Behavioral/Systems/Cognitive

Sleep Deprivation Increases A_1 Adenosine Receptor Binding in the Human Brain: A Positron Emission Tomography Study

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It is currently hypothesized that adenosine is involved in the induction of sleep after prolonged wakefulness. This effect is partially reversed by the application of caffeine, which is a nonselective blocker of adenosine receptors. Here, we report that the most abundant and highly concentrated A_1 subtype of cerebral adenosine receptors is upregulated after 24 h of sleep deprivation. We used the highly selective A_1 adenosine receptor (A_1AR) radioligand [^{18}F]CPFPX ([^{18}F]8-cyclopentyl-3-(3-fluoropropyl)-1-propylxanthine) and quantitative positron emission tomography to assess cerebral A_1ARs before and after sleep deprivation in 12 healthy volunteers and a control group ($n = 10$) with regular sleep. In sleep deprived subjects, we found an increase of the apparent equilibrium total distribution volume in a region-specific pattern in all examined brain regions with a maximum increase in the orbitofrontal cortex (15.3%; $p = 0.014$). There were no changes in the control group with regular sleep. This is the first molecular imaging study that provides *in vivo* evidence for an A_1AR upregulation in cortical and subcortical brain regions after prolonged wakefulness, indicating that A_1AR expression is contributing to the homeostatic sleep regulation.

Key words: **imaging; adenosine A_1 receptor; positron emission tomography; [^{18}F]CPFPX; sleep deprivation; human**

Introduction

There are numerous reports indicating that endogenous adenosine is a candidate for the homeostatic sleep factor theory inducting sleep after prolonged wakefulness (for review, see Basheer et al., 2004). Evidence supporting the role of adenosine derives from the wakefulness inducing effects of caffeine, which are mediated through the blockade of cerebral adenosine receptors. Continuous monitoring of adenosine levels during a sleep-wake cycle of freely moving cats showed that adenosine accumulates during prolonged wakefulness (6 h) in cats in the basal forebrain and, to a lower degree, in the cortex (Porkka-Heiskanen et al., 1997). Subsequent recovery-sleep restores the adenosine concentrations to baseline levels. These experiments have been repeated in rats with similar results (Basheer et al., 1999; Murillo-Rodriguez et al., 2004). An experimental elevation of the adenosine concentration in the cholinergic zones of the basal forebrain (Portas et al., 1997) or by inhibiting equilibrative nucleoside transporters (Porkka-Heiskanen et al., 2000) mimicked the electroencephalographic and behavioral effects of sleep deprivation.

Whereas adenosine is widely accepted as a sleep factor, the mediating subtype of adenosine receptors is highly debated. The A_1 subtype has the widest distribution in the CNS with particularly high concentrations in cortex, hippocampus, striatum, and thalamus (Fredholm, 1995). Direct application of a selective A_1 adenosine receptor (A_1AR) agonist increases the propensity to sleep in rats (Benington et al., 1995; Schwierin et al., 1996) and correspondingly a selective A_1AR antagonist decreases sleep propensity in cats (Strecker et al., 2000). Furthermore, 3 and 6 h of sleep deprivation increased A_1AR mRNA in the basal forebrain (Basheer et al., 2001). Consequently, microdialysis perfusion of A_1AR antisense oligonucleotides, which inhibit the translation of the A_1AR mRNA, significantly decreased non-rapid eye movement (REM) sleep and increased wakefulness in rats (Thakkar et al., 2003). However, A_1AR knock-out mice did not show different reactions to sleep deprivation than their wild-type littermates (Stenberg et al., 2003). Similarly it has been reported for the adenosine A_{2A} receptor (A_{2A}AR), that subarachnoidal infusion of a selective agonist (Satoh et al., 1999) promoted deep sleep.

Data on sleep-related changes in the human adenosine system are rare because neither adenosine nor its receptors are easily accessible *in vivo* in humans. A recent microdialysis study in epileptic patients revealed no increase in adenosine concentration in amygdala during 40 h of sleep deprivation (Zeitzer et al., 2006).

We have previously proposed and evaluated a method to quantify cerebral A_1ARs with receptor positron emission tomography (PET) and the radioligand [^{18}F]8-cyclopentyl-3-(3-

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fluoropropyl)-1-propylxanthine ([^{18}F]CPFPX) (Holschbach et al., 2002; Bauer et al., 2003; Meyer et al., 2004, 2005), which now allows to assess changes of A_1ARs *in vivo*. [^{18}F]CPFPX is a highly affine (dissociation constant, $K_D = 1.26$ nmol/L to cloned human A_1AR) and selective ($K_D = 940$ nmol/L for A_{2A}AR) compound, which shows a rapid brain uptake (time to peak within 5 min). The aim of the present study was to investigate whether prolonged wakefulness alters A_1AR availability in the human brain. For this purpose, volunteers were examined with PET on 2 subsequent days. One group of subjects was deprived of sleep for 24 h, another group had regular 8 h per night sleep between the two scans and served as a control.

Materials and Methods

Subjects. All procedures were approved by the Ethics Committee of the Medical Faculty of the University of Duesseldorf, Germany, and the German Federal Office for Radiation Protection. Twenty-two healthy male volunteers participated after giving written informed consent. Volunteers were screened for the following exclusion criteria: history of neurological and psychiatric diseases, sleep disorders, shift work, night work, head injury, and alcohol or substance abuse. All subjects were nonsmokers and not on any current or chronic medication. Caffeine intake was not allowed for at least 36 h before PET scanning. All subjects underwent two [^{18}F]CPFPX PET studies at the same time of day on consecutive days under identical conditions. Time of injection of the radioligand was between 10:00 A.M. and 2:00 P.M. in all subjects. The plasma metabolite analyses failed in one of the measurements of two subjects. Therefore, the data of these two subjects could only be analyzed using a reference region model. Thus, a total 22 subjects (control group, $n = 10$; sleep-deprivation group, $n = 12$) could be analyzed by the reference region analysis while 20 subjects (control group, $n = 8$) could also be analyzed using plasma data.

Before and after each PET scan, the subjects were asked to give a rating of their actual sleepiness on the Stanford Sleepiness Scale (SSS). The results were then averaged per scan.

Sleep-deprived subjects were monitored by staff members throughout the time between both scans, who supported them during the night to stay awake. During the 120 min period of PET data acquisition, the subjects were requested to stay awake and keep the eyes open. Via video monitoring system, it was assured that no subjects closed their eyes longer than usual. In this case, the subjects were addressed not to sleep and open their eyes again.

Detailed results about nine subjects of the control group will be published in a separate report addressing the test–retest stability of A_1AR quantification using [^{18}F]CPFPX.

[^{18}F]CPFPX PET was performed as described previously (Meyer et al., 2005) using a bolus/infusion schedule with a slightly different K_{bol} value (denotes the amount of bolus equaling an infusion of a certain length) of 55 min. The scan duration was prolonged from 90 to 120 min. Three subjects (two of the sleep deprivation and one of the control group) were scanned according to the original 90 min protocol ($K_{bol} = 48$ min).

PET acquisition and blood sampling. PET measurements were performed in three-dimensional mode on a Siemens (Knoxville, TN) ECAT EXACT HR+ scanner. Emission scans were started with the injection of 259 ± 4.3 MBq (specific activity, 105 ± 79 GBq/μmol).

Arterialized venous blood samples were collected at 1, 5, and 10 min and in 10 min intervals thereafter. It has been validated previously for [^{18}F]CPFPX bolus/infusion experiments that during equilibrium, venous and arterial concentrations equilibrate and venous blood sampling can substitute arterial (Meyer et al., 2005). Radioactivity determinations in whole blood and plasma, and plasma metabolite analyses and assessment of the fraction of free [^{18}F]CPFPX in plasma (denoted by f_1) were performed as described previously (Meyer et al., 2005). Plasma caffeine levels were assessed by HPLC.

Image analysis. Segmentation, realignment, normalization, and coregistration of PET and the individual magnetic resonance image (MRI) were done with SPM2 (Statistical Parametric Mapping, Wellcome Department of Cognitive Neurology, London, UK). Regions of interest (ROIs) were defined by freehand drawing of polygonal ROI on the individual MRI according to anatomical landmarks on transversal planes using the software PMOD (version 2.5; PMOD Group, Zurich, Switzerland). Within these ROIs, only PET voxels, which were classified as gray matter in the MRI, were included into time–activity curve (TAC) generation. This image-analysis procedure is adopted from the method proposed by Abi-Dargham et al. (2000). TACs were corrected for the contribution of intracerebral blood volume to the regional activity assuming a fractional blood volume of 5%.

The cholinergic basal forebrain and the nucleus basalis of Meynert are of particular interest for comparing the results of this study to animal experiments. The anatomical definition was done as proposed by Herholz et al. (2004), referring to landmarks [anterior commissure (ac), third ventricle, optic tract] identifiable on the MRI by positioning rectangular ROIs (7 × 3 mm) on three coronal planes on the right and left hemisphere. The localization was 2 mm caudal to ac on the ac–posterior commissure line, ventral to the ac and dorsolateral to the optic tract, at the base of the forebrain.

Outcome parameters. Although receptor density is the parameter of interest, the maximal number of receptors available for binding (B_{max}') cannot be determined in a single PET study. Therefore, outcome parameters which are directly proportional to B_{max}' have been proposed. For this report, two outcome measures with specific advantages and disadvantages for this setting have been chosen: equilibrium total distribution volume (DV_t') and binding potential (BP_2). DV_t' is composed of the specific distribution volume (DV_s, equal to B_{max}'/K_D) and the distribution volume of free and nonspecifically bound ligand (DV_{f+ns}). In the case of [^{18}F]CPFPX, the relatively low fraction of specific binding (~2/3 of DV_t') decreases the sensitivity for possible changes in DV_t'. Another shortcoming is the dependence on error-prone individual plasma input functions, which increases variability. These drawbacks can be avoided by using an approach independent on blood sampling by using a reference region input as for BP_2. Herein, the ratio of $DV_s/DV_{f+ns} - 1$ is also related to B_{max}'. The cerebellar cortex is used as reference region for [^{18}F]CPFPX PET (Meyer et al., 2007). Although the cerebellar cortex is the region in the human brain with the lowest A_1AR density, one third of the DV_t' is displaceable with unlabeled CPFPX (Meyer et al., 2006). Therefore, changes in BP_2 deserve careful consideration because of their dependency on DV_s changes in the reference region. BP_2 was determined by Logan's noninvasive graphical analysis (Logan et al., 1996). Under equilibrium conditions (time span from 50 to 100 min), DV_t' can be calculated by the ratio of TAC to plasma activity (C_p): $DV_t' = f_1\, DV_t = TAC/C_p$. The annex prime (′) was used to indicate that the DVt′ is not corrected for the free fraction of ligand in plasma (f_1). As discussed previously (Meyer et al., 2005), f_1 was not included into the analysis because it introduces a substantial error. [The f_1 value (2.01 ± 0.58%) correlated with DV_t (e.g., temporal cortex, $r^2 = 0.63$), but not with DV_t' ($r^2 = 0.24$).]

Statistical analyses. Average values are given as mean ± SD. Statistical significance was assessed with an ANOVA for repeated measures. If the day by group interaction indicated a significant effect ($p < 0.05$), *post hoc* Tukey–Kramer tests were used for pairwise comparisons.

Results

Group characteristics

The sleep-deprivation ($n = 12$) and the control ($n = 8$) groups were matched with regard to age (26.7 ± 2.5 and 27.6 ± 1.3 years, respectively; $p = 0.34$; Student's t test), gender (all male), and constitution (body mass index, 24.3 ± 2.5 and 24.8 ± 3.72 kg/m^2, respectively; $p = 0.68$). Average chronic caffeine consumption (in 0.15 L cups of coffee) was ~1.4 ± 1.4 and 2.2 ± 1.6 cups/d, respectively ($p = 0.48$). Sleep durations before the first and second scan of the control (8.3 ± 1 and 8.18 ± 0.5 h, respectively) and the first scan of the sleep-deprivation group (7.95 ± 0.6 h) did not differ significantly. Likewise, the time spent awake before these three scans (237.2 ± 74.2, 229.5 ± 84.0, and 246.1 ± 65.9 min, respectively) was not statistically different. The groups and conditions were not significantly different with regard to

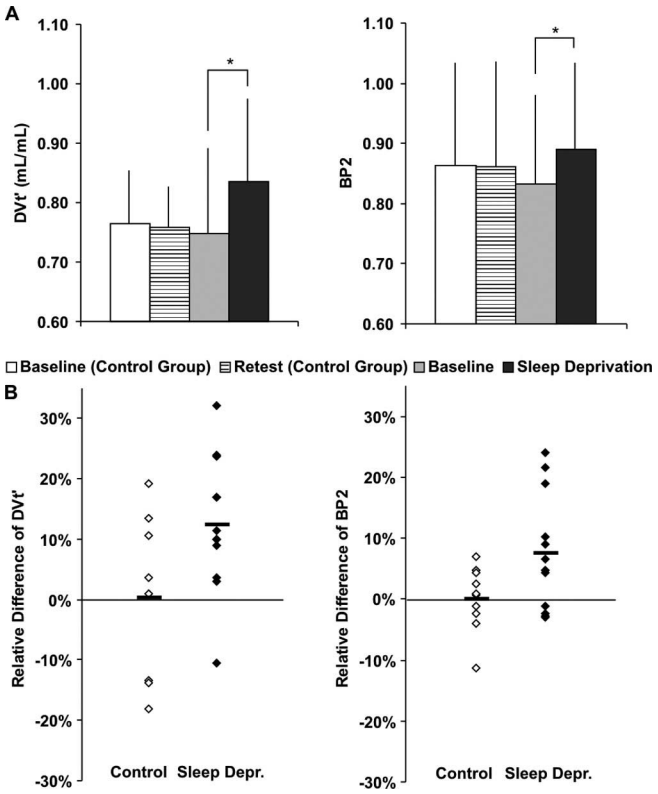

Figure 1. Adenosine receptor DV_t' and BP_2 in temporal cortex of 10 control (baseline and retest condition) and 12 sleep-deprived subjects (baseline and after sleep deprivation). ***A***, Absolute changes [significant interaction of day by group ($p = 0.04$ and $p = 0.04$ for DV_t' and BP_2, respectively), ANOVA for repeated measures, followed by Tukey–Kramer test (*) ($p = 0.02$ for DV_t'; $p = 0.03$ for BP_2)]. Error bars depict SD. ***B***, Scatterplot of relative changes [(day 2 − day 1)/day 1]. Horizontal bars show the mean.

injected activity or mass of injected CPFPX, specific activity, f_1 value, or mean rates of change of the parent compound in plasma.

Imaging quantification

No between-group differences in DV_t' and BP_2 were observed between controls and experimental group in the examined regions at the first day. Figure 1 displays the average DV_t' and BP_2 values per group and condition for the temporal cortex as a representative ROI. The A_1AR availability of the second day of the control group was not significantly different from the first scans of both groups, although the average BP_2 values of the control group were slightly higher than the average BP_2 values of the sleep-deprivation group. The regional DV_t' and BP_2 values and the results of the test for a significant interaction of day by group by repeated measures ANOVA are presented in Table 1. In case of significant interactions, pairwise comparison of groups and conditions were subsequently performed. This *post hoc* comparison revealed only significant differences between the first and second scan of the sleep-deprivation group. Therefore, only the results of these comparisons are displayed. Figure 1 shows the distribution of the relative difference between both days [(day 2 − day 1)/day 1] of both groups for the temporal cortex. As can be seen from this scatterplot, the test–retest variability is more prominent for DV_t' than for BP_2. The mean DV_t' values were elevated ~12.5% and the mean BP_2 values ~7.5% after sleep deprivation in the temporal cortex.

Mean parametric images of BP_2 are depicted in Figure 2. The increase in cortical binding is already appreciable in various regions by visual inspection. The corresponding average relative differences of the examined ROIs (except basal forebrain) are presented in Figure 3. The average regional change in the control group ranged between a decrease of −4.8% (BP_2) and −1.2% (DV_t') (occipital cortex), and an increase of 2.0% (BP_2) and 2.5% (DV_t') (thalamus). In contrast, in the sleep-deprivation group the average regional change of DV_t' and BP_2 ranged from 10.6% (cerebellum) to 15.3% (orbitofrontal cortex) and 4.7% (parietal cortex) to 13.2% (orbitofrontal cortex), respectively. The most pronounced difference was found in the orbitofrontal cortex (DV_t', 15.3%; BP_2, 13.2%). The average DV_t' increase was lowest in the cerebellum (10.6%), which is the cerebral region with the smallest amount of A_1AR in the human brain. The temporal cortex is the only region in the analysis of BP_2, which was significantly different after correction for multiple comparisons, although several other regions like the frontal and occipital cortex and striatum showed a trend toward significance ($p < 0.1$).

No significant changes in the outcome parameters were found in the nucleus basalis of Meynert. There was a weak tendency for an increase of DV_t' (and BP_2) after sleep deprivation of 6.3% (7.7%) for this ROI.

Looking at the relative change of sleepiness, the rating scale used (SSS) revealed a weak correlation between the increase in sleepiness and the change in DV_t' ($r = 0.303$; $p = 0.34$) but no correlation in case of BP_2 ($r = -0.004$; $p = 0.99$).

Discussion

This study demonstrates that a single night of total sleep deprivation leads to a significant increase of A_1AR availability in the human brain. This effect was observed regardless of the applied quantification method. The present findings point to an upregulation of A_1ARs by prolonged wakefulness because the reported outcome measures DV_t' and BP_2 are linearly related to the maximum concentration of available A_1ARs. This so far unknown mechanism could sustain long-term sleep-inducing effects of adenosine.

Both groups did not differ in chronic caffeine consumption, which was moderate in both groups. The withdrawal of caffeine before the study, to eliminate caffeine from plasma, had apparently no ongoing effects between 36 and 60 h after caffeine restriction on the A_1AR availability. There is no difference of DV_t' and BP_2 detectable in the control group between both days. The sleep durations of the subjects reported here were based on standardized self-reportings. If the self-reporting were somehow inaccurate, this could increase the variation of the results, but not affect the proposition per se.

Most of the subjects had only moderate difficulties to stay awake during the scanning and had rarely to be asked to keep their eyes open. One subject, however, had severe problems to maintain wakefulness during the scan after sleep deprivation and fell asleep for approximately one-third of the scanning period. It was not excluded from the study, because it is rather unlikely that 40 min of fragmented sleep could restore the effects of overnight sleep deprivation. When DV_t' data are evaluated without this subject, the results of the repeated-measures ANOVA show additional significant increases to those reported in Table 1 in the parietal cortex and cerebellum. For BP_2, the frontal, temporal, and occipital cortex as well as striatum still show the same magnitude of increase, but changes in the temporal cortex are no longer significant ($p = 0.075$).

These results in humans are in line with previous evidence

Table 1. Regional A_1AR DV_t' (in milliliter/milliliter) and BP_2 in control and sleep-deprived subjects

Region	Control		Sleep deprivation		ANOVA	Pairwise
	Day 1	Day 2	Day 1	Day 2	*p*	adjusted *p*
DV_t'						
Frontal ctx	0.80 ± 0.09	0.78 ± 0.08	0.77 ± 0.16	0.87 ± 0.15	0.041	0.033
Orbitofrontal ctx	0.75 ± 0.08	0.75 ± 0.06	0.72 ± 0.13	0.82 ± 0.13	0.047	0.014
Cingulate gyrus	0.74 ± 0.09	0.75 ± 0.08	0.73 ± 0.15	0.80 ± 0.15	0.118	-
Parietal ctx	0.78 ± 0.09	0.77 ± 0.08	0.77 ± 0.16	0.85 ± 0.15	0.074	-
Occipital ctx	0.80 ± 0.11	0.78 ± 0.09	0.80 ± 0.16	0.89 ± 0.16	0.035	0.036
Temporal ctx	0.76 ± 0.09	0.76 ± 0.07	0.75 ± 0.14	0.84 ± 0.14	0.041	0.021
Thalamus	0.77 ± 0.09	0.78 ± 0.07	0.78 ± 0.16	0.86 ± 0.16	0.157	-
Striatum	0.78 ± 0.11	0.77 ± 0.07	0.79 ± 0.17	0.89 ± 0.17	0.050	0.037
Cerebellum	0.41 ± 0.06	0.40 ± 0.02	0.40 ± 0.08	0.44 ± 0.08	0.127	-
Nucleus basalis	0.60 ± 0.07	0.63 ± 0.10	0.63 ± 0.10	0.67 ± 0.14	0.477	-
BP_2						
Frontal ctx	0.96 ± 0.17	0.94 ± 0.18	0.91 ± 0.15	0.97 ± 0.14	0.023	0.073
Orbitofrontal ctx	0.83 ± 0.16	0.84 ± 0.13	0.77 ± 0.15	0.85 ± 0.12	0.086	-
Cingulate gyrus	0.83 ± 0.16	0.83 ± 0.16	0.78 ± 0.12	0.82 ± 0.14	0.179	-
Parietal ctx	0.95 ± 0.15	0.92 ± 0.18	0.89 ± 0.14	0.93 ± 0.13	0.038	0.203
Occipital ctx	0.98 ± 0.16	0.94 ± 0.20	0.97 ± 0.14	1.03 ± 0.13	0.008	0.088
Temporal ctx	0.86 ± 0.16	0.86 ± 0.14	0.83 ± 0.12	0.89 ± 0.12	0.042	0.028
Thalamus	0.89 ± 0.11	0.91 ± 0.16	0.90 ± 0.15	0.95 ± 0.12	0.622	-
Striatum	0.93 ± 0.15	0.91 ± 0.12	0.96 ± 0.18	1.03 ± 0.18	0.033	0.064
Nucleus basalis	0.17 ± 0.22	0.11 ± 0.17	0.13 ± 0.10	0.14 ± 0.17	0.872	-

Values are mean ± SD. ANOVA *p*, Probability value of a repeated-measures ANOVA for the interaction of the effects day by group; Pairwise adjusted *p*, multiple-comparison-adjusted probability value of day 1 versus day 2 of the sleep-deprivation group according to the method of Tukey–Kramer; pairwise *p* is the result of a paired *t* test of this contrast. ctx, Cortex.

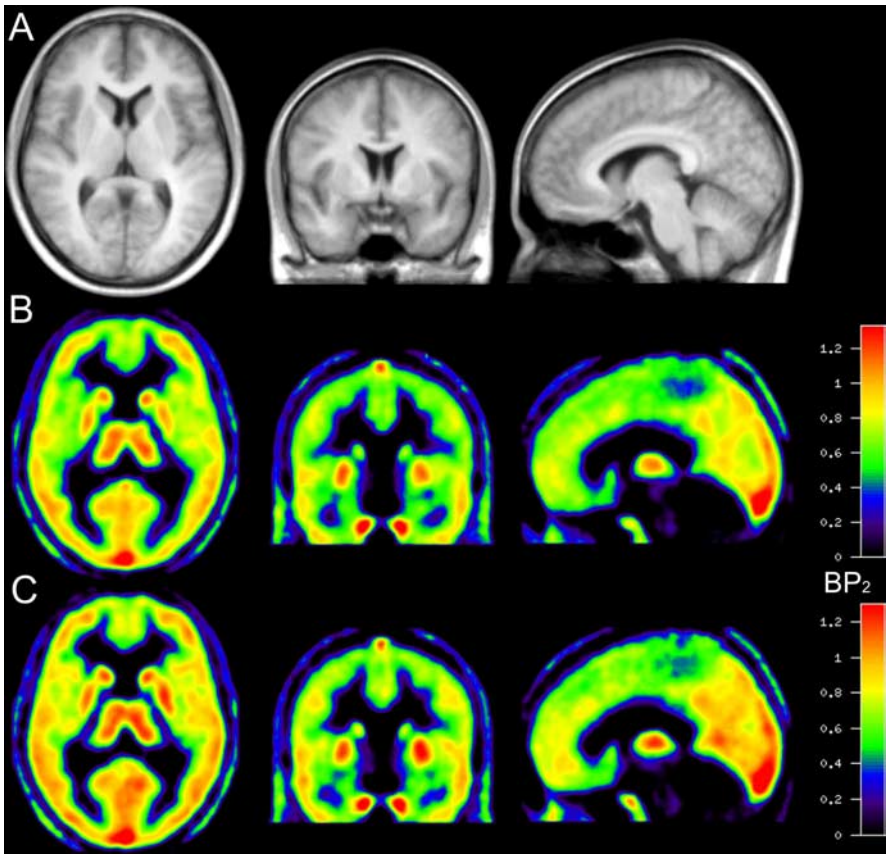

Figure 2. Average images of the sleep-deprivation group ($n = 12$) after spatial normalization. ***A***, MRIs. ***B***, Parametric image of BP_2 before sleep deprivation. ***C***, Image after sleep deprivation.

from animal experiments. Basheer et al. (2001) showed that the A_1AR mRNA levels in rats that were exposed to 6 h of sleep deprivation increased in the basal forebrain (78%), but not in the cingulate gyrus. Nevertheless, this increase did not lead to similar alterations in A_1AR density in the same samples measured with [^{3}H]DPCPX ([^{3}H]-8-cyclopentyl-1,3-dipropylxanthine) autoradiography. Yanik and Radulovacki (1987) detected a significant increase of A_1AR after 48 h of REM sleep deprivation in the cortex (15%) and striatum (23%) of rats with autoradiography. More recent findings in rats kept awake for 12 and 24 h showed an upregulation of A_1AR density after this prolonged wakefulness using [^{3}H]CPFPX autoradiography. This increased density was found in the basal forebrain but not in the cingulate cortex (McCarley et al., 2005). However, other cortical areas showed an increase in a range of ~10% (our unpublished observation).

In several animal experiments, adenosine accumulated during prolonged wakefulness (Porkka-Heiskanen et al., 1997, 2000; Basheer et al., 1999). This raises the question why the consequence of elevated levels of agonists is an upregulation and not a downregulation as commonly observed in G-protein-coupled receptors (Bohm et al., 1997). There is evidence that receptor density regulation is varying according to the time of agonist stimulation. In the early phase (minutes to hours) receptors are internalized, but after long-term stimulation (>2 h) receptor mRNA increases, which consequently leads to increased receptor densities (Souaze, 2001). The physiological relevance could be to maintain responsiveness to stimuli and/or to adapt cellular responses to external stimuli.

An endogenous displacement of the radioligand caused by

elevated adenosine concentrations, as found in animal experiments during sleep deprivation, could have mitigated the observed effects in this study. Given this fact, the underlying receptor upregulation would be even larger. At present, however, there is no experimental evidence that [^{18}F]CPFPX is displaceable *in vivo* by endogenous adenosine.

As stated previously, there is evidence that adenosine modulates cholinergic cell clusters in the basal forebrain (Ch4 or basal nucleus of Meynert). This target region is characterized by both a small volume and a low regional A_1AR density so that the given scanner resolution and the low DV_s'/DV_{f+ns}' ratio impede precise quantification by [^{18}F]CPFPX PET. The observed tendency of A_1AR increase is therefore probably related to technical and physiological limitations.

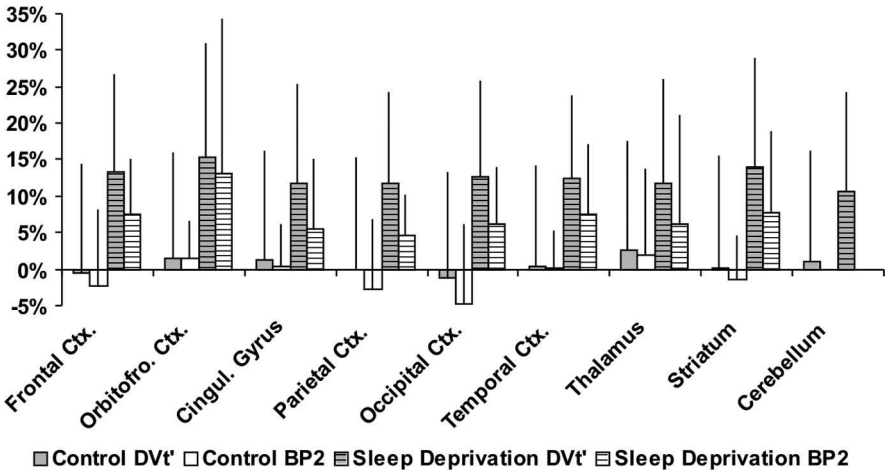

Figure 3. Average relative changes [(day 2 − day 1)/day1] of adenosine receptor DV_t' and BP_2 in various brain regions for the control and the sleep-deprivation group. Error bars depict SD.

There are numerous theories on a potential function of sleep, which point to a role in activity-dependent synaptic reorganization (for review, see Benington, 2000). It has, for instance, been proposed that sleep is directly linked to synaptic homeostasis and regulation of synaptic weight (Tononi and Cirelli, 2006). According to this hypothesis, the function of sleep is to downscale synaptic strength to a baseline level that is physiologically stable. Sleep deprivation would thus impair the recalibration of synaptic strength resulting in elevated levels of synaptic formations and, thus, synaptic receptors compared with the postsleep condition, which would also be in line with our observations.

What consequences might the observed increase of cerebral A_1AR density have? First, it might be an alternative way to enhance adenosine functions besides an increase of adenosine itself. Modulating sleep by varying concentrations of adenosine alone would be highly dynamic. In contrast, longer-lasting changes of receptor density could modulate the level of adenosine efficacy in a stable and robust manner.

Second, the regional distribution of adenosine receptors is an important aspect regarding the local control of sleep–wake organization. Another important finding is that the increase of A_1AR density occurs all over the brain, which is consistent with a global effect, linked to basic cell functions. In man, the cerebral A_1AR shows highest densities in the thalamus and the neocortex, both of which are important structures in the induction and maintenance of slow-wave sleep.

In conclusion, this study provides *in vivo* evidence of higher levels of A_1AR availability in the human brain after 24 h of prolonged wakefulness as demonstrated with [^{18}F]CPFPX PET. Our data suggest that the A_1 subtype of adenosine receptors could be addressed as a potential sleep factor besides the already well established role of adenosine itself.

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