



## Wide awake at bedtime? Effects of caffeine on sleep and circadian timing in male adolescents – A randomized crossover trial

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### ABSTRACT

Adolescents often suffer from short and mistimed sleep. To counteract the resulting daytime sleepiness they frequently consume caffeine. However, caffeine intake may exaggerate sleep problems by disturbing sleep and circadian timing. In a 28-hour double-blind randomized crossover study, we investigated to what extent caffeine disturbs slow-wave sleep (SWS) and delays circadian timing in teenagers. Following a 6-day ambulatory phase of caffeine abstinence and fixed sleep-wake cycles, 18 male teenagers (14–17 years old) ingested 80 mg caffeine vs. placebo in the laboratory four hours prior to an electro-encephalographically (EEG) recorded nighttime sleep episode. Data were analyzed using both frequentist and Bayesian statistics. The analyses suggest that subjective sleepiness is reduced after caffeine compared to placebo. However, we did not observe a strong caffeine-induced reduction in subjective sleep quality or SWS, but rather a high inter-individual variability in caffeine-induced SWS changes. Exploratory analyses suggest that particularly those individuals with a higher level of SWS during placebo reduced SWS in response to caffeine. Regarding salivary melatonin onsets, caffeine-induced delays were not evident at group level, and only observed in participants exposed to a higher caffeine dose relative to individual bodyweight (i.e., a dose > 1.3 mg/kg). Together, the results suggest that 80 mg caffeine are sufficient to induce alertness at a subjective level. However, particularly teenagers with a strong need for deep sleep might pay for these subjective benefits by a loss of SWS during the night. Thus, caffeine-induced sleep-disruptions might change along with the maturation of sleep need.

### 1. Introduction

Around 80% of teenagers consume caffeine [1–3], a psychoactive stimulant which is present in a variety of foods, over-the-counter beverages, and medications [4]. The amounts of daily intake strongly vary from 40 mg reported by adolescents in the UK up to 350 mg in Austria [5–9]. However, due to a lack of empirical studies [10,11], the consequences of caffeine-intake on the developing neuronal and cardiovascular system are rather unclear. Accordingly, evidence-based limits for safe caffeine consumption in children and adolescents are missing up to

date [10,11], and the American Academy of Pediatrics strongly recommends to eliminate the stimulant from the daily diet in this age group [12]. At the same time caffeine-containing beverages, in particular so-called energy-drinks, are aggressively marketed towards teenagers and young adults [13], often promising a boost in physical and mental capacities [13,14]. These claims indeed reach their target population [15], as mirrored in the teenagers' motivation to consume caffeine-containing drinks in order to enhance performance and reduce sleepiness [1,16].

As conceptualized in the 'perfect storm model' [17,18], high daytime sleepiness of teenagers can arise from a conflict between social

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constraints and developmental changes in both sleep-homeostatic and circadian components of sleep-wake regulation [19]. While school times often require early rise times, the teenagers' sleep-wake regulation supports bedtimes late at night, as the increase of homeostatic sleep need slows down [20] and the circadian timing system becomes phase-delayed [21,22] as compared to childhood. Hence, it is not surprising that in the US around 75% of teenagers do not get the required 9 h of sleep [23], and around 40% of teenagers suffer from excessive daytime sleepiness [23–25].

At first glance caffeine intake appears to be well-suited to counteract sleepiness. As an adenosine receptor antagonist in the central nervous system, it can disturb the homeostatic regulation of sleep need [26]. In adults, the acute intake of the stimulant has repeatedly been shown to reduce duration and intensity of deep sleep [27] and subjective sleepiness [28]. Moreover, recent evidence in adults suggests that caffeine consumption particularly in the evening suppresses melatonin secretion [29,30] and delays the circadian onset of the biological night [31]. In teenagers, caffeine intake might thus exaggerate developmental changes in both homeostatic and circadian components of sleep-wake regulation. Indeed, caffeine consumption in teenagers has been associated with a higher sleepiness [32,33]. This could potentially be linked to caffeine-induced disturbances in sleep-wake regulation, as caffeine-consuming teenagers also report a shorter sleep duration [23,34–37], less restorative sleep [34,38], and show a reduced depth of sleep [39] in cross-sectional studies. However, such study designs are not optimally suited to identify cause-effect-relationships and experimental laboratory studies are missing so far. Thus, we examined the consequences of caffeine intake in adolescents using a placebo-controlled double-blind crossover design. We hypothesized that one-time caffeine intake reduces the amount of deep sleep (i.e., slow-wave sleep, SWS) and delays the circadian melatonin onset in teenagers. A secondary objective was to test the assumption that caffeine acutely reduces subjective sleepiness and sleep quality.

## 2. Materials and methods

### 2.1. Sample size

The available evidence in adults indicated that caffeine intake in the evening elicits large effects on both SWS duration (assumed  $d = 1.14$  on the basis of [40]; and  $d = 1.38$  on the basis of [41]) and circadian phase ( $d = 0.93$ , [31]). Thus, accepting  $\alpha = 0.05$  and  $\beta = 0.20$ , a sample size of  $N = 18$  could be considered to be sufficient to detect significant caffeine-induced effects of this size when assuming calculation of a  $t$ -test for dependent samples (analyses done with G\*Power 3.1.9.2., [42]). Completion of data collection of the 18th full data set defined the end of data collection for the study.

### 2.2. Subjects

Participants were recruited between February 2018 and December 2018 via online platforms at the University of Basel, posts at sports clubs and presentations at schools in Basel, Switzerland, and the surrounding area. Overall, 68 teenagers completed a telephone interview (with CFR or SV), to check inclusion criteria (14–17 years of age, sex: male, BMI: 16.2–25.4, right handedness (due to MRI assessments, which are not subject of the present manuscript), sleep duration during school days: 6–10 h, moderate chronotype [according to the Munich Chronotype Questionnaire [43] [MSF-Sc]: 2–6.99). The latter two criteria were set to avoid abnormal sleep pressure levels and extreme timing of circadian phase during the laboratory part of the study. Individuals were only included if habitual caffeine intake ranged between 80 and 300 mg per week (assessed by [44]) in order to avoid inclusion of naïve and/or highly sensitive individuals. The upper limit of 300 mg/week was set to keep potential withdrawal symptoms at low levels during the week of abstinence preceding the laboratory part. Interested teenagers were

asked to fill in questionnaires in order to quantify pubertal stage (Pubertal Development Scale (PDS) [45]), to exclude chronic diseases (such as diseases of the respiratory or coronary system), and to check on sleep behavior (Pittsburgh Sleep Quality Index [46]). In addition, the physician in charge (CG, MM, or HS) conducted the neuropsychiatric interview MINI KID [47] in order to exclude participants suffering from psychiatric disorders. In a last step before start of study, participants were invited for an adaption night in the sleep laboratory, to allow habituation to the sleep rooms and electro-encephalographic (EEG) instruments, to explain the study procedures, and to conduct a urinary drug screening (Drug-Screen-Multi 6, nal von minden GmbH, Germany) in order to exclude recent drug use.

Overall, of 68 individuals that had been contacted by phone, 15 met one of the exclusion criteria and 28 could not be contacted anymore or lost interest in participation during the recruitment procedure. From the remaining 25 individuals, one had to be excluded due to medical reasons, five dropped out after the habituation night and one dropped out during the first laboratory condition. The final sample consisted of  $N = 18$  caucasian participants, completing both conditions. Demographics of this sample are summarized in Table 1.

### 2.3. Study protocol

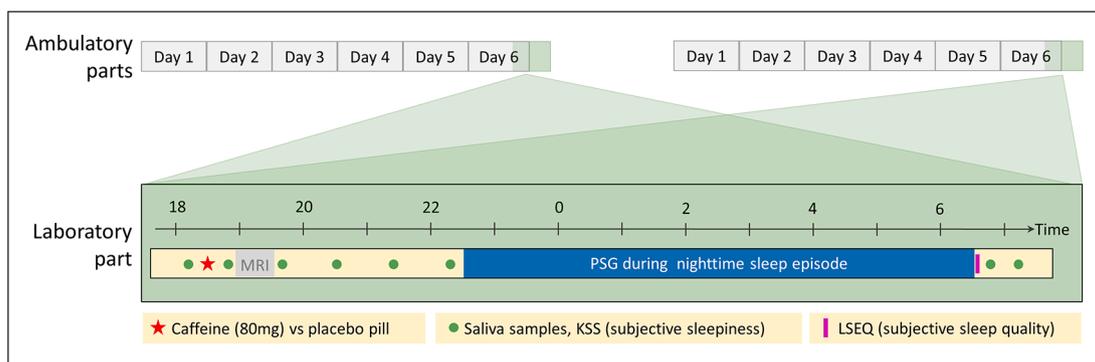
The procedures were conducted in accordance with the ethical standards of the responsible regional ethical committee (Ethikkommission Nordwest- und Zentralschweiz) and in accordance with the Helsinki Declaration of 1975 as revised in 1983. Every participant and one legal representative of each participant signed informed consents. Participants received a compensation for participation (vouchers). Data collection took place between March 2018 and December 2018.

Every participant took part in two conditions (caffeine and placebo). The within-subject design was chosen to reduce non-treatment-related variance between conditions. Conditions were separated on average by 8.5 days (range: 6–35 days, median: 7 days). Participants were randomly assigned by JW to the order of conditions (55% of the sample had placebo first), according to a simple computer-generated allocation schedule. Salivary caffeine and paraxanthine values before treatment did not significantly differ between participants who were assigned to the caffeine condition first and participants who were assigned to the placebo condition first ( $P > 0.5$ ). This indicates a sufficient washout period between conditions. As illustrated in Fig. 1, both caffeine and placebo conditions started with an ambulatory period of six days, to

**Table 1**

Demographic information. Means and standard deviations are given per variable, over the total sample and split by order of conditions. T-tests for independent samples indicated no significant difference in the variables listed between groups according to the order of condition (two-sided  $P_{all} > 0.118$ ). Self-reported habitual caffeine intake was assessed by a survey tool [44] and adapted according to [101]. Regular sleep duration and regular bedtime were extracted from the Munich Chronotype Questionnaire [43]. MCTQ MSF-Sc: Chronotype according to Munich Chronotype Questionnaire [43]; PDS: Pubertal Development Scale [45].

Variable (unit)	Caffeine-Placebo (n = 8)	Placebo-Caffeine (n = 10)	Total sample (n = 18)
Age (y)	16.03 ± 1.09	16.17 ± 1.14	16.11 ± 1.09
Regular sleep duration at schooldays (h:min)	7:48 ± 46 min	7:42 ± 32 min	7:45 ± 36 min
Regular bedtime during schooldays (hh:min)	22:42 ± 41 min	22:27 ± 33 min	22:32 ± 36 min
Bedtime during study (hh:min)	22:52 ± 39 min	22:24 ± 33 min	22:36 ± 37 min
BMI (kg/m <sup>2</sup> )	20.91 ± 2.70	20.71 ± 1.71	20.80 ± 2.13
Habitual caffeine intake (mg/wk)	195.81 ± 70.69	277.71 ± 146.51	241.31 ± 123.19
Chronotype (MCTQ MSF-Sc)	3.57 ± 0.76	3.99 ± 0.79	3.80 ± 0.78
Pubertal stage (PDS)	2.83 ± 0.38	2.71 ± 0.34	2.76 ± 0.35

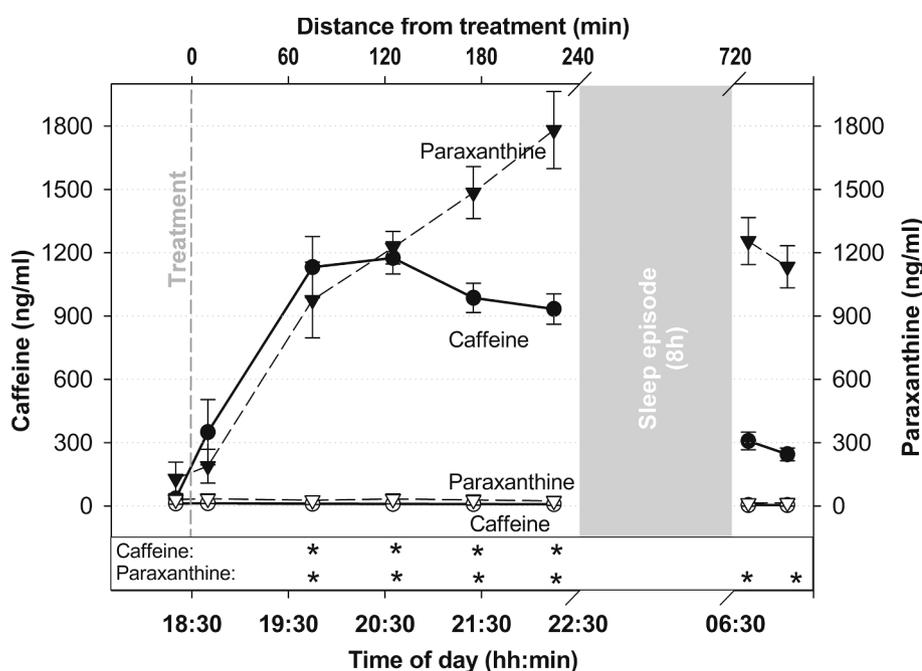


**Fig. 1.** Illustration of study protocol. In each of the two conditions (caffeine and placebo), the protocol started with an ambulatory part of 6 days during which participants were asked to abstain from caffeine and to adhere to a fixed sleep-wake cycle (8 h sleep per day, no naps allowed). Sleep-times were adjusted to the participant’s individual and social demands, and participants were allowed to deviate by ± 1 h from the times set. In the evening of day six of the ambulatory period, the laboratory part started 5 h prior to bedtime (i.e., on average at 17:36). Before and after treatment (caffeine vs. placebo), administered 4 h before habitual bedtime, we assessed subjective sleepiness (by the Karolinska Sleepiness Scale, KSS, [52]), conducted a magnetic resonance imaging (MRI) session, and collected saliva to determine caffeine, paraxanthine, and melatonin levels. Polysomnography (PSG) was conducted during the scheduled sleep episode. Subjective sleep quality was measured by the Leeds Sleep Evaluation Questionnaire (LSEQ, [57]) right after awakening.

control for sleep debt and allow circadian entrainment as well as washout from prior habitual caffeine intake. To do so, participants were asked to adhere to a fixed sleep-wake cycle (8 h sleep window, no naps allowed; allowed deviation from bed- and wake-time ± 60 min) and to abstain from all caffeine-containing foods and beverages. Bed- and wake-times for the ambulatory weeks were set individually according to the individual preferences and social constraints (i.e., school times), and compliance was checked by means of wrist actigraphy (Actiwatch®, Cambridge Neurotechnology Ltd., Cambridge, UK). Salivary caffeine levels and levels of the main caffeine metabolite paraxanthine (depicted in Fig. 2) were checked at outset of the laboratory parts.

Both laboratory parts started in the evening 5 h before habitual bedtime (bedtime *M*: 10:36 p.m., *SD*: 37 min) and did only vary with regard to the treatment (see Fig. 1). The caffeine or placebo capsules looked identically and were given 4 h before habitual bedtimes and contained either 80 mg of caffeine and mannitol or mannitol only (Hänseler AG, Herisau, Switzerland). A container with the respective capsule was prepared by JW for each condition and participant, so that

any person actively involved in recruitment and data collection was blind to the treatment during these study phases. The dose of 80 mg roughly equals not only the daily average intake of adolescents in the US [3,5] but also the content per serving in a range of common energy drinks (e.g., ~0.25 l of Bomb Energy Drink®, Burn®, Hell Energy®, RedBull®, Rockstar® Energy Drink, etc.). We set the timing of administration to the evening hours, as evening intake in teenagers is not only common [5], but might also maximally impact on both sleep [40,48,49] and circadian rhythms [31,50]. Until habitual bedtime and also after nighttime sleep, we regularly assessed subjective sleepiness and took saliva samples to quantify melatonin levels. At 1,85 h before bedtime, participants received a standardized meal. Starting at habitual bedtime, participants were asked to sleep during an 8-hour sleep episode, to assess our primary endpoint (deep sleep) by polysomnography. Bedrooms were located at the Centre for Chronobiology, Basel, Switzerland, and controlled for ambient temperature, light, and sound. After the 8-hour sleep episode, participants were woken up, completed the Leeds Sleep Evaluation Questionnaire (LSEQ, [51]) and two final assessments



**Fig. 2.** Course of salivary caffeine and paraxanthine levels per condition. Black symbols indicate the course of caffeine (circles) and paraxanthine (triangles) during the caffeine condition, while white symbols indicate the levels (caffeine: circles, paraxanthine: triangles) assessed during the placebo condition. Time-of-day values on the x-axis represent group means, as time of treatment was adjusted to the individual bedtime of each participant. Stars indicate significant differences between conditions per time of measurement for caffeine and paraxanthine, respectively ( $P < 0.05$ , adjusted for multiple comparisons).

of sleepiness and salivary melatonin. During the entire study, adverse or serious adverse events did not occur.

#### 2.4. Subjective sleepiness and sleep quality

During scheduled wakefulness, subjective sleepiness was assessed every 45 min with the Karolinska Sleepiness Scale (KSS, [52]), a scale frequently used in teenagers to measure the sleepiness response to sleep-wake manipulations such as total [53] or partial sleep deprivation [54–56]. Volunteers rated their sleepiness within the past 10 min using a 9 point verbally anchored scale from 1 (extremely alert) to 9 (extremely sleepy, fighting sleep). Due to technical problems, we lost one data set in the caffeine condition and replaced missing values by the mean KSS score per time of assessment of all participants over both conditions.

To assess subjective sleep quality, we used the LSEQ [51], administered in the morning right at wake-up. The evaluation of the questionnaire results in the four dimensions *Getting to Sleep* (covering sleep latency), *Quality of Sleep* (covering stability of sleep), *Awake Following Sleep* (covering easiness and duration of waking-up), and *Behavior Following Wakening* (covering alertness after waking-up) [57]. Due to technical problems, we lost the data of one participant in the caffeine condition and replaced missing values by the sample mean of each scale over both conditions.

Expectations might particularly influence subjective ratings of sleepiness and sleep. However, participants who correctly identified the caffeine condition at the end of the laboratory part, did not significantly differ on average in ratings of sleepiness or sleep quality ( $P_{all} > 0.14$ ) compared to other participants.

#### 2.5. Salivary caffeine and melatonin

We took saliva samples every 45 min during scheduled wakefulness under dim-light conditions. Caffeine levels as well as levels of the main caffeine metabolite paraxanthine were determined with liquid chromatography coupled to tandem mass spectrometry.

Melatonin levels were measured using a direct double-antibody radio immunoassay [58]. Dim-light melatonin onset (DLMO) was quantified using the hockey-stick method [59] with an ascending level of 2.5 pg/ml. We calculated the area under the curve (AUC) of melatonin levels in the evening (i.e., all measures assessed before the sleep episode) with respect to the ground (i.e., AUC<sub>G</sub>) using the trapezoid formula described in [60]. A missing value ( $n = 1$  in the caffeine condition) was replaced by the mean across groups and conditions.

#### 2.6. Sleep EEG

For recordings of sleep electroencephalogram (EEG), electrooculogram (EOG), and electromyogram (EMG), we used Live-Amp devices (Brain Products GmbH, Gilching, Germany) in combination with electrode caps (32Ch LiveAmp Cap with Multitrodes, EasyCap GmbH, Herrsching, Germany). Each of the two nights of every participant was recorded with the same device. We recorded signals with a sampling rate of 250 Hz, applying an online notch filter at 50 Hz, at derivations from the frontal, central, parietal, and occipital regions (F3, FZ, F4, C3, CZ, C4, P3, PZ, P4, O1, OZ, and O2) referenced against FCz. Offline the signals were re-referenced against the contra-lateral mastoid (A1 or A2) according to the guidelines of the American Academy of Sleep Medicine (AASM, <https://aasm.org/clinical-resources/scoring-manual/>) and downsampled to 128 Hz for automatic sleep staging by a validated [61] algorithm (Somnolyzer 24 × 7, The Siesta Group, Vienna, Austria). Scorings were visually controlled epoch-by-epoch by an expert scorer according to the AASM criteria [62]. All of the automatic scorings were classified as correct. This approach combines the reliability of standardized automatic scoring with the validity of human expert scoring and has successfully been applied in earlier studies on sleep of adolescents (e.g., [63,64]). In our analyses we focus on proportion of SWS

(percentage of SWS of total sleep time). We did not include analyses of non-rapid eye movement (NREM) sleep slow-wave activity (SWA) in the present report as these will be published elsewhere.

Due to technical problems, the recordings of two individuals in one night each (1 × placebo condition, 1 × caffeine condition) were incomplete. These data do not correspond to the same individuals in which we lost KSS and LSEQ data. The data were not included into the analyses but replaced by the means of each parameter across the group and the two conditions.

#### 2.7. Statistical analyses

We analyzed the course of salivary caffeine and salivary paraxanthine values between conditions as a manipulation check, using the procedure PROC MIXED (SAS 9.4 software, SAS Institute, Cary, USA). In the mixed model, we included subject as a random factor, condition, and time (with covariance structure AR(1)) as fixed factors. *P*-values were based on corrected degrees of freedom according to Kenward and Roger [65]. Post-hoc comparisons were calculated using the LSMEANS statement and were adjusted for multiple comparisons according to the Tukey-Kramer method.

For analyses of our primary and secondary outcomes, we used the software package SPSS (Version 25, IBM Corp., Armonk, USA). KSS scores were analyzed with an repeated measures ANOVA to assess both differences between conditions (caffeine vs. placebo) and condition-specific differences in the time course (interaction between factors condition and time) within one model. Baseline differences between conditions were taken into account by calculating the difference of each value assessed after treatment to the baseline (value before treatment) in the same condition. Degrees of freedom were adjusted according to Greenhouse-Geisser when appropriate. Confidence intervals of post-hoc comparisons were adjusted for multiple comparisons using the Dunn-Šidák correction. Confidence intervals of effect sizes (90%CI, according to [66]) were determined using the CI-R2-SPSS package [67].

Differences in subjective sleep quality, proportion of SWS (primary outcome), and DLMO were examined by t-tests for dependent measures. If not otherwise indicated, we report *P*-values based on one-sided testing as we tested directed hypotheses. For calculation of effect sizes (Cohen's  $d_{unbiased}$  according to [68]) and their precision (95%CI) we used ESCI [Exploratory Software for Confidence Intervals [68]].

To explore potential factors which might contribute to the high inter-individual variance in caffeine-induced responses in SWS need, we calculated Pearson correlations between the individual difference in SWS between conditions (i.e., SWS proportion placebo - SWS proportion caffeine) and variables which might contribute to variance in SWS proportion, that is pubertal stage (assessed by PDS [45]), self-reported habitual intake levels (assessed by [44]), relative caffeine dose ([69,70] quantified by mg/kg body weight), actual state of caffeine metabolism (assessed by the ratio paraxanthine/caffeine in saliva [71] taken 15 min before start of sleep episode in the caffeine condition), and an approximation of individual SWS need (i.e., SWS proportion during the placebo night). Thresholds for significance (two-sided testing) were adjusted for multiple testing according to Bonferroni. Based on these analyses (please see results for more details) we finally tested by an ANCOVA whether the effect of condition (caffeine vs. placebo) on SWS proportion interacts with a covariate (i.e., the difference in SWS proportion between conditions).

Similarly, regarding caffeine-induced differences in DLMO, we aimed at exploring reasons for our results diverging from our hypothesis and earlier studies in adults [29–31]. Thus, we calculated Pearson correlations between the individual difference in DLMO between conditions (i.e., DLMO in caffeine – DLMO in placebo condition) and those variables clearly diverging from earlier studies, that is age, habitual intake levels (assessed by [44]), relative given dose (quantified by mg/kg body weight), and distance of treatment to DLMO. Thresholds for significance (two-sided testing) were adjusted according to Bonferroni.

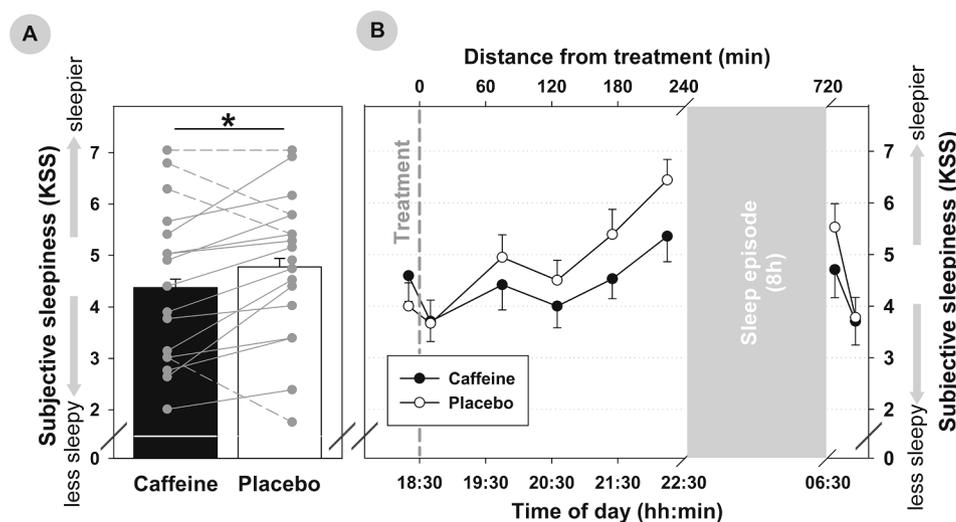
During the process of peer-review, all frequentist statistics of primary and secondary endpoints were complemented by Bayesian statistics. To conduct these statistics, we used the software JASP (version 0.13.1, JASP Team (2020)). More specifically, for analysis of KSS values, we used a Bayesian ANOVA for repeated measures including the factors subject, condition, and time. To account for potential baseline differences between conditions, we subtracted from each KSS value assessed after time of treatment the KSS value of this individual assessed before treatment. We compared the Bayes Factors ( $BF_{10}$ ) between different models: a null model including the factor subject only ( $M_0$ ), a model including the factors subject and time ( $M_t$ ), a model including the factors subject and condition ( $M_c$ ), a model including the factors subject, time, and condition ( $M_{c+t}$ ), and a model including the factors subject, time, condition, and the interaction of condition\*time ( $M_{c+t+ct}$ ). To evaluate the influence of caffeine on subjective sleep quality, sleep, and melatonin (DLMO and AUC), we calculated Bayesian  $t$ -tests for dependent samples. Accordingly, we used Bayesian Pearson correlations to complement our exploratory analyses mentioned above. Finally, using a Bayesian ANCOVA, we tested whether the association of caffeine-induced changes in SWS proportion (i.e., SWS proportion placebo - SWS proportion caffeine) and SWS proportion is specific for the placebo condition. We compared a null model (including condition and caffeine-induced changes in SWS proportion) with a model additionally including the interaction term of condition\*caffeine-induced changes in SWS proportion.

Please note that replacement of the missing values did not substantially change the results neither regarding frequentist nor Bayesian statistics.

### 3. Results

#### 3.1. Salivary caffeine

Fig. 2 shows the course of caffeine and paraxanthine levels over time in both conditions. Before treatment, caffeine levels in the caffeine condition did not differ from those in the placebo condition, but were significantly increased from 75 min after intake until bedtime compared to the placebo condition ( $F_{[7,135]} = 22.89, P < 0.001$ ). Similarly, paraxanthine levels (i.e., levels of the main metabolite of caffeine) did not differ between conditions before treatment, and were increased in the caffeine condition compared to the placebo condition starting from 75 min after intake until the end of the study (i.e., 12.7 h after intake ( $F_{[7,92.5]} = 17.17, P < 0.001$ )).



**Fig. 3.** Differences in subjective sleepiness between conditions. A) Subjective sleepiness (as assessed by the Karolinska Sleepiness Scale, KSS [52]) was on average lower (\*:  $P < 0.05$ ) in the caffeine (black bar, mean + standard error) compared to the placebo condition (white bar, mean + standard error). This caffeine-induced reduction of sleepiness was present in ~75% of individuals (solid lines). Other cases are indicated by dashed lines. B) The typical increase in subjective sleepiness towards bedtime is shown as group mean with standard error per time of assessment. The increase was not significantly modulated by condition (interaction time  $\times$  condition:  $P = 0.300$ ). As time of treatment was adjusted to the individual bedtime of each participant, time-of-day values on the x-axis represent group means.

#### 3.2. Subjective sleepiness and sleep quality

As illustrated by absolute KSS values in Fig. 3A, on average participants indicated lower sleepiness values in the caffeine compared to the placebo condition ( $F_{[1,17]} = 10.585, P = 0.005, \eta^2 = 0.384, 90\%CI: 0.085-0.577; M_{caff} \pm SE = -0.20 \pm 0.24, M_{plac} \pm SE = 0.88 \pm 0.32$ ). However, the increase of sleepiness towards bedtime ( $F_{[3.58,60.81]} = 9.538, P = 0.00001; \eta^2 = 0.359, 90\%CI: 0.171-0.462$ ) did not significantly differ between conditions ( $F_{[3.39,57.66]} = 1.250, P = 0.300; \eta^2 = 0.069, 90\%CI: 0.000-0.146$ ; Fig. 3B). Similarly, a Bayesian ANOVA for repeated measures indicated that subjective sleepiness was substantially influenced by both time and condition ( $M_t$  vs.  $M_0$ :  $BF_{10} = 963208.132$ ;  $M_c$  vs.  $M_0$ :  $BF_{10} = 115729.533$ ;  $M_{c+t}$  vs.  $M_0$ :  $BF_{10} = 2.201 \times 10^{12}$ ). A  $BF_{10} = 0.053$  does not support the inclusion of the interaction term into the model ( $M_{c+t+ct}$  vs.  $M_{c+t}$ :  $BF_{10} = 0.053$ ).

The analyses of subjective sleep quality revealed a significant difference for the scale *Getting to Sleep* only ( $t_{(17)} = -2.27, P = 0.018; d = -0.549, 95\%CI: -1.099- -0.036; M_{caff} \pm SD = 42.04 \pm 11.54, M_{plac} \pm SD = 50.09 \pm 16.09$ ), indicating that participants noticed a little more problems with falling asleep in the caffeine condition compared to placebo. In the Bayesian  $t$ -test, this was mirrored in a  $BF_0 = 3.61$ , based on the alternative directional hypothesis (i.e., subjective sleep quality is worse after caffeine compared to placebo). The other scales (i.e., *Quality of Sleep [QOS]*, *Awake Following Sleep [AFS]*, and *Behavior Following Wakening [BFW]*) did not significantly differ between conditions (all  $P > 0.165$ ; effect sizes: QOS:  $M_{caff} \pm SD = 41.02 \pm 14.06, M_{plac} \pm SD = 43.29 \pm 16.25, d = -0.143; 95\%CI: -0.444-0.148$ ; AFS:  $M_{caff} \pm SD = 57.61 \pm 14.72, M_{plac} \pm SD = 56.74 \pm 17.70, d = 0.051; 95\%CI: -0.459-0.565$ ; BFW:  $M_{caff} \pm SD = 48.29 \pm 28.04, M_{plac} \pm SD = 45.99 \pm 21.95, d = 0.087; 95\%CI: -0.257-0.437$ ). Bayesian  $t$ -test revealed a  $BF_0 = 0.621$  regarding QOS, for AFS and BFW a  $BF_0 = 0.21$  and  $BF_0 = 0.173$  respectively, that is moderate evidence suggesting that the quality of awakening in teenagers is not affected by caffeine intake in the evening.

#### 3.3. Sleep patterns

For completeness, the proportion of all sleep stages and sleep latencies during the placebo and caffeine condition are summarized in Table 2. Here, we focus on condition-specific differences in SWS proportion. SWS proportion was slightly reduced after caffeine compared to placebo ( $t_{(17)} = -2.093, P = 0.026; d = -0.339, 95\%CI: -0.703-0.002; M_{caff} \pm SD = 36.91 \pm 8.69, M_{plac} \pm SD = 40.51 \pm 11.41$ ; Fig. 4A) by 3.6% on average, as mirrored in ~21 min less time spent in SWS in the caffeine condition compared to placebo. Similarly, a Bayesian  $t$ -test does not indicate strong evidence in favor for our hypothesis of caffeine-

**Table 2**

Amount of sleep, sleep stages, and latencies to sleep stages per condition. *P*-values for each sleep parameter refer to *t*-tests for dependent samples, conducted for each variable separately. A # indicates exploratory analyses and two-sided testing, while † refers to a one-sided *t*-test based on a directed a-priori hypothesis. Accordingly, Bayes Factors (*BF*) refer either to undirected hypotheses (#,  $BF_{10}$ ) or a directed hypothesis (†,  $BF_{-0}$ ). Total sleep time (TST): Sum of time in any sleep stage; SWS: Slow-wave sleep; REM: Rapid eye movement sleep; latency to sleep stages: Time from lights out to the first occurrence of each stage; latency to persistent sleep: Time from lights out to the first occurrence of 10 min in any sleep stage; sleep efficiency: Total sleep time/time in bed\*100.

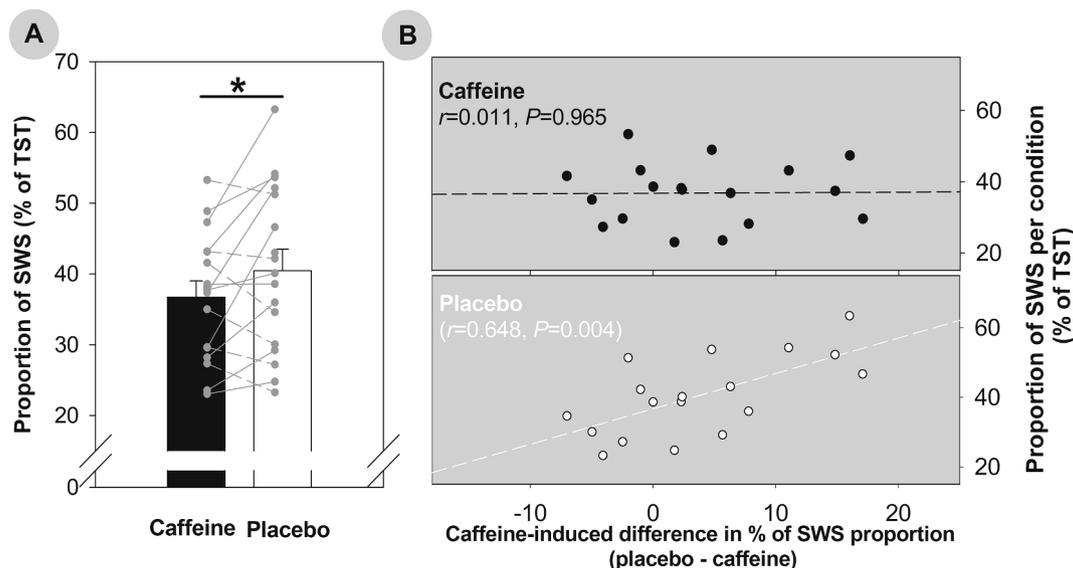
Parameter	Condition		<i>P</i> -values	<i>BF</i>
	Caffeine ( <i>M</i> ± <i>SD</i> )	Placebo ( <i>M</i> ± <i>SD</i> )		
Total sleep time ( <i>min</i> )	454.56 ± 30.97	467.06 ± 8.68	0.07#	1.158#
Wake after sleep onset ( <i>min</i> )	16.55 ± 25.71	6.60 ± 6.46	0.07#	1.083#
Stage 1 (% of TST)	11.46 ± 4.93	8.98 ± 6.48	0.02#	2.785#
Stage 2 (% of TST)	31.98 ± 6.69	31.06 ± 9.13	0.54#	0.289#
SWS (% of TST)	36.91 ± 8.69	40.51 ± 11.41	0.03†	2.723†
Stage REM (% of TST)	19.66 ± 3.71	19.46 ± 3.81	0.86#	0.246#
Latency to stage 1 ( <i>min</i> )	8.88 ± 7.38	5.57 ± 3.54	0.08#	1.023#
Latency to stage 2 ( <i>min</i> )	12.39 ± 8.51	8.86 ± 6.05	0.15#	0.648#
Latency to SWS ( <i>min</i> )	22.33 ± 7.91	16.78 ± 8.02	0.03#	2.021#
Latency to REM ( <i>min</i> )	118.13 ± 33.36	132.63 ± 39.41	0.11#	0.809#
Latency to persistent sleep ( <i>min</i> )	10.56 ± 7.54	6.70 ± 6.09	0.09#	0.958#
Sleep efficiency (%)	94.70 ± 6.40	97.46 ± 1.87	0.05#	1.401#

induced decreases in SWS proportion ( $BF_{-0} = 2.723$ ). This is also mirrored in the variance between participants in caffeine-induced changes in SWS proportion (see Fig. 4A for individual values). To explore potential sources for this variance, we correlated the difference in SWS between conditions with variables potentially associated with a differential SWS response. Neither frequentist analyses (with an adjusted significance threshold for multiple testing to  $P < 0.01$ ) nor Bayesian correlations indicated strong evidence for an association of

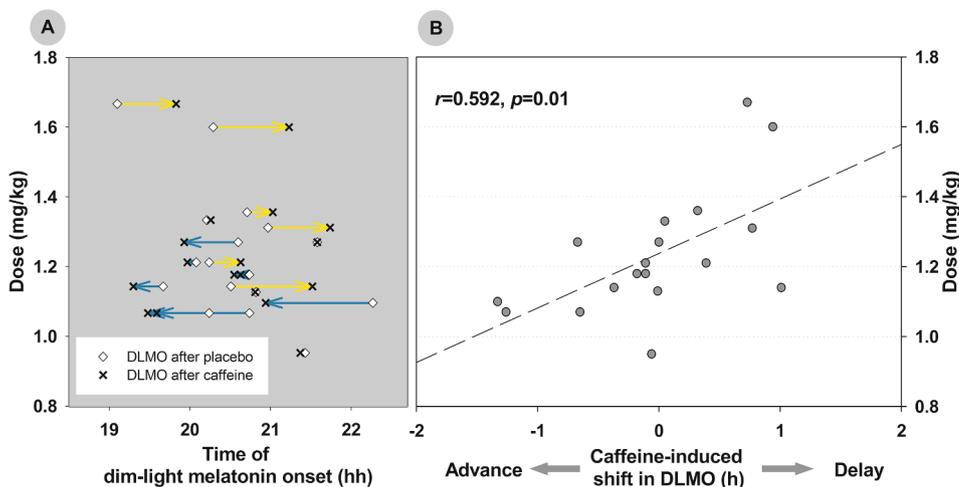
caffeine-induced reductions in SWS proportion with pubertal stage ( $r = -0.527$ ,  $P = 0.025$ ;  $BF_{10} = 3.031$ ), self-reported habitual intake levels ( $r = -0.444$ ,  $P = 0.065$ ;  $BF_{10} = 1.420$ ), relative caffeine dose ( $r = 0.132$ ,  $P = 0.603$ ;  $BF_{10} = 0.331$ ) or actual state of caffeine metabolism ( $r = 0.275$ ,  $P = 0.269$ ;  $BF_{10} = 0.514$ ). However, we observed an association with the individual's SWS proportion assessed during placebo ( $r = 0.648$ ,  $P = 0.004$ ;  $BF_{10} = 14.517$ ), such that the higher the SWS proportion was during placebo, the stronger were caffeine-induced changes in this parameter (Fig. 4B). In a final step, we explored whether this association is specific for SWS proportion in the placebo condition. Indeed, an ANCOVA revealed a significant interaction of condition and caffeine-induced changes in SWS proportion ( $F_{[1,33]} = 7.303$ ,  $P = 0.011$ ,  $\eta^2 = 0.181$ , 90%CI: 0.025–0.358, see Fig. 4B for illustration). Similarly, Bayesian analyses indicate moderate evidence that caffeine-induced changes in particular occur in those individuals with a high level of SWS proportion during the placebo night (null model (including condition and changes in SWS proportion) vs. null model plus additional interaction term:  $BF_{10} = 3.268$ ).

### 3.4. Melatonin

The DLMO was detectable in all subjects in both conditions and showed the typical [21,72] maturational delay (correlation DLMO × PDS,  $r = 0.701$ ,  $P = 0.001$ , two-sided) in the placebo condition. However, in contrast to our hypothesis, the analysis did not reveal a significant delay in DLMO in the caffeine compared to the placebo condition ( $t_{(17)} = -0.189$ ,  $P = 0.43$ ;  $d = -0.039$ , 95%CI:  $-0.461$ – $0.381$ ;  $M_{\text{caff}} \pm SD = 20:35 \pm 46$  min,  $M_{\text{plac}} \pm SD = 20:37 \pm 43$  min; Fig. 5A). Bayesian statistics moreover indicate moderate evidence that there is no influence of the given caffeine dose on DLMO ( $BF_{+0} = 0.212$ ). Similarly, the AUC did not differ between conditions ( $t_{(17)} = 0.835$ ,  $P = 0.415$  two-sided;  $d = 0.091$ , 95%CI:  $-0.062$ – $0.130$ ;  $M_{\text{caff}} \pm SD = 909.572 \pm 682.48$  pg\*min/ml,  $M_{\text{plac}} \pm SD = 843.38 \pm 695.66$  pg\*min/ml; Bayesian *t*-test  $BF_{10} = 0.331$ ). As illustrated in Fig. 5A, there was a high inter-individual variance in the response to the treatment, as mirrored in a similar frequency of observed delays (in 44% of the sample) and advances (in 56% of the sample) of melatonin onsets after caffeine compared to placebo.



**Fig. 4.** Proportion of slow-wave sleep (SWS) per condition and its relation to caffeine-induced changes in SWS. A) The proportion of SWS (as percentage of total sleep time, TST) was significantly reduced in the caffeine condition compared to placebo (\*:  $P = 0.026$ , one-sided *t*-test). The proportion of SWS per condition is depicted both as group mean plus standard error and per individual. Solid lines indicate individuals with reductions of SWS proportion, dashed lines indicate individuals with increases in caffeine compared to placebo. B) Association of SWS proportion per condition with difference in SWS proportion between conditions. While there was no association in the caffeine condition (upper panel), the SWS proportion in the placebo condition was positively associated with caffeine-induced changes of this variable (lower panel). That is, the higher the SWS proportion was during the placebo condition, the higher was the caffeine-induced reduction in the caffeine condition. Lines represent correlations; *P*-values are based on two-tailed tests.



**Fig. 5.** Timing of melatonin onset per participant, condition, and in relation to the relative given dose. A) Each individual's dim-light melatonin onset (DLMO) is depicted for the placebo (as diamond) and caffeine condition (as cross), according to the caffeine dose (relative to the individual's bodyweight) on the y-axis. Arrows indicate the shifting direction of DLMO from the placebo towards the caffeine condition within the same individual (yellow arrows represent a delay, blue arrows an advance in DLMO after caffeine intake compared to placebo). B) Caffeine-induced shifts in DLMO (calculated as the difference in timing of the DLMO in the caffeine condition minus DLMO in the placebo condition) were associated with the relative given caffeine dose (i.e., 80 mg relative to the bodyweight of each participant). The *P*-value is based on a two-tailed test.

Thus, we explored potential variables explaining this variance as well as the contradiction to the previously reported results in adults [29,31]. Correlational analyses indicated that of those factors, differing with regard to earlier studies, only the relative dose was associated with the extent of caffeine-induced shifts in DLMO ( $r = 0.592$ ,  $P = 0.010$ ;  $BF_{10} = 6.506$ , see Fig. 5B), while we have no evidence that age, habitual intake levels, and distance of treatment to DLMO play an important role (age:  $r = -0.257$ ,  $P = 0.304$ ,  $BF_{10} = 0.476$ ; habitual intake:  $r = -0.223$ ,  $P = 0.374$ ,  $BF_{10} = 0.421$ ; distance of treatment to DLMO:  $r = -0.383$ ,  $P = 0.117$ ,  $BF_{10} = 0.916$ ). Regarding frequentist analyses, the threshold for significance was adjusted for multiple testing using the Bonferroni method (to  $P < 0.013$ ).

#### 4. Discussion

Caffeine is a strong sleep-wake regulator [27,73] and part of the daily diet of most teenagers [1–3]. The present study is the first to investigate the effects of caffeine on sleep and circadian timing during adolescence using a placebo-controlled randomized crossover design. Our data indicate that 80 mg of the stimulant in the evening can be sufficient to elicit the typical wake-promoting effects at the costs of subsequent sleep. More specifically, caffeine intake reduced feelings of sleepiness, and thus meets one of the main motivations to consume the substance [1]. However, it also slightly suppressed SWS during nighttime, that is a sleep feature characterized by slow cortical oscillations that are crucial for neuronal recovery [74] and brain maturation [75]. Critically, the degree of this caffeine-induced SWS reduction was more pronounced in individuals with a relatively high SWS proportion during the placebo night. Thus, caffeine may be particularly sleep-disruptive in those teenagers with a higher sleep need. Finally, in the present sample, caffeine intake did not delay melatonin secretion as has been observed in adults [29–31], potentially due to the comparably low dose of caffeine used in the present study. Together, the results show the acute stimulating potential of caffeine and thereby underline the need to investigate long-term consequences of the drug on the developing sleep-wake regulatory system.

Already 80 mg of caffeine, that is the content in 0.25 l of common energy drinks, were sufficient to effectively promote alertness in teenagers at a subjective level. At the same time, the slightly reduced SWS indicates a potential deficit to recover during nighttime from the burden of prior wakefulness. This deficit could explain previous observations [25,33,76] that daily caffeine intake is associated with higher daytime sleepiness as has been assessed retrospectively for different daily life situations [25,33,76]. In comparison, our participants rated sleepiness in regard to the past minutes and under the acute influence of the drug. Caffeine might thus acutely counteract sleepiness but loose this wake-

promoting property if consumed repeatedly, or even express as a withdrawal-related higher subjective sleepiness occurring within a certain time after last intake [77,78]. Given that one of the main reasons for caffeine intake amongst teenagers is to combat sleepiness and enhance alertness [1], caffeine-induced differences in these variables between acute on-time and habitual daily caffeine intake represent an important target in the future regarding both research and education.

In line with a decrease in subjective sleepiness, we observed some indications for a slightly reduced amount of SWS after caffeine intake, a sleep stage characterized by cortical slow oscillations and a high slow-wave-activity (SWA) [62]. It appears reasonable that reducing SWS by caffeine interferes with the expression of SWA and potentially as well with the related processes of brain maturation [79,80]. Accordingly, chronic caffeine treatment in pubertal rats delayed the developmental course of SWA and structural markers of brain maturation [81]. While consequences of long-term caffeine intake on human brain maturation during adolescence remain to be established, data in human adults indicate that chronic caffeine consumption may indeed affect cortical morphology [82]. Thus, as long as experimental long-term or longitudinal studies in human adolescents are missing, teenagers may be advised to limit caffeine intake to a minimum.

On the other hand it is important to note that the acute caffeine-induced decrease in SWS in the present study seems to be rather low compared to SWS reductions for instance in clinical samples (e.g., [83,84]) independent of caffeine. However, at present we cannot exclude that even subtle caffeine-induced changes could add on or interact with other sleep-disturbing factors to which teenagers are exposed to (e.g., media use [85]) such that even a small caffeine-induced change could make a clinically relevant difference. Together with a caffeine-induced increase in the amount of stage N1 (see Table 2) during the nighttime sleep episode, our data indicate that the given dose induced rather light and superficial sleep. These differences in sleep structure might be one reason for self-reported sleep disturbances in caffeine-consuming adolescents [27,34,38]. Such subjective impressions of bad sleep could not only drive the motivation to in turn combat fatigue by caffeine [1] but also lead to chronic intake and potential long-term adaptations in sleep [39,81].

Importantly, caffeine intake might particularly affect the homeostasis of sleep in those individuals with a (trait-like) high need for sleep and a higher sleep pressure. Also adults show stronger caffeine-induced changes in waking EEG and vigilance the more sensitive they are for high sleep pressure [86]. Complementary, our data revealed that the acute amount of caffeine-induced SWS reduction is strongly related to the individual amount of SWS during a drug-free (i.e., a placebo) night, but not significantly associated with pubertal stage, habitual caffeine intake, or relative given dose. However, one might criticize the

predictive potential of the SWS amount under placebo as a trait-like indicator for sleep need. For instance, inter-individual variations of SWS duration are only moderately determined by genetic factors [87] and only to some extent stable between conditions. In fact, the amount of SWS is strongly dependent on recent sleep-wake history, such as the duration of prior wakefulness [20] and accumulated sleep debt [88], mirroring the actual need for SWS. However, in the present study, we aimed at controlling the latter by implementing a fixed sleep-wake schedule for six days before start of each laboratory condition. Although limited by a rather small sample size, the present data strongly suggest to further examine whether caffeine-induced sleep-disruptions are particularly strong in populations characterized by a higher need for SWS (e.g., in children), thereby identifying individuals particularly vulnerable for the stimulating potential of the drug.

Beside the individual need for SWS, we also tested whether the individual caffeine-induced reduction of SWS is related to the individual state of caffeine metabolism. We focused on the ratio paraxanthine/caffeine, a reliable indicator for CYP1A2 activity [71], which in adults metabolizes a large proportion of the caffeine ingested [89] and is most likely not fully developed before the end of puberty [90]. Together with the available data in human adults [40,91], the data at present do not support a strong relation of the absolute metabolic ratio paraxanthine/caffeine and caffeine-induced changes in sleep, but rather suggest a differential adenosinergic signal transduction to be involved in the individual caffeine-induced reduction of SWS (e.g., a differential sensitivity of receptors [92]). Such differences might also contribute to inter-individual differences in SWS under placebo conditions [93,94], which were closely associated with the caffeine-induced SWS response to caffeine.

In contrast to earlier reports, we did not observe a clear shift in the circadian onset of melatonin secretion, considered as a key to the nocturnal gate of sleep [94]. Evening caffeine intake may thus not exacerbate the circadian delay of teenagers [21] in any case. However, apart from the group mean, the results also indicate that higher doses have indeed the potential to do so. In fact, studies in adults indicating a caffeine-induced phase delay [31] or evening melatonin suppression [29,30] utilized doses which were more than two-fold higher as in the present design (i.e., 2.9 mg/kg in [31] and ~2.65 mg/kg in [29] vs. ~1.2 mg/kg in the present study), and a lower relative dose has been considered as one reason for a weakening in the suppressive effect of evening caffeine on melatonin [30]. It remains to be elucidated which component within the human circadian timing system is affected by caffeine and majorly regulates caffeine-induced shifts in melatonin secretion. A blockade of adenosine receptors might change light responses at the level of the retina [95] or the central circadian master-clock [50,96] or may even affect melatonin release at the pineal gland [97].

#### 4.1. Limitations

Despite of our rigid study design, this study bears some limitations. First, we included male teenagers only in order to avoid increased variance of sleep and circadian rhythms induced by the menstrual cycle [98] and by the use of oral contraceptives on caffeine metabolism [99,100]. Second, we have no data of an adult control group to specify whether the reported effects of caffeine are specific for maturation during adolescence. In order to approach a potential maturational influence we used pubertal development scores (PDS) and age as continuous variables, and explored whether they systematically vary with caffeine-induced responses in the dependent variables. Third, our results are derived from a rather small sample and require replication in a larger sample. Please note however, that sample size was carefully determined before implementation, based on effect sizes reported in earlier studies and the commonly accepted  $\alpha$ - and  $\beta$ -thresholds. Fourth, based on earlier studies we derived directed hypotheses and report *P*-values according to one-sided *t*-tests (except for exploratory analyses).

However, the diversity of individual reactions to caffeine in the present results (e.g., see Figs. 3–5) suggests considering undirected hypotheses and two-sided testing for future studies. Fifth, we administered caffeine in the evening hours and administered one dose only such that the results might not be generalizable to other settings. Sixth, we focused on the effects of caffeine on sleep structure only. Future analyses will focus on the caffeine-induced changes of NREM sleep SWA as a more precise marker for sleep pressure and indicator for brain maturation.

## 5. Conclusion

In conclusion, the results are the first to systematically show the janus-faced wake-promoting effects of evening caffeine intake in teenagers particularly in the homeostatic process of sleep-wake regulation. The effects were characterized by the typical reduction in subjective sleepiness at the costs of nighttime sleep quality after a relatively low dose of the stimulant. As these costs were higher in teenagers expressing more deep sleep under baseline conditions, our data raise the question whether caffeine could be particularly harmful in individuals with a high homeostatic sleep need, such as children. As long as further empirical studies on consequences and underlying adenosinergic mechanisms are missing, caffeine should thus be consumed with caution.

### CRedit authorship contribution statement

**Carolyn F. Reichert:** Conceptualization, Methodology, Formal analysis, Investigation, Data curation, Writing - original draft, Writing - review & editing, Visualization, Project administration, Funding acquisition. **Simon Veitz:** Investigation. **Miriam Bühler:** Investigation. **Georg Gruber:** Resources. **Gunnar Deuring:** Formal analysis. **Sophia S. Rehm:** Resources. **Katharina Rentsch:** Resources. **Corrado Garbazza:** Investigation. **Martin Meyer:** Investigation. **Helen Slawik:** Investigation. **Yu-Shiuan Lin:** Investigation, Writing - review & editing. **Janine Weibel:** Investigation, Writing - review & editing.

### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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