Neurobiological and functional consequences of chronic partial sleep deprivation

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Chapter 1

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1. WHY DO WE SLEEP?

The fact that we spend every day approximately eight hours asleep which, added up, is the third of our lives, suggests that sleep must have an important physiological function. Similar to humans, all animals show cycles of behavioural rest and activity of varying length, and the resting phase often proves to be a sleep-like condition comparable to our sleep (Shaw et al., 2000; Horne, 2002). Since sleep is specific for animals with complex nervous structures, the generally accepted idea is that sleep is primarily for the brain. This idea is supported by the effects of sleep deprivation: the loss of sleep leads to clear changes in the brain’s electrophysiological activity (Borbély and Neuhaus, 1979, Dijk et al., 1987). On top of such a fundamental neurobiological change, the first symptoms of sleep loss concern the higher executive functions of the brain comprising mood, cognition and motor control (Maquet, 2001; Durmer and Dinges, 2005). Next to its effects on the nervous system, sleep restriction also influences peripheral functions including adverse circulatory, metabolic, endocrine and immunological effects (Spiegel et al., 1999; Vgontzas et al., 2004; Gangwisch et al., 2006). Since sleep deprivation leads to complex changes in several systems, and since we do not know which of these is more important than the other, the exact function of sleep is still a hot and fiercely debated topic in the life sciences.

Hypotheses on the function of sleep are numerous and many of them try to explain the function of sleep as a homeostatic process. Some of the hypotheses focus on different aspects of brain homeostasis such as restoration, energy balance, temperature regulation or detoxification (Walker and Berger, 1980; McGinty and Szymusiak, 1990; Inoué et al., 1995; Maquet, 1995). The restorative hypothesis has been further developed by Benington and Heller (1995), who suggested that sleep is a state that allows the replenishment of the brain’s glycogen stores, which serve as an important energy buffer supporting neuronal activity during waking (Gruetter, 2003; Brown, 2004). Another leading hypothesis champions sleep as a state that supports neuronal plasticity and synaptic organization (Benington and Frank, 2003; Tononi and Cirelli, 2006), which would support basic neuronal function and communication but also help learning and memory processes (Crick and Mitchinson, 1983; Walker and Stickgold, 2004).

What makes the quest for the function of sleep difficult is that sleep is not a homogenous state. The brain’s electrical activity changes throughout sleep following a cyclic pattern, where non-rapid eye movement (NREM) sleep and rapid eye movement (REM) sleep alternate. As sleep has these two forms, it may be that sleep has multiple and stage-specific functions (Benington and Heller, 1995; Siegel, 1995; Walker and Stickgold, 2004). A recent hypothesis discusses NREM sleep in particular as a state which primarily serves synaptic homeostasis (Tononi and Cirelli, 2006). According to this hypothesis, high neuronal activity during waking promotes synaptic potentiation and increases the strength, size and number of synapses. This prolonged waking-induced synaptic potentiation would reduce available space and energy reserves in the brain and conversely, sleep would serve to downscale overall synaptic strength and bring it down to lower levels. Importantly, synaptic downscaling during NREM sleep would decrease the overall synaptic
strength but conserve the relative differences in synaptic strength between specific neuronal populations with different levels of activity.

REM sleep, on the other hand, may function to support neuronal plasticity and promote synaptic strength, particularly in neuronal circuits involved in memory formation (Walker and Stickgold, 2004). Such a role of REM sleep is supported by the fact that after waking, which is associated with information processing and learning, the subsequent REM sleep stages are characterized by the re-activation of learning-specific neuronal firing patterns and re-expression of genes involved in synaptic plasticity and memory formation (Graves et al., 2001; Benington and Frank, 2003).

Acting like this, NREM sleep and REM sleep together may serve to maintain overall synaptic homeostasis and at the same time promote the relative synaptic strength in specific circuits that are actively used for memory traces and cognitive functions. Thereby, the two sleep states together might increase synaptic efficacy and signal-noise ratio.

Whatever the exact function(s) of sleep may be, sleep is certainly needed for a proper and well-balanced mental functioning. Indeed, without sleep, mood, cognitive function, and motor performance are the most prominently impaired functional outputs in humans (Pilcher and Huffcutt, 1996; Dinges et al., 1997; Van Dongen et al., 2003).

2. CHRONIC PARTIAL SLEEP LOSS

Among humans living in modern society, chronic partial sleep deprivation is increasingly common (Bonnet and Arand, 2003; Alvarez and Ayas, 2004; Malik and Kaplan, 2005). Sleep loss is due to several factors comprising lifestyle, work-related factors and stress. (1) Lifestyle-like reasons include the element of personal choice, which is influenced by the unlimited access to television and the internet (Vioque et al., 2000; Ohida et al., 2001; Thompson and Christakis, 2005). Next to the personal choice element, the pressure of the 24/7 society, where everything is available at any time during the day and where circadian rhythms and geographical time zones are ignored, further threatens normal and healthy sleep schedules (Rosekind, 2005). (2) Shiftwork is also a prominent factor that leads to chronic loss of sleep in a large segment of modern society (Rajaratnam and Arend, 2001). Not only does doing shifts lead to the loss of sleep, but it also seriously and persistently damages the timing and architecture of sleep that people working in such schedules are allowed (Dumont et al., 1997; Akerstedt, 2003). Finally, (3) stress and sleep loss make up a kind of vicious circle, where the one is able to induce the other and vice versa (Riemann and Voderholzer, 2003; Taylor et al., 2005). In other words, daytime feelings associated with everyday stressors are likely to continue as unsettling thoughts in bed making it difficult to fall asleep, and conversely, insomnia is mostly experienced as feeling stressed. The inability to fall asleep is common among humans: insomnia has been reported in 10-35% of the population (Ohayon, 2002; Ohayon and Partinen, 2002). All in all, these factors, mostly in combination, have led to major changes in human sleep hygiene, illustrated by the fact that during the last century our average
General introduction

sleep time has been reduced by approximately 20%, which means hours of sleep lost (Ferrara and De Gennaro, 2001).

Importantly, the incidence of sleep loss and excessive daytime sleepiness are high not only among working adults but are becoming an important issue among children and adolescents as well (Fredriksen et al., 2004; Teixeira et al., 2004; Amschler and McKenzie, 2005; Thompson and Christakis, 2005; Gibson et al., 2006).

Interestingly, humans adapt to increasing sleepiness which is inherent to chronic partial sleep deprivation (Carskadon and Dement, 1981), and tend to disregard the profound cognitive deficits that accumulate over time and view sleep as a luxurious item in their daily routine (Durmer and Dinges, 2005). However, the question remains whether chronic loss of sleep results in gradual and persistent neurobiological changes that may, on the long-term, have consequences for physical and mental well-being.

3. CHRONIC PARTIAL SLEEP LOSS AND MOOD

Several human studies show that sleep deprivation has detrimental effects on mood and emotionality, which may even exceed the impact on cognition and motor performance (Pilcher and Huffcutt, 1996; Dinges et al., 1997; Lieberman et al., 2002; Bonnet and Arand, 2003; Haack and Mullington, 2005; Scott et al., 2006). A meta-analytical study has demonstrated that chronic partial sleep deprivation (less than 5 hours sleep deprivation per day) has even more profound functional effects on cognitive function than either short or long-term total sleep deprivation (less than 45 hour continuous sleep deprivation or more, respectively) (Pilcher and Huffcutt, 1996). This finding underscores the importance of the cumulative feature of changes caused by sleep loss.

In the long run, chronically restricted or disrupted sleep may even play a role in the development of psychopathologies. According to the traditional view, sleep loss or insomnia is a symptom of depressive episodes or mood disorders (Adrien, 2002). The inability to sleep is possibly caused by increased anxiety, disturbed circadian rhythms or by the neurobiological impairments linked to depression (Benca, 2000). On the other hand, an increasing number of studies suggest that sleep problems may in some cases be primary to mood disorders (for review see Riemann and Voderholzer, 2003). It still may be that insomnia is both cause and symptom of depression however, the exact causal relationship between insomnia and mood disorders remains yet unclear (Abad and Guilleminault, 2005; Taylor et al., 2005; Turek, 2005). Nevertheless, the idea of sleep loss being primary to mood disorders is supported by mounting evidence showing that insufficient sleep may precede mood disorders including depressive or manic episodes, clinical depression and anxiety (Wehr et al., 1987; Wright, 1993; Taylor et al., 2003; Taylor et al., 2005; Kaneita et al., 2006). Sleep loss or disturbances in adolescents may even signal an increased risk for future suicidal behaviour (Liu and Buysse, 2005). Epidemiological studies have shown that insomnia may be a risk factor for depression over a few years (follow-up period ranging from 6 month to 3 years) in young as well as older adults (Ford and Kamerow, 1989; Livingston et al.,
Chapter 1

1993; Breslau et al., 1996; Foley et al., 1999; Perlman et al., 2006). These mood changes not only occur within a few years, but also on much longer time scales: the sensitivity to or occurrence of sleep disturbances can predict depression even over several decades. One particular longitudinal study with a follow-up time of 30 years, the Johns Hopkins Precursors Study, showed that insomnia in young men indicates a significantly greater risk for clinical depression later in life (Chang et al., 1997).

Next to depression, sleep loss can also be a predictor of other mood disorders such as mania or anxiety. This issue has been explored in a study where individuals were asked to identify the prodromes of their mood disorder, and almost 80% of the subjects identified sleep disturbance as the most robust predictor of mania (Jackson et al., 2003). Another study with a follow-up period of 10-15 years demonstrated that childhood sleep problems are associated with anxiety disorders in adulthood (Gregory et al., 2005).

A further psychopathology for which sleep disturbances have been suggested as a mediating factor, is the posttraumatic stress disorder (PTSD). PTSD is caused by a major life event (such as war combat, car accident, catastrophe, rape, etc.) and one of its main symptoms is insomnia along with other sleep disturbances (Singareddy and Balon, 2002). However, sleep changes prior to or shortly after the stressful event can also predict the development of the disorder. In one study, 77% of rape victims who developed PTSD reported sleep problems before the sexual assault (Krakow et al., 2001). This suggests that disturbed sleep may change the brain in a way that makes it more vulnerable to PTSD, should a major life event occur. Another study showed that sleep complaints within one month of a vehicle accident can predict which of the victims will develop PTSD over a year (Koren et al., 2002). All these results indicate that the relationship between sleep and stress is complex and probably bidirectional (Krakow et al., 2001; Abad and Guilleminault, 2005).

4. Chronic Partial Sleep Loss and Stress

Animal studies indicate that sleep loss activates the hypothalamo-pituitary-adrenal axis (HPA-axis) and results in increased plasma levels of the adenocorticotropic hormone (ACTH) and corticosteroids. The extent of HPA-axis activation depends on the amount of stress involved in the sleep deprivation protocol: more stressful techniques such as the flower pot technique used for REM sleep deprivation result in a more profound activation of the HPA-axis (Patchev et al., 1991; Suchecki et al., 1998; Andersen et al., 2005), while forced locomotion and gentle handling which are less stressful lead to a milder activation (Tobler et al., 1983; Meerlo et al., 2001a; Meerlo et al., 2002; Sgoifo et al., 2006). In humans however, the relationship between sleep loss and HPA-axis activation is not so clear-cut as it is in rodents. A few human studies have shown that sleep deprivation results only in a mild activation of the HPA-axis (Leproult et al., 1997; Vgontzas and Chrousos, 2002; Alexander, 2003; Voderholzer et al., 2004). A study found a burst in cortisol and ACTH secretion at the beginning of a sleep fragmentation schedule, but then plasma cortisol levels
decreased below baseline sleep levels (Spath-Schwalbe et al., 1991). Also, even a decrease or no significant change have been described in the awakening-linked cortisol response after disturbed sleep (Backhaus et al., 2004; Williams et al., 2005). In a further study, no increase has been found in cortisol levels at the end of a 24-hour sleep deprivation experiment (Gonzalez-Ortiz et al., 2000).

While data concerning effects of sleep loss on basal activity of the HPA-axis remains inconclusive, virtually nothing is known regarding effects of sleep loss on the reactivity of this system to subsequent new stressors. A number of animal studies suggest that the reactivity of the HPA-axis is changed in chronically sleep restricted rats (Meerlo et al., 2002; Sgoifo et al., 2006). The study by Meerlo et al. (2002) demonstrated that rats subjected to chronic partial sleep loss have a blunted ACTH response to a heterotypic stressor. In contrast, other stressors including immobilization, cold or noise exposure and exercise lead to the sensitization of the ACTH response to subsequent new stressors (Bhatnagar et al., 1995; White-Welkley et al., 1995; Van Raaij et al., 1997; Ma et al., 1999). The contrast between these findings underscores the fact that sleep loss is a special kind of stressor (Meerlo et al., 2002).

Similarly to sleep deprivation, in PTSD the responsiveness of the HPA-axis to a novel stressor is blunted (Heim et al., 2000). Also, damage to the amygdala, reduces the ACTH response to immobilization stress (Beaulieu et al., 1986). The limbic system is of great importance in the modulation of HPA-axis function, since many nervous structures within the limbic system project to the central regulatory nuclei of the HPA-axis and are highly involved in the regulation of the stress system (Herman et al., 1996; Herman et al., 2005).

One of the systems that might mediate effects of sleep loss on the HPA-axis is the serotonergic system. This idea is supported by the fact that the HPA-axis is under the stimulatory regulation of this neurotransmitter system (Calogero et al., 1990; Feldman and Weidenfeld, 1991; Dinan, 1996b). The interaction between the two systems is not exclusively unidirectional (that is, the serotonergic modulation of the stress system), since stress hormones can induce changes in the serotonin system too (Dinan, 1996a; Porter et al., 2004). Beside the stress systems, the serotonergic system is also intimately linked to sleep (Adrien, 2002; Ursin, 2002): this interaction will be discussed in the next section.

5. THE SEROTONERGIC SYSTEM, SLEEP AND MOOD

The serotonergic system originates in the raphe nuclei of the brain stem (Hornung, 2003), and its fibres extend to almost all structures of the central nervous system including the limbic system, the cortical mantle, the cerebellum, and the spinal cord (Tohoyama and Takatsuji, 1998). This widespread innervation pattern ensures an exquisite position for the serotonergic system to modulate other systems (Hensler, 2006). The modulatory role is further supported by the presence of 14 different serotonin receptor subtypes which are linked to various signal transduction pathways (Barnes and Sharp, 1999; Raymond et al., 2001). On this basis, the serotonergic system effectively
modulates the central regulation of many physiological and behavioural functions including mood, stress, and sleep (Adrien, 2002; Neumeister and Charney, 2002; Ursin, 2002).

The release of serotonin changes across the day (Portas et al., 1998; Park et al., 1999; Portas et al., 2000). The serotonergic neurons show highest firing and release rates during wakefulness. These rates decrease during NREM sleep and the serotonergic neurons almost completely cease to fire and stop releasing serotonin during REM sleep. It is believed that different amounts of serotonin released in the projection areas (for example in the thalamus) are in part responsible for the differential sleep stage-specific electroencephalographic activity of the brain (Ursin, 2002). In depression, which is characterized by a diminished serotonin production, sleep architecture is abnormal and sleep is disrupted. The abnormalities include decreased amounts of deep NREM sleep, decreased REM sleep latency and increased amounts of REM sleep, frequent or early awakenings (Benca et al., 1992).

However, the interaction between the serotonergic system and sleep is not a one-way process: the loss of sleep has a profound effect on the functioning of the serotonergic system too (Seifritz, 2001). In line with the findings on normal wakefulness, sleep deprivation activates serotonergic neurons and increases serotonergic neuronal firing rates (Santos and Carlini, 1983; Maudhuit et al., 1996; Prevot et al., 1996; Gardner et al., 1997; Adell et al., 2002; Evrard et al., 2006). This increased serotonin transmission (Lopez-Rodriguez et al., 2003; Penalva et al., 2003; Senthilvelan et al., 2006) is believed to counteract the diminished functioning of the serotonergic system which is one aspect in the pathophysiology of affective disorders, and contribute to the improvement of mood (Cryan and Leonard, 2000; Sobczak et al., 2002; Stockmeier, 2003). Decreased serotonergic function in mood disorders is supported by a large body of evidence. Firstly, a decrease in serotonin-1A receptor-mediated signalling in depressed patients has been shown by pharmacological challenges (Lesch, 1991; Mann et al., 1995; Shapira et al., 2000) and PET studies (Drevets et al., 1999; Sargent et al., 2000). Secondly, a number of studies on postmortem brain material of suicide victims are consistent with a decrease in serotonin-1A receptor function in depression (Stockmeier, 2003). Finally, antidepressant medication such as the selective serotonin reuptake inhibitor-based treatment, is based on drugs that enhance serotonergic neurotransmission by increasing the amount of serotonin present in the synaptic cleft (Blier and De Montigny, 1994; Middlemiss et al., 2002).

Restricted sleep might affect the brain and its functional outputs directly on the level of serotonin receptors but it might also modulate intracellular signalling pathways downstream the receptors. The majority of different serotonin receptor subtypes are G-protein-coupled metabotropic receptors, with the exception of one ionotropic subtype, the serotonin-3 receptor (Barnes and Sharp, 1999; Raymond et al., 1999; Raymond et al., 2001). The activated G-proteins associated to the receptors may either activate or inhibit intracellular signalling cascades, depending on the receptor subtype. The most widely studied serotonin-1 receptors are coupled to a multitude of signalling pathways including the cAMP, phosphoinositol, calcium, and arachidonic acid second messenger systems (Raymond et al., 2001). The production of second messengers leads to the activation of ion channels and protein kinases, and the latter ones are able to phosphorylate a
number of substrates. These intracellular changes are later translated into genomic changes by transcription factors such as phospho-CREB, c-Fos and zif-268 (Tilakaratne and Friedman, 1996; Kovács, 1998; Knapska and Kaczmarek, 2004; Josselyn and Nguyen, 2005).

Important in this context is the increasing awareness that affective disorders may not just be related to alterations on the level of receptors for serotonin or other neurotransmitters but, rather, may at least in part be due to impairments in the signal transduction pathways beyond the neurotransmitter receptors (Duman, 1998). Potential candidates for impairments in mood disorders are the G-proteins (Donati and Rasenick, 2003), the enzymes adenylyl cyclase and protein kinase A (Duman, 1998) as well as the transcription factor CREB (Nestler et al., 2002; Berton and Nestler, 2006). In depressed suicide victims the amount of inhibitory G-proteins and the activity of adenylyl cyclase in the brain is diminished in comparison with postmortem material obtained from non-suicidal humans (Pacheco et al., 1996; Dwivedi et al., 2002; Dowlatshahi et al., 1999). Similarly, in the same condition, both the expression and the activity of PKA are altered (Dwivedi et al., 2003; Dwivedi et al., 2004). Other studies have shown that the expression of CREB and its phosphorylation are decreased in limbic areas in mood disorders (Dwivedi et al., 2003; Akin et al., 2005) and antidepressant treatment can counteract this decrease (Dowlatshahi et al., 1998; Thome et al., 2000). Given these new insights in the neurobiology of psychopathologies, it is an important question how these intracellular signalling mechanisms may be affected by restricted and disrupted sleep. Yet, the effects of sleep loss on these elements of signalling pathways, except a few studies, have not been extensively examined yet (Cirelli and Tononi, 2000; Alanko et al., 2004).

6. SLEEP LOSS AND NEURONAL PLASTICITY

One neurobiological process that is not only under serotonergic control but also forms a potential link between restricted sleep and alterations in cognitive function and mood is hippocampal plasticity, in particular, adult hippocampal neurogenesis (Gould, 1999; Radley and Jacobs, 2002; Banasr et al., 2004; Huang and Herbert, 2005). It has been found in depressed humans or subjects with PTSD that the volume of the hippocampus decreases (Bremner et al., 2000; Manji et al., 2001), but it is not clear yet whether this is due to dendritic atrophy, cell loss or decreased neurogenesis. Nevertheless, chronic stress and corticosterone treatment, two animal models of depression, result in diminished hippocampal cell proliferation and neurogenesis (Gould et al., 1997; Gould et al., 1998). Moreover, pro-serotonergic antidepressant treatments restore the rate of neurogenesis (Malberg et al., 2000; Malberg and Duman, 2003; Malberg, 2004). Other animal studies have demonstrated that also the beneficial behavioural effects of antidepressants are paralleled by restored neurogenesis (Santarelli et al., 2003).

With respect to possible effects of restricted and disturbed sleep, a few studies have shown that sleep deprivation leads to a diminished production of new cells in the hippocampus (Guzman-Marin et al., 2003; Guzman-Marin et al., 2005; Hairston et al., 2005; Tung et al., 2005). Such effects only seem to occur with a more prolonged deprivation of sleep for several days and not with
acute and short sleep deprivation during a normal single resting phase (Van der Borght et al., 2006). The results of the long-term sleep deprivation studies suggest that sleep loss-induced reductions in hippocampal plasticity may contribute, along with other factors, to impaired hippocampal functions such as learning and mood (Graves et al., 2001; McDermott et al., 2003; Marks and Wayner, 2005).

7. THE CONCEPT OF THE THESIS

While frequently restricted and disrupted sleep is a rapidly increasing problem in modern society, very little is known on the long-term consequences of chronic sleep loss for brain function and health. This thesis describes a series of studies that examined the neurobiological consequences of restricted sleep in rats, with special emphasis on changes in serotonergic signalling and brain systems that are involved in the regulation of stress and emotionality. The background of these studies was the hypothesis that restricted and disrupted sleep may gradually alter the brain and sensitize individuals for mood disorders such as depression. If sleep loss were indeed a potential causal factor in the development of psychopathologies, we expected that chronic sleep restriction would gradually induce changes in the brain that are similar as what is seen in such psychopathologies.

Figure 1. The conceptual background of the present thesis. Sleep loss is a commonly occurring and growing problem in human society. It is mostly related to work, stress and other factors associated with an overactive modern life style (top pictures). A number of studies have indicated that sleep loss may be a risk factor for psychopathologies including depression. Mood disorders are often linked to alterations in neurotransmitter systems. In particular, an impaired serotonergic signalling has been implicated as an underlying mechanism for depression (the figure includes two positron emission tomography scans: the right is a depressed brain with a lower serotonin function, the left is a healthy brain with a normal serotonergic system). The present work aims to establish whether sleep loss leads to neurobiological changes which are also characteristic to mood disorders. Therefore, the major questions of this project are: does sleep loss affect the brain’s serotonergic system, and does it eventually lead to impairments in the functional outputs of the brain in a manner that is similar to mood disorders? In this framework, we studied how sleep loss affects the serotonin-1A receptor system; our findings about this system and its interactions with other systems (muscarinic cholinergic and adenosinergic) are discussed in Chapters 2, 3, 5 and 7. As functional outputs of the brain, we examined neuroendocrine stress reactivity, emotionality (illustrated by a rat in a fear conditioning box) and neuronal plasticity (depicted by a newly produced – lighter in colour – granule cell of the hippocampal dentate gyrus). The results of these studies are discussed in Chapters 4, 6 and 8, respectively. The numbers next to the pictures in the figure represent the chapters of the thesis related to the respective topics.
Although indirect evidence and epidemiological studies in humans suggest that disrupted sleep may be a risk factor for psychopathologies, few attempts have been made to study the relationship between chronic sleep loss and brain function with a realistic experimental model. Since experiments with human beings have various limitations, we have chosen a rat model of chronic partial sleep deprivation as our experimental approach to mimic sleep loss as it often occurs in human society. Although there have been numerous animal studies on the effects of sleep deprivation, most of these studies were based on total sleep deprivation for a short period of time, unlike the situation that occurs in real life. Instead of using total sleep deprivation which is not typical for human individuals, we used a novel approach including animals subjected to a protocol of partial sleep deprivation over a longer period of time. The protocol allowed the rats 4 hours of sleep every day, which is less than the 10 to 12 hours that they normally sleep, and presumably would not be sufficient for full recovery. Our question then was how such insufficient recovery over the course of many days would affect the brain.

8. THE OUTLINE OF THE THESIS

In this paragraph, I will shortly introduce the contents and rationale of each chapter in the present thesis. The study described in Chapter 2 examined the effects of chronic sleep restriction on the functional sensitivity of the serotonin-1A receptor system, a receptor system that has long been implicated in mood disorders. The sensitivity of the receptors in sleep restricted and control rats was studied by subjecting them to pharmacological challenges with a serotonin-1A receptor agonist and measuring body temperature responses with a radio telemetry set-up. We assessed how changes in receptor sensitivity develop over time and how persistent these changes are. Since glucocorticoid stress hormones are capable of affecting the serotonin-1A receptor system, in Chapter 3, we investigated whether the sleep loss-induced decreased serotonin-1A receptor sensitivity found in the previous chapter might be due to elevated adrenal stress hormone levels. To this end, we used adrenalectomized animals. Next to this, we studied whether the effects of chronic partial sleep loss are specific for the serotonergic receptors or they concern a further receptor system which also has implications in mood disorders; the muscarinic cholinergic system.

In Chapter 4, we studied how sleep loss changes the reactivity of the HPA-axis if experimentally challenged. We did this by measuring pituitary hormone (ACTH) and corticosterone responses to pharmacological challenges with a serotonergic agonist and corticotropin releasing hormone. Based on our earlier findings of a sleep loss-induced desensitization of the serotonin-1A receptor system, in Chapter 5, we investigated whether sleep loss alters the number of serotonin-1A receptors in a way so that it could explain the diminished 1A receptor-related physiological responses. Additionally, we examined the inhibitory G-proteins coupled to the 1A receptors as potential molecular substrates of receptor sensitivity. Our investigations included a number of cortical, limbic and basal forebrain areas. Chapter 6 then reports how sleep loss changes the
behaviour of rats upon re-exposure to a fearful environment in a contextual fear conditioning paradigm. Furthermore, we tried to correlate emotional behaviour with neuronal activation in the amygdala and the hippocampus. In Chapter 7, we set out to examine whether cross-talk between different receptor populations could be the cause of decreased serotonin-1A receptor sensitivity. Here, our question was whether chronic pharmacological activation of the adenosine receptors, as it may occur under conditions of chronic sleep restriction, would affect the functional sensitivity of the serotonin-1A receptors. The rationale for this particular study was based on data suggesting that adenosine accumulates in the brain during wakefulness as well as during sleep deprivation. Also, a number of studies indicate that there a cross-talk between the adenosinergic and serotonergic receptors exists. Chapter 8 describes the effects of chronic partial sleep loss on a form of adult neuronal plasticity; cell proliferation in the dentate gyrus of the hippocampus. It is known that sleep loss impairs cognitive functions and mood, and some studies link adult neurogenesis to both of these functional outputs of the brain. However, data on the impact of sleep loss on hippocampal cell production has been scarce. Finally, in Chapter 9 the results of all experiments are summarized and discussed.