A Genetic Variation in the Adenosine A_{2A} Receptor Gene (*ADORA2A*) Contributes to Individual Sensitivity to Caffeine Effects on Sleep

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Caffeine is the most widely used stimulant in Western countries. Some people voluntarily reduce caffeine consumption because it impairs the quality of their sleep. Studies in mice revealed that the disruption of sleep after caffeine is mediated by blockade of adenosine A_{2A} receptors. Here we show in humans that (1) habitual caffeine consumption is associated with reduced sleep quality in self-rated caffeine-sensitive individuals, but not in caffeine-insensitive individuals; (2) the distribution of distinct c.1083T>C genotypes of the adenosine A_{2A} receptor gene (*ADORA2A*) differs between caffeine-sensitive and -insensitive adults; and (3) the *ADORA2A* c.1083T>C genotype determines how closely the caffeine-induced changes in brain electrical activity during sleep resemble the alterations observed in patients with insomnia. These data demonstrate a role of adenosine A_{2A} receptors for sleep in humans, and suggest that a common variation in *ADORA2A* contributes to subjective and objective responses to caffeine on sleep.

The requirements of the modern 24-hour society, with professional and social activities around the clock, lead many individuals to prolong wakefulness at the expense of sleep.[1,2] Short habitual sleep may cause cumulative sleep debt associated with decrements in alertness and performance.[3,4] The potency to promote alertness and performance is the prime reason why people consume caffeine, the most frequently used stimulant in the Western world.[5,6] Some authors have suggested that caffeine counteracts the detrimental effects of sleep debt similarly to nap sleep.[7]

In doses typically contained in coffee, tea, energy drinks, foods, and pharmaceutical formulations,[6] caffeine acts as an adenosine receptor antagonist.[5] Adenosinergic mechanisms appear to be critically involved in wake–sleep processes in humans.[8,9] Consistent with the "adenosine hypothesis" of sleep, caffeine prolongs sleep latency, decreases the deep stages of non-rapid-eye movement (nonREM) sleep, reduces sleep efficiency, and alters the waking and sleep electroencephalogram (EEG) in frequencies, which reliably reflect sleep need.[10–13] These changes in sleep and the sleep EEG are reminiscent of patients with primary insomnia (*i.e.*, insomnia not related to another sleep, medical, or psychiatric disorder), and caffeine intake was proposed to produce a model of insomnia in healthy volunteers.[14] Unsatisfactory sleep quality can indeed be a reason for some people to voluntarily reduce or abstain from caffeine consumption.[15] Nevertheless, the first scientific examinations of caffeine in humans have already revealed that the effects on sleep are highly variable among individuals.[16] The pharmacokinetic and/or pharmacodynamic mechanisms underlying these differences are unknown and a matter of an ongoing debate.[17–21]

Both the anxiogenic and stimulant properties of caffeine contribute to individual differences in the subjective response to the drug.[19,22,23] More specifically, a c.1083T>C polymorphism in the adenosine A_{2A} receptor gene (*ADORA2A*) modulates individual differences in symptoms of anxiety after caffeine.[24] In addition, the perceived stimulation after caffeine depends on the level of arousal at the time of drug intake.[19] We found that optimal performance on a psychomotor vigilance task was more impaired after one night without sleep in self-rated caffeine-sensitive individuals when compared with caffeine-insensitive individuals.[9] Moreover, the improvement in performance by caffeine was inversely related to the impairment by sleep debt. Studies in knockout mice showed that the wakefulness-promoting effect of caffeine requires functional A_{2A} receptors.[25] Because the

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ADORA2A c.1083T>C polymorphism affects the waking EEG in frequencies that are functionally related to the wake–sleep continuum,[8] we hypothesized that this genetic variation also modulates subjective and objective effects of caffeine on sleep.

We combined epidemiologic, genetic, pharmacokinetic, and polysomnographic methods and first addressed more than 20,000 individuals with a brief questionnaire about self-rated caffeine sensitivity and sleep. A total of 4,329 people responded. Caffeine consumption was associated with subjectively reduced sleep quality in caffeine-sensitive respondents, but not in caffeine-insensitive respondents. The distribution of the *ADORA2A* C/C and T/T genotypes differed between subgroups of caffeine-sensitive and -insensitive individuals. A subsequent sleep deprivation study showed that the perceived psychostimulant action of caffeine was more pronounced in caffeine-sensitive men than in caffeine-insensitive men. Conversely, the caffeine concentration in saliva was similar in both groups. The stimulant induced changes in rhythmic brain activity in recovery sleep after prolonged waking, which reflect reduced sleep intensity. Intriguingly, the increase in higher frequency EEG activity, which is reminiscent of patients with primary insomnia, was prominent in the *ADORA2A* C/C genotype, whereas no increase was present in the T/T genotype. These results demonstrate a role of the adenosine A_{2A} receptor for sleep in humans, and suggest that genetic variation in this receptor contributes to individual sensitivity to subjective and objective effects of caffeine on sleep.

RESULTS

A total number of 2,308 men and 2,021 women responded to the internet questionnaire (response rate: 21%). Subjective caffeine sensitivity was normally distributed ($n = 4{,}329$, $\Pr > D > 1.50$, $D = 0.247$, Kolmogorov–Smirnov). Approximately one-third of respondents rated themselves as being caffeine sensitive (4.0% very sensitive and 27.4% rather sensitive), one-third as caffeine insensitive (4.3% very insensitive and 25.7% rather insensitive), and one-third as average caffeine sensitive (38.6%). Approximately half of the respondents ($n = 2{,}093$, 48.3%) reported to habitually abstain from caffeine, whereas the other half ($n = 2{,}236$, 51.7%) consumed either average or high amounts of caffeine. Caffeine consumption was less prevalent in caffeine-sensitive subjects ($n = 1{,}357$) than in caffeine-insensitive subjects ($n = 1{,}301$; $\chi^2 = 132.4$, $\mathrm{df} = 1$, $P < 0.001$), and negatively associated with subjective caffeine sensitivity ($\mathrm{df} = 8$, $\gamma = -0.273$).

First, we investigated whether caffeine consumption affects subjective sleep quality. A self-estimated sleep latency (*i.e.*, the time between lights-out and sleep onset) of longer than 20 min and the perception of frequent awakenings from sleep were considered as reduced sleep quality ("insomnia"). The prevalence of "insomnia" in caffeine-sensitive and -insensitive individuals is illustrated in **Figure 1**. Interestingly, "insomnia" was more prevalent in sensitive subjects than in insensitive subjects ($\chi^2 = 13.3$, $\mathrm{df} = 1$, $P < 0.001$, χ^2 probability test). Moreover, in the sensitive group, the proportion of individuals with "insomnia" was higher in those who consumed caffeine than in those who habitually abstained from caffeine ($\chi^2 = 6.2$, $\mathrm{df} = 1$, $P < 0.02$). This was not the case in the insensitive group.

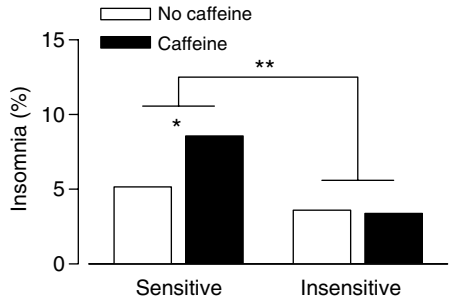

Figure 1 Habitual caffeine consumption induces insomnia-like reduced sleep quality in caffeine-sensitive individuals, but not in caffeine-insensitive individuals. The frequency of reduced sleep quality (*i.e.*, sleep latency longer than 20 min and frequent awakenings from sleep) was assessed in 1,357 caffeine-sensitive and 1,301 -insensitive respondents to an internet questionnaire. $^{**}P < 0.001$ (sensitive vs insensitive subjects, χ^2 probability test). $^{*}P < 0.02$ (no caffeine vs caffeine, χ^2 probability test).

Next, we determined whether the distribution of the *ADORA2A* c.1083T>C alleles and genotypes differed with respect to caffeine sensitivity. We genotyped two subgroups of self-rated caffeine-sensitive and -insensitive subjects (**Table 1**). We found allele and genotype distributions, which were comparable to previous studies.[24] Nonetheless, the C allele appeared to be more frequent in sensitive individuals than in insensitive individuals (66.4 vs 53.6%) ($\chi^2 = 3.4$, $\mathrm{df} = 1$, $P = 0.06$, χ^2 probability test). Moreover, a higher proportion of sensitive subjects had the C/C genotype, whereas a higher proportion of insensitive subjects had the T/T genotype ($\chi^2 = 5.5$, $P < 0.03$, Fisher's exact test, two-sided). The prevalence of the C/T genotype was the same in sensitive and insensitive subjects.

To examine whether objective effects of caffeine on sleep are related to the *ADORA2A* genotype, we studied the action of the stimulant in two matched groups of self-rated caffeine-sensitive and -insensitive men (**Table 2**) during sleep deprivation and subsequent recovery sleep. The sensitive subjects had a much higher "caffeine effect score" after caffeine than after placebo (**Figure 2a**). This was not the case in the insensitive group. Thus, the caffeine effect (difference between caffeine and placebo) differed between the groups ($P < 0.001$, Wilcoxon two-sample, two-sided exact test). In contrast, the scores after placebo were virtually the same, indicating that the sensitive subjects did not just pretend to reflect higher effect scores even in the absence of caffeine. Moreover, the caffeine-induced improvement in optimal performance on a psychomotor vigilance task after sleep loss[9] correlated positively with the subjective effects of caffeine ($r_s = 0.48$, $P = 0.02$, $n = 22$, Spearman rank-correlation analysis).

Table 1 Distribution of *ADORA2A* c.1083T>C alleles and genotypes in caffeine-sensitive and -insensitive individuals

	Allele		Genotype		
Sensitivity	C	T	C/C	C/T	T/T
Sensitive (*n*=58)	77 (66.4%)	39 (33.6%)	23 (39.7%)	31 (53.4%)	4 (6.9%)
Insensitive (*n*=84)	90 (53.6%)	78 (46.4%)	22 (26.2%)	46 (54.8%)	16 (19.0%)

Caffeine sensitivity was based upon a detailed subjective caffeine effects questionnaire.

Table 2 Demographic characteristics of caffeine-sensitive and caffeine-insensitive men

	Sensitive (*n*=12)	Insensitive (*n*=10)	*P*-value
Age (years)	24.1±0.9	25.5±0.7	0.26
Body mass index (kg/m^2)	22.6±0.4	23.6±0.8	0.26
Trait Anxiety Inventory	34.0±1.6	32.1±2.8	0.54
Epworth sleepiness scale	7.5±1.0	6.5±0.9	0.48
Habitual sleep duration (h)	7.4±0.2	7.3±0.2	0.63
Alcohol consumption (drinks/week)	2.5±0.5	3.1±0.7	0.50
Caffeine consumption (mg/day)	58.3±24.4	96.0±27.1	0.31
ADORA2A c.1083T>C genotype			
1083C/C	4/12	3/10	
1083C/T	7/12	5/10	
1083T/T	1/12	2/10	

Values represent means±SEM. *P*-values: unpaired two-tailed *t*-tests. The estimates of caffeine consumption were based on the following average caffeine contents per serving.[47] Filter coffee: 120 mg; espresso: 70 mg; ceylon tea: 40 mg; green tea: 20 mg; 1 l cola drink: 100 mg; 2 dl energy drink: 80 mg.

Given the possibility that subjective caffeine sensitivity reflects anxiogenic effects of the drug,[24] state anxiety according to Spielberger *et al.*[26] was quantified. Anxiety symptoms (average over all four assessments) tended to be higher in sensitive men than in insensitive men (**Figure 2b**). However, they were not related to the *ADORA2A* c.1083T>C genotype. State anxiety was similar after caffeine and after placebo ($P>0.6$, Wilcoxon matched-pairs signed-ranks test), yet higher in the morning than in the evening (38.8±2.0 vs 34.4±2.2, $P<0.001$).

Because there exist large interindividual differences in caffeine metabolism in the liver,[27] we determined whether the different subjective effects reflected different caffeine levels in saliva. The area under the caffeine concentration curve (**Figure 3**) did not differ between the groups (sensitive: 255.0±47.5 μmol/l, $n=11$; insensitive: 206.3±33.7 μmol/l, $n=9$). Moreover, in both groups, 2×200 mg of caffeine led to a peak of roughly 16 μmol/l after the second dose (sensitive: 16.4±2.5 μmol/l; insensitive: 15.7±2.7 μmol/l). The concentration declined later and reached almost zero shortly before the beginning of the recovery night. The kinetics of caffeine was similar in subjects with distinct *ADORA2A* c.1083T>C genotypes ("genotype"×"time" interaction: $F_{2,26}=1.2$, $P>0.2$).

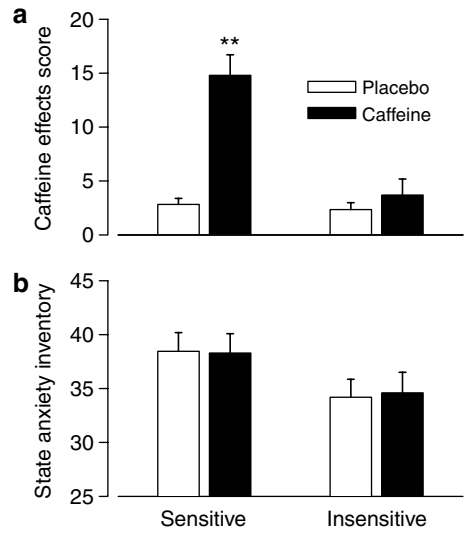

Figure 2 Perceived differences in caffeine effects do not reflect differences in state anxiety. (**a**) A caffeine effects score and (**b**) state anxiety were assessed 105 min after capsule intake. The scores after the two capsules in each condition were averaged. The caffeine effects score was based on a 20-item questionnaire (range: 0–60). The possible answers to the questions whether common caffeine effects[42] were present were: not at all (scored as 0), a little (1), quite a bit (2), and very much (3). State anxiety was quantified with a German translation of the State-Trait Anxiety Inventory of Spielberger *et al.*[26] State anxiety in the context of sleep deprivation (average of all four assessments) tended to be higher in the sensitive men than in the insensitive men ($P<0.09$, Wilcoxon two-sample, two-sided exact test). **$P=0.001$ (caffeine vs placebo, two-tailed paired *t*-test).

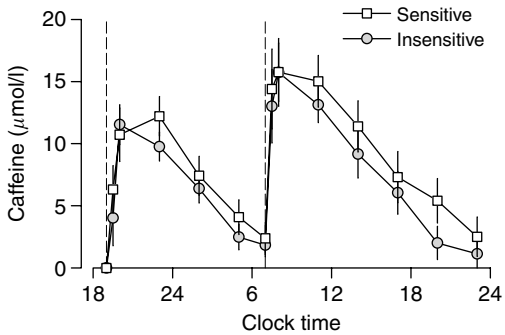

Figure 3 Similar time course of saliva caffeine concentration in caffeine-sensitive ($n=11$, open squares) and caffeine-insensitive subjects ($n=9$, gray circles). Values represent means±SEM.

Polysomnographic baseline sleep recordings demonstrated that sensitive and insensitive men were good sleepers with normal sleep architecture (data not shown) and high sleep

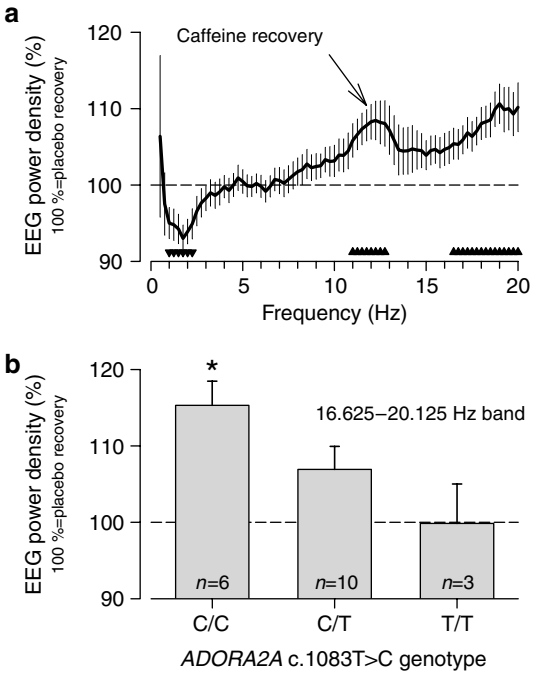

Figure 4 The *ADORA2A* c.1083T>C polymorphism modulates the effect of caffeine on the nonREM sleep EEG in the recovery night after sleep deprivation. (**a**) Reduced nonREM sleep (stages 2, 3, and 4) intensity after caffeine when compared with placebo. For each frequency bin, EEG power density after caffeine (caffeine recovery) was expressed as a percentage of the corresponding values after placebo (placebo recovery, horizontal dashed line at 100%). Means ± SEM ($n = 19$) are plotted at the center of each 0.25-Hz bins. Triangles above the abscissa denote frequency bins for which power differed significantly from the placebo recovery condition ($P < 0.05$, two-tailed paired *t*-tests). Orientation of triangles indicates the direction of deviation. (**b**) Caffeine induces insomnia-like EEG pattern in subjects with the *ADORA2A* C/C genotype. Spectral power in the beta band (16.625–20.125 Hz) after caffeine was expressed as a percentage of the corresponding value after placebo. The rise after caffeine was significantly higher in the C/C genotype than in the T/T genotype. *$P < 0.03$ (C/C vs T/T, Kruskal–Wallis test, exact approximation).

efficiency (>90%). Caffeine affected sleep and the sleep EEG in the recovery night following sleep deprivation. Relative to placebo, the stimulant slightly reduced the typical sleep deprivation-induced increase in nonREM sleep (40.9 ± 4.6 vs 49.0 ± 6.3 min, $P < 0.05$, two-tailed paired *t*-test) and in sleep efficiency (4.6 ± 0.9 vs 5.1 ± 0.9%, $P < 0.01$). Moreover, caffeine attenuated EEG activity in low-delta frequencies (0.625–2.125 Hz), and enhanced activity in the high-alpha/low-sigma range (10.625–12.625 Hz; minimum $F_{1,17} = 4.7$, $P < 0.05$) (**Figure 4a**). These drug-induced changes during sleep, which indicate reduced sleep intensity possibly because of attenuated buildup of sleep propensity during wakefulness,[10–12] did not differ between sensitive and insensitive subjects.

In contrast to the changes in delta and alpha/sigma frequencies, we found that the effect of caffeine on the EEG beta band (16.625–20.125 Hz) differed among individuals with distinct *ADORA2A* c.1083T>C genotypes ("genotype": $F_{2,16} = 6.0$, $P < 0.02$). Increased beta activity in nonREM sleep may characterize patients with insomnia when compared with healthy good sleepers.[28,29] Consistent with the observation that the C allele is more prevalent in caffeine-sensitive individuals who report more sleep disturbances after caffeine than insensitive individuals, the increase was highest in the C/C genotype (**Figure 4b**). Suggesting a functional relationship between the *ADORA2A* genotype and the effect of caffeine on EEG beta activity in nonREM sleep, the rise was approximately half in the C/T genotype, whereas no change was present in the T/T genotype.

DISCUSSION

The main finding of this study is that a common gene polymorphism in *ADORA2A* is associated with subjective and objective differences in the effect of caffeine on sleep. The same genetic variation was previously reported to contribute to the susceptibility to panic disorder,[30,31] to differences in spectral characteristics of the EEG in waking and sleep,[8] and to the increase in anxiety after acute caffeine intake in healthy subjects.[24] Although the direct consequences of the genetic variation for receptor function and protein expression are unknown and influences of other closely linked polymorphisms cannot be excluded, these studies suggest that the *ADORA2A* c.1083T>C polymorphism is functionally relevant.

The prevalence of insomnia disorders in young European adults equals roughly 4%.[32] This estimate is very close to the overall prevalence of reduced sleep quality ("insomnia") in the respondents to our internet survey. It is lower, however, than the prevalence of "insomnia" in self-rated caffeine-sensitive people, particularly in those who habitually consume caffeine. In contrast, habitual caffeine consumption was not associated with increased "insomnia" in the caffeine-insensitive group. Together with the finding that caffeine intake was less frequent in caffeine-sensitive subjects than in caffeine-insensitive subjects, these data support previous research indicating that healthy people who voluntarily reduce or avoid caffeine might do so because they experience disturbed sleep after the stimulant.[15]

The large interindividual variation in the subjective response to caffeine on sleep has long been recognized.[16,19,22,23] The underlying causes, however, remain elusive. Studies looking for pharmacokinetic differences between caffeine-sensitive and -insensitive subjects provided inconsistent results.[18,20,22,33] The different subjective "caffeine effects scores" in our double-blind study confirmed the correct classification in sensitive and insensitive subjects based on screening questionnaires. Because the two groups showed similar caffeine concentration in saliva, this finding suggests that pharmacodynamic rather than pharmacokinetic differences modulate subjective caffeine effects. In other words, our study supports the hypothesis proposed more than 40 years ago that endogenous diversity at the site of action of caffeine could influence the effects of the drug on sleep.[17]

Recent studies in mice provided strong evidence that caffeine promotes wakefulness and also stimulates locomotor activity by blocking adenosine A_{2A} receptors.[25,34,35] Large differences in these effects exist in distinct mouse strains that exhibit genetically determined differences in A_{2A} receptor function.[25,35] Our findings are consistent with the data in mice and support a role of the adenosine A_{2A} receptor for sleep in humans. They show that the relative distributions of the C/C and T/T genotypes of the *ADORA2A* c.1083T>C polymorphism differ in people who experience subjective sleep disturbances after caffeine when compared with people who perceive no caffeine-induced sleep problems. These epidemiologic data suggest that individuals with the C/C genotype are particularly susceptible to disturbed sleep after caffeine.

This notion is further supported by the distinct caffeine-induced increase in EEG beta oscillations in nonREM sleep after sleep deprivation. Enhanced beta activity during sleep was proposed to reflect cortical hyperarousal, which might underlie primary insomnia.[36] This idea was challenged because experimentally induced arousal in a caffeine model of insomnia does not consistently enhance beta activity.[37] Our pharmacogenetic findings may reconcile the controversy. They demonstrate that the increase in higher frequency activity in nonREM sleep after caffeine depends on the *ADORA2A* genotype. A low dose of caffeine, which was virtually undetectable in saliva before sleep, increased higher frequency oscillations in individuals with the c.1083T>C C/C genotype. Supporting a functional gene–effect relationship, the enhancement was half in individuals with the C/T genotype and not present in individuals with the T/T genotype.

The *ADORA2A* c.1083T>C polymorphism not only modulates the effects of caffeine on brain oscillations during sleep, but also on symptoms of anxiety in healthy subjects. It was previously noticed that caffeine can have an anxiogenic action in certain individuals.[23,38] A recent study showed that 150 mg caffeine induced anxiety in infrequent caffeine users with the *ADORA2A* T/T genotype, but not in subjects with the C/C and C/T genotypes.[24] Although the experimental procedures (*e.g.*, caffeine dose, absence or presence of sleep deprivation) and habitual caffeine intake patterns of subjects differ between the study of Alsene *et al.*[24] and ours, it is interesting to note that the drug-induced increase in sleep beta oscillations and in anxiety appear to be distinctly favored by the C allele and the T allele, respectively. This pharmacogenetic interrelation could explain why the caffeine-sensitive and -insensitive groups of our sleep deprivation experiment showed similar distributions of the *ADORA2A* genotypes. Nevertheless, the number of subjects who participated in this part of the study is too low to allow a reliable statistical analysis of the distribution of *ADORA2A* genotypes.

The heritability of insomnia symptoms may reach up to 50%.[39] Thus, genetic mutations and polymorphisms, in interaction with exogenous factors, probably underlie this disorder. Cortical hyperarousal, as reflected in higher frequency EEG activity during sleep, may neurophysiologically characterize acute insomnia.[36,40] Our epidemiologic and neurophysiological data consistently suggest that the *ADORA2A* c.1083T>C polymorphism modulates individual susceptibility to cortical hyperarousal, induced by caffeine a common exogenous factor of acute insomnia and the world's most popular stimulant.

METHODS

Subject recruitment and study procedures. The study protocol and all experimental procedures were approved by cantonal and local ethics committees for research on human subjects, and carried out in accordance with the Declaration of Helsinki Principles. To recruit participants with low and high subjective caffeine sensitivity, moderate habitual caffeine consumption (to minimize the influence of tolerance), and regular sleep–wake habits, an internet questionnaire about caffeine sensitivity and sleep was distributed among 20,343 university students. A total of 4,329 individuals (2,308 men, 2,021 women) responded. On average, they were 23.6 ± 3.6 (mean ± SD) years of age.

One hundred and twenty-one respondents to the internet survey with very high or very low subjective caffeine sensitivity (89 men, 32 women), and 36 older men (mean age: 65.7 ± 3.4 years) who were evaluated for participation in a study on age-related changes in sleep–wake regulation[41] were selected for genetic investigations. All participants were without health or sleep complaints. After being informed about the goals and risks, they signed a consent form and gave 10 ml blood for genotyping of the A_{2A} receptor (*ADORA2A*) c.1083T>C single-nucleotide polymorphism (SNP-ID: rs5751876; formerly referred to as 1976T>C: GenBank accession no. X68486). All subjects filled in a detailed questionnaire containing 108 questions about subjective caffeine sensitivity and sleep habits.[42,43] On the basis of this questionnaire, 58 individuals were considered as being caffeine sensitive and 84 individuals as being caffeine insensitive (**Table 1**); 15 individuals could not be unambiguously classified. Among the most important selection criteria was the answer to the question whether or not caffeine intake in the afternoon disturbs subjective sleep quality at night.

Genomic DNA was extracted from the blood samples, and the genotypes were determined with allele-specific polymerase chain reaction[44] using allele-specific primers designed for selective amplification of each allele: AR2A_for_T (forward primer specific for allele T: 5′-CGG AGG CCC AAT GGC TAT-3′), AR2A_for_C (forward primer specific for allele C: 5′-CGG AGG CCC AAT GGC TAC-3′), and AR2A_rev (reverse primer: 5′-GTG ACT GGT CAA GCC AAC CA-3′). The polymerase chain reaction was performed in volumes of 25 μl containing 50 ng genomic DNA, 250 μM dNTPs, 400 nM forward primer, 400 nM reverse primer, 0.02 U HotStar Taq DNA Polymerase (Qiagen, Basel, Switzerland) in 4 mM MgCl2 reaction buffer. Fragments were amplified using a "hot start" procedure. Specifically, an initial denaturating step (15 min, 95°C) was followed by 35 cycles of denaturation (1 min, 95°C), annealing (1 min, 66°C for the primer pair AR2A_for_C/AR2A_rev, 68°C for the primer pair AR2A_for_T/AR2A_rev), and elongation (30 s, 72°C), as well as a final extension step (5 min, 72°C). The polymerase chain reaction products were analyzed by electrophoresis on 1% agarose gels containing 10 μg/ml ethidium bromide. The expected size of the polymerase chain reaction product was 243 bp.

Among all genotyped individuals, 13 caffeine-sensitive and 10 caffeine-insensitive healthy male good sleepers without sleep disturbances completed a laboratory study on the effects of caffeine during sleep deprivation. The stimulant action of caffeine is stronger and more variable after prolonged waking than when fully rested.[6,12] Because one sensitive subject did not comply with task instructions

and protocol specifications, his data were excluded from the analyses. The effects of sleep deprivation and caffeine in the remaining subjects on neurobehavioral performance and the regional EEG power distribution in waking and sleep were reported elsewhere,[9,45] where the study protocol and the recruitment and pre-experimental procedures are described in detail. In brief, all subjects participated in two blocks of four consecutive nights separated by 1 week. The first and second nights of each block served as 8-h adaptation and baseline nights, respectively. The volunteers then stayed awake for 40 h (*i.e.*, for two days, skipping one night of sleep) until bedtime of a 10.5 h recovery night. Two doses of 200 mg caffeine and placebo were administered in the form of capsules to all subjects after 11 and 23 h of extended waking according to a randomized, double-blind, crossover design. Approximately 105 min after capsule intake, subjects filled in a detailed "caffeine effects questionnaire" based on the typical effects of caffeine as reported by Griffiths *et al.*,[42] and completed the State-Trait Anxiety Inventory of Spielberger *et al.*[26] To ensure wakefulness, the subjects remained under continuous supervision of a member of the research team. They were allowed to read, study, play games, watch films, and occasionally take a walk outside the laboratory. In 14 sessions, at 3-h intervals, they completed 8-min waking EEG recordings followed by 10-min sessions of a psychomotor vigilance task and a random number generation task.

All participants refrained from all sources of caffeine for 2 weeks before the study to minimize the possible effects of tolerance and withdrawal. They were also requested to abstain from ethanol and to maintain regular 8:16-h sleep–wake cycles for 3 days before and during the experiment. Sleep during the study was scheduled from 23 to 07 h ($n = 3$) or from 24 to 08 h (remaining subjects) according to the participants' habitual sleep times. During the 3 days preceding each study block, a deviation of more than 1 h from these bedtimes was not allowed. Compliance with the pre-study instructions was verified by determining the level of caffeine in saliva and breath ethanol concentration upon arrival in the sleep laboratory, and by inspecting the records from rest–activity monitors worn on the wrist of the non-dominant arm.

Caffeine kinetics. The kinetics of the caffeine concentration was determined in saliva. Samples were collected 5 min before and 30 and 60 min after caffeine intake, and at 3-h intervals before each waking EEG recording that followed the first dose of caffeine. All saliva samples were stored at $\sim 6^{\circ}$C and later analyzed for caffeine with a homogenous enzyme immunoassay (Emit®-Caffeine Test, Syva Company, Palo Alto, CA). The data of one sensitive and one insensitive individual could not be analyzed because of insufficient saliva volumes.

Polysomnography

Continuous polysomnographic recordings (EEG, electrooculogram (EOG), electromyogram (EMG), electrocardiogram) were performed during all experimental nights. Sleep stages were visually scored for 20-s epochs according to the rules of Rechtschaffen and Kales.[46] Power spectra of consecutive 20-s epochs (FFT, Hanning window, average of five 4-s epochs, 0–20 Hz) were computed for the C3A2 and six bipolar EEG derivations.[9] Artifacts were identified by visual inspection and a semiautomatic algorithm (moving average threshold) to separately exclude high (20–40 Hz) and low (0.75–4.5 Hz) frequency artifacts. For calculation of the all-night power spectra in nonREM sleep, all artifact-free 20-s spectral values of sleep stages 2, 3, and 4 were averaged. Only power spectra derived from the C3A2 derivation are reported, and in the recovery nights only the first 8 h of the 10.5-h sleep opportunity were considered. Power was computed for consecutive 0.25-Hz bins and for distinct frequency bands. The frequency bins and bands are indicated by the encompassing frequency range (*i.e.*, the 1.0 Hz bin denotes the 0.875–1.125 Hz range). Because of a system breakdown, the data of the recovery nights of two caffeine-sensitive subjects and one caffeine-insensitive subject were lost.

Data analyses and statistics. Statistical analyses were performed with SPSS® 11.5 (SPSS, Chicago, IL) and SAS® 8.02 software (SAS Institute, Cary, NC). The significance level for statistical tests was set at $\alpha < 0.05$. If not stated otherwise, only significant effects of factors and interactions were reported. Variables that were not normally distributed were either log-transformed to approximate a normal distribution (absolute EEG power densities) or analyzed by non-parametric testing. If appropriate, repeated-measures analyses of variance (general linear model) with the between-subject factors "sensitivity" (caffeine sensitive, caffeine insensitive) and "genotype" (C/C, C/T, T/T), and the within-subject factors "deprivation" (BL, SD), "caffeine" (caffeine, placebo), and "time" (assessments 1–14) were performed before testing contrasts. *Post hoc* tests were only used if the main factor or interaction of the repeated-measures analyses of variance reached significance.

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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