

Recovery Sleep after Extended Wakefulness Restores Elevated A₁ Adenosine Receptor Availability in the Human Brain

Abbreviated title: Effect of Recovery Sleep on Adenosine Receptors

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Abstract

Background: Adenosine and functional A₁ adenosine receptor (A₁AR) availability are supposed to mediate sleep-wake regulation and cognitive performance.

We hypothesized that cerebral A₁AR availability after an extended wake-period is decreasing to a well-rested state after recovery sleep.

Methods: [¹⁸F]CPFPX positron emission tomography was used to quantify A₁AR availability in 15 healthy male adults after 52 h of sleep deprivation and followed by 14 h of recovery sleep. Data were additionally compared to A₁AR values after 8 h of baseline sleep from an earlier dataset. Polysomnography, cognitive performance and sleepiness were monitored.

Results: Recovery from sleep deprivation was associated with a decrease in A₁AR availability in several brain regions, ranging from 11% (insula) to 14% (striatum). A₁AR availabilities after recovery did not differ from baseline sleep in the control group. The degree of performance impairment, sleepiness and homeostatic sleep pressure response to sleep deprivation correlated negatively with the decrease in A₁AR availability.

Conclusion: Sleep deprivation resulted in a higher A₁AR availability in the human brain. The increase that was observed after 52 h of wakefulness was restored to control levels during a 14-h recovery sleep episode. Individuals with a large increase in A₁AR availability were more resilient to sleep loss effects than those with a subtle increase. This pattern implies that differences in endogenous adenosine and A₁AR availability might be causal for individual responses to sleep loss.

Significance statement

Our study reveals that prolonged sleep deprivation is accompanied by an A₁ adenosine receptor (A₁AR) upregulation in the human brain. Recovery sleep quickly restores A₁AR availability to control levels. High individual A₁AR availability is related to a low sleep pressure and good cognitive performance.

Sleep deprivation is an efficient but short lasting therapeutic strategy in depression. A causal sleep-wake dysregulation has been proposed, possibly mediated by cerebral adenosine and its A₁AR. The restoration of the A₁AR availability after recovery from sleep deprivation mimics the rapid relapse following the end of therapeutic sleep deprivation.

Understanding the adenosine regulation under sleep restriction, especially regarding individual characteristics, might improve the rationale for the individual indication and design of therapeutic sleep modulation in depression.

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Introduction

Sleep loss is known to impair almost every aspect of cognition such as learning (1), long-term memory consolidation (2), attention and psychomotor vigilance (PVT) (3), and executive functions (4) including decision making (5) and emotional control (6). Sleep deprivation further typically alters the frequency distribution of the waking electroencephalogram (EEG) as an indicator of alertness corresponding to cognitive performance (7). Large inter-individual differences exist however in the degree of cognitive performance decline during sleep deprivation (3). In a trait-like process some individuals keep high-level performance during sustained wakefulness while others suffer from severe performance loss (3). The neuro-molecular mechanisms in the brain responsible for these different vulnerabilities are still largely unknown. Caffeine, commonly consumed for fighting fatigue, promotes wakefulness via adenosine receptor antagonism. It seems likely that the adenosinergic system is a neurochemical link between performance and sleep (8). Adenosine is contributing to the homeostatic process of sleep-wake regulation (for review see (9-12)). As has been shown in cats and rats, extracellular adenosine concentration fluctuates rhythmically in many brain regions such as the basal forebrain, increasing during wakefulness and decreasing during sleep. It thereby induces sleep after wake extension and is in turn restored to baseline levels after recovery sleep (13). For additional information on adenosine see supplemental information.

According to the two-process model of sleep-wake regulation (14), homeostatic sleep pressure increases with time awake according to a saturating exponential function, and declines exponentially during sleep. It has been proposed that the development of depressive symptoms is associated with a dysfunction in this homeostatic sleep drive (15). Recently a synaptic plasticity model of therapeutic sleep deprivation in major depression has been proposed (16). The model integrates the synaptic plasticity hypothesis of depression (17) and

the synaptic homeostasis hypothesis (18). According to this model, therapeutic sleep deprivation strengthens synapses, thereby shifting the deficient long-term potentiation in patients with major depressive disorder in a more favorable range of associative plasticity. Sleep deprivation and sleep restriction are effective, but short-lasting treatments (19) in depression. In contrast, healthy individuals show negative effects concerning mood, alertness and cognition. Adenosine-related interactions are also crucial in astrocyte-neuron communication, which underlies both cortical sleep (20) and also antidepressive effects of sleep deprivation (21). Apart from extracellular adenosine itself, evidence exists for the mediating subtype of adenosine receptors to regulate sleep-wake rhythmicity. In the central nervous system the A₁ subtype shows the widest distribution among adenosine receptors, with particularly high densities in various areas of the cortex, striatum and thalamus (22). The neurophysiological and behavioral effects of sleep deprivation in cats were mimicked by increasing the adenosine concentration experimentally (13, 23). Several studies in cats and rodents revealed that activation of the A₁ adenosine receptor (A₁AR) by an agonist and blockage by an antagonist up- and downregulated sleep propensity (24, 25). Moreover, A₁AR mRNA was shown to increase in the basal forebrain under sleep restriction (11). Inhibiting the A₁AR mRNA translation in rats decreased non-REM sleep and increased wakefulness (26). An upregulation of A₁AR density in the human and in the rat brain in response to acute sleep loss (10, 27, 28) has been shown. Neither adenosine nor adenosine receptors can easily be studied in vivo in the human brain. However, positron emission tomography (PET) is a tool that allows for exploring adenosine receptors in vivo. In earlier experiments we already found evidence that A₁AR availability is stable after repeated 8-h sleep episodes (29) and increased after 24 h of sleep deprivation (27). In the present study, we intended to increase sleep pressure even further to examine if A₁AR availability is satiating as predicted by the two-process-model and whether the exponential discharge of sleep pressure during recovery sleep is reflected in A₁AR availability.

The aims of this study were therefore to determine in healthy volunteers 1) to what extent 14 h of recovery sleep reduces cerebral A₁AR availability as measured following 52 h of sleep deprivation (primary outcome parameter), 2) if such recovery sleep restores A₁AR availability to the rested levels found in an independent control group after an 8-h sleep episode without preceding sleep deprivation, and 3) if impairment of cognitive performance under sleep deprivation compared to following recovery sleep is correlated with a higher cerebral A₁AR availability (exploratory analyses). A₁AR availability was measured in 14 participants using PET after 52 h (SD52) of sustained wakefulness followed by 14 h of recovery sleep (REC14) and compared to A₁AR availability after an 8-h sleep episode in a control group of 20 participants.

For reasons of radiation protection, it was not possible to investigate each participant more than twice. Instead of measuring baseline A₁AR availability after an 8 h sleep episode we performed a scan after sleep deprivation and after recovery sleep. As shown previously there is a high test-retest reliability of A₁AR availability after an 8-h sleep episode (29) which is also comparable between groups of the same age (30). Receptor binding data of earlier experiments after 8 h of sleep at night (27, 29) were therefore integrated into the present analyses as independent control group values (Table 1).

Results

Group characteristics: Table S1 provides an overview of participants' demographic data and scanning parameters. Both groups were not significantly different in these aspects.

Imaging Quantification: Regionalized A₁AR availability values and statistics are presented in Table 1. Under sleep deprivation the time spent awake before the scans was 52:26 h:min ± 1:45 h:min. A₁AR availability was significantly higher after 52 h of sleep deprivation in all examined brain regions compared to the scan after recovery sleep and in some regions compared to the control group. A₁AR availability did not differ between recovery and the

control group. Figure 1 displays the average parametric images of A₁AR availability for both conditions. A higher cortical binding after sleep deprivation in comparison to recovery sleep is apparent in various regions. The average regional decrease of A₁AR availability after recovery from sleep deprivation (i.e. SD52-REC14) ranged from 14% (striatum) to 10% (temporal cortex). Figure 2 shows the distribution of the relative difference between both days $[(SD52-REC14)/SD52]$ of the examined regions of interest (ROI).

Cognitive performance, sleep, and sleepiness: Mixed linear regression showed that performance in PVT and N-back declined, and sleepiness increased significantly with time awake (PVT: response speed and lapses $p < 0.0001$, N-back: correct response (sum 1-, 2-, and 3-back) $p < 0.0001$, Karolinska sleepiness scale KSS $p < 0.0001$). The 14-h recovery sleep period restored performance and sleepiness (Table S2).

A₁AR availability and cognitive performance in response to sleep deprivation varied considerably among individuals. Contrary to our third hypothesis, Figure 3 illustrates that across individuals larger decreases in A₁AR availability (SD52-REC14) were correlated with smaller decrements in PVT and N-back task performance as well as with less sleepiness (significant correlations for other brain regions can be found in Table S3). Furthermore, A₁AR availability correlated also negatively with the percent time spent in slow wave sleep (i.e. stage N3) during the first sleep cycle of recovery sleep.

Two subgroups were identified based on inter-individual differences in A₁AR decrease between 52 h of sleep deprivation and the recovery condition. A PET- A₁AR availability test-retest evaluation study revealed that in the striatal region the average of the absolute difference between scans was 0.1 mL/mL (29). This value was used as cut off criterion to divide subjects into two groups, one group (n=8) with a large difference (>0.1 mL/mL) in A₁AR availability between sleep deprivation and recovery, and one group (n=6) with small difference (≤ 0.1 mL/mL). Individuals with large differences in A₁AR availability proved

resilient to the effects of sleep loss on performance whereas individuals with minor differences in A₁AR availability showed strong degradations in performance (Figure 3 and Figure 4). Such group differences were not found for sleepiness.

The comparison of the subjects with minor and predominant A₁AR decrease from SD52 to REC14 did not reveal any significant difference in receptor binding at SD52 after correction for multiple testing (Benjamini and Hochberg method, $n=10$). On the other hand, when comparing the changes within the subjects with minor or predominant A₁AR availability decrease all brain regions (except orbitofrontal and temporal cortex) are significantly different between SD52 and REC14 for the group that showed predominant A₁AR availability decreases (Benjamini and Hochberg method, $p<0.02$, $n=20$ t-tests) (Table S4).

Discussion

This study reveals for the first time that 14 h of recovery sleep after 52 h sleep deprivation decreases elevated A₁AR availability in the human brain. This decrease in A₁AR was predominant in striatum and thalamus, but also evident in other brain regions including the orbitofrontal cortex, amygdala, occipital cortex, frontal cortex, anterior cingulate cortex, insula, parietal, and temporal cortex (in descending order). In comparison to a well-rested independent control group we observed an increase in A₁AR availability after 52 h sleep deprivation which was significant in frontal, occipital and parietal cortex. Our human data are confirming earlier autoradiography experiments in rats which were kept under 48 h of sleep deprivation (31). The authors observed an upregulation in A₁AR availability of up to 23% in the striatum and 13% in the cortex. Own studies in rats which were sleep deprived for 12 h or 24 h showed also an increase in A₁AR availability in the basal forebrain and in cortical areas (28). Nevertheless it should be kept in mind that the impact of prolonged sleep deprivation (~50 h) cannot easily be compared between rats and humans given the sizable differences in the kinetics (i.e. time constants, triggers, metabolic processes) of the homeostatic build up

and continuity of sleep between the species. Moreover, there are large differences in the procedure to apply sleep deprivation in humans and rats that may impact the results as well. Common methods for sleep deprivation in rodents impose varying levels of stimulation, physical activity or stress on the animals that is fundamentally different from voluntary wakefulness in human subjects or patients.

Compared to a previous human study in which we investigated A₁AR availability after 28 h of sleep deprivation (27), there was no significant additional increase in receptor availability in the present study with 52 h of sleep deprivation, although the sample sizes might have been too small to detect subtle differences. The results, however, appear to be consistent with the two-process model, which due to the saturating kinetics of sleep pressure only predicts a small additional increase between 28 h and 52 h of wakefulness. A single night of 14 h recovery sleep was sufficient to restore A₁AR availability to levels that were observed in the well-rested control group, consistent with the rapid exponential discharge of sleep pressure during sleep. Taken together, the data support the assumption that the sleep-wake-dependent fluctuations of homeostatic sleep pressure are mediated – at least in part – by the amount of A₁AR available.

Another key but counterintuitive finding of the present study was that the decrease in A₁AR availability was highly, but negatively correlated with 1) the degradation of cognitive performance in the PVT and in the N-back task, 2) the rise in subjective sleepiness during prolonged wakefulness as well as 3) sleep pressure reflected in the amount of N3 in the first sleep cycle of recovery sleep. The brain regions in which we observed decreases in receptor availability have previously been identified as highly relevant for cognitive performance. In functional neuroimaging studies a widespread pattern of frontal and parietal cortical as well as thalamic brain areas was found to be active during ‘good’ cognitive performance (i.e. in the absence of lapses of attention), in contrast to lower activity in these areas during poor

performance in a visual, selective attention task (not PVT) (32). In an investigation of the PVT frontal and parietal activations were required to assure a good task performance after sleep deprivation versus baseline (33). From cognitive performance under sleep deprivation conditions it is known that the degree of impairment varies highly among individuals (34). Differences in caffeine effects have been linked to sleep loss induced performance impairments (35) and to genetic variants of the adenosinergic system (36, 37). Along these lines, we defined two subgroups based on the A₁AR availability decrease, of which one group showed a strong decline in receptor availability whereas the other revealed only a minor decline. The group with predominant decreases in A₁AR availability proved resilient to the effects of sleep deprivation on cognitive performance. Participants with a minor A₁AR availability decrease, however, were vulnerable and reacted with performance decline to sleep deprivation. These observations seem paradoxical at first glance. Although speculative, they might be explained by individual differences in the interplay of both a sleep loss-dependent increase in endogenous adenosine levels and a sleep loss-dependent upregulation of adenosine receptors, which both have been shown in animal experiments. If both groups experienced A₁AR upregulation in response to the prolonged time awake, but in the vulnerable group this upregulation was accompanied by a considerable increase in endogenous adenosine levels, increased receptor activation could have mediated the large performance impairing effects. In the resilient group, in contrast, the increase in adenosine levels may have been less pronounced, thus mediating smaller performance impairments, but leaving more A₁AR available for binding with the PET receptor ligand [¹⁸F]CPFPX. This interpretation is supported by several observations from animal experiments. First, adenosine concentrations were found to be increased in specific brain sites with prolonged wake-time (13). In vitro, we found evidence, that adenosine competes with CPFPX binding at the A₁AR (38). However, so far it has not been shown in humans that adenosine levels increase with prolonged wake-time. On the contrary, in medicated epilepsy patients with

pharmacologically refractory seizures no significant increase was detected with microdialysis in preparation for surgical resections in amygdala (n=7), hippocampus (n=1) or motor cortex (n=1) (39). Second, after an initial internalization of receptors, long-term agonist stimulation led to an increase in receptor mRNA and higher receptor availability (40). These findings imply that different from other downscaled G-protein coupled receptors (41), A₁AR are upregulated during prolonged wakefulness. This effect seems to enable sustained responsiveness of the system and to amplify the sleep-inducing function of adenosine.

In the current dataset, we further tried to link the differences in adenosine receptor availability to genetic polymorphisms that have been reported to explain resiliency to sleep deprivation and caffeine effects on sleep and performance (ADORA2A single nucleotide polymorphism (SNP) rs5751876, ADORA2A haplotype 4 (42)) and anxiety (43) or sleep (adenosine deaminase SNP rs73598374 (44)). ADORA2A SNPs might be relevant as we previously found an association between A₁AR availability under baseline condition and ADORA2A SNP (rs5751876 and rs2236624) in another population (43). However, presumably due to the rather small sample size we could not detect a significant association here. There was also no relationship between adenosine receptor availability and subjective caffeine sensitivity based on a previously evaluated questionnaire (35). Further, no association was found between the caffeine sensitivity subtype and cognitive performance or sleep parameters. Interestingly, subjective sleepiness (SD52-REC14) differed between the two subgroups indicating that caffeine sensitive subjects felt sleepier (Mann-Whitney-U test: $p = 0.008$, KSS median category 6) than insensitive ones (category 7).

At the time of the PET scans the subjects were off caffeine for at least 5 days, but duration of withdrawal might be up to 9 days (45). Saliva samples at the beginning of the study proved caffeine abstinence. None of the subjects reported withdrawal related symptoms like headache during the study period.

The negative correlation between the sleep loss-dependent decreases in A₁AR availability and increase in N3 in the first sleep cycle of recovery sleep seemingly contradicts animal findings on the involvement of the adenosinergic system in the homeostatic regulation of sleep (24-26, 46). This negative correlation – similar to the correlations for the cognitive performance impairments and sleepiness – is most likely due to a wake-dependent increase of adenosine release/concentration, outweighing the homeostatic upregulation of A₁AR and thus leaving fewer sites available for binding with the PET receptor ligand. In our human data set the recovery night did not only restore A₁AR availability to control levels, it also recovered cognitive performance and sleepiness ratings. Our findings are consistent with the concept of activity-dependent local sleep of groups or single neurons (47, 48) that integrates the synaptic homeostasis theory and metabolic theories, based on the occurrence of local neuromodulators like ATP and adenosine.

It is a robust finding that sleep deprivation improves depressive symptoms in a large proportion of human patients (49). Shortly after the first description of the two-process model of sleep-wake regulation it has been hypothesized that in depressed patients the homeostatic regulation might be deficient as reflected in a lower build-up of sleep pressure during wakefulness (15). A key candidate mediating both the homeostatic process and the antidepressant effect is adenosine. Interestingly, S-adenosylmethionine (S-AdoMet), a precursor of adenosine, is a widely used over-the-counter medication of major depression (50). More directly, it was recently shown in a readout-model of antidepressive effects (forced swim test in mice) that astrocytic adenosine signaling to A₁AR during sleep deprivation is necessary to reduce depressive-like behaviors (21). Upregulating A₁AR in a transgenic mouse model of conditionally enhanced forebrain A₁AR expression promoted resilience against depression-resembling reactions in various behavioral tests (51). Conversely A₁AR knockout mice had an increased depressive-like behavior and lacked the antidepressant effects of sleep

deprivation. The rapid relapse following the end of therapeutic total sleep deprivation is in line with our findings of a normalization of A₁AR availability following a single episode of recovery sleep. It is tempting to speculate that potential interventions that induce a chronic upregulation of A₁AR may have a longer-lasting therapeutic effect. In fact, continued sleep restriction following total sleep deprivation was reported to extend the antidepressant effect in some patients (52). Our findings have therefore potential clinical implications. The subtle differences in the settings of the adenosine receptor system that we found to be associated with different behavioural responses (vulnerable or resilient against sleep loss) might also serve as indicators for the outcome of therapeutic sleep deprivation in patients with major depression. Even more, there might be a specific ‘depression-type’ of cerebral receptor/enzyme settings which accounts for differential therapy efficacy, but also represents a primary neurochemical basis for depression-associated patterns of sleep disturbances and disease-associated behavioural phenotypes. In line with this assumption the beneficial therapeutic effects of sleep deprivation in major depression could arise from an adjustment of the pathological receptor/enzyme setting.

In conclusion we found that sleep deprivation resulted in a higher A₁AR availability in the human brain. The increase that was observed over 52 h of wakefulness was restored to control levels during a 14-h sleep episode. Individuals with a large increase in A₁AR availability were more resilient to sleep loss effects than those with a subtle increase. This pattern implies that differences in the endogenous adenosine and A₁AR availability might be causal for individual responses to sleep loss. We therefore propose that endogenous adenosine and its receptors are key players in the individual regulation of sleep-wake behaviour and cognitive performance. Understanding the mechanistic link between mood and adenosine regulation under sleep restriction, especially in the light of individual

characteristics, might improve the rationale for the individual indication and design of therapeutic sleep modulation in depression.

Methods

Participants. The study was approved by the Ethics Committee of the Medical Faculty of the University of Duesseldorf and the German Federal Office for Radiation Protection. 15 healthy, male volunteers gave written informed consent of which 14 (mean age 27.7 ± 5.4 years) were included in the analyses. For details on participant selection see SI Methods.

Study design. One week prior to the arrival in the laboratory, subjects maintained a sleep log and routine (bed time 11 pm to 7 am). Four days prior to the arrival they abstained from caffeine which was checked with saliva samples upon arrival and by plasma samples at the time of PET scans. The last three days before the laboratory stay they wore an actigraph to check compliance. After an adaptation night (11 pm – 7 am), polysomnographic measurements were recorded during one baseline night (11 pm – 7 am). From Monday morning until Wednesday afternoon (5 pm) two participants at a time were sleep deprived. The two participants completed the neuropsychological test batteries one hour apart of each other. Starting at 9 pm on Monday participants completed the test battery and a 3-min recording of waking EEG at 6-h intervals. Out of testing sessions, subjects were allowed to do non-vigorous activities. Subjects were continuously monitored by at least one study staff member in order to ensure wakefulness and adherence to the protocol. After SD52 and REC14 (5 pm – 7 am) participants were scanned with the two scans scheduled 24 h apart. First subject was scanned at 10 am and the second one at 12 am (mean scanning clock times: $11:42 \text{ am} \pm 1:16 \text{ h}$). The control group underwent the same scanning protocol but was allowed to sleep for 8 h during the night prior to the scan (baseline) but without neuropsychological testing. The study design is further presented in Figure 1 and SI Methods.

Polysomnography and neurobehavioral testing. See SI Methods.

PET. [^{18}F]CPFPX PET were performed as previously reported (27, 29). See SI Methods.

Statistical analyses. The sleep loss response in the PET (regional $A_1\text{AR}$ distribution volumes (V_T) in mL/mL) was quantified in reference to 1) control and 2) recovery condition with a one-way mixed analysis of variance (ANOVA) with subject as random factor ($p < 0.05$). Post hoc t-tests were used for pairwise comparisons with a false discovery rate corrected significance level. Spearman rank correlation and regression analyses were used to evaluate associations between adenosine receptor availability and 1) performance measurements, 2) self-ratings of sleepiness, and 3) sleep parameters. The effect of recovery sleep on performance measures and sleepiness ratings was evaluated with two-tailed paired t-tests. Average values are reported as mean $\pm$ standard deviation. For all analyses, significance was assumed at $p < 0.05$ if not stated differently.

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Figure Legends

Figure 1: A: Study design. Black arrows indicate times of the 6 hourly neuropsychological testings. Grey arrows indicate time points of PET scans. Abbreviation: TIB = time in bed. Average images of anatomy (B, MRI) and A₁AR availability (C and D, PET) after spatial normalization. Left, middle, right column, axial, sagittal, coronal view, respectively; coordinates according to the Montreal Neurological Institute brain atlas were 22, -17, 0 (x, y, z); n = 14.

Figure 2: Average relative differences [(52h sleep deprivation condition – recovery sleep condition)/52h sleep deprivation condition] of A₁AR availability (distribution volume V_T) in brain regions. Error bars indicate standard errors of the mean (SEM). * significant differences between sleep deprivation and recovery (paired t-test, $p < 0.022$, see Table 1), n = 14.

Figure 3: Significant correlations (Spearman) and regressions between the difference of A₁AR distribution volumes (V_T) after sleep deprivation and recovery sleep. A) Difference in psychomotor vigilance task (PVT) performance (number of lapses of attention) in the striatum. B) Difference in 3-back performance (number of omissions) in the insula. C) Fraction of slow-wave sleep in the first sleep cycle in the insula. D) Subjective sleepiness rating in the temporal cortex.

Figure 4: Time course of A) psychomotor performance (PVT); B) 3-back omissions and C) KSS-sleepiness during 58 h of sleep deprivation and after 14 h of recovery sleep. Based on high or low A₁AR availability the subjects were divided into two subgroups. The absolute

difference of the test-retest evaluation revealed that in the striatal region the average of the absolute difference between scans was 0.1 (29). This variance was selected as cut-off criterion for selecting groups with high and low receptor availability. The small inserts indicate average parametric receptor maps of subgroup high or low A₁AR availability at corresponding time points. Error bars indicate standard error of the mean (SEM). * significant differences in unpaired t-tests between subgroups at corresponding time points. For visualization purposes N-back time courses have been normalized to the respective baseline values at 14 h awake. Abbreviations: PVT = psychomotor vigilance task; KSS = Karolinska sleepiness scale.

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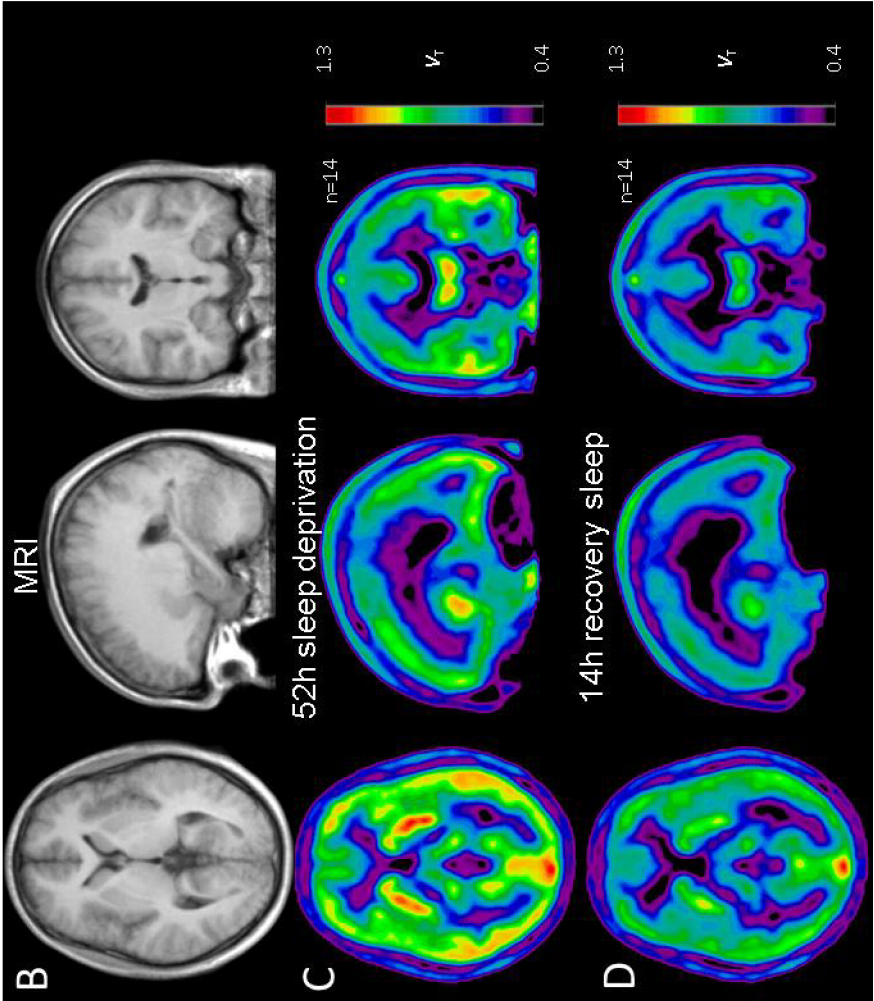
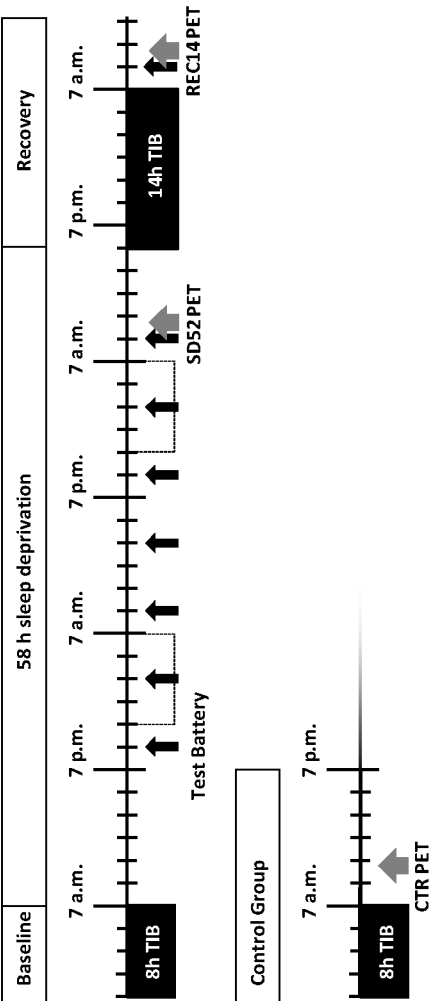
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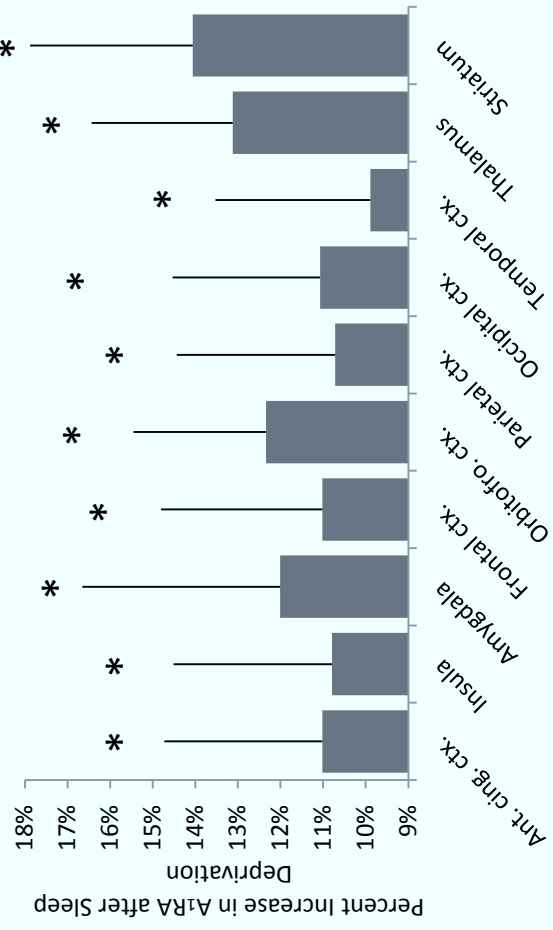
Table 1. Regional A₁AR distribution volumes (V_T) in mL/mL in two groups after 8 h control sleep, 52 h of sleep deprivation and 14 h recovery sleep.

Region	Receptor binding VT			ANOVA	unpaired	unpaired	paired t-test
	CTR	SD52	REC14		CTR vs. SD52	CTR vs. REC14	SD52 vs. REC14
Ant. cing. ctx.	0.77 ± 0.11	0.78 ± 0.11	0.69 ± 0.12	0.0094	0.7129	0.0490	0.0057
Insula	0.80 ± 0.11	0.86 ± 0.12	0.76 ± 0.13	0.0171	0.1484	0.3634	0.0083
Amygdala	0.75 ± 0.10	0.78 ± 0.11	0.67 ± 0.10	0.0162	0.3789	0.0397	0.0103
Frontal ctx.	0.78 ± 0.13	0.91 ± 0.12	0.80 ± 0.11	0.0082	0.0101	0.5659	0.0063
Orbitofrontal ctx.	0.73 ± 0.12	0.81 ± 0.12	0.71 ± 0.13	0.0031	0.0460	0.9001	0.0020
Occipital ctx.	0.80 ± 0.14	0.92 ± 0.13	0.81 ± 0.13	0.0058	0.0144	0.6582	0.0042
Parietal ctx.	0.77 ± 0.13	0.91 ± 0.13	0.80 ± 0.13	0.0061	0.0059	0.5323	0.0074
Temporal ctx.	0.75 ± 0.12	0.85 ± 0.12	0.76 ± 0.12	0.0130	0.0272	0.7261	0.0089
Thalamus	0.78 ± 0.13	0.88 ± 0.14	0.75 ± 0.12	0.0025	0.0328	0.8381	0.0015
Striatum	0.79 ± 0.15	0.88 ± 0.13	0.75 ± 0.12	0.0050	0.0599	0.4405	0.0020

Values are given as mean ± standard deviation; ANOVA p is the probability value of a mixed one-way ANOVA with subject as random; statistical comparisons that exceed the multiple-comparison-adjusted threshold (false discovery rate, Benjamini and Hochberg method, $p < 0.022$, $n=30$ t-tests) are in bold. Abbreviations: CTR = 8 h control sleep ($n=20$); SD52 = 52 h sleep deprivation ($n=14$); REC = 14 h recovery sleep; V_T = A₁AR distribution volume; ant = anterior; cing = cingulate; ctx = cortex.

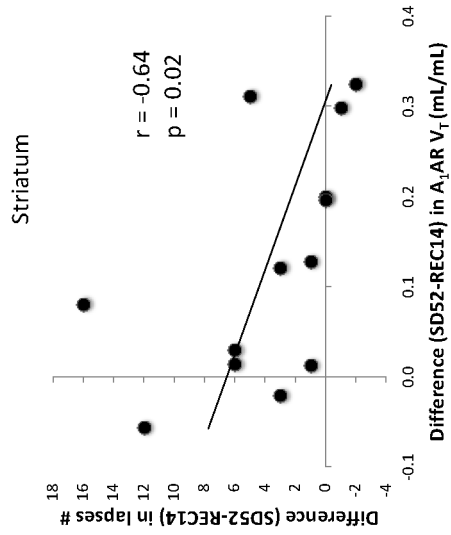
A





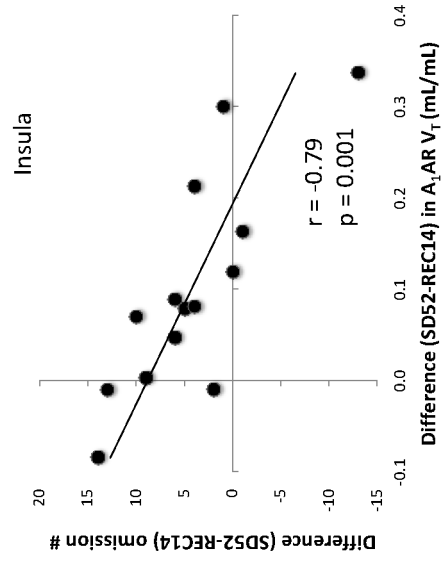
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PVT Lapses



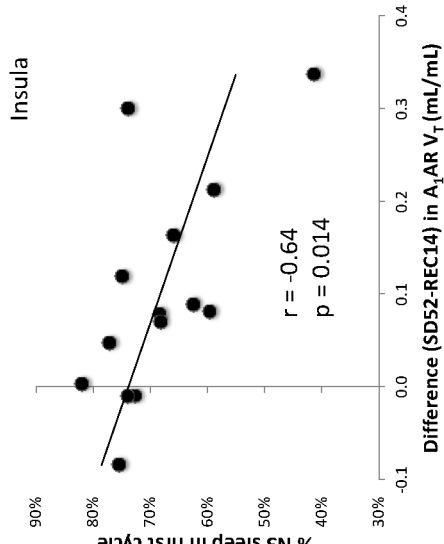
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3-back Omissions



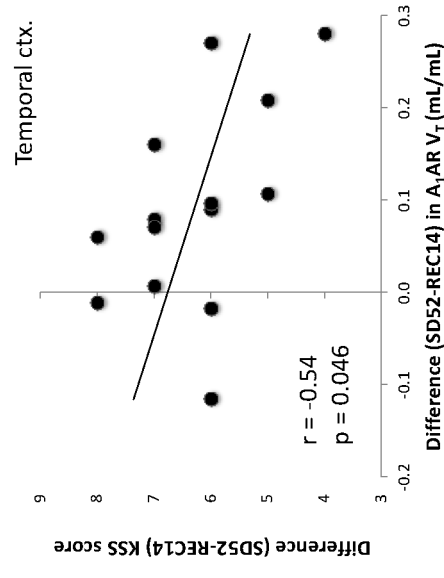
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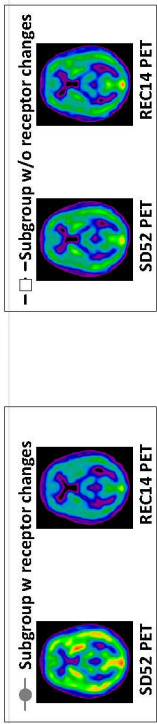
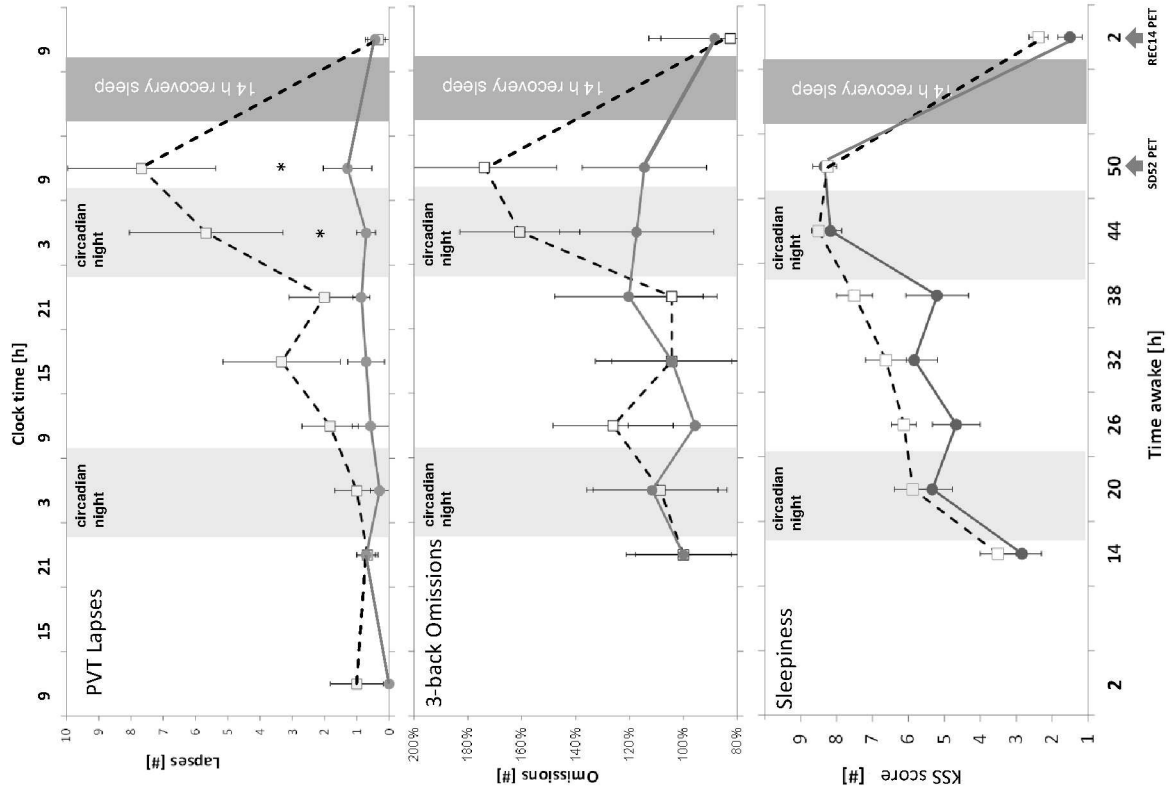
Sleep



D

Sleepiness rating





Supplemental Information

Introduction

Background on adenosine:

Adenosine is an important neuromodulator in the central nervous system and a key substance for the energetic homeostasis in cells (10). In times of high energy demand adenosine triphosphate (ATP) as an ubiquitous energy storage is dephosphorylated and adenosine is generated via adenosine di- and monophosphate by ATPase, ADPase and 5'-nucleotidase. Adenosine is not stored in vesicles, but atypically released into the extracellular space via transporters. These bidirectional nucleoside transporters ensure adenosinergic equilibrium between the extra- and intracellular space via concentration-driven passive replacement. Adenosine is rapidly converted to inosine by adenosine deaminase or to AMP by adenosine kinase, both intra- and extracellularly. Additionally, the extracellular adenosine level is regulated via SNARE-dependent gliotransmission of ATP from astrocytes (53). ATP is rapidly metabolized by ectonucleotidases into adenosine which in turn depresses synaptic activity on local neurons (54).

In general, adenosine decreases the activity of wakefulness/vigilance-promoting neurons in several brain regions, including basal forebrain and brainstem. Additionally, it might account for general cortical inhibition by attenuating input from ascending excitatory cholinergic and monoaminergic pathways. Especially, adenosinergic action in the basal forebrain might serve as the link to neurocognitive implications during sleep loss as this region has been shown to be involved in the control of sustained attention. Thus, the cortical sustained attention network which is closely linked to PVT performance in humans receives input from basal forebrain neurons and is likely modulated by them (for review see (55)).

Methods

Participants. Detailed interviews ensured that volunteers did not have a history of neurological and psychiatric diseases, sleep disorders, shift work, jet-lag, night work, head injury, and alcohol or substance abuse. Only non-smoking subjects without any medication were included. Caffeine intake was restricted 84 h prior to the arrival at the laboratory which was controlled by saliva samples upon arrival at the laboratory and by plasma samples at the time of the PET scans (below detection limits in all subjects - < 0.5 mg/L). One subject suffered from apneas and was excluded from all analyses. According to the caffeine sensitivity questionnaire (35) six participants reported sleep problems after caffeine intake in the afternoon indicating sensitivity to caffeine, six participants had no sleep impairment indicating insensitivity to caffeine and two participants did not consume caffeine at all. The average Becks depression inventory (BDI II) score sum of the subjects was 0.43 ± 0.94 at the beginning of the in-patient phase of the study.

During the beginning of the study, participants filled out a chronotype questionnaire (D-MEQ) (56). Based on the questionnaire, participants were classified as morningness, eveningness or intermediate type. Our evaluation shows that most of our sample belongs to the intermediate chronotype. Only two participants were classified as evening type and one as morning type.

Protocol. The subjects were asked to maintain a sleep log and adhere to a constant sleep-wake routine (bed time 11 pm to 7 am) in the week before the arrival at the laboratory. For the three days prior to the arrival the subjects wore an actigraph on their wrists checking compliance.

Between the adaptation night and the baseline night, participants were allowed to leave the laboratory during the day (between 9:15 am and 7:30 pm) with an actigraph attached to the non-dominant wrist. Upon arrival in the evening, saliva was tested for caffeine and actigraphs

were checked to exclude daytime napping. Apart from the previously described daytime, participants spent the scheduled time awake, including the testing sessions, in the research facilities of the Forschungszentrum Jülich and in the sleep laboratory of the German Aerospace Center. In between the testing sessions, non-vigorous activities (e.g. talking with each other, watching TV, playing calm games, reading, surfing the internet) were allowed. Under constant monitoring of at least one study staff member, two participants at a time were sleep deprived. Participants weren't allowed to close their eyes to ensure wakefulness. Whenever they closed their eyes they were addressed by the experimenter and encouraged to stay awake.

Polysomnography and neurobehavioral testing. During baseline and recovery sleep EEG (F4/A1, C4/A1, O2/A1), EOG, electromyography, electrocardiography, finger pulse, and breathing were recorded. Recordings were scored according to conventional criteria (57).

Every 6 h during wakefulness cognitive performance was assessed with a 3 min version of the PVT (58) on a portable, handheld computer. Reaction times in response to the lighting up of the battery lamp as trigger signal were recorded. Lapses in attention were defined as reactions longer than 500 ms.

Working memory capacity was tested with an N-back task in which the spatial position of a circle had to be remembered for 1, 2, and 3 positions back (59).

Sleepiness was measured on the Karolinska sleepiness scale (KSS) (60) and fatigue with the Samn and Perelli fatigue checklist.

PET. A Siemens ECAT EXACT HR+ scanner (Siemens-CTI, Knoxville, TN, USA) was used for 3D PET data acquisition. The radiotracer was injected as a bolus followed by a constant infusion with a K_{bol} value of 55 min. Scan duration was 100 min (start time see Table S1).

Arterialized venous blood sampling took place at min-timepoints 1, 5, and 10, and every 10

min subsequently. The total distribution volume V_T in the equilibrium (between 50 and 100 min) can be expressed as $V_T = TAC / C_p$ with TAC being the tissue activity concentration and C_p the plasma activity (27).

Realignment, co-registration, segmentation, and normalization of PET data and corresponding MRI (acquired on a 3T Siemens Magnetom Trio with MPRAGE sequence) were done with PMOD (version 3.305, PMOD Group, Zuerich, Switzerland).

To ensure comparability with previous own data and other neuroreceptor imaging studies of sleep-wake regulation, the selection of regions of interest (ROI) was done as described in Elmenhorst et al. (27) (individually drawn ROI) and Hefti et al. (61) (standard ROI from the Montreal Neurological Institute Atlas, anterior cingulate cortex, insula, and amygdala). In detail, the ROI that previously showed a significant increase in A_1AR after 28 h of sleep deprivation compared to 4 h of wakefulness were selected for the current analysis.

Activity data from PET voxels, which were classified as gray matter (probability higher than 10%) based on the anatomical MRI were corrected for the contribution (5%) of activity from blood within the tissue and used for further analysis.

The A_1AR availability which we will refer to in the following is directly proportional to the equilibrium total distribution volume. V_T in the equilibrium (between 50 and 100 min) can be expressed as $V_T = TAC / C_p$ with TAC being the tissue activity concentration and C_p the plasma activity (27).

Participants' constant wakefulness during PET scans was checked by video monitoring of subjects' eye blink behavior. Two participants were examined per day with a 2 h delay. The time slot of the scan (i.e. first or second scan of the day) did not have any significant effect on the outcome parameters ($p > 0.47$). Vigilance during the PET scanning was determined with

simultaneous EEG and electrooculography (EOG). At signs of drowsiness or sleep-like EEG patterns the subjects were requested to open their eyes.

The quantification of the receptor availability is based on an equilibrium pharmacokinetic model. Under steady state conditions the diffusion of the radioligand between the blood plasma compartment (input from the computerized infusion pump) and the brain tissue compartment (tissue ‘behind’ the blood brain barrier) results in a (temporally constant) ratio of these compartments which reflects the distribution volume which is directly proportional to the receptor density. Therefor the applied model is independent of changes in the amount of injected radioactivity. The difference of injected radioactivity (Table S1) between both studies is about 6% but the injected dose is slightly albeit not significantly lower. In both studies tracer conditions, e.g. conditions excluding any pharmacological effects, were guaranteed. We previously showed, that at these conditions the mass of injected CFPX had no effect on the binding parameters (62).

The Figure S1 shows for the parietal cortex the distribution volume in relation to the amount of injected activity (open circles denote the baseline group and the filled circles the experimental group).

Figure Legends

Figure S1: Distribution volume in relation to the amount of injected activity (open circles denote the control group and the filled circles the experimental group).

Table S1. Study participants' demographic and experimental parameters.

	Control group	SD52/REC14 group	p-value
N	20	14	n.a.
Age (years)	26.2 ± 1.1	27.7 ± 5.4	0.4
BMI (kg/m ²)	22.5 ± 3.6	24.5 ± 3.5	0.9
Chronic caffeine consumption (in 0.15 L cups of coffee)	1.3 ± 1.4	1.2 ± 1.7	0.87
Clock time of scanning	11:38 AM	11:41 AM	0.87
Injected radioactivity (MBq)	260.1 ± 6.2	277.1 ± 6.9	<0.001
Injected mass (nmol)	4.5 ± 2.2	3.1 ± 1.8	0.06

Values are given as mean ± standard deviation. Unpaired t-test. Abbreviation: BMI = body mass index.

Table S2. Effect of recovery sleep after prolonged sleep deprivation on cognition

	SD52	REC14	p
Karolinska Sleepiness Scale	8.3 ± 0.8	2.0 ± 0.9	< 0.0001
Fatigue Checklist (0-20)	15.1 ± 2.4	5.4 ± 2.5	< 0.0001
N-back Task: Correct responses			
- 3-back	8.4 ± 5.4	12.9 ± 5.5	0.0309
- sum (1- 3-back)	36.8 ± 11.3	48.9 ± 10.9	0.0083
Psychomotor Vigilance Task			
- Mean reaction speed (1/s)	3.91 ± 0.61	4.74 ± 0.69	0.0001
- Lapses	4.23 ± 5.1	0.38 ± 0.77	0.0213

p values represent two-tailed paired t-test. Values are given as mean ± standard deviation. n=14 (except for PVT, n=13).

Table S3. Correlations (Spearman) between the difference of A₁AR distribution volume (V_T) after sleep deprivation and recovery sleep and 1) the difference in psychomotor vigilance task performance (number of lapses of attention), 2) the difference in 3-back performance (number of omissions), 3) the fraction of slow wave sleep in the first sleep cycle and 4) the difference in subjective sleepiness rating.

	Performance PVT	Perf. 3-back	Sleep	Sleepiness rating
Δ VT	Δ Lapses #	Δ omissions #	% N3 in 1st cycle	Δ KSS
Ant. cing. ctx.	r=-0.65; p=0.016	r=-0.58; p=0.029	r=-0.60; p=0.023	r=-0.44; p=0.120
Insula	r=-0.30; p=0.327	r=-0.79; p=0.001	r=-0.64; p=0.014	r=-0.32; p=0.273
Amygdala	r=-0.46; p=0.110	r=-0.60; p=0.022	r=-0.49; p=0.078	r=-0.47; p=0.087
Frontal ctx.	r=-0.58; p=0.040	r=-0.64; p=0.014	r=-0.53; p=0.054	r=-0.49; p=0.078
Orbitofro. ctx.	r=-0.01; p=0.986	r=-0.55; p=0.040	r=-0.41; p=0.149	r=-0.53; p=0.052
Parietal ctx.	r=-0.46; p=0.117	r=-0.77; p=0.001	r=-0.60; p=0.022	r=-0.33; p=0.248
Occipital ctx.	r=-0.39; p=0.192	r=-0.74; p=0.002	r=-0.48; p=0.085	r=-0.50; p=0.068
Temporal ctx.	r=-0.45; p=0.125	r=-0.67; p=0.009	r=-0.44; p=0.114	r=-0.54; p=0.046
Thalamus	r=-0.54; p=0.055	r=-0.62; p=0.017	r=-0.51; p=0.061	r=-0.53; p=0.053
Striatum	r=-0.64; p=0.020	r=-0.57; p=0.035	r=-0.60; p=0.023	r=-0.42; p=0.140

Abbreviations: ant = anterior; cing = cingulate; ctx = cortex; PVT = psychomotor vigilance task, KSS = Karolinska Sleepiness Scale; V_T = A₁AR distribution volume.

Table S4. Subgroup analysis. Regional A₁AR distribution volumes (V_T) in mL/mL in two groups after 8 h control sleep, 52 h of sleep deprivation and 14 h recovery sleep and subgroups with minor and predominant changes.

Region	Receptor binding VT				Students ttest p value		
	n=6	n=8	n=6	n=8	unpaired	paired	paired
	SD52	SD52	REC14	REC14	SD52 low diff vs.	SD52 low diff vs.	SD52 high diff vs.
	low diff	high diff	low diff	high diff	SD52 high diff	REC14 low diff	REC14 high diff
Ant. Cing. Ctx	0.73	0.83	0.71	0.67	0.119	0.849	0.010
Insula	0.79	0.91	0.78	0.74	0.090	0.846	0.011
Amygdala	0.69	0.87	0.70	0.67	0.007	0.863	0.007
Hippocampus	0.71	0.82	0.70	0.66	0.040	0.890	0.005
Frontal Ctx.	0.84	0.95	0.83	0.77	0.076	0.936	0.003
Orbitofrontal Ctx.	0.76	0.86	0.72	0.72	0.152	0.606	0.039
Occipital Ctx.	0.86	0.97	0.84	0.79	0.131	0.782	0.012
Temporal Ctx.	0.79	0.92	0.78	0.75	0.072	0.972	0.023
Thalamus	0.81	0.93	0.78	0.74	0.105	0.704	0.007
Striatum	0.80	0.92	0.79	0.70	0.058	0.911	0.0003

Values are given as mean; statistical comparisons that exceed the multiple-comparison-adjusted threshold (false discovery rate, Benjamini and Hochberg method) are in bold.

Abbreviations: SD52 = 52 h sleep deprivation; REC = 14 h recovery sleep; V_T = A₁AR distribution volume; ant = anterior; cing = cingulate; ctx = cortex.

