Regular Caffeine Intake Delays REM Sleep Promotion and Attenuates Sleep Quality in Healthy Men

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Abstract Acute caffeine intake can attenuate homeostatic sleep pressure and worsen sleep quality. Caffeine intake—particularly in high doses and close to bedtime—may also affect circadian-regulated rapid eye movement (REM) sleep promotion, an important determinant of subjective sleep quality. However, it is not known whether such changes persist under chronic caffeine consumption during daytime. Twenty male caffeine consumers (26.4 ± 4 years old, habitual caffeine intake 478.1 ± 102.8 mg/day) participated in a double-blind crossover study. Each volunteer completed a caffeine (3 × 150 mg caffeine daily for 10 days), a withdrawal (3 × 150 mg caffeine for 8 days then placebo), and a placebo condition. After 10 days of controlled intake and a fixed sleep-wake cycle, we recorded electroencephalography for 8 h starting 5 h after habitual bedtime (i.e., start on average at 04:22 h which is around the peak of circadian REM sleep promotion). A 60-min evening nap preceded each sleep episode and reduced high sleep pressure levels. While total sleep time and sleep architecture did not significantly differ between the three conditions, REM sleep latency was longer after daily caffeine intake compared with both placebo and withdrawal. Moreover, the accumulation of REM sleep proportion was delayed, and volunteers reported more difficulties with awakening after sleep and feeling more tired upon wake-up in the caffeine condition compared with placebo. Our data indicate that besides acute intake, also regular daytime caffeine intake affects REM sleep regulation in men, such that it delays circadian REM sleep promotion when compared with placebo. Moreover, the observed caffeine-induced deterioration in the quality of awakening may suggest a potential motive to reinstate caffeine intake after sleep.

Keywords caffeine, withdrawal, sleep, electroencephalography, REM sleep, circadian

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Caffeine has been used for centuries (Camandola et al., 2019) and is considered to be today's most popular stimulating substance around the globe (Fredholm et al., 1999). Approximately 80% of the world's population consumes caffeinated aliments day by day (Heckman et al., 2010). The common pattern of caffeine intake in the morning and afternoon (Martyn et al., 2018; Lieberman et al., 2019) most likely originates from the motive to achieve both benefits in daytime alertness (Smith, 2002; Einöther and Giesbrecht, 2013) and a good subjective nighttime sleep quality despite of previous stimulant consumption (Snel and Lorist, 2011). These double-edged alerting but sleep-disrupting effects of caffeine have been attributed to its impact on the homeostatic component of sleep-wake regulation (Landolt, 2008). By antagonizing adenosine receptors (Fredholm et al., 1999), caffeine acutely reduces homeostatic sleep pressure (Landolt, 2008), as evident in caffeine-induced reductions of waking electroencephalography (EEG) theta activity (Landolt et al., 2004), slow-wave sleep (SWS), and slow-wave activity (SWA), while it increases activity in the sigma range (Landolt, Dijk, et al., 1995).

Importantly, structure and intensity of sleep are determined not only by homeostatic sleep need but also by the circadian timing system (Borbély, 1982; Lazar et al., 2015). One of the most prominent circadian sleep features is rapid eye movement (REM) sleep propensity, which usually peaks in the morning around 2 h after the nadir of core body temperature (Dijk and Czeisler, 1995). This natural peak right before usual wake time may facilitate the rearousal of the brain from sleep (Dijk and Czeisler, 1995) and may contribute to REM sleep's substantial promotion of good subjective sleep quality (Akerstedt et al., 1994; Della Monica et al., 2018). Since the choice to consume caffeine might also strongly depend on an individual's sleep quality, it remains to be established how daily caffeine intake affects REM sleep and its role in promoting sleep quality.

There is evidence for acute effects of caffeine on REM sleep. In animal models, the perfusion of the *sleep factor* adenosine increased the time spent in REM sleep (Portas et al., 1997; Basheer et al., 1999). Regarding nighttime sleep in humans, some studies—particularly those utilizing high caffeine dosages (but see Bonnet and Arand, 1992; Drake et al., 2013)—report a caffeine-induced reduction of REM sleep duration (Brezinova, 1974; Nicholson and Stone, 1980; Robillard et al., 2015) or shift in REM sleep episodes (Karacan et al., 1976; Nicholson and Stone, 1980), while others did not show any differences in REM sleep after caffeine administration (Bonnet and Arand, 1992; Landolt, Dijk, et al., 1995; Landolt, Werth, et al., 1995; Drapeau et al., 2006; Drake et al., 2013). When sleep was initiated around the peak of REM sleep propensity (i.e., 1 h after habitual wake time) following one night of sleep loss, caffeine intake right before bedtime increased wakefulness at the cost of REM sleep (Carrier et al., 2007, 2009). It remains unknown whether such effects during daytime recovery sleep can also be observed under conditions of regular daily daytime caffeine intake.

Regular daytime caffeine intake can lead to adaptations and thus mitigate both caffeine-induced wake promotion and nighttime sleep disturbances. Such adaptations are represented in the occurrence of withdrawal symptoms within around 36 h after acute cessation of caffeine, and comprise increased sleepiness (James, 1998; Weibel, Lin, Landolt, Garbazza, et al., 2020), enhanced waking EEG theta activity (Sigmon et al., 2009), and reduced sleep EEG sigma activity (Weibel, Lin, Landolt, Kistler, et al., 2020). Thus, comparing withdrawal-induced effects on sleep against long-term abstinence and habitual use in the same individuals represents a valid tool to reliably estimate the consequences of daily caffeine intake. For this report, we took advantage of an existing data set with exactly these conditions, in which the start of each of the sleep episodes was individually scheduled around the circadian peak of REM sleep promotion. Previous sleep-wake history, light input, posture, meal intake, and circadian phase (estimated by dim-light melatonin onset) were carefully controlled and did not differ between conditions or within individuals (Weibel, Lin, Landolt, Garbazza, et al., 2020). Based on the evidence summarized above, we explored whether the duration and timing of REM sleep change in response to daily daytime caffeine intake (over 10 days) and acute caffeine withdrawal, compared with a long-term (10-day) placebo baseline. In a second step, we tested whether changes in REM sleep relate to differences in subjective sleep quality.

METHODS

Volunteers

Data sets were available from a total of 20 male study volunteers between 18 and 35 years old. All participants were regular caffeine consumers with a daily intake between 300 and 600 mg assessed by a survey tool based on Bühler et al. (2013) and its caffeine content classified according to Snel and Lorist (2011). Prior to study participation, all volunteers were screened for good health assessed by self-reports

and a medical check performed by a study physician. Individuals reporting a body mass index (BMI) <18 or >26 were excluded. Good sleep quality was ensured by the Pittsburgh Sleep Quality Index (PSQI; score ≤5) (Buysse et al., 1989) and a polysomnography (PSG) during which we screened for sleep apnea (index >10/h), period leg movements (index >15/h), and poor sleep efficiency (SE < 70%). Smoking, drug use, or extreme chronotype (score ≤30 or ≥70 in the Morningness-Eveningness Questionnaire (Horne and Ostberg, 1976)) resulted in the exclusion of participants. In addition, volunteers were not allowed to engage in shift work (<3 months prior to study) or to travel across more than two time zones (<1 month prior to study). To minimize the potential confounding influence by the menstrual cycle and the use of oral contraceptives on sleep (Shechter and Boivin, 2010) as well as caffeine elimination (Abernethy and Todd, 1985; Balogh et al., 1995), female individuals were not included in this study. Demographical data of the study sample can be found in Table 1.

Table 1. Demographical data of the study sample.

Sample Characteristics (N = 20)	M ± SD
Years of age	26.4 ± 4.0
Habitual caffeine intake (mg/day)	478.1 ± 102.8
BMI (kg/m^2)	22.7 ± 1.4
Chronotype (MEQ)	52.8 ± 8.7
Sleep quality (PSQI)	2.8 ± 1.4
Habitual bedtime[a] (hh:mm)	23:21 ± 00:49
Habitual sleep duration[a] (h)	7.5 ± 0.4

Abbreviations: BMI = body mass index; MEQ = Morningness-Eveningness Questionnaire (Horne and Ostberg, 1976); PSQI = Pittsburgh Sleep Quality Index (Buysse et al., 1989).
a. Self-reported.

Design and Protocol

We employed a double-blind crossover study with three treatments: a caffeine, a withdrawal, and a placebo condition. Random permutations were performed to assign the volunteers to the order of the three conditions, for more details, see Weibel, Lin, Landolt, Garbazza, et al. (2020). As depicted in Figure 1a, each condition comprised an ambulatory part of 9 days which was followed by an in-lab part of 43 h. On average, the three conditions were separated by 9.6 days (range: 1-33 days). In each condition, volunteers swallowed identical-appearing gelatin capsules 3 times daily (45, 255, and 475 min after awakening) containing either caffeine (150 mg; Hänseler AG, Herisau, Switzerland) or placebo (mannitol; Hänseler AG). To induce caffeine withdrawal in the withdrawal condition, the first capsule on day 9 of treatment contained caffeine but was followed by placebo capsules for the remaining administrations. The dosage and timing of administrations were chosen based on previous studies which investigated tolerance development to the effects of caffeine and its cessation (James, 1998; Keane and James, 2008) and to represent an everyday situation of most coffee consumers (Martyn et al., 2018; Lieberman et al., 2019). Volunteers were instructed to abstain from all other caffeine sources during the entire study duration. Compliance to the aforementioned regimen was verified by assessing caffeine levels in fingertip sweat collected prior to habitual bedtime during the ambulatory part of 9 days (see supplemental material of Weibel, Lin, Landolt, Garbazza, et al., 2020). Moreover, volunteers were instructed to keep a regular sleep-wake rhythm (±30 min of self-selected bedtime and waketime, 8 h in bed) and to avoid naps. Their compliance was monitored by wrist actimetry (Actiwatch, Cambridge Neurotechnology, Cambridge, United Kingdom) with concurrent sleep logs. Sleep schedule remained constant across all three conditions except for three volunteers (two volunteers: +30 min in caffeine compared with placebo and withdrawal conditions; one volunteer: −30 min in placebo compared with caffeine and withdrawal conditions).

As illustrated in Figure 1b, on day 9 of treatment, volunteers reported to the laboratory 5.5 h prior to habitual bedtime. Upon arrival, PSG electrodes were fitted and a sleep episode of 8 h took place at the volunteer's habitual bedtime. The following day, saliva was regularly collected to quantify the levels of caffeine and its main metabolites. In the evening, we scheduled a 1-h nap (for details, see Weibel, Lin, Landolt, Garbazza, et al. (2020)), which was followed by another wakefulness episode. To minimize masking effects during the in-lab protocol, no time-of-day information was provided, communication was restricted to staff members, and light (scheduled wakefulness: <8 lux; sleep episode: 0 lux) and posture (scheduled wakefulness: semirecumbent; sleep episode: supine) were controlled.

Approximately 5 h after volunteers' habitual bedtime (mean: 04:22 h; range: 03:15-05:15 h), a sleep episode of 8 h was scheduled, which corresponds to the expected circadian peak of REM sleep promotion (Dijk and Czeisler, 1995; Münch et al., 2005), and was 13.5 and 44.5 h after the last caffeine intake in the caffeine and withdrawal condition, respectively. Upon awakening, volunteers rated their subjective sleep quality by the Leeds Sleep Evaluation Questionnaire (LSEQ) (Parrott and Hindmarch, 1978).

Approval was obtained from the local ethics committee (Ethikkommission Nordwest- und Zentralschweiz), and the study was conducted in

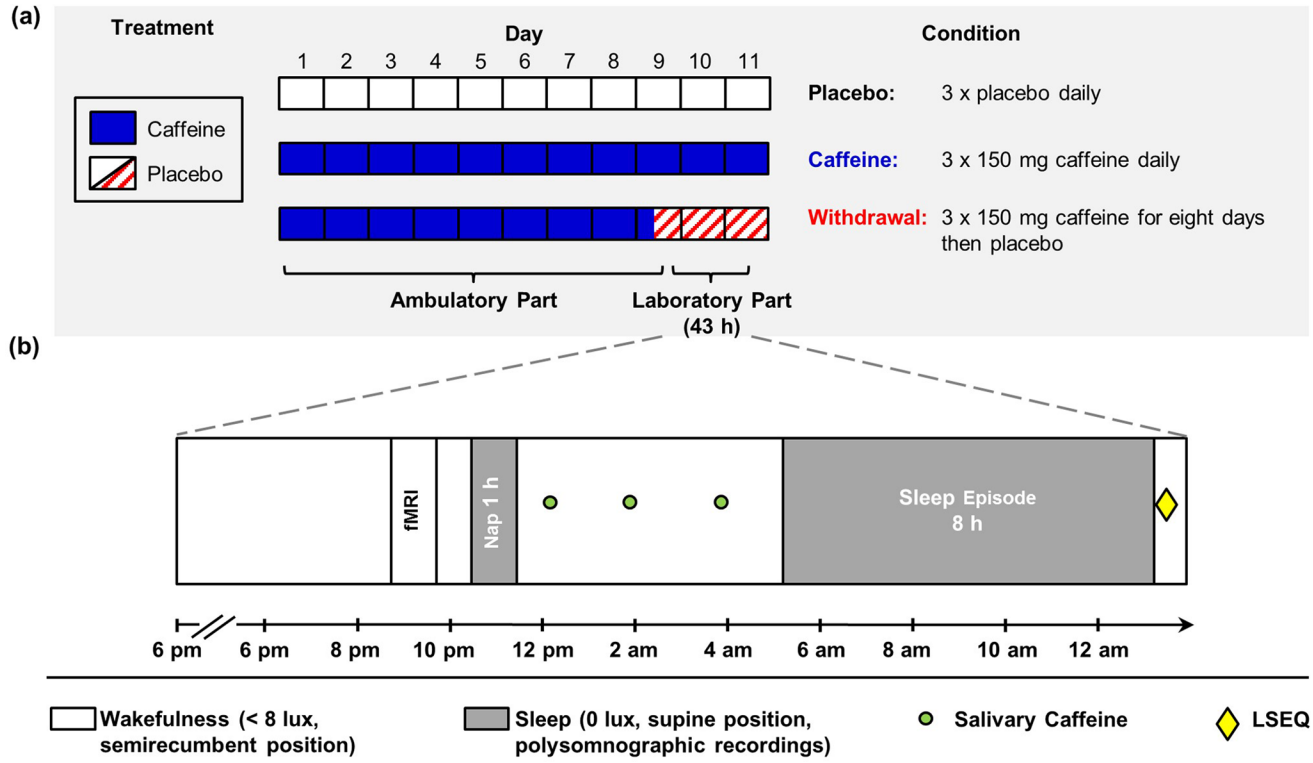

Figure 1. Illustration of the research protocol. Adapted from Weibel, Lin, Landolt, Garbazza, et al. (2020).
(a) Each participant took part in a placebo, a caffeine, and a withdrawal condition consisting of an ambulatory part of 9 days and an in-lab part of 43 h. (b) The in-lab protocol started with a baseline night scheduled to volunteers' habitual bedtime. On the following day, we scheduled a 1-h nap in the evening and salivary caffeine levels were collected in regular intervals. Five hours after usual bedtime, an 8-h sleep episode was scheduled, and subjective sleep quality was assessed afterwards. Abbreviation: LSEQ = Leeds Sleep Evaluation Questionnaire.

accordance with the Declaration of Helsinki. All volunteers provided a written informed consent and received financial compensation for study participation.

Caffeine Levels

Salivary caffeine levels were repeatedly assessed in intervals of approximately 2 h throughout the in-lab protocol to check compliance with the treatment requirements. Here, we focus on the levels within 5 h prior to the sleep episode. After saliva collection, samples were immediately stored at 5 °C, later centrifuged at 3000 r/min for 10 min, and subsequently frozen at −24 °C until assayed by liquid chromatography coupled to tandem mass spectrometry. The data of one volunteer collected in the withdrawal condition were lost.

Subjective Sleep Quality

To assess subjective sleep quality, volunteers completed the LSEQ (Parrott and Hindmarch, 1978) right after the end of the 8-h nighttime sleep episode. Volunteers rated 10 items on visual analog scales consisting of four different scales, that is, *getting to sleep*, *quality of sleep*, *awake following sleep*, and *behavior following wakening*.

EEG Recordings

We utilized EEG recordings to assess sleep structure. Six EEG derivations (F3, F4, C3, C4, O1, and O2), two electrooculographic, two electromyographic, and two electrocardiographic electrodes were placed according to the international 10-20 system and referenced online against the linked mastoids (A1, and A2). The EEG signal was recorded using V-Amp devices (Brain Products, Gilching, Germany) with a sampling rate of 500 Hz and a filter applied online at 50 Hz.

Sleep staging was performed with an automatic scoring algorithm (ASEEGA, version 4.4.23, PHYSIP, Paris, France) which has been successfully used in previous studies (Reichert et al., 2017; Gaggioni et al., 2019) and has been shown to reach good agreement with manual sleep scoring (Berthomier et al., 2020).

The concordance of a subset of manually scored nights (50 nights of this study sample) with the automatic sleep scoring reached >80%. The derivation (C4O2) was used for sleep autoscoring. Sleep latencies to specific stages were defined based on the first epoch scored in the respective sleep stage. While in this article we focus on REM sleep, we report all other sleep stages as well. All sleep stages are expressed as percentage of total sleep time (TST). Spectral analyses were performed by employing a fast Fourier transform with a Hanning window on consecutive epochs of 30 s. Artifacts were automatically rejected, and power spectra in the delta band (0.1-4 Hz) during non-REM (NREM) sleep are reported as a measure for sleep pressure.

Three data sets were excluded from analyses of all-night variables concerning the entire sleep episode due to technical issues (placebo condition: $n = 2$; caffeine condition: $n = 1$) and one volunteer was excluded from all analyses of REM sleep parameters (placebo condition) based on potential missed REM sleep episode by the algorithm and disagreement with visual scorers.

Statistical Analyses

Data analyses were performed using SAS (version 9.4, SAS Institute, Cary, USA). Values exceeding 3 times the interquartile range (IQR) from the first and third quartile were treated as extreme outliers and removed from subsequent analyses when attributed to errors in data collection or subsequent handling. Based on these criteria, we observed $n = 2$ outliers in the data (outlier regarding REM sleep latency and delta power). We applied mixed model analyses of variance with the factors condition (placebo, caffeine, and withdrawal) and time (levels differ per variable). The degrees of freedom were based on the approximation of Kenward and Roger reported in Kenward and Roger (1997). Contrasts were calculated by applying the LSMEANS statement and were adjusted for multiple comparisons based on Tukey-Kramer. To investigate whether the ratings of sleep quality can be explained by REM sleep characteristics, we performed general linear models with the statistics software SPSS (version 24, IBM Corporation, Armonk, NY, USA) including REM sleep variables as covariates and subject as a random factor. A value of $p < 0.05$ was used to determine statistical significance. The thresholds for the sleep stages and subjective sleep quality, however, were adjusted according to the Bonferroni method (to $p < 0.0055$ and <0.0125, respectively) due to multiple testing. One volunteer was excluded from all analyses due to noncompliance with the treatment requirements (caffeine condition: $n = 1$).

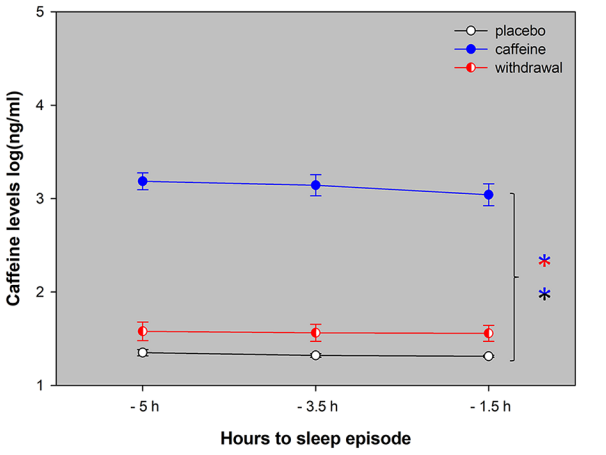

Figure 2. Depicted are the salivary caffeine levels collected within 5 h prior to the sleep episode in the placebo (black open circles), caffeine (blue filled circles), and withdrawal (red semi-filled circles) conditions (means ± standard errors). Overall, caffeine levels were increased in the caffeine condition compared with both placebo and withdrawal conditions (*$p < 0.05$).

RESULTS

Salivary Caffeine Levels

The analyses of caffeine levels assessed within 5 h prior to the sleep episode revealed a significant main effect of condition ($F_{2,55.9} = 21.79$; $p < 0.0001$). Post hoc comparisons indicated that caffeine levels were still elevated in the caffeine compared with the placebo and withdrawal conditions ($p_{all} < 0.0001$), as presented in Figure 2. Caffeine levels in the withdrawal condition did not significantly differ from placebo ($p = 0.984$).

Sleep

EEG Recordings. We analyzed TST, sleep latencies, and sleep architecture of the 8-h sleep episode, as summarized in Table 2. TST and the proportion of each sleep stage did not significantly differ among the three conditions ($p_{all} > 0.1$). In the caffeine condition, however, it took participants longer to enter REM sleep compared with the placebo and withdrawal conditions ($p_{all} < 0.05$).

In a next step, we investigated whether the accumulation of REM sleep proportion across the sleep opportunity differs among the three conditions. As shown in Figure 3, on average, REM sleep proportion was reduced in the caffeine compared with the placebo condition (main effect of condition: $F_{2,39.1} = 6.75$, $p = 0.003$). This effect was modulated by time (interaction of condition x time: $F_{14,195} = 1.77$, $p = 0.046$) indicating

Table 2. Sleep variables and results in the electroencephalographic variables.

Variable	Placebo	Caffeine	Withdrawal	Condition
TST (min)	366.19 ± 16.71	393.89 ± 13.94	393.20 ± 11.23	$F_{2,35.2} = 2.32$, $p = 0.113$
SE (%)	76.79 ± 3.44	82.36 ± 2.79	82.46 ± 2.30	$F_{2,35.3} = 2.27$, $p = 0.118$
N1 (% of TST)	3.78 ± 0.52	4.49 ± 0.79	4.20 ± 0.48	$F_{2,35.9} = 0.40$, $p = 0.676$
N2 (% of TST)	44.93 ± 1.80	45.57 ± 1.39	44.69 ± 1.45	$F_{2,34.6} = 0.06$, $p = 0.942$
N3 (% of TST)	24.21 ± 1.25	25.22 ± 1.25	24.40 ± 1.31	$F_{2,35.4} = 0.27$, $p = 0.763$
REM (% of TST)	27.82 ± 1.31	24.73 ± 1.55	26.72 ± 1.11	$F_{2,34.9} = 1.87$, $p = 0.169$
SL2	11.13 ± 2.29	9.13 ± 0.89	8.13 ± 1.11	$F_{2,37.3} = 1.62$, $p = 0.212$
RL	53.63 ± 5.53	78.74 ± 10.21*	53.95 ± 6.42	$F_{2,36.4} = 6.30$, $p = 0.005$
NA	8.61 ± 1.30	9.22 ± 1.18	8.50 ± 1.27	$F_{2,35.4} = 0.14$, $p = 0.871$

Abbreviations: TST = total sleep time (sum of N1, N2, N3, and REM); SE = sleep efficiency (TST/time in bed); N1 = stage 1; N2 = stage 2; N3 = slow-wave sleep; REM = rapid eye movement; SL2 = time from lights-off to first epoch of N2; RL = time from lights-off to first epoch of REM sleep; NA = number of awakenings. Depicted are the means and standard errors per condition.
*Significant post hoc comparisons compared with placebo and withdrawal conditions ($p < 0.05$, adjusted according to Tukey-Kramer) of significant main effects ($p < 0.0055$, as threshold was adjusted according to Bonferroni).

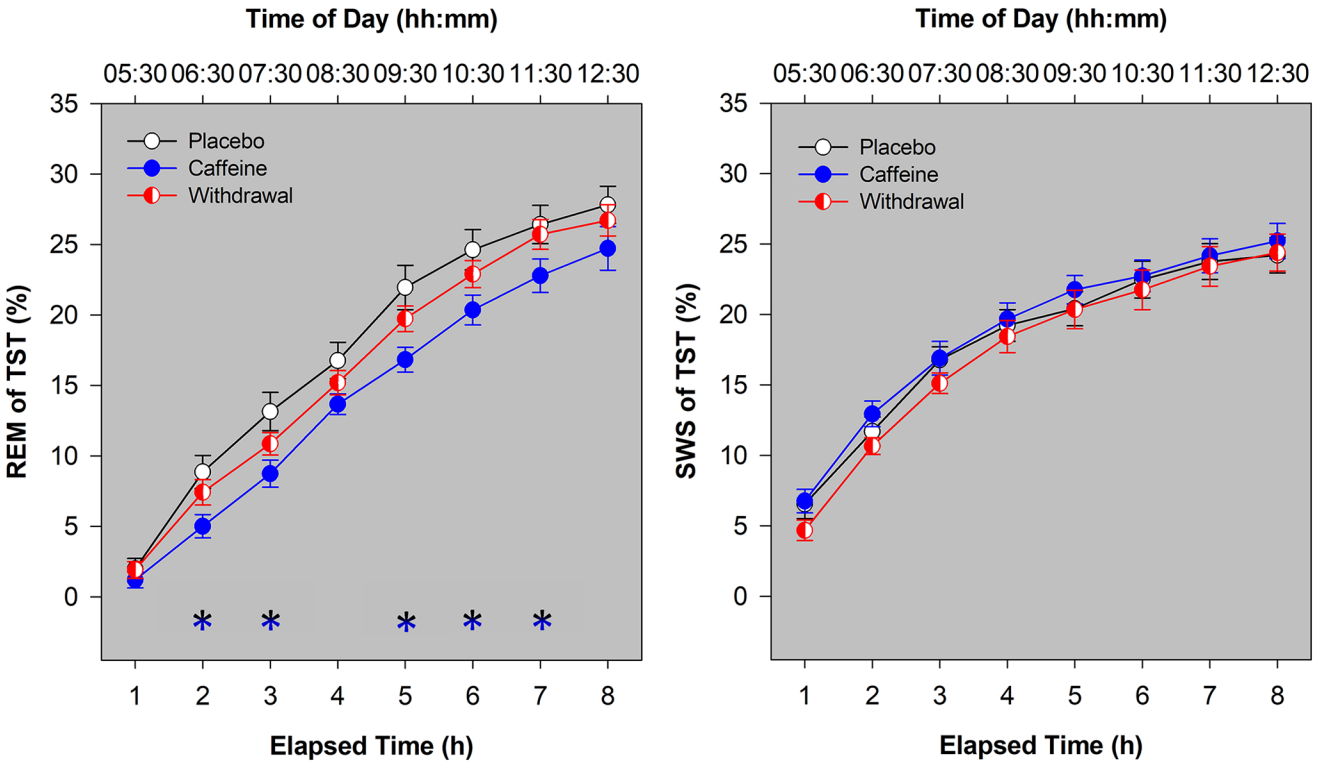

Figure 3. Accumulation of REM sleep and SWS proportion across the sleep opportunity of 8 h. REM sleep (% of TST) and SWS (% of TST) were collapsed into bins of 1 h and accumulated across the sleep episode. Depicted are means and standard errors of the placebo (black open circles), caffeine (blue filled circles), and withdrawal conditions (red semi-filled circles). The color-coded asterisks represent significant (*$p_{all} < 0.05$) differences between the placebo and caffeine conditions corrected for multiple comparisons according to Curran-Everett (2000). Abbreviations: REM = rapid eye movement; TST = total sleep time; SWS = slow-wave sleep.

that this caffeine-induced reduction in the caffeine condition was particularly present in bin 2, 3, 5, 6 and 7, that is, in the second and third hour of sleep as well as in the fifth, sixth and seventh hour of the given sleep window. To control whether the observed changes in REM sleep accumulation are mainly driven by the prolonged latency to REM sleep, we analyzed the amount of REM sleep per hour of sleep (Figure 4, for illustration). In this analysis, the amount of REM sleep did neither significantly differ among the three conditions ($F_{2,411} = 0.23$; $p = 0.796$) nor was it modulated by the hour spent asleep (interaction of condition x time: $F_{14,398} = 1.44$; $p = 0.130$) which underlines the impact of REM sleep latency on its accumulation.

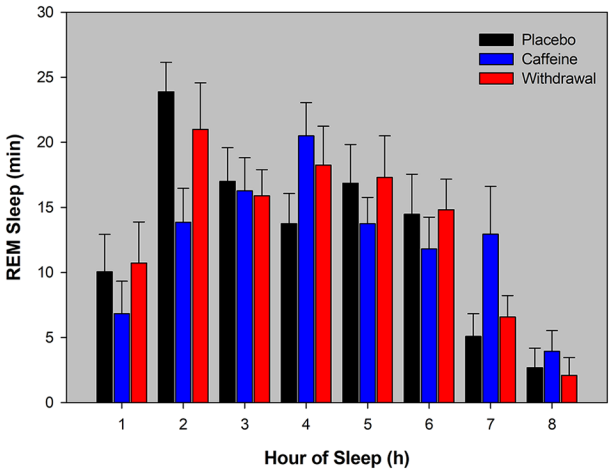

Figure 4. Amount of REM sleep in each hour spent asleep. Depicted are means and standard errors of the placebo (black), caffeine (blue), and withdrawal conditions (red) across the sleep opportunity of 8 h. Abbreviation: REM = rapid eye movement.

Importantly, the analyses of the accumulation of wakefulness and SWS (see Fig. 3) did neither yield a significant main effect of condition (wakefulness: $F_{2,36.4} = 0.49$, $p = 0.615$; SWS: $F_{2,38.6} = 0.94$, $p = 0.401$) nor a significant interaction of the factors condition and time (wakefulness: $F_{14,205} = 0.86$, $p = 0.603$; SWS: $F_{14,196} = 0.54$, $p = 0.908$).

Finally, we analyzed the spectral power in the delta band (0.1-4 Hz) across the first three NREM sleep cycles as an indicator for sleep pressure. There were no significant differences between the three conditions in NREM sleep delta power (main effect of condition: $F_{2,26.8} = 1.19$; $p > 0.3$) nor was it modulated by the NREM sleep cycles (interaction condition × time: $F_{4,45.1} = 0.99$; $p > 0.4$).

Subjective Sleep Quality. The parameters and results of subjective sleep quality are presented in Table 3. While the domains *getting to sleep* and *quality of sleep* did not significantly differ between the three conditions ($p_{all} > 0.05$), volunteers reported more difficulty in *awake following sleep* and *behavior following wakening*, that is, increased tiredness in the caffeine compared with the placebo condition ($p_{all} < 0.01$). Neither REM sleep duration nor REM (% of TST) had a significant effect on the ratings of *awake following sleep* ($p_{all} > 0.4$) and *behavior following wakening* ($p_{all} > 0.3$).

DISCUSSION

To the best of our knowledge, this is the first study to explore the effects of regular daytime caffeine intake on REM sleep promotion and subjective quality of sleep. An 8-h sleep window scheduled in the early morning hours allowed to investigate REM sleep expression at its circadian maximum (Dijk and Landolt, 2019) in a highly controlled laboratory setting. The volunteers in the caffeine condition entered REM sleep later compared with both placebo and withdrawal conditions, and its accumulation across the night was delayed compared with placebo. As a similar pattern was not observed during regular nighttime sleep (Weibel, Lin, Landolt, Kistler, et al., 2020), that is, at another circadian time, we speculate that these findings may potentially indicate caffeine-induced differences in circadian REM sleep promotion. Thus, similar to the earlier reported REM sleep reductions after caffeine right before bedtime (Carrier et al., 2007, 2009; Robillard et al., 2015), specific features of REM sleep (such as onset and accumulation) might be sensitive to caffeine intake, even if it is restricted to daytime and the last intake is 13.5 h apart from lights-off. Moreover, volunteers reported more difficulties in awakening from sleep and feeling more tired after daily caffeine intake compared with continuous placebo intake. Such subtle changes in subjective sleep quality may promote the maintenance of regular caffeine intake under conditions of delayed or shifted sleep.

While daily caffeine intake is highly popular, the effects of regular daytime consumption on sleep-wake regulation are not well understood. One reason might be difficulties to standardize the history of prior caffeine consumption and to control its daily intake. Moreover, a potential adaptation to the continuous availability of caffeine (Bonnet and Arand, 1992; Weibel, Lin, Landolt, Garbazza, et al., 2020) requires a design sophisticated enough to disentangle an inherent insensitivity from a habituation to the stimulant's effect (James, 1998). In this report, for which we took advantage from an existing highly controlled laboratory data set, we suggest that regular daytime caffeine intake has the potential to alter REM sleep, indexed as prolonged onset and delayed accumulation of REM sleep. As a few studies suggest an adaptation of several nighttime sleep features (Bonnet and Arand, 1992) and of the circadian timing of melatonin onset (Weibel, Lin, Landolt, Garbazza, et al., 2020) in response to regular caffeine intake, the present results indicate that the habituation of REM sleep promotion to caffeine might either follow another (potentially slower) time course or is even absent. Moreover, caffeine-induced REM sleep differences were statistically not detectable after withdrawal over 44.5 h, indicating that a potential caffeine-related change in REM sleep promotion seems to be reversible. As withdrawal symptoms commonly reach peak intensity between 20 and 51 h after acute caffeine cessation (Juliano and Griffiths,

Table 3. Parameters and results of subjective sleep quality as assessed by the Leeds Sleep Evaluation Questionnaire.

Variable	Placebo	Caffeine	Withdrawal	Condition
GTS	73.65 ± 3.41	66.60 ± 4.49	68.22 ± 3.77	$F_{2,37.5} = 1.20$, $p = 0.313$
QOS	46.78 ± 4.66	41.03 ± 5.59	47.10 ± 4.28	$F_{2,37.5} = 0.74$, $p = 0.485$
AFS	75.50 ± 2.84	58.61 ± 4.74*	67.88 ± 3.49	$F_{2,37.1} = 6.35$, $p = 0.004$
BFW	74.75 ± 3.49	56.92 ± 5.74*	66.73 ± 3.17	$F_{2,37.4} = 7.04$, $p = 0.003$

Abbreviations: GTS = getting to sleep; QOS = quality of sleep; AFS = awake following sleep; BFW = behavior following wakening. Reported are the means and standard errors per condition. Lower values represent poorer subjective sleep quality.
*Significant post hoc comparisons to placebo ($p < 0.05$, adjusted according to Tukey-Kramer) of significant main effects ($p < 0.0125$, as threshold was adjusted according to Bonferroni).

2004), volunteers have presumably already overcome the acute phase of caffeine withdrawal at the time of sleep initiation.

Caffeine-induced reductions of REM sleep propensity have earlier been demonstrated after administration of caffeine right before daytime sleep (Carrier et al., 2007, 2009). However, the same outcome observed from 13.5 h after the last intake in this study suggests that caffeine concentration is not the only determinant to induce REM sleep changes. This notion receives further support as high dosages do not consistently evoke an effect on REM sleep ((Karacan et al., 1976; Nicholson and Stone, 1980; Robillard et al., 2015) vs. (Bonnet and Arand, 1992; Drake et al., 2013)). However, in line with the present data, caffeine-induced reductions of REM sleep features have consistently been observed when sleep was scheduled in the morning hours or at daytime (Carrier et al., 2007, 2009). Thus, caffeine effects on REM sleep seem to depend on circadian factors and may be particularly prominent if sleep is scheduled around the peak of REM sleep promotion. This might be comparable to the impact of caffeine on the homeostatic component of sleep-wake regulation, which seems to be particularly sensitive to the effects of caffeine under conditions of high sleep pressure (Roehrs and Roth, 2008; Snel and Lorist, 2011). Taken together, an effect of caffeine on sleep appears to be more easily detectable if the experimental design allows the sleep feature, that is, REM sleep, to reach a certain level.

Changes in sleep pressure have been shown to interact with the circadian expression of REM sleep (Dijk and Czeisler, 1995; Wyatt et al., 1999) and might thus have modulated the present effects as the sleep episode was scheduled 5 h after volunteer's habitual bedtime. However, it is important to note that we scheduled a 1-h nap episode in the evening prior to the 8-h sleep episode. In this nap, participants slept for around 30 min, and sleep pressure as indexed by EEG SWA did not significantly differ between conditions (Weibel, Lin, Landolt, Garbazza, et al., 2020). Moreover, additional analyses revealed no difference in all-night SWA and SWA of the first sleep cycle during nocturnal sleep after 16 h of wakefulness and the present reported sleep episode after 21 h of wakefulness among the three conditions (statistics not presented). Thus, our results suggest that the potential occurrence of increased sleep pressure did not strongly affect the present findings which can therefore likely be traced back to the circadian component of sleep regulation.

Interestingly, volunteers in the caffeine condition indicated worse sleep quality indexed as more difficulties with awakening following sleep and feeling more tired compared with placebo. While SWS is commonly related with recuperation and subjectively perceived good sleep quality (Keklund and Akerstedt, 1997), the duration of REM sleep has been recently reported to be an important determinant for the perception of sleep quality (Della Monica et al., 2018). Thus, it is tempting to associate the changes of REM sleep with the perception of poorer sleep quality, although REM sleep did not account for the perceived sleep quality in the present sample. Moreover, the changes in sleep quality which were particularly evident with awakening might also be influenced by sleep inertia. Thus, the observed perception of nonrestorative sleep might promote daily caffeine consumption to compensate the lack of feeling refreshed upon wake-up and therefore act as a negative reinforcer.

Our results need to be interpreted taking the following limitations into careful consideration. First, based on the potential influence of the menstrual cycle on sleep-wake regulation (Shechter and Boivin, 2010) and the use of oral contraceptives on caffeine elimination (Abernethy and Todd, 1985; Balogh et al., 1995), female individuals were excluded which limits the generalizability of the present findings. Second, the effects of caffeine on sleep vary with age (Drapeau et al., 2006; Carrier et al., 2009; Robillard et al., 2015), and thus, the present results only refer to young healthy individuals. Third, previous studies reported that a specific variation in adenosine receptor gene modulates the effects of caffeine on sleep (Rétey et al., 2007) which was however not controlled in this study. Fourth, while we investigated REM sleep propensity at one circadian phase only, the optimal design would

involve several circadian phases and a control for sleep pressure to allow conclusions on circadian regulation of sleep (such as in so-called forced desynchrony studies, see (Wyatt et al., 2004)).

In summary, we have first evidence that regular caffeine intake during daytime delays REM sleep promotion, as evident in prolonged REM sleep latency and delayed REM sleep accumulation. In the light of the evidence at present, these caffeine-induced changes in REM sleep promotion may particularly occur when sleep is delayed and centered around the morning hours such as at the weekend or during shift work. Caffeine-induced REM sleep changes may contribute to a worse quality of awakening and thus reinforce the maintenance of daytime caffeine intake.

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CONFLICT OF INTEREST STATEMENT

The author(s) have no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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