Neurobiological and functional consequences of chronic partial sleep deprivation

Roman, Viktor

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Document Version
Publisher's PDF, also known as Version of record

Publication date: 2007

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Chapter 9

General discussion
Chapter 9

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1. NEUROBIOLOGICAL CONSEQUENCES OF CHRONIC PARTIAL SLEEP DEPRIVATION

The present thesis aimed to make a link between two sets of observations: [1], the observation that sleep problems may be associated with increased sensitivity to psychopathology; and [2], the observation that mood disturbances are associated with a decreased serotonergic neurotransmission. The data of the present thesis confirms that chronic partial sleep deprivation gradually alters serotonin 1A receptor sensitivity in a direction that is similar to what is seen in affective disorders (Chapters 2, 3 and 4). The sleep loss-induced receptor desensitization has been shown by two different functional read-outs of the serotonergic 1A receptor system in response to an injection of the serotonin-1A receptor agonist 8-OH-DPAT, that is, body temperature (hypothermic response) and pituitary hormones (ACTH release). A further important observation is that the reduction in 1A receptor sensitivity not only develops gradually but also is persistent and several days of undisturbed rest are needed for the complete recovery and normalization of receptor sensitivity (Chapter 2).

Since sleep restriction in the present studies was achieved by forced locomotion, a condition that is associated with physical activity and stress, we performed additional experiments and measurements that would control for these two factors. Chapters 2 and 3 discuss one of these control experiments, where we not only looked at sleep restriction by forced locomotion but also included a control group that walked at twice the speed for half the time, thereby covering the same distance but still having sufficient time to sleep. This forced activity control group did not show the desensitization in serotonin-1A receptor-mediated response, which indicates that forced activity alone does not explain the desensitization of the serotonin-1A receptor system.

The other factor that might have contributed to the reduction in serotonin-1A receptor sensitivity in sleep restricted animals is potential stress and release of glucocorticoid stress hormones associated with forced wakefulness. The results of Chapter 2 showed that rats subjected to the sleep restriction protocol, if anything, had only mildly elevated plasma corticosterone levels at the end of the daily sleep deprivation sessions. However, since we did not have information on adrenal stress hormone levels at earlier time points of the day during sleep deprivation, we could not rule out elevated levels of stress hormones as a potential factor in the serotonin-1A system desensitization. In order to examine the possible involvement of adrenal stress hormones, we performed a further control experiment with adrenalectomized animals (Chapter 3). By using such animals, we demonstrated that the adrenal stress hormones do not play a role in the desensitization of the serotonin-1A receptors. In conclusion, the desensitization of the 1A receptor system in chronically sleep restricted rats is most likely due to the loss of sleep and not due to forced activity or elevated stress hormone levels.

In contrast to a downregulated serotonergic system in depression, opposite changes have been described for cholinergic signalling, in particular a hypersensitive muscarinic receptor system. Within this framework, we investigated how chronic partial sleep deprivation affects the muscarinic cholinergic system by using the muscarinic cholinergic receptor agonist oxotremorine-induced
hypothemic response as read-out of functional receptor sensitivity (Chapter 3). While sleep loss did not significantly change the sensitivity of the muscarinic cholinergic receptors, forced activity, a condition that is associated with increased levels of stress hormones, hypersensitized these receptors. This hypersensitization was indeed due to an elevation in adrenal stress hormones, since it disappeared in adrenalectomized animals. Thus, the findings of Chapters 2 and 3 demonstrate that chronic partial sleep deprivation and stress exert differential effects on serotonin-1A and muscarinic cholinergic receptor sensitivity, with sleep loss decreasing serotonin-1A receptor sensitivity and stress increasing muscarinic acetylcholine receptor sensitivity. Importantly, whereas in our experimental set-up, we tried to separate the effects of sleep loss and stress, in real life the two are often experienced together. In fact, stress is considered as one of the most important causes of disrupted sleep (Drake et al., 2003). Thus, the two factors together, sleep loss and chronic stress may enhance each others effects resulting in a brain condition that is more vulnerable to malfunction and disease.

In Chapters 5 and 7 we tried to unravel what the molecular mechanism behind the sleep loss-induced serotonin-1A receptor desensitization might be. Two possible mediators of a reduction in serotonin-1A receptor sensitivity are the neurotransmitters serotonin and adenosine.

It has been demonstrated that serotonin can desensitize its own receptors by repeatedly and continuously stimulating them (Li et al., 1999). The release of serotonin changes throughout the day, with higher levels during wakefulness and considerably lower levels during sleep (Portas et al., 1998; Park et al., 1999; Portas et al., 2000). Similar to wakefulness, sleep deprivation also results in high serotonin levels in the projection areas of this transmitter system (Lopez-Rodriguez et al., 2003; Penalva et al., 2003). Thus, chronic partial sleep deprivation may yield a neurochemical condition which repeatedly overstimulates the serotonin receptor system and thereby causes desensitization. Chapter 5 shows that the 1A receptor desensitization that we found earlier was not matched by significantly altered receptor numbers in the brain areas that we examined by receptor autoradiography. This was not completely unexpected, given the fact that a number of studies have shown that even selected strains of rats with different sensitivity to 8-OH-DPAT have equal numbers of the 1A receptors. Importantly, in the context of stress and depression we were particularly interested in limbic forebrain areas. It may be however, that receptor numbers change in the brain stem thereby leading to alterations in physiological responses. On the other hand, it might very well be that in the face of unaltered receptor numbers, the ability of receptors to transmit signals to the cells’ interior decreases (i.e. molecular changes of the receptor itself) but this remains undetected by receptor autoradiography. Perhaps the phosphorylation state of the 1A receptors changes, which might affect sensitivity of the receptor cascade while leaving ligand binding properties of the receptor itself unchanged.

Another possible mediator of a serotonin receptor system desensitization, as suggested by the literature, is adenosine. A number of studies have suggested that an increased adenosine turnover and frequent stimulation of adenosine receptors, which may happen with prolonged wakefulness (for review see Porkka-Heiskanen et al., 1997; Basheer et al., 2004), might ultimately affect intracellular signalling pathways associated with the serotonin-1A receptor (Zgombick et al.,
However, our study on possible cross-talk between the adenosinergic and serotonergic receptor systems in Chapter 7 failed to provide evidence for such an interaction. Chronic treatment with an adenosine agonist resulted in a clear desensitization of the adenosine A1 receptor system, but did not result in a desensitized serotonin-1A signalling as we saw after chronic sleep restriction. Thus, it is more likely that chronic sleep restriction may be a condition that chronically elevates the levels of serotonin which, in the long run, may be responsible for the receptor desensitization reported in Chapters 2, 3 and 4. Although the release of serotonin can change rapidly, dependent on the behavioural state, subsequent microdialysis studies could answer the question whether chronic sleep loss indeed changes the amount of released serotonin in the long-term as described for conditions of acute sleep deprivation (Lopez-Rodriguez et al., 2003; Penalva et al., 2003).

As receptor sensitivity not only depends on receptor number or phosphorylation state, but also on the proper functioning of the downstream signal transduction cascades, we further investigated the G-proteins linked to the serotonin-1A receptors. These studies described in Chapter 5 showed that sleep loss or forced activity did not change the number of inhibitory G-proteins in various forebrain areas except for one limbic area, the amygdala, which showed increased numbers of serotonin-1A associated inhibitory G-proteins.

Since the amygdala is a crucial brain structure for behavioural stress-reactivity and its function is under serotonergic control, in Chapter 6 we studied whether chronic partial sleep deprivation alters amygdala-related behaviour. The results of this experiment show that partial sleep deprivation for 8 days diminishes amygdala-related fear behaviour in a contextual fear conditioning paradigm. When control rats and sleep restricted rats were re-exposed to a shockbox in which they had previously received a series of mild electric shocks, sleep restricted animals displayed an attenuated freezing response. Likewise, a number of studies have shown that rapid eye movement or unselective sleep deprivation results in diminished fear, reduced neophobia and impaired consolidation of contextual fear (Hicks and Moore, 1977; Moore et al., 1979; Mogilnicka et al., 1985; Graves et al., 2003; Ruskin et al., 2004). Interestingly, the attenuated fear response was not accompanied by a reduction in the number of activated neurons in the amygdala, as indicated by the neuronal activation marker c-Fos (Chapter 6). Instead, there were more activated cells in the dentate gyrus of the hippocampus, which suggests an overactivation of the hippocampus in sleep restricted rats.

Our study described in Chapter 8 examined another aspect of hippocampal plasticity; adult hippocampal cell proliferation. The results of this chapter show that sleep deprivation reduces hippocampal glial cell proliferation in the hilus of the dentate gyrus. However, there is no indication of reduced neurogenesis or altered phenotypical differentiation of newly produced cells. As recent studies have suggested an important role for glia in tissue homeostasis, it is still possible that the reduction of hilar cell proliferation has later consequences on hippocampal function. As Chapters 6 and 8 demonstrate, the effects of sleep loss on the hippocampus are rather complex and difficult to bring together into one coherent theory.

The results of Chapter 6 showing reduced behavioural stress reactivity are supported by Chapter 4 which demonstrates reduced neuroendocrine reactivity in sleep restricted rats. In this
study, the release of pituitary ACTH in response to an injection of CRH was reduced. Thus, based on the results of Chapters 4 and 6, chronic partial sleep loss proves to be a condition that reduces both neuroendocrinological and behavioural stress reactivity. These findings support our hypothesis on chronic sleep loss as a condition increasing the vulnerability to disease since compromised neuroendocrine and emotional states are both characteristic features of psychopathologies.

Taken together, the present studies clearly indicate that chronic partial sleep deprivation functionally desensitizes the serotonin-1A receptor system measured by pharmacological challenges inducing changes in body temperature or hormone levels. On the other hand, the cell biological correlates of these changes remain unclear. It is very likely that functional desensitization does not derive from the receptor level, but from more downstream elements of signal transduction pathways such as the G-proteins.

2. IS RECOVERY POSSIBLE?

An important question that emerges is whether it is possible to recover from the effects of sleep loss. Based on EEG data, it seems that after a short episode of sleep deprivation a quick and complete recovery can take place. However, a number of studies indicate that recuperation after long-term total sleep loss may not be so straightforward. After long-term total sleep deprivation the sleep rebound does not contain as much NREM sleep that was lost during the treatment (Rechtschaffen et al., 1999). In other words, after heavy sleep loss animals do not make up anymore for the NREM sleep that was lost previously. Animal experiments showed that recovery is impossible after prolonged total sleep deprivation: after 2-3 weeks of treatment there is a point of no return leading to the death of the animal even with allowing undisturbed rest (Everson et al., 1989a; 1989b; Everson, 1995).

On the other hand, chronic partial sleep deprivation does not lead to such irreversible physiological changes. In humans, adverse effects in this case include mild psychological and cognitive alterations. Furthermore, it is known from animal studies that chronic sleep loss also leads to changes in stress reactivity (Meerlo et al., 2002). Studies that involved chronic partial sleep deprivation in humans indicated that subjects need several days before their learning abilities or their sleepiness returns to normal levels (Van Dongen et al., 2003; Belenky et al., 2003). Thus, it seems that long-term partial loss of sleep is a more serious challenge for an organism than a short-lasting episode total sleep deprivation and, if anything, only a prolonged recovery is possible.

This difference between the ability to recover from acute and chronic sleep loss makes sense from an evolutionary point of view. Acute sleep loss may challenge animals irregularly and infrequently, with which they have to be able to cope readily. In contrast, chronic partial sleep loss may be more restricted to certain periods of animal life. One such period can be the taking care of the offspring which is continuously time-demanding for mothers. In human populations, this kind of sleep loss is characteristic to much wider segments of the society than only mothers. On top of this,
many people experience chronic partial sleep loss over long periods of time, usually years or even decades. Since all of this may be a consequence of living in a modern society, we might not be equipped with the physiological mechanisms developed by natural selection that would enable us to efficiently cope with such a challenge on the long-term. One example of evidence supporting this idea is the lack of habituation in terms of corticosterone levels to chronic sleep loss as a stressor (Meerlo *et al.*, 2002). A consequence of chronic partial sleep loss may be maladaptive processes, such as the aforementioned lack of habituation, that lead to the development of a depressed state.

### 3. Does Chronic Partial Sleep Loss Make Subjects More Vulnerable to Depression?

In the present thesis we suggest that chronic partial sleep deprivation is a condition that may make individuals more vulnerable to mood disorders, in particular, depression. This was based on a leading theory of depression, the neurochemical theory. This theory explains the pathogenesis of depression through a reduced serotonergic and an enhanced cholinergic signalling (Dilsaver, 1986). In concert with this, our own findings showed that chronic partial sleep deprivation for a week indeed induced a desensitization of postsynaptic serotonin-1A receptors and chronic stress resulted in a sensitization of muscarinic cholinergic receptors. Acting like this, chronic partial sleep deprivation affects serotonergic neurotransmission opposite to what is seen after treatment with certain antidepressants (*Blier et al.*, 1987). In particular, tricyclic antidepressants and electroconvulsive therapy increase the responsiveness of the postsynaptic serotonin-1A receptors (*Blier et al.*, 1994).

Another, more recently developed theory discusses depression as a disorder of plasticity and information processing (*Manji et al.*, 2001; *Castren*, 2005; *Berton* and *Nestler*, 2006). Our results show that chronic sleep loss does not significantly alter adult hippocampal neurogenesis, which is a form of neuronal plasticity. This finding is not in support of chronic sleep loss being depressogenic, since sleep loss in our experiment did not reduce the number of newly produced hippocampal cells and the number of cells that differentiate into neurons, while depression is believed to be associated with decreased neurogenesis. On the other hand, chronic partial sleep deprivation significantly affected presumably glial plasticity which may be an important factor contributing to the homeostasis of neural tissue. Glial cells have recently started to receive more attention as a factor that possibly plays a key role also in the etiology of depression (*Miller* and *O’Callaghan*, 2005).

Thus, most results of the present thesis support the notion that chronic sleep loss is depressogenic or at least changes the brain in a direction that is similar to what is seen in depression. Although it does not seem likely that in human beings one week of restricted sleep would result in fully developed mood disorders, in the long-run it may act as a factor that sensitizes the individual to depression. The latter may be true especially when chronically restricted sleep occurs in concert with other depressogenic factors such as stress. Also, sleep restriction may
perhaps only act as depressogenic in individuals with a predisposition for this disorder. Yet, along these lines, human studies have indeed shown an increased risk for mood disorders in subjects with sleep problems (Chang et al., 1997; Perlman et al., 2006).

4. FUTURE PERSPECTIVES

The experiments presented here provided novel data on what happens in the brain when it is chronically sleep restricted, but they also led to a series of new questions. Future studies could go basically in two distinct directions. Firstly, our current findings could be extended into carefully planned and controlled human studies. And, secondly, we could follow new questions with our animal model.

Our results have been obtained in animals, and it would be interesting to extrapolate them and validate whether similar changes in receptor sensitivity measured by pharmacological challenges are present in humans after chronic partial sleep deprivation. Using pharmacological challenges in humans would not be novel, since this technique has been used in humans to test their serotonin receptor sensitivity within the framework of depression research (Meltzer and Maes, 1995; Shapira et al., 2000). A similar experiment after sleep loss in humans would make a stronger link with earlier human data on the effects of sleep loss on mood (Pilcher and Huffcutt, 1996; Dinges et al., 1997; Lieberman et al., 2002; Bonnet and Arand, 2003; Haack and Mullington, 2005; Scott et al., 2006).

Following new issues and questions with our animal model could include various experiments. First, investigations of behavioural indices of depression and anxiety. The effects of human sleep loss are mostly assessed by examining behavioural read-outs (e.g. psychomotor vigilance, memory, mood, sleepiness, sleep latency, etc). Thus, behavioural studies in our settings could also serve to establish a stronger link to the results of human research. Behavioural studies measuring levels of anxiety, various aspects of depressed mood and motivation may provide us with more evidence supporting the idea that chronic partial sleep loss is depressogenic.

Second, studies on the sensitivity of other serotonin receptor subtypes. The pharmacological challenge studies with 8-OH-DPAT showed a clear-cut sleep loss-induced desensitization of the postsynaptic serotonin-1A system in rats. However, it would also be useful to see whether there are changes in the sensitivity of the presynaptic serotonin-1A receptor population or in other serotonin receptor subtypes. One obvious candidate for this type of studies is the serotonin-7 receptor, since it is activated by 8-OH-DPAT, just as the 1A receptor is (Hedlund et al., 2004). The serotonin-2 receptor subtype should also be studied because its assumed involvement in the pathophysiology of depression (Cheetham et al., 1988).

Third, further explorations of intracellular serotonin-1A signalling pathways. The present data do not give us unequivocal evidence for the biological substrates underlying the observed serotonergic alterations. Western blot analysis of serotonin-1A receptors in different brain areas would answer questions about possible posttranslational modifications and the phosphorylation
state of receptor proteins. A further element of serotonergic signalling are the recently discovered RGS proteins (regulators of G-protein signalling), which are positioned at a very important station in the transduction pathway. These proteins enhance the GTPase activity of G-proteins, thereby making the refractory period of G-proteins shorter (Chasse and Dohlman, 2003). Sleep loss may also interfere with this particular class of regulatory proteins.

Finally, experiments could be extended in the direction of looking for gender differences. It would be interesting to extend the research with our model of chronic sleep restriction to female animals. It is known that women more often than men suffer from disturbed sleep (Voderholzer et al., 2003). This is due to bringing up children, the menopause and the menstrual cycle (Miller, 2004; Soares, 2005). Another factor, that would make this line of research attractive, is that depression is much more prevalent among women than men (Piccinelli and Wilkinson, 2000). It may very well be that men and women are different in terms of vulnerability to certain consequences of sleep restriction. Applying our model in female animals could give us clues as to whether there are gender-specific changes in EEG or serotonin receptor sensitivity after chronic sleep loss.

5. CONCLUDING REMARKS

The data presented in this thesis indicate that chronic partial sleep loss, as it often occurs in modern human society, desensitizes the brain’s serotonergic receptor system and alters stress reactivity. The most important finding of the present work is that all these physiological changes develop gradually over time and may be persistent for days even with undisturbed sleep. Since similar changes in serotonergic receptor sensitivity have been found in depressed subjects, our data suggests that restricted sleep may change the brain in a direction that might increase the vulnerability of the individual for psychopathologies such as depression. The gradual and persistent nature of the changes may have far-reaching implications given the general sleep hygiene of human societies. In light of this, one commonly exercised habit, oversleeping in the weekends, might possibly be not enough to catch up on the physiological effects of sleep loss gathered during the week.

Taken together, the present thesis points at the fact that sleep loss, which is generally viewed by human society as not harmful, may have serious consequences on the well-being of the individual. These serious physiological consequences include the aforementioned increased risk for psychopathologies but also an increased risk for other diseases such as hypertension, metabolic and gastrointestinal disorders. In conclusion, awareness about these adverse effects should increase in the modern world and sleep should be recognized as an important and indispensable item in our life.