Coffee effectively attenuates impaired attention in ADORA2A C/C-allele carriers during chronic sleep restriction

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Abstract: Many people consume coffee to attenuate increased sleepiness and impaired vigilance and attention due to insufficient sleep. We investigated in genetically caffeine sensitive individuals whether 'real world' coffee consumption during a simulated busy work week counteracts disabling consequences of chronically restricted sleep. We subjected homozygous C-allele carriers of ADORA2A (gene encoding adenosine A2A receptors) to 5 nights of only 5 h time-in-bed. We administered regular coffee (n = 12; 200 mg caffeine at breakfast and 100 mg caffeine after lunch) and decaffeinated coffee (n = 14) in double-blind fashion on all days following sleep restriction. At regular intervals 4 times each day, participants rated their sleepiness and performed the psychomotor vigilance test, the visual search task, and the visuo-spatial and letter n-back tasks. At bedtime, we quantified caffeine and the major caffeine metabolites paraxanthine, theobromine and theophylline in saliva. The 2 groups did not differ in age, body-mass-index, sex-ratio, chronotype and mood states. Subjective sleepiness increased in both groups across consecutive sleep restriction days and did not differ. By contrast, regular coffee counteracted the impact of repeated sleep loss on sustained and selective attention, as well as executive control when compared to decaffeinated coffee. The coffee induced benefits on different aspects of performance lasted for 4–5 days of insufficient sleep. All differences between the groups disappeared after the recovery night and the cessation of coffee administration. The data suggest that 'real world' coffee consumption can efficiently attenuate sleep restriction-induced impairments in vigilance and attention in genetically caffeine sensitive individuals.

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Coffee effectively attenuates impaired attention in *ADORA2A* C/C-allele carriers during chronic sleep restriction

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ABSTRACT

Many people consume coffee to attenuate increased sleepiness and impaired vigilance and attention due to insufficient sleep. We investigated in genetically caffeine sensitive men and women whether 'real world' coffee consumption during a simulated busy work week counteracts disabling consequences of chronically restricted sleep. We subjected homozygous C-allele carriers of *ADORA2A* (gene encoding adenosine A_{2A} receptors) to five nights of only 5 h time-in-bed. We administered regular coffee ($n = 12$; 200 mg caffeine at breakfast and 100 mg caffeine after lunch) and decaffeinated coffee ($n = 14$) in double-blind fashion on all days following sleep restriction. At regular intervals four times each day, participants rated their sleepiness and performed the psychomotor vigilance test, the visual search task, and the visuo-spatial and letter n-back tasks. At bedtime, we quantified caffeine and the major caffeine metabolites paraxanthine, theobromine and theophylline in saliva. The two groups did not differ in age, body-mass-index, sex-ratio, chronotype and mood states. Subjective sleepiness increased in both groups across consecutive sleep restriction days and did not differ. By contrast, regular coffee counteracted the impact of repeated sleep loss on sustained and selective attention, as well as executive control when compared to decaffeinated coffee. The coffee also induced initial or transient benefits on different aspects of baseline performance during insufficient sleep. All differences between the groups disappeared after the recovery night and the cessation of coffee administration. The data suggest that 'real world' coffee consumption can efficiently attenuate sleep restriction-induced impairments in vigilance and attention in genetically caffeine sensitive individuals.

German Clinical Trial Registry: # DRSK00014379.

1. Introduction

Undisturbed sleep of sufficient duration is a prerequisite for personal well-being and health and is essential for alertness and cognitive performance necessary for safe and effective functioning. Despite this knowledge, representative national surveys indicate that more than 30% of the adult population in Western societies report sleeping less than the commonly recommended 7–8 h on weekday nights, and roughly 15% regularly sleep less than 6 h (Basner et al., 2014; Tinguely et al., 2014). Consistent with the prevalent co-occurrence of insufficient sleep and excessive daytime sleepiness (Ohayon, 2008), increased sleepiness belongs to the first signs of experimentally induced insufficient sleep (Lo et al., 2012).

Similarly, a general slowing in response speed and an increased number of attentional lapses on a psychomotor vigilance test (PVT) is typically observed in normal sleepers when time-in-bed is restricted to 5–6 h over several nights (Van Dongen et al., 2003; Balkin et al., 2004; Lo et al., 2012). These findings corroborate the notion that simple, reaction-time based tasks of vigilance such as the PVT are very sensitive to insufficient sleep (Tkachenko and Dinges, 2018). By contrast, more demanding waking functions such as working memory and response inhibition, which also rely on underlying aspects of attention, appear to

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be less affected.

A currently prevailing model posits that three separate but interacting attentional networks regulate vigilance (alerting network), orienting (orienting and selection network), and executive attention (executive control network) (Petersen and Posner, 2012). These networks respond in concert to environmental stimuli but are largely independent (Fan et al., 2005). It is assumed that the alerting network prepares and maintains responses to salient stimuli, the orienting network isolates desired stimuli for processing and directs attention to a target stimulus, and the executive control network allocates attentional resources to manage cognitive workload (Petersen and Posner, 2012). Sleep deprivation, as well as acute and chronic sleep restriction impair not only vigilance but also executive control, albeit with a lower effect size (Lo et al., 2012). Conversely, tasks assessing orienting appear largely unaffected by acute sleep restriction (Cunningham et al., 2018). These findings suggest that insufficient sleep differently affects distinct attentional network systems which may provide distinct targets for pharmacological interventions to mitigate sleep-loss induced attentional impairments (Dijk and Landolt, 2019).

To enhance wakefulness in response to sleep restriction, intake of caffeine is highly common, particularly in the morning and early afternoon (Martyn et al., 2018). It is estimated that more than 80% of the world's population consume caffeine on a daily basis, with coffee being the most common dietary source (Clark and Landolt, 2017; Martyn et al., 2018). The average daily caffeine intake per adult equals ~300 mg in Europe and South America, and ~200 mg in the US (Heckman et al., 2010; Urry et al., 2017; Frozi et al., 2018; Martyn et al., 2018). By blocking A_1 and A_{2A} receptors of the sleep promoting neuromodulator adenosine, caffeine facilitates cholinergic and monoaminergic neurotransmission in brain regions that regulate vigilance and higher-order attentional processes (Fan et al., 2005). Consistent with this mode of action, acute caffeine administration between ~200–300 mg preserves vigilance and all aspects of attention, in particular when performance degrading factors such as insufficient sleep are present (Jarvis, 1993; Lieberman et al., 2002). Nevertheless, it is currently unclear whether the evidence from studying acute caffeine effects can be translated to real world consumption, where caffeinated beverages are commonly consumed every day. Indeed, it was recently reported that repeated administration of 300–450 mg caffeine per day failed to improve vigilance performance in rested and sleep restricted individuals (Bartrim et al., 2020; Weibel et al., 2020). Remarkably, after a short-lived initial benefit, sleepiness and attentional lapses were even enhanced after caffeine in comparison to placebo when sleep was restricted for more than three nights (Doty et al., 2017). In conclusion, it is currently not known whether daily coffee intake in a dose and timing that mimics 'real world' European habits maintains simple and complex attentional processes during repeated sleep restriction.

Not only dose and frequency of administration, but also pronounced inter-individual differences determine the subjective and objective responses to caffeine and may hamper conclusions on its potency to enhance vigilance and attention. These inter-individual differences are robust and in part genetically determined. More specifically, genetic variants of the adenosine A_{2A} receptor gene (*ADORA2A*), in particular the c.1976T>C variant, were consistently found to modulate neurobehavioral performance during sleep restriction (Bodenmann et al., 2012; Rupp et al., 2013), as well as individual effects of caffeine on self-reported alertness (Rogers et al., 2010), attention network functions (Renda et al., 2015; Geiger et al., 2016) and sleep (Rétey et al., 2007; Bodenmann et al., 2012). These findings suggest that prospective genotyping of the c.1976T>C variant of *ADORA2A* could provide clearer outcomes on the potential usefulness of coffee as a countermeasure against impaired attention due to insufficient sleep.

To tackle this question, we subjected two carefully matched groups of homozygous C-allele carriers of *ADORA2A* to repeated sleep restriction and studied the effects of standardized regular coffee (300 mg caffeine per day) or decaffeinated coffee (< 3 mg caffeine per day) on subjective sleepiness and different facets of attention. We hypothesized that daily coffee consumption in genetically caffeine sensitive individuals attenuates sleepiness and the impairment of performance on all attentional domains during a five-day simulated busy workweek of only 5 h time-in-bed each night.

2. Materials and methods

All study procedures were approved by the ethics committee of North Rhine ("Ärztekammer Nordrhein"), the German Federal Office for Radiation Protection ("Deutsches Bundesamt für Strahlenschutz") and carried out in accordance with the Declaration of Helsinki. All participants gave written informed consent before participating in the study.

2.1. Participants

Prospective study participants aged between 20 and 40 years were recruited through the internal test subject database of the Institute of Aerospace Medicine of the German Aerospace Center (Deutsches Zentrum für Luft- und Raumfahrt e.V.; DLR), as well as advertisements on public websites. Individuals interested in study participation were provided with more details via e-mail and asked to complete a dedicated screening questionnaire. Exclusion criteria included a reported body-mass-index (BMI) > 30, presence of sleep-wake disorders and any chronic diseases, habitual nightly sleep duration outside the range of 6–9 h, current shift work and jet-lag, history of head injury, and alcohol or substance abuse. Participants were only evaluated further if they reported no current medication (except contraceptives) and nicotine intake and an estimated habitual caffeine consumption below 450 mg/day. To eligible volunteers, we sent by mail a parcel containing detailed information and a saliva self-collection kit (DNA Genotek Inc., Ottawa, Canada). They were asked to provide a saliva sample for determination of the c.1976T>C polymorphism (SNP-ID: rs5751876) of the gene *ADORA2A*. A total of 309 OraGene-500 test-kits was genotyped. A detailed flow chart of participant recruitment is provided in Supplementary Fig. S1.

2.2. Determination of the c.1976T>C genotype

According to the manufacturer's instructions, genomic DNA was extracted following an ethanol precipitation protocol using prepIT reagent (prepIT-L2P), such as described previously (Urry et al., 2017). Allele-specific primers were used for selective amplification of each allele (forward primer specific for allele T: 5′-CGG AGG CCC AAT GGC TAT-3′, forward primer specific for allele C: 5′-CGG AGG CCC AAT GGCTAC-3′, and reverse primer: 5′-GTG ACT GGT CAAGCC AAC CA-3′). Fragments containing 10 ng genomic DNA were amplified using a TaqDNA Polymerase (Thermo Fisher Scientific, Whaltham, USA) and a "hot start" procedure. Specifically, an initial denaturating step (10 min, 95 °C) was followed by 40 cycles of denaturation (1 min, 92 °C), annealing and elongation (1 min, 60 °C), using an Applied Biosystems GeneAmp PCR System 2700 thermal cycler (Applied Biosystems, Foster City, USA). An Applied Biosystems PRISM 7900HT with SDS software version 2.2 was used for allelic discrimination and fluorescence detection. Consistent with the expected allele frequencies of a Western European study sample (Rétey et al., 2007), 105 homozygous C-allele carriers (34.0%) were identified.

2.2.1. Pre-study procedures and experimental protocol

During at least one week preceding the study, participants were asked to adhere to a regular sleep-wake schedule of 9 h of sleep, starting at 22:00/23:00, and 15 h of wakefulness. They wore a wrist activity monitor and completed a sleep-wake diary, to verify compliance with this instruction. Naps and caffeine, alcohol and medication intake were not permitted (occasional medication intake unknown to interfere with sleep or performance could be allowed upon consultation).

The experimental portion of the study was conducted under controlled conditions in a dedicated, state-of-the-art research facility of the Institute for Aerospace Medicine at the DLR's headquarter in Cologne (https://www.dlr.de/envihab/). Upon arrival, prospective study participants were pseudo-randomly assigned to one of two coffee groups, stratified by sex, age and BMI, to either receive standardized regular coffee or decaffeinated coffee during chronic sleep restriction. A member of the medical staff informed all volunteers about the objectives, as well as the risks of the study. Adherence to all pre-study instructions, including urine toxicological screening and saliva caffeine quantification, was verified. In addition, in the first night following arrival at the laboratory the absence of sleep-wake disturbances was confirmed in an adaptation/screening night. Individuals with an apnea-hypopnea index higher than 10 and a periodic leg movement index higher than 15 were excluded from study participation.

The experimental protocol consisted of 9 consecutive days and nights (Fig. 1), aimed at investigating the effects of coffee intake on subjective state, attention, declarative memory, decision making, risk taking, sleep architecture, the sleep and waking EEG, as well as cerebral adenosine A_1 receptor binding in vivo before, during, and after recovery from sleep restriction. After the adaptation/screening and two baseline nights (BS1 & BS2; either from 23:00–07:00 [n = 14] or from 00:00–08:00 [n = 12], according to each participant's self-selected sleep-wake schedule during the pre-study weeks), sleep was restricted to 5 h time-in-bed during five consecutive days (CS1-CS5; either from 02:00–07:00 [n = 14] or from 03:00–08:00 [n = 12]). The study was concluded with an 8-h recovery night (RN; either from 23:00–07:00 [n = 14] or from 00:00–08:00 [n = 12]). All measurements and recordings were scheduled at identical times awake.

All participants slept in their own single bedroom, where during the day test sessions at the computer took place at regular time intervals. When not engaged in sleep or cognitive testing, participants remained in a common living area to read, eat, play games, or watch television and movies. Light intensity was set at <100 lx during waking hours to minimize light's acute alerting effect and impact on circadian rhythms. The volunteers were not allowed to leave the research facility during the entire experimental period. During the study, naps, smoking, caffeine (except experimental coffee intake), alcohol, medications, and sports were not allowed. Violation of protocol instructions lead to exclusion from further participation in the study.

During the pre-study procedures, at screening and in the course of the experiment, 42 individuals needed to be excluded (referred to as "late drop-outs") (Supplementary Fig. S1). The typical reasons for exclusion included non-compliance to the imposed sleep-wake schedule, positive toxicological screening, and increased apnea-hypopnea or periodic-leg-movement index in the screening night. Twenty-seven homozygous rs5751876 C-allele carriers completed the study. Because one participant of the regular coffee group performed outside the normal range on virtually all cognitive tasks and her data differed by >2 standard deviations from the values of the other participants, this dataset was excluded from the analyses. The demographic characteristics of the remaining 26 individuals are summarized in Table 1. The two experimental groups did not differ on any demographic criteria considered.

2.3. Coffee preparation and administration

Two batches of coffee and high-quality, electric drip filter coffee machines (Tchibo type 5794 and 2855) were obtained from the same manufacturer (Tchibo GmbH, Coffee Technology, Hamburg, Germany). The coffee was brewed according to detailed instructions provided by the manufacturer, whose pre-study analytics confirmed that adherence to the brewing instructions produced a content of 101 ± 0.6 mg (SD) caffeine per 200 g of regular coffee and 2.4 ± 0.05 mg caffeine per 200 g

Table 1
Demographic characteristics of study participants.

	Regular coffee	Decaffeinated coffee	p value
n	12	14	
Gender ratio	6 female/6 male	6 female/8 male	
Age (years)	29.9 ± 5.3	28.6 ± 5.4	0.42
Chronotype (MCTQ)	04:25 ± 56 min	04:28 ± 51 min	0.82
Epworth Sleepiness Scale	5.9 ± 3.1	4.9 ± 2.7	0.37
PANAS (Positive Affect Schedule)	35.2 ± 3.2	33.8 ± 10.6	0.62
PANAS (Negative Affect Schedule)	13.8 ± 2.9	13.7 ± 3.2	0.96
Beck Depression Inventory (BDI-II)	1.8 ± 2.4	1.4 ± 1.6	0.68
Body mass index (kg/m^2)	23.2 ± 3.2	23.6 ± 2.5	0.85
Habitual caffeine intake (mg/day)	156 ± 124	113 ± 91	0.25

Values represent means and standard deviations. All study participants were ADORA2A c.1976T>C homozygous C/C allele carriers. MCTQ = Munich ChronoType Questionnaire, values represent time of mid-sleep on free days corrected for sleep debt, ESS = Epworth Sleepiness Scale, PANAS = Positive and Negative Affect Schedule Questionnaire, Body Mass Index and habitual caffeine intake per day were estimated from recruitment survey, Genotype was determined by analyzing DNA from a saliva sample. P-values are derived from a two-tailed, unpaired *t*-test.

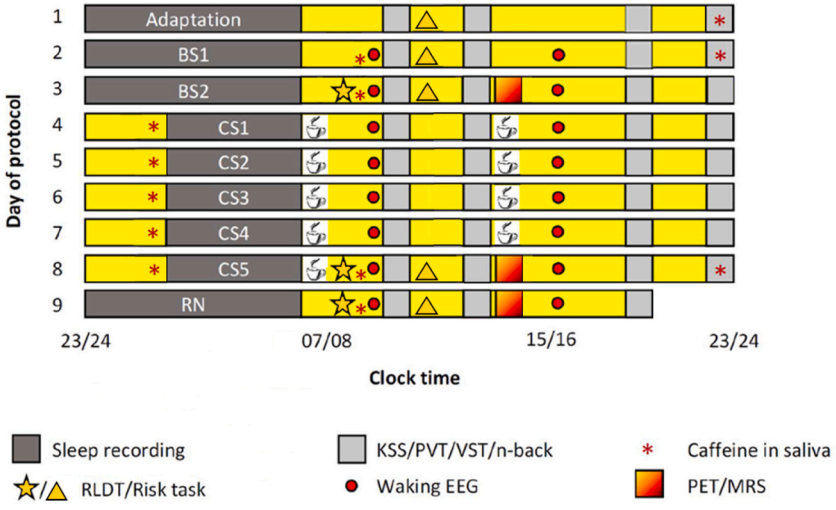

Fig. 1. Experimental protocol. Dark grey shading: sleep opportunities recorded with standard polysomnography. Adaptation: adaptation/screening night; BS1, BS2: baseline nights; CS1-5: sleep restriction nights; RN: recovery night. Yellow shading: monitored wakefulness under controlled laboratory conditions. Light grey shading: neurobehavioral test battery consisting of Karolinska Sleepiness Scale (KSS), psychomotor vigilance test (PVT), visual search task (VST), and visuo-spatial and letter n-back tasks. Red asterisks indicate the times of saliva collection for caffeine quantification. Coffee mugs indicate coffee administration. Red circles: high-density waking EEG recordings. Yellow asterisks: reversal learning decision task (RLDT). Yellow triangles: risk task. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of decaffeinated coffee (data available on file). On all days following sleep restriction, 400 and 200 g regular (coded as batch "162") or decaffeinated coffee (coded as batch "833"), respectively, were administered in double-blind fashion at 07:30/08:30 and 14:00/15:00. A daily dose of ~300 mg caffeine roughly matches the estimated habitual caffeine intake patterns from wastewater analyses in Zurich, Switzerland (Gracia-Lor et al., 2017). On sleep restriction day 5, the midday coffee intake was omitted to avoid interference with the scheduled positron emission tomography (PET) scanning (Fig. 1). All members of the research team were blind to the identity of the two coffee batches throughout data collection and statistical analyses of the subjective and behavioral data.

2.4. Subjective sleepiness

To quantify the evolution of subjective sleepiness throughout the experimental protocol, the Karolinska Sleepiness Scale (KSS) was administered before each test session devised to assess the different components of attention (see below). The KSS is a widely validated 9-point Likert scale to estimate the subjects' self-reported momentary level of drowsiness/sleepiness. It ranges from "1" (extremely alert) to "9" (extremely sleepy, fighting sleep) (Åkerstedt and Gillberg, 1990).

2.5. Testing attention network functions

To study the effects of coffee intake during chronic sleep restriction on different functional aspects of attention, a 35-min test battery was administered on all experimental days at 09:00/10:00, 12:00/13:00, 18:00/19:00, and 21:00/22:00 (Fig. 1). To examine all three attentional networks, each test session included validated versions of a psychomotor vigilance test, a visual search task, and a visuo-spatial and letter (verbal) n-back task.

2.5.1. Vigilance and alerting network

The psychomotor vigilance test (PVT) is a gold-standard measurement of sustained vigilant attention in sleep and chronobiology research (Dinges and Powell, 1985). This test relies on measuring the reaction time (RT) to a digital ms-counter on a computer screen that starts to scroll randomly ~100 times with variable inter-stimuli intervals between 2 and 10 s over a test duration of 10 min. The RTs below 100 ms were defined as errors of commission and excluded, whereas the RT above 500 ms were defined as errors of omission (lapses) and also omitted from the analyses of mean RT. Speed (1/RT), the number of lapses, accuracy, and the log-transformed signal-to-noise ratio (LSNR) on the PVT were analyzed. Accuracy was defined as the sum count of true positives over the total number of stimuli, thus also taking into account lapses (RT > 500 ms) and false positive responses (RT < 100 ms). The LSNR was previously suggested to quantify PVT performance with high sensitivity, stability, normal distribution, and absence of floor and ceiling effects (Chavali et al., 2017). This novel measure of PVT performance is independent of the absolute position on the metric scale (Chavali et al., 2017).

The signal-to-noise-ratio (SNR) of PVT performance was approximated with the following formula (Chavali et al., 2017):

$$SNR \approx \frac{N\left(\sum_{i=1}^{N} w_i S_i\right)^2}{\sum_{i=1}^{N}\left[w_i\left(S_i \sum_{i=1}^{N} w_i - \sum_{i=1}^{N} w_i S_i\right)^2\right]} + 1,$$

where $S_i = 1/(RT_i - C)$, $w_i = 1/(r^2 S_i + 1)$, $C = 100$ ms, $r^2 = 196$ ms, RT_i is the i^{th} RT (in ms), and N is the number of trials in the PVT session. RT < 100 ms (i.e., false starts) were not included. The log-transformed form of this metric as $LSNR = 10\log_{10}(SNR)$ expressed in decibel (dB) was analyzed.

2.5.2. Orienting and selection network

The visual search task probes the distinct subsystems of the attention network, which relate to searching and selecting stimuli for further processing (orienting) (Pashler, 1987). In the visual search paradigm employed here, the study participants were instructed to find a target item as fast and precisely as possible (i.e., the digit '2') on a display cluttered with distractor items (i.e., the digit '5') (Santhi et al., 2007). The target was either present or not present and the set size of search items varied between 10, 20, 30 and 40. Speed (excluding RTs < 100 ms) and accuracy (i.e., sum of true positives and true negatives divided by total amount of responses) were analyzed.

2.5.3. Executive control network

A visuo-spatial and a letter n-back task were employed to probe working memory and the executive control network responsible for the allocation of attentional resources (Owen et al., 2005). With increasing cognitive workload, these tasks require the short-term managing and updating of information and as such tap into the executive control of attention. Participants were presented with a series of dot positions and letters on a computer screen and asked to press a button if the current stimulus was presented 1, 2, or 3 steps back. Each of the 3 cognitive workload levels consisted of 20 targets and 40 non-targets. The data were analyzed for speed (including only correct answers and RTs > 100 ms for calculating average values) and accuracy of task responses. Accuracy was defined as the sum of hits and correct rejections, divided by the amount of total responses.

2.6. Quantification of caffeine and caffeine metabolites in saliva

Saliva samples for the quantification of caffeine and its main metabolites, paraxanthine, theobromine, and theophylline were collected in Salivette® tubes (Sarstedt, Germany) on all experimental days at bedtime (except for RN where no coffee was administered), as well as in the morning of experimental days B1, B2, CS5 and RN (Fig. 1). The samples were stored at −20 °C and only quantified when the analyses of the subjective and behavioral data were completed. For metabolite quantification, internal standards (IS) had to be prepared consisting of caffeine, theophylline, theobromine and caffeine-$^{13}C_3$, purchased from Sigma-Aldrich (St. Louis, USA), and paraxanthine purchased from Cerilliant (Texas, USA). All chemicals used were of the highest grade available. For sample preparation, 280 µl of saliva, 70 µl of the IS (8 µM caffeine-$^{13}C_3$) and 1000 µl of ethyl acetate were added to a tube. Samples were shaken for 10 min and centrifuged (5 min, 10′000 rpm). 800 µl of the supernatant was transferred into an auto-sampler vial and evaporated to dryness under a gentle stream of nitrogen and reconstituted in 250 µl of an eluent-mixture (95:5, v/v). Calibrator (Cal) and quality control (QC) samples were prepared with the same sample preparation, but 70 µl of the Cal or QC solutions were added before adding 930 µl of ethyl acetate. The saliva samples were analyzed using an ultra-high-performance liquid chromatography (UHPLC) system (Thermo Fisher, San Jose, CA), coupled to a linear ion trap quadrupole mass spectrometer 5500 (Sciex, Darmstadt, Germany). The mobile phases of the UHPLC consisted of water (eluent A) and a mixture (70:30 v/v) of methanol and acetonitrile (eluent B), both containing 0.1% of formic acid (v/v). The flow rate was set to 0.45 ml/min with the following gradient: start conditions 95% of eluent A, decreasing in 3 min to 80%, and a quick decrease to 2% A within 0.5 min, holding these conditions for 1 min and then switch to the starting conditions for a 1 min re-equilibration. Injection volume was 5 µl. A Kinetex Biphenyl column (50 × 2.1 mm, 1.7 µm) (Phenomenex, Aschaffenburg, Germany) was used for the separation of the analytes. Mass spectrometer (MS) was operated in positive electrospray ionization mode with scheduled multiple reaction monitoring (MRM) (Kondrat et al., 1978) with a detection window of 35 s and a target scan time of 1.1 s. Three MRM transitions were used for each analyte. For analyte quantification, peak areas were integrated and divided by the peak area of the IS. Cal samples were fitted

with a least-squares fit and weighted by 1/x.

The caffeine and metabolite concentrations in the final five study participants (two members of the regular coffee group and three members of the decaffeinated coffee group) could not be analyzed because the UHPLC/MS system was not available. The results of the pharmacokinetic analyses thus rely on ten volunteers who received regular coffee and eleven volunteers who received decaffeinated coffee.

2.7. Data analyses

In this manuscript, we report the effects of common coffee intake on subjective sleepiness and the distinct components of attention (vigilance, orienting and executive control) during chronic sleep restriction. All analyses were conducted using R version 4.0.0 (R Core Team, 2018) and RStudio Version 1.2.5042 (RStudio, Inc.). Data were analyzed via linear mixed effects models (R package lme4 v.1.1.23 and lmerTest v. 3.1.2) using residual maximum likelihood estimates to fit the model and maximum likelihood for omnibus analysis of variance (ANOVA). Factors included 'day' (BL [mean of BS1 and BS2], CS1, CS2, CS3, CS4, CS5, RN), 'group' (coffee batch '162, '833'), cognitive 'workload' (1-, 2-, 3-back) and their interactions as fixed effects, whereas 'study participant' and 'set size of search items' (visual search task) were added as random effects when appropriate. Distribution of residuals and goodness of fit was checked in all models and compared to the results of quantile and robust regression methods (R package MASS v.7.3.51.6). In all Figures, group means and 95% confidence intervals are presented, based on 1000 bootstrap replicates (Efron and Tibshirani, 1993). Post-hoc general linear hypothesis tests were computed to compare groups on each day of the study, when 'day' x 'group' interaction terms were significant. To correct for multiple comparison, the Benjamini-Hochberg procedure was applied (R package multcomp v. 1.4.13). To quantify the effect size, Cohen's d measures were computed for each day of the study (Cohen, 1988).

All results of the linear mixed effects model ANOVAs are summarized in Supplementary Tables S1 (self-rated sleepiness and PVT), S2 (visual search task), S3 (visuo-spatial and letter n-back tasks), and S4 (caffeine and metabolites). The statistical analyses testing differences from baseline are illustrated in Supplementary Tables S5-S8.

3. Results

The baseline assessments of self-rated sleepiness did not differ between the two groups. By contrast, cognitive performance was not uniformly distributed across all subjects. To avoid overestimating the effects of sleep restriction and coffee intake, individual performance measures were linearly centered to the mean baseline value of all participants (referred to as "normalized to baseline"). Moreover, deviations across subjects, were modeled during statistics as random effect. The behavioral data were averaged on each study day and the deviations from the normalized baseline across the experimental protocol are illustrated.

3.1.1. Subjective sleepiness

Self-rated sleepiness scores increased after sleep restriction day 2 in both groups, irrespectively of whether participants received regular coffee or decaffeinated coffee ('day': $F_{6,672} = 17.50$, $p < 0.001$) (Supplementary Fig. S2; Supplementary Table S1). Although the mean KSS rating was slightly attenuated on sleep restriction day 1 after regular coffee administration and appeared to rise less steeply in the regular coffee group when compared to the decaffeinated coffee group ('day' x 'group' interaction: $F_{6,672} = 3.64$, $p = 0.001$), no significant difference between the groups was detected on any day of the experimental protocol. Interestingly, self-rated sleepiness remained elevated after the recovery night in the regular coffee group, but not in the decaffeinated coffee group (Fig. S2).

3.1.2. Psychomotor vigilance test

Speed, lapses and accuracy on the PVT deteriorated with increasing sleep restriction, yet the impairment was attenuated in the regular coffee group when compared to the decaffeinated coffee group ('day' x 'group' interactions: speed: $F_{6,672} = 7.72$; lapses: $F_{6,672} = 3.69$; accuracy: $F_{6,672} = 4.52$; $p_{all} < 0.001$) (Supplementary Table S1). When compared to baseline, the performance impairment in the decaffeinated coffee group started between sleep restriction day 1 (speed) and 3 (lapses and accuracy) and persisted until the day after the recovery night (Fig. 2). By contrast, in the group receiving regular coffee, PVT speed was faster than in baseline on sleep restriction days 1 and 2 but fell below baseline on sleep restriction days 4 and 5. The slowing in response speed persisted after recovery sleep. A moderate performance impairment operationalized as an increased number of lapses and reduced accuracy on the PVT was evident on sleep restriction day 5.

The differences between the two groups were not uniform throughout the protocol. Mean speed was faster in the regular coffee group than in the decaffeinated coffee group on day 1 through 3 of chronic sleep restriction, while we observed no differences in PVT speed on sleep restriction days 4 and 5. By contrast, on sleep restriction days 3 and 4, the regular coffee group produced less attentional lapses and performed more accurately than the decaffeinated coffee group. More specifically, simultaneously correcting for errors of commission and errors of omission, accuracy remained stable in both groups on sleep restriction days 1 and 2. Afterwards, it steeply decreased with accumulating sleep loss in the decaffeinated coffee group, whereas this decrease was delayed and attenuated in the regular coffee group. The benefit of regular coffee intake was no longer statistically significant on sleep restriction day 5 (Fig. 2; Supplementary Table S5).

To further characterize the performance differences between the groups, the fidelity of information processing in cognition was operationalized as LSNR, a recently proposed novel measure of PVT performance (Chavali et al., 2017). The LSNR was increased in the regular coffee group when compared to baseline on sleep restriction days 1 through 4 and higher than in the decaffeinated coffee group on restriction days 1 through 3 ('day' x 'group' interactions: $F_{6,672} = 9.54$, $p < 0.001$; (Supplementary Table S5). Cognitive information processing fidelity slightly increased in both groups from baseline to sleep restriction day 1, was highest in the regular coffee group on day 2, and decreased in both groups thereafter ('day': $F_{6,672} = 7.99$, $p < 0.001$).

All group differences on the PVT showed large effect sizes ($d_{all} > 0.8$). No differences in any variable were present following the recovery night (Fig. 2).

3.1.3. Visual search task

Mean response speed on the visual search task was dependent on the set size of visual distractors (not shown) and the presence of the visual target. Both, when the target was present and when it was absent, response speed remained stable throughout the experiment in the regular coffee group, while response speed in the decaffeinated coffee group was slower than in baseline on virtually all days of sleep restriction and after the recovery night (Fig. 3). The regular coffee group performed moderately faster than the decaffeinated coffee group on sleep restriction day 5 when the target was present ('day' x 'group' interaction: $F_{6,2759} = 3.08$, $p = 0.005$, 'group': $F_{1,26} = 3.28$, $p = 0.082$) and on days 4 and 5 of sleep restriction when the target was absent ('day' x 'group' interaction: $F_{6,2759} = 4.83$, $p < 0.001$, 'group': $F_{1,26} = 4.29$, $p = 0.049$) (Supplementary Tables S2 & S6). Interestingly, the ability to correctly recognize the target among the distractors was better than in baseline in the regular coffee group on restriction days 3 and 4 and impaired in the decaffeinated coffee group on restriction days 2 through 5. Thus, the regular coffee group performed more accurately throughout extended sleep restriction ('group': $F_{1,26} = 8.11$, $p = 0.008$; 'day' x

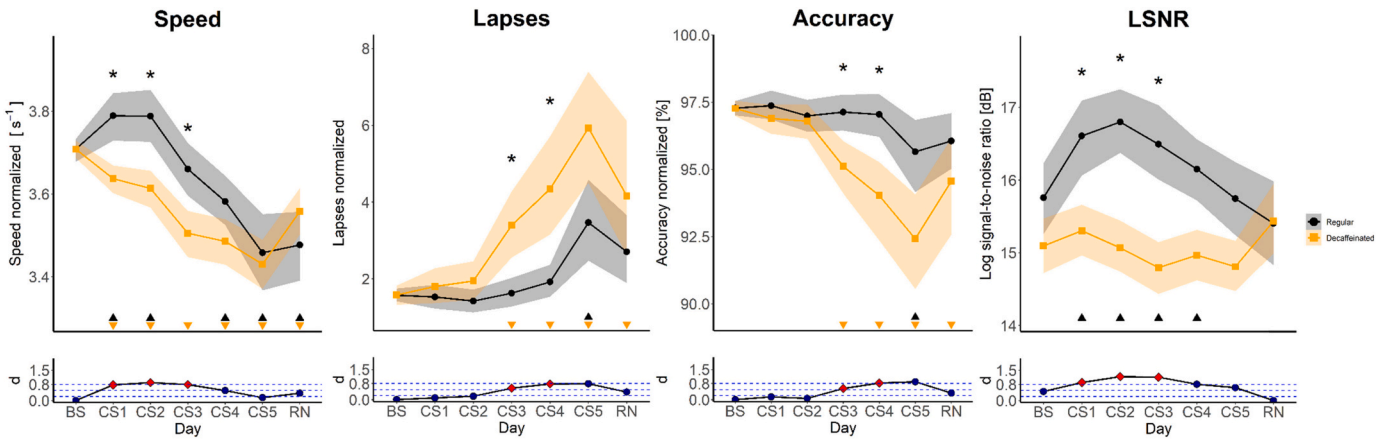

Fig. 2. Evolution of vigilance across the experimental protocol. On each study day, performance on four psychomotor vigilance test (PVT) sessions spread over the entire day were averaged. Lines and shaded areas represent means ±95% confidence intervals in the groups receiving either regular (black; n = 12) or decaffeinated coffee (orange; n = 14). Black (regular coffee group) and orange triangles (decaffeinated coffee group) above the x-axis represent Benjamini-Hochberg corrected differences ($p_{corr} < 0.05$) from baseline (Supplementary Table S5). Stars indicate significant differences between the groups: *p < 0.05. The corresponding effect sizes (Cohen's d) are represented underneath. Dashed blue lines separate regions of "small", "medium" or "large" effect size. Red dots indicate a significant group difference (p < 0.05). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

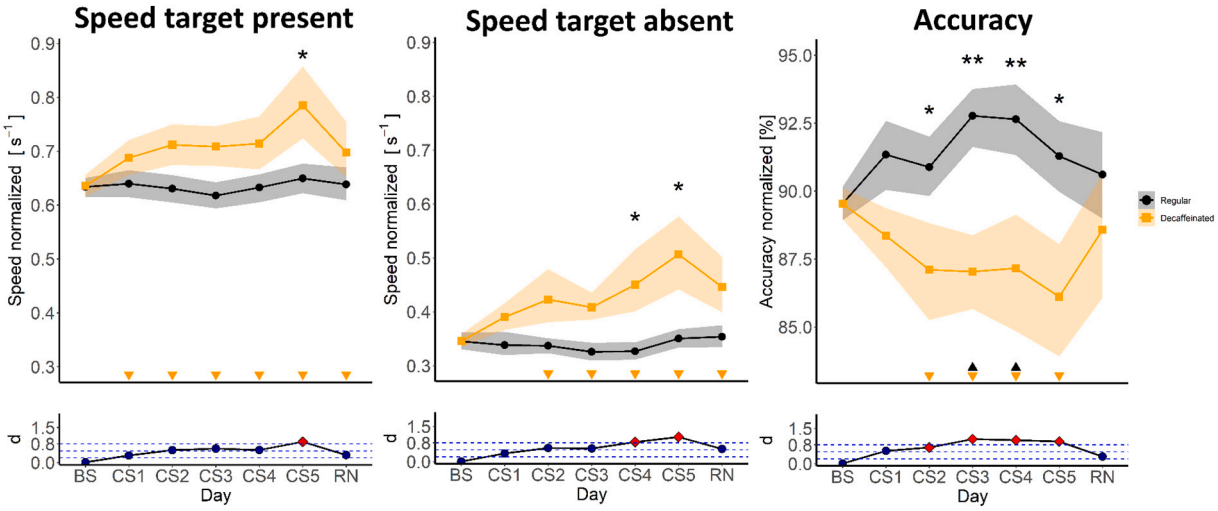

Fig. 3. Evolution of orienting/selection across the experimental protocol. On each study day, performance on four visual search task (VST) sessions spread over the entire day were averaged. Lines and shaded areas represent means ±95% confidence intervals in groups receiving either regular (black; n = 12) or decaffeinated coffee (orange; n = 14). Accuracy quantified the numbers of correct hits and correct rejections over total responses. Black (regular coffee group) and orange triangles (decaffeinated coffee group) above the x-axis represent Benjamini-Hochberg corrected differences ($p_{corr} < 0.05$) from baseline (Supplementary Table S6). Stars indicate significant differences between the groups: **p < 0.005, *p < 0.05. The corresponding effect sizes (Cohen's d) are represented underneath. Dashed blue lines separate regions of "small", "medium" or "large" effect size. Red dots indicate a significant group difference (p < 0.05). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

'group' interaction: $F_{6,672} = 4.35$, $p < 0.001$). All group differences were of medium to large effect size and no longer present after the recovery night.

3.1.4. *Visuo-spatial n-back task*

Response speed and accuracy on the visuo-spatial n-back task depended on cognitive workload (speed: $F_{2,2062} = 17.96$; accuracy: $F_{2,2062} = 465.51$; $p_{all} < 0.001$) and day of the protocol (speed: $F_{6,2062} = 8.16$; accuracy: $F_{6,2062} = 16.23$; $p_{all} < 0.001$). Performance speed was faster than in baseline on many experimental days in the regular coffee group while it fell below the baseline level in the 1-back task on sleep restriction days 4 and 5 in the group receiving decaffeinated coffee (Fig. 4). Accuracy also deteriorated across sleep restriction on all cognitive workloads in the latter group whereas accuracy remained virtually unchanged in the former. Thus, the regular coffee group performed faster (except on the 3-back task) and/or more accurately than the decaffeinated coffee group on the majority of days during sleep restriction ('day' x 'group' interaction: speed: $F_{6,2062} = 9.52$; accuracy: $F_{6,2062} = 5.13$; $p_{all} < 0.001$) (Supplementary Tables S3 & S7). The differences between the groups generally were of medium to large effect size and no longer present after the recovery night.

3.1.5. *Letter n-back task*

The visuo-spatial n-back task alike, response speed and accuracy on the letter n-back task depended on cognitive workload (speed: $F_{2,2065} = 191.32$; accuracy: $F_{2,2065} = 285.36$; $p_{all} < 0.001$) and day of the protocol (speed: $F_{6,2065} = 6.43$; accuracy: $F_{6,2065} = 9.82$; $p_{all} < 0.001$). The effects of sleep restriction and coffee administration were similar to those on the visuo-spatial n-back task, except that speed also on the 3-back version of the task was enhanced on sleep restriction days 1 through 3

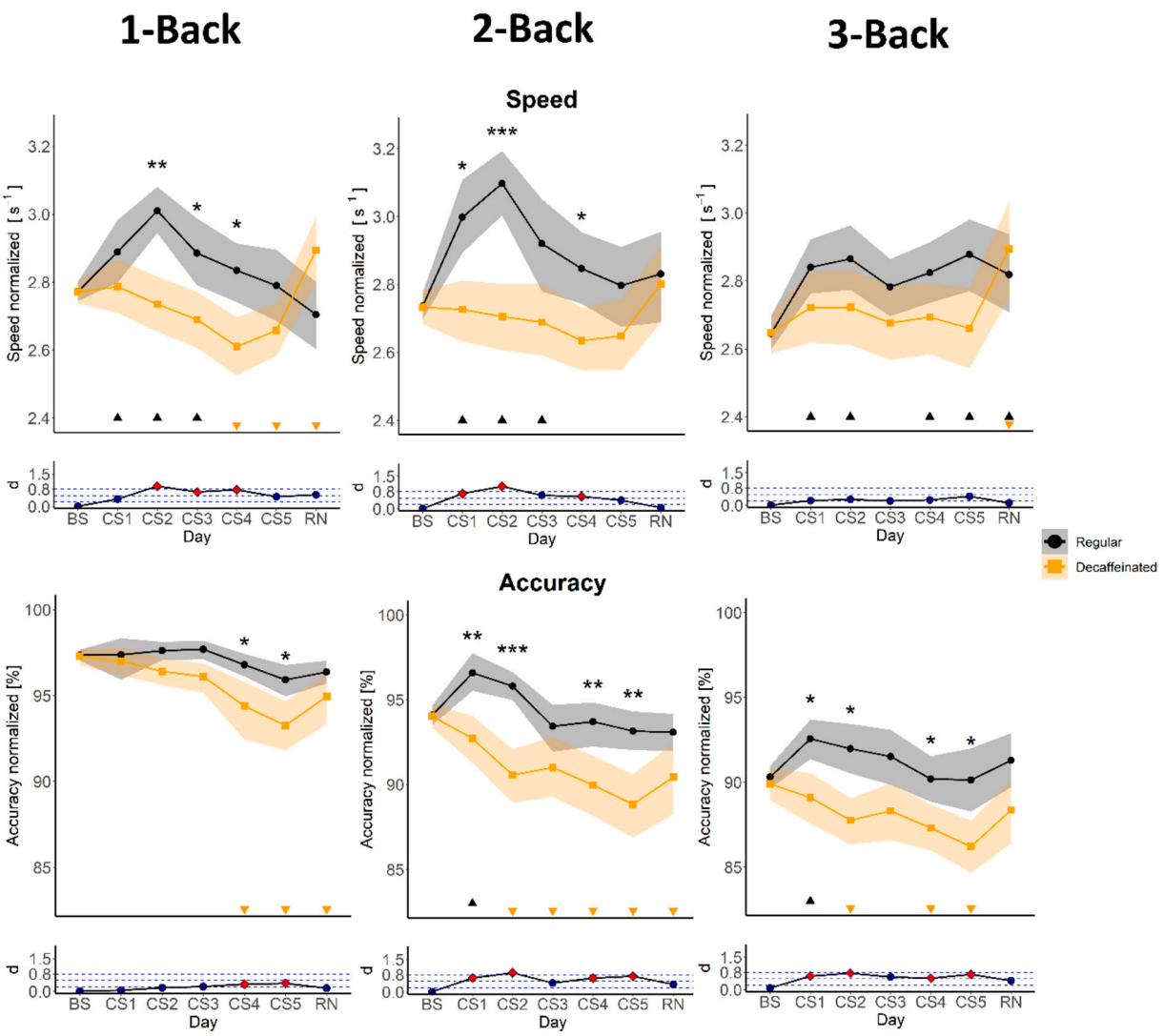

Fig. 4. Evolution of visuo-spatial working memory and executive control across the experimental protocol. On each study day, performance on four n-back task sessions spread over the entire day were averaged. Lines and shaded areas represent means ±95% confidence intervals in groups receiving either regular (black; n = 12) or decaffeinated coffee (orange; n = 14). Normalized mean speed and accuracy, calculated via sum of hits and correct rejections over total responses are shown for each step of cognitive load. Black (regular coffee group) and orange triangles (decaffeinated coffee group) above the x-axis represent Benjamini-Hochberg corrected differences ($p_{corr} < 0.05$) from baseline (Supplementary Table S7). Stars indicate significant differences between the groups: ***$p < 0.005$, **$p < 0.01$, *$p < 0.05$. The corresponding effect sizes (Cohen's d) are represented underneath. Dashed blue lines separate regions of "small", "medium" or "large" effect size. Red dots indicate a significant group difference ($p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

in the regular coffee group when compared to the decaffeinated coffee group (Fig. 5). The evolution of performance differed between the two groups ('day' x 'group' interaction: speed: $F_{6,2065} = 8.11$; accuracy: $F_{6,2062} = 4.23$; $p_{all} < 0.001$), such that the regular coffee group performed faster and/or more accurately than the decaffeinated coffee group on all 3 workload levels on many of sleep restriction days 1 through 4 (Supplementary Table S8). The differences between the groups generally were of large effect size, yet absent on sleep restriction day 5 and after the recovery night.

3.1.6. *Caffeine and metabolites*

Confirming that the study participants adhered to the instruction to abstain from all sources of caffeine prior to the experiment, caffeine and caffeine metabolites were undetectable at bedtime in both groups at baseline. Among the group receiving regular coffee, the mean caffeine levels increased until sleep restriction day 4, reaching a maximum of roughly 6 μmol/l (Fig. 6). Afterwards, the concentration decreased because coffee was served only in the morning on sleep restriction day 5 and no coffee was administered after the recovery night. A similar time course, albeit less variable, was seen for the three primary caffeine metabolites, paraxanthine, theobromine, and theophylline. Very low concentrations of caffeine and metabolites were detected in saliva of the control group who received coffee '833', confirming that this batch contained only negligible amounts of caffeine ('day' x 'group' interaction: $F_{6,112} \geq 10.01$, $p_{all} < 0.001$; Supplementary Table S4).

4. Discussion

We addressed the question whether the prevalent habit of drinking morning and midday coffee and thus ingesting roughly 300 mg caffeine per day ensures optimal attention during chronic sleep restriction. Such a coffee intake pattern is common in Europe and South America. We found that regular coffee effectively attenuated the repercussions of five nights of time-in-bed restricted to 5 h on all three attentional domains (vigilance, orienting, executive control) when compared to decaffeinated coffee. Our data suggest that 'real world' coffee consumption is able

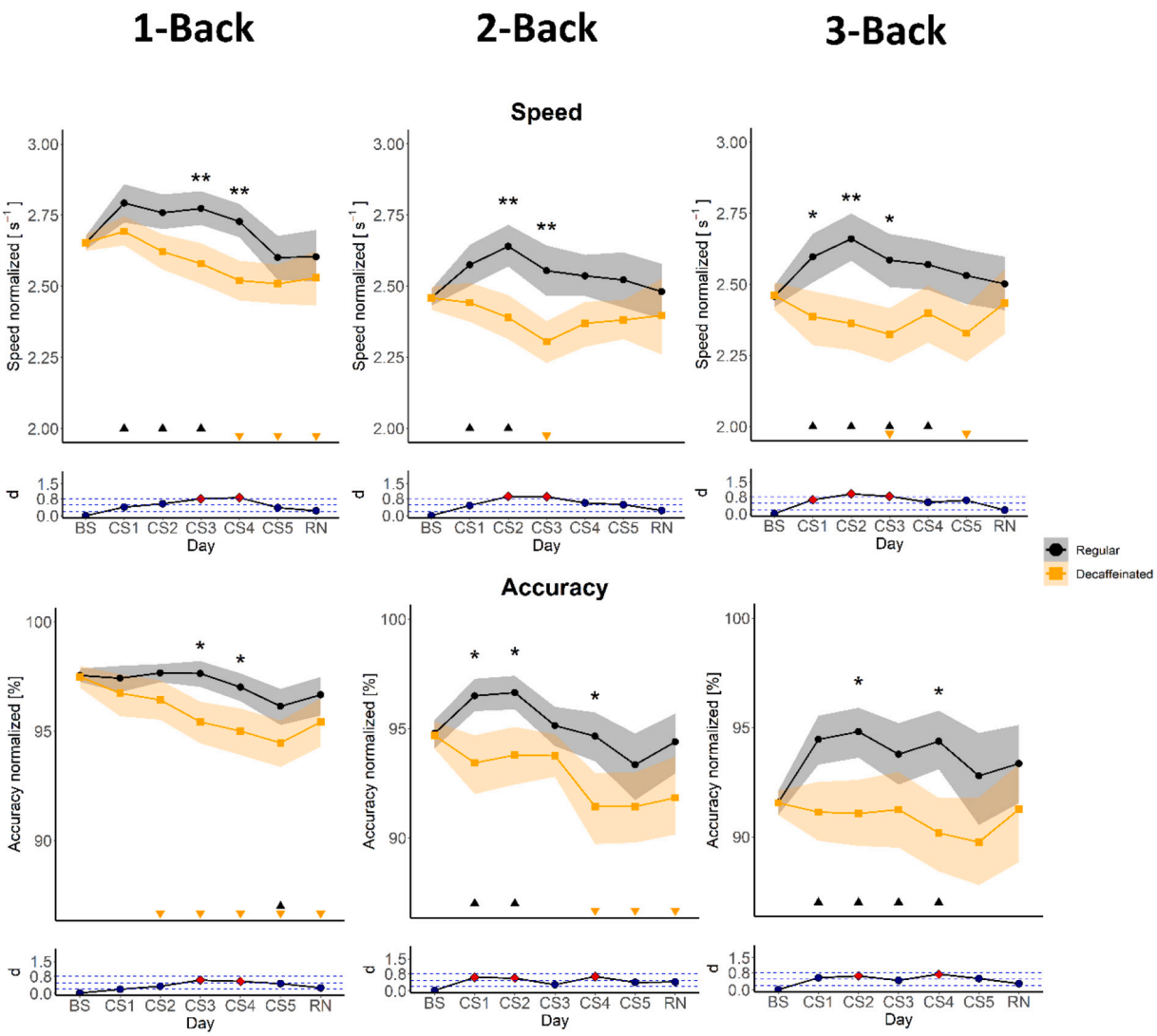

Fig. 5. Evolution of verbal working memory and executive control across the experimental protocol. On each study day, performance on four n-back task sessions spread over the entire day were averaged. Lines and shaded areas represent means ±95% confidence intervals in groups receiving either regular (black; n = 12) or decaffeinated coffee (orange; n = 14). Normalized mean speed and accuracy, calculated via sum of hits and correct rejections over total responses are shown for each step of cognitive load. Black (regular coffee group) and orange triangles (decaffeinated coffee group) above the x-axis represent Benjamini-Hochberg corrected differences ($p_{corr} < 0.05$) from baseline (Supplementary Table S7). Stars indicate significant differences between the groups: **p < 0.01, *p < 0.05. The corresponding effect sizes (Cohen's d) are represented underneath. Dashed blue lines separate regions of "small", "medium" or "large" effect size. Red dots indicate a significant group difference (p < 0.05). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to counteract – at least in part - attentional performance impairment in genetically caffeine sensitive adults due to repeated sleep restriction, which is common in many societies.

Our findings partly contrast with recent work suggesting that 2 × 200 mg caffeine per day loses efficacy to counteract the repercussions of sleep restriction on vigilance (Doty et al., 2017). In both studies, the evolution of subjective sleepiness, PVT speed, and PVT lapses across sleep restriction in the groups receiving placebo and decaffeinated coffee was highly similar. In addition, the slowing of mean PVT response speed in the active treatment groups was consistently attenuated in both experiments during the initial three restriction days. Afterwards, however, the findings diverged and in the prior report, tolerance to caffeine developed. In fact, the number of PVT lapses in the caffeine group was even higher than in the placebo group on sleep restriction days 4 and 5 (Doty et al., 2017). We observed no such tolerance and further performance impairment in the present experiment. By contrast, the number of lapses in the regular coffee group virtually remained close to the baseline level up until sleep restriction day 5 when a slight increase was observed. Together with more in-depth analyses of accuracy and cognitive information processing fidelity underlying PVT performance (Chavali et al., 2017), the data confirmed that regular coffee indeed provided a benefit over decaffeinated coffee and preserved task performance for 3–4 days of sleep restriction (Fig. 2).

An important difference between the two studies is the prospective genotyping and selective enrolment of participants based on polymorphism rs5751875 of *ADORA2A* in the present experiment. The A_{2A} receptor constitutes the primary target structure for caffeine effects on vigilance, attention, and sleep-wake regulation (Huang et al., 2005; Rétey et al., 2007; Bodenmann et al., 2012; Rupp et al., 2013; Renda et al., 2015). According to a new paradigm in the design of human studies to test the effects of caffeine and A_{2A} receptor antagonists on sleep-wake processes and cognitive functions with decreased variability (Holst et al., 2016; Satterfield et al., 2019; Chen and Cunha, 2020), only homozygous C-allele carriers of *ADORA2A* here were examined. Supporting the notion that these individuals are sensitive to the effects of caffeine on vigilance and executive control in rested and sleep deprived

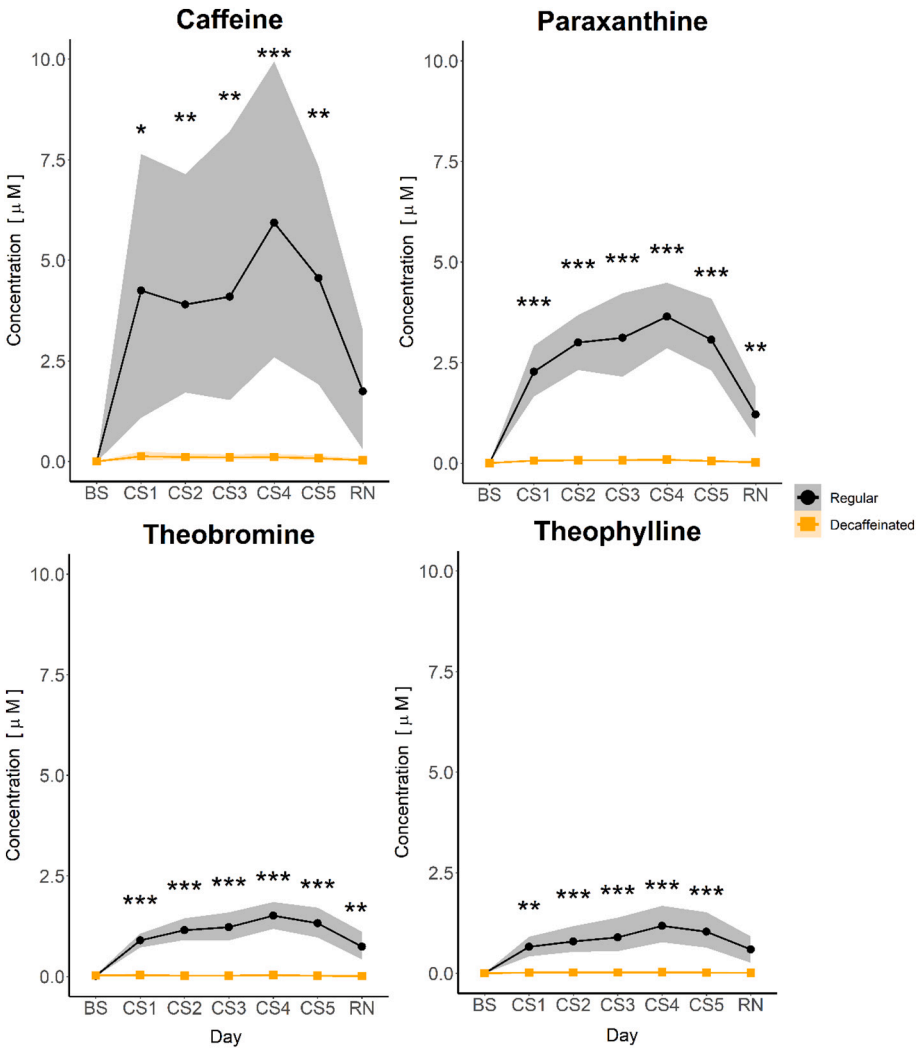

Fig. 6. Evolution of caffeine and caffeine metabolite (paraxanthine, theobromine, and theophylline) concentrations in saliva at bedtime across the experimental protocol. The last portion of coffee was administered in the morning after CS5. The labels BS through RN refer to bedtimes of the respective sleep episodes, whereas in Figure 2, 3, 4 and 5 the labels refer to the wake periods after the respective sleep episodes. Lines and shaded areas represent means ±95% confidence intervals in groups receiving either regular (black; n = 10) or decaffeinated coffee (orange; n = 11). Stars indicate significant differences between the groups: ***p < 0.005; **p < 0.01, *p < 0.05. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

state (Rétey et al., 2007; Renda et al., 2015), clear benefits of coffee on objective measures of attentional performance were found. By contrast, recent findings suggest that C-allele carriers of this polymorphism exhibit reduced interoceptive accuracy when compared to T-allele homozygotes (Geiger et al., 2016). It may be speculated that the reduced processing of interoceptive information in C-allele homozygotes underlies the lack of coffee effects on subjective sleepiness during repeated sleep restriction.

The orienting network may be relatively resilient against the detrimental effects of repeated sleep loss (Cunningham et al., 2018). Indeed, response speed on the visual search task was only moderately slowed in the decaffeinated coffee group and remained unaffected by sleep restriction in the regular coffee group. Furthermore, when performance accuracy was analyzed, we found a consistent benefit of regular coffee over decaffeinated coffee (Fig. 3). This finding adds supporting evidence to the notion that caffeine can normalize attention when stressors dampen performance and that this benefit can persist for multiple days.

In accordance with previous findings (Lo et al., 2012), both cognitive workload as well as sleep restriction reduced speed and accuracy on visuo-spatial and verbal n-back tasks. In general, regular coffee intake improved performance with medium to high effect size when compared to decaffeinated coffee. Similar to the effect on the alerting network, improved response speed on the visuo-spatial n-back task was restricted to the initial 3–4 sleep restriction days. Speed on the task with the highest cognitive workload (3-back) was the only metric probing executive control that was not improved by coffee. By contrast, task accuracy on all workload levels was boosted by coffee until the final day of sleep restriction (Fig. 4). The results on the letter n-back task basically corroborated these observations, yet the beneficial effects of coffee only lasted to the fourth sleep restriction day (Fig. 5). Together, the findings confirm the conclusions of previous work in rested individuals showing that caffeine increases executive control functions of the brain (Brunyé et al., 2010; Einöther and Giesbrecht, 2013). Functional imaging studies showed that caffeine up-regulates prefrontal brain areas in concert with anterior cingulate cortex that provide the executive control of visual attention (Koppelstaetter et al., 2008). Some evidence in mice suggests that caffeine-targeted A_{2A} receptors control information flow in prefronto-cortical circuits by synergizing with dopamine D_2 receptors (Real et al., 2018). Caffeine-induced increased dopaminergic neurotransmission in prefrontal cortex may thus support the executive functioning of the brain.

We intended to investigate the effects of habitual coffee intake which in dose and timing is similar to typical human behavior, as a countermeasure to impaired attention caused by prevailing sleep restriction. To standardize the experimental conditions and to avoid withdrawal reactions in the decaffeinated group, all study participants abstained from caffeine intake for at least 10 days prior to the first sleep restriction day. However, in the "real world", most people consume caffeinated beverages every day, regardless of prior partial sleep loss. Although still a matter of discussion, findings in rats and mice indicate that chronic caffeine intake increases the number of adenosine receptors and their sensitivity both in vitro as well as in vivo, and caffeine withdrawal

decreases locomotor activity in rats for several days (reviewed by Nehlig et al., 1992). In humans, the regulation of cerebral adenosine receptors by chronic caffeine and changes in attentional performance over multiple days after caffeine abstinence in habitual moderate consumers have not been explored. It is intriguing to note that in the present study, regular coffee improved several attentional measures, particularly speed on the PVT and the n-back tasks, during the first two to three sleep restriction days when compared to baseline. This observation could indicate that due to a highly sensitive adenosine system, the re-introduction of caffeine not only attenuated the impairment by sleep restriction, but initially improved baseline performance despite increasing sleep debt. Future research is warranted to study the possible underpinnings of this unexpected observation.

The caffeine and metabolite levels in saliva confirmed that the decaffeinated coffee contained negligible amounts of caffeine. The concentrations of the caffeine metabolites in the regular coffee group reflect the different ratios at which they are formed in the liver (Camandola et al., 2019). Among many other phytochemicals, coffee contains theobromine and theophylline, whereas paraxanthine is not present in plant extracts (Camandola et al., 2019). The psychostimulant effects of coffee are commonly attributed to caffeine, which acutely affects neuronal network activity and promotes alertness and attention over a wide dose range (McLellan et al., 2016). In humans, caffeine is metabolized in the liver through the cytochrome P450 isoenzyme, CYP1A2, which accounts for almost all primary metabolism of caffeine. The CYP1A2 enzymatic activity exhibits pronounced inter-individual variation, likely underlying the large variation in individual saliva caffeine concentrations (Fig. 6). Paraxanthine is formed by demethylation of caffeine. Roughly 10 h after coffee intake, the paraxanthine concentration reaches levels comparable to or even higher than those of caffeine (Urry et al., 2016; Camandola et al., 2019). The circulating concentrations of both these methylxanthines were sufficient to block adenosine receptors (Müller and Jacobson, 2011). Both chemicals should be considered when interpreting the beneficial effects of coffee consumption against the detrimental consequences of repeated sleep restriction on vigilance and attention. On the other hand, caffeine easily crosses the blood-brain barrier, whereas much less paraxanthine enters the brain (Camandola et al., 2019). Because saliva was only sampled at bedtime, whereas sleepiness and cognitive performance were tested throughout the day, it is not possible to reliably estimate the respective contributions of sleep restriction, caffeine, and caffeine metabolites on the observed time courses of individual behavioral changes across the sleep restriction. Future studies for that purpose are warranted.

In conclusion, we found that daily 300 mg caffeine intake in coffee effectively reduced impairments of vigilance and attention across five days/nights of sleep restriction in genetically caffeine sensitive individuals. The C/C genotype of *ADORA2A* is present in roughly 35% of individuals in European populations. The selective enrolment of this genotype thus limits the generalizability of the present results. Nevertheless, the findings support the conclusion that dietary inhibition of adenosine A_{2A} receptors can persistently benefit vigilance and attention during repeated sleep restriction. The coffee-induced blockade of these receptors may potentiate cholinergic and monoaminergic neurotransmission in thalamus, anterior cingulate and other cortical regions that regulate vigilance, alerting and executive control (Fan et al., 2005). In ongoing analyses of our data that will be published elsewhere, we examine whether the repeated coffee consumption also affects the consequences of sleep restriction on reversal learning decision making (Whitney et al., 2015) and risk taking (Maric et al., 2017). In addition, we investigate whether this highly prevalent behavior attenuates or accelerates the evolution of waking and sleep electroencephalographic markers of sleep need across sleep curtailment and recovery sleep (Landolt et al., 2004), as well as possible changes in the availability of cerebral adenosine A_1 receptors (Elmenhorst et al., 2018).

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Ethical statement

All study procedures were approved by the ethics committee of North Rhine ("Ärztekammer Nordrhein"), the German Federal Office for Radiation Protection ("Deutsches Bundesamt für Strahlenschutz") and carried out in accordance with the Declaration of Helsinki. All participants gave written informed consent before participating in the study.

Declaration of Competing Interest

The work was made possible by the Institute for Scientific Information on Coffee (ISIC), a not-for-profit organization devoted to the study and disclosure of science related to coffee and health.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2020.110232.

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