-training SD impairs memory consolidation in a manner that is dependent on the exact time after training during which animals are sleep deprived. SD performed for 5 hours immediately post training impairs memory consolidation of hippocampus-dependent contextual fear memory, whereas delayed SD, starting 5 hours after training has no effect (Graves et al., 2003; Vecsey et al., 2009). This supports the hypothesis that SD might act on memory consolidation by interfering with pathways that involve pCREB (Graves et al., 2003). Indeed, it was recently shown in mice that brief SD by itself impairs cAMP- and PKA signaling in the hippocampus (Vecsey et al., 2009) and also negatively affects phosphorylation of the transcription factor CREB. However, so far no studies examined whether SD immediately following training in a learning task affects the increase in pCREB expression induced by training. Therefore, in several chapters of this thesis we examined whether SD following training disrupts the normal training-induced increases in pCREB expression.

In addition, so far few studies examined whether SD-induced behavioral impairments seen during the testing for memory are associated with changes in neuronal activation and pCREB expression in relevant and specific brain areas. Since it is known that pCREB expression is up-regulated following testing for memory (Hall et al., 2001; Mamiya et al., 2009), we also examined in this thesis whether an attenuated behavioral response during testing for memory was associated with a reduced increase in pCREB expression in relevant brain areas.

Figure 2. Schematic representation of two of the intracellular signaling pathways known to be involved in synaptic plasticity and memory formation. In the present thesis we specifically focused on AMPA receptor functioning and activation of CREB during the investigation of possible molecular mechanisms underlying the effects of SD on memory processes.
7. AIM AND OUTLINE OF THE THESIS

SD is a common problem in our society that may have detrimental effects on our lives. Therefore, understanding the cellular and molecular pathways affected by SD is of social and clinical importance. The major aim of this thesis was to assess effects of SD on memory processes and the possible mechanisms underlying these effects. Emphasis was given to the effects of SD on hippocampus functioning, being one of the brain regions involved in memory processes that is particularly sensitive to sleep loss. Since animal studies rely on forced SD, it is sometimes argued that stimulation and stress involved in the SD method might contribute to the SD-induced memory impairments. We therefore performed several experiments in this thesis to address this issue.

So far, most animal studies that focused on the effects of SD prior to learning on subsequent behavioral performance applied long, multiple-day periods of SD (at least 72 hours). Therefore, in chapter 2 we aimed to assess the consequences of relatively short periods of SD within the range of the normal resting phase. We examined the consequences of SD prior to learning on the processing of new information and hippocampus-dependent working memory. In addition, we determined the effects of SD on hippocampal glutamate AMPA receptor function, which is crucially involved in working memory but so far received little attention in relation to the consequences of sleep loss. Adult mice were subjected to different durations of SD (0, 6, or 12 hours) followed by a novel arm recognition task in which spatial working memory performance was assessed. In a different batch of mice SD was immediately followed by brain collection for determinations of AMPA receptor protein levels and phosphorylation, as well as for the assessment of proteins that regulate AMPA receptor phosphorylation. In this experiment we also measured levels of the stress hormone corticosterone (CORT) at the end of the SD period to assess whether the effects of SD might be related to stress.

To further assess the effects of SD on different memory stages, we performed a number of experiments aimed at the consolidation of new memories as well as the adaptation of existing memories. Particularly, the effects of SD on memory adaptation and flexibility so far received little attention. In chapter 3, a study was performed with a dual solution maze paradigm in which animals had to learn to locate a food reward. Animals had the opportunity to solve the task based on visual cues (i.e., hippocampus-dependent spatial learning strategy) or by learning to always make the same turning response (i.e., striatum-based response learning strategy). Part of the animals was subjected to 5 hours of SD after each daily training session. We further assessed the effects of SD on memory adaptation by relocating the food reward to a previously non-baited arm once the animals had learned the first location. Since distinct cognitive strategies are paralleled by brain region-specific increases in the phosphorylation of the transcription factor CREB (pCREB), we also examined whether SD following training affected pCREB levels in the hippocampus and striatum. Finally, control experiments were conducted to determine effects of SD on stress hormone levels and anxiety in order to establish whether these potential factors might contribute to the changes in CREB expression and memory performance.

In chapter 4 we continued on the findings of chapter 3. In the latter chapter animals could use different learning strategies to solve the maze task. Any SD-induced hippocampal deficit could
potentially be compensated for by applying a striatal, non-spatial learning strategy to solve that maze task. Therefore, in the next experiment we forced animals to use a hippocampal strategy by applying a single-solution, spatial version of the T-maze paradigm. In this case we aimed to test whether SD after training would immediately result in a learning impairment by lack of compensatory mechanisms.

In the following chapters we used the contextual fear conditioning paradigm. Since this is a single-trial task, it has the advantage that one can more easily and directly examine the effects of SD following training on selectively memory consolidation. In addition, one can specifically focus on the consolidation of hippocampus-dependent memories. In chapter 5 we investigated whether brief SD following training impairs memory consolidation for contextual fear in rats and attenuates the subsequent behavioral freezing response. In addition, we aimed to assess whether a SD-induced memory impairment and attenuated freezing response would be associated with attenuated endocrine and neuronal responses. Animals were subjected to 6 hours of SD immediately following training. During training an animal was placed in a chamber and exposed to the conditioning context for 3 minutes followed by a mild electric foot shock. Twenty-four hours later the animal was re-exposed to the same shock context, without receiving a shock. In one part of the study freezing behavior and the corticosterone response were assessed upon re-exposure to the shock context. In a different batch of animals testing for contextual fear memory was followed by brain collection to investigate neuronal activation in relevant brain areas, as examined by immunoreactivity for phosphorylated CREB.

In chapter 6 we continued on the findings of chapter 5. In the latter chapter, as well as in many other experimental studies that examined the role of sleep in memory consolidation, the learning task is performed near or in their main resting phase and subjects are sleep deprived immediately following training. However, in real life, subjects do not only learn right before they go to sleep, but often learn during their active phase. Therefore, in chapter 6, we examined the effects of SD on memory consolidation for contextual fear in rats, not only when the task was performed at the beginning of the resting (light) phase, but also right before the onset of the active (dark) phase. Furthermore, we assessed whether effects of SD might be related to stress hormones or the amount of stimulations the animals received during the SD period. Finally, Chapter 7 discusses all findings of the thesis.
Chapter 1

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General introduction


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