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The influence of daily daytime caffeine intake on human sleep-wake regulation

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Table of contents

Summary

Caffeine is the most widely consumed natural stimulant in history. While caffeine is commonly used to mitigate sleepiness and to boost performance, it is intentionally avoided to prevent adverse consequences on nocturnal sleep. The latter has been particularly investigated under conditions of acute evening intake. Sleep disrupting effects are mainly attributed to caffeine's impact on the homeostatic component of sleep-wake regulation as caffeine antagonizes the sleep factor adenosine. This results in delayed sleep onset and superficial sleep. Furthermore, evidence accumulates that acute caffeine intake in the evening impacts also on the circadian axis of sleep-wake regulation by reducing and delaying melatonin secretion, which is the primary endogenous marker of the internal timekeeping system. The overarching aim of the present thesis was to investigate whether these caffeine-induced alterations in the homeostatic and circadian sleep-wake features can also be detected under chronic exposure to caffeine timed to morning and afternoon hours, which presents a common intake pattern in coffee drinkers.

Twenty male young habitual caffeine consumers participated in a double-blind, crossover study comprising a placebo (3 x placebo daily), a caffeine (3 x 150 mg caffeine daily), and a withdrawal (3 x 150 mg caffeine for eight days then change to placebo) condition, each lasting 11 days. After nine days of continuous treatment, volunteers were studied during a 43-h laboratory part under strictly controlled conditions. During scheduled wakefulness, we regularly assessed sleepiness, vigilance performance, and circadian hormonal markers (i.e., melatonin and cortisol) while polysomnography was recorded during scheduled sleep episodes at different times of the day to quantify sleep structure and intensity.

First, we were interested whether daily caffeine intake in the morning and afternoon hours disrupts nighttime sleep structure and intensity, indexed as reduced slow-wave sleep (SWS) duration and decreased slow-wave activity (SWA; 0.75-4.5 Hz) **(chapter 4.1.)**. Surprisingly, neither daytime caffeine consumption nor its acute cessation strongly impaired nighttime sleep architecture or subjective sleep quality in comparison to placebo. Nevertheless, during both caffeine and withdrawal conditions spectral power density in the sigma frequencies (12-16 Hz) was reduced, starting 8 and 15 hours after the last caffeine intake in the caffeine and withdrawal condition, respectively. Opposite to the reported higher sigma power after acute caffeine intake in other studies, the observed reduction in the sigma frequencies might point to early signs of caffeine withdrawal which occur as soon as regular caffeine intake is stopped.

Second, we investigated whether daily daytime caffeine intake impacts on circadian hormonal rhythms and wake-promotion as well as waking performance **(chapter 4.2.)**. Interestingly, our results indicate that habitual caffeine consumption in the morning and afternoon hours does not strongly affect the diurnal secretion of melatonin and cortisol nor enhances circadian wake-promotion in the evening. Moreover, in contrast to the common perception of the stimulating

properties of caffeine, such a common intake pattern did not go along with clear-cut benefits in sleepiness or vigilance performance when comparing it to the placebo condition. However, the abrupt cessation from caffeine was associated with increased subjective sleepiness, worse vigilance performance, and increased sleep pressure in the evening as indexed by shortened sleep latency, increased total sleep time, and longer SWS. Together, these findings suggest an adaptation after repeated intake, presumably in the homeostatic aspect of sleep-wake regulation, which manifests itself as soon as chronic caffeine intake is ceased. In contrast to previous studies, we did not find evidence for circadian phase shifts.

In a last step, we explored the impact of daily caffeine intake and its acute cessation on circadian-regulated rapid eye movement (REM) sleep promotion in a sleep episode timed to the morning hours **(chapter 4.3.)**. Similar to nighttime sleep, total sleep time, and sleep architecture at this time of day were not strongly affected by caffeine or its withdrawal. Nonetheless, after daily daytime caffeine intake it took volunteers longer to enter REM sleep, its accumulation was slower, and subjective quality of awakening was worse compared to continuous placebo intake. As the latter might be counteracted in turn by caffeine intake, it might encourage caffeine consumption particularly in people who shift their sleep to morning or daytime hours which often occurs in shift-workers.

Taken together, we have first evidence that repeated daytime caffeine intake in the morning and afternoon hours does not strongly disrupt nocturnal sleep nor hormonal markers of the circadian timing system such as the diurnal secretion of melatonin and cortisol. However, daily daytime caffeine intake might still weaken the circadian sleep signal under conditions of strong circadian REM sleep promotion. Moreover, the daily exposure to caffeine bears the risk of developing withdrawal symptoms as early as 8 hours after its last intake, such as increased sleepiness, worse performance, and subtle changes in nighttime sleep. Together these withdrawal-induced alterations point to changes primarily in the homeostatic component of sleep-wake regulation and might be attributed to differences in adenosine signaling. The circadian timekeeping system, however, stays rather stable under conditions of daily daytime caffeine intake. In summary, this thesis provides novel insights into the consequences of daily presence and nightly abstinence of the world’s most popular psychoactive substance on homeostatic and circadian measures of sleep-wake regulation.

List of abbreviations

ACh	Acetylcholine
ATP	Adenosine triphosphate
BF	Basal forebrain
CBT	Core body temperature
DMH	Dorsomedial nucleus of the hypothalamus
EEG	Electroencephalography
GABA	Y-aminobutyric acid
LC	Locus coeruleus
LH	Lateral hypothalamus
LDT	Laterodorsal tegmental nuclei
MCH	Melanin-concentrating hormone
NREM	Non-rapid eye movement
ORX	Orexin
PPT	Pedunculopontine
PSG	Polysomnography
REM	Rapid eye movement
SCN	Suprachiasmatic nucleus
SE	Sleep efficiency
SPZ	Subparaventricular zone
SWA	Slow-wave activity
SWS	Slow-wave sleep
TMN	Tuberomammillary nucleus
VLPO	Ventrolateral preoptic nucleus
WMZ	Wake-maintenance zone

1. Introduction

Caffeine is among the most widely consumed psychoactive substances around the globe (Fredholm, Bättig, Holmén, Nehlig, & Zvartau, 1999). Around 80% of the worldwide population consume caffeine on a daily basis (Heckman, Weil, & Gonzalez de Mejia, 2010). Empirical evidence suggests that caffeine intake is increasing in the population with respect of daily intake dosage and with earlier age of regular substance consumption (Roehrs & Roth, 2008). Caffeine containing aliments such as coffee or tea (Fredholm et al., 1999) are well-known for their wake-promoting (Einöther & Giesbrecht, 2013) but sleep-disrupting effects (Clark & Landolt, 2017). In line with this common perception, caffeine has been shown to increase alertness (James, 1998), to result in delayed and more superficial sleep (Landolt, Dijk, Gaus, & Borbély, 1995), and to phase delay melatonin secretion (Burke et al., 2015). These effects have been well documented in placebo-controlled studies during the past decades, particularly under the condition of acute and evening administration.

However, evening caffeine intake is not common in most caffeine consumers (Martyn, Lau, Richardson, & Roberts, 2018; Lieberman, Agarwal, & Fulgoni, 2019). Thus, the question arises whether caffeine-induced effects on sleep-wake regulatory processes also occur when consumption is timed to daytime hours, and whether these effects persist after daily caffeine consumption, an intake pattern which is highly prevalent (Heckman, Weil, et al., 2010). Moreover, repeated caffeine intake bears the risk of developing withdrawal symptoms once regular caffeine intake is ceased (Juliano & Griffiths, 2004). However, such potential consequences on sleep-wake regulation are not yet fully elaborated.

Based on the extremely high number of habitual caffeine consumers around the globe (Heckman, Weil, et al., 2010), it is crucial for public health to have a better understanding of the benefits and consequences of this highly prevalent stimulant on human sleep-wake regulation. Thus, the aim of the present thesis was to probe the influence of regular caffeine intake in the morning and afternoon hours and its acute cessation on subjective and objective measures of homeostatic and circadian sleep-wake regulation in healthy young regular consumers. In the following chapters, I will briefly describe how sleep and wakefulness are regulated and how its features are modulated by caffeine intake.

2. Theoretical background

2.1. Sleep-wake regulation

Sleep and wakefulness are regulated by a fine-tuned interplay of homeostatic and circadian processes as initially introduced by the two-process model of sleep regulation by Borbély (1982). Accordingly, the combined action of these two opponent processes allow maintaining wakefulness during the day and sleep during the night (Dijk & Czeisler, 1994) and together determine quality, quantity, and timing of wakefulness and sleep (Borbély, 1982; Daan, Beersma, & Borbély, 1984), see Figure 1.

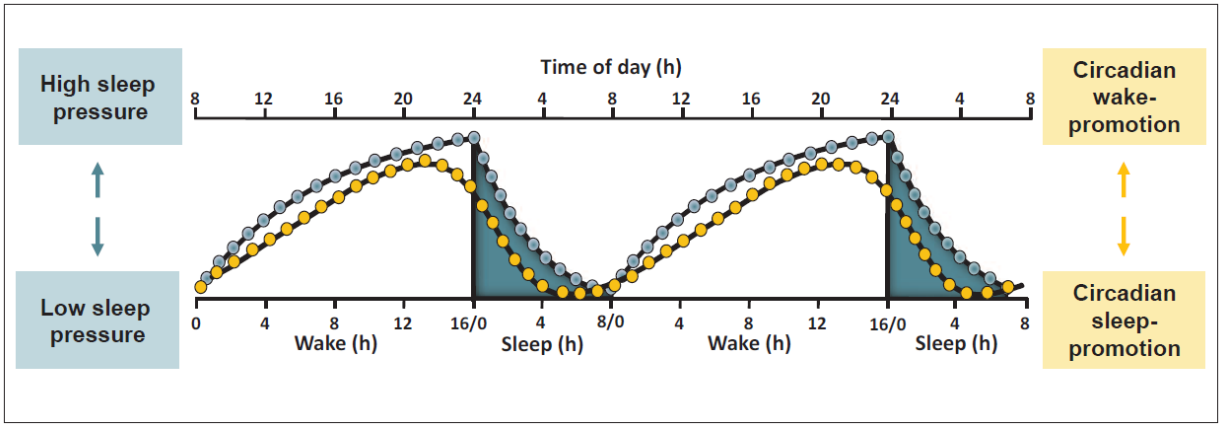

Figure 1. Illustration of sleep-wake regulation by the two-process model.
Sleep and wakefulness are regulated by a homeostatic and a circadian process which together determine timing, duration, and quality. The homeostatic process (process S; blue) increases depending on prior wake time and declines during sleep, relatively independent of the 24-hour cycle. In contrast, the circadian process (process C; yellow) oscillates with a periodicity of around 24 hours independent of prior sleep-wake history. Figure from (Reichert, Maire, Schmidt, & Cajochen, 2016) and legend adapted from (Maire, Reichert, & Schmidt, 2013).

2.1.1. Homeostatic process

Sleep homeostasis (also known as process S) is mirrored in increasing sleep need depending on prior waking time while dissipating during subsequent sleep, and thus determines sleep propensity and sleep intensity (Borbély, 1982), see Figure 1. Accordingly, sleep intensity is highest at the beginning of the night indexed as polysomnography (PSG)-derived slow-wave sleep (SWS) or more specifically electroencephalography (EEG)-derived slow-wave activity (SWA; power density in the range of 0.75-4.5 Hz), which shows a steady decline across the course of non-rapid eye movement (NREM) sleep episodes (Dijk & Czeisler, 1995). Sigma activity, also called spindle frequency activity (power density in the range of 12-16 Hz), represents a second marker of sleep intensity which however increases in the course of NREM sleep (Dijk & Czeisler, 1995; Dijk, Shanahan, Duffy, Ronda, & Czeisler, 1997) and thus shows reduced activity under enhanced sleep pressure (Borbély, Baumann, Brandeis, Strauch, & Lehmann, 1981; Dijk, Hayes, & Czeisler, 1993; Finelli, Borbély, & Achermann, 2001; Knoblauch, Martens, Wirz-Justice, & Cajochen, 2003). During wakefulness, EEG power in the theta range (5-8 Hz) reliably mirrors the homeostatic process showing an increase under

enhanced sleep pressure (Cajochen, Khalsa, Wyatt, Czeisler, & Dijk, 1999; Finelli, Baumann, Borbély, & Achermann, 2000; Cajochen, Knoblauch, Kräuchi, Renz, & Wirz-Justice, 2001) which is associated with the increase in SWA during sleep (Finelli et al., 2000). The build-up and degradation of homeostatic sleep need is linked with the neuromodulator adenosine, a so-called sleep factor (Porkka-Heiskanen, 2013), which mediates the somnogenic effects of prior time awake (Porkka-Heiskanen et al., 1997). Its functions have been attributed to energy metabolism, neural plasticity, and cellular defense (Porkka-Heiskanen, 2013).

2.1.2. Circadian process

The timing of wakefulness and sleep is set by the circadian (latin: circa = about, diem = day) pacemaker in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus (Moore & Eichler, 1972; Lydic, Schoene, Czeisler, & Moore-Ede, 1980). As depicted in Figure 1, this self-sustaining biological clock promotes wakefulness during the day and sleep during the night (Dijk & Czeisler, 1994) and is daily synchronized to the 24-hour day by zeitgebers (= time givers) such as light (Duffy & Wright, 2005), food intake (reviewed in Stephan, 2002), physical activity (Van Reeth et al., 1994; Buxton et al., 1997), or social cues (reviewed in Mistlberger & Skene, 2004).

This circadian process (also called process C) is present in many aspects of behaviour and physiology such as core body temperature (CBT) and the secretion of cortisol and melatonin (Lack & Wright, 2007; Broussard et al., 2017), which represent typical markers of the human pacemaker (Klerman, Gershengorn, Duffy, & Kronauer, 2002). Accordingly, CBT peaks in the evening and declines to a temperature nadir during the late biological night prior to awakening (Monk et al., 1997). In contrast, the levels of cortisol increase across the night reaching peak levels in the morning close to the end of the biological night and decrease during daytime (Broussard et al., 2017). The primary marker of the human timing system is the hormone melatonin (Klerman et al., 2002; Benloucif et al., 2005), also occasionally called the hormone of darkness (Arendt, 2005). Melatonin is synthesized in the pineal gland (Moller & Baeres, 2002) and starts to rise approximately two hours prior to habitual bedtime in entrained individuals (Wright, Gronfier, Duffy, & Czeisler, 2005). This so-called dim-light melatonin onset is measured under low light levels (Broussard et al., 2017). It serves as a reliable marker of the circadian phase (Klerman et al., 2002) and presents the key to the nocturnal gate of sleep (Shochat, Haimov, & Lavie, 1998). Accordingly, high melatonin levels mark the biological night and decrease to low levels after awakening (Wright et al., 2013).

Under entrained conditions, circadian wake-promotion ensures stable and continuous periods of wakefulness and is optimally tuned to counteract the increasing homeostatic sleep need during the course of the day (Dijk & Czeisler, 1994). Thereof, circadian wake-promotion is highest in the evening approximately one to three hours before habitual bedtime when sleep

pressure is high (Strogatz, Kronauer, & Czeisler, 1987; Dijk & Czeisler, 1994). During this time window, called wake-maintenance zone (WMZ) (Strogatz et al., 1987) or forbidden zone for sleep (Lavie, 1986), it is particular difficult to fall asleep and to maintain sleep (Strogatz et al., 1987; Dijk & Czeisler, 1994). Accordingly, sleep latency and sleep efficiency (SE) during the WMZ have been characterized as markers of the strength of the circadian wake-promoting signal (Münch et al., 2005; Reichert et al., 2017). On the contrary, the circadian system promotes sleep at the end of the biological night to counteract the decreasing sleep pressure by the homeostatic system (Dijk & Czeisler, 1995). In contrast to SWS and SWA depending on prior waking (Achermann, Dijk, Brunner, & Borbély, 1993), rapid eye movement (REM) sleep is strongly controlled by the circadian oscillator showing an increase across the night (Dijk & Czeisler, 1995) which peaks around two hours after the CBT nadir (Dijk & Czeisler, 1995).

2.1.3. Neural mechanisms of sleep-wake regulation

2.1.3.1. Wakefulness and sleep

In this chapter, I will briefly describe the underlying neural mechanisms of sleep and wakefulness to provide a better understanding of how these processes are modulated by caffeine. First mentioned by von Economo in 1916 and extensively reviewed in Saper, Scammell, and Lu (2005), the discrete bouts of sleep and wakefulness are regulated by an interaction of arousal and sleep-promoting systems. Wakefulness is regulated by an ascending arousal system which comprises specific cell groups and neurotransmitters located in the brainstem, hypothalamus, and basal forebrain (BF) (Saper et al., 2005). This system consists of two primary ascending pathways which activate the thalamus and cerebral cortex (Saper et al., 2005). The first arm originates in the upper brainstem, specifically in the cholinergic (ACh) pedunculopontine (PPT) and the laterodorsal tegmental nuclei (LDT), and arouses the thalamic relay and reticular nuclei (Hallanger, Levey, Lee, Rye, & Wainer, 1987), which transmit thalamic information to the cortex (Saper et al., 2005). The second major pathway arouses the cerebral cortex by several cell groups located in the upper brainstem and caudal hypothalamus (i.e., noradrenergic locus coeruleus (LC), serotoninergic raphe nuclei, dopaminergic ventral periaqueductal grey matter, and histaminergic tuberomammillary nucleus (TMN)) (Saper et al., 2005). The cortex receives further input from neurons in the lateral hypothalamus (LH; peptidergic neurons containing orexin (ORX) and melanin-concentrating hormone (MCH)), and from neurons in the BF (i.e., containing y-aminobutyric acid (GABA) or ACh) which together foster the processing of thalamic input (Saper et al., 2005).

In contrast to the two ascending pathways which support wakefulness, sleep is promoted by the ventrolateral preoptic nucleus (VLPO) in the anterior hypothalamus (Saper et al., 2005) of

which cells are particularly active during sleep (Sherin, Shiromani, McCarley, & Saper, 1996; Szymusiak, Alam, Steininger, & McGinty, 1998). These specific neurons in the VLPO contain the inhibitory neurotransmitters galanin and GABA and suppress the arousal promoting neurons in the hypothalamus and brainstem (i.e., ORX neurons, A10 cell groups, TMN, Raphe, LC, PPT, LDT) (Saper et al., 2005).

The transitions from sleep and wakefulness are regulated by a so-called flip-flop switch mechanism (Saper et al., 2005). According to this concept, the monoaminergic cell groups (i.e., LC, TMN, Raphe) promote wakefulness by inhibiting the sleep promoting neurons in the VLPO (Saper et al., 2005). In addition, the ORX neurons located in the LH act as a reinforcer of the arousal system and promote consolidated bouts of wakefulness (Saper et al., 2005). In contrast, during sleep, the neurons in the VLPO inhibit the arousal promoting monoaminergic cell groups and reinforce its own activation (Saper et al., 2005). Thus, the reciprocal inhibition of these arousal and sleep promoting systems allow discrete states of wakefulness and sleep while ORX neurons act as a stabilizer (Saper et al., 2005).

2.1.3.2. The role of adenosine in sleep homeostasis

Several key molecules have been proposed to be involved in sleep homeostasis such as the neuromodulator adenosine, the cytokine tumor necrosis factor-alpha, and the neuronal growth factor BDNF (Porkka-Heiskanen, 2013). Evidence accumulates that adenosine plays a crucial role in the homeostatic regulation of sleep and wakefulness (Landolt, 2008) acting as an endogenous somnogen (Porkka-Heiskanen, Alanko, Kalinchuk, & Stenberg, 2002). Based on the energy hypothesis of sleep, during wakefulness neuronal activity requires energy in form of adenosine triphosphate (ATP) which results in energy depletion and as a consequence increases extracellular adenosine levels, a byproduct of the breakdown of ATP (Porkka-Heiskanen, Zitting, & Wigren, 2013). In line with this hypothesis, in animals extracellular adenosine levels have been shown to increase in the brain with prior waking, particularly in the BF, and to decrease during recovery sleep (Porkka-Heiskanen et al., 1997; Basheer, Porkka-Heiskanen, Stenberg, & McCarley, 1999) while the injection of exogenous adenosine promoted sleep (Portas, Thakkar, Rainnie, Greene, & McCarley, 1997). These effects of adenosine on sleep and wakefulness have been mainly attributed to its impact on the A_1 adenosine receptors (Porkka-Heiskanen et al., 2002; Landolt, 2008). Accordingly, by binding to the inhibitory A_1 adenosine receptor, the neuromodulator blocks the wake-promoting neurons such as the cholinergic cells groups in the BF or the ORX neurons in the LH which reduces cortical activity (Strecker et al., 2000; Thakkar, Delgiacco, Strecker, & McCarley, 2003; Liu & Gao, 2007; Porkka-Heiskanen et al., 2013). Moreover, the A_1 adenosine receptor might excite the sleep promoting neurons in the VLPO by reducing inhibitory GABAergic input (Chamberlin et al., 2003; Morairty, Rainnie, McCarley, & Greene, 2004). However, evidence is

growing that also the excitatory A_{2A} adenosine receptor plays a role in the regulation of sleep and wakefulness. In animals, adenosine excited the sleep-promoting neurons in the VLPO via A_{2A} adenosine receptors (Gallopin et al., 2005) and also the injection of an agonist of this receptor type promoted sleep (Scammell et al., 2001; Hong et al., 2005). In summary, adenosine presumably acts as a sleep-substance accumulating during time awake and inhibits the wake-promoting cell groups while disinhibits the sleep-promoting cell groups, which allows in the end the initiation of sleep (Saper et al., 2005).

2.1.3.3. The projections of the SCN

The circadian process is controlled by the master clock in the SCN of the anterior hypothalamus (Moore & Eichler, 1972; Lydic et al., 1980), which neurons fire with a rhythm of 24 hours based on a transcriptional/translational feedback loop (Reppert & Weaver, 2002). The SCN is synchronized to the solar day by environmental cues such as light, which serves as the strongest zeitgeber (Golombek & Rosenstein, 2010). Accordingly, our “internal master clock” receives photopic stimuli through the retinohypothalamic tract by intrinsically photosensitive retinal ganglion cells which contain the photopigment melanopsin (Hattar, Liao, Takao, Berson, & Yau, 2002).

Importantly, the SCN has only modest and primarily indirect projections to the wake and sleep-promoting neurons (Saper, 2013). As illustrated in Figure 2, signals which originate in the SCN project to the ventral and dorsal subparaventricular zone (SPZ), the latter one regulating body temperature (Saper et al., 2005). The vSPZ relays information for the dorsomedial nucleus of the hypothalamus (DMH) which regulates sleep by GABAergic projections to the VLPO and wakefulness by glutamatergic projections to the ORX neurons and MCH neurons in the LH (Saper et al., 2005). The DMH also regulates feeding, locomotor activity, and the secretion of corticosteroids (Saper et al., 2005).

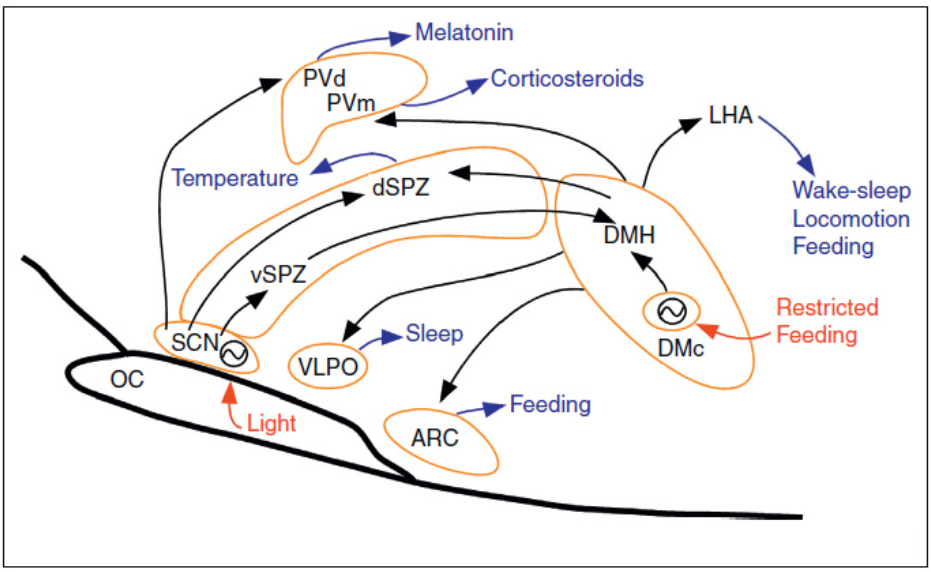

Figure 2. A schematic illustration of the main components of the circadian timing system in the mammalian brain. Arrows depict the neural pathways which convey these influences, however they do not imply whether these pathways are excitatory or inhibitory. The neurons of the suprachiasmatic nucleus (SCN) represent a genetically based clock which is daily reset by the light cycle. The SCN regulates certain circadian rhythms, i.e. melatonin secretion, by direct outputs to target cell groups such as the paraventricular nucleus (PV), however most circadian rhythms are mediated by relays through the subparaventricular zone (SPZ). Body temperature is regulated by the dorsal SPZ, but its pathway is unknown. The ventral SPZ (vSPZ) drives the dorsomedial nucleus of the hypothalamus (DMH), which in turn regulates circadian rhythms of wake-sleep, locomotion, feeding, secretion of corticosteroids but can also reset body temperature. ARC, arcuate hypothalamic nucleus; DMc, compact part of the DMH; OC, optic chiasm; LHA, lateral hypothalamic area; PV, paraventricular hypothalamic nucleus; PVd, dorsal parvicellular PV; PVm, medial parvicellular PV; nucleus VLPO, ventrolateral preoptic nucleus. Zeitgebers are marked in red, cell groups in tan and circadian functions in blue. Figure and legend adapted from (Saper, 2013).

2.2. Caffeine

Caffeine derives from the German word *Kaffee* (= coffee) based on its first chemical isolation by the German chemist Friedrich Ferdinand Runge on behalf of his friend Johann Wolfgang von Goethe in 1819 (Clark & Landolt, 2017). It is a natural occurring compound present in beans, leaves, and fruits of over 60 plants (Heckman, Weil, et al., 2010), and today commonly known as the stimulating property of coffee.

2.2.1. Sources and consumption

Historically, the use of caffeine dates back to 2737 BC when a Chinese Emperor consumed caffeine in the form of tea (Arab & Blumberg, 2008) while coffee is said to be discovered in the 9th century by a shepherd consuming wild coffee berries in Ethiopia (Heckman, Weil, et al., 2010). Today, caffeine is among the most popular psychoactive substances around the world (Fredholm et al., 1999). Up to 85% of the population in the United States (Mitchell, Knight, Hockenberry, Teplansky, & Hartman, 2014) and 90% in Switzerland report its intake (Tinguely, Landolt, & Cajochen, 2014) while approximately 80% of the worldwide population consume caffeine on a daily basis (Heckman, Weil, et al., 2010). Caffeine is found in a wide variety of

common dietary products (i.e., coffee, tea, soda drinks, energy drinks, chocolate) as well as over the counter medications (i.e., pain relievers) (Snel & Lorist, 2011). The compound is additionally added to various nutritional products such as bottled water, ice cream, yoghourt, cereals, milk, beef, or to body care products (i.e., cosmetics, soap, or shampoo) (James, 2014). On average a standard filtered cup of coffee (125 ml) contains 80 mg; an espresso (50 ml) 55 mg; regular soda drinks (300 ml) 22 mg, and energy drinks (300 ml) approximately 90 mg of caffeine based on (Snel & Lorist, 2011). However, the caffeine contents found in food and beverages varies largely based on the brand, preparation method, and plant source (Mandel, 2002; McCusker, Goldberger, & Cone, 2003). In adults, most of the caffeine sources derive from coffee and tea while in children and adolescents tea and soda drinks are among the most consumed caffeinated products (Verster & Koenig, 2018). At the same time the consumption of so-called energy drinks is increasing since the last century, products which are predominantly consumed by teenagers and young adults (Heckman, Sherry, & de Mejia, 2010). However, the consumption pattern differs largely between countries such as North Americans consume around 200 mg of caffeine/day while the average caffeine intake in Switzerland and in Northern European countries is estimated at ≥ 300 mg/day (Heckman, Weil, et al., 2010). In adults, caffeine is commonly consumed in the morning and/or afternoon hours (Lieberman et al., 2019), a consumption pattern which stays fairly stable across the week (Martyn et al., 2018). Thus, caffeine presents an ubiquitous component of most people's daily dietary around the globe, presumably due to its easy accessibility and affordability.

2.2.2. Pharmacological and neural underpinnings of caffeine intake

In humans, caffeine is rapidly absorbed from the gastrointestinal tract into the bloodstream (Nawrot et al., 2003), already detectable in plasma within 5 minutes (Grab & Reinstein, 1968), with concentrations peaking between 30 and 75 minutes after oral ingestion (Mandel, 2002). Following absorption, caffeine is distributed throughout the body and crosses all biological membranes such as the blood-brain-barrier (Snel & Lorist, 2011). In the liver caffeine is metabolized via the cytochrome P-450 enzymes (i.e., CYP1A2) to the three primary dimethylxanthines paraxanthine (84%), theobromine (12%), and theophylline (4%) and is later excreted in the urine (Nehlig, 2018). The half-life of caffeine varies between 2 to 10 hours with an average of 4 hours (Snel & Lorist, 2011) which is modulated by several endogenous and exogenous factors such as nicotine use (Joeres et al., 1988), oral contraceptives (Abernethy & Todd, 1985; Balogh et al., 1995), menstrual cycle (Lane, Steege, Rupp, & Kuhn, 1992), pregnancy (Grosso & Bracken, 2005), and genetic polymorphisms (Han et al., 2001).

The stimulatory effects of caffeine are mainly ascribed to its influence on the adenosinergic system (Fredholm et al., 1999) and thus related to sleep homeostasis (Landolt, 2008). As described in an earlier chapter, adenosine is proposed to be involved in the regulation of

arousal and sleep by inhibiting arousal promoting neurons while exciting sleep promoting neurons in the VLPO (Saper et al., 2005). Caffeine acts as a non-selective adenosine receptor antagonist and primarily targets the A_1 and A_{2A} adenosine receptors of the four known subtypes (i.e., A_1, A_{2A}, A_{2B}, A_3) (Fredholm et al., 1999; Ferré, 2008). Both targeted receptors show high affinity for adenosine (Borea, Gessi, Merighi, Vincenzi, & Varani, 2018) and are predominantly located in the central nervous system (Ferré, 2008). While the A_1 adenosine receptor is found in almost all brain regions (particularly in the hippocampus, cerebral and cerebellar cortex, thalamic nuclei), the A_{2A} adenosine receptor is primarily expressed in dopaminergic areas (i.e., striatum, nucleus accumbens, and olfactory tubercle) (Snel & Lorist, 2011). Regular dietary caffeine intake has the potential to block 50% of the A_1 and A_{2A} adenosine receptors found in the brain (Fredholm, Chen, Masino, & Vaugeois, 2005; Elmenhorst, Meyer, Matusch, Winz, & Bauer, 2012). Importantly, as caffeine primarily antagonizes the inhibitory (A_1) and facilitatory (A_{2A}) adenosine receptors (Ferré, 2008), the stimulant indirectly modulates the release of various neurotransmitters such as noradrenaline, dopamine, acetylcholine, serotonin, glutamate, and GABA (Fredholm et al., 1999).

2.3. The impact of caffeine on sleep-wake regulation

Caffeine is used since centuries to modulate sleep and wakefulness (Camandola, Plick, & Mattson, 2019). However, its empirical research gained momentum in 1909 when the federal government of the United States filed a lawsuit against the company Coca-Cola accusing them of adding the deleterious ingredient caffeine to their products (Benjamin, Rogers, & Rosenbaum, 1991). This lawsuit led to the first well-controlled studies about the effects of caffeine on performance conducted by Hollingworth who concluded that caffeine does not have negative consequences (Benjamin et al., 1991) except that in some individuals caffeine may lead to poorer sleep quality and reduced sleeping time (Hollingworth, 1912). Since then, the effects of caffeine on subjective and objective measures of sleep-wake regulation under various conditions have been extensively studied. In the following chapters, I will outline the present empirical evidence on its influence on waking performance (i.e., sleepiness and vigilance performance), sleep, and measures of the circadian timing system.

2.3.1. Waking performance

Caffeine is widely known for its alerting and performance enhancing effects (Snel & Lorist, 2011; Einöther & Giesbrecht, 2013), which are one reason for the daily use of caffeine by the worldwide population (Heckman, Weil, et al., 2010) such as by athletes (Del Coso, Muñoz, & Muñoz-Guerra, 2011), students (Franke et al., 2011; Norton, Lazev, & Sullivan, 2011), and shift workers (Dekker, Paley, Popkin, & Tepas, 1993). In line with this common perception, empirical evidence indicates that acute caffeine intake enhances alertness and performance

in low- or non-consumers (Childs & de Wit, 2006; Smith, Christopher, & Sutherland, 2013), and in habitual caffeine consumers after a wash-out period of several days (James, 1998; Smith et al., 2013) but see (James, 1998; Keane, James, & Hogan, 2007). These wake-promoting effects are primarily attributed to the antagonism of adenosine (Fredholm et al., 1999), which presumably dampens the increasing homeostatic sleep need across waking hours (Landolt, 2008). Hence, the stimulating properties have been particularly reported after sleep restriction or sleep deprivation when sleep pressure is increased (Roehrs & Roth, 2008; Snel & Lorist, 2011). Accordingly, acute caffeine consumption affected the dynamics of sleep pressure as evident in reduced EEG theta activity, an objective marker of sleepiness, which was stronger after 23 hours compared to 11 hours of wakefulness in comparison to placebo intake (Landolt et al., 2004). In line with this finding, caffeine intake between 42 to 64 hours of continuous wakefulness improved vigilance performance (Kamimori et al., 2000; Wesensten et al., 2002; Wesensten, Killgore, & Balkin, 2005; Killgore et al., 2008; Paech et al., 2016) and reduced subjectively rated sleepiness (Penetar et al., 1993; Kamimori et al., 2000; Wesensten et al., 2002) compared to placebo in volunteers who abstained from caffeine for minimum three days. However, commonly low to moderate caffeine consumers were studied which have been caffeine-deprived prior to the assessments while the administered caffeine doses commonly exceeded volunteer's habitual caffeine consumption (Wesensten et al., 2002; Wesensten et al., 2005; Killgore et al., 2008; Paech et al., 2016). There is growing evidence that the stimulating effects might vanish after repeated caffeine exposure. After one week of caffeine intake, the alerting effect diminished in well-rested volunteers compared to acute caffeine intake (James, 1998) and no benefits in sleepiness were perceived compared to placebo intake in sleep-restricted volunteers (Keane & James, 2008). Moreover, the lack of improvement in sleepiness and vigilance in moderate compared to low consumers provides some indications for tolerance development to the stimulating effects of caffeine (Rogers, Heatherley, Mullings, & Smith, 2013) but see (Haskell, Kennedy, Wesnes, & Scholey, 2005).

2.3.2. Sleep

Beside caffeine's proposed beneficial effects on alertness and performance (reviewed in Smith, 2002; Einöther & Giesbrecht, 2013), the stimulant can have detrimental consequences on subsequent sleep (reviewed in Clark & Landolt, 2017). Caffeine intake, particularly when consumed close to nighttime sleep, has been repeatedly shown to delay sleep initiation (Bonnet & Arand, 1992; Landolt, Dijk, et al., 1995; Drapeau et al., 2006; Carrier et al., 2007; Robillard, Bouchard, Cartier, Nicolau, & Carrier, 2015) and to reduce total sleep time (Nicholson & Stone, 1980; Bonnet & Arand, 1992; Landolt, Dijk, et al., 1995; Drapeau et al., 2006; Drake, Roehrs, Shambroom, & Roth, 2013; Robillard et al., 2015). Moreover, in line with the proposed role of adenosine in sleep homeostasis (Landolt, 2008), there is compelling

evidence that caffeine interferes with sleep pressure resulting in shorter deep sleep (Nicholson & Stone, 1980; Bonnet & Arand, 1992; Landolt, Dijk, et al., 1995; Carrier et al., 2007; Drake et al., 2013; Robillard et al., 2015), reduced EEG activity in the delta frequencies (Landolt, Dijk, et al., 1995; Drapeau et al., 2006) while increased activity in the sigma frequencies (Landolt, Dijk, et al., 1995; Drapeau et al., 2006; Robillard et al., 2015), which represent typical markers of homeostatic sleep intensity (Landolt, 2008). These sleep-disrupting effects were observed after evening dosages ranging from the equivalent of one cup of coffee to approximately four cups of coffee (Nicholson & Stone, 1980; Landolt, Dijk, et al., 1995; Drapeau et al., 2006; Carrier et al., 2007; Drake et al., 2013; Robillard et al., 2015). However, adults consume caffeine predominantly in the morning and afternoon hours while evening consumption is not common (Martyn et al., 2018; Lieberman et al., 2019), particularly after 9 pm (Lieberman et al., 2019), presumably to prevent adverse consequences on nocturnal sleep (Snel & Lorist, 2011). Whether caffeine consumed in the morning and/or afternoon hours disrupts nighttime sleep similarly as evening caffeine consumption is not fully established yet.

In addition to the caffeine-induced impact on the typical sleep features of sleep homeostasis, i.e. SWA and SWS, there are as well indications that caffeine interferes with REM sleep, which is strongly controlled by the circadian oscillator (Dijk & Czeisler, 1995). Caffeine-induced reductions or shifts in REM sleep episodes were particularly seen when utilizing high caffeine dosages (i.e., the equivalent of ≥ 3 cups of coffee) (Karacan et al., 1976; Nicholson & Stone, 1980; Robillard et al., 2015) or when sleep was scheduled to daytime hours (Carrier et al., 2007; Carrier et al., 2009).

These caffeine-induced alterations on sleep architecture seem to be a good determinant for the perceived sleep quality. Volunteers in four out of five studies which observed an acute caffeine-induced impact on PSG-derived sleep measures (Karacan et al., 1976; Nicholson & Stone, 1980; Bonnet & Arand, 1992; Landolt, Dijk, et al., 1995; Drake et al., 2013), reported an deterioration of subjectively perceived sleep upon awakening (Karacan et al., 1976; Nicholson & Stone, 1980; Bonnet & Arand, 1992; Drake et al., 2013) which might play a crucial role in promoting daily caffeine consumption.

So far, it is rather unclear if caffeine-induced sleep disruptions persist if caffeine is taken repeatedly every day. After one week of daily caffeine consumption, the sleep disrupting effects mainly vanished, even under the condition of high evening caffeine intake with only sleep stage 4 remaining strongly affected while no difference in sleep quality was perceived (Bonnet & Arand, 1992). Whether more sensitive markers such as EEG-derived sleep markers adapt as well to the long-term exposure of the stimulant has to the best of our knowledge not yet been investigated. This is important as repeated daytime intake is very common in regular consumers (Martyn et al., 2018; Lieberman et al., 2019). An adaptation would represent an endogenous coping mechanism and eventually disrupt the vicious cycle of caffeine-induced

nighttime sleep disturbances leading to increased daytime sleepiness which in turn promotes caffeine consumption the next day (Roehrs & Roth, 2008; Snel & Lorist, 2011).

2.3.3. Circadian timing system

Homeostatic and circadian processes interact on various physiological and behavioural levels and there is evidence that adenosinergic mechanisms mediate the effects on the circadian timing system (Reichert et al., 2016). In animal models, the A_3 adenosine receptor plays a role in the circadian regulation of temperature (Yang, Wang, Garcia-Roves, Bjornholm, & Fredholm, 2010) while the A_1 adenosine receptor is involved in the circadian phase shifting response to light (Watanabe et al., 1996; Elliott, Todd Weber, & Rea, 2001). Moreover, in rats adenosine and adenosine receptor agonists increased melatonin levels in the pineal gland (Gharib et al., 1989). Thus, the adenosine receptor antagonist caffeine might substantially alter the timekeeping system.

In humans, acute caffeine intake in the evening and during nighttime hours suppressed melatonin secretion (Wright, Badia, Myers, Plenzler, & Hakel, 1997; Wright, Myers, Plenzler, Drake, & Badia, 2000) and delayed the melatonin onset on average by 40 minutes (Burke et al., 2015). Such caffeine-induced phase shifts could have detrimental consequences on sleep initiation and consequently reduce sleep duration when adhered to fixed wake times due to social and professional demands. These circumstances may further contribute to the vicious cycle of disrupted sleep which in turn promotes daily caffeine intake. However, repeated hourly caffeine intake during the waking hours did not alter melatonin levels and CBT but it strengthened circadian wake-promotion, indexed in reduced SE when sleep was timed during the WMZ (Wyatt, Cajochen, Ritz-De Cecco, Czeisler, & Dijk, 2004). Importantly, data in the latter study were averaged over four weeks, thus it cannot be excluded that melatonin and CBT developed tolerance across the laboratory stay. However, empirical evidence on cortisol secretion suggests that the circadian system does not fully adapt to chronic caffeine exposure. While an acute one-time caffeine administration clearly affected cortisol secretion by elevating its level up from 60 to 120 minutes after oral administration in well-rested volunteers (Lovallo, Al'Absi, Blick, Whitsett, & Wilson, 1996), repeated intake over five days led to partial tolerance development such as cortisol response was diminished but not absent (Lovallo et al., 2005). However, it is important to note that tolerance development was more complete when the prior intake dose and the challenge dose were similar (Lovallo et al., 2005), which presumably represents a common intake pattern in coffee drinkers (Martyn et al., 2018). Thus, whether the circadian timing system adapts to the long-term changes in the adenosinergic system is not yet fully understood. Based on the growing trend to consume the stimulant (Roehrs & Roth, 2008), it is crucial to have a better understanding about the effects of chronic caffeine intake

on circadian measures, particularly when administered in the morning and afternoon hours, in order to mimic an everyday situation in most consumers.

2.4. The impact of caffeine withdrawal

Importantly, not only caffeine per se bears the risk of affecting human behaviour and physiology but also its concomitantly associated caffeine withdrawal. The abrupt cessation of regular caffeine intake induces a wide range of withdrawal symptoms such as headache, decreased alertness, drowsiness, reduced contentedness, depressed mood, irritability, and impaired performance while the former one represents the most frequent one with an incidence of approximately 50% (Juliano & Griffiths, 2004). These symptoms occur after as little as 100 mg of caffeine per day (i.e., the equivalent of one cup of coffee) (Griffiths et al., 1990) but the severity depends on the dose of prior intake (Evans & Griffiths, 1999). Typically, symptoms develop 12 to 24 hours after the last caffeine intake reaching peak levels between 20 and 51 hours of caffeine abstinence and last for maximal 9 days (Juliano & Griffiths, 2004). During wakefulness, the observed increases in EEG theta activity after acute caffeine abstinence (Keane et al., 2007; Sigmon, Herning, Better, Cadet, & Griffiths, 2009) may be linked with the commonly observed enhanced drowsiness and impaired alertness (Sigmon et al., 2009) and presumably mirror increased homeostatic sleep need due to altered or adapted adenosine sensitivity following chronic caffeine intake (Ferré, 2008). Withdrawal symptoms thus express a state of reduced arousal and presumably contribute to the reinforcing properties of caffeine and promote its daily use which in turn alleviate withdrawal symptoms (Juliano & Griffiths, 2004). Nonetheless, in contrast to the well-investigated effects of caffeine withdrawal during waking hours, its impact on sleep remains fairly unknown. Although volunteers reported a better subjective sleep quality in terms of longer sleep duration and more sound sleep after approximately 32 hours of caffeine abstinence (James, 1998), Bonnet and Arand (1992) did not systematically report any effect of nighttime sleep architecture after about 25 hours of caffeine abstinence following seven days of high caffeine use (1200 mg/day) while more sensitive measures such as spectral power were not assessed. Moreover, the impact of caffeine withdrawal on markers of the circadian timing system i.e., hormonal rhythms of melatonin and cortisol have to the best of our knowledge not yet been investigated. Therefore, in the present thesis the focus was also set on caffeine withdrawal as a) the influence of caffeine withdrawal on measures of homeostatic and circadian processes are not yet fully elaborated and b) withdrawal symptoms likely represent a sign of adaptation to regular intake of caffeine which comes to light as soon as consumption is stopped.

3. Research questions and approach of the thesis

The overarching aim of the present thesis was to investigate whether regular caffeine consumption in the morning and afternoon hours change homeostatic and circadian sleep-wake measures, and how they are influenced by caffeine's acute cessation. To answer this question, we recruited twenty male, habitual caffeine consumers (mean age: 26.4 ± 4 years, BMI: 22.7 ± 1.4 kg/m^2, daily caffeine consumption: 478.1 ± 102.8 mg) and employed a double-blind randomized within-subject study design. Each volunteer completed three conditions: a caffeine (3 x daily caffeine), a withdrawal (3 x daily caffeine followed by a switch to placebo), and a placebo condition (3 x daily placebo), each lasting 11 days, see Figure 3. After nine days of continuous ambulatory caffeine (vs placebo) intake, we assessed subjective sleepiness and vigilance performance during scheduled wakefulness in a 43-h laboratory protocol as depicted in Figure 3. These data were combined with the evaluation of the diurnal profiles of the circadian hormones melatonin and cortisol, which allow assessing changes in the circadian timing system. In addition, physiological measures i.e. EEG-derived sleep structure and sleep intensity during sleep episodes scheduled at different times of the day were recorded (i.e., habitual nighttime sleep, nap during WMZ, or sleep shifted to morning hours). The combination of these techniques, measured during scheduled wakefulness and scheduled sleep under stringently controlled laboratory conditions, offered a unique and comprehensive view on the consequences of long-term daytime exposure to caffeine and its cessation on behavioural, hormonal, and physiological parameters of homeostatic and circadian sleep-wake regulation. Based on the described available literature, the aim of the present thesis was to answer the following main questions:

1. Does habitual caffeine intake in the morning and afternoon hours interfere with sleep homeostasis by reducing sleep depth, indexed by shortened SWS duration and reduced SWA assessed during nighttime sleep compared to placebo? Moreover, how does acute caffeine cessation affect nighttime sleep structure and intensity compared to placebo and caffeine? **(chapter 4.1.)**

2. Does daily daytime caffeine consumption and its cessation affect circadian timing (i.e., diurnal secretion profiles of melatonin and cortisol), and does it impact circadian wake-promotion assessed in the evening compared to placebo? **(chapter 4.2.)**

3. Does daily caffeine intake lead to benefits in waking performance (i.e., subjective sleepiness and psychomotor vigilance), and how is waking performance affected by acute caffeine cessation compared to continuous placebo intake? **(chapter 4.2.)**

4. Does regular daytime caffeine intake and its acute cessation change circadian regulated REM sleep promotion when sleep is shifted to morning hours in comparison to continuous placebo intake? Furthermore, do potential changes in REM sleep relate to perceived subjective sleep quality compared to placebo intake? **(chapter 4.3.)**

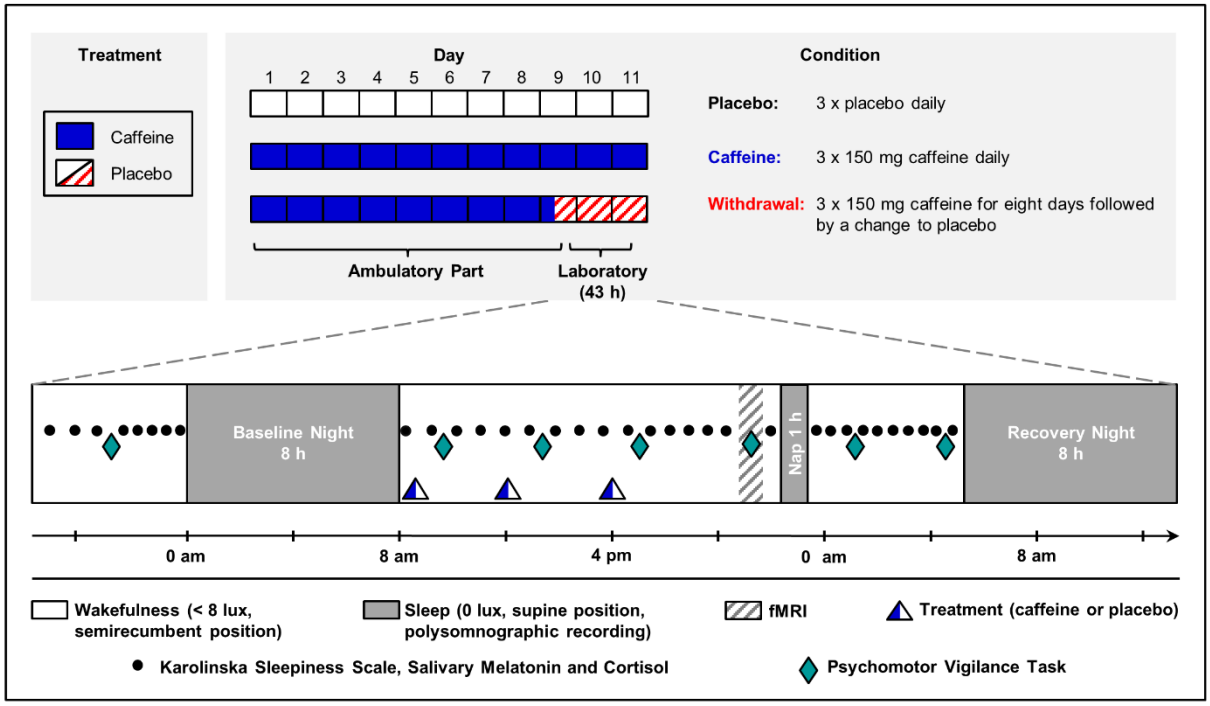

Figure 3. Illustration of the study protocol.
The present within-subject design comprised three treatments: a caffeine, a placebo, and a withdrawal condition consisting of an ambulatory part of nine days and an in-lab part of 43 hours. In each condition, volunteers either ingested caffeine or placebo capsules 3 x daily over a period of 11 days. Starting in the evening of day nine of treatment, behavioural, hormonal, and physiological measures during both wakefulness and sleep were continuously assessed under controlled laboratory conditions. Figure modified from (Weibel, Lin, Landolt, Garbazza, et al., 2020).

4. Research publications

In the following chapter, I will present three manuscripts on the effects of regular daytime caffeine intake and its cessation on subjective and objective measures of sleep-wake regulation. To all manuscripts I contributed as a first-author, which included: preparing and conducting the experimental study, collecting and analyzing the data as well as writing of the manuscripts.

4.1. The impact of daily caffeine intake on nighttime sleep in young adult men: Signs of overnight withdrawal?

Weibel, J., Lin, Y.-S., Landolt, H.-P., Kistler, J., Rehm, S., Rentsch, K.M., Slawik, H., Borgwardt, S., Cajochen, C., Reichert, C.F.

Submitted for publication, 2020
Published on the preprint server bioRxiv: https://doi.org/10.1101/2020.05.26.114769

4.2. Caffeine-dependent changes of sleep-wake regulation: Evidence for adaptation after repeated intake

Weibel, J., Lin, Y.-S., Landolt, H.-P., Garbazza, C., Kolodyazhniy, V., Kistler, J., Rehm, S., Rentsch, K., Borgwardt, S., Cajochen, C., Reichert, C.F.

Published in Progress in Neuropsychopharmacology & Biological Psychiatry, 2020
https://doi.org/10.1016/j.pnpbp.2019.109851

4.3. Regular caffeine intake attenuates REM sleep promotion and sleep quality in healthy men

Weibel, J., Lin, Y.-S., Landolt, H.-P., Berthomier, C., Brandewinder, M., Kistler, J., Rehm, S., Rentsch, K.M., Meyer, M., Borgwardt, S., Cajochen, C., Reichert, C.F.

Submitted for publication, 2020
Published on the preprint server bioRxiv: https://doi.org/10.1101/2020.09.18.291039

4.1. The impact of daily caffeine intake on nighttime sleep in young adult men: Signs of overnight withdrawal?

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Submitted for publication, 2020

Abstract

Acute caffeine intake can delay sleep initiation and reduce sleep intensity, particularly when consumed in the evening. However, it is not clear whether these sleep disturbances disappear when caffeine is continuously consumed during daytime, which is common for most coffee drinkers. To address this question, we investigated the sleep of twenty male young habitual caffeine consumers during a double-blind, randomized, crossover study including three 10-day conditions: caffeine (3 x 150 mg caffeine daily), withdrawal (3 x 150 mg caffeine for eight days, then switch to placebo), and placebo (3 x placebo daily). After nine days of continuous treatment, electroencephalographically (EEG)-derived sleep structure and intensity were recorded during a scheduled 8-h nighttime sleep episode starting 8 (caffeine condition) and 15 h (withdrawal condition) after the last caffeine intake. Upon scheduled wake up time, subjective sleep quality and caffeine withdrawal symptoms were assessed. Unexpectedly, neither polysomnography-derived total sleep time, sleep latency, sleep architecture, nor subjective sleep quality differed among placebo, caffeine, and withdrawal conditions. Nevertheless, EEG power density in the sigma frequencies (12-16 Hz) during non-rapid eye movement sleep was reduced in both caffeine and withdrawal conditions when compared to placebo. These results indicate that daily caffeine intake in the morning and afternoon hours does not strongly impair nighttime sleep structure or subjective sleep quality in healthy good sleepers who regularly consume caffeine. The reduced EEG power density in the sigma range might represent early signs of overnight withdrawal from the continuous presence of the stimulant during the day.

Keywords

Caffeine, habitual, caffeine cessation, polysomnography, EEG, spectral analysis

1. Introduction

Caffeine is the most popular psychoactive substance in the world (Fredholm et al., 1999), consumed daily by around 80% of the population (Heckman, Weil, et al., 2010). While caffeine is frequently used to counteract sleepiness and boost performance (Snel & Lorist, 2011), its consumption is commonly avoided in the evening (Martyn et al., 2018; Lieberman et al., 2019) to prevent adverse consequences on nocturnal sleep (Snel & Lorist, 2011). The sleep disrupting effects of caffeine are mainly attributed to its influence on the homeostatic component of sleep-wake regulation. Sleep homeostasis describes the increase in sleep pressure during time awake and its dissipation during the following sleep episode (Borbély, 1982), which has been suggested to be related to rising and decreasing concentrations of adenosine (Porkka-Heiskanen, 2013). Caffeine is an adenosine receptor antagonist, which blocks the A_1 and A_{2A} adenosine receptors in the central nervous system (Fredholm et al., 1999). It may, thus, attenuate the increase in sleep pressure during wakefulness (Landolt, 2008) and lead to delayed sleep initiation and more superficial sleep (Clark & Landolt, 2017).

The effects of caffeine intake on the quality and quantity of sleep depend on the timing of its consumption. More specifically, caffeine consumed in the evening hours prolongs sleep latency (Bonnet & Arand, 1992; Landolt, Dijk, et al., 1995; Drapeau et al., 2006; Carrier et al., 2007; Robillard et al., 2015), reduces total sleep time (TST) (Bonnet & Arand, 1992; Landolt, Dijk, et al., 1995; Drapeau et al., 2006; Drake et al., 2013; Robillard et al., 2015), shortens deep sleep (Bonnet & Arand, 1992; Landolt, Dijk, et al., 1995; Carrier et al., 2007; Drake et al., 2013; Robillard et al., 2015), and decreases electroencephalographically (EEG)-derived slow-wave activity (SWA) (Landolt, Dijk, et al., 1995), while activity in the sigma range is increased (Landolt, Dijk, et al., 1995). However, evening caffeine intake only accounts for approximately 10-20% of the total daily caffeine intake in regular consumers (Martyn et al., 2018; Lieberman et al., 2019). It needs to be elucidated whether habitual caffeine intake restricted to the morning and afternoon hours similarly affects nighttime sleep.

Furthermore, not only the timing but also the frequency of preceding caffeine intake prior to sleep may be an important factor for the repercussions on sleep. The majority of the worldwide population consumes caffeine on a daily basis (Heckman, Weil, et al., 2010), which can lead to tolerance development due to the recurrent supply of the psychostimulant (Fredholm et al., 1999). In line with these results, the sleep-disrupting effects of continuous high-dose caffeine intake in the morning, afternoon, and evening (3 x 400 mg) vanished and only stage 4 sleep remained reduced after one week of caffeine intake (Bonnet & Arand, 1992). However, whether more sensitive markers for sleep intensity such as spectral sleep EEG measures, adapt to the long-term exposure to the stimulant has to our best knowledge not yet been investigated.

Importantly, not only caffeine *per se*, but also the state of acute abstinence to which regular consumers expose themselves every night, might affect sleep. This so-called overnight

abstinence represents the start of a caffeine withdrawal phase (James & Rogers, 2005). Withdrawal symptoms such as increased tiredness (Juliano & Griffiths, 2004), longer sleep duration and better sleep quality (James, 1998) can be observed at a subjective level starting roughly 12 h after last caffeine intake (Juliano & Griffiths, 2004). However, the influence of caffeine withdrawal on objective EEG-derived sleep variables were not systematically reported up to date and remain to be compared against a placebo-baseline.

Here we aimed at determining whether daily caffeine intake during morning and afternoon hours impairs nighttime sleep structure and sleep intensity after continuous daytime caffeine intake over nine days. We hypothesized a reduced depth of sleep after caffeine intake, indexed in shortened slow-wave sleep (SWS) duration and a decrease in SWA compared to placebo. Moreover, we hypothesized that the abrupt cessation from the daily intake generates acute subjective withdrawal symptoms, and changes sleep structure and intensity compared to both the daily caffeine intake and the placebo-baseline.

2. Methods

2.1. Participants

Twenty male volunteers were recruited into the present study through online advertisements and flyers distributed in public areas. Interested individuals aged between 18 and 32 years old (mean age ± SD: 26.4 ± 4 years) and reporting a daily caffeine consumption between 300 and 600 mg (mean intake ± SD: 478.1 ± 102.8 mg) were included. The self-rating assessment for the daily amount of caffeine intake was structured based on Bühler et al. (Bühler, Lachenmeier, Schlegel, & Winkler, 2013), and the amount of caffeine content was defined according to Snel and Lorist (Snel & Lorist, 2011). To ensure good health, volunteers were screened by self-report questionnaires and a medical examination conducted by a physician. Additionally, all volunteers reported good sleep quality assessed with the Pittsburgh Sleep Quality Index (PSQI; score ≤ 5) (Buysse, Reynolds, Monk, Berman, & Kupfer, 1989) and showed no signs of sleep disturbances in a polysomnography (PSG) recorded during an adaptation night in the laboratory (sleep efficiency (SE) > 70%, periodic leg movements < 15/h, apnea index < 10). To control for circadian misalignment, volunteers who reported shiftwork within three months and transmeridian travels (crossing > 2 time zones) within one month prior to study admission were excluded. Further exclusion criteria comprised body mass index (BMI) < 18 or > 26, smoking, drug use, and extreme chronotype assessed by the Morningness-Eveningness Questionnaire (MEQ; score ≤ 30 and ≥ 70) (Horne & Ostberg, 1976). To reduce variance in the data incurred by the effect of menstrual cycle on sleep (Shechter & Boivin, 2010) and the interaction between caffeine metabolism and the use of oral contraceptives (Abernethy & Todd, 1985; Balogh et al., 1995), only male volunteers were studied. A detailed description of the study sample can be found in Weibel et al. (Weibel, Lin, Landolt, Garbazza, et al., 2020).

All volunteers signed a written informed consent and received financial compensation for study participation. The study was approved by the local Ethics Committee (EKNZ) and conducted according to the Declaration of Helsinki.

2.2. Design and protocol

We employed a double-blind randomized crossover study including a caffeine, a withdrawal, and a placebo condition. Volunteers were allocated to the order of the three conditions based on pseudo-randomization, for more details see Weibel et al. (Weibel, Lin, Landolt, Garbazza, et al., 2020). As illustrated in Figure 1, each condition started with an ambulatory part of nine days, followed by a laboratory part of 43 h. In each condition, participants took either caffeine (150 mg) or placebo (mannitol) in identical appearing gelatin capsules (Hänseler AG, Herisau, Switzerland) three times daily, scheduled at 45 min, 255 min, and 475 min after awakening, for a duration of 10 days. This regimen was applied based on a previous study investigating tolerance to the effects of caffeine and caffeine cessation (James, 1998). To enhance caffeine withdrawal in the withdrawal condition, treatment was abruptly switched from caffeine to placebo on day nine of the protocol (255 min after wake-up, 15 h before sleep recording).

During the nine days of the ambulatory part, volunteers were asked to maintain a regular sleep-wake rhythm (± 30 min of self-selected bedtime/wake-up time, 8 h in bed, no daytime napping), verified by wrist actimetry (Actiwatch, Cambridge Neurotechnology Ltd., Cambridge, United Kingdom), and to keep subjective sleep logs. The duration of the ambulatory part was set for nine days based on the maximum duration of withdrawal symptoms (Juliano & Griffiths, 2004) and thus, to avoid carry-over effects from the previous condition. Furthermore, volunteers were requested to refrain from caffeinated beverages and food (e.g. coffee, tea, soda drinks, and chocolate), alcohol, nicotine, and medications. Caffeine abstinence and compliance to the treatment requirements were checked by caffeine levels from the daily collection of fingertip sweat.

On day nine, volunteers admitted to the laboratory at 5.5 h prior to habitual sleep time. Upon arrival, a urinary drug screen (AccuBioTech Co., Ltd., Beijing, China) was performed to ensure drug abstinence. Electrodes for the PSG were fitted and salivary caffeine levels collected. An 8-h nighttime sleep episode was scheduled at volunteers' habitual bedtime starting 8 and 15 h after the last caffeine intake in the caffeine and withdrawal condition, respectively. The next day, volunteers rated their subjective sleep quality by the Leeds Sleep Evaluation Questionnaire (LSEQ) (Parrott & Hindmarch, 1978) and potential withdrawal symptoms by the Caffeine Withdrawal Symptom Questionnaire (CWSQ) (Juliano, Huntley, Harrell, & Westerman, 2012).

To reduce potential masking effects on our outcome variables, volunteers were housed in single apartments under dim-light (< 8 lx) during scheduled wakefulness and 0 lx during sleep.

Volunteers were asked to maintain a semi-recumbent position during wakefulness, except for restroom breaks. Social interactions were restricted to team members and no time-of-day cues were provided throughout the in-lab protocol.

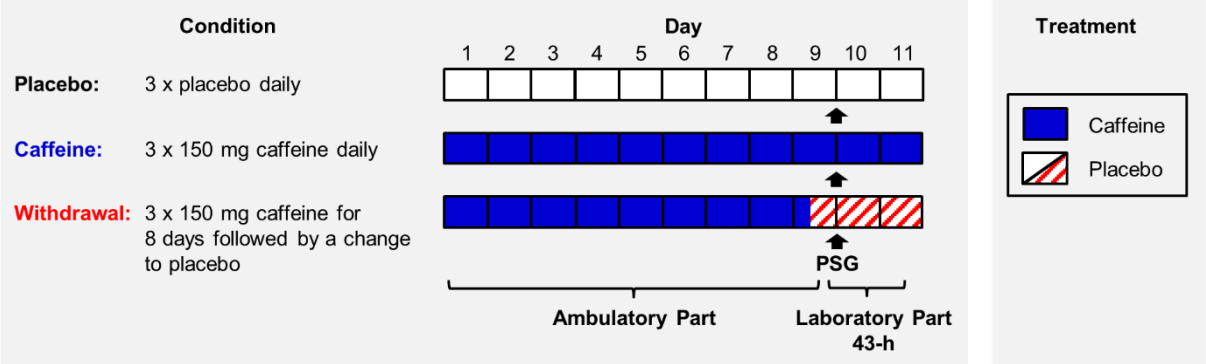

Figure 1. Illustration of the study design. Twenty volunteers participated in a placebo, a caffeine, and a withdrawal condition during which they ingested either caffeine or placebo capsules three times daily (wake-up +45 min, +255 min, and +475 min). Each condition started with an ambulatory part of nine days and was followed by a laboratory part of 43 h. After nine days of continuous treatment, we recorded 8 h of polysomnography (PSG), indicated as arrows, during nighttime sleep under controlled laboratory conditions. The sleep episode was scheduled to volunteers' habitual bedtime.

2.3. Salivary caffeine

To characterize individual caffeine levels during nighttime sleep, we report salivary caffeine levels assessed 3 h prior to the scheduled sleep episode and 5 min after wake-up. Samples were stored at 5°C following collection, later centrifuged (3000 rpm for 10 min) and subsequently kept at -28°C until analyses. Liquid chromatography coupled to tandem mass spectrometry was used to analyze the levels of caffeine. One dataset in the withdrawal condition was lost.

2.4. Subjective sleep quality

Subjective sleep quality was assessed 10 min upon scheduled wake-up time with a paper and pencil version of the LSEQ (Parrott & Hindmarch, 1978). Volunteers were asked to rate 10 items on visual analogue scales which are grouped into four domains (getting to sleep (GTS), quality of sleep (QOS), awake following sleep (AFS), and behavior following wakening (BFW)).

2.5. Polysomnographic recordings

PSG was continuously recorded during 8 h of nighttime sleep using the portable V-Amp device (Brain Products GmbH, Gilching, Germany). Grass gold cup electrodes were applied according to the international 10-20 system including two electrooculographic, two electromyographic, two electrocardiographic, and six electroencephalographic derivations (F3, F4, C3, C4, O1, O2). Channels were referenced online against the linked mastoids (A1, A2). Signals were recorded with a sampling rate of 500 Hz and a notch filter was online applied at 50 Hz.

Each epoch of 30 sec of the recorded PSG data was visually scored according to standard criteria (Berry et al., 2012) by three trained team members blind to the condition. SWS was additionally classified into stage 3 and 4 based on Rechtschaffen and Kales (Rechtschaffen & Kales, 1968). The scoring agreement between the three scorers was regularly confirmed to reach > 85%.

TST was defined as the sum of the time spent in sleep stages 1-4 and rapid eye movement sleep (REM). Sleep latencies were calculated as minutes to the first occurrence of the corresponding sleep stage following lights off. Non-rapid eye movement (NREM) sleep was calculated as sum of sleep stages 2, 3 and 4. All sleep stages are expressed as relative values (%) of TST.

Spectral analysis was performed by applying fast Fourier transformation (FFT; hamming, 0% overlapped, 0.25 Hz bins) on 4-s time windows. Artifacts were manually removed based on visual inspection, and data were log-transformed prior to spectral analyses. All-night EEG power density during NREM sleep was analyzed for each 0.25 Hz frequency bin in the range of 0.75-32 Hz recorded over the central derivations (C3, C4). SWA was defined as EEG power density between 0.75-4.5 Hz and sigma activity between 12-16 Hz. Sleep cycles were defined based on adapted rules developed by Feinberg and Floyd (Feinberg & Floyd, 1979) and divided into 10 NREM and four REM intervals within each cycle. Ten nights were excluded from sleep analyses due to technical problems (placebo: $n = 3$; caffeine: $n = 4$; withdrawal: $n = 3$).

2.6. Caffeine withdrawal symptoms

Withdrawal symptoms were first assessed 35 min after wake-up and subsequently prior to each treatment administration with the self-rating CWSQ (Juliano et al., 2012). Twenty-three items are grouped into seven factors (fatigue/drowsiness, low alertness/difficulty concentrating, mood disturbances, low sociability/motivation to work, nausea/upset stomach, flu-like feelings, headache) and were rated on a 5 point scale by choosing between 1 (not at all) and 5 (extremely). Prior to analyses, eight items have been reversed scored as they were positively worded (e.g. alert or talkative) in the questionnaire. To assess caffeine withdrawal, we first calculated a sum score comprising all 23 items of the caffeine withdrawal questionnaire. Missing responses to single items were replaced by the median response of each condition over all volunteers in the respective time of assessment. In a next step, we calculated relative withdrawal symptoms in the caffeine and withdrawal condition (i.e. the difference of the withdrawal score in the caffeine and withdrawal condition respectively minus the score of the placebo condition). The data of one volunteer was lost due to technical difficulties.

2.7. Statistical analyses

Analyses were performed with the statistical package SAS (version 9.4, SAS Institute, Cary, NC, USA) by applying mixed model analyses of variance for repeated measures (PROC MIXED) with the repeated factors 'condition' (placebo, caffeine, withdrawal) and/or 'time' (levels differ per variable) and the random factor 'subject'. The LSMEANS statement was used to calculate contrasts and degrees of freedom were based on the approximation by Kenward and Roger (Kenward & Roger, 1997). Post-hoc comparisons were adjusted for multiple comparisons by applying the Tukey-Kramer method. A statistical significance was defined as $p < 0.05$. One dataset has been excluded from all the analyses due to non-compliance with the treatment requirements (caffeine: $n = 1$).

3. Results

3.1. Salivary caffeine levels

Caffeine levels significantly differed between each of the three conditions (main effect of condition: $F_{2,90.7} = 46.12$; $p < 0.001$) with the highest levels in the caffeine condition and the lowest in the placebo condition (post-hoc comparisons: $p_{all} < 0.01$). In addition, a significant interaction of the factors condition and time ($F_{2,89.6} = 10.65$; $p < 0.001$) confirmed that caffeine levels were modulated by time with levels decreasing during nighttime sleep in the caffeine condition only (post-hoc comparison: $p < 0.001$), see Figure 2.

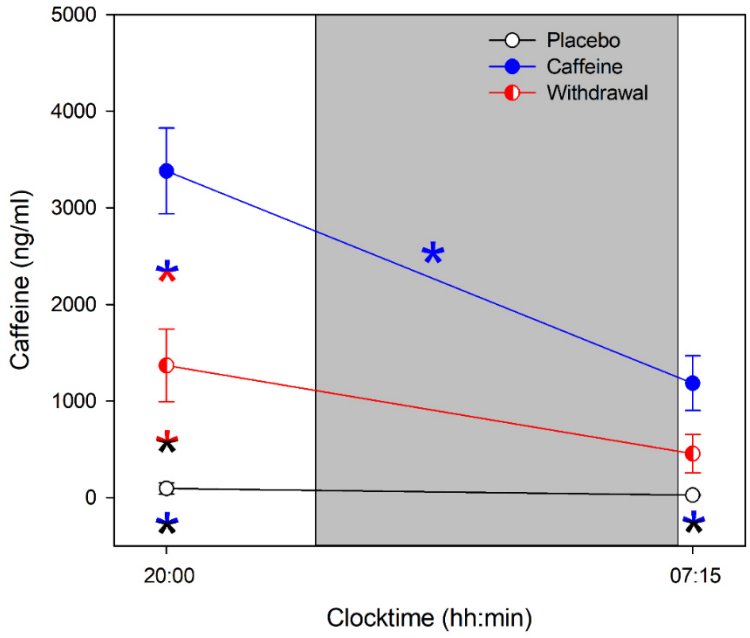

Figure 2. Average caffeine levels collected prior to and after nighttime sleep (grey bar) in the placebo (black open circles), caffeine (blue filled circles), and withdrawal (red semi-filled circles) condition (mean values ± standard errors). The x-axis indicates the mean time of day of sample collection and color-coded asterisks represent significant ($p < 0.05$) post-hoc comparisons of the interaction effect condition x time.

3.2. Sleep

Table 1 summarizes the statistical analyses of subjective sleep quality and objective sleep structure assessed during nighttime sleep. Analyses of subjective sleep quality assessed with the LSEQ did not reveal significant differences among the three conditions in any of the four domains of sleep quality ($p_{all} > 0.05$).

In line with these results, the analyses of the PSG did not reveal significant differences in total sleep time (TST), sleep efficiency (SE), sleep latencies (SL), or the relative amount of sleep stages among the three conditions ($p_{all} > 0.05$).

Table 1. Subjective and objective sleep parameters per condition and results of the analyses.

Parameter	Placebo	Caffeine	Withdrawal	Factor Condition
Subjective (LSEQ)				
Getting to sleep	47.00 ± 3.20	51.39 ± 3.95	57.02 ± 4.82	$F_{2,37.6} = 2.30$, $p = 0.11$
Quality of sleep	42.25 ± 2.72	42.71 ± 3.55	45.05 ± 4.42	$F_{2,37.5} = 0.22$, $p = 0.81$
Awake following sleep	50.18 ± 3.10	44.53 ± 3.43	45.03 ± 4.10	$F_{2,36.7} = 0.99$, $p = 0.38$
Behavior following wakening	47.98 ± 3.57	42.37 ± 4.25	39.63 ± 4.44	$F_{2,37.7} = 1.32$, $p = 0.28$
Objective (PSG)				
TST (min)	440.88 ± 8.16	450.97 ± 5.13	447.59 ± 10.13	$F_{2,31.3} = 0.83$, $p = 0.45$
SE (%)	91.85 ± 1.70	93.94 ± 1.08	93.31 ± 2.11	$F_{2,31.2} = 0.86$, $p = 0.43$
SL1 (min)	13.18 ± 3.22	10.33 ± 1.39	8.67 ± 1.88	$F_{2,31.8} = 1.47$, $p = 0.25$
SL2 (min)	16.56 ± 3.47	16.67 ± 2.08	16.82 ± 4.53	$F_{2,31.9} = 0.02$, $p = 0.98$
RL (min)	76.65 ± 10.85	85.21 ± 18.86	86.56 ± 13.86	$F_{2,28.5} = 0.33$, $p = 0.72$
Stage 1 (%)	9.62 ± 1.36	7.79 ± 0.89	8.75 ± 1.12	$F_{2,31.6} = 1.19$, $p = 0.32$
Stage 2 (%)	48.90 ± 1.24	49.70 ± 2.09	48.08 ± 1.57	$F_{2,29.0} = 0.42$, $p = 0.66$
Stage 3 (%)	9.38 ± 0.70	10.15 ± 1.20	10.98 ± 1.00	$F_{2,29.8} = 1.03$, $p = 0.37$
Stage 4 (%)	7.11 ± 1.58	5.24 ± 1.64	6.85 ± 1.62	$F_{2,28.1} = 0.53$, $p = 0.59$
SWS (%)	16.49 ± 1.73	15.39 ± 2.13	17.83 ± 1.75	$F_{2,28.3} = 0.62$, $p = 0.55$
NREM (%)	65.39 ± 1.19	65.09 ± 1.06	65.91 ± 1.34	$F_{2,28.9} = 0.14$, $p = 0.87$
REM (%)	24.99 ± 1.60	27.11 ± 1.12	25.34 ± 1.81	$F_{2,30.1} = 0.73$, $p = 0.49$
WASO (%)	6.49 ± 2.18	4.35 ± 1.26	6.49 ± 3.50	$F_{2,31.4} = 0.49$, $p = 0.62$

LSEQ: Leeds Sleep Evaluation Questionnaire; PSG: polysomnography. Values represent means ± standard errors for each variable and condition. Lower values in the four scales of the subjective ratings indicate worse sleep quality. TST: total sleep time (stages 1-4 + REM sleep); SE: sleep efficiency (TST/time in bed x 100); SL1: latency to first occurrence of stage 1; SL2: latency to first occurrence of stage 2; RL: latency to first occurrence of REM sleep; SWS: slow-wave sleep (stages 3-4); NREM: non-rapid eye movement sleep (stages 2-4); REM: rapid eye movement sleep; WASO: wakefulness after sleep onset.

In a next step, we analyzed all-night EEG power density in the range of 0.75-32 Hz over the central derivations recorded during NREM sleep. In contrast to our assumptions, we did not find any significant differences among the three conditions in the lower frequency bins (0.75-13.25 Hz; $p_{all} > 0.05$). However, power density was significantly reduced compared to placebo in the sigma range during both withdrawal (frequency bins 13.5-17.25 Hz and 18-18.5 Hz, $p_{all} < 0.05$) and caffeine (frequency bins 13.5-16 Hz, $p_{all} < 0.05$).

In a second step, we were interested in the temporal dynamics of both SWA and sigma activity across the night assessed during NREM sleep. As depicted in Figure 3 (top panel), SWA showed a typical temporal pattern with increased activity during the first NREM cycle followed by a steady decline across the night (main effect of time: $F_{39,613} = 26.28$, $p < 0.001$). However, differences among the three conditions did not reach significance (main effect of condition: $F_{2,178} = 1.33$, $p = 0.27$). Also, the interaction of condition and time was not significant ($F_{78,1060} = 0.89$, $p = 0.74$).

As illustrated in Figure 3 (bottom panel), sigma activity was significantly reduced in both the caffeine and withdrawal conditions compared to placebo intake (main effect of condition: $F_{2,209} = 19.96$, $p < 0.001$; post-hoc comparisons: $p < 0.001$) and the interaction of condition and time tended to be significant ($F_{78,1049} = 1.25$, $p = 0.08$).

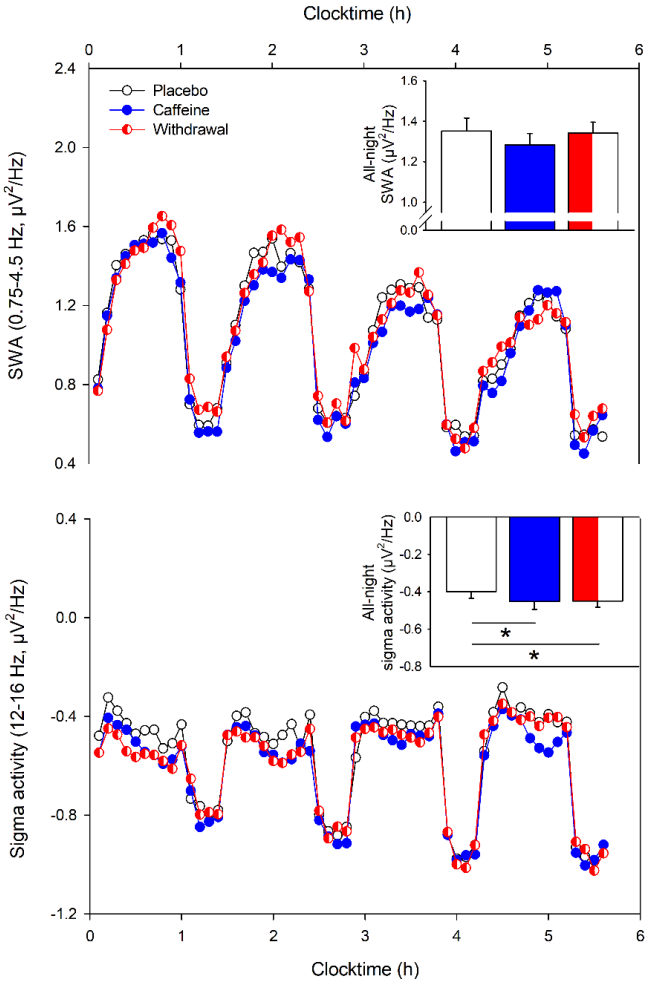

Figure 3. Temporal dynamics of SWA (top) and sigma activity (bottom) during the first four sleep cycles in the placebo (black open circles), caffeine (blue filled circles), and the withdrawal (red semi-filled circles) condition (mean values). The x-axis indicates the mean time of day. While SWA (0.75-4.5 Hz) was not significantly affected by the treatment, sigma activity (12-16 Hz) showed reduced activity during both caffeine and withdrawal compared to the placebo condition ($p_{all} < 0.05$). The inset in each right upper corner represents the mean values ± standard errors of the all-night SWA and sigma activity respectively during NREM sleep in the placebo, caffeine, and withdrawal condition. While all-night SWA (0.75-4.5 Hz) did not differ among the conditions, sigma activity (12-16 Hz) was lower in the caffeine and withdrawal condition compared to placebo ($p < 0.05$). All analyses are based on log-transformed data.

Taken together, we could not confirm our assumption of a caffeine-induced reduction of sleep depth, neither in terms of shorter SWS nor in terms of reduced SWA in the caffeine compared to the placebo condition. Based on the discrepancies between the present results and a previous study about the effects of chronic caffeine intake on sleep (Bonnet & Arand, 1992), we thus explored whether differences in the individual levels of caffeine before sleep could explain the variance within SWS and SWA. However, no significant effects were observed when controlling for dependent observations within subjects ($p > 0.05$).

3.3. Subjective caffeine withdrawal symptoms

Analyses of the relative withdrawal symptoms yielded a significant main effect of condition ($F_{2,20.2} = 11.30$, $p < 0.01$) indicating more withdrawal symptoms during the withdrawal compared to the caffeine condition (post-hoc comparison: $p < 0.01$), depicted in Figure 4. This effect was modulated by time (interaction of condition x time: $F_{2,37.2} = 3.43$, $p = 0.04$), such that the increase in symptoms during the withdrawal compared to caffeine condition was particularly present during the last measurement ($p < 0.01$), i.e. 31 h after the last caffeine intake in the withdrawal condition.

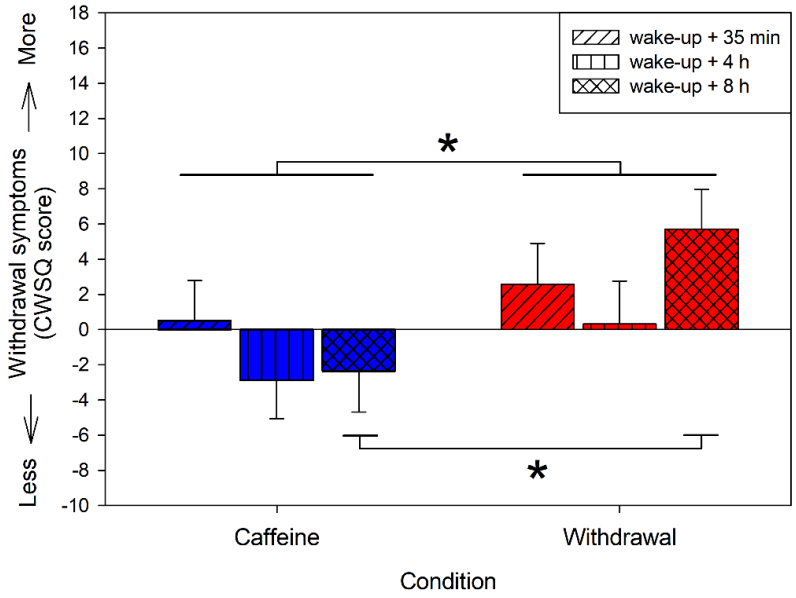

Figure 4. Relative withdrawal symptoms in the caffeine and withdrawal condition (i.e. withdrawal score of the caffeine and withdrawal condition respectively minus the score of the placebo condition) assessed 35 min, 4 h, and 8 h after wake-up on day ten of treatment. Depicted are mean values and standard errors of the relative values (i.e. difference to placebo). Overall, volunteers reported more withdrawal symptoms in the withdrawal condition compared to the caffeine condition ($p < 0.05$). This difference was particularly present 8 h after wake-up during withdrawal compared to caffeine ($p < 0.001$).

4. Discussion

The aim of the present study was to investigate the influence of daily daytime caffeine intake and its cessation on nighttime sleep in habitual caffeine consumers under strictly controlled laboratory conditions. Strikingly, caffeine consumption did not lead to clear-cut changes in

nighttime sleep structure nor in subjective sleep quality when assessed 8 and 15 h after the last intake in the caffeine and withdrawal condition, respectively. The evolution of subjective withdrawal symptoms indicates that withdrawal becomes perceivable at earliest between 27-31 h after intake. However, compared to placebo, EEG power density was reduced in the sigma range during both caffeine and withdrawal conditions. We conclude that daily daytime intake of caffeine does not strongly influence nighttime sleep structure nor subjective sleep quality in healthy men when consumed in the morning, midday, and in the afternoon. In contrast to the reported increases in sigma activity after acute caffeine intake (Landolt, Dijk, et al., 1995), the observed changes in the sigma frequencies might point to early signs of caffeine withdrawal which occur due to overnight abstinence and presumably derive from preceding caffeine-induced changes in adenosine signaling.

To quantify the influence of caffeine on sleep, the stimulant is commonly administered close to the onset of a sleep episode (Bonnet & Arand, 1992; Landolt, Dijk, et al., 1995; Drapeau et al., 2006; Carrier et al., 2007; Robillard et al., 2015), for instance within one hour prior to bedtime (Landolt, Dijk, et al., 1995; Drapeau et al., 2006; Carrier et al., 2007; Robillard et al., 2015). Taking into account that caffeine plasma levels peak within 30 to 75 min following caffeine ingestion (Mandel, 2002), consumption within one hour prior to sleep allows the stimulant to exert its maximum effects at sleep commencement. Indeed, the sleep disrupting effects of caffeine are frequently reported to affect sleep initiation or the first half of the sleep episode (Bonnet & Arand, 1992; Landolt, Dijk, et al., 1995; Drapeau et al., 2006; Carrier et al., 2007; Robillard et al., 2015). Moreover, sleep intensity, which is usually strongest at the beginning of the night (Dijk & Czeisler, 1995), was particularly disrupted during the first sleep cycle, as indexed in reduced SWS and SWA (Landolt, Dijk, et al., 1995). However, caffeine intake in the evening, particularly after 9 pm is rare (Lieberman et al., 2019), presumably to avoid impairment of subsequent sleep (Snel & Lorist, 2011). Up to date it remained fairly unclear whether caffeine intake in the morning and afternoon still bears the potential to disrupt nighttime sleep. Thus, our data provide first evidence that daily daytime caffeine intake does not necessarily alter subsequent sleep structure and SWA when consumed > 8 h prior to sleep. Importantly, our findings do not preclude potential impairments of nighttime sleep after morning caffeine intake, if preceded by several days of abstinence from the stimulant (Landolt, Werth, Borbély, & Dijk, 1995). It rather appears likely that the duration of preceding caffeine consumption drives the discrepancies between acute and chronic effects of caffeine on sleep. Chronic caffeine intake induces some tolerance development in both physiological measures such as cortisol (Lovallo et al., 2005), blood pressure (Lovallo et al., 2004), heart rate (Denaro, Brown, Jacob, & Benowitz, 1991), and also subjective measures such as alertness (James, 1998). Over time, the stimulatory effects of the substance vanish potentially due to changes in adenosine levels (Conlay, Conant, deBros, & Wurtman, 1997) and/or adenosine receptors

(Fredholm, 1982; Shi, Nikodijevic, Jacobson, & Daly, 1993; Johansson, Georgiev, Lindström, & Fredholm, 1997; Varani et al., 1999). Accordingly, an one-week treatment of caffeine reduced the sleep disrupting effects, even under conditions of high evening dosages (Bonnet & Arand, 1992). Thus, the available evidence and the absence of clear-cut changes in the present study point to adaptive processes in sleep initiation, sleep structure, and subjective sleep quality due to the long-term exposure to the stimulant.

However, chronic caffeine consumption bears the risk of withdrawal symptoms when abruptly ceased. These symptoms have been reported to occur as early as 6 h but with peak intensity being reached within 20 to 51 h after last caffeine intake (Juliano & Griffiths, 2004). While 25 h of caffeine abstinence might not affect nighttime sleep structure (Bonnet & Arand, 1992), 32 h of abstinence improved subjective sleep quality (James, 1998). Thus, scheduling the start of the sleep episode to 15 h after the last caffeine intake, as in our withdrawal condition, was probably too early to detect changes in sleep structure or subjective sleep quality. In line with this assumption, volunteers subjectively indicated withdrawal symptoms 31 h after caffeine abstinence in the withdrawal condition compared to caffeine. Thus, our findings support the notion that the alterations in sleep structure and subjective sleep quality induced by caffeine abstinence potentially develop at a later stage (> 27 h) of caffeine withdrawal.

Most strikingly and unexpectedly, a reduction in NREM sigma activity during both the withdrawal and caffeine conditions was observed, a phenomenon which is commonly reported under conditions of enhanced sleep pressure (Borbély et al., 1981; Dijk et al., 1993; Finelli et al., 2001; Knoblauch et al., 2003). Thus, it seems at first glance in contrast to the reported increases in this frequency range (Landolt, Dijk, et al., 1995; Landolt, Werth, et al., 1995) and the well-known alerting effects after acute caffeine intake (James, 1998). However, during conditions of chronic caffeine intake, mice showed a deeper sleep compared to placebo (Panagiotou, Meijer, Meijer, & Deboer, 2018). Moreover, repeated caffeine intake enhances the sensitivity of adenosine binding (Ferré, 2008) presumably due to increased adenosine plasma levels (Conlay et al., 1997), upregulated adenosine receptors (Fredholm, 1982; Shi et al., 1993; Johansson et al., 1997; Varani et al., 1999) or changes in the functions of adenosine receptor heteromers (Ciruela et al., 2006). These neuronal alterations in the adenosinergic system might drive the commonly observed changes in the homeostatic sleep-wake regulation such as increased sleepiness when caffeine intake is suddenly ceased (Juliano & Griffiths, 2004). As reported previously, we also observed in the present study higher subjective sleepiness following caffeine withdrawal when compared to the placebo and caffeine conditions (Weibel, Lin, Landolt, Garbazza, et al., 2020). Thus, the reduction in sigma activity might reflect adenosinergic changes which already emerge 8 and 15 h after the last caffeine intake in the caffeine and withdrawal condition, respectively. This reduction might reflect withdrawal symptoms which chronic consumers reverse daily by the first caffeine dose. Given

the high prevalence of daily caffeine consumers in the society, these findings stress the importance to carefully control for prior caffeine intake when assessing sleep in order to exclude potential confounding by induced withdrawal symptoms which are only detectable in the microstructure of sleep.

Our study has some limitations which must be taken into careful consideration when interpreting the present findings. First, age moderates the effects of caffeine on sleep (Drapeau et al., 2006; Robillard et al., 2015). Thus, the present results cannot be generalized to other age groups. Second, only a limited number of participants were studied. However, a well-controlled study design was employed and power calculation on the basis of an earlier study (Bonnet & Arand, 1992) indicated a sufficient sample size. Third, no genetic information of the study participants is available. A genetic variation of the ADORA2A genotype has been linked with caffeine sensitivity to the effects on sleep (Rétey et al., 2007). Thus, carriers of this genetic variance are more likely to curtail caffeine consumption and are consequently excluded from the present study leading to a selection bias. However, the focus of the present study was to investigate habitual caffeine consumers as they represent the majority of the worldwide population (Heckman, Weil, et al., 2010). Fourth, to reduce variance in the data incurred by the influence of the menstrual cycle on sleep (Shechter & Boivin, 2010) and the interaction between caffeine metabolism and the use of oral contraceptives (Abernethy & Todd, 1985; Balogh et al., 1995), only male volunteers were included which clearly reduces the generalizability of the findings.

In conclusion, we report evidence that daily daytime intake of caffeine and its cessation has no strong effect on sleep structure or subjective sleep quality. However, the quantitative EEG analyses revealed reduced activity in the sigma range during both caffeine and withdrawal. These subtle alterations point to early signs of caffeine withdrawal in the homeostatic aspect of sleep-wake regulation which are already present as early as 8 h after the last caffeine intake. Thus, habitual caffeine consumers constantly expose themselves to a continuous change between presence and absence of the stimulant. Around the clock, their organisms dynamically adapt and react to daily presence and nightly abstinence.

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Author contributions

CR, CC and SB designed the study; JW, YS and HS collected the data; JW, CR and CC analyzed and interpreted the data; JW and CR drafted the manuscript; CC, YL and HPL critically revised the manuscript regarding its intellectual content; JK, SR and KR provided the resources for the caffeine measurements and performed its analyses; all authors reviewed the present article.

Competing interests

The authors declare no competing interests.

4.2. Caffeine-dependent changes of sleep-wake regulation: Evidence for adaptation after repeated intake

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Abstract

Background: Circadian and sleep-homeostatic mechanisms regulate timing and quality of wakefulness. To enhance wakefulness, daily consumption of caffeine in the morning and afternoon is highly common. However, the effects of such a regular intake pattern on circadian sleep-wake regulation are unknown. Thus, we investigated if daily daytime caffeine intake and caffeine withdrawal affect circadian rhythms and wake-promotion in habitual consumers.

Methods: Twenty male young volunteers participated in a randomised, double-blind, within-subject study with three conditions: i) caffeine (150 mg 3 x daily for 10 days), ii) placebo (3 x daily for 10 days) and iii) withdrawal (150 mg caffeine 3 x daily for eight days, followed by a switch to placebo for two days). Starting on day nine of treatment, salivary melatonin and cortisol, evening nap sleep as well as sleepiness and vigilance performance throughout day and night were quantified during 43 h in an in-laboratory, light and posture-controlled protocol.

Results: Neither the time course of melatonin (i.e. onset, amplitude or area under the curve) nor the time course of cortisol was significantly affected by caffeine or withdrawal. During withdrawal, however, volunteers reported increased sleepiness, showed more attentional lapses as well as polysomnography-derived markers of elevated sleep propensity in the late evening compared to both the placebo and caffeine condition.

Conclusions: The typical pattern of caffeine intake with consumption in both the morning and afternoon hours may not necessarily result in a circadian phase shift in the evening nor lead to clear-cut benefits in alertness. The time-of-day independent effects of caffeine withdrawal on evening nap sleep, sleepiness and performance suggest an adaptation to the substance, presumably in the homeostatic aspect of sleep-wake regulation.

Keywords

Caffeine, withdrawal, circadian, alertness, sleep

1. Introduction

Caffeine is the most commonly consumed psychoactive substance in the world (Fredholm et al., 1999). Around 80% of the worldwide population consume caffeine regularly on a daily basis (Heckman, Weil, et al., 2010) and intake is increasing in terms of daily dosages and earlier age of regular substance intake (Roehrs & Roth, 2008). Caffeine containing aliments, e.g. coffee, tea, soda drinks and chocolate (Fredholm et al., 1999), are used since centuries to modulate sleep and wakefulness (Camandola et al., 2019).

Timing, quality and quantity of sleep and wakefulness are regulated by the interplay of a homeostatic and a circadian process (Borbély, 1982). Caffeine interferes with sleep homeostasis by antagonising adenosine (Landolt, 2008), a proposed mediator of the increase of homeostatic sleep pressure during time spent awake and its decrease during sleep (Porkka-Heiskanen, 2013). By blocking the A_1 and A_{2A} adenosine receptors (Fredholm et al., 1999), which are expressed in wide-spread areas of the human central nervous system (Elmenhorst et al., 2012; Naganawa et al., 2014), acute caffeine administration reduces the effects of sleep pressure, as mirrored in reduced sleepiness (James, 1998), improved behavioural performance (Einöther & Giesbrecht, 2013) and dampened sleep depth during nighttime sleep (Landolt, 2008), particularly when sleep pressure is high (Roehrs & Roth, 2008; Snel & Lorist, 2011).

Furthermore, evidence accumulates that caffeine also impacts on human circadian rhythms, as indexed by changes in salivary melatonin levels following administrations at a certain circadian phase. Acute caffeine intake in the evening and at night has been shown to delay the onset of melatonin secretion (Burke et al., 2015) and decrease nighttime melatonin levels (Wright et al., 1997; Wright et al., 2000). However, evening intake of caffeine is not common in the society nowadays (Martyn et al., 2018). Considering the average half-life of caffeine with a duration of around 4 h (Snel & Lorist, 2011), the question arises whether caffeine-induced circadian effects also occur when consumption is timed to morning and afternoon, as observed in habitual caffeine consumers (Martyn et al., 2018).

Besides the timing of caffeine intake, the duration of prior repeated daily use moderates the impact of caffeine-induced changes on sleep-wake regulation. There is evidence that consumers develop tolerance to the substance already after several days, such that effects of a particular dose of caffeine, for instance on sleep (Bonnet & Arand, 1992) or alertness (James, 1998) become weakened. However, in line with a recent study in animals (Panagiotou et al., 2018), it has also been shown that continuous hourly caffeine intake over four weeks strengthens circadian wake-promotion, as indicated by a reduced ability to sleep prior to habitual bedtime (Wyatt et al., 2004). Interestingly, timing of melatonin secretion was not shifted by the long-term treatment. An open question remains, whether the absence of phase

shifts can be traced back to the continuous timing of caffeine administration around the entire circadian cycle or due to neuroadaptations in response to long-term treatment.

One of the typical indicators of neuronal and systemic alterations in response to long-term caffeine use is the occurrence of withdrawal symptoms when intake is ceased (Juliano & Griffiths, 2004). Caffeine withdrawal symptoms include decreased alertness (James, 1998; Rogers et al., 2005), impaired cognitive performance (James, 1998; Rogers et al., 2005) and changes in waking electroencephalogram (EEG) such as enhanced theta power (Sigmon et al., 2009), starting 12 to 24 h after last caffeine intake with peak intensity between 20 to 51 h and a maximal duration of nine days (Juliano & Griffiths, 2004). Based on changes in adenosine-signaling (Ferré, 2008), caffeine withdrawal thus constitutes a state of low arousal which may help triggering the maintenance of daily caffeine intake in habitual consumers (Juliano & Griffiths, 2004). However, to our best knowledge the impact of caffeine-withdrawal on human circadian sleep-wake regulation has not yet been examined.

Thus, we investigated the effects of daily daytime caffeine consumption and its withdrawal on human waking performance, circadian rhythms and wake-promotion. To establish both tolerance and withdrawal, and to enable a comparison to a withdrawal-free baseline, caffeine and placebo, respectively, were administered over 10 days in a crossover design with three conditions (caffeine, placebo and withdrawal). Starting at day nine of treatment, sleepiness and vigilance performance was assessed as well as salivary melatonin, cortisol and nap sleep during high circadian wake-promotion within a 43 h-laboratory protocol under controlled light, posture and meal intake.

2. Methods

The present study was approved by the Ethics Committee northwest/central Switzerland (EKNZ) and conducted in accordance with the declaration of Helsinki. All volunteers provided written informed consent and received a monetary compensation for study participation.

2.1. Volunteers

In total, 179 male healthy habitual caffeine consumers underwent a thorough screening procedure. Exclusion criteria comprised age < 18 or > 35 years, body mass index (BMI) < 18 or > 26, drug dependency, shiftwork within three months prior to study admission, transmeridian travels within one month prior to study, extreme chronotype (Morningness-Eveningness Questionnaire (Horne & Ostberg, 1976), score ≤ 30 and ≥ 70) and poor sleep quality (Pittsburgh Sleep Quality Index (Buysse et al., 1989), score > 5). Female volunteers were excluded in order to reduce variance in our data due to the menstrual cycle (Shechter & Boivin, 2010) and use of oral contraceptives (Abernethy & Todd, 1985; Balogh et al., 1995). Volunteers were included when habitual daily caffeine intake was between 300 and 600 mg,

assessed with a survey tool based on (Bühler et al., 2013) and adapted to the caffeine content according to (Snel & Lorist, 2011). Twenty-nine volunteers were invited for a habituation night and a physical examination by a physician in charge to exclude poor sleep efficiency (SE < 70%), clinical sleep disturbances (apnea index > 10, periodic leg movements > 15/h) and chronic or debilitating medical conditions. Demographic characteristics of the 20 participants who completed all three conditions can be found in the supplementary materials (Table S1).

2.2. Design and protocol

The protocol is illustrated in Fig. 1(a). A double-blind, crossover study comprising three conditions was conducted: a caffeine, a placebo and a withdrawal condition. This within-subject design was chosen to reduce variance in the data due to expectancies or inter-individual variability in caffeine metabolism. For calculation of sample size and pseudo-random allocation of volunteers to the order of the three conditions, see supplementary materials (Table S3). In each condition, participants ingested gelatin capsules over 10 days three times daily (45 min, 255 min, 475 min after wake-up), containing either placebo (mannitol, Hänseler AG, Herisau, Switzerland) or caffeine (150 mg, Hänseler AG, Herisau, Switzerland). Participants were instructed to refrain from caffeinated beverages and food. Compliance was verified by assessing caffeine metabolites from fingertip sweat collected prior to habitual bedtime (see supplementary materials). The length of the treatment of 10 days was based on the maximum duration of withdrawal symptoms (Juliano & Griffiths, 2004), occurring during placebo treatment in habitual consumers. Timing and dose were based on an earlier study investigating tolerance to caffeine and its withdrawal (James, 1998).

The caffeine and placebo conditions included one type of pill exclusively (caffeine or placebo, respectively). The withdrawal condition comprised a switch from caffeine to placebo pills in the late morning of day nine (255 min after wake-up). In scheduling the caffeine to placebo switch to the late morning, we aimed at a coincidence of the peak of withdrawal symptoms (after around 35 h after last caffeine intake) with a window of high circadian wake-promotion. Dim light melatonin onset (DLMOnset) was used as marker of circadian timing.

Each condition started with an ambulatory part of nine days during which participants ingested capsules according to the regimen described above. In addition, participants kept a fixed sleep-wake rhythm (within ± 30 min of self-selected bedtime, time in bed 8 h, no naps) verified by wrist actimetry (Actiwatch, Cambridge Neurotechnology, Cambridge, United Kingdom). The average deviation from the targeted bedtime was ± 21 min in the evening and ± 26 min in the morning with an average time in bed of 8 h and 3 min. The sleep-wake schedule was consistent across the conditions except for three volunteers (twice: +30 min in caffeine compared to placebo and withdrawal conditions; once: -30 min in placebo compared to caffeine and

withdrawal conditions). Compliance to drug abstinence was checked prior to each laboratory part by a urinary toxicology screen (AccuBioTech Co., Ltd., Beijing, China).

In the evening on day nine of treatment, 5.5 h before individual habitual bedtime, a 43-h laboratory part started, illustrated in Fig. 1(b). Volunteers were accommodated in single apartments, isolated from external time cues and communication was restricted to team members. Additionally, light conditions (< 8 lux), posture and meal intake was controlled.

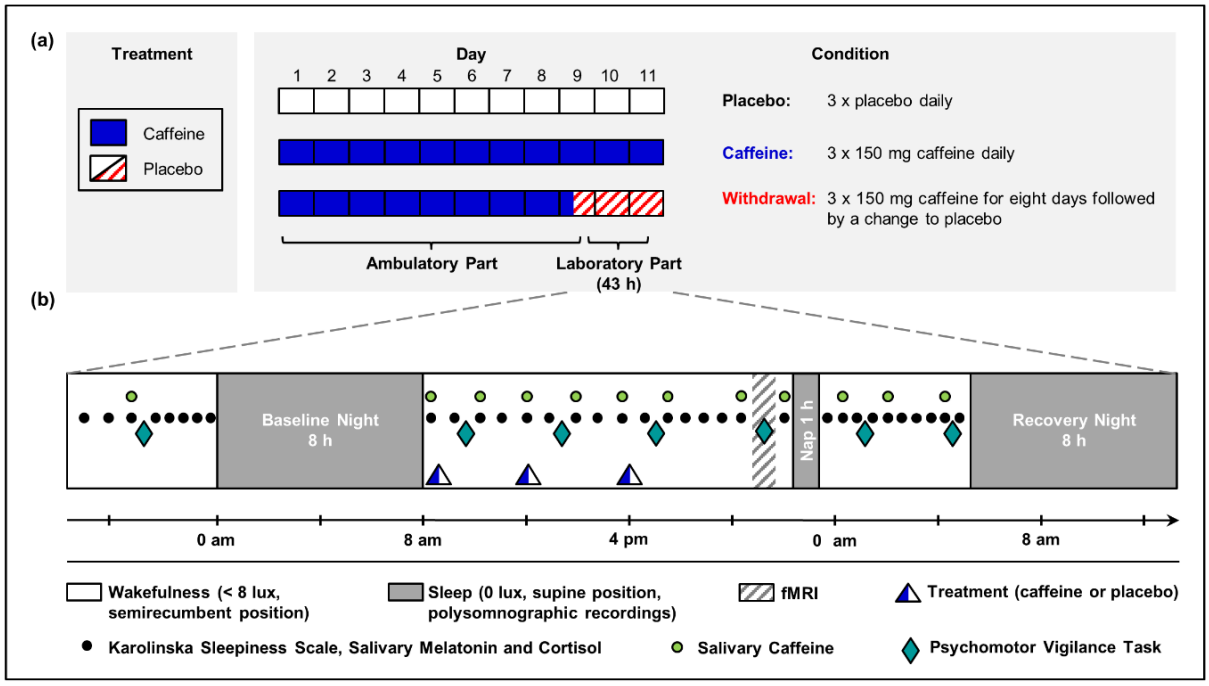

Fig. 1. Illustration of study protocol.
(a) Each volunteer participated in a placebo, a caffeine and a withdrawal condition, each comprising a 43-h laboratory stay, preceded by an ambulatory part. In each condition either caffeine or placebo capsules were administered three times daily. (b) The laboratory stay started in the evening of day nine with assessments of subjective sleepiness, vigilance performance, salivary melatonin, cortisol and caffeine levels, which were continued on the next day after an 8-h baseline night. In the evening of day 10, 14.5 h after wake-up, a 1-h nap was scheduled during a time of high circadian wake-promotion (Strogatz et al., 1987; Dijk & Czeisler, 1994) followed by 5.75 h of scheduled wakefulness and an 8-h recovery night.

2.3. Salivary caffeine

Caffeine levels were quantified in saliva samples collected in intervals of approximately 2 h during scheduled wakefulness during the laboratory stay. Subsequently, caffeine levels were analysed with liquid chromatography coupled to tandem mass spectrometry. One dataset was not included due to its non-availability.

2.4. Neurobehavioural assessments

Subjective sleepiness was assessed regularly with the Karolinska Sleepiness Scale (KSS) (Akerstedt & Gillberg, 1990) every 30 to 60 min during scheduled wakefulness. For analyses, values were binned to 4-h intervals. Vigilance performance was measured by a visual 10-

minute psychomotor vigilance task (PVT) (Dinges & Powell, 1985), every 4 h during scheduled wakefulness. Volunteers were instructed to focus on a white cross displayed on a black screen and to respond as fast as possible by a key press as soon as a millisecond counter appeared. The inter-stimulus interval was randomised between 2 and 10 s. Here, we focus on the number of lapses (reaction time > 500 ms), the most sensitive measure for the interaction of caffeine with both sleep pressure and circadian phase (Wyatt et al., 2004). As one of the tests was conducted in a magnetic resonance scanner with a compatible button box, lapses were z-transformed before analyses according to this change in environment.

2.5. Salivary melatonin and cortisol

Saliva samples were collected regularly in intervals of 30 to 60 min. For handling, see supplementary materials. Melatonin and cortisol levels were detected using a direct double-antibody radio immunoassay (Weber, Schwander, Unger, & Meier, 1997) and an enzyme-linked immunosorbent assay (ALPCO, Salem, NH, USA), respectively. For analyses, data were collapsed into bins of 1.5 h.

Four datasets were excluded from melatonin analyses due to insufficient data quality (placebo condition: one; caffeine condition: two; withdrawal condition: one). For analyses of melatonin, data were resampled every minute by applying linear interpolation. Subsequently, a bimodal skewed baseline cosine function (BSBCF) curve (Van Someren & Nagtegaal, 2007) was fitted to the data based on (Kolodyazhniy et al., 2012) with the modified cost function proposed in (Gabel et al., 2017). Goodness of fit (R^2) was acceptable for all datasets ($R^2 > 0.6$) except for two volunteers (placebo condition: one; caffeine condition: one). DLMOnset and dim light melatonin offset (DLMOffset) were determined for the fitted BSBCF curve applying a threshold of 0.1 of its amplitude which was defined by the difference between peak to baseline levels (Van Someren & Nagtegaal, 2007). In order to estimate condition-specific changes in the melatonin profile, the amplitude and the area under the curve (AUC) were calculated including samples following wake-up at day 10 of treatment.

2.6. Polysomnography during nap sleep

To test caffeine-induced differences in circadian wake-promotion, EEG was recorded during a one hour nap episode in the evening, starting 14.5 h after wake-up. It has repeatedly been shown that the ability to sleep is lowest in the evening (Strogatz et al., 1987; Dijk & Czeisler, 1994), mirroring maximal wake-promoting strength at the end of the day. Recorded EEG data were visually scored according to (Berry et al., 2012) by scorers who were blind to the condition. Slow-wave sleep (SWS) was further classified into stages 3 and 4 (Rechtschaffen & Kales, 1968). For details on recording procedure and scoring, see supplementary materials.

Total sleep time (TST) was calculated as sum of sleep stages 1, 2, 3, 4 and rapid eye movement (REM) sleep. Sleep efficiency (SE) was calculated as TST divided by time in bed. SWS comprises the sum of sleep stages 3 and 4. Sleep latency 1 and 2 were defined as latency to the first occurrence of sleep stage 1 and 2, respectively. Non-REM (NREM) sleep was calculated as sum of stages 2, 3 and 4. Duration of REM sleep was not analysed as most participants (n = 16; corresponding to 93%) did not reach this sleep state. To test condition-specific differences in the time course of nap sleep, time spent asleep (stages 1, 2, 3, 4 and REM sleep) and SWS were collapsed into 5-min time bins.

Spectral analysis of NREM sleep was conducted using a fast Fourier transformation (FFT) on 4-s time windows (hamming, 0% overlapped) resulting in 0.25 Hz bins. Frequency bins from 0.5 – 32 Hz of NREM sleep, recorded from frontal derivations (F3, F4), were analysed. Data were collapsed into 1 Hz bins and log-transformed. Note that condition-specific analyses are based on a reduced number of datasets because 10 participants did not initiate NREM sleep in at least one of the three conditions (four participants in placebo, six participants in caffeine, none in the withdrawal condition).

2.7. Statistical analyses

Data analyses were conducted with the statistical analyses software (SAS Institute, Cary, NC, USA) version 9.4 using mixed model analysis of variance (PROC MIXED) with *subject* as a random factor and the two repeated factors *condition* (three levels: placebo, caffeine and withdrawal) and *time* (levels differ per variable). To account for correlations between adjacent points within *time*, we used AR(1) as covariance structure (i.e. autoregressive (1)). In analyses without the factor *time*, we assumed CS (i.e. compound symmetry) to model the covariance structure most properly. Degrees of freedom were adjusted based on Kenward-Roger (Kenward & Roger, 1997). *P*-levels of post-hoc comparisons, derived from the LSMEANS statement, were corrected for multiple comparisons with the Tukey-Kramer method. Data of one participant in the caffeine condition have been excluded from all analyses due to incompliance with the treatment.

3. Results

3.1. Caffeine levels

As expected, caffeine levels were higher in the caffeine condition compared to both the withdrawal and placebo condition (main effect of condition: $F_{2,121}$ = 185.16; $p < 0.0001$; post-hoc tests: $p < 0.0001$) while levels during withdrawal were increased compared to the placebo condition (post-hoc tests: p = 0.035; mean ± SD: placebo: 18.52 ± 84.80 ng/ml; caffeine: 3024.18 ± 2163.68 ng/ml; withdrawal: 296.81 ± 683.42 ng/ml). As illustrated in Fig. 2, this general pattern was modulated by time (interaction condition x time: $F_{22,237}$ = 2.76; $p < 0.0001$),

mirroring both a decrease of caffeine levels during withdrawal condition and an increase in the caffeine condition after administration of treatment.

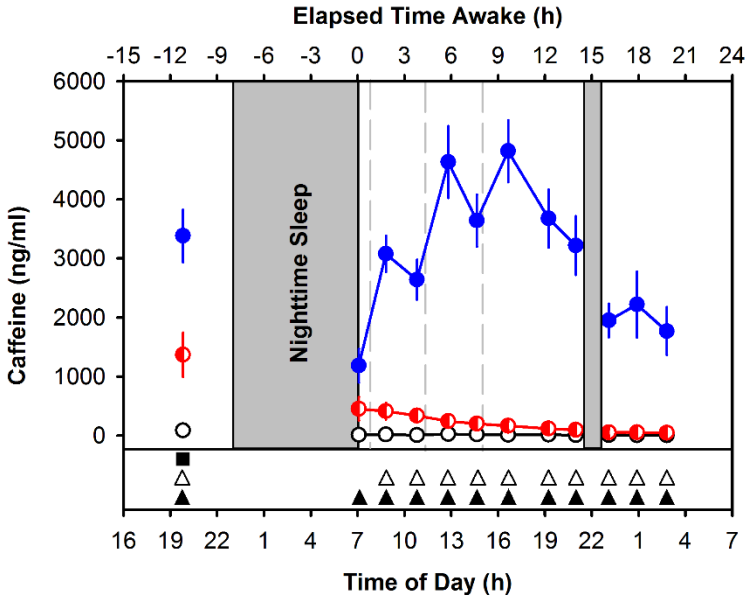

Fig. 2. Time course of caffeine levels across the 43 h under controlled laboratory conditions in the placebo (black open circles), caffeine (blue circles) and withdrawal (red semi-filled circles) condition. Pill administrations (caffeine or placebo) are depicted with dashed lines and the bottom x-axis indicates the mean time of day during which samples have been collected. Symbols mark significant ($p < 0.05$) post-hoc comparisons of the interaction effect condition x time, corrected according to (Curran-Everett, 2000) for multiple comparisons (black triangles: placebo compared to caffeine, open triangles: withdrawal compared to caffeine, black square: withdrawal compared to placebo).

3.2. Subjective sleepiness and vigilance performance

The time course of subjective sleepiness values for each of the three conditions is illustrated in Fig. 3(a) and the number of lapses in the PVT in Fig. 3(b).

The results of the mixed model analysis of variance indicated higher subjective sleepiness in the withdrawal condition compared to the placebo and caffeine conditions (main effect of condition: $F_{2,58.2} = 9.71$; $p < 0.001$, post-hoc tests: $p < 0.01$; mean ± SD: placebo: 4.72 ± 1.78; caffeine: 4.66 ± 1.91; withdrawal: 5.27 ± 1.81). A significant main effect of time ($F_{7,127} = 54.62$; $p < 0.0001$) confirmed a diurnal profile with higher sleepiness during the biological night compared to daytime.

The analysis of the number of lapses on the PVT yielded a significant main effect of condition ($F_{2,50.5} = 6.66$; $p = 0.0027$; post-hoc tests: $p < 0.01$) revealing more lapses during the withdrawal condition compared to the placebo and caffeine conditions (mean ± SD: placebo: -0.18 ± 0.77; caffeine: -0.09 ± 1.04; withdrawal: 0.27 ± 1.11). A significant main effect of time ($F_{6,98.2} = 7.55$; $p < 0.0001$) indicated more lapses during the night compared to daytime.

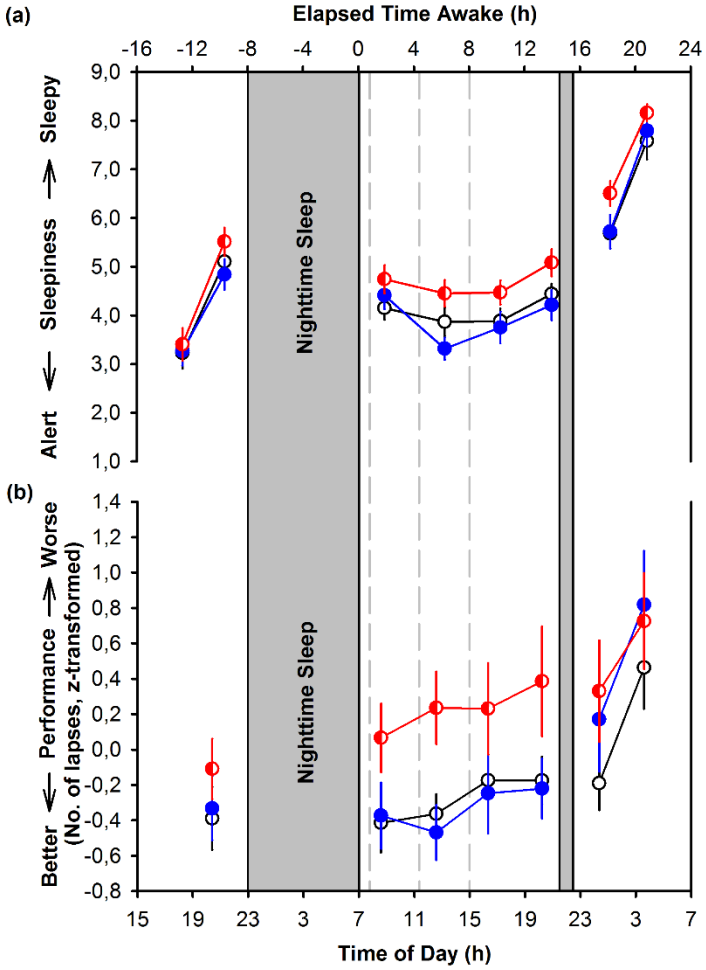

Fig. 3. Time course of subjective sleepiness (a) and attentional lapses assessed during PVT (b) in the placebo (black open circles), caffeine (blue circles) and withdrawal (red semi-filled circles) condition across 43-h (means ± standard errors). Pill administrations (caffeine or placebo) are depicted with dashed lines and the bottom x-axis indicates the mean time of day during which samples have been collected. Both subjective sleepiness and vigilance performance were impaired during withdrawal compared to placebo and caffeine.

3.3. Melatonin and cortisol

In the analysis of melatonin levels, only the main effect of the factor time ($F_{17,290}$ = 33.44; p < 0.0001) was significant, confirming a diurnal profile of higher melatonin levels during the night compared to daytime (Fig. 4(a)). Neither DLMOnset ($F_{2,33.4}$ = 1.16; p = 0.325) nor DLMOffset ($F_{2,35.3}$ = 1.32; p = 0.280) significantly differed between conditions. Similarly, the effect of condition on amplitude ($F_{2,33.8}$ = 0.57; p = 0.573) and AUC ($F_{2,33.8}$ = 1.77; p = 0.185) on day 10 of treatment did not reach significance. For means and standard errors of the melatonin outcomes, see Table S4.

The analysis of cortisol levels did not show significant differences between conditions ($F_{2,129}$ = 0.43; p = 0.653). However, a significant main effect of the factor time ($F_{17,303}$ = 31.66; p < 0.0001) demonstrated a normal diurnal pattern with higher cortisol levels in the morning and lower levels during the night. The time course of salivary cortisol levels is illustrated in Fig. 4(b).

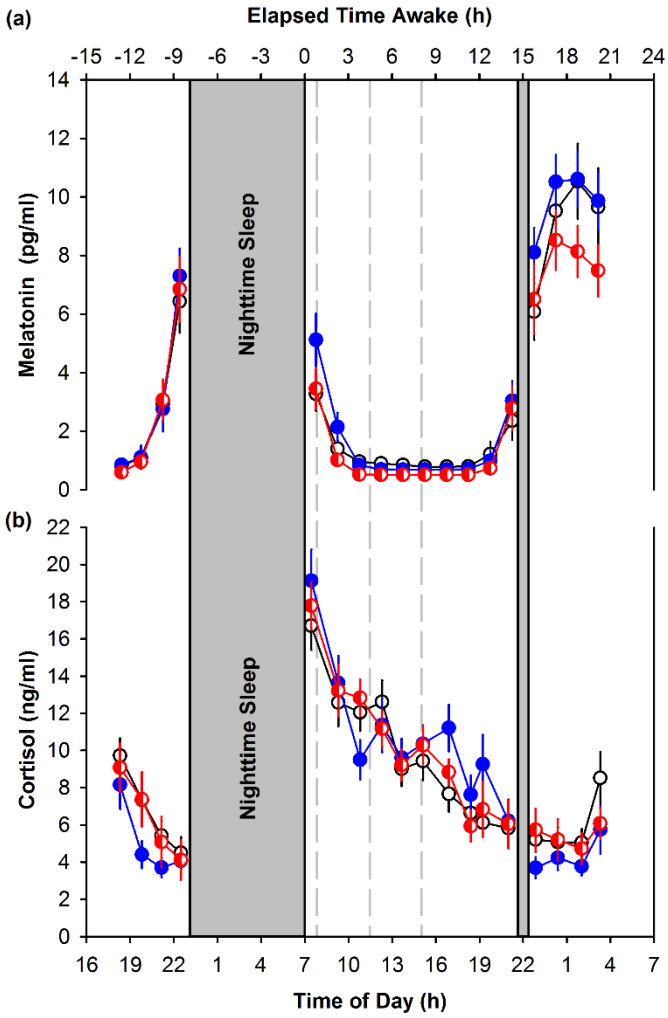

Fig. 4. Time course of salivary melatonin (a) and cortisol (b) across the 43-h laboratory stay. The means ± standard errors are depicted for placebo (black open circles), caffeine (blue circles) and withdrawal (red semi-filled circles) conditions and pill administrations (caffeine or placebo) are indicated with dashed lines. The melatonin profile depicts the fitted data and the bottom x-axis indicates the mean time of day during which samples have been collected. The typical course of both melatonin and cortisol across a day is clearly visible, however being independent of the treatment.

3.4. Evening nap sleep

A summary of sleep variables per condition and results of the statistical analyses are presented in Table 1. TST and SWS were longer, and SE was higher during withdrawal compared to placebo and caffeine conditions (post-hoc tests: $p < 0.05$). Furthermore, sleep latency to sleep stage 1 and stage 2 were shorter during withdrawal compared to placebo and caffeine conditions (post-hoc tests: $p < 0.05$). Generally, initiation of NREM sleep was less frequent in the caffeine and placebo conditions compared to withdrawal (no NREM sleep in placebo: $n = 4$, caffeine: $n = 6$, and withdrawal condition: $n = 0$, Cochrans Q-test: $p < 0.05$).

Table 1. Sleep parameters derived from visual scoring assessed during evening nap sleep.

Sleep Parameter	Placebo	Caffeine	Withdrawal	Factor Condition
TST (min)	31.63 ± 3.90	24.26 ± 4.55	43.88 ± 2.83[a]	$F(2,37.3) = 9.64$, $p < 0.001$
SE (%)	52.71 ± 6.50	40.44 ± 7.58	73.12 ± 4.72[a]	$F(2,37.3) = 9.64$, $p < 0.001$
Stage 1 (min)	4.95 ± 0.73	4.66 ± 0.83	5.80 ± 0.85	$F(2,37.6) = 0.67$, $p = 0.519$
Stage 2 (min)	12.10 ± 1.97	9.82 ± 2.22	15.30 ± 1.57[b]	$F(2,37.2) = 3.85$, $p = 0.030$
Stage 3 (min)	6.53 ± 1.48	5.74 ± 1.73	10.20 ± 1.96[b]	$F(2,37.4) = 4.09$, $p = 0.025$
Stage 4 (min)	6.80 ± 2.16	3.74 ± 2.01	11.48 ± 2.69[b]	$F(2,37.2) = 6.06$, $p = 0.005$
SWS (min)	13.33 ± 2.82	9.47 ± 2.70	21.68 ± 3.00[a]	$F(2,37.3) = 8.22$, $p = 0.001$
NREM (min)	25.43 ± 4.01	19.29 ± 4.27	36.98 ± 3.34[a]	$F(2,37.1) = 8.87$, $p < 0.001$
SL1 (min)	25.70 ± 3.94	28.51 ± 4.57	12.09 ± 2.11[a]	$F(2,37.4) = 7.59$, $p = 0.002$
SL2 (min)	31.16 ± 4.11	37.94 ± 4.51	18.39 ± 2.87[a]	$F(2,37.3) = 9.80$, $p < 0.001$

Values are for means ± standard errors. TST: total sleep time; SE: sleep efficiency; SWS: slow-wave sleep (stage 3 + 4); NREM: non-rapid eye movement sleep; SL1: sleep latency to sleep stage 1; SL2: sleep latency to sleep stage 2.

[a] $p < 0.05$ compared to placebo and caffeine conditions.

[b] $p < 0.05$ compared to caffeine condition.

In a next step, we were interested whether time spent asleep and SWS accumulate differently depending on the treatment. As depicted in Fig. 5, particularly in the beginning of the nap, time spent asleep and SWS accumulated faster during the withdrawal condition compared to caffeine and placebo conditions (interaction condition x time for time spent asleep: $F_{22,354} = 5.40$; $p < 0.0001$; and SWS: $F_{22,354} = 3.82$; $p < 0.0001$).

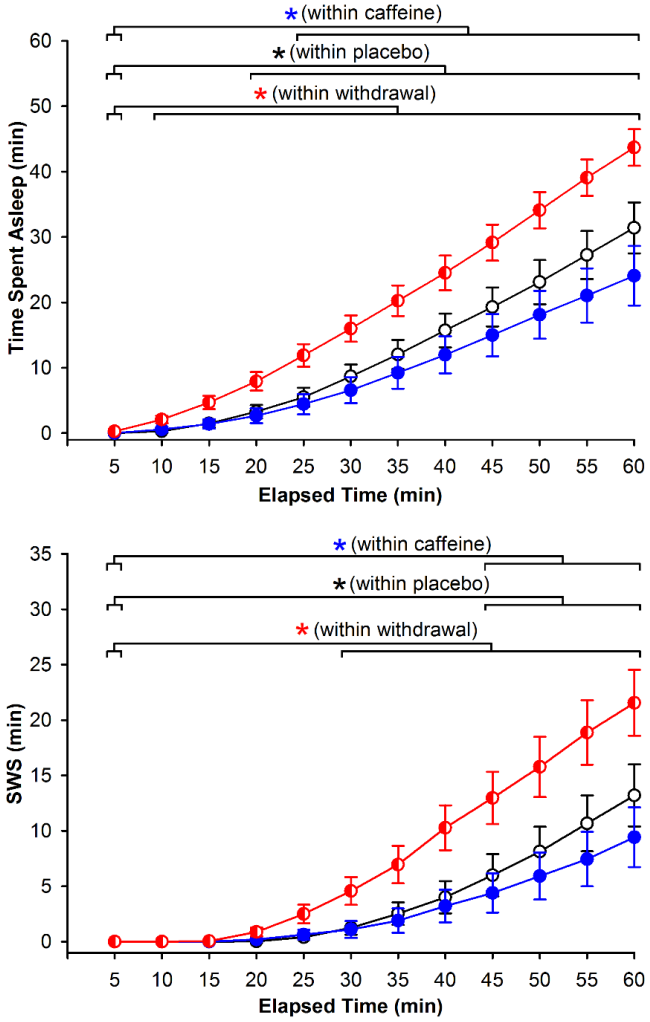

Fig. 5. Accumulation curves of time spent asleep and SWS during evening nap sleep. Data were collapsed into bins of 5 min and accumulated across the 1 h. Means and standard errors are represented for placebo (black open circles), caffeine (blue circles) and withdrawal (red semi-filled circles). Asterisks mark significant differences to the first bin within the same condition ($p_{all} < 0.05$).

In a final step, differences in spectral power density between conditions were analysed. There were no significant differences in spectral power density between caffeine and placebo. However, spectral power density was reduced in the sigma range during withdrawal compared to placebo condition (15 Hz: $F_{2,27.4} = 3.48$; $p = 0.045$; 16 Hz: $F_{2,27.6} = 3.15$; $p = 0.059$).

4. Discussion

The aim of the present study was to examine the effects of daily caffeine intake and its cessation on circadian timing, wake-promotion and the course of neurobehavioural indices in habitual consumers under entrained conditions. Making use of a carefully controlled within-subject design, we focused on the effects of repeated daily consumption in the morning and afternoon, as this is the typical pattern in the society nowadays. Under these conditions, neither caffeine consumption nor its cessation affected the diurnal profile of melatonin or cortisol

secretion. However, for more than 20 h, cessation of intake induced a state of withdrawal, characterised by higher subjective sleepiness, impaired vigilance performance and a higher drive to sleep even at a phase of high circadian wake-promotion. It is concluded that daily daytime intake of caffeine throughout the morning and afternoon does not strongly affect human circadian rhythms under entrained conditions. Still, it induces adaptations, potentially at the level of the adenosine receptors, which come to light as a state of reduced arousal and alertness as soon as caffeine intake is ceased.

In animal studies, long-term treatment with caffeine leads to a different timing of circadian rest-activity rhythms, specifically to a lengthening of circadian period under constant lighting conditions (Oike, Kobori, Suzuki, & Ishida, 2011; van Diepen et al., 2014). Similar observations in unmasked human rest-activity cycles are missing so far. However, under conditions of forced desynchronisation between the timing of circadian rhythms and sleep-wake cycles, circadian plasma melatonin rhythms were not altered in response to hourly administration of caffeine over four weeks (Wyatt et al., 2004). The results of the present study add that – under entrained conditions – the timing of both melatonin and cortisol does not necessarily change by daily caffeine consumption in the morning, midday and afternoon, a temporal pattern which is commonly observed in habitual consumers (Martyn et al., 2018). Importantly, based on the absence of a clear-cut shift in circadian timing in the withdrawal condition, it seems unlikely that the participants might have developed tolerance to the phase-shifting effects of the drug. Please note, however, that apart from statistics on the group's average, visual inspection revealed a delay in DLMOffset during caffeine in more than half of the volunteers ($n = 13$, see supplementary materials). Thus, interindividual differences in response to the stimulant (Fulton et al., 2018) as well as the small sample size might have hampered statistical condition-specific differences at $p < 0.05$. Interestingly, one (Panagiotou et al., 2018) of three (Oike et al., 2011; Narishige et al., 2014; Panagiotou et al., 2018) animal studies on the effects of repeated caffeine intake suggests a slight delay in activity onset of mice under normal light-dark cycles. However, in naïve animals no phase-shift in locomotor activity pattern has been found if caffeine was administered at activity onset (Jha et al., 2017). In humans, a one-time treatment before regular wake-up time could have the potential to prevent dim-light induced phase delays (Burke and Wright, personal communication (Burke et al., 2018)). Together, the effects of caffeine intake on circadian timing in the morning hours might thus differ according to treatment continuity (i.e. acute vs chronic). Moreover, the available evidence indicates that the effects of repeated daytime caffeine intake on human circadian timing when consumed in the morning, midday and afternoon under entrained conditions seem to be small.

Most likely, however, the effects of caffeine on circadian rhythms depend on the time of intake. While there is evidence that morning caffeine intake prevents dim-light induced phase delays (Burke and Wright, personal communication (Burke et al., 2018)), caffeine administration in

the evening delays circadian phase (Burke et al., 2015). As a consequence caffeine taken throughout the day as in the present study may have cancelled out any potential phase shifting effects. So far, a delay (Burke et al., 2015) or reduction in melatonin (Wright et al., 1997) in humans, either abstinent (Burke et al., 2015) or potentially under withdrawal (Wright et al., 1997), was specifically induced after a caffeine treatment in the evening or at night. In contrast, repeated caffeine intake in the morning did not successfully entrain three blind patients (St Hilaire & Lockley, 2015). Furthermore, a recent animal study under constant conditions suggests that caffeine treatment does only potentiate light-induced phase shifts when given at the end of the active phase or during rest, but not at the start of the active phase (Jha et al., 2017). Together with the present results, the evidence suggests that the circadian system seems to be particularly sensitive to caffeine when given at the end of the biological day. Future studies might disentangle circadian and sleep-homeostatic contributions to this effect. Independent of time-of-day, we observed clear-cut effects induced by caffeine withdrawal. In line with earlier studies (Juliano & Griffiths, 2004), the acute challenge of cessation from caffeine was associated with signs of increased sleep pressure, such as increased subjective sleepiness and worse vigilance performance during day and nighttime as well as a faster initiation of NREM sleep even at a time of high circadian wake-promotion. Thus, the preceding repeated presence of caffeine might have induced compensatory adaptations at the neuronal level (Ferré, 2008), which modulate the stimulatory effects of caffeine and underlie the effects of withdrawal as soon as consumption is stopped. Several changes have been associated with long-term caffeine intake, e.g. upregulation of adenosine receptors (Fredholm, 1982; Shi et al., 1993; Johansson et al., 1997; Varani et al., 1999), increased plasma adenosine concentrations (Conlay et al., 1997) or modulations in the function of adenosine heteromers (Ciruela et al., 2006). These neuronal changes in the adenosinergic system might alter the homeostatic sleep need. In the present study, an increased sleep pressure experienced during caffeine withdrawal might have overruled the circadian drive for wake-promotion in the evening, a phenomenon which has already been shown after sleep restriction in humans (Sargent, Darwent, Ferguson, Kennaway, & Roach, 2012).

In contrast to the clear-cut symptoms of caffeine withdrawal in behaviour, we do not have any indication for a significant difference in either of the measured variables during daily caffeine consumption compared to placebo. Importantly our study was designed to focus on the effects of caffeine after a certain period of repeated intake under normal sleep-wake conditions. In earlier studies that showed a caffeine-induced sleep disruption during circadian wake-promotion after continuous daily intake, measurements were taken under relatively high levels of sleep pressure (i.e. after 25 h (Carrier et al., 2009) or 28 h (Wyatt et al., 2004) of wakefulness). Interestingly, reviews indeed suggest that the stimulating properties of caffeine are most prominently under high sleep pressure such as after sleep deprivation or sleep

restriction (Roehrs & Roth, 2008; Snel & Lorist, 2011). Therefore, we may not exclude that caffeine intake would have induced sleep disruption and alertness under a longer duration of wakefulness as was applied in the present study. However, studies controlling for withdrawal reversal, similarly applied in our study, failed to show a caffeine-induced improvement in performance in sleep-restricted subjects (James, Gregg, Kane, & Harte, 2005; Keane & James, 2008), indicating that improvements by caffeine cannot solely be explained by sleep-wake-history but probably also depend on preceding caffeine intake. Applying repeated caffeine administrations and an ambulatory period of nine days prior to each assessment phase, potential effects in the caffeine condition deriving from withdrawal reversal and carry-over effects can likely be excluded.

Moreover, one might argue that the lack of improvement in subjective alertness and performance during caffeine intake compared to placebo condition is due to a floor effect. In other words, the low sleepiness and high performance level occurring during the placebo condition did not leave much room for additional improvement by caffeine. However, our measurements took place also during the biological night, in which we observed the typical nighttime decrease in alertness and performance. As we did not observe a significant caffeine-induced improvement under these conditions, our results suggest that the effects of daytime caffeine intake on alertness and performance are either short-lasting, small or not present under conditions of habitual daily caffeine intake.

Finally, the development of tolerance could have been induced by regular daily caffeine consumption. There are convincing indicators for tolerance to occur after 3-5 days of habitual caffeine intake (James, 2014) during both wakefulness and sleep with the potential of complete (Evans & Griffiths, 1992) or partial tolerance (Bonnet & Arand, 1992; Watson, Deary, & Kerr, 2002). Moreover, the current results in subjective sleepiness and vigilance performance during caffeine cessation provide evidence for an adaptation to the daily exposure of caffeine, however, circadian timing and amplitude remain mainly unaffected. Within this context, it is important to note that the present study design does not include a condition assessing the effects of acute caffeine intake after long-term abstinence. Thus, we cannot provide a strict measure of tolerance by comparing acute effects of caffeine with the effects after daily treatment in the same individuals. This is of particular interest, as the habitual consumption level in the present sample might be an indicator for a reduced sensitivity to react to caffeine (Rétey et al., 2007). Nevertheless, doses of 400 mg have been shown to induce certain performance benefits in habitual consumers (Brunye, Mahoney, Lieberman, Giles, & Taylor, 2010) and it is still debatable whether there is a dose-dependent saturation of caffeine-induced performance benefits at 200 mg as suggested by (Brunye, Mahoney, Lieberman, & Taylor, 2010) (but see (Childs & de Wit, 2006; Brunye, Mahoney, Lieberman, & Taylor, 2010)).

We are aware that this study may have several limitations which have to be taken into careful consideration. First, no washout phase was scheduled between the conditions. However, we implemented an ambulatory part with a fixed sleep-wake schedule and a constant treatment during nine days preceding data collection in the lab. Thus, circadian and sleep-wake systems might have returned to a normal state and recovered from previous conditions. Second, the exclusion of female volunteers clearly limits the generalisability of the present results. We studied male participants only in order to avoid potential confounding by the menstrual cycle on caffeine elimination (Lane et al., 1992) and sleep-wake regulation and thus on our main outcome variables melatonin, cortisol and sleep (Shechter & Boivin, 2010). Moreover, we aimed at reducing variance in the data due to the potential use of oral contraceptives which have been shown to change caffeine clearance (Abernethy & Todd, 1985; Balogh et al., 1995) and its effect depending on the duration of oral contraceptive use (Rietveld, Broekman, Houben, Eskes, & van Rossum, 1984). Third, we studied volunteers with an age range of 18-35 years. As evidence suggests an age-related modulation of caffeine-effects on cognition (Jarvis, 1993; Hogervorst, Riedel, Schmitt, & Jolles, 1998) and sleep (Drapeau et al., 2006; Robillard et al., 2015) our results are not necessarily transferable to other age groups such as teenagers or adults older than 40 years. Fourth, in the present study regular caffeine consumers were studied of which some reported habitual caffeine consumption in the evening (see supplementary materials). However, evening caffeine intake is not common (Martyn et al., 2018) presumably due to potential sleep-disruption (Landolt, 2008) and thus might be an indicator of a certain insensitivity to the effects of caffeine. While the observed clear-cut withdrawal-induced effects make it unlikely that our sample was entirely unresponsive to the stimulant, the impact of caffeine might be stronger as compared to our sample in more sensitive individuals. Last, no genetic information including variations in the adenosine A_{2A} receptor gene (ADORA2A) were collected, which have been previously associated with habitual caffeine intake and insensitivity to the effects of the stimulant (Rétey et al., 2007). However, based on the clear-cut changes in performance and sleepiness when caffeine was ceased, it is unlikely that volunteers were insensitive to the effects of caffeine but rather developed tolerance due to the repeated intake of the stimulant.

Taken together, this is the first study investigating the impact of habitual caffeine consumption on human circadian rhythms under entrained conditions. The study was designed to focus on the effects of a typical pattern of caffeine consumption with daily intake in the morning, midday and afternoon. We provide first evidence that this type of exposure to the stimulant does not considerably shift circadian markers such as melatonin and cortisol nor does it lead to an increased wake-promotion in the evening. However, the acute challenge of cessation from caffeine was associated with signs of increased sleep pressure. Together, our data point to an adaptation of waking-performance to habitual exposure to the stimulant while circadian

markers remain fairly stable. These mechanisms of both adaptation and robustness might enable normal sleep-wake states during constant supply of a stimulating agent in the central nervous system.

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Declaration of competing interest

The authors declare that there is no conflict of interest.

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Supplemental Material

Description of study sample

Table S1. Demographic characteristics of study sample.

Demographics	Mean ± SD
Age (years)	26.4 ± 4.0
Habitual daily caffeine intake (mg)	478.1 ± 102.8
Body Mass Index (kg/m^2)	22.7 ± 1.4
Morningness-Eveningness Questionnaire [1]	52.8 ± 8.7
Munich ChronoType Questionnaire (MSF sc) [2]	4.2 ± 0.7
Epworth Sleepiness Scale [3]	3.6 ± 3.4
Pittsburgh Sleep Quality Index [4]	2.8 ± 1.4
Beck Depression Inventory-II [5]	1.4 ± 2.3

Timing of habitual daily caffeine intake

As illustrated in the following table, caffeine was almost equally often consumed in the morning, during lunchtime and in the afternoon, which is similar to the timing of the caffeine administrations in the current study. Eight volunteers consumed caffeine additionally after dinner, however, mostly aliments containing low levels of caffeine such as soda drinks (n = 1), tea (n = 1), chocolate (n = 3) or cacao (n = 1). Only two volunteers consumed coffee after dinner. The information about the timing of caffeine intake of one volunteer was missing.

Table S2. Timing of at least one caffeinated beverage or food.

Breakfast	Between Breakfast and Lunch	Lunch	Between Lunch and Dinner	Dinner	After Dinner
n = 18	n = 17	n = 15	n = 18	n = 6	n = 8

Rationale for sample size

Calculation of sample size was done with G*Power 3.1 [6], planning the calculation of an ANOVA for repeated measures (three conditions) with an accepted α error probability = 0.05 and power (1-β) = 0.8. For an estimation regarding circadian phase, the assumed correlation between measures (r = 0.7) was based on a previous study, in which melatonin was assessed in two differential sleep pressure conditions in young healthy volunteers after seven days of a fixed sleep-wake cycle [7]. The expected effect size (f^2 = 0.26) is assumed on the basis of [8] investigating the influence of caffeine on melatonin levels.

Frequency of order of conditions

Volunteers were pseudo-randomly allocated to the order of the three conditions based on random permutation controlling for block size.

Table S3. Number of participants per order of conditions.

Caffeine - Placebo - Withdrawal	Caffeine - Withdrawal - Placebo	Withdrawal - Placebo - Caffeine	Withdrawal - Caffeine - Placebo	Placebo - Caffeine - Withdrawal	Placebo - Withdrawal - Caffeine
$n = 3$	$n = 3$	$n = 4$	$n = 3$	$n = 4$	$n = 3$

Caffeine levels during ambulatory phase

In order to verify volunteers' compliance to the regimen prior to laboratory admission, samples containing fingertip sweat were collected approximately 8 h after the last pill intake on days one to eight and 5 h after the last pill intake on day nine of treatment. Subsequently, caffeine levels were analysed by triple-quadrupole mass spectrometry.

A significant main effect of condition ($F_{2,51.8} = 21.70$; $p < 0.0001$) confirmed increased caffeine levels during the caffeine and withdrawal conditions compared to the placebo condition ($p_{all} < 0.0001$), depicted in Figure S1. Although these values suggest compliance, note that we cannot fully exclude that caffeine levels were due to the intake of caffeine-containing beverages and food rather than the treatment capsules.

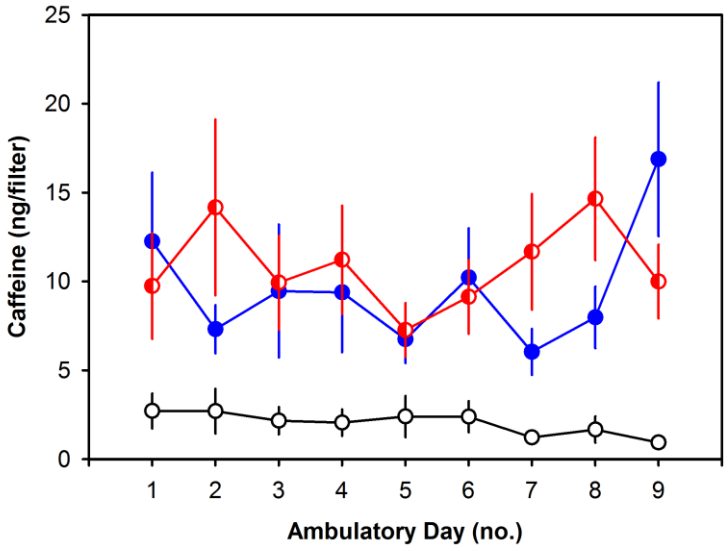

Figure S1. Time course of caffeine levels collected during the ambulatory phase in the placebo (black open circles), caffeine (blue circles) and withdrawal (red semi-filled circles) conditions confirming volunteers' compliance to the regimen.

Handling and analyses of saliva samples

Saliva samples were collected regularly in intervals of 30 to 60 min under dim light conditions (< 8 lux). Following collection, samples were stored at maximum 5°C, later centrifuged (3000 rpm for a duration of 10 min) and subsequently stored at -24°C until data analyses. Melatonin and cortisol levels were analysed by a trained staff member (at Bühlmann Laboratories AG, Schönenbuch, Switzerland) using a direct double-antibody radio immunoassay [9] and an enzyme-linked immunosorbent assay (ALPCO, Salem, NH, USA), respectively.

To test caffeine-dependent shifts in circadian timing, we analysed condition-specific differences in dim light melatonin onset (DLMOnset) and dim light melatonin offset (DLMOffset). In order to account for slightly different bedtimes between conditions in three volunteers (twice: +30 min in caffeine compared to placebo and withdrawal conditions; once: -30 min in placebo compared to caffeine and withdrawal conditions), we further tested condition-specific effects in phase angle (difference of bedtime – DLMOnset). However, phase angle did not significantly differ among the three conditions on day nine or on day ten of treatment (p_{all} > 0.2). A summary of melatonin parameters per condition and results of the statistical analyses are depicted in Table S4.

Table S4. Melatonin parameters assessed on day 10 of treatment.

Melatonin Parameter	Placebo	Caffeine	Withdrawal	Factor Condition
DLMOnset (h)	21.74 ± 0.33	21.15 ± 0.18	21.60 ± 0.31	$F(2,33.4) = 1.16$, $p = 0.325$
Phase angle (h)	1.32 ± 0.31	1.91 ± 0.16	1.48 ± 0.25	$F(2,33.6) = 1.46$, $p = 0.246$
DLMOffset (h)	8.35 ± 0.45	9.04 ± 0.26	8.69 ± 0.22	$F(2,35.3) = 1.32$, $p = 0.280$
Amplitude (pg/ml)	11.00 ± 1.44	10.71 ± 1.02	9.92 ± 1.32	$F(2,33.8) = 0.57$, $p = 0.573$
AUC (pg*h/(24*ml))	3.17 ± 0.36	3.78 ± 0.41	2.96 ± 0.39	$F(2,33.8) = 1.77$, $p = 0.185$

DLMOnset: dim light melatonin onset; DLMOffset: dim light melatonin offset; AUC: area under the curve. Values represent means ± standard errors for each condition.

For a more detailed inspection, the individual values of the DLMOnset, DLMOffset and the amplitude on day 10 of treatment are presented in Figure S2.

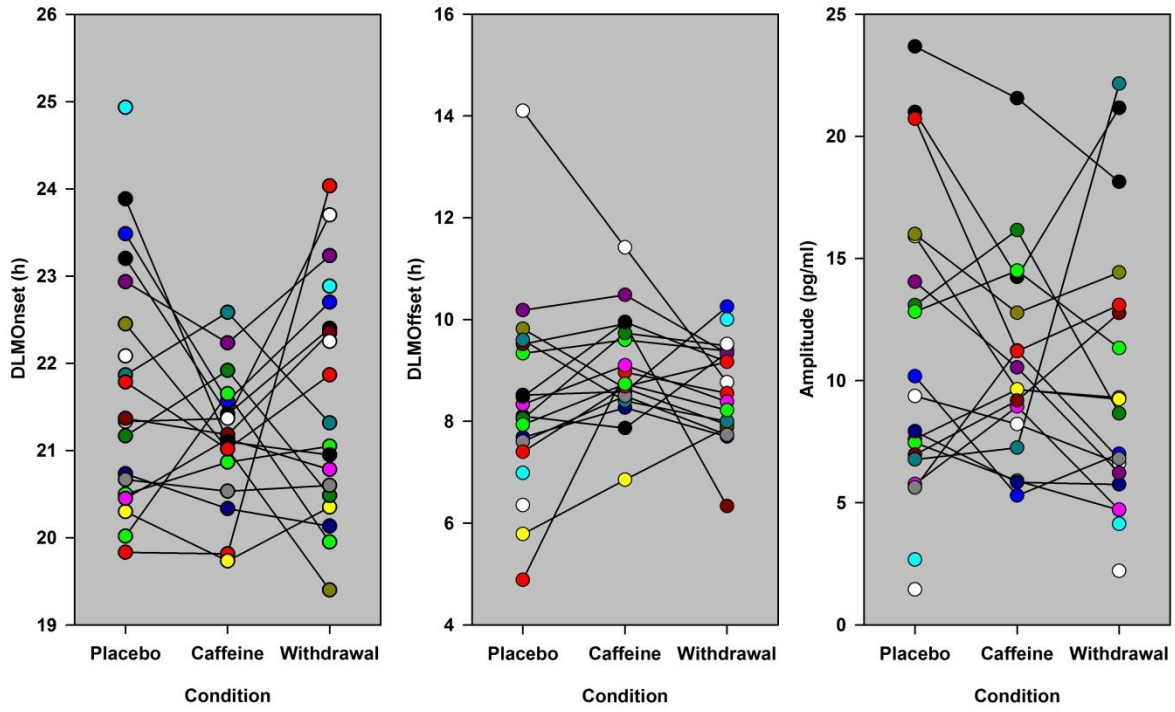

Figure S2. Depicted are the individual values for the melatonin parameters DLMOnset (left), DLMOffset (centre) and amplitude (right) assessed on day 10 of treatment. The exclusion of a visually identified extreme value (> 1.5 x interquartile range) in the analyses of DLMOffset (depicted as white point in the figure) reveals a significant delay in the caffeine condition compared to placebo (main effect of condition: $F_{2,34.5} = 3.75$; $p = 0.034$; post-hoc tests: $p = 0.030$).

Polysomnographic recordings

For recordings, we used a portable V-Amp device (Brain Products GmbH, Gilching, Germany) and Grass gold cup electrodes. Applied according to the standard international 10-20 system, two electro-oculargraphic, two electro-myographic and two electro-cardiographic signals were recorded, together with six derivations from the frontal, central and occipital regions (F3, F4, C3, C4, O1, O2) referenced against the linked mastoids (A1, A2). Data were recorded with a sampling rate of 500 Hz and filtered online by applying a notch filter at 50 Hz.

Epochs of 30 seconds were visually scored. Additionally, one third of the data have been scored by a second trained staff member in order to ensure a continuous scoring agreement of at least 85%.

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4.3. Regular caffeine intake attenuates REM sleep promotion and sleep quality in healthy men

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Abstract

Acute caffeine intake can attenuate homeostatic sleep pressure and worsen sleep quality. Besides, caffeine intake - particularly in high doses and close to bedtime - may also affect circadian-regulated 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 x 150 mg caffeine daily), a withdrawal (3 x 150 mg caffeine for eight days then placebo), and a placebo condition. After ten days of controlled intake and a fixed sleep-wake cycle, we recorded 8 h of electroencephalography starting 5 h after habitual bedtime (i.e., start on average at 04:22 am 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 latency was longer after daily caffeine intake compared to both placebo and withdrawal. Moreover, the accumulation of REM sleep proportion was slower, and volunteers reported more difficulties at awakening after sleep and feeling more tired upon wake-up in the caffeine condition compared to placebo. Our data indicate that besides acute also regular daytime caffeine intake affects REM sleep regulation in men. We have evidence that regular caffeine intake during daytime weakens circadian sleep promotion when compared to 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, circadian

1. Introduction

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 consume caffeinated aliments day by day (Heckman, Weil, 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 & Giesbrecht, 2013) and a good subjective nighttime sleep quality despite of previous stimulant consumption (Snel & Lorist, 2011). These double-edged alerting but sleep-disrupting effects of caffeine have been traced back to its impact on the homeostatic component of sleep-wake regulation (Landolt, 2008). By antagonizing adenosine (Fredholm et al., 1999), caffeine dampens homeostatic sleep need (Landolt, 2008), as evident in caffeine-induced reductions of waking 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 not only determined by homeostatic sleep need but also by the circadian timing system (Borbély, 1982; Lazar, Lazar, & Dijk, 2015). One of the most prominent circadian sleep features is REM sleep propensity, which usually peaks in the morning around 2 h after the nadir of core body temperature (Dijk & Czeisler, 1995). This natural peak right before usual wake time may facilitate the re-arousal of the brain from sleep (Dijk & Czeisler, 1995) and may contribute to REM-sleep's substantial promotion of good subjective sleep quality (Akerstedt, Hume, Minors, & Waterhouse, 1994; Della Monica, Johnsen, Atzori, Groeger, & Dijk, 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 impacts on 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 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 & Arand, 1992; Drake et al., 2013)) - report a caffeine-induced reduction of REM sleep duration (Brezinova, 1974; Nicholson & Stone, 1980; Robillard et al., 2015) or shift in REM sleep episodes (Karacan et al., 1976; Nicholson & Stone, 1980) while others did not report any differences in REM sleep after caffeine administration (Bonnet & 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 reduced REM sleep at the cost of wakefulness (Carrier et al., 2007; Carrier et al., 2009). Whether such effects during daytime recovery sleep can also be observed under conditions of regular daily daytime caffeine intake remains unknown.

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 to 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.

2. Methods

2.1. Volunteers

Data sets were available from a total of 20 male study volunteers between 18 and 32 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 & Lorist, 2011). Prior to study participation, all volunteers were screened for good health assessed by self-report questionnaires and a medical check performed by a study physician. Individuals reporting a 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), 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 & Ostberg, 1976)) resulted in the exclusion of participants. In addition, volunteers were not allowed to engage in shift work (< three months prior to study) or to travel across more than two time zones (< one month prior to study). In order to minimize the potential confounding by the menstrual cycle and the use of oral contraceptives on sleep (Shechter & Boivin, 2010) and caffeine elimination (Abernethy & Todd, 1985; Balogh et al., 1995), female individuals were not included in the present 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)	Mean ± 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 (hh:mm)[1]	23:21 ± 00:49
Habitual sleep duration (hh:mm)[1]	07:28 ± 00:25

Notes. BMI: Body Mass Index; MEQ: Morningness-Eveningness Questionnaire (Horne & Ostberg, 1976); PSQI: Pittsburgh Sleep Quality Index (Buysse et al., 1989); [1]self-reported.

2.2. 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 Fig. 1a, each condition comprised an ambulatory part of nine days which was followed by an in-lab part of 43 h. In each condition, volunteers swallowed identical appearing gelatin capsules three times daily (45 min, 255 min, and 475 min after awakening) containing either caffeine (150 mg; Hänseler AG, Herisau, Switzerland) or placebo (mannitol; Hänseler AG, Herisau, Switzerland). To induce caffeine withdrawal in the withdrawal condition, the first capsule on day nine of treatment contained caffeine but was followed by placebo capsules for the remaining administrations. The dosage and timing of administrations was chosen based on previous studies which investigated tolerance development to the effects of caffeine and its cessation (James, 1998; Keane & 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 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. 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 to placebo and withdrawal conditions; one volunteer: -30 min in placebo compared to caffeine and withdrawal conditions).

As illustrated in Fig. 1b, on day nine 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 was scheduled at the volunteer's habitual bedtime. The following day, saliva was regularly

collected in order to quantify the levels of caffeine and its main metabolites. In the evening, we scheduled a one-hour 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, light (scheduled wakefulness: < 8 lux; sleep episode: 0 lux), and posture (scheduled wakefulness: semirecumbent; sleep episode: supine) was controlled.

Approximately 5 h after volunteers' habitual bedtime (mean: 04:22 am; range: 03:15 - 05:15 am), a sleep episode of 8 h was scheduled, which corresponds to the expected circadian peak of REM sleep promotion (Dijk & 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 & Hindmarch, 1978).

Approval was obtained from the local ethics committee (Ethikkommission Nordwest- und Zentralschweiz) and the study was conducted in accordance with the declaration of Helsinki. All volunteers provided a written informed consent and received financial compensation for study participation.

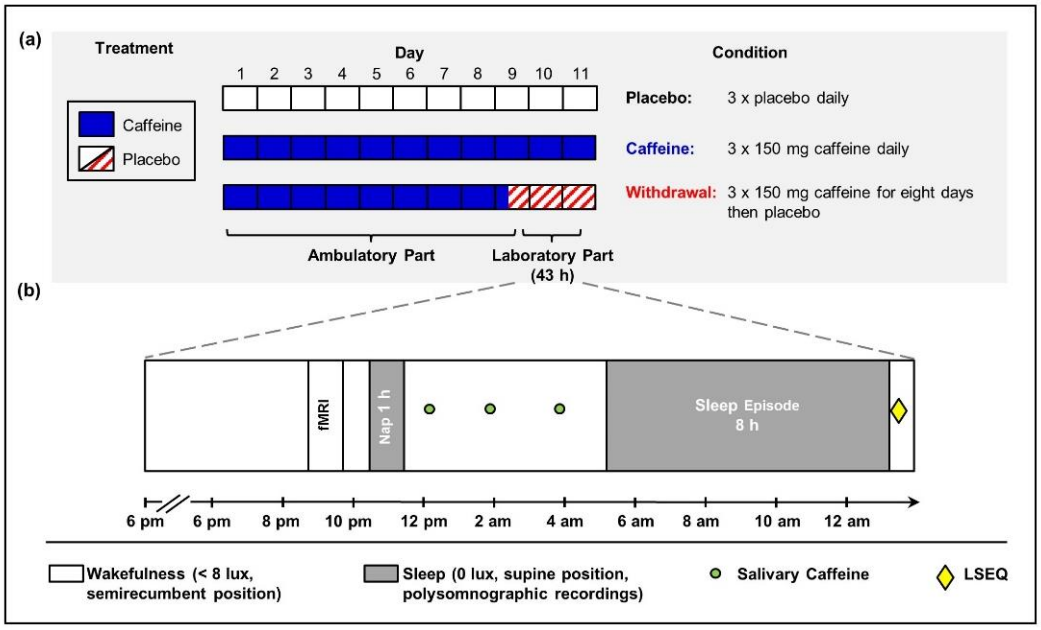

Fig. 1. Illustration of the research protocol (adapted from (Weibel, Lin, Landolt, Garbazza, et al., 2020)). **(a)** Each participant took part in a placebo, a caffeine, and a withdrawal condition consisting of an ambulatory part of nine days and an in-lab part of 43 h. **(b)** The in-lab protocol started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

2.3. Caffeine levels

Salivary caffeine levels were repeatedly assessed in intervals of approximately two hours 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 rpm 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 was lost.

2.4. Subjective sleep quality

To assess subjective sleep quality, volunteers completed the LSEQ questionnaire (Parrott & Hindmarch, 1978) right after the end of the 8-h nighttime sleep episode. Volunteers rated 10 items on visual analogue scales consisting of four different scales, i.e. getting to sleep (GTS), quality of sleep (QOS), awake following sleep (AFS), and behavior following wakening (BFW).

2.5. Electroencephalographic recordings

We utilized electroencephalographic recordings to assess sleep structure. Six EEG derivations (F3, F4, C3, C4, O1, 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, 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 the present 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 we focus in the present paper 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 seconds. Artefacts were automatically rejected, and power spectra in the delta band (0.1-4 Hz) during NREM sleep is reported as a measure for sleep pressure.

Three datasets were excluded from analyses of all-night parameters 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 parameters (placebo condition) based on potential missed REM sleep episode by the algorithm and disagreement with visual scorers.

2.6. Statistical analyses

Data analyses were performed using SAS (version 9.4, SAS Institute, Cary, United States). Values exceeding three 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 (REM latency = 1). 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 & 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 parameters, we performed general linear models with the statistics software SPSS (version 24, IBM Corporation, Armonk, NY, USA) including REM sleep parameters as covariates and subject as a random factor. A p-value < 0.05 was used to determine statistical significance. However, the thresholds for the sleep stages and subjective sleep quality were adjusted according to the Bonferroni method (to $p < 0.0055$ and $p < 0.0125$ respectively) due to multiple testing. One volunteer was excluded from all analyses due to noncompliance with the treatment requirements (caffeine condition: $n = 1$).

3. Results

3.1. 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 to the placebo and withdrawal conditions ($p_{all} < 0.0001$), as presented in Fig. 2. Caffeine levels in the withdrawal condition did not significantly differ from placebo ($p = 0.984$).

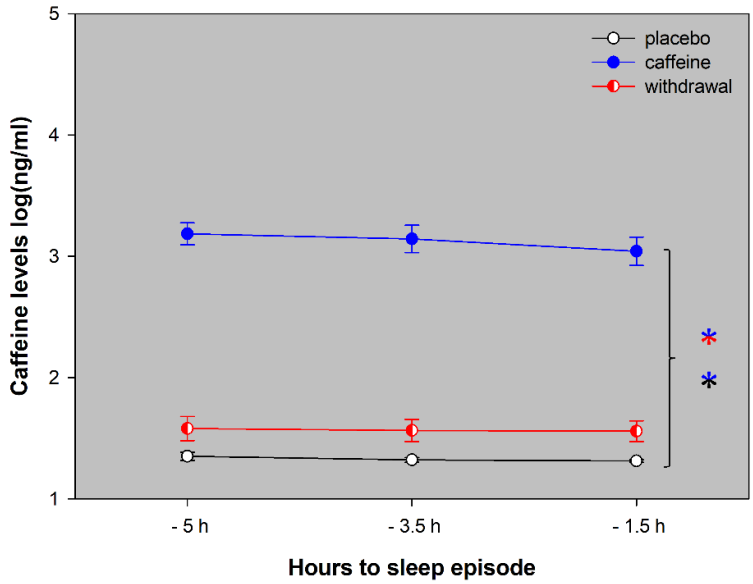

Fig. 2. Depicted are the salivary caffeine levels collected within 5 h prior to the sleep episode in the placebo (black open circles), caffeine (blue filled circles), and withdrawal (red semi-filled circles) conditions (means ± standard errors). Overall, caffeine levels were still increased in the caffeine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

3.2. Sleep

3.2.1. Electroencephalographic recordings

We analyzed total sleep time (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 ($p_{all} > 0.1$). However, in the caffeine condition it took participants longer to enter REM sleep compared to the placebo and withdrawal conditions ($p_{all} < 0.05$).

Table 2. Sleep variables and results of 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	$F_{2,35.4} = 0.14$, $p = 0.871$

Depicted are the means and standard errors per condition. 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 sleep; 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.
*significant post-hoc comparisons ($p < 0.0055$; threshold adjusted according to Bonferroni) compared to placebo and withdrawal conditions.

Based on the reductions and shifts of REM sleep reported in previous studies (Karacan et al., 1976; Nicholson & Stone, 1980; Carrier et al., 2007; Robillard et al., 2015), we investigated in a next step whether the accumulation of REM sleep proportion across the sleep opportunity differs among the three conditions. As shown in Fig. 3, on average REM proportion was reduced in the caffeine compared to 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 that this caffeine-induced reduction in the caffeine condition was particularly present between 1 to 3 and 4 to 7 h into the sleep opportunity. 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: $F_{14,196} = 0.54$, $p = 0.908$).

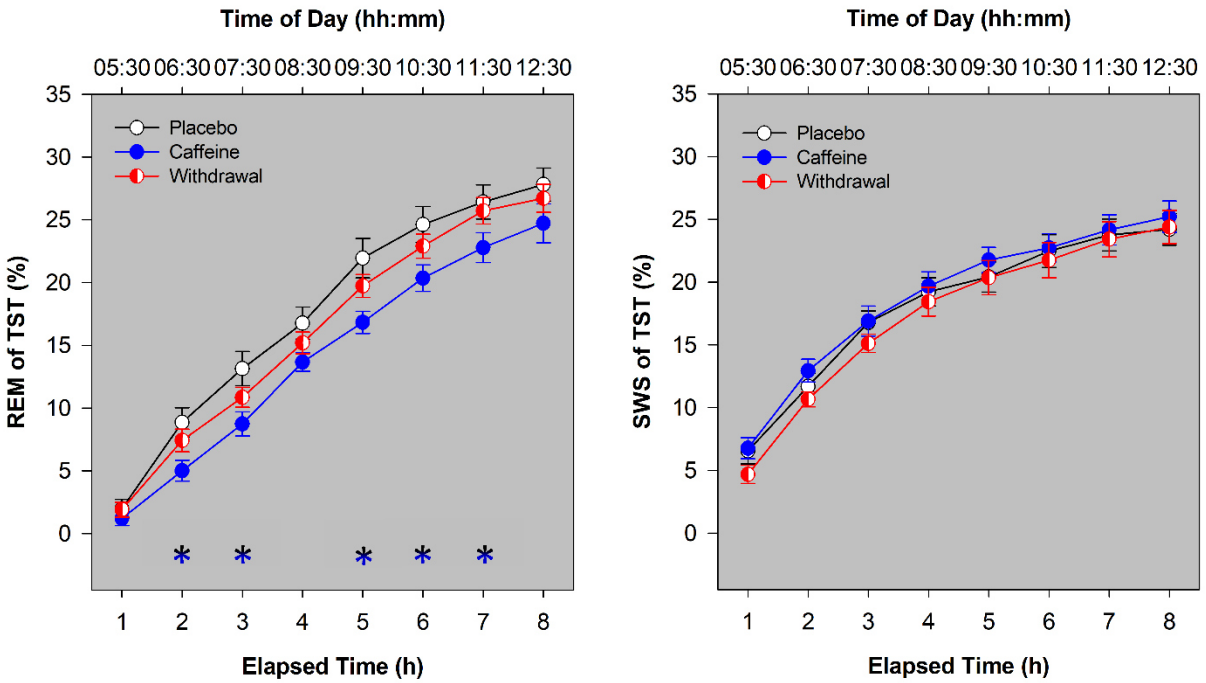

Fig. 3. Accumulation of REM and SWS proportion across the sleep opportunity of 8 h. REM (of TST) and SWS (of TST) were collapsed into bins of one hour and accumulated across the sleep episode. Depicted are means and standard errors of the placebo (black open circles), caffeine (blue filled circles), 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).

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

3.2.2. 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 awakening following sleep (AFS) and feeling more tired following wakening (BFW) in the caffeine compared to placebo condition ($p_{all} < 0.01$). Neither REM sleep duration nor REM (% of TST) had a significant effect on the ratings of AFS ($p_{all} > 0.4$) and BFW ($p_{all} > 0.3$).

Table 3. Parameters and results of subjective sleep quality as assessed by the LSEQ.

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	$F_{2,37.4} = 7.04$, $p = 0.003$

Reported are the means and standard errors per condition. GTS: Getting to sleep; QOS: Quality of sleep; AFS: Awake following sleep; BFW: Behavior following wakening; lower values represent poorer subjective sleep quality; *significant ($p < 0.0125$; threshold adjusted according to Bonferroni) post-hoc comparisons to the placebo condition.

4. Discussion

To the best of our knowledge, we explored for the first time the effects of regular daytime caffeine intake on REM sleep promotion and subjective quality of sleep. An 8-hour sleep window scheduled in the early morning hours allowed to investigate REM sleep expression at its circadian maximum (Dijk & Landolt, 2019) in a highly controlled laboratory setting. Indicating a reduced circadian promotion of sleep (Dijk & Landolt, 2019), volunteers in the caffeine condition entered REM sleep later compared to both placebo and withdrawal conditions and the accumulation of REM sleep across the night was slower compared to placebo. Thus, the earlier reported REM sleep reductions after caffeine right before bedtime (Carrier et al., 2007; Carrier et al., 2009; Robillard et al., 2015) seem to persist, even if caffeine intake 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 to 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 & 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 the present 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 delayed onset and reduced accumulation. As a few studies suggest an adaptation of several nighttime sleep features (Bonnet & 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 partly reversible. As withdrawal symptoms commonly reach peak intensity between 20 and 51 h after acute caffeine cessation (Juliano & Griffiths, 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; Carrier et al., 2009). However, the same outcome observed from 13.5 h after the last intake in the present study suggest that caffeine concentration is not the only determinant to induce REM sleep changes. This notion receives further support as high dosages do not consistently evolve an effect on REM sleep [(Karacan et al., 1976; Nicholson & Stone, 1980; Robillard et al., 2015) vs (Bonnet & Arand, 1992; Drake et al., 2013)]. On the other hand, 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; Carrier et al., 2009). Thus, caffeine effects on REM sleep seem to depend on circadian factors. The importance of the circadian phase (i.e., during strong circadian REM sleep promotion in the early morning) might be comparable to the impact 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 & Roth, 2008; Snel & Lorist, 2011). Taken together, an effect of caffeine on sleep appears to be more easily detectable if the experimental design allows the sleep feature, i.e. REM sleep, to reach a certain level.

Changes in sleep pressure have been shown to interact with the circadian expression of REM sleep (Dijk & Czeisler, 1995; Wyatt, Ritz-De Cecco, Czeisler, & Dijk, 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 one-hour nap episode in the evening prior to the 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 thus 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 at awakening following sleep and feeling more tired compared to placebo. While SWS is commonly related with recuperation and subjectively perceived good sleep

quality (Keklund & 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 at awakening in the present sample might also influence sleep inertia. Thus, the observed perception of non-restorative sleep might promote daily caffeine consumption in order to compensate the lack of feeling refreshed upon wake-up and thus 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 & Boivin, 2010) and the use of oral contraceptives on caffeine elimination (Abernethy & 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 the present 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 weakens REM sleep promotion, as evident in prolonged REM latency and reduced REM sleep accumulation. In the light of the evidence at present, these caffeine-induced changes in the circadian axis of sleep promotion may specifically occur when sleep is delayed and centered around the morning hours such as at the weekend or during shiftwork. 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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Declaration of conflicting interests

The authors declare that there is no conflict of interest.

5. General discussion

5.1. Summary of the main findings

The main aim of the present thesis was to investigate the influence of daily caffeine use timed to morning and afternoon hours on sleep-wake regulation in young habitual caffeine consumers, and to further examine how homeostatic and circadian measures are affected by its abrupt cessation.

As reported in the first manuscript, such a common caffeine intake pattern did neither strongly affect nocturnal PSG-derived sleep structure nor perceived sleep quality compared to the placebo and withdrawal conditions **(chapter 4.1.)**. However, EEG activity in the sigma frequencies (12-16 Hz) was reduced during both the caffeine and withdrawal condition compared to placebo. These subtle alterations in EEG sigma activity point to early signs of caffeine withdrawal which occur as early as 8 hours after the last caffeine intake and presumably reflect prodromal changes in the sleep homeostatic process.

Moreover, daily caffeine consumption did not alter the diurnal profile of melatonin and cortisol secretion (i.e., phase or amplitude) and did not lead to benefits in alertness, as reported in the second manuscript **(chapter 4.2.)**. However, the acute cessation of caffeine intake resulted in increased sleepiness, worse vigilance performance, and a better PSG-derived sleep ability in the late evening compared to both placebo and caffeine conditions. These withdrawal-induced effects indicate an adaptation to the stimulatory effects of caffeine in the homeostatic regulation of sleep and wakefulness as soon as regular caffeine consumption is ceased.

Such a pattern of adaptation was however not evident in REM sleep during the morning hours, as reported in the last manuscript **(chapter 4.3.)**. Circadian regulated REM sleep was delayed and accumulated slower when timed to morning hours, even under the condition of daytime caffeine use compared to placebo. Moreover, volunteers reported more difficulties upon awakening after sleep and felt more tired. These findings suggest an attenuated circadian REM sleep promotion under regular daytime caffeine consumption. A worse quality of awakening indicates caffeine-induced changes in sleep inertia and might reinforce caffeine consumption, particularly in consumers who delay or shift their sleep to the morning hours.

In summary, we could not confirm our hypothesis that daily daytime caffeine intake strongly interferes with measures of the homeostatic and circadian sleep-wake regulation. Unexpectedly, when sleep was delayed into the morning hours, circadian regulated REM sleep was clearly affected by regular daytime caffeine intake which highlights the importance of the circadian phase of assessment. Importantly, the acute cessation from such a common intake pattern resulted in clear-cut alterations mainly in the homeostatic component of sleep-wake regulation which presumably reinforce the daily use of caffeine intake in regular consumers.

5.2. Discussion

The influence of the timing of caffeine intake on sleep-wake regulation

A cup of coffee belongs to the morning routine of most people in order to have a fresh start into the day, while it is avoided later in the day to have a good night's sleep (Martyn et al., 2018; Lieberman et al., 2019). In the same vein, to abstain from caffeine belongs to the most frequent sleep hygiene recommendations (Sin, Ho, & Chung, 2009). Indeed, caffeine intake 6 hours prior to bedtime impacted both sleep duration and deep sleep (Drake et al., 2013). This thesis provides first evidence that caffeine consumed > 8 hours prior to nighttime sleep does not necessarily disrupt total sleep time or sleep architecture in regular consumers **(chapter 4.1.)**. The average half-life of caffeine is around 4 hours (Snel & Lorist, 2011), and a daily intake pattern promotes faster caffeine clearance (Nehlig, 2016). Thus, if the last caffeine intake was in the afternoon, the majority of the caffeine ingested might have already been eliminated at the time of sleep initiation in the present study, which provides one explanation of the observed discrepancies between the present results and the commonly reported findings when caffeine was ingested close to bedtime (Bonnet & Arand, 1992; Landolt, Dijk, et al., 1995; Drapeau et al., 2006; Robillard et al., 2015). Thus, our data indicate that the timing of caffeine intake plays a key role in whether caffeine is sleep-disrupting or not. Importantly, the present findings were reported in young healthy good sleepers. Caffeine-induced disruptions of sleep is among the main reasons why people voluntarily abstain from caffeine (Landolt, 2008). In line with this observation, caffeine-sensitive individuals indicate worse sleep quality (Rétey et al., 2007), show slower caffeine elimination (Levy & Zylber-Katz, 1983), and report lower caffeine intake (Levy & Zylber-Katz, 1983; Bchir, Dogui, Ben Fradj, Arnaud, & Saguem, 2006; Rétey et al., 2007) compared to caffeine tolerant consumers. Based on the rigid study design, we only included individuals who did not show any signs of sleep disturbances and who additionally reported a moderate to high habitual caffeine consumption pattern (300-600 mg/day), thereof particularly caffeine-sensitive individuals were most likely excluded. Moreover, there are indices that young individuals (i.e., 20-30 years of age) are less sensitive to the effects of caffeine on sleep compared to older individuals (i.e., 40-60 years of age) (Robillard et al., 2015) which warrants further investigations which populations might be specifically vulnerable to the effects of caffeine.

The timing of administration might also play a role in the phase shifting effects of caffeine, commonly measured with the hormonal secretion of melatonin. Based on in-vitro studies (Zatz & Heath, 1995; Diaz-Munoz et al., 1999), one might assume a similar phase response curve of caffeine and the zeitgeber light, which phase delays the circadian clock when administered in the evening while exposure in the morning advances the circadian clock (Khalsa, Jewett, Cajochen, & Czeisler, 2003). Thus, caffeine has been considered to be of particular use when facing jetlag (Burke et al., 2015; Landolt, 2015). Consequently, based on the timing in the

morning and afternoon hours in the present study, potential phase-shifts might have been cancelled out by the administrations at different circadian phases **(chapter 4.2.)**. However, evidence from animal and human studies do not unequivocally support the phase shifting properties of caffeine per se. In adult humans, acute caffeine intake in the evening or at night resulted in a phase delay (Burke et al., 2015) and reduced melatonin levels (Wright et al., 1997; Wright et al., 2000), however in teenagers a comparably low dose did not induce any circadian effect (Reichert et al., 2020). Moreover, caffeine administration in the morning was not sufficient to fully prevent a phase delay in response to dim-light conditions (personal communication (Burke et al., 2018)) nor did repeated morning intake entrain three blind patients (St Hilaire & Lockley, 2015). Similar evidence rises from animal models in which acute caffeine administration and A_1 adenosine receptor antagonists did not phase advance or delay locomotor activity (Elliott et al., 2001; Jha et al., 2017; Ruby et al., 2018). Thus, rather than acting as a zeitgeber per se, caffeine might alter the sensitivity to circadian timing cues such as light (Ruby et al., 2018). Indeed, acute caffeine potentiated light-induced phase delays and phase advances (Jha et al., 2017; Ruby et al., 2018) but see in humans (Burke et al., 2015). One possible mechanism is that A_1 adenosine receptors which are located on the hypothalamic tract reduce the release of glutamate in response to retinal illuminance and thus mediate light-induced changes in the circadian system (Elliott et al., 2001).

The influence of prior caffeine history on sleep-wake regulation

Beside the influence of the timing of intake on sleep-wake regulation, the observed findings in waking performance (i.e., sleepiness and vigilance), nighttime sleep, and hormonal secretion (i.e., melatonin and cortisol) might be particularly attributed to tolerance development. A phenomenon which has already been reported for subjective alertness (James, 1998) and visually scored sleep stages (Bonnet & Arand, 1992), the latter also under the condition of high daytime and evening caffeine intake (namely the dose of around 22 espressi per day). On a neural level, the adenosinergic system responds differently to acute and chronic exposure to caffeine, presumably due to alterations in the sensitivity of adenosine binding (Ferré, 2008). Several changes have been reported after repeated caffeine intake such as upregulation of A_1 (Shi et al., 1993) and A_{2A} adenosine receptors (Johansson et al., 1997; Varani et al., 1999) but also altered functions of the adenosine receptor heteromers (Ciruela et al., 2006), and increased levels of adenosine (Conlay et al., 1997). The latter ones are suggested to be responsible for the development of tolerance to caffeine (Ferré, 2008). Accordingly, chronic caffeine use increases adenosine levels extracellularly which thus particularly stimulate the A_{2A} adenosine receptors as this receptor subtype shows a lower affinity for caffeine (Ciruela et al., 2006). Moreover, the binding to the A_{2A} adenosine receptor in turn inhibits the A_1 adenosine receptors which can thus no longer be blocked by caffeine (Ciruela et al., 2006). This difference

in the adenosine receptors might provide some indices why some measures adapt to chronic caffeine intake while others remain responsive. However, further research is needed to causally relate neural changes in the adenosinergic system to specific observations in behaviour and physiology.

Importantly, tolerance to a drug is defined as the decreasing responsiveness to a substance with repeated intake or that a higher dose is needed to exert the desired effects (WHO, 2004). While some studies still revealed caffeine-induced effects on sleep (Drapeau et al., 2006; Drake et al., 2013; Robillard et al., 2015) or cortisol (Lovallo et al., 2005) in regular caffeine consumers, it is important to note that commonly a larger dosage than the prior habitual intake level was administered. Thus, the observed effects might be attributed to the high dosage administered and does not preclude any tolerance development under the conditions of a stable intake pattern which is assumed in regular consumers (Martyn et al., 2018).

Potential influence of the phase of assessment on sleep

Interestingly, REM sleep assessed during daytime was the only measure which showed a clear alteration in the caffeine condition compared to placebo although the last intake was longer than 13.5 hours ago **(chapter 4.3.)**. This change might be likely attributed to the time of sleep assessment rather than the timing of intake. Support for this interpretation derives from studies which did not report changes in REM sleep after high caffeine dosages close to habitual bedtime (Bonnet & Arand, 1992; Drake et al., 2013) while caffeine-induced reductions in REM sleep were particularly observed when sleep was timed to morning hours (Carrier et al., 2007; Carrier et al., 2009), which comprises the time window of strong circadian REM sleep promotion (Dijk & Czeisler, 1995). Such an effect might be comparable to the homeostatic component of sleep-wake regulation which is particularly sensitive to the effects of caffeine under conditions of increased sleep pressure (Roehrs & Roth, 2008; Snel & Lorist, 2011). Thus, the impact of chronic caffeine intake on sleep features might particularly emerge when sleep is challenged such as by shifts of the sleep episode to another circadian phase.

The influence of acute caffeine cessation on sleep-wake regulation

The acute cessation of caffeine intake leads to a wide range of withdrawal symptoms including decreased alertness and impaired performance (Juliano & Griffiths, 2004). Our data strongly corroborate these findings showing reduced sleepiness and more attentional lapses independent of time of day as well as increased sleep pressure during an evening nap **(chapter 4.2.)**. Moreover, we provide first evidence that early signs of withdrawal, indexed in reduced sigma activity, are presumably already evident as early as 8 hours after the last caffeine intake **(chapter 4.1.)**. In contrast to the reported increase in the sigma frequencies after acute caffeine administration (Landolt, Dijk, et al., 1995; Landolt, Werth, et al., 1995), we

observed in our data the opposite (i.e., decreased sigma activity) under conditions of repeated daily use. As lower sigma activity is commonly observed under the conditions of enhanced sleep pressure (Borbély et al., 1981; Dijk et al., 1993; Finelli et al., 2001; Knoblauch et al., 2003), they might represent a kind of rebound phenomenon under conditions of daily caffeine use, commonly referred to as overnight-withdrawal.

As proposed by the withdrawal reversal hypothesis (James, 2014), overnight caffeine abstinence occurs naturally in habitual consumers as caffeine is commonly consumed in separate portions across the day while primarily avoided in the evening or during the night. This acute cessation from caffeine during nighttime has been suggested to result in degraded performance and mood the next morning, impairments which are however reversed by caffeine intake, which suggests that caffeine does not enhance waking performance but rather restores it (James, 1994; James & Rogers, 2005; James, 2014). Such overnight-induced withdrawal symptoms presumably promote caffeine consumption the next day and thus act as a negative reinforcer (James & Rogers, 2005). Potential consequences of acute overnight abstinence were commonly investigated the next morning, however the present study provides first indications that subtle withdrawal symptoms might already manifest itself during the night **(chapter 4.1.)**. While sleepiness was increased in the withdrawal condition but not in the caffeine condition in the present study, it is tempting to assume that adverse effects were reversed by the first caffeine capsule in the morning **(chapter 4.2.)**. Thereof, in contrast to the earlier proposed vicious cycle (Roehrs & Roth, 2008; Snel & Lorist, 2011), not caffeine per se but the associated occurrence of withdrawal symptoms, which presumably already emerge during nighttime sleep, might increase sleepiness and worsen performance the next day and thus promote the maintenance of daily caffeine intake by negative reinforcement, i.e. withdrawal avoidance.

Importantly, typical measures of the circadian timing system such as melatonin and cortisol secretion or circadian regulated REM sleep were not altered in the withdrawal condition. When considering the timing of caffeine administration in relation to the morning sleep episode, it can however not be excluded that changes in REM during morning sleep in the caffeine condition (i.e., after 13.5 h from last intake) could potentially be as well interpreted as a sign of withdrawal **(chapter 4.3.)**. Within this line of reasoning, withdrawal-induced changes in the circadian axis of sleep-wake regulation could only come to light if the refrained caffeine intake hits the circadian promotion onset of a specific variable. Generally, withdrawal-induced alterations, which might already be present after a relative short period of abstinence, in combination with the extremely high number of caffeine consumers around the globe strongly recommend to take this peril into account when planning sleep studies and at best implement a washout period of several days to minimize or eliminate this risk of withdrawal-induced sleep alterations.

In summary, the present thesis strongly implies that caffeine-induced effects on sleep-wake regulation depend on the timing of intake and on prior caffeine history, factors which should be taken into account when interpreting findings on the effects of this highly prevalent stimulant.

5.3. Strengths and limitations

The present study has several strengths. First, by making use of a multimodal approach combining behavioural, hormonal, and physiological data allowed to uniquely investigate the effects of this highly prevalent stimulant on various subjective and objective levels and to gain a broader view on its potential benefits and consequences. Second, in contrast to a number of previous studies, data collection was conducted under stringently controlled conditions, particularly controlling for the amount and frequency of preceding caffeine intake. Together with the ambulatory part of nine days, we can thus rule out that the observed effects occurred due to carry-over effects from the previous condition. Moreover, by controlling food intake, light exposure, and posture change during the assessment phase in the laboratory, it can be excluded that particularly the results on circadian timing were confounded by these typical masking factors. Third, by administering capsules instead of caffeine diluted in beverages, the present effects can be likely attributed to caffeine per se and are not confounded by additional ingredients which are commonly found in coffee or energy drinks nor by the sensory properties or expectancies induced by the type of beverage. Finally, in contrast to a great number of previous studies, we investigated measures of alertness repeatedly across the day following multiple caffeine administrations, and not exclusively during the morning hours.

Despite the highly controlled design, the present study has several limitations which have to be taken into careful consideration when interpreting the observed findings. First, we did not implement an additional washout period prior to each ambulatory part. However, by scheduling an ambulatory part of nine days including fixed sleep-wake cycle and controlled treatment preceding each laboratory stay, potential influences from the preceding condition have likely vanished. Second, only young healthy participants (age range: 18-35 years) were studied and thus the findings cannot be referred to other age groups which are known to modulate the caffeine-mediated effects on sleep (Drapeau et al., 2006; Robillard et al., 2015) and cognition (Jarvis, 1993; Hogervorst et al., 1998). Third, female volunteers were excluded to reduce variance in our data which however clearly reduces the generalizability of the reported findings. In this context, it is important to note that caffeine clearance is modulated by the menstrual cycle (Lane et al., 1992) and by the use of oral contraceptives (Abernethy & Todd, 1985; Balogh et al., 1995) which can result in a twofold caffeine clearance time (Rietveld et al., 1984). Last, a specific polymorphism in the ADORA2A genotype has been linked with habitual caffeine intake and individual sensitivity to the effects of caffeine on sleep (Rétey et al., 2007). In the present study, we did not control for genotypes, however due to the inclusion of regular

caffeine consumers and the clear-cut effects following caffeine cessation, it can likely be excluded that only sensitive or insensitive volunteers, respectively, were studied.

5.4. Conclusions and outlook

The present thesis might suggest at first glance that daily daytime caffeine consumption does not seem to heavily disrupt homeostatic and circadian sleep-wake mechanisms in young healthy regular consumers. This is most likely due to the timing of its intake and tolerance development. Particularly the latter indicates however that caffeine-induced changes under conditions of regular use might simply happen “under the surface”, i.e. more specifically at the level of signal transduction at the receptor itself. In line with this reasoning, the acute cessation of regular daily intake brought such potential molecular changes to light, as decreased alertness, vigilance performance, and increased PSG-measured sleep pressure. In a next step, it remains to be elucidated what the specific underlying changes are on the receptor level in response to caffeine intake and how they relate to the observed changes in behaviour and physiology. Importantly, no strong detrimental effects on sleep-wake regulation were seen after daily caffeine intake, however the stimulant should still be consumed with some precaution. Although chronic moderate caffeine intake has been associated with a reduced risk of developing Parkinson’s disease (reviewed in Costa, Lunet, Santos, Santos, & Vaz-Carneiro, 2010), Alzheimer’s disease (reviewed in Santos, Costa, Santos, Vaz-Carneiro, & Lunet, 2010), or type 2 diabetes (reviewed in Carlstrom & Larsson, 2018), its intake has also been related to the risk of cardiovascular diseases (reviewed in Ding, Bhupathiraju, Satija, van Dam, & Hu, 2014) or reduced grey matter volume, as shown in the present sample (Lin et al., 2019). Thus, it needs further research to tackle potential long-term consequences of daily presence and nightly abstinence on sleep-wake regulation as seen in regular consumers. Moreover, in the present study young, male good sleepers were studied. Thus, it is important to study how such a common intake pattern might affect populations which diverge from the present sample. Although some studies suggest that men consume more caffeine than women ((Demura et al., 2013; Lieberman et al., 2019) but see (Tinguely et al., 2014)), they are commonly excluded from studies about the effects of caffeine to reduce variance in the data incurred by menstrual cycle (Lane et al., 1992) and oral contraceptives (Abernethy & Todd, 1985; Balogh et al., 1995) which both modulate caffeine elimination. Thus, it needs further research to investigate whether the present findings can be referred to women and whether for example the use of hormonal contraceptives enhances the sensitivity to the effects of caffeine. Moreover, caffeine consumption occurs most frequent in middle aged consumers (Mitchell et al., 2014; Tinguely et al., 2014; Martyn et al., 2018) which have been shown to be more sensitive to the sleep disrupting effects by caffeine (Robillard et al., 2015). Whether they are as well more sensitive to the caffeine-induced effects on the circadian timing system remains open and needs to be

elaborated. Future studies should focus on factors which modulate the effects of chronic caffeine intake on sleep-wake regulation and which might help to identify individuals which are particularly vulnerable to this highly common stimulant.

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