British Journal of Pharmacology

DOI:10.1111/j.1476-5381.2011.01689.x
www.brjpharmacol.org

RESEARCH PAPER

Polymorphisms of *ADORA2A* modulate psychomotor vigilance and the effects of caffeine on neurobehavioural performance and sleep EEG after sleep deprivation

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Keywords
adenosine; sleep homeostasis; vigilance; stimulant; pharmacogenetics; modafinil

Received
16 July 2011
Revised
10 August 2011
Accepted
30 August 2011

BACKGROUND AND PURPOSE

Prolonged wakefulness impairs sustained vigilant attention, measured with the psychomotor vigilance task (PVT), and induces a compensatory increase in sleep intensity in recovery sleep, quantified by slow-wave activity (SWA) in the sleep electroencephalogram (EEG). These effects of sleep deprivation are counteracted by the adenosine receptor antagonist caffeine, implying involvement of the adenosine neuromodulator/receptor system. To examine a role for adenosine A_{2A} receptors, we investigated whether variation of the A_{2A} receptor gene (*ADORA2A*) modified effects of caffeine on PVT and SWA after sleep deprivation.

EXPERIMENTAL APPROACH

A haplotype analysis of eight single-nucleotide polymorphisms of *ADORA2A* was performed in 82 volunteers. In 45 young men carrying five different allele combinations, we investigated the effects of prolonged waking and 2 × 200 mg caffeine or 2 × 100 mg modafinil on psychomotor vigilance, sleepiness, and the waking and sleep EEG.

KEY RESULTS

Throughout extended wakefulness, the carriers of haplotype HT4 performed faster on the PVT than carriers of non-HT4 haplotype alleles. In haplotype HT4, caffeine failed to counteract the waking-induced impairment of PVT performance and the rebound of SWA in recovery sleep. However, caffeine was effective in non-HT4 allele carriers, and modafinil reduced the consequences of prolonged waking, independently of *ADORA2A* haplotype.

CONCLUSIONS AND IMPLICATIONS

Common genetic variation of *ADORA2A* is an important determinant of psychomotor vigilance in rested and sleep-deprived state. It also modulates individual responses to caffeine after sleep deprivation. These findings demonstrate a role for adenosine A_{2A} receptors in the effects of prolonged wakefulness on vigilant attention and the sleep EEG.

Abbreviations

ADORA2A, gene encoding the adenosine A_{2A} receptor; EMG, electromyogram; EOG, electrooculogram; FFT, fast Fourier transform; HT, haplotype; LD, linkage disequilibrium; NREM, non-rapid-eye-movement; PLMS, periodic limb movements in sleep; PVT, psychomotor vigilance task; SNP, single-nucleotide polymorphism; SWA, slow-wave activity; VLPO, ventro-lateral pre-optic

Introduction

Caffeine promotes vigilance and reduces sleepiness by blocking adenosine receptors (Fredholm *et al.*, 2005; Huang *et al.*, 2005; Landolt, 2008). This mode of action, as well as the potency of caffeine to restore performance in ecological situations, such as highway-driving during the night (Philip *et al.*, 2006), support the notion that the adenosine neuromodulator/receptor system is importantly involved in sleep–wake regulation. Wakefulness and sleep are modulated by a circadian process that underlies endogenous diurnal variations in sleep propensity, as well as a homeostatic process that tracks accumulating sleep need (Borbély, 1982). These two processes underlie the evolution of objective, subjective and electroencephalographic (EEG) markers of vigilance during prolonged wakefulness, sleep structure and the repercussions of sleep loss on recovery sleep.

Our previous studies showed that prolonged wakefulness impaired sustained vigilant attention more in self-rated caffeine-sensitive individuals than in caffeine-insensitive individuals (Rétey *et al.*, 2006). The difference was revealed by measuring reaction times on the psychomotor vigilance task (PVT) and the effects of caffeine (Rétey *et al.*, 2006). Psychomotor speed relies on the ability to respond, rapidly and reliably, to randomly occurring stimuli and involves the striatum. Accordingly, optimal PVT performance (i.e. fastest 10th percentile of reaction times) was associated with increased activation of putamen, caudate nucleus and globus pallidus (Drummond *et al.*, 2005). These structures exhibit high mRNA and radioligand binding of adenosine A_{2A} receptors (Martinez-Mir *et al.*, 1991; Svenningsson *et al.*, 1998; Bauer and Ishiwata, 2009; receptor nomenclature follows Alexander *et al.*, 2011), which modulate the effects of caffeine on psychomotor functions (El Yacoubi *et al.*, 2000; Fisone *et al.*, 2004; Cauli and Morelli, 2005).

Apart from behavioural measures, mechanisms underlying sleep–wake regulation are best examined by studying sleep–wake-dependent changes in the electrical activity of the brain. Low-frequency (<8 Hz) EEG activity in wakefulness and, especially, in non-rapid-eye movement (NREM) sleep increases after sleep deprivation and is thought to provide a physiological marker of sleep homeostasis (Borbély *et al.*, 1981; Finelli *et al.*, 2000; Cajochen *et al.*, 2002; Landolt *et al.*, 2004). More specifically, slow-wave (or delta) activity (0.75–4.5 Hz) at the beginning of a sleep episode is predictably correlated with the duration of preceding wakefulness (Dijk *et al.*, 1987). The actions of adenosine contribute to inter-individual differences in the waking and sleep EEG, both in baseline and after sleep deprivation (Rétey *et al.*, 2005; 2006; Bachmann *et al.*, 2011). Findings in transgenic mice suggest that at least some of the effects are mediated via adenosine A_{2A} receptors (Urade *et al.*, 2003).

In humans, genetic variation of the adenosine A_{2A} receptor gene (*ADORA2A*) modulates susceptibility to panic disorder and individual differences in anxiety-related personality, arousal and habitual caffeine consumption (Deckert *et al.*, 1998; Hamilton *et al.*, 2004; Cornelis *et al.*, 2007; Hohoff *et al.*, 2010). Moreover, individual responses to the stimulant, sleep-disrupting and anxiogenic responses to caffeine have been consistently associated with a common C-to-T substitution at nucleotide 1976 of *ADORA2A* (Alsene *et al.*, 2003; Rétey *et al.*, 2007; Childs *et al.*, 2008; Rogers *et al.*, 2010). The T-allele of this single-nucleotide polymorphism (SNP; SNP-ID: rs5751876) predisposes Caucasian individuals to caffeine-induced anxiety (Alsene *et al.*, 2003; Childs *et al.*, 2008; Rogers *et al.*, 2010), whereas the C-allele appears to confer sensitivity towards caffeine-induced sleep disturbance (Rétey *et al.*, 2007).

Here we examined the impact of genetic variation of *ADORA2A* and sleep deprivation on sustained vigilant attention, subjective sleepiness, waking and sleep EEG, and the pharmacogenetic response to the stimulants caffeine and modafinil. We performed a haplotype analysis of 8 SNP variants of *ADORA2A* in 82 healthy subjects and re-analysed the data obtained in 45 young men who recently completed controlled sleep deprivation studies in our laboratory (Rétey *et al.*, 2006; Bodenmann and Landolt, 2010). The carriers of a distinct *ADORA2A* haplotype referred to as haplotype HT4 showed higher vigilance during prolonged waking than carriers of non-HT4 haplotype alleles. Moreover, caffeine failed to counteract the consequences of sleep loss on psychomotor speed and EEG delta activity in the carriers of haplotype HT4. By contrast, modafinil, which does not interfere with A_{2A} receptors, mitigated the effects of prolonged wakefulness irrespective of *ADORA2A* haplotype. Taken together, the findings demonstrate that genetic variation of *ADORA2A* affects psychomotor response speed and modulates the effects of caffeine on neurobehavioural and neurophysiological markers of sleep–wake regulation.

Methods

Study participants and haplotype determination

The study protocol was approved by the local ethics committees for research on human subjects, and carried out in accordance with the principles of the Declaration of Helsinki.

Genomic DNA was extracted from 3 mL of fresh blood samples of 82 healthy participants of recent sleep studies (73 men, 9 women; age range: 20–70 years). Eight polymorphic

variants spanning the entire *ADORA2A* gene and its flanking regions were chosen and genotyped based on previous work (see supporting material). Overall genotyping yielded 100% completion rate for all eight SNP. The genotypes were determined by investigators who were unaware of the behavioural results, subjective sleepiness, trait anxiety, and sleep and waking EEG. High linkage disequilibrium (LD, $D'_{all} > 0.90$) resulted in one haploblock spanning all eight SNP (solid spine of LD method) and containing eight different haplotype alleles (Supporting Information Table S1). Therefore, genotypes were not assessed separately. Individually reconstructed haplotypes could be assigned to 81 subjects.

Participants of sleep studies

Sleep and homeostatic sleep regulation were studied in a subgroup of 45 healthy Caucasian men (age range: 20–30 years) (Rétey *et al.*, 2006; Bodenmann and Landolt, 2010). The experimental protocol included performance measurements, subjective sleepiness ratings and waking EEG recordings during prolonged wakefulness, as well as all-night polysomnography before and after sleep deprivation. All participants were non-smokers and good sleepers with regular bedtimes. They denied any history of neurological or psychiatric diseases and intake of medications or illicit drugs for at least 2 months before the study. They were paid for participation. Before inclusion, potential participants were screened in the laboratory to exclude sleep apnea and periodic limb movements in sleep (PLMS). Volunteers with a sleep apnea index and/or a PLMS index of 10 or more per hour of sleep, or a sleep efficiency lower than 80% were excluded. Reported habitual alcohol and caffeine intake, daytime sleepiness, and body mass index were moderate and normal (Supporting Information Table S2).

Sleep study protocol

All screening and pre-study procedures (see Supporting Information), sleep study protocol, neurobehavioural testing and waking EEG and polysomnographic recordings have been previously described in detail (Rétey *et al.*, 2006; 2007; Bodenmann *et al.*, 2009b; Bodenmann and Landolt, 2010).

In brief, subjects completed two experimental blocks separated by 1 week, each consisting of four consecutive nights and 2 days in the sleep laboratory. Upon arrival, breath alcohol concentration was measured and saliva samples for caffeine determination were collected. The first and second night of each block served as adaptation and baseline nights, respectively. The following 2 days and one night, until bedtime of the recovery night, volunteers stayed awake under constant supervision by members of the research team. After 11 and 23 h of wakefulness, 200 mg caffeine ($n = 23$) or 100 mg modafinil ($n = 22$) were administered in the form of capsules to half of the subjects in placebo-controlled, randomized, double-blind, cross-over fashion. The capsules were produced by homogenizing anhydrous caffeine (Siegfried Ltd., Zofingen, Switzerland) and commercial Modasomil 100® tablets (Globopharm AG, Küsnacht, Switzerland) with mannitol (Siegfried Ltd., Zofingen, Switzerland). Identical placebo capsules contained only mannitol. The doses of caffeine and modafinil were based on earlier results (Rétey *et al.*, 2007; Bodenmann *et al.*, 2009b). During prolonged wakefulness, the participants completed at 3 h intervals 14 sessions comprising PVT, Stanford Sleepiness Scale (validated German translation) and standardized waking EEG recording.

Assessment of psychomotor vigilance

The PVT provides a valid measure of sustained vigilant attention. Subjects were instructed to press a button on a response box as quickly as possible, to stop a digital millisecond counter starting at variable intervals of 2–10 s. They performed the task either in its original version (Dinges and Powell, 1985) or on a personal computer (Bodenmann *et al.*, 2009b). A total of 100 stimuli was presented during 10 min, requiring sustained attention to detect the randomly occurring stimuli. Here the 90th (slowest) percentile of reaction times is reported because it is most strongly affected by sleep deprivation and may represents the best neurobehavioural marker of homeostatic sleep pressure (Cajochen *et al.*, 1999). The PVT data in one subject with non-HT4 haplotype recorded at 14:15 after sleep loss in the placebo condition was lost.

Waking EEG recordings

The waking EEG (data of the C3A2 derivation are reported here) was recorded, conditioned, digitized and stored as previously described (Rétey *et al.*, 2006; Bodenmann *et al.*, 2009a). When signs of drowsiness were detected (e.g. reduced alpha activity or rolling eye movements), subjects were alerted by addressing them over the intercom. One hour before each recording, subjects had to stay in the laboratory (constant temperature, light intensity <150 lux), and at least 15 min before each recording, they were by themselves in their bedrooms. Each recording consisted of 3 min with eyes closed, followed by 5 min with eyes open. Artefacts were visually identified. The power spectra of artefact-free, 2 s epochs were computed with MATLAB® (The MathWorks Inc, Natick, MA, USA) (fast Fourier transform [FFT] routine, Hanning window, frequency resolution 0.5 Hz). The present analyses were restricted to the placebo condition and the recordings with eyes open. Relative delta/theta activity (1–8 Hz) was expressed as a percentage of the mean value in the waking EEG at 3, 6 and 9 h waking. The data in 1 subject with HT4 haplotype recorded at 05:00 after sleep loss had to be excluded because of severe artefacts.

Polysomnographic sleep recordings

Polysomnographic data consisting of EEG (C3A2 derivation), electrooculogram (EOG), mental electromyogram (EMG), and electrocardiogram (ECG) were recorded, conditioned, digitized and stored as previously described (Rétey *et al.*, 2006; Bodenmann *et al.*, 2009a). Sleep stages were visually scored in 20 s epochs according to standard criteria (Rechtschaffen and Kales, 1968). The EEG power spectra were calculated with MATLAB® (The MathWorks Inc.) (average of five, 4 s epochs, FFT routine, Hanning window, frequency resolution 0.25 Hz) and matched with the sleep scores. Epochs with movement- and arousal-related artefacts were visually identified and excluded. The all-night power spectra in NREM sleep (stages 1–4) did not differ between the two baseline nights and were averaged. To control for differences in absolute EEG activity

recorded with the two different polygraphic amplifiers PSA24® (Braintronics Inc., Almere, the Netherlands) (Rétey *et al.*, 2006) and Artisan® (Micromed, Mogliano Veneto, Italy) (Bodenmann *et al.*, 2009a), slow-wave activity (SWA, EEG power within 0.75–4.5 Hz) in the first 4 NREM sleep episodes in baseline and recovery nights was expressed as a percentage of the all-night value in baseline. Because of computer breakdown, the data of the recovery night in one subject with HT4 haplotype and in two subjects with non-HT4 haplotype were lost.

Statistical analyses

All data were analysed with SAS® 9.1 software (SAS Institute, Cary, NC, USA). One- to three-way, mixed-model ANOVA included the between-subjects factor 'haplotype' and the within-subjects factors 'session' (14 assessments during prolonged waking), 'condition' (placebo, stimulant), 'NREM sleep episode' (1–4), 'night' (baseline, recovery), and 'treatment' (placebo, caffeine or modafinil), as well as their interactions. Significance level was set at $\alpha < 0.05$. If not stated otherwise, only significant effects of factors and interactions are reported. EEG spectral data were log-transformed prior to statistical analyses. Two-tailed paired and unpaired *t*-tests to localize differences within and between groups were only performed if respective main effects or interactions of the ANOVA were significant.

Results

Genotyping of eight SNP variants from a single haplotype block spanning the entire *ADORA2A* gene and its flanking regions revealed the presence of eight different allele combinations (HT1–HT8). Five haplotypes (HT1–HT5) occurred with a frequency of over 10% and were assessed in the present study (Supporting Information Table S1).

ADORA2A haplotype predicts stable difference in psychomotor vigilance throughout sleep deprivation

Given the proposed role for adenosine A_{2A} receptors in regulating psychomotor speed and the strong genetic contribution to simple reaction times (Simonen *et al.*, 1998), we hypothesized that the *ADORA2A* haplotype would influence PVT performance. The evolution of the slowest 10th percentile of response speed throughout prolonged wakefulness was virtually the same in all five haplotype groups and reflected circadian and homeostatic influences (Figure 1). Nevertheless, the carriers of haplotype HT4 performed faster on the PVT than the HT1, HT2, HT3 and HT5 haplotype groups (Table 1). Similar results were found for mean and fastest 10th percentile of reaction times (see Supporting Information Figure S1 and Supporting Information Table S3). Because no other haplotype differed consistently from the others, HT1, HT2, HT3 and HT5 allele carriers were averaged for subsequent analyses (and referred to as non-HT4 allele carriers). The HT4 and non-HT4 haplotype groups were indistinguishable in habitual alcohol and caffeine intake, self-rated sleepiness at the beginning of the study, and body mass index (Supporting Information Table S2). Age was slightly less and

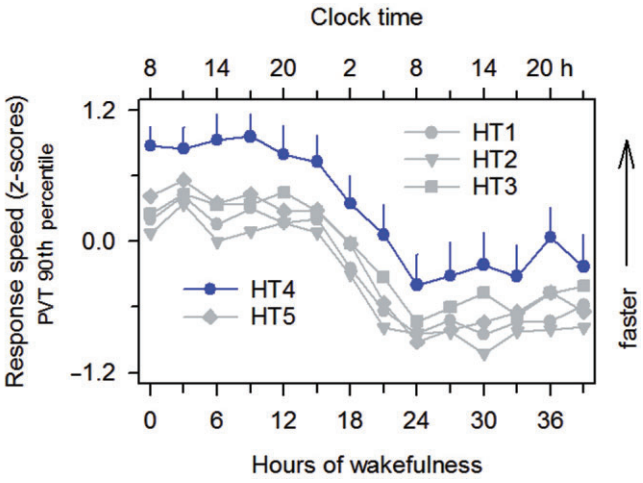

Figure 1

Genetic variation of *ADORA2A* determines inter-individual difference in psychomotor vigilance during prolonged wakefulness. Mean values (+ SEM) in carriers of HT4 ($n = 14$) and non-HT4 haplotype alleles of *ADORA2A* are plotted. HT1: $n = 21$, HT2: $n = 16$, HT3: $n = 15$, HT5: $n = 13$. A majority of subjects were heterozygous HT allele carriers (see Supporting Information Table S1). Ticks on the *x*-axes were rounded to the previous hour. Starting 30 min after wake-up from the baseline night, a 10 min PVT was administered at 3 h intervals during 40 h prolonged wakefulness. The 90th percentile of reaction times on the PVT per session was expressed as speed (1/reaction time). Two-way mixed-model ANOVA with the between-subjects factor 'haplotype' (HT1–HT5) and the within-subjects factor 'session' (1–14) and the co-variate 'age' revealed that individuals with haplotype HT4 performed faster than non-HT4 allele carriers throughout sleep deprivation ('haplotype': $F_{4,1033} = 15.41$, $P < 0.0001$; 'session': $F_{13,1033} = 22.03$, $P < 0.0001$; 'haplotype' × 'session': $F_{52,1034} = 0.14$, $P > 0.9$).

trait anxiety was slightly higher in the former when compared with the latter, but were not significantly different after Bonferroni correction for multiple testing.

Because each volunteer completed two independent experimental blocks in random order (placebo condition and stimulant condition), this study offered the unique opportunity to examine whether the effect of allelic variation in *ADORA2A* on psychomotor vigilance was robust and reliable. The PVT performance in placebo and stimulant conditions was averaged across those test sessions that occurred before placebo and stimulant intake, and compared with each other. This analysis demonstrated that the difference between HT4 and non-HT4 haplotype groups was the same between placebo and stimulant conditions (Figure 2). In other words, PVT performance consistently differed between *ADORA2A* allele carriers in two independent measurements separated by 1 week.

Next, we asked whether the observed differences between carriers of HT4 and non-HT4 haplotypes reflected differences in A_{2A} receptor-mediated signal transduction. To address this question, we investigated the effects of caffeine on the sleep loss-induced impairment of sustained vigilant attention. Consistent with genotype-dependent differences in A_{2A} receptor function, we found that intake of 2 × 200 mg caffeine during prolonged wakefulness improved PVT performance in

Table 1

Carriers of haplotype HT4 perform faster on the PVT than carriers of non-HT4 alleles of *ADORA2A*

Contrast	$F_{1,1033}$	*P*
HT1 vs. HT2: –0.28 ± 0.18 vs. –0.38 ± 0.18	1.79	0.1809
HT1 vs. HT3: –0.28 ± 0.18 vs. –0.11 ± 0.26	3.7	0.0548
HT1 vs. **HT4**: –0.28 ± 0.18 vs. 0.29 ± 0.23	43.26	<0.0001
HT1 vs. HT5: –0.28 ± 0.18 vs. –0.18 ± 0.17	1.15	0.283
HT2 vs. HT3: –0.38 ± 0.18 vs. –0.11 ± 0.26	8.84	0.003
HT2 vs. **HT4**: –0.38 ± 0.18 vs. 0.29 ± 0.23	53.26	<0.0001
HT2 vs. HT5: –0.38 ± 0.18 vs. –0.18 ± 0.17	5.11	0.024
HT3 vs. **HT4**: –0.11 ± 0.26 vs. 0.29 ± 0.23	19.37	<0.0001
HT3 vs. HT5: –0.11 ± 0.26 vs. –0.18 ± 0.17	0.46	0.4957
HT4 vs. HT5: 0.29 ± 0.23 vs. –0.18 ± 0.17	22.07	<0.0001

Mean values (±SEM) reflect z-scores of the 90th percentile (slowest) reaction times on the PVT. HT1: $n = 21$, HT2: $n = 16$, HT3: $n = 15$, HT4: $n = 14$, HT5: $n = 13$. Contrasts among haplotypes were calculated following two-way mixed-model ANOVA with the between-subjects factor 'haplotype' (HT1–HT5) and the within-subjects factor 'session' (1–14) and the co-variate 'age' (for results, see legend to Figure 1). The difference of HT4 from all non-HT4 haplotype groups remained significant after Bonferroni correction to control for multiple comparisons. Comparisons referring to haplotype HT4 are in bold.

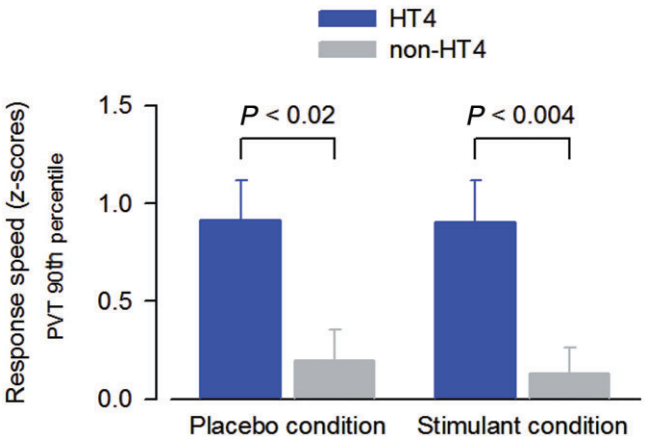

Figure 2

Stable difference in psychomotor vigilance between carriers of HT4 ($n = 14$) and non-HT4 haplotype ($n = 31$) alleles of *ADORA2A*. The 90th percentile of reaction times (expressed as speed, 1/reaction time) on the PVT in test sessions at 3, 6 and 9 h awake (prior to first placebo/stimulant administration) was averaged. Means + SEM are shown. Individuals with HT4 haplotype performed consistently faster than non-HT4 allele carriers in placebo and stimulant conditions, occurring in random order 1 week apart (ANOVA: 'haplotype': $F_{1,43} = 9.3$, $P < 0.004$; 'condition': $F_{1,43} = 0.19$, $P > 0.6$; 'haplotype' × 'condition': $F_{1,43} = 0.13$, $P > 0.7$). *P*-values refer to unpaired two-tailed *t*-tests.

non-HT4 carriers, but was ineffective in carriers of HT4 alleles (Figure 3A & 3B). These data demonstrate that genetic variation of *ADORA2A* modulated the effects of pharmacological blockade of adenosine A_{2A} receptors on waking performance after sleep loss.

To further support this conclusion, the effects of caffeine were compared with those of modafinil. This compound has no known affinity to adenosine A_{2A} receptors (Minzenberg and Carter, 2008). Consistent with the distinct mode of action, modafinil (2 × 100 mg) similarly mitigated the wakefulness-induced reduction in sustained attention in both *ADORA2A* haplotype groups (Figure 3C & 3D). The different pharmacogenetics of caffeine and modafinil strongly suggest that the differences between the haplotypes reflect genetically determined differences in A_{2A} receptor-mediated signals.

Similar build-up of sleep pressure during wakefulness in HT4 and non-HT4 haplotypes

The carriers of HT4 and non-HT4 haplotypes were good sleepers with high sleep efficiency, short sleep latency and normal sleep structure (Supporting Information Table S4). To examine the build-up of sleep pressure during prolonged wakefulness, the evolution of subjective sleepiness and EEG low-frequency oscillations in waking were quantified. The time course of subjective sleepiness during sleep deprivation was remarkably similar in both haplotype groups (Supporting Information Figure S2). To analyse the effects of prolonged wakefulness on the waking EEG, averaged spectral power in three recording sessions after sleep loss (1100, 1400 and 1700 h) was compared with the corresponding values before the night without sleep. This analysis showed that sleep deprivation increased delta/theta oscillations in all bins below 8.0 Hz ($F_{1,43} \geq 6.0$, $p_{all} < 0.02$), irrespective of *ADORA2A* haplotype (data not shown). The evolution of this increase during sleep loss was very similar in HT4 and non-HT4 allele carriers (Supporting Information Figure S2). Taken together, neurobehavioural, subjective and neurophysiological data confirm that the build-up of homeostatic sleep pressure during sleep deprivation was the same in both haplotypes.

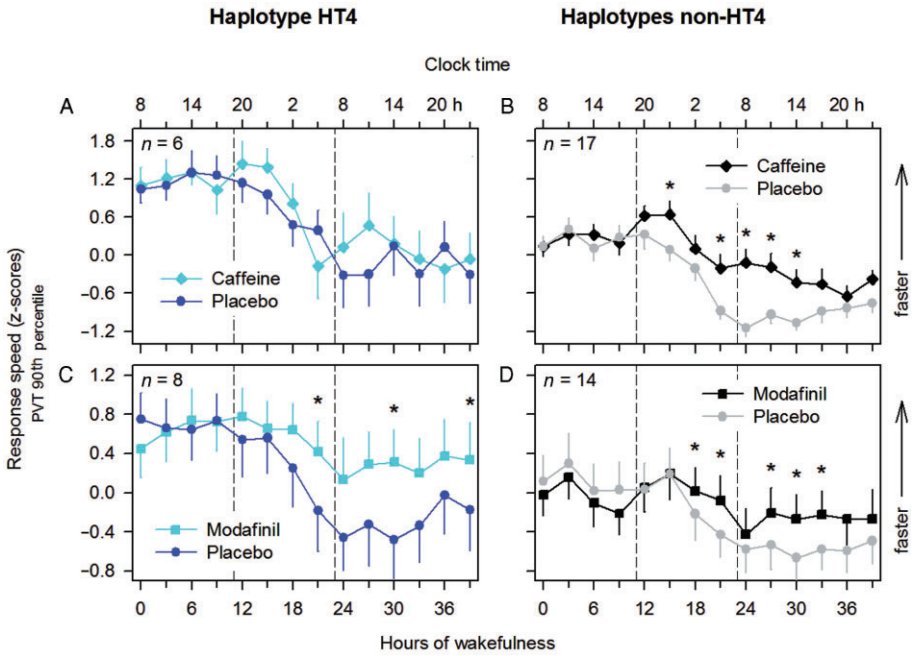

Figure 3

Genetic variation of *ADORA2A* differently modulates the effects of caffeine and modafinil on impaired psychomotor vigilance after sleep loss. The PVT was administered at 3 h intervals throughout prolonged wakefulness, beginning 30 min after wake-up from the baseline nights. Mean values (±SEM) of the 90th percentile of reaction times (expressed as speed, 1/reaction time) in carriers of HT4 (left panels) and non-HT4 haplotype (right panels) alleles of *ADORA2A* are plotted. Ticks on the *x*-axes were rounded to the previous hour. Study participants received 2 × placebo, and 2 × 200 mg caffeine or 2 × 100 mg modafinil during two 40 h periods of prolonged wakefulness, separated by 1 week. (A and B) Caffeine improved PVT response speed after sleep loss in non-HT4 haplotype carriers of *ADORA2A* only (ANOVA: 'haplotype': $F_{1,21.2} = 7.91$, $P < 0.02$; 'session': $F_{13,205} = 13.43$, $P < 0.0001$, 'treatment': $F_{1,68} = 8.45$, $P < 0.005$; 'haplotype' × 'treatment' × 'session': $F_{26,219} = 2.1$, $P < 0.003$). (C and D) Modafinil improved PVT response speed after sleep loss independently of *ADORA2A* haplotype ('session': $F_{13,198} = 10.24$, $P < 0.0001$; 'treatment': $F_{1,64.9} = 12.45$, $P = 0.0008$; 'haplotype' × 'session': $F_{13,198} = 1.87$, $P < 0.04$; 'haplotype' × 'treatment' × 'session': $F_{26,205.} = 1.35$, $P > 0.12$). *$P < 0.05$ (stimulant vs. placebo, paired two-tailed *t*-tests).

ADORA2A haplotype modulates effect of caffeine on sleep EEG response to sleep deprivation

Both *ADORA2A* haplotype groups also showed a strong increase in EEG SWA in recovery sleep after sleep deprivation (Supporting Information Figure S3). To investigate whether the rebound involved adenosine A_{2A} receptor-mediated signal transduction, we studied the effects of caffeine and modafinil in the two haplotype groups. Caffeine significantly reduced SWA in non-HT4 carriers, whereas the stimulant had no effect in carriers of HT4 alleles (Figure 4). By contrast, modafinil failed to affect the rebound in SWA in both haplotype group ($p_{all} > 0.08$; data not shown). The distinct pharmacogenetics of caffeine and modafinil demonstrate a role for adenosine A_{2A} receptors in mediating the effect of prolonged wakefulness on EEG SWA in NREM sleep.

Discussion and conclusions

Our study demonstrates that genetic variation of *ADORA2A* modulates psychomotor vigilance and the effects of caffeine in counteracting the repercussions of prolonged wakefulness on waking performance and EEG delta oscillations in NREM sleep. The findings strongly suggest that adenosine A_{2A} receptors contribute to wakefulness-induced changes in neurobehavioural and neurophysiological markers of sleep–wake regulation.

Previous work has shown that psychomotor speed exhibits high *inter*-individual variation and high *intra*-individual stability, which is strongly controlled by genetic factors (Simonen *et al.*, 1998; Van Dongen *et al.*, 2004). The underlying genes, however, remained unknown. Here we found that *ADORA2A* contributed to trait-like, inter-individual differences in psychomotor vigilance in rested and sleep-deprived state. In other words, HT4 allele carriers consistently performed on a higher level on the sustained vigilant attention task than non-HT4 allele carriers. By contrast, prolonged wakefulness induced a similar slowing in response speed in both haplotype groups. Thus, even under physiological conditions, genetic variation of *ADORA2A* is an important determinant of waking quality including vigilance and neurobehavioural performance. Importantly, caffeine failed to mitigate the waking-induced impairment in individuals carrying HT4 alleles. By contrast, carriers of HT4 and of non-HT4 haplotypes benefited from modafinil. These findings demonstrate that a mechanism involving adenosine A_{2A} receptors underlies the sleep loss-induced reduction in sustained attention and individual responses to commonly used counter-

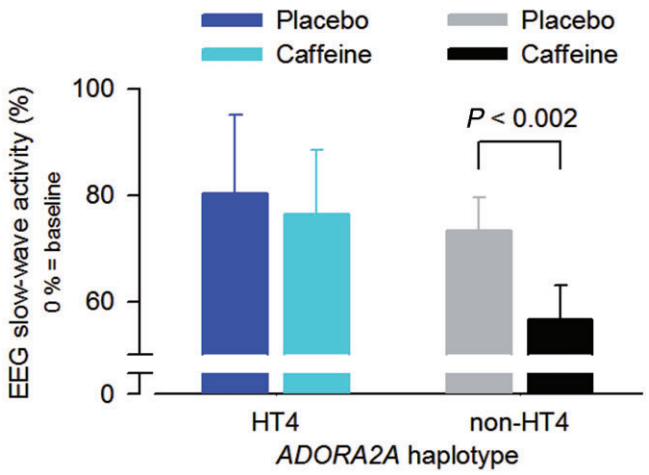

Figure 4

Caffeine attenuates the rebound of EEG delta oscillations (0.75–4.5 Hz) after sleep loss in subjects with non-HT4 haplotype of *ADORA2A* only. Bars represent the mean increase (+SEM) in slow-wave activity in the first NREM sleep episode (stages 1–4) of the recovery night, expressed as a percentage of the corresponding value in the baseline night. Individuals with HT4 (n = 5) and non-HT4 haplotype (n = 15) alleles received 2 × placebo and 2 × 200 mg caffeine during two 40 h periods of prolonged wakefulness, separated by one week. Caffeine reduced the rebound in delta activity when compared with placebo (ANOVA: 'treatment': $F_{1,18}$ = 5.59, P < 0.03; 'haplotype': $F_{1,18}$ = 1.13, P = 0.3; 'treatment' × 'haplotype': $F_{1,18}$ = 2.23, P = 0.15). P-value refers to paired two-tailed t-test.

measures against the detrimental effect of prolonged wakefulness. Nevertheless, because cognition is based on multiple components, our observations on psychomotor speed may not be generalized to other components of cognition, such as memory, decision-making and divided attention.

Adenosine A_{2A} receptors are predominantly expressed in the striatum and globus pallidus (Martinez-Mir *et al.*, 1991). Activation of these structures allows optimal responses to salient stimuli (Drummond *et al.*, 2005), whereas lesions, particularly in the right hemisphere, impair response times (Howes and Boller, 1975). GABA-containing medium spiny neurons are the most abundant type of neurons in the striatum. These cells form antagonistic heteromers containing adenosine A_{2A} and dopamine D_2 receptors, and play a critical role in controlling psychomotor responses (Hauber, 1998; Cauli and Morelli, 2005). It is thought that increased extracellular adenosine impairs psychomotor activity by disinhibiting the dopamine D_2 receptor mediated inhibition of an indirect striato-pallidal motor pathway (Hauber, 1998; Fisone *et al.*, 2004; Cauli and Morelli, 2005). On the other hand, by blocking A_{2A} receptors, caffeine indirectly potentiates dopaminergic signalling and leads to motor activation (El Yacoubi *et al.*, 2000; Higgins *et al.*, 2007). Taken together, while the different pharmacogenetics of caffeine and modafinil suggest that genetic differences in A_{2A} receptor function determine the robust difference in psychomotor speed between HT4 and non-HT4 allele carriers, dopaminergic mechanisms and other possible influences may also be involved.

We previously observed that mechanisms involving adenosine also contribute to individual differences in sleep loss-induced changes in rhythmic brain oscillations and suggested that the A_{2A} receptor is involved (Rétey *et al.*, 2006; 2007; Landolt, 2008). In accordance with this hypothesis, here we show that the normal caffeine-induced reduction in EEG SWA in recovery sleep after sleep deprivation (Landolt *et al.*, 2004) was abolished in carriers of HT4 haplotype alleles. Animal studies support a role for adenosine A_{2A} receptors in sleep–wake regulation. Local administration of the selective A_{2A} receptor agonist, CGS-21680, to the subarachnoid space adjacent to basal forebrain and lateral preoptic region increased *c-fos* expression in the ventro-lateral preoptic (VLPO) area and enhanced NREM sleep (Scammell *et al.*, 2001). Direct activation of sleep-promoting VLPO neurons upon stimulation of adenosine A_{2A} receptors may underlie this effect (Gallopin *et al.*, 2005). In mice without functional A_{2A} receptors, CGS-21680 did not induce sleep and caffeine failed to promote wakefulness (Huang *et al.*, 2005; Huang *et al.*, 2007). Further supporting a role for adenosine A_{2A} receptors in sleep regulation, our human study indicated that genetic variation of *ADORA2A* slightly altered the rebound after sleep deprivation of SWA in NREM sleep (Supporting Information Figure S3). While the different pharmacogenetics of caffeine and modafinil strengthen the validity of this finding, future studies are needed to determine whether distinct polymorphisms of *ADORA2A* modulate the dynamics of sleep homeostasis and contribute to differential vulnerability to the consequences of sleep loss.

It is currently not known how distinct combinations of *ADORA2A* alleles interact with psychomotor speed and sleep–wake regulation, and whether the investigated polymorphisms alter expression, structure and/or affinity of adenosine A_{2A} receptors. Nevertheless, it is informative to note that haplotypes HT1 and HT4 only differ in rs2236624 (Supporting Information Table S1), but behave very differently on the PVT (Figure 1). This observation may suggest that the combination present in HT4 of a C-allele at position rs2236624 and a T-allele at position rs5751876 is required for high psychomotor performance and reduced efficacy of caffeine to offset the consequences of sleep loss on PVT and EEG SWA. Thus, a ceiling effect caused by the T-allele or a U-shaped gene-effect relationship of rs5751876 in combination with the C-allele at position rs2236624 could underlie the observed differences between the haplotype groups. Because only a subset of all known polymorphic variants of *ADORA2A* could be determined in the present study and other possibly existing allele combinations may alter *ADORA2A* expression, further work is needed to pinpoint the underlying mechanism. Other limitations include the small sample size, in particular of individuals with haplotype HT4 who received caffeine, and the fact that only young men were investigated. The present findings may not be generalizable to women and older age groups. Moreover, independent replication with an *a priori* hypothesis with respect to HT4 haplotype will be needed to ascertain that the observed differences are indeed related to the *ADORA2A* haplotype. Our work provides the basis for future genetic and genomic studies of sleep–wake regulation in animal models and sleep-disordered patients. It also suggests a molecular mechanism underlying the individual psychostimulant response to caf-

feine, which is relevant for many individuals trying to counteract impaired vigilance as a consequence of, for instance, shiftwork or jetlag.

Acknowledgements

We thank Prof A. Borbély and Dr C. Kopp for helpful discussions and comments on an earlier version of the manuscript. This research was supported by Swiss National Science Foundation grants # 3100A0-107874 and 310000–120377.

Conflicts of interest

All authors declare that they have no competing interests, financial or otherwise.

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Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Genetic variation of *ADORA2A* determines inter-individual difference in psychomotor vigilance during prolonged wakefulness. Mean values (+SEM) in carriers of HT4 (black circles, $n = 14$) and non-HT4 haplotype (grey symbols) alleles of *ADORA2A* are plotted. HT1: $n = 21$, HT2: $n = 16$, HT3: $n = 15$, HT5: $n = 13$. A majority of subjects were heterozygous HT allele carriers (see Table S1). Ticks on the x-axes were rounded to the previous hour. Starting 30 min after wake-up from the baseline night, a 10-min psychomotor vigilance task (PVT) was administered at 3-hour intervals during 40 h prolonged wakefulness. The mean and fastest 10th percentile of reaction times on the PVT per session were expressed as speed (1/reaction time). Two-way mixed-model ANOVA with the between-subjects factor 'haplotype' (HT1-HT5) and the within-subjects factor 'session' (1–14) and the co-variate 'age' revealed that individuals with haplotype HT4 performed faster than non-HT4 allele carriers throughout sleep deprivation. (A) Mean: 'haplotype': $F_{4,1033} = 14.37$, $P < 0.0001$; 'session': $F_{13,1033} = 16.85$, $P < 0.0001$; 'haplotype' × 'session': $F_{52,1034} = 0.13$, $P = 1.0$. (B) Fastest 10th percentile: 'haplotype': $F_{4,1033} = 10.56$, $P < 0.0001$; 'session': $F_{13,1033} = 9.97$, $P < 0.0001$; 'haplotype' × 'session': $F_{52,1034} = 0.18$, $P = 1.0$.

Figure S2 Genetic variation of *ADORA2A* determines inter-individual difference in sustained attention during prolonged wakefulness. Starting 15 min after wake-up from the baseline night, the Stanford Sleepiness Scale, a standardized waking EEG recording (Landolt *et al.*, 2004), and a 10-min psychomotor vigilance task (PVT) were administered at 3-hour intervals during 40 h prolonged wakefulness. Mean values (±SEM) in carriers of HT4 (black symbols, $n = 14$) and non-HT4 haplotype (grey symbols, $n = 31$) alleles of *ADORA2A* are plotted. Ticks on the x-axes were rounded to the previous hour. The evolution of subjective sleepiness, response speed on the PVT, and EEG delta/theta oscillations suggest the same dynamics of sleep homeostasis. (A) Time course of 90th percentile of reaction times (expressed as speed, 1/reaction time) on the PVT. Individuals with HT4 haplotype performed faster than non-HT4 allele carriers throughout sleep deprivation ('haplotype': $F_{1,43.2} = 6.33$, $P < 0.02$; 'session': $F_{13,305} = 20.43$, $P < 0.0001$; 'haplotype' × 'session': $F_{13,305} = 1.85$, $P < 0.04$). (B) Subjective sleepiness evolved similarly during prolonged time awake in carriers of HT4 and non-HT4 haplotype alleles ('session': $F_{13,293} = 20.35$, $P < 0.0001$; 'haplotype': $F_{1,43.7} = 0.09$, $P > 0.76$, 'haplotype' × 'session': $F_{13,295} = 0.69$, $P > 0.76$). (C) EEG 1–8 Hz activity expressed as a percentage of the mean value at 3, 6 and 9 h waking. Activity increased non-monotonically during prolonged wakefulness, independently of haplotype ('session': $F_{13,310} = 11.39$, $P < 0.0001$; 'haplotype': $F_{1,43.8} = 1.89$, $P > 0.17$; 'haplotype' × 'session': $F_{13,310} = 0.77$, $P > 0.6$). *$P < 0.04$ (HT4 vs. non-HT4, unpaired 2-tailed *t*-tests).

Figure S3 Genetic variation of *ADORA2A* modulates the rebound of EEG delta oscillations (0.75–4.5 Hz) after sleep loss (placebo condition). Delta activity in baseline (circles) and recovery (triangles) nights across consecutive NREM sleep episodes was expressed as a percentage of the all-night value in baseline (NREM sleep stages 1–4, horizontal dashed line). Data represent means ± SEM in carriers of HT4 (black symbols, n = 14) and non-HT4 haplotype (grey symbols, n = 31) alleles of *ADORA2A*. Forty hours prolonged wakefulness induced larger rebound in delta activity in HT4 haplotype than in non-HT4 haplotype ('haplotype' × 'night' × 'NREM sleep episode': $F_{6,125} = 68.95$, $P < 0.0001$). *$P < 0.02$ (HT4 vs. non-HT4, unpaired 2-tailed t-test).

Table S1 Adenosine A_{2A} receptor gene (*ADORA2A*) haplotypes

Table S2 Demographic characteristics of HT4 and non-HT4 haplotype carriers

Table S3 Carriers of haplotype HT4 perform faster on the psychomotor vigilance task (PVT) than carriers of non-HT4 alleles of *ADORA2A*

Table S4 Visually scored sleep variables in baseline and recovery nights

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