Neurobiological and functional consequences of chronic partial sleep deprivation

Roman, Viktor

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Chapter 2

Too little sleep gradually desensitizes the serotonin-1A receptor system

Viktor Román, Irene Walstra, Paul G. M. Luiten, Peter Meerlo

Department of Molecular Neurobiology, University of Groningen,

P.O. Box 14, 9750 AA Haren, The Netherlands

ABSTRACT

In our 24h society, frequently disrupted and restricted sleep is a rapidly increasing problem that may contribute to the development of diseases such as depression. One of the proposed neurobiological mechanisms underlying depression is a disturbance in the brain's serotonergic neurotransmission, particularly a desensitization of the serotonin-1A receptor system. However, a relationship between chronic sleep loss and changes in serotonin receptors has not been established yet. Therefore, in the present study we experimentally tested the hypothesis that chronic sleep restriction leads to desensitization of the serotonin-1A receptor system. Rats were subjected to a schedule of restricted sleep allowing them 4h of sleep per day. Sleep restriction was achieved by placing the animals in slowly rotating wheels. The sensitivity of the 1A receptor system was examined by measuring the hypothermic response to a standard injection of a 1A agonist. The results show that 2 days of restricted sleep had not yet affected the sensitivity of the serotonin-1A receptor system whereas the system was desensitized after 8 days of sleep restriction. Control experiments indicated that the effect of sleep restriction was not due to forced activity or stress. The effect of sleep loss persisted for many days even with unlimited recovery sleep. The desensitization of the 1A system was still present after 1, 2, and even 7 days of recovery. These findings provide a link between chronic sleep loss and sensitivity for disorders that are associated with deranged serotonergic neurotransmission.

INTRODUCTION

A rapidly increasing number of people in our modern society experiences regular sleep loss due to our modern around-the-clock lifestyle. Concerns have been raised that, in the long run, chronically restricted sleep may have serious repercussions for health and well being (Rajaratnam and Arendt, 2001). Controlled studies have provided evidence that acute sleep deprivation strongly affects cognitive performance and emotionality (Pilcher and Huffcutt, 1996). Also, recent experiments in healthy subjects showed that successive nights of restricted sleep result in a gradually accumulating decline in cognitive function (Dinges *et al.*, 1997; Van Dongen *et al.*, 2003). Whereas subjects may initially recover from these effects after subsequent sleep, frequent or chronic sleep loss may induce neurobiological changes that are not immediately evident but accumulate over time, ultimately with serious health consequences. One long-term prospective study that clearly suggested such a link between inadequate sleep and sensitivity to disease showed that insomnia and sleeping problems in otherwise healthy young people were associated with an increased risk for clinical depression 20 to 40 years later (Chang *et al.*, 1997).

Several lines of evidence indicate that the neurotransmitter serotonin is involved in the regulation of mood and that serotonergic neurotransmission is impaired in affective disorders (Cryan and Leonard, 2000; Sobczak *et al.*, 2002; Stockmeier, 2003). 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). Although postmortem studies have yielded various results, some of them are consistent with a decrease in serotonin-1A receptor function in depression (Stockmeier, 2003). Finally, antidepressant medication is often based on drugs that enhance serotonergic neurotransmission (Blier and De Montigny, 1994; Middlemiss *et al.*, 2002).

Given the evidence for a role of the serotonergic system in clinical depression, a gradual alteration in this system seems a candidate mechanism by which disrupted and restricted sleep might increase the risk for this disease. However, this potential link between sleep loss and sensitivity to psychopathology has not been established. Therefore, the aim of our study was to experimentally test the hypothesis that restricted sleep gradually causes a desensitization of the serotonin-1A receptor system and thereby changes the brain in a direction that makes it more vulnerable to psychopathology.

MATERIALS AND METHODS

Animals and housing

In a series of 3 experiments, we used 76 adult male Wistar rats (± 250 g at the start of the experiments) bred at the local animal facility of the University of Groningen, Haren, The Netherlands. Animals were housed under a 12h light/12h dark cycle, with lights on from 09.00 h to 21.00 h. The average temperature of the room was $21 \pm 1^{o}C$. Rats were provided with food and water *ad libitum* in all experiments. The experiments were approved by the Ethical Committee of Animal Experiments of the University of Groningen.

Experiment 1. Sleep restriction

Rats were subjected to a sleep restriction protocol allowing them 4h of undisturbed rest per day at the beginning of the light phase (09.00-13.00 h), their normal resting phase. The remainder of the time, the animals were kept awake by placing them in slowly rotating wheels (40 cm in diameter) driven by an engine at constant speed (0.4 m/min). Since rats normally sleep about 10 to 12h per day (Borbély and Neuhaus, 1979) the 4h of rest would not be sufficient to fully recover from the 20h of wakefulness. Animals had free access to food and water inside the wheels. A total of 28 rats was used: 8 rats were subjected to 2 days of sleep restriction and 8 rats underwent 8 days of sleep restriction. Six rats in both sessions served as controls and remained in their home cage.

Figure 1. Experimental set-up of sleep restriction protocol and forced activity control. Top bar: rats in Experiment 1 were sleep restricted by forced locomotion for 20h each day (grey section of the bar) and were allowed 4h of rest in their home cage (first 4 hours of the light phase). Middle bar: rats in Experiment 2 were subjected to a protocol of forced activity at double speed for half the time. The forced activity was divided in 5 blocks of 2h (dark sections of the bar) separated by 2.5h of rest (white sections of the bar). Lower bar depicts the 24h LD cycle. (*) Serotonin 1A agonist injections on day 2 and day 8 took place between the third and fourth hour of the light phase. (●) Blood samples after 1 or 7 days of sleep restriction / forced activity were collected at the beginning of the light phase (the end of the daily sleep deprivation or forced activity session) and after the 4th hour of the light phase (after the daily 4h recovery phase).

Experiment 2. Forced activity control

Since the procedure of sleep deprivation included mild forced locomotion, we performed an additional experiment to establish whether effects of sleep restriction were partly due to forced activity rather then sleep loss per se. A second group of rats was subjected to a schedule of forced activity in the same drums that were used for the sleep restriction. However, these new animals were forced to walk at double speed for half the time (0.8 m/min for 10h per day). In other words, the housing conditions were the same and the animals covered the same distance as the sleep restricted rats in Experiment 1, however, they had to walk at a higher intensity and had more time to sleep (14h of rest per day versus 4h in the sleep restricted animals). The 10h of daily forced activity was divided in 5 blocks of 2h separated by 2.5h of rest, thus covering a time frame of 20h that corresponded to the 20h time frame of sleep restriction in the first experiment (see Fig. 1). For the 20h period of alternating rest and activity, the rotation of the wheels was controlled by a timer. Only for the first 4h of the light phase were the animals returned to their standard home cage, similar to the sleep restricted animals in Experiment 1. In the forced activity experiment, 32 rats were used. Eight rats were subjected to a 2-day and 8 rats to an 8-day forced activity schedule. Eight animals served as controls in both sessions.

Experiment 3. Recovery

After establishing sleep loss-induced changes in serotonin-1A sensitivity, another important question was how long such changes would persist with unrestricted recovery. In a third group of rats, we examined the hypothermic response to 8-OH-DPAT after 8 days of sleep restriction followed by different durations of unrestricted recovery sleep: 1, 2 and 7 days of recovery. The protocol of sleep restriction was similar to that in Experiment 1. After 8 days, the animals were returned to their home cage for undisturbed recovery. In this third experiment a total of 32 rats were used. In one group of rats, the sensitivity of the serotonin-1A receptor system was measured after 8 days of sleep restriction and after 2 days of recovery (8 sleep restricted and 8 control rats). In a second series of rats the 1A sensitivity was measured after 1 and 7 days of recovery (8 sleep restricted and 8 control rats). Thus, in this experiment, each individual rat received 2 pharmacological challenges to test the serotonin-1A sensitivity. We did not perform more than two challenges in each animal to prevent desensitization of the receptors as a consequence of the pharmacological challenges themselves.

Serotonergic challenge

To examine the effect of chronic sleep loss on the sensitivity of the serotonin-1A receptor system, we measured the physiological response to a subcutaneous injection with the serotonin-1A agonist (±)-8-hydroxy-2-(di-*n*-propyl-amino) tetralin hydrobromide (8-OH-DPAT; 0.25 mg/kg body weight; Sigma, St. Louis, MO, USA). This drug causes an acute hypothermic response that can be used as an indicator of central serotonin-1A neurotransmission, as has been shown in rats (Hjorth, 1985) as well as humans (Blier *et al.*, 2002). Importantly, in depressed patients, this serotonin-1A mediated hypothermia is attenuated, in accordance with other evidence of decreased serotonin-1A signalling (Lesch, 1991; Mann *et al.*, 1995; Shapira *et al.*, 2000). The pharmacological challenges were performed between 11.00 h and 13.00 h, the third and fourth hour of the light phase, when all animals were in their home cage see Fig. 1). The sensitivity to the drug was determined by measuring the acute hypothermic response by means of radio telemetry.

Radio telemetry of body temperature

To record the serotonin-1A receptor mediated drop in body temperature we applied radio telemetry with chronically implanted transmitters (model TA10TA-F40; Data Sciences, St. Paul, MN, USA). Implantation of the transmitters in the abdominal cavity was performed under full anaesthesia (inhalation anaesthesia with a mixture of N_2O, O_2, and isoflurane). After surgery, the animals were allowed at least 10 days of recovery. The transmitters measured core body temperature and transformed temperature values into frequency coded radio signals. These radio signals were relayed to a PC by receivers placed under home cages (model RPC-1; Data Sciences, St. Paul, MN, USA). Body temperature was sampled for 5 sec every 5 min and processed with Dataquest Labpro[TM] system (Data Sciences).

Blood sampling and corticosterone measurements

It has been reported that serotonin-1A receptor sensitivity can be attenuated by stress and elevated levels of glucocorticoids (Meijer and De Kloet, 1994; Bush *et al.*, 2003; Leitch *et al.*, 2003). We therefore sought to determine whether our sleep restriction protocol might attenuate serotonin-1A receptor sensitivity by increased levels of stress hormones. In Experiment 1 and Experiment 2, blood samples were collected to measure effects of sleep restriction and forced activity on plasma levels of corticosterone. The blood samples were taken on the first and the seventh day of the protocol, thereby not interfering with the 8-OH-DPAT challenges on day 2 and 8. On both days, 0.3 ml blood samples were collected by a making a small incision in the tail, one sample at 09.00 h (the end of the daily sleep deprivation or forced activity session) and another sample at 13.00 h (after the daily 4h recovery phase; see Fig. 1). The blood was collected in pre-chilled Eppendorf tubes containing EDTA as anti-coagulant. The samples were centrifuged at 2600 g for 15 min and the supernatant was stored at -80 ^{o}C for later analysis. Corticosterone levels were determined by radioimmunoassay (ICN Biomedicals, Costa Mesa, CA, USA).

Data analysis and statistics

To test for effects of sleep restriction or forced activity on the hypothermic response to 8-OH-DPAT injection, body temperature data were subjected to analysis of variance (ANOVA) with repeated measures. When appropriate, *post hoc* t-test was applied to establish at which time points after injection the experimental and control groups differed. Plasma levels of corticosterone were analyzed with ANOVA.

RESULTS

Experiment 1: Sleep restriction

The subcutaneous injection of 8-OH-DPAT caused an immediate hypothermia that reached its lowest value within 20-30 min, approximately 2°C below baseline temperature. Body temperature values returned to baseline 90 min after the injection (Fig. 2). In rats that were sleep restricted for 2 days, the hypothermic response was not different from control animals that were allowed unrestricted sleep (Fig. 2A). However, after 8 days of restricted sleep the serotonin-1A receptor-mediated response was significantly attenuated (Fig. 2B).
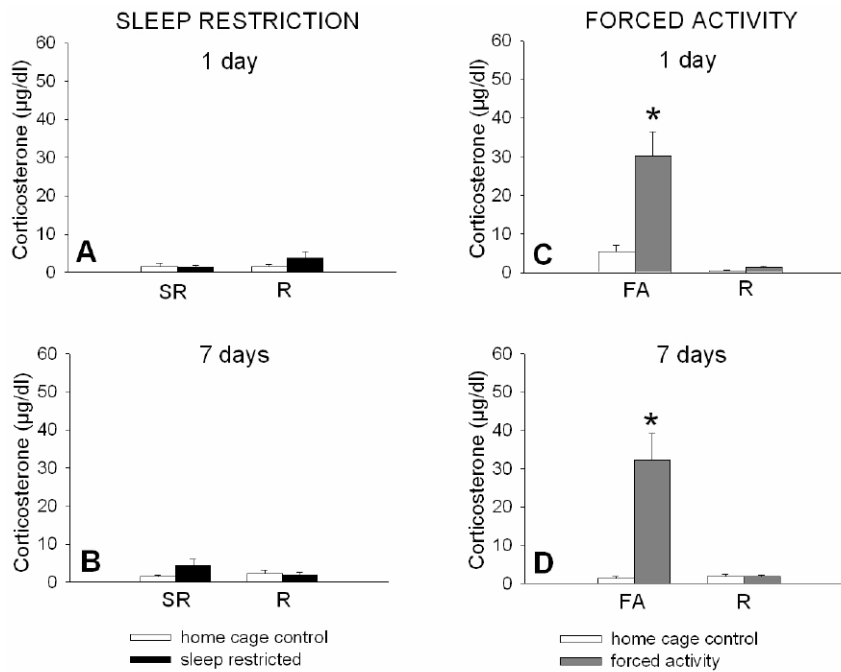
No differences were observed in serum corticosterone levels between sleep restricted and home cage control rats, neither after 1 day nor after 7 days of sleep restriction (Fig. 3A and B).

Experiment 2: Forced activity control

Contrary to the sleep restricted animals in Experiment 1, the rats subjected to the protocol of forced activity at higher intensity did not show significant changes in the temperature response to 8-OH-DPAT (Fig. 2C and D). Whereas sleep restricted rats did not show significantly elevated levels of the stress hormone corticosterone, the animals that were subjected to the forced activity protocol had increased levels of corticosterone at the end of the activity session, which returned to baseline levels during the rest periods (Fig. 3C and D). The elevation in corticosterone in these animals was similar after the first and seventh day of the protocol.
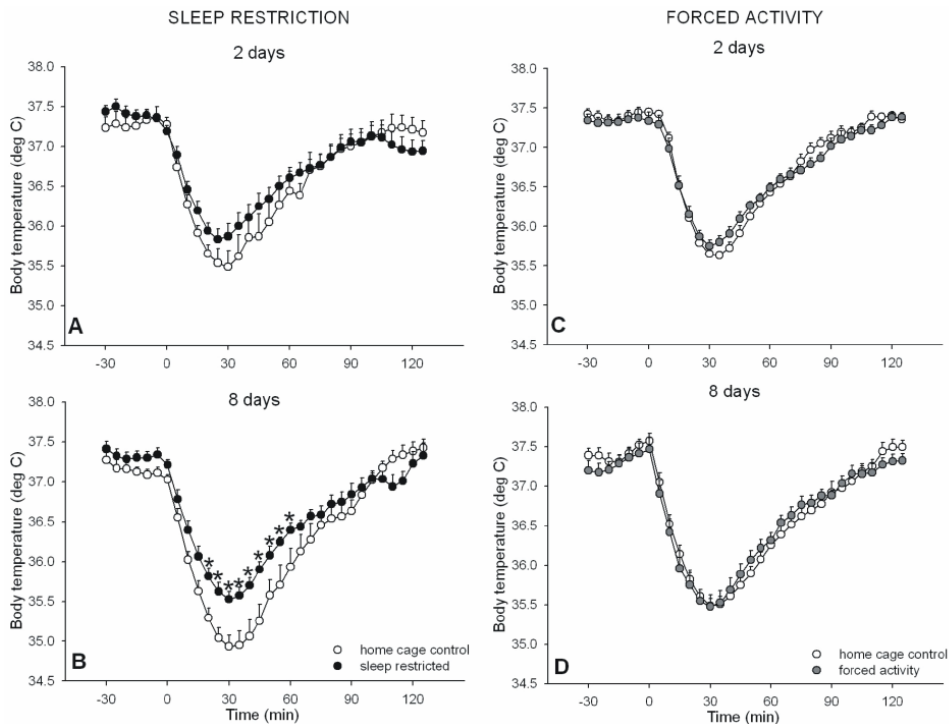

Figure 2. Chronic sleep restriction gradually desensitizes serotonin-1A receptors in the brain. Rats received injections of the serotonin-1A agonist 8-OH-DPAT (0.25 mg/kg). The sensitivity to the drug was measured by recording the acute hypothermic response by means of radio telemetry with implanted transmitters. [A and B] The hypothermic response to 8-OH-DPAT after 2 or 8 days of restricted sleep. After 8 days of restricted sleep the serotonin-1A receptor-mediated hypothermic response was significantly attenuated (repeated measures ANOVA: treatment effect: $F_{(1,12)}$ = 7.615, p = 0.017; treatment x time interaction: $F_{(20, 240)}$ = 3.78, p<0.001). On each day, n=8 for sleep restriction, n=6 for control). [C and D] The hypothermic response to 8-OH-DPAT after 2 or 8 days of forced activity at double speed for half the time. No significant differences were found between animals subjected to forced activity and home cage controls. On each day, n=8 for sleep restriction and n=8 for control.

Figure 3. *Plasma levels of the stress hormone corticosterone in rats subjected to sleep restriction and forced activity. Blood samples were collected by tail bleeding after the first and after the seventh day of the sleep restriction or forced activity protocol. [A and B] No differences were observed in serum corticosterone levels between sleep restricted and home cage control rats, neither at the end of the daily sleep deprivation session (SR), nor at the end of the daily 4h resting phase (R). [C and D] Animals subjected to a protocol of forced activity at double speed had significantly elevated corticosterone levels compared to home cage rats, both after 1 day ($F_{(1,14)}$=14.326, p<0.01) and after 7 days ($F_{(1,14)}$=20.506, p<0.01). On each day, the elevations that occurred immediately after the forced activity (FA) had disappeared after 4h of rest (R).*
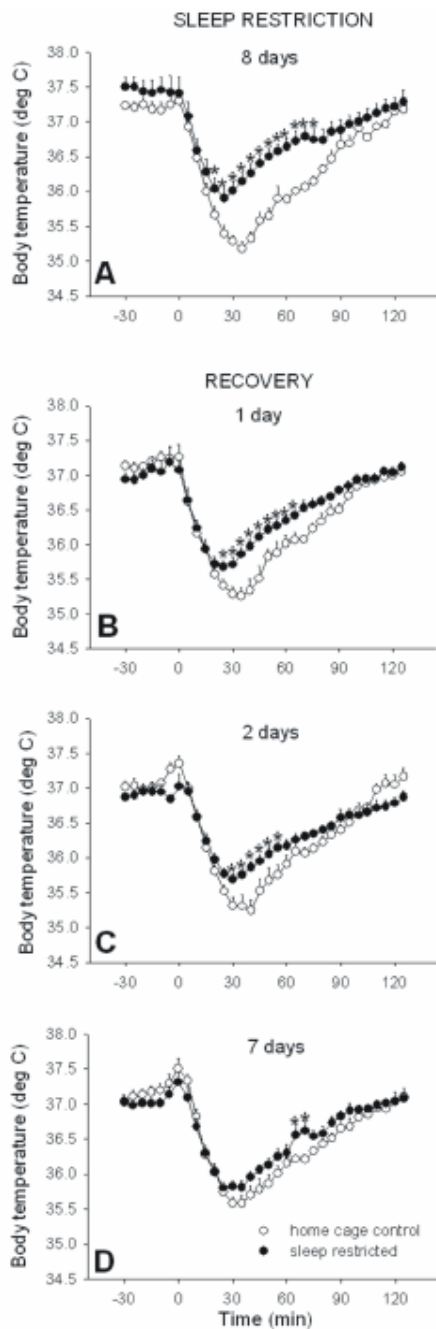
Figure 4. *The reduced hypothermic response to serotonin 1A stimulation after 8 days of sleep restriction persists for several days even with unlimited recovery sleep. (A) 8 days of sleep restriction (treatment effect: $F_{(1,13)} = 8.462$, $p = 0.012$; treatment x time interaction: $F_{(20, 260)} = 5.351$, $p<0.001$). (B) 8 days of sleep restriction followed by 1 day of unlimited recovery sleep (treatment effect: $F_{(1,13)} = 5.440$, $p = 0.036$; treatment x time interaction: $F_{(20, 280)} = 3.274$, $p<0.001$). (C) 8 days of sleep restriction followed by 2 days of unrestricted recovery sleep (treatment x time interaction: $F_{(20, 260)} = 3.351$, $p<0.001$). (D) 8 days of sleep restriction followed by 7 days of unrestricted recovery sleep (treatment x time interaction: $F_{(20, 280)} = 2.721$, $p<0.001$). On each day, $n=8$ for sleep restriction and $n=8$ for control.*

Experiment 3: Recovery

Confirming the results of the first experiment, rats had a significantly attenuated response to 8-OH-DPAT after 8 days of restricted sleep (Fig. 4A). The attenuated serotonin-1A response did not rapidly normalize with unrestricted recovery sleep but persisted for many days (Fig. 4B-D). Even after 7 days, the serotonin 1A mediated response had not fully normalized (Fig. 4D).

DISCUSSION

The present study aimed to make a link between two sets of observations: one, the observation that sleep problems may be associated with increased sensitivity to psychopathology; and two, the observation that mood disturbances are associated with decreased serotonergic neurotransmission. The data confirm that chronic sleep restriction gradually alters serotonin-1A receptor sensitivity in a direction that is similar to what is seen in affective disorders. Along with other evidence of attenuated serotonergic neurotransmission, depressed patients show a blunted temperature response to serotonin-1A receptor stimulation similar to our chronically sleep restricted rats (Lesch, 1991; Mann *et al.*, 1995; Shapira *et al.*, 2000).

In the present study, sleep restriction was achieved by forced locomotion. Therefore, changes in serotonin-1A receptor sensitivity might have been partly due to physical activity or to stress associated with the protocol. We performed a control experiment with rats that were forced to walk at double speed for half the time. These rats covered the same distance as the sleep restricted rats, however, they walked at a higher intensity and had more time to sleep (14h of rest per day versus 4h in the sleep restricted animals). Contrary to the sleep restricted animals, these rats did not show significant changes in the serotonin-1A response. The latter finding is in accordance with other studies showing that neither acute exercise nor chronic training affected postsynaptic serotonin-1A receptor sensitivity measured by behavioural responses such as forepaw treading and flat body posture in rats (Chauoloff, 1994).

We also measured plasma levels of the stress hormone corticosterone to examine the possible involvement of stress in the effects of sleep loss. In the sleep restricted animals, corticosterone levels were not significantly elevated compared to home cage control rats, suggesting that the sleep disruption procedure was not particularly stressful for these rats. These data are in line with other studies showing that sleep deprivation by forced locomotion does not or only mildly increase corticosterone levels (Tobler *et al.*, 1983; Meerlo *et al.*, 2002). In contrast, the animals that were subjected to forced activity at double speed for half the time had elevated levels of corticosterone at the end of their activity sessions. Together these results suggest that sleep restriction attenuates serotonin-1A receptor sensitivity by a mechanism that does not involve glucocorticoids and that is independent of stress and forced activity.

A potential explanation for the gradual desensitization of the serotonin-1A receptor system in the sleep restricted rats is a direct effect of serotonin itself, that is, a chronically enhanced serotonergic load on the serotonin-1A receptors. Microdialysis studies have shown that the release of serotonin during wakefulness and sleep deprivation is higher than during sleep (Park *et al.*, 1999; Lopez-Rodriguez *et al.*, 2003; Penalva *et al.*, 2003), and it is a common phenomenon that continued or frequent stimulation of receptors gradually diminishes their functional reactivity. Indeed, it has been shown that repeated injections of an agonist result in 1A receptor desensitization (Kreiss and Lucki, 1992). Also, in serotonin transporter knock-out mice with tonically increased extracellular serotonin levels, the serotonin-1A receptor-mediated temperature and neuroendocrine responses are reduced (Li *et al.*, 1999). Thus, chronic sleep restriction may be

a condition with chronically elevated levels of serotonin which, in the long run, may be responsible for the receptor desensitization here reported.

Alternatively, the desensitization of the serotonin-1A receptor population after chronic sleep restriction may be an indirect consequence of cross-talk between this receptor system and other neurotransmitter systems, for example, the adenosine system. Adenosine in particular is an important homeostatic molecule that signals neuronal activity and wakefulness (Porkka-Heiskanen *et al.*, 1997; for review see Basheer *et al.*, 2004). Adenosine is a metabolite of ATP, the main source of fuel in our body, and is thereby directly coupled to cellular energy use, including neuronal energy use in a highly active waking brain. Release of adenosine, via stimulation of its widespread G-protein-coupled A1 receptors, inhibits neuronal activity and protects the brain against overactivity. However, chronic stimulation of these receptors may result in desensitization (Olah and Stiles, 2000). Such desensitization may not be restricted to the receptors themselves but may involve downstream elements of the signalling pathway, which could eventually also affect the serotonin-1A receptor system. In various brain regions, adenosine A1 and serotonin-1A receptors are co-localized and share elements of their signal transduction pathways, including the G-proteins via which these receptors act on intracellular signalling cascades (Zgombick *et al.*, 1989). A number of studies have demonstrated that G-protein levels associated with adenosine A1 receptors may decrease in response to chronic agonist exposure (Zgombick *et al.*, 1989). It might be that increased adenosine turn-over and frequent stimulation of adenosine receptors under conditions of chronic prolonged wakefulness ultimately affects intracellular signalling pathways associated with the serotonin 1A receptor.

In our experiment, desensitization of the serotonin-1A receptor system developed gradually. The sleep restricted rats displayed normal temperature responses to 8-OH-DPAT after two days, but after 8 days of restricted sleep a significant attenuation of the response had developed. This finding of an accumulated effect of sleep loss is in line with studies in humans showing that successive nights of restricted sleep cause a gradually accumulating decline in cognitive function (Dinges *et al.*, 1997; Van Dongen *et al.*, 2003). Importantly, in our experiment the serotonin-1A receptor desensitization persisted for several days, despite unrestricted recovery sleep. In fact, after 8 days of restricted sleep, complete normalization of serotonin 1A receptor sensitivity almost required a similar period of recovery. The important implication of these data is that, sleep loss-induced changes in the brain not only accumulate over time but are also far more persistent than is generally assumed. Chronically restricted sleep causes gradual and persistent alterations in the serotonergic system, thereby providing a mechanism via which disrupted and restricted sleep may alter the sensitivity to psychopathologies such as depression (Chang *et al.*, 1997).

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