The impact of sleep pressure, circadian phase and an ADA-polymorphism on working memory: a behavioral, electrophysiological, neuroimaging approach

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Abstract

The need for sleep, the so-called sleep pressure, increases continuously during wakefulness and decreases during sleep again, in particular during intense deep sleep (Borbely, 1982). This sleep homeostatic process is mediated by the increase and degradation of adenosine in frontal brain structures (Porkka-Heiskanen, 2013). At the behavioural level, it is commonly mirrored in declines of performance under high sleep pressure (Cajochen, Blatter, & Wallach, 2004).

Adenosine is degraded by adenosine deaminase (ADA; Landolt, 2008). Due to a polymorphism (rs73598374), ADA activity differs inter-individually. Lower ADA activity in G/A- compared to G/G-allele carriers (Battistuzzi, Iudicone, Santolamazza, & Petrucci, 1981)has been associated with a trait-like higher sleep pressure level, indicated by deeper sleep and worse vigilance performance (Bachmann et al., 2012).

However, the impact of sleep pressure on several sleep and waking functions depends on circadian phase (Dijk & Franken, 2005): It is potentiated during the night while counteracted during daytime by circadian wake promoting mechanisms. Also, the influence of sleep pressure on neuro-behavioral performance depends on cognitive domain (Van Dongen, Baynard, Maislin, & Dinges, 2004). Performance relying on the frontal lobes, such as executive aspects of working memory (WM), has been suggested to be particularly vulnerable to high sleep pressure (Harrison & Horne, 2000).

In a multi-methodological approach we compared thus circadian variations in sleep and in a set of waking functions according to the ADA-genotype. To capture both circadian variations and their interaction with sleep pressure, we compared two 40-h conditions, in which sleep pressure was either kept low by multiple napping (low sleep pressure) or accumulated during sleep deprivation (high sleep pressure). Nap sleep electroencephalographic (EEG) activity, vigilance, WM performance and underlying blood oxygen level-dependent (BOLD) activity was assessed in regular time intervals.

Vigilance and WM performance was worse during high as compared to low sleep pressure, particularly during the night. Specifically in executive aspects of WM, sleep pressure-dependent performance modulations were evident in G/A- but not in G/G-allele carriers (Reichert, Maire, Gabel, Viola, et al., 2014). WM performance of G/A-allele carriers benefited during napping in particular from rapid eye movement (REM) sleep duration (Reichert, Maire, Gabel, Hofstetter, et al., 2014). At times of high circadian wake promotion G/A-allele carriers showed a reduced sleep ability, indicating changes of circadian arousal promotion in response to lower ADA activity. Accordingly, we observed at a cerebral level during high circadian sleep promotion, that G/A-allele carriers showed more cortical compensatory mechanisms during WM performance to cope with high sleep pressure at night.

Overall, the data suggest that the impact of sleep pressure on performance, whether state- or trait-like, is modulated by circadian mechanisms. These mechanisms contribute to a differential resistance or vulnerability to sleep deprivation according to cognitive domain.

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Abbreviations

ADA: Adenosine deaminase

BOLD: Blood oxygen level-dependent

DMH: Dorsomedial hypothalamus

EEG: Electroencephalography

fMRI: Functional magnetic resonance imaging

GABA: γ-Aminobutyric acid

LC: Locus coeruleus

LH: Lateral hypothalamus

NP: Nap protocol

REM: Rapid eye movement

NREM: Non rapid eye movement

PFC: Prefrontal cortex

SCN: Suprachiasmatic nuclei

SD: Sleep deprivation

SWA: Slow-wave activity

SWS: Slow-wave sleep

TMN: Tuberomammillary nuclei

VLPO: Ventrolateral preoptic nucleus

WM: Working memory

Figure Index

1. Introduction

Sleep and wakefulness are different states of consciousness, whose timing, duration and quality is mainly regulated by sleep homeostatic and circadian mechanisms (Borbely, 1982). Homeostatic sleep pressure accumulates during time spent awake and dissipates during intense deep sleep, mirrored in the increase and degradation of adenosine, predominantly in frontal brain areas (Cajochen, Foy, & Dijk, 1999; Cajochen, Wyatt, Czeisler, & Dijk, 2002; Porkka-Heiskanen, 2013). Adenosine is degraded by several enzymes, among them adenosine deaminase (ADA; Landolt, 2008). ADA activity varies in humans systematically according to a single nucleotid polymorphism (rs73598374) in the ADA-gene (Battistuzzi et al., 1981). Interestingly, lower ADA activity in G/A- compared to G/G-allele carriers has been linked to a trait-like higher sleep pressure level, as mirrored in higher deep sleep electroencephalographic (EEG) activity and impaired vigilance performance during sleep deprivation (SD; Bachmann et al., 2012; Retey et al., 2005), reflecting the negative impact of sleep pressure on behavioural performance.

Sleep and wakefulness also crucially depend on circadian phase. This is due to circadian arousal-regulating systems, promoting wakefulness during daytime, and sleep during the night (Dijk & Czeisler, 1994). At a neurobehavioral level, high sleep pressure attenuates the beneficial impact of wake-promotion, but amplifies circadian night-time troughs, indicating an interaction between circadian and homeostatic processes (Dijk & Franken, 2005). However, it is not yet known whether trait-like higher sleep pressure levels in G/A-allele carriers are differentially expressed according to time of day.

Importantly, behavioural declines under high sleep pressure have been shown to be strongly dependent on cognitive domain (Van Dongen et al., 2004). Furthermore, it has been suggested that performance relying crucially on frontal brain areas, such as working memory (WM) performance, is particularly vulnerable to sleep pressure (Harrison & Horne, 2000). However, whether the trait-like difference in sleep pressure due to the ADA-genotype impact on WM and its underlying cerebral correlates has not yet been investigated.

Thus, we compared in a multi-methodological approach circadian variations in G/A- and G/G-allele carriers under low and high sleep pressure. In a randomized within-subject design with two 40-h conditions, sleep pressure was either kept low by multiple napping (low sleep pressure condition) or accumulated during SD (high sleep pressure condition). EEG nap-sleep and waking patterns, hormonal levels, subjective sleepiness, well-being, vigilance and WM performance as well as underlying cerebral blood oxygen level-dependent (BOLD) correlates were assessed in regular time intervals across the day.

In the paper entitled “Insights into behavioral vulnerability to differential sleep pressure and circadian phase from a functional ADA-polymorphism” (chapter 4.1.) we report changes in circadian phase according to genotype. Furthermore, we show that G/A-allele carriers benefited from nap sleep specifically in executive functions of WM compared to performance under SD, while this pattern was neither observed in vigilance performance nor in G/G-allele carriers.

We next focused on nap sleep patterns and their relation to WM in G/A- and G/G-allele carriers (chapter 4.2). Compared to G/G-allele carriers, G/A-allele carriers benefited more in WM performance from the amount of REM (rapid eye movement) sleep in the early morning. They further exhibited problems to sleep during times of high circadian wake promotion (i.e., the late evening). This is indicative for a stronger circadian arousal signal and points to an adaptive change of circadian wake promoting mechanisms to alterations in the adenosinergic modulations of sleep pressure. We published the results in the paper entitled “The circadian regulation of sleep: Impact of a functional ADA-polymorphism and its association to working memory improvements”.

Finally we studied WM performance underlying BOLD activity, in order to investigate the impact of sleep pressure and circadian phase at a cerebral level (chapter 4.3). As summarized in our manuscript entitled “Time of day matters: circadian modulation of sleep loss-related changes in cognitive brain functions”, typical sleep loss-related decreases in cerebral BOLD activity are dependent on circadian phase, and occur particularly during nighttime, independent of genotype. As briefly outlined within this thesis (chapter 5.3.2), the data revealed further a pronounced impact of high sleep pressure at night in the G/A-genotype, mainly in parietal and parahippocampal regions. Interestingly, the implicated brain regions and activity patterns mirror an engagement of G/A-allele carriers in adaptive compensatory mechanisms in order to cope with high sleep pressure at night.

Overall, our findings substantially add to the current literature by incorporating the influence of circadian mechanisms on sleep pressure-dependent modulations, particularly in the domain of WM. The multi-methodological approach allows an integration of behavioural, electrophysiological and cerebral activity data, differentially sensitive for the impact of circadian phase and sleep pressure. The results generate future research questions regarding adaptive changes in sleep-wake regulation in response to chronic internal or external alterations in sleep pressure.

2. Theoretical background

2.1 Sleep-wake regulation at a conceptual level

The timing, duration, and quality of sleep and wakefulness have been majorly explained by the combined action of two processes (Borbely, 1982). The sleep homeostatic process can be basically described as a rise of sleep pressure during wakefulness and its dissipation during sleep, as measured by slow electroencephalographic (EEG) activity (Borbely, 1982; Cajochen, Khalsa, Wyatt, Czeisler, & Dijk, 1999). The term 'homeostasis' refers to the compensatory facilitation of deep, continuous and long sleep episodes when sleep is initiated after a long period wakefulness (Borbely & Achermann, 1999). At the neuropharmacological level, several substances, so-called sleep factors, have been identified, mediating the dynamics of sleep homeostatic effects during wakefulness and sleep, especially in frontal brain areas (Porkka-Heiskanen, 2013). The specific function of sleep homeostatic mechanisms in the brain have been mainly discussed in terms of energy restoration and cellular defence (Porkka-Heiskanen, 2013) as well as synaptic plasticity (Tononi & Cirelli, 2014).

The second process refers to circadian oscillations (lat. circa diem= about a day), which superimpose a nearly 24-hour pattern on the sleep-wake cycle: In diurnal beings, the circadian system actively promotes wakefulness during the biological day, while it promotes sleep during the biological night, i.e., during phases of melatonin secretion by the pineal gland (Dijk & Czeisler, 1994; Edgar, Dement, & Fuller, 1993). This rhythm is triggered and adjusted to the external light dark-cycle by the brains' main circadian pacemaker, the suprachiasmatic nuclei (SCN) of the anterior hypothalamus (Saper, 2013a). Within the SCN, a genetic clockwork determines the endogenous rhythm by a self-sustaining feedback loop with a duration of nearly 24 h (Franken, 2013). The synchronization to the external light-dark cycle is mainly based on the ocular perception and transduction of environmental light information to the SCN (Cajochen, Chellappa, & Schmidt, 2010). This general mechanism to synchronize with the rhythm of environmental signals, so-called zeitgebers, is evolutionary highly conserved and can be observed in almost all species (Hut & Beersma, 2011).

Along the 24-hour cycle, circadian and sleep homeostatic mechanisms act either in synchrony or in opposition to each other (Figure 1). When wakefulness of diurnal organisms is scheduled to occur during day- and sleep during nighttime (i.e., under so-called entrained conditions), circadian arousal promoting mechanisms oppose rising sleep pressure levels during daytime (Dijk & Czeisler, 1994). This opposing action enables a consolidated episode of wakefulness under accumulating sleep need (Edgar et al., 1993). With the onset of melatonin secretion in the late evening hours, the circadian wake promoting impact breaks down and the "gate for sleep" opens (Lavie, 1986). Together with

high sleep pressure levels, this time can be considered as an optimal window for sleep initiation. Towards the end of a night-sleep episode, when sleep pressure is degraded to a minimum, sleep is presumably maintained due to active circadian sleep promoting mechanisms (Dijk & Czeisler, 1994).

Overall, circadian and sleep homeostatic mechanisms thus contribute together to a consolidation of wake and sleep bouts under entrained conditions. Consequently, disruption of the interplay of both processes, for instance due to shift-work or travelling across time-zones, reduces optimal sleep and wake quality. Importantly, this is not only due to a simple shift of one process with regard to the state of the other, but also to their interdependence. At several behavioural and physiological levels, the impact of circadian modulations on sleep and wakefulness turned out to be crucially dependent on sleep pressure levels (Dijk & Franken, 2005). When sleep pressure is at low levels, the circadian arousal peak in the late evening hours is particularly pronounced (Wyatt, Cajochen, Ritz-De Cecco, Czeisler, & Dijk, 2004; Wyatt, Ritz-De Cecco, Czeisler, & Dijk, 1999), while typical circadian nighttime troughs in cognitive performance have been shown to be enhanced under high sleep pressure (Dijk, Duffy, & Czeisler, 1992; Wyatt et al., 2004; Wyatt et al., 1999).

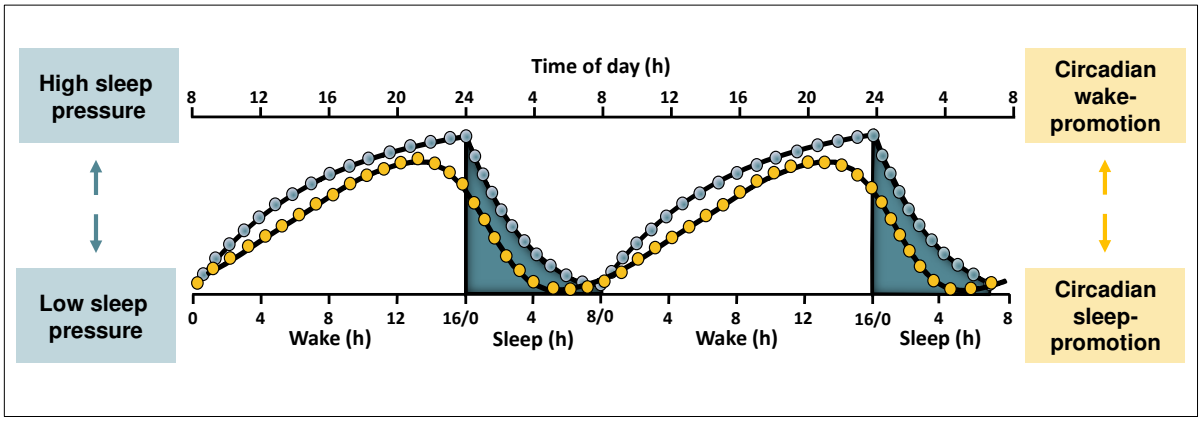

Figure 1.Schematic illustration of sleep-wake regulation by circadian and homeostatic mechanisms under entrained conditions (modified from (Daan, Beersma, & Borbély, 1984)). Under entrained conditions wakefulness is scheduled to daytime, and sleep to the biological night. The homeostatic sleep need (blue) increases with enduring wakefulness and declines during sleep. Circadian oscillations (yellow) promote wakefulness during the day and sleep during the night, and are relatively independent of prior sleep-wake history. *Figure and legend adapted from* (Maire, Reichert, & Schmidt, 2013).

2.2 Investigating circadian and sleep homeostatic mechanisms

Several laboratory protocols have been developed to investigate the influence of circadian and sleep homeostatic mechanisms on behaviour and physiology (Maire et al., 2013). The most sophisticated design is the so-called forced desynchrony protocol. In such a study, participants are separated from the natural environment for several weeks and scheduled on a specific sleep-wake

cycle. This artificial sleep-wake cycle corresponds to the usual 1:2 ratio of sleep and wakefulness, but is considerably longer or shorter than the regular 24-hour cycle (e.g., Dijk et al., 1992; Wyatt et al., 1999). As a consequence, sleep and wakefulness occur systematically at differential times of the biological day or night and are desynchronized from the endogenous circadian rhythm, all by controlling prior duration of wakefulness. The influence of differential sleep pressure levels can thus be assessed at virtually all circadian phases, or conversely, circadian influences can be measured under differential sleep pressure conditions. Consequently, a forced desynchrony protocol allows to investigate the interaction between circadian and sleep homeostatic processes and to quantify their separate contribution in the assessed variables (e.g., electroencephalographic activity during sleep or cognitive performance).

A less time-consuming way to study the impact of differential sleep pressure levels at the same circadian phase is the implementation of a so-called constant routine protocol with a duration of more than 24 h. In a constant routine protocol, participants stay continuously awake while the influence of potential zeitgebers such as light, body posture, meal intake, or sleep and wakefulness is kept constant (Minors & Waterhouse, 1983). The protocol was originally developed to investigate unmasked circadian rhythms. When extending wakefulness to more than 24 h, it enables also to assess dependent variables at the same circadian phase under differential sleep pressure levels. However, it has to be taken into account that SD per se might delay circadian phase position (Cajochen, Jewett, & Dijk, 2003). Also, a separation of circadian and sleep homeostatic influences is not possible, as a certain level of sleep pressure does not systematically occur at all circadian phases. Circadian variations are rather confounded with rising sleep pressure.

To control for this confound, multiple-nap protocols (NP) have been developed, in which regularly scheduled naps serve to keep the homeostatic sleep pressure at a rather low level throughout the 24-hour cycle. Such a design allows to study the circadian course of several waking functions without the confounding rise in sleep pressure (Birchler-Pedross et al., 2009; Blatter, Opwis, Munch, Wirz-Justice, & Cajochen, 2005; Cajochen, Knoblauch, Krauchi, Renz, & Wirz-Justice, 2001; Graw, Krauchi, Knoblauch, Wirz-Justice, & Cajochen, 2004; Sagaspe et al., 2012). Importantly, the regularly scheduled sleep episodes enable further to assess circadian variations in differential sleep features (Knoblauch, Martens, Wirz-Justice, Krauchi, & Cajochen, 2003; Lavie, 1986; Munch et al., 2005). A major disadvantage is that the fragmentation of sleep prevents ultradian processes requiring long and continuous sleep-episodes. Nonetheless, a combination of a constant routine and a NP appears to be a useful alternative to the much more laborious forced desynchrony protocol. Additionaly, sleep pressure levels reached in constant routine protocols are usually higher than those in a forced desynchrony. Thus, the combination of a constant routine and a NP allows the study of homeostatic and circadian effects under extremely challenging conditions.

2.3 Circadian and homeostatic regulation in sleep and waking functions

2.3.1 Circadian and homeostatic regulation of sleep features

Sleep is classically assessed by a combination of EEG, electrooculography, and electromyography. The assessed data are analysed qualitatively by visual scoring of different sleep stages and quantitatively by spectral analysis. In various sleep features, forced desynchrony and multiple- nap studies revealed circadian and homeostatic patterns (Dijk & Czeisler, 1994, 1995; Dijk, Shanahan, Duffy, Ronda, & Czeisler, 1997; Knoblauch et al., 2003; Munch et al., 2005; Wyatt et al., 1999). Slow-wave sleep (SWS)duration (Wyatt et al., 1999) and NREM sleep spectral power in the range of 0.7-4 Hz (Dijk et al., 1997) mirror the dynamics of homeostatic sleep pressure (Borbély & Acherman, 2005), particularly in frontal areas (Cajochen, Foy, et al., 1999). These features are more pronounced the longer the time spent wake before initiation of sleep, and decrease over the course of a sleep episode. Also, NREM EEG power density in the range of 12-16 Hz (sigma activity) shows a sleep homeostatic pattern as well, but is also strongly modulated by circadian phase (Dijk et al., 1997).

On the other hand, sleep latency (Figure 2) and sleep efficiency for example follow a clear-cut circadian pattern. They mirror the course of circadian arousal promotion, with difficulties to initiate and maintain sleep during daytime, specifically at the end of the biological day (Dijk & Czeisler, 1994; Munch et al., 2005). During the late evening hours at the end of a day, circadian wake-promotion reaches peak-levels (see Figure 1). Accordingly, this time window has been labelled as the 'wake-maintenance zone' (Strogatz, Kronauer, & Czeisler, 1987). Similarly, peak levels of active circadian sleep promotion in the early morning (see Figure 1) have been proposed to be mirrored in prominent circadian peaks of REM sleep duration (Dijk & Czeisler, 1995; Munch et al., 2005;Dijk & Edgar, 1999).

Generally, it should be noted that a strong circadian or homeostatic control of a specific sleep feature might not be understood as exclusive, but rather as a predominance of one of the two sleep-wake regulatory mechanisms under specific conditions. For instance, sleep latency is shortened under high sleep pressure (Borbely, Baumann, Brandeis, Strauch, & Lehmann, 1981; Knoblauch, Krauchi, Renz, Wirz-Justice, & Cajochen, 2002), and sleep efficiency decreases according to time spent asleep (Wyatt et al., 1999). Furthermore, REM sleep duration is modulated by time spent asleep, in a circadian phase-dependent manner (Dijk & Czeisler, 1995). Finally, the core marker of NREM sleep homeostasis, slow-wave activity (SWA), exhibits a "small but significant" circadian variation (Dijk & Czeisler, 1995). Taken together, these observations strengthen the assumption of an inherent connection between circadian and sleep homeostatic mechanisms in the regulation of sleep features.

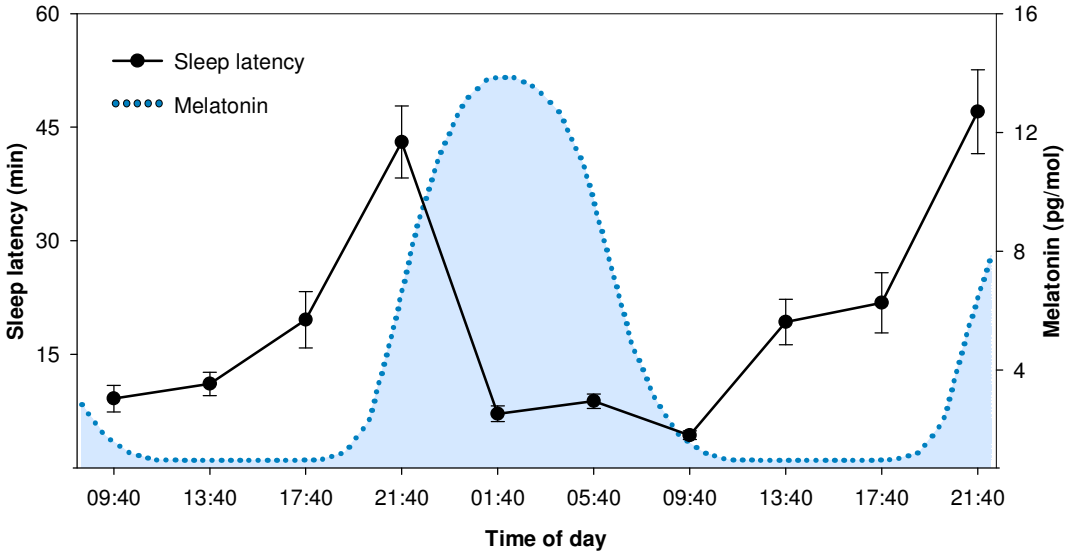

Figure 2. Sleep latency over the 24-hour cycle. Sleep latency to sleep stage 1, assessed during regular naps of 80 min (NP), shows a striking circadian pattern. Longest durations occur in the evening hours shortly before habitual bedtime and mirror highest levels of circadian wake promotion at the end of the biological day (Dijk & Czeisler, 1994). Shortest durations were measured during the biological night, which is illustrated by the blue dotted curve of melatonin secretion. Melatonin was analysed in saliva samples collected in the same study and modelled according to (Kolodyazhniy et al., 2012).

2.3.2 Circadian and homeostatic regulation of waking functions

Circadian and homeostatic profiles have also been observed in waking functions, ranging from waking EEG, to behavioural performance, and in both subjective and objective sleepiness. For instance, alpha activity (8-12 Hz) decreases (Cajochen et al., 2002), and also performance deteriorates with time spent awake (Dijk et al., 1992; Silva, Wang, Ronda, Wyatt, & Duffy, 2010; Wyatt et al., 1999). Similarly frontal EEG delta activity (1-4.5 Hz) increases (Cajochen et al., 2002), and subjective sleepiness rises continuously the longer the time spent awake (Wyatt et al., 1999).

Most of these measures are as well affected by circadian phase. Generally, the impact of circadian phase has been shown as nighttime trough in waking EEG alpha activity (8-12 Hz; Cajochen et al., 2002) and cognitive performance (Dijk et al., 1992; Silva et al., 2010; Wyatt et al., 1999). Also sleepiness is enhanced during nighttime, both subjectively (Figure 3; Wyatt et al., 1999) and objectively as measured by electrooculographic slow rolling eye movements (Maire et al., 2014).

Finally both, circadian and homeostatic mechanisms act in a combined manner on waking quality. The typical interaction of these processes can be nicely observed during SD (see Figure 3). During the first day, that is under usual sleep pressure levels, frontal low EEG activity, sleepiness, well-being and performance are relatively preserved. However as soon as passing into the biological night, frontal low EEG activity and sleepiness steeply increase, while performance and well-being deteriorate concomitantly. Intriguingly, once passing into the biological day, the values stabilize or even approach baseline levels, even though wakefulness is further extended (Birchler-Pedross et al.,

2009; Cajochen et al., 2001; Graw et al., 2004; Maire et al., 2014; Sagaspe et al., 2012). This daytime stabilization under high sleep pressure is most presumably due to circadian arousal promoting mechanisms which oppose high sleep pressure levels during daytime (Cajochen et al., 2004) .

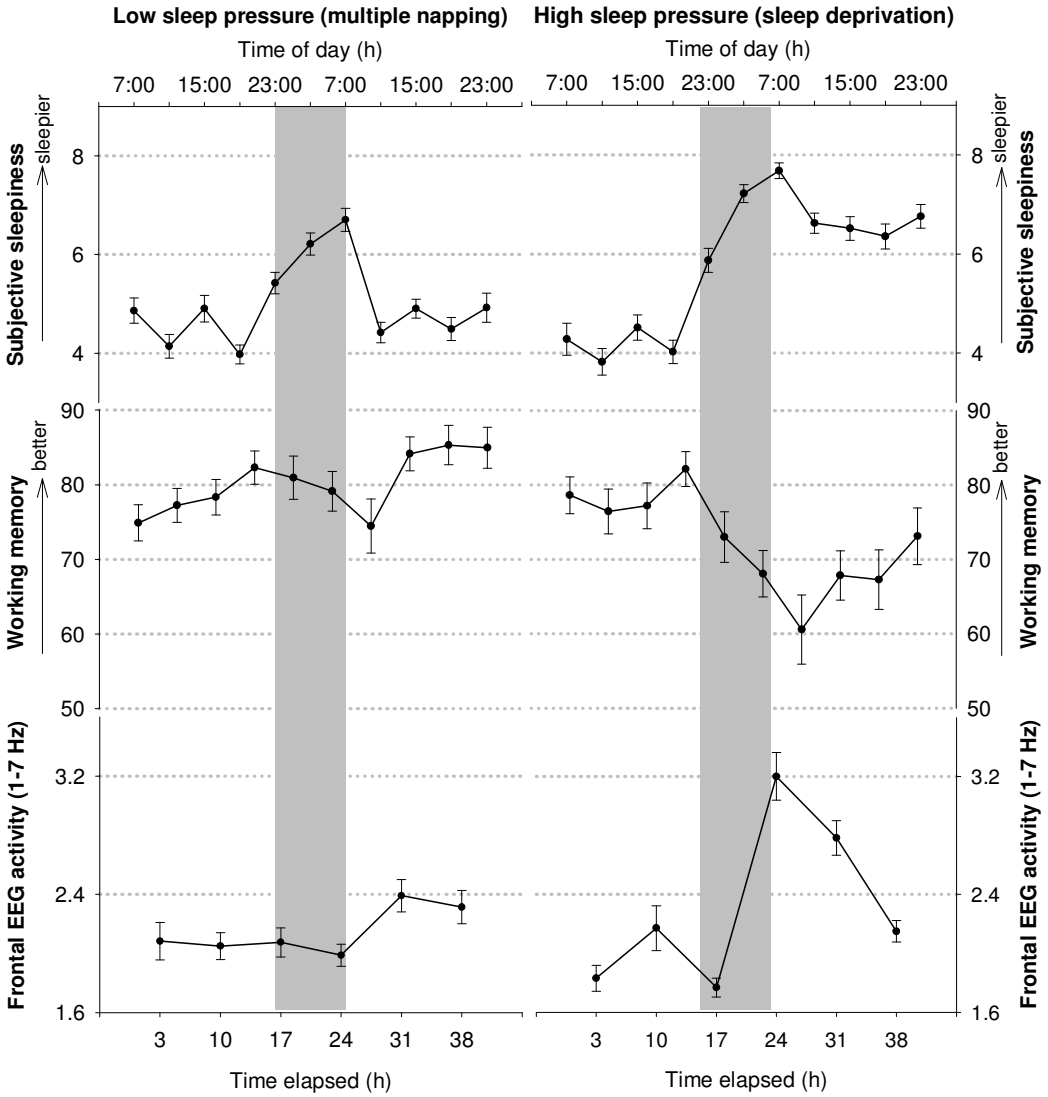

Figure 3.Circadian and homeostatic influences on subjective sleepiness, WM performance and waking EEG. Values assessed during a low sleep pressure condition (NP) are depicted on the left panel (naps are indicated by black bars at the top x-axis), and mirror circadian influences under rather low sleep pressure conditions. On the right side, the impact of rising sleep pressure during night- and daytime is illustrated, as these values were assessed during a SD of 40 h. The grey bars indicate nighttime. Subjective sleepiness was assessed by a questionnaire [Karolinska Sleepiness Scale (Akerstedt & Gillberg, 1990)], WM performance by a n-back task (depicted is the percentage of hits). The waking EEG was analysed over three frontal derivations (F3,F4, FZ).

While it is tempting to assume that all these measures are closely correlated, underlining evidence is mixed so far. Most studies focused on the relationship between subjective and objective sleepiness, assessed under rising and high sleep pressure (reviewed for the Karolinska Sleepiness Scale in Akerstedt, Anund, Axelsson, & Kecklund, 2014). However, under high sleep pressure, people

react differentially according to cognitive domain (Van Dongen et al., 2004). Given the same person, subjective and objective sleepiness might thus not be affected to the same extent by high sleep pressure. This might hamper a striking correlation between differential measures assessed under such conditions.

2.4 Neuronal underpinnings of sleep and wakefulness and the role of adenosine

2.4.1 Adenosinergic regulation of sleep homeostasis

Sleep homeostatic mechanisms in the brain have been associated to the increase and decrease of substances, so-called sleep-factors, in widespread cerebral networks (Porkka-Heiskanen, 2013). Here, the focus will be on evidence underlining the role of adenosine and its metabolism. Its important role in human sleep-wake regulation is underlined by the world-wide common use of the non-selective adenosine antagonist caffeine (Landolt, 2008).

2.4.1.1 A role of adenosine in sleep homeostasis – Implicated brain regions

The nucleoside adenosine is intra- and extracellularly ubiquitous in the central nervous system. It acts on sleep-wake regulation mainly via its widely distributed inhibitory A_1receptors (Landolt, 2008; Porkka-Heiskanen & Kalinchuk, 2011). In animals, adenosine levels increase in several brain areas during extended wakefulness, and decrease during recovery sleep from SD. Moreover, adenosine inhibits arousal and induces sleep, modulated by receptors in the basal forebrain (Basheer, Strecker, Thakkar, & McCarley, 2004; Hawryluk, Ferrari, Keating, & Arrigoni, 2012; Porkka-Heiskanen et al., 1997; Thakkar, Delgiacco, Strecker, & McCarley, 2003). Evidence suggests further inhibitory influences on other structures crucially involved in arousal promotion (for an overview Figure 4a) such as the tuberomamillary nuclei (TMN), or orexin containing neurons in the lateral hypothalamus (LH; Porkka-Heiskanen & Kalinchuk, 2011). Conversely, adenosine has an excitatory influence via A_{2A} receptors in the sleep promoting neurons in the ventrolateral preoptic area (VLPO) of the hypothalamus (Figure 4b; Szymusiak & McGinty, 2008). In sum, adenosine appears to be a powerful modulator of arousal promoting structures.

2.4.1.2Why does adenosine increase with time spent awake? Contributions of its metabolization

Adenosine is the end-product of the hydrolysis of adenosine triphosphate, the so-called “energy currency” (Porkka-Heiskanen & Kalinchuk, 2011). Consequently, it has been related to the energy consumption of a cell (Porkka-Heiskanen & Kalinchuk, 2011). However, an increase and decrease of adenosine, linked to sleep homeostasis, is not inevitably or exclusively due to increased or decreased

energy demands. It can also strongly depend on adenosine metabolization and transport. Extracellular clearance of adenosine is mostly regulated via nucleoside transporters (Latini & Pedata, 2001) or ecto-ADA (Landolt, 2008). Intracellularly, adenosine is converted by adenosine kinase, or metabolized by ADA to inosine (Landolt, 2008). The ADA-dependent degradation plays a presumably crucial role under conditions of high adenosine concentrations (Latini & Pedata, 2001).

There is evidence that adenosine degradation plays a role in sleep wake-regulation. For instance, the activity of several adenosine metabolizing enzymes shows a diurnal rhythm (Mackiewicz et al., 2003). During the active phase, ADA activity has been observed to peak in the VLPO, while exhibiting troughs in the basal forebrain. Also, pharmacological inhibition of ADA leads to a rise in extracellular adenosine and prolongs NREM sleep (Oishi, Huang, Fredholm, Urade, & Hayaishi, 2008; Okada et al., 2003; Radulovacki, Virus, Djuricic-Nedelson, & Green, 1983). Moreover, Franken and colleagues demonstrated that a region encoding ADA in mice is associated with the rate of NREM sleep need accumulation (Franken, Chollet, & Tafti, 2001). However, ADA activity remained unchanged after SD in several sleep-wake regulatory brain areas, such as the LC, TMN, VLPO and basal forebrain (Mackiewicz et al., 2003).

In humans, individual differences in ADA activity due to a polymorphism (rs73598374) have been shown to have an impact on sleep homeostatic markers in EEG activity as well as on neurobehavioral functions in both well rested and sleep deprived states. In the present thesis, the impact of this polymorphism plays a key role, and is thus described more specifically in the following section.

2.4.1.3.Impact of a human ADA-polymorphism on sleep-wake regulation

According to the Online Mendelian Inheritance in Man (OMIM) database, more than 30 allelic variants of ADA (association no. 608958, cytogenetic location: 20q13.12) are known so far, most of which are not functional. The functional single-nucleotide polymorphism (rs73598374) in the focus of the present thesis, has been located at nucleotide 22. A G>A transition causes a substitution of asparagine for aspartic acid at the 8^{th} codon of the ADA protein (Hirschhorn, Yang, & Israni, 1994). This substitution has been linked to differences in enzymatic activity of ADA in human blood cells (Battistuzzi et al., 1981; Riksen et al., 2008). Compared to G/G-allele carriers, the catalytic ADA activity is reduced in G/A-allele carriers by around 20%. Enzymatic activity in A/A-allele carriers is unknown so far. Homozygosity for the G-allele can be expected in about 90% of the population, heterozygosity in about 7.9% (Mazzotti et al., 2011; Persico et al., 2000).

The first study, associating this ADA-polymorphism to differences in sleep-wake regulation, was published by Retey and colleagues (Retey et al., 2005). They reported that G/A-allele carriers exhibited higher NREM EEG delta and theta power, REM theta power, sleep efficiency, and longer SWS. Further, G/A-allele carriers reported subjectively fewer awakenings than G/G-allele carriers

(Retey et al., 2005). These first indications of a role of ADA in sleep architecture and intensity were specified in a study by the same group, in which participants were sleep-deprived for 40 h (Bachmann et al., 2012). Similarly, NREM and REM sleep power in different frequency ranges were enhanced and SWS prolonged in G/A-allele compared to G/G-allele carriers in both baseline and recovery night from SD. Furthermore, G/A-allele carriers felt sleepier and performed worse in a vigilance task throughout wakefulness. Thus, a role of ADA in sleep-wake regulation could be confirmed. However, a specific sleep homeostatic response, implying a more pronounced reaction to SD, has not been shown. The genotype-dependent differences were rather consistently exhibited over 40 h of continuous wakefulness. This is in line with a twin-study assessing the slope of the vigilance decrease during SD, a measure which mirrors a kind of homeostatic response at the behavioural level (Kuna et al., 2012). While in general a strong heritability was demonstrated, G/A- and G/G-allele carriers did not significantly differ in this variable. Furthermore, the authors did not find any indications for baseline differences between genotypes (Kuna et al., 2012).

Another line of evidence supporting the role of the ADA-polymorphism in sleep-wake regulation comes from a recent epidemiological study. In a sample of around 900 participants, sleep efficiency of G/A- and A/A-allele carriers was shown to be higher in a habitual night sleep episode compared to G/G-allele carriers. However, this difference was not significant anymore in a subsample of 226 participants, who were not consuming caffeine during the day before sleep was recorded (Mazzotti et al., 2011). While the authors did not report whether caffeine consumption differed between genotypes in the day before the study, habitual caffeine consumption did not systematically vary according to the ADA-polymorphism. In a subsequent publication, Mazzotti and colleagues reported that G/A-allele carriers exhibited a higher SWS delta power specifically in occipital derivations. Frequency analysis of the less deep sleep stages and REM sleep revealed higher theta and beta power in G/A- and A/A-allele carriers as well in occipital areas (Mazzotti et al., 2012).

In sum, these data underline the potential impact of the ADA-polymorphism on the regulation of sleep-intensity and EEG-generating mechanisms. It can be assumed that G/A- and G/G-allele carriers differ in sleep ability and baseline sleep pressure levels, while the dynamics of sleep homeostasis appear to be similar between genotypes. Differential genotype-dependent sleep pressure levels have been proposed to be due to differences in the adenosinergic tone at the synapse (Bachmann et al., 2012). The present evidence further indicates that genotypes differ in the perception and/or subjective ratings of sleepiness and sleep quality. Regarding behavioural performance, the evidence is less consistent. This might not only be due to small sample sizes, but also to different statistical methods applied. In contrast to Bachmann and colleagues, Kuna and colleagues accounted for the impact of circadian effects on vigilant attention (Bachmann et al., 2012; Kuna et al., 2012).

Accumulating evidence suggests that sleep pressure acts differentially on sleep- and wakefulness according to circadian phase (Dijk & Franken, 2005). Differences in sleep pressure levels, as assumed in G/A- and G/G-allele carriers, might thus be exhibited in a circadian phase-dependent manner. Before physiological mechanisms will be discussed, which mediate this interaction, the next section briefly summarizes brain structures and neurotransmitters involved in circadian arousal promotion during daytime and sleep-promotion during the night.

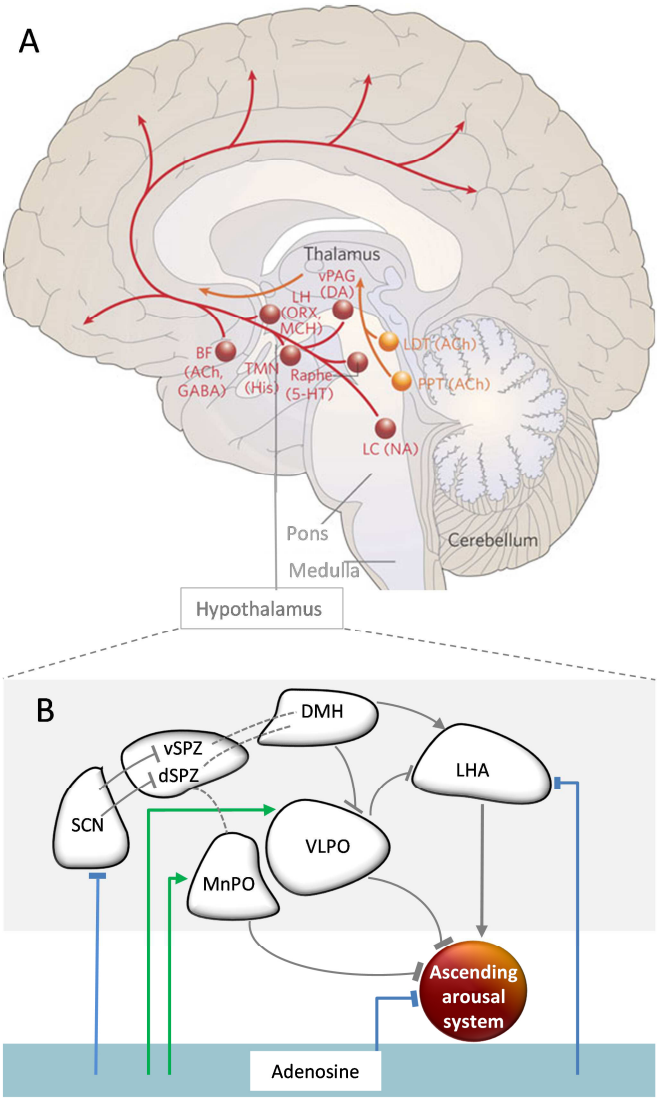

Figure 4. Hypothalamic regulation of the ascending arousal system and the impact of adenosine. (A) The ascending arousal system. One of the main pathways (red) activating the cortex arises from neurons in the monoaminergic cell groups, including the locus coeruleus (LC) containing noradrenaline (NA), the dorsal and median raphe nuclei containing serotonin (5-HT), the A10 cell group containing dopamine (DA), and the tuberomammillary nucleus (TMN) containing histamine (His). This pathway receives contributions from peptidergic neurons in the lateral hypothalamus (LHA) containing orexin (ORX) or melanin-concentrating hormone (MCH), and from basal forebrain (BF) neurons that contain γ-aminobutyric acid (GABA) or acetylcholine (Ach). The red pathway activates the cerebral cortex to facilitate the processing of inputs from the thalamus. Orange lines represent input to the thalamus originating from cholinergic (ACh) cell groups in the upper pons, the pedunculopontine (PPT) and laterodorsal tegmental nuclei (LDT). *Figure and legend modified from* (Saper, Scammell, & Lu, 2005)

(B) Hypothalamic and adenosinergic arousal modulation. The SCN innervates by GABA-ergic neurons of the ventral supraventricular zone, projecting to the dorsomedial hypothalamus (DMH), in turn providing glutamatergic input to the orexin-containing neurons in the lateral hypothalamus (LH). These target finally the locus coeruleus (LC), a major player in the ascending arousal system (Saper, 2013a). The ascending arousal system is inhibited by GABA-ergic input of the ventrolateral and median preoptic area (VLPO and MnPO), and in part by adenosine. Adenosine acts inhibitory (blue lines) via A_1 or disinhibitory (green lines) via A_{2A} receptors on several structures within this network. Striped lines show neural projections of which implicated neurotransmitters are currently unknown. *Figure adapted from* (Saper, 2013a)

2.4.1 Pathways of circadian arousal promotion

The SCN has a central role in the regulation of circadian rhythmicity. It has often been labelled as the director of an orchestra of circadian rhythms ticking in most cells of the body (e.g., Davidson, Yamazaki, & Menaker, 2003; Schibler & Sassone-Corsi, 2002). SCN lesions in animals (Saper, 2013a) and humans (Cohen & Albers, 1991; DelRosso, Hoque, James, Gonzalez-Toledo, & Chesson, 2014) indicate that the SCN is not only crucially involved in the timing of sleep and wakefulness, but also in its consolidation (reviewed by Mistlberger, 2005). However, it has to be noted that all lesions might have involved a destruction of SCN adjacent areas (Mistlberger, 2005; Saper, 2013a).

The SCN receives light-dark information via the retinohypothalamic tract (Cajochen et al., 2010). Downstream from the SCN, circadian arousal promotion during daytime is most likely mediated via several interfaces, including the dorsomedial hypothalamus (DMH) and orexinergic neurons in the LH (Figure 4b). The latter have been shown to be crucially important to consolidate wakefulness (Saper et al., 2005). They target the noradrenergic neurons of the locus coeruleus (LC; Saper, 2013a). Together with other projections (Figure 4a), the LC provides excitatory input to a widespread cortical network (Figure 4a; Alexandre, Andermann, & Scammell, 2013; Aston-Jones, 2005; Aston-Jones, Chen, Zhu, & Oshinsky, 2001; Gompf & Aston-Jones, 2008; Mahoney, Brewer, & Bittman, 2013; Saper, 2013b; Saper et al., 2005).

During the biological night, circadian arousal promotion is reduced. The circadian phase information is again, via several interfaces (Figure 4b), transduced to arousal inhibiting brain structures (Saper, 2013a). Particularly important in arousal inhibition are sleep-active neurons of the VLPO. They inhibit by GABA-ergic input not only orexinergic LH neurons, but also nearly all brainstem structures, mediating arousal, such as the TMN, raphe nuclei, pedunculopontine and laterodorsal tegmental nuclei and the LC (Saper et al., 2005). In turn, the activity of the VLPO is inhibited by the ascending monoaminergic projections, for instance from the LC, and by GABA-ergic input from the DMH. This forms a reciprocal system between arousal promoting and reducing brain areas (Samuels & Szabadi, 2008; Saper, 2013a).

In the present thesis, it is of particular interest how circadian sleep-wake promoting mechanisms interact with sleep homeostatic processes, mediated by adenosine. The next section will summarize where such an interaction might take place at the neuronal level.

2.5 Interactions of circadian and sleep homeostatic mechanisms

2.5.1 The genetic clockwork and sleep homeostasis

Evidence for an interaction between homeostatic and circadian processes has been reported at the very basis, within the genetic clockwork. As summarized recently by Franken (Franken, 2013), the disruption of genes implicated in the circadian feedback loop leads to differences in the sleep homeostatic rebound in different species. Further, messenger ribonucleic acid (mRNA) levels of clock genes in the cerebral cortex and other tissues are altered according to the sleep-wake history. Studies in humans focusing on a polymorphism in the clock gene PERIOD3 have also been listed as evidence for a cross-talk of the circadian clock and homeostatic processes (Franken, 2013). NREM sleep SWA (Viola et al., 2007) and neurobehavioral vulnerability to high sleep pressure have been shown to vary systematically according to the genotype (Groeger et al., 2008; Lo et al., 2012; Maire et al., 2014).

2.5.2 Brain regions and substances mediating interaction

In addition to research at the molecular level, electro-physiological studies underline an interaction between circadian and sleep homeostatic mechanisms, amongst others directly in the SCN. Evidence indicates that firing rates of SCN-neurons are reduced during NREM as compared to REM sleep (Deboer, Vansteensel, Detari, & Meijer, 2003). Also, SCN-activity correlates negatively with sleep pressure (Deboer et al., 2003) and the amplitude of SCN activity is reduced after SD (Deboer, Detari, & Meijer, 2007). Also after SD, the SCN response to light is reduced (Mistlberger, Landry, & Marchant, 1997; van Diepen et al., 2014), but can be reinstated by treatment with the adenosine antagonist caffeine (van Diepen et al., 2014). Furthermore, caffeine treatment lengthened the circadian period under normal waking conditions, while the amount of physical activity remained unchanged (van Diepen et al., 2014). Thus, it has been suggested that adenosinergic A_1 receptors might be involved in a sleep homeostatic modulation of the activity of the main circadian pacemaker (van Diepen et al., 2014). In humans, a differential modulation of the SCN according to sleep pressure is underlined by BOLD activity assessed by functional magnetic resonance imaging (fMRI). In line with the results derived from animal studies, activity in a SCN encompassing region was negatively associated to SWA (Schmidt et al., 2009).

Beside the SCN, an integration of circadian and sleep homeostatic inputs is also reasonable in other hypothalamic areas, such as the orexin-containing LH (Silver & Lesauter, 2008). Orexin-levels show a circadian rhythm, but are also influenced by the sleep homeostatic mechanisms (Deboer et al., 2004). The impact of sleep pressure has been suggested to be regulated by adenosinergic A_1

receptors in the LH. Adenosine inhibits orexinergic LH activity and has a potential sleep inducing effect (Liu & Gao, 2007; Thakkar, Engemann, Walsh, & Sahota, 2008).

Finally, given the widespread projections of circadian arousal promotion and the distribution of adenosinergic receptors all over the brain, the integration of circadian and homeostatic signals is reasonable in various brain regions at the single neuronal level. It has been shown in rats that the density of adenosinergic A_1 receptors in the basal forebrain is upregulated in response to SD (Basheer, Bauer, Elmenhorst, Ramesh, & McCarley, 2007) and similarly, in humans, A_1 receptor binding is increased after SD in several cortical and subcortical regions (Elmenhorst et al., 2007). Interestingly, the binding potential of these receptors has been proposed to show a circadian pattern in the cerebral cortex in animals (Florio, Rosati, Traversa, & Vertua, 1997), a finding however, which needs replication under constant lighting conditions.

Overall, the evidence strongly supports an interaction between sleep homeostatic and circadian mechanisms based on complex and widely distributed neuronal mechanisms.

2.6 Working memory

In our study we assessed the circadian and sleep homeostatic variations of several waking functions, including working memory (WM) performance in two groups, presumably differing in the adenosinergic modulation of sleep pressure (Bachmann et al., 2012). WM performance has been proposed to be particularly sensitive to the effects of high sleep pressure due to a certain dependence on the prefrontal cortex (Harrison & Horne, 2000). Therefore, among the assessed waking functions, a focus on the domain of WM appeared as particularly interesting.

The main process characterizing WM performance is generally considered as the successful manipulation of information in a kind of short term storage. Importantly, WM is distinct from short term memory in that it not only refers to a brief storage of information, but also to its manipulation. Irrespective of a limited storage capacity to a specific 'magical' number of items (Cowan, 2001; Miller, 1956), WM performance can be trained successfully by practicing executive aspects of WM (Backman & Nyberg, 2013; Buschkuehl, Jaeggi, & Jonides, 2012; Morrison & Chein, 2011). Executive aspects refer to processes apart from storage, for instance to the inhibition of interference (Collette & Van der Linden, 2002). Following a brief summary of how WM is understood at a conceptual level, the current knowledge will be outlined about which neuronal processes underlie WM performance and how they vary according to sleep-wake regulatory mechanisms.

2.6.1 Working memory at a conceptual level

The currently most well-known conceptualisation of WM was originally published by Baddeley and Hitch first in 1974 (Baddeley & Hitch, 1974), and has been continuously refined since then. According to this multicomponent theory, the WM system is constituted of several modules (Baddeley, 2012): Two capacity-limited storage modules, at least, termed phonological loop and visual-spatial sketchpad, are assumed to store information in a modality specific manner over short terms. These storage modules are linked to an executive control system. The central executive regulates manipulation of information within the storage modules. It is assumed to control the focus and the division of attention, and guides decision making and switching between tasks. Thus it is central for processes commonly labelled as executive functions (Baddeley, 2012).

2.6.2 Assessment of working memory by the n-back task

Mirroring the diversity of WM processes, there is a wide range of tasks assessing WM functions. We measured WM and underlying cerebral correlates by means of a visual verbal n-back task, frequently used in neuroimaging studies (Jaeggi, Buschkuehl, Perrig, & Meier, 2010; Owen, McMillan, Laird, & Bullmore, 2005). This tasks, first introduced by Mackworth in 1959 [(Mackworth, 1959) cited in (Jaeggi et al., 2010)], consists of the visual presentation of a series of verbal stimuli, such as letters, separated each by a short interstimulus interval. Participants are asked to decide and indicate whether the stimulus currently presented is the same as n trials before. Accordingly, the task requires a range of different cognitive operations, such as encoding, monitoring, maintenance and updating of stimuli in the short term storage, stimuli manipulation (i.e., temporal ordering and matching), and finally the inhibition of pre-potent responses as well as execution of the response (Jonides et al., 1997; Kane, Conway, Miura, & Colflesh, 2007).

Jaeggi and colleagues summarized that reliability indices of the n-back crucially depend on the difficulty level, the so-called load (i.e., the size of n). Coefficients exceeding .80 were specifically reported for the more difficult versions (i.e., 2-back or 3-back; Jaeggi et al., 2010). Concerning construct validity, several studies reported n-back performance to be associated to WM span measures, to performance in specific tasks measuring several executive functions as well as to fluid intelligence (Jaeggi et al., 2010). Low validity indices, observed in some studies, might be traced back to the impact of familiarity based responses on performance (Kane et al., 2007).

2.6.3 Neuronal underpinnings

2.6.3.1 Brain activity patterns

Brain activity during a WM task typically involves widespread networks, ranging from prefrontal areas to parietal regions as well as the occipital lobe (see Figure 5). The temporal patterns of the activity distributions appear to fit well to the conceptual models of WM: Broadly speaking, prefrontal areas, reminiscent of the central executive, control activity in sensory regions, representing modality specific storage modules (Jonides et al., 2008). Several functional aspects of WM, such as protection against interference, updating or switching mechanisms have been linked to distinct brain regions (Collette, Hogge, Salmon, & Van der Linden, 2006; Nee et al., 2013).

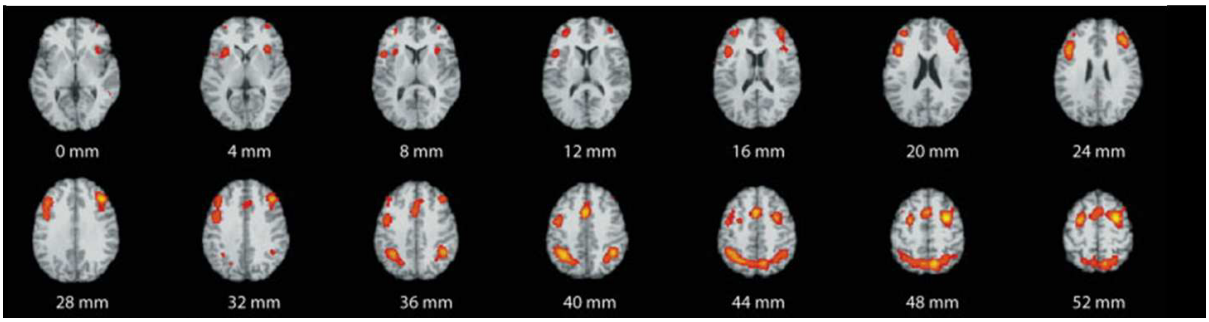

Figure 5. Activity differences during n-back performance. Meta-analytic activation maps for n-back performance. Regions consistently activated across studies are color-coded according to the probability of false discovery (voxelwise P < 0.01; FDR corrected). The right side of each section represents the right side of the brain; the z-coordinate in Talairach space is indicated below each section. Regions of activation highlighted by these selected slices include dorsolateral (z = 28) and ventrolateral (z =4) prefrontal cortex and frontal poles (z =4); lateral and medial premotor cortex (z =52, 40), and lateral and medial posterior parietal cortex (z =52, 40). *Figure and legend adapted from* (Owen et al., 2005)

Generally, persistent activity in lateral prefrontal neurons mirrors top-down control of those regions, which maintain sensory information. The lateral prefrontal cortex (lateral PFC) presumably exerts its top-down control by both active promotion of relevant information and active suppression of irrelevant information (Sander, Lindenberger, & Werkle-Bergner, 2012). The ventrolateral part of the PFC has been suggested to mediate a controlled access to memory contents and their maintenance (Badre & Wagner, 2007), while the dorsolateral region appears to be more implicated in the organisation of WM contents into higher-order units of information, so-called chunks (Owen et al., 2005).

To regulate interference reduction, the pre-supplemental motor area appears to be particularly important (Irlbacher, Kraft, Kehrer, & Brandt, 2014). This area has also been suggested to play a role in the capacity limits of WM, and linked to the limits in selective attention (Linden, 2007). A further

limiting factor for capacity, is activity in more posterior parietal areas, which are crucially involved in formation and maintenance of information (Sander et al., 2012; Linden, 2007).

Finally, subcortical areas, such as the striatum or cerebellum are involved in successful WM performance, for instance in the suppression of irrelevant information (Sander et al., 2012) or maintenance of information and guiding attention (Stoodley, 2012).

2.6.3.2 Neurotransmitters and Neuromodulators

Numerous neurotransmitters are involved in the regulation of WM performance. So far, the presumably largest body of research targets the role of dopamine in the PFC (Dash, Moore, Kobori, & Runyan, 2007; Khan & Muly, 2011). The effects of dopamine are mirrored in an inverted u-shaped function, such that a dysregulation in any direction has a detrimental impact on performance (Clark & Noudoost, 2014). In humans, strong evidence for a dopaminergic modulation of WM arises from impairments of WM functions following pathophysiological changes in the dopaminergic system (for instance in schizophrenia (Barch & Ceaser, 2012)). However, other neurotransmitters in the PFC, such as acetylcholine, norephinephrine or serotonin have also been shown to be implicated in WM functions (Robbins & Roberts, 2007).

Intriguingly, also the adenosinergic system plays a role in WM performance modulation. A reduction of the adenosinergic tone appears beneficial for performance under pathophysiological conditions (Chen, 2014). However, in healthy adults, performance did not significantly change after caffeine administration, even though differences in underlying brain activity patterns were observed (Haller et al., 2013; Klaassen et al., 2013; Koppelstaetter et al., 2008). Notably, the latter studies have been conducted under normal waking conditions. After 64 h of continuous wakefulness, caffeine has been shown to improve WM performance (Wesensten, Killgore, & Balkin, 2005). Also, caffeine impacts on short term memory performance not until a certain sleep pressure level is reached (Wyatt et al., 2004). Furthermore, there is evidence for an impact of high sleep pressure on WM performance, as discussed in the next section.

2.6.5 Impact of sleep loss

As recently summarized by a meta-analysis, WM performance is robustly affected by sleep loss (Lim & Dinges, 2010). However, it is still a matter of debate in which of the various processes constituting WM performance these decreases specifically occur (Killgore, 2010). According to the so-called vigilance hypothesis (Lim & Dinges, 2010), decrements in performance might be traced back to a general decline in basic attentional processes, such as arousal, required to perform in a WM task. Tucker and colleagues, for instance, disentangled executive from non-executive WM components

and showed that specifically the latter were mainly affected by extended wakefulness (Tucker, Whitney, Belenky, Hinson, & Van Dongen, 2010).

In parallel, sleep loss-related declines in WM have been proposed to be due to their particular dependence on activity in the PFC. Harrisons and Horne (Harrison & Horne, 2000) suggested that the PFC, continuously challenged during wakefulness, is specifically sensitive for the effects of SD. This so-called neuropsychological hypothesis (Lim & Dinges, 2010) is underlined by a predominance of delta and theta power EEG in frontal areas during recovery sleep from SD (Cajochen, Foy, et al., 1999). Also, in animals, the wake-dependent increase in adenosine has been specifically observed in the basal forebrain, located frontally (Basheer et al., 2004). Further, the up-regulation of human A_1-receptors after 24 h SD has been reported to be most pronounced in the orbito-frontal cortex (Elmenhorst et al., 2007). In further support for the neuropsychological hypothesis, Drummond and colleagues showed that performance in the inhibition of prepotent responses, a specific executive aspect of WM, was impaired by SD while the general ability to correctly respond to frequent trials was not affected (Drummond, Paulus, & Tapert, 2006). A similar specific effect of high sleep pressure has been reported regarding the executive WM component of switching (Couyoumdjian et al., 2010).

However, several studies report stable levels of WM performance, specifically for higher order executive functions, over the course of SD. Roughly a decade ago it was even considered as "prevailing view in SD research […] that high-level complex skills are relatively unaffected by SD […]" (Harrison & Horne, 2000), p. 236. This view was based on the idea that higher order cognitive tasks generate a kind of motivation or interest, which leads to compensatory effort to perform well even under high sleep pressure(Harrison & Horne, 2000).

Accordingly, neuroimaging studies investigating the impact of sleep loss on WM-related brain activity revealed a complex pattern of increases and decreases in several brain regions (Chee & Chuah, 2008). Compared to baseline, activity decreases have been observed after sleep loss in a fronto-parieto-occipital network and associated to declines in WM performance (Chee & Choo, 2004; Chee et al., 2006; Choo, Lee, Venkatraman, Sheu, & Chee, 2005; Chuah, Venkatraman, Dinges, & Chee, 2006; Habeck et al., 2004; Mu, Nahas, et al., 2005; Thomas et al., 2000 but see Lythe, Williams, Anderson, Libri, & Mehta, 2012). The maintenance of stable WM performance in sleep deprived states has been traced back to compensatory increases at the brain activity level in frontal, anterior cingulate and thalamic areas (Chee & Choo, 2004; Choo et al., 2005; Chuah et al., 2006; Habeck et al., 2004; Mu, Mishory, et al., 2005). One factor modulating compensatory increases has been suggested to be task complexity: Better performance after SD was observed in the more complex tasks and proposed to be related to increases in prefrontal and thalamic activity (Chee & Choo, 2004). Importantly, it has been observed that individuals highly differ in compensatory brain activity

patterns. These variations presumably underlie stable inter-individual differences in vulnerability to sleep loss at the behavioural level (summarized in section 2.7; Chee & Van Dongen, 2013).

Finally, it is important to consider that the impact of sleep loss on WM performance is dependent on circadian phase. How circadian phase acts on WM performance, and how this pattern changes according to homeostatic sleep pressure, will be summarized in the next section.

2.6.4 Circadian modulation

The present evidence indicates that partial aspects of WM performance deteriorate at night, such as processing speed, focused attention and short term memory functions (Dijk et al., 1992; Grady, Aeschbach, Wright, & Czeisler, 2010; Lee et al., 2009; Silva et al., 2010; Wyatt et al., 2004; Wyatt et al., 1999). However, higher executive functions have been observed to remain at stable levels during nighttime, as for instance inhibition (Sagaspe et al., 2012) or planning performance (Blatter et al., 2005). Correspondingly, Monk reported a negative correlation of the circadian variation in cortisol and WM speed, while WM accuracy was not significantly associated (Monk, 1997).

Intriguingly, under conditions of sleep loss, higher order executive functions have been observed to decrease at night. This pattern is not simply due to a rise in sleep pressure, as performance stabilized or increased again when wakefulness was extended to the following day (Blatter et al., 2005; Sagaspe et al., 2012). Thus, the impact of sleep pressure is enhanced during the night, but counteracted during the day. This interaction of sleep homeostatic and circadian mechanisms, was generally shown as well in tasks assessing more basic functions, however not in a consistent manner (Dijk et al., 1992; Grady et al., 2010; Lee et al., 2009; Silva et al., 2010; Wyatt et al., 2004; Wyatt et al., 1999). These inconsistencies are most likely due to study designs, in which wakefulness was restricted to less than 16 h. Under these conditions, sleep pressure levels might not be high enough to exert a clear-cut influence at night (Dijk et al., 1992; Grady et al., 2010; Wyatt et al., 2004).

In sum, the emerging picture suggests that the more basic processes required for WM decrease during nighttime. These nighttime troughs are pronounced under high sleep pressure, and can only be observed under these adverse conditions in higher order executive functions. Under low sleep pressure, complex tasks might trigger motivational resources which help to overcome circadian nighttime troughs in cognitive performance (Harrison & Horne, 2000). Alternatively, compensatory brain mechanisms might operate in a more successful manner, the more widespread the implicated network and the more complex the cognitive process (Chee & Van Dongen, 2013). Such compensatory mechanisms might contribute to the vulnerability to sleep loss at night. The vulnerability to sleep loss, its inter-individual variability and also the stability over time will be discussed in the next section.

2.7 Individual differences in sleep-wake regulation

Twin-studies and multiple recordings within the same individuals suggest that sleep and waking EEG as well as sleep architecture are highly heritable traits. In distinct frequency bands, up to 90% of the variance can be traced back to genetic influences (Landolt, 2011). However, compared to the fairly stable values measured *within* one subject, the variability *between* subjects is comparatively large (Chua et al., 2014; Landolt, 2011; Tucker, Dinges, & Van Dongen, 2007). The magnitude of robust differences between individuals has been shown to even exceed the effects of SD in several sleep features, including the classical marker of sleep homeostasis, NREM sleep delta power (Tucker et al., 2007).

A similar pattern has been observed in neurobehavioral performance. Individuals differ stably and highly in their ability to cope with extended wakefulness at the behavioural level (Van Dongen et al., 2004). These substantial differences in reaction to sleep loss have persistently been shown even if controlling for prior sleep history, duration of wakefulness, time of day, task duration, posture changes, physical activity level, or light exposure (Chee & Van Dongen, 2013; Van Dongen et al., 2004). Importantly, inter-individual patterns in sleep loss-dependent cognitive impairments are specific for a cognitive domain (Frey, Badia, & Wright, 2004; Van Dongen et al., 2004). A factor analytic approach revealed three different cognitive domains or clusters, classified as self-evaluation of sleepiness, cognitive processing capability and behavioural alertness (Van Dongen et al., 2004). As these clusters are differentially modulated at the cerebral level, the results point to inter-individual differences in vulnerability of particular neuronal networks (Van Dongen et al., 2004) Thus, in the present context the term 'trait-like vulnerability to sleep loss' should not be misunderstood as a kind of general inability to cope with sleep loss, but concerns specific cognitive clusters and the respective neuronal underpinnings.

In several aspects of circadian rhythmicity, a certain stability within individuals has been demonstrated as well. For instance, melatonin or the core body temperature curve during constant routine conditions have been shown to be stable over time (Chua et al., 2014; Leproult et al., 2003).

Not much is known about time of day-dependent patterns in trait-like vulnerability to sleep loss. Compared to homeostatic contributions to performance under sleep loss, the extent of individual differences in the circadian process appears to be less pronounced, at least for vigilance performance (Van Dongen, Bender, & Dinges, 2012). Interestingly, inter-individual differences in vulnerability to sleep loss can be traced back to a genetic variation in the clock gene PERIOD3. Specifically under high sleep pressure at night, genotypes differed in vigilance (Maire et al., 2014) and higher order cognitive performance (Lo et al., 2012; Viola et al., 2007). Underlying cerebral correlates modulating this genotype-specific and trait-like response have not been published so far. During the biological day, however, Vandewalle and colleagues observed a potentially compensatory increase in brain activity

after sleep loss in the genotype which has been reported previously to be less vulnerable to SD (Vandewalle et al., 2009).

3. Research questions and design

Based on the findings summarized above three key conclusions can be drawn: 1.) The impact of sleep pressure on sleep and waking functions strongly depends on time of day. 2.) The adenosinergic system modulates the dynamics of sleep pressure (i.e., the increase during wakefulness and its decrease during sleep), and 3.) a change in the adenosinergic balance due to the ADA-polymorphism contributes to trait-like differences in sleep and waking functions. However, it is not yet known whether circadian modulations of sleep and waking functions and their interaction with the sleep-wake homeostat vary according to genotype.

We focused first on the following research question: **Do circadian modulations in physiology and behaviour differ according to the ADA-genotype?** We assessed circadian variations in EEG, melatonin, BOLD activity, and neurocognitive behaviour in two groups of G/A- and G/G-allele carriers during a 40 h of multiple napping (NP). By 10 regularly scheduled 80-min naps the state of sleep pressure was kept at constantly low levels (Figure 6). Further we aimed at clarifying: **Do genotype-specific circadian modulations contribute to differences in response to sleep loss?** Thus, we implemented a second condition, a 40-h SD (Figure 6), in which sleep pressure rose continuously during wakefulness. The two conditions (low vs. high sleep pressure) were combined in a within-subjects design, and differed exclusively with regard to the scheduled nap sleep episodes. The assessment of salivary melatonin, sleep EEG, subjective sleepiness, cognitive performance as well as underlying cerebral correlates was scheduled exactly to the same time of day in both the multiple nap and SD protocol.

Physiological effects of SD, particularly in the adenosinergic system (Porkka-Heiskanen, 2013), are pronounced in frontal brain areas (Cajochen, Foy, et al., 1999; Cajochen et al., 2002). Consequently, tasks relying on these regions have been proposed to be specifically susceptible to SD (Harrison & Horne, 2000). In parallel, sleep loss-dependent declines in performance under SD are dependent on cognitive domain and expressed individually in a stable, trait-like manner (Van Dongen et al., 2004). We therefore assessed not only WM performance, but also vigilance in order to investigate: **Are genotype-specific modulations in sleep-wake regulation differentially expressed according to neurobehavioural domain?**

In the first publication (chapter 4.1), we answered the three outlined questions. Among others, we report that specifically in G/A-allele carriers profited from nap sleep in WM compared to performance under SD. Based on this result, we wondered: **Do genotype-specific differences in WM relate to differences in circadian nap sleep patterns?** Earlier reports point to circadian and sleep pressure-dependent modulations of WM and suggest sleep-dependent improvement of WM during

the night (Kuriyama, Mishima, Suzuki, Aritake, & Uchiyama, 2008). However, neither the role of circadian variations in sleep nor their genotype-dependent modulation has been considered so far. Our second publication (chapter 4.2) targets this missing link.

Finally we investigated BOLD activity underlying WM performance according to sleep pressure and circadian phase. Even though extensive research activities were dedicated to study the effects of sleep pressure at a BOLD level, the impact of the circadian system on these effects is unknown. The intensification of nighttime performance troughs under high sleep pressure suggests a pronounced impact of sleep pressure at night, also in performance underlying cerebral correlates. In our third manuscript (chapter 4.3) we deal with the following question: **Does circadian phase act on the typical sleep loss-related activity declines?** In a final step, we analysed the influence of genotype on circadian modulations of sleep loss-related activity declines. The results well be outlined in chapter 5.3.

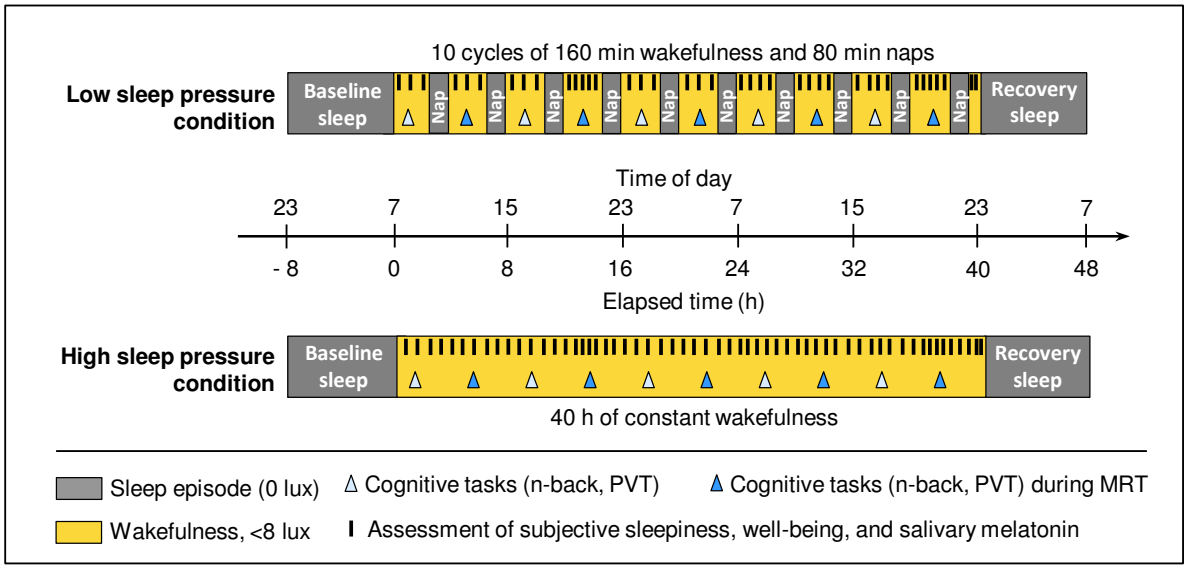

Figure 6. Illustration of the laboratory study. In a within-subjects design, sleep pressure levels were varied by multiple napping (low sleep pressure) vs. constant wakefulness (high sleep pressure). In healthy young adults (for recruitment and demographic information please see chapter 4), we assessed several sleep and waking functions, explained in detail in chapter 4. PVT: psychomotor vigilance task; MRT: magnetic resonance tomography.

4. Original research papers

This chapter consists of three research papers, to which I contributed by planning the experimental design, recruitment of volunteers, study conduction and data acquisition, data processing, statistical analyses, and manuscript writing.

The titles of the three papers are:

1) Insights into Behavioral Vulnerability to Differential Sleep Pressure and Circadian Phase from a Functional ADA Polymorphism
2) The Circadian Regulation of Sleep: Impact of a Functional ADA-Polymorphism and Its Association to Working Memory Improvements
3) Time of Day Matters: Circadian Modulation of Sleep loss-related Changes in Cognitive Brain Functions

4.1 Original research paper 1

Insights into Behavioral Vulnerability to Differential Sleep Pressure and Circadian Phase from a Functional ADA Polymorphism.

Reichert, C. F., Maire, M., Gabel, V, Viola, A.U., Kolodyazhniy, V., Strobel, W.,Götz, T., Bachmann, V., Landolt, H.-P., Cajochen, C. & Schmidt, C. (2014). *J Biol Rhythms, 92*(2), 119-130.

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Insights into Behavioral Vulnerability to Differential Sleep Pressure and Circadian Phase from a Functional *ADA* Polymorphism

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Insights into Behavioral Vulnerability to Differential Sleep Pressure and Circadian Phase from a Functional *ADA* Polymorphism

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Abstract Sleep loss affects human behavior in a nonuniform manner, depending on the cognitive domain and also the circadian phase. Besides, evidence exists about stable interindividual variations in sleep loss–related performance impairments. Despite this evidence, only a few studies have considered both circadian phase and neurobehavioral domain when investigating trait-like vulnerability to sleep manipulation. By applying a randomized, crossover design with 2 sleep pressure conditions (40 h sleep deprivation vs. 40 h multiple naps), we investigated the influence of a human adenosine deaminase (*ADA*) polymorphism (rs73598374) on several behavioral measures throughout nearly 2 circadian cycles. Confirming earlier studies, we observed that under sleep deprivation the previously reported vulnerable G/A-allele carriers felt overall sleepier than G/G-allele carriers. As expected, this difference was no longer present when sleep pressure was reduced by the application of multiple naps. Concomitantly, well-being was worse in the G/A genotype under sleep loss when compared to the nap protocol, and n-back working memory performance appeared to be specifically susceptible to sleep-wake manipulation in this genotype. When considering psychomotor vigilance performance, however, a higher sensitivity to sleep-wake manipulation was detected in homozygous participants, but specifically at the end of the night and only for optimal task performance. Although these data are based on a small sample size and hence require replication (12 G/A- and 12 G/G-allele carriers), they confirm the assumption that interindividual differences regarding the effect of sleep manipulation highly depend on the cognitive task and circadian phase, and thus emphasize the necessity of a multimethodological approach. Moreover, they indicate that napping might be suitable to counteract

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endogenously heightened sleep pressure depending on the neurobehavioral domain.

Keywords adenosine deaminase, sleep pressure, circadian phase, interindividual variability, well-being, cognition

Interindividual differences in sleep-wake regulation, particularly in neurobehavioral functioning in response to sleep loss, have been shown in several studies (Chee and Chuah, 2008; Maire et al., 2013) and may play an important role in how an individual performs at night or under condtions of extended wakefulness, such as during shiftwork. These trait-like differences can be traced back to variability among individuals in 2 interacting processes underlying sleep-wake regulation (Van Dongen et al., 2012). Circadian oscillations originating in cells of the suprachiasmatic nuclei modulate subjective sleepiness (Wyatt et al., 1999), well-being (Birchler-Pedross et al., 2009), and cognition (Schmidt et al., 2007) in a nearly 24-h fashion. An additional important factor impinging on these variables is the homeostatic sleep drive (Birchler-Pedross et al., 2009; Schmidt et al., 2007; Wyatt et al., 1999). It increases during wakefulness and dissipates during sleep, and may reflect at the molecular level the accumulation and subsequent degradation of so-called sleep factors, such as adenosine (Porkka-Heiskanen and Kalinchuk, 2011). Importantly, evidence for a mutual interaction between homeostatic and circadian aspects has been detected in multiple dimensions, ranging from molecular (Franken and Dijk, 2009) and electrophysiological levels (Cajochen and Dijk, 2003; Wyatt et al., 1999) to the modulation of cognitive functions (Dijk et al., 1992; Wyatt et al., 1999, 2006) and their underlying cerebral correlates (Schmidt et al., 2009; Vandewalle et al., 2009).

Increasing evidence indicates that there are substantial interindividual differences in performance declines secondary to total sleep deprivation, which have been associated with the polymorphic nature of genes implicated in circadian and sleep homeostatic mechanisms (Franken et al., 2001; King et al., 2009; Landolt, 2011). Among others, the human c.22G>A polymorphism (rs73598374) in the gene encoding adenosine deaminase (ADA) has been studied. This enzyme degrades the sleep factor adenosine (Porkka-Heiskanen and Kalinchuk, 2011) and differs in its activity depending on the genotype (Battistuzzi et al., 1981; Riksen et al., 2008). In humans, genetically reduced enzymatic activity of ADA has been linked to a longer duration of deep sleep and an enhanced electroencephalographic (EEG) activity in the slow-wave range, both indexes of elevated homeostatic sleep pressure levels (Bachmann et al., 2012; Mazzotti et al., 2012; Retey et al., 2005). Moreover, G/A-allele carriers, associated with less active ADA, indicated higher subjective sleepiness, thus appearing more susceptible to the detrimental effects of sleep loss, and showed impaired vigilance throughout sleep deprivation (SD; Bachmann et al., 2012).

Interindividual differences in neurobehavioral vulnerability to SD do not appear to be homogeneously reflected in all behavioral variables. Van Dongen and colleagues (2004) identified 3 distinct dimensions of sleep loss–related interindividual variability in neurobehavioral performance, clustering around self-evaluation of sleepiness, mood, and fatigue; cognitive processing; and behavioral alertness as measured by the psychomotor vigilance task (PVT). These findings point to distinct underlying neurocognitive subsystems, being differentially affected by extended wakefulness as also evidenced by neuroimaging studies (Chee and Chuah, 2008).

Even though the literature suggests that the behavioral impact of sleep-wake manipulation highly depends on circadian phase, individual constitution of sleep-wake-regulation, and the investigated cognitive task, only a few studies applied a multimethodological approach to simultaneously investigate these variables. Here, we studied 2 matched groups of healthy young individuals, solely differing with regard to their c.22G>A genotype of *ADA*, to compare the influence of 2 different levels of trait-like vulnerability to sleep pressure on subjective sleepiness, well-being, and on performance in 2 different cognitive tasks, challenging either mainly attentional vigilance (PVT) or working memory storage performance (n-back). A combined application of a 40-h SD and a 40-h multiple-nap protocol (Cajochen et al., 2001; Knoblauch et al., 2002; Sagaspe et al., 2012) served to systematically investigate not only conditions and times of day at which an endogenously heightened sleep pressure appears to be specifically detrimental but also if, when, and in which neurobehavioral tasks a counteraction by napping might be possible. According to previous literature reports, we expected higher subjective sleepiness levels as well as impaired vigilance performance under SD in the G/A genotype. Moreover, we hypothesized that, compared to SD, differences among genotypes will be reduced when participants are allowed to sleep during the nap

protocol, at least during the biological night. As interindividual differences in sleep-wake-specific modulations of cognitive performance depend on the according cognitive domain (Van Dongen et al., 2004), we expected that, compared to vigilance and subjective sleepiness, higher order working memory performance, as measured by the n-back task, might be differentially affected by the experimental condition, time of day, and genotype. Finally, since sleep homeostatic mechanisms can majorly affect the circadian timing system, it might be expected that genotypes also differ in circadian regulation at both the physiological and behavioral levels.

Table 1. Demographic data, questionnaire scores, and actimetrical data (means and standard deviations) split by genotype.

Sample characteristics	G/A-allele carriers	G/G-allele carriers	*p*
N (f, m)	12 (8, 4)	12 (8, 4)	1.00
Age (y)	24.33 (3.87)	24.75 (2.49)	.757
BMI (kg/m^2)	21.8 (2.9)	21.6 (2.0)	.791
PSQI	3.58 (1.16)	2.83 (1.11)	.121
ESS	4.46 (2.83)	4.29 (2.04)	.870
MEQ	54.8 (9.7)	57.6 (10.8)	.505
MCTQ sleep duration	7.92 (0.58)	7.87 (0.68)	.824
MCTQ MSF sc	4.34 (1.08)	4.26 (1.03)	.837
MCTQ MSF sac	7.29 (2.39)	7.62 (2.71)	.754
Wake time (hh:min) during study	07:08 (57 min)	07:13 (57 min)	.832
Habitual caffeine consumption (mg/day)	108.01 (60.78)	87.31 (60.80)	.469
Habitual sleep time (hh:min), work days	23:39 (57 min)	23:19 (58 min)	.444
Habitual wake time (hh:min), work days	07:42 (43 min)	07:43 (72 min)	.921
Habitual sleep duration (min), work days	460.92 (36.97)	487.04 (47.90)	.198
Habitual sleep time (hh:min), free days	23:48 (54 min)	23:04 (47 min)	.069
Habitual wake time (hh:min), free days	08:06 (38 min)	08:18 (34 min)	.454
Habitual sleep duration (min), free days	488.41 (38.11)	511.99 (24.23)	.124

F = female; m = male; y = years; BMI = Body Mass Index; PSQI = Pittsburgh Sleep Quality Index; ESS = Epworth Sleepiness Scale (Johns, 1981); MEQ = Morningness-Eveningness Questionnaire (Horne and Östberg, 1976); MCTQ = Munich Chronotype Questionnaire (Roenneberg et al., 2003); MSF sc = mid-sleep-free days, sleep corrected; MSF sac = mid-sleep-free days, sleep and age corrected. Habitual caffeine consumption was assessed by sleep diaries during 3 weeks; wake time, sleep time, and sleep duration were derived by actimetrical data collected during 3 weeks. *p*-values were derived from χ^2-(gender ratio) and *t*-tests (all other variables).

MATERIALS AND METHODS

The study was approved by the local ethics committee (Ethikkommission beider Basel) and performed according to the Declaration of Helsinki. All participants gave written informed consent before participation. For procedures of recruitment and genotyping see the supplemental online material.

Study Protocol

A total of 12 heterozygous and 12 homozygous volunteers agreed to participate and completed the laboratory part of the study. All participants indicated good subjective sleep quality (Pittsburgh Sleep Quality Index [PSQI] ≤ 5; Buysse et al., 1989), a habitual sleep duration of 8 ± 1 h, and no symptoms of clinical depression (Beck Depression Inventory [BDI-II] < 9; Beck et al., 1996). The genotype groups were matched for the sex distribution within the groups, and did not differ according to age, BMI, sleep quality, and chronotype, variables possibly confounding sleep-wake regulation (see Table 1). As well, groups were balanced according to the PERIOD3 polymorphism, shown to affect behavioral vulnerability in response to sleep loss (Viola et al., 2007). To allow habituation to laboratory conditions and to screen for major sleep disturbances, volunteers slept in the laboratory for 1 night before study participation. They underwent a medical check and a drug screen (Drug-Screen-Multi 6, nal von minden, Regensburg, Germany) to guarantee basic physical and mental health. One week before starting the laboratory part, participants kept a fixed sleep-wake cycle for 7 days (8 h sleep at night and no daytime naps) to ensure sufficient sleep and stable circadian entrainment before starting the laboratory part. Sleep-wake times were derived from a 3-week actimetry field study and individually adapted to the participants' professional duties. Compliance to the regimen was verified by means of actigraphic recordings. Participants were instructed to abstain from alcohol and caffeine during this week to prevent withdrawal effects, especially in the adenosinergic system. All women were tested for pregnancy before the laboratory study started and were required to participate during their luteal phase (3 of 16 participating women) unless they were taking hormonal contraceptives.

The laboratory part comprised 2 conditions of 40 h each, separated by at least 1 week and implemented as a within-subject, randomized, crossover design. While the high sleep pressure condition consisted of 40 h of total SD, the low sleep pressure condition encompassed 10 short sleep-wake cycles each of 80 min of a sleep opportunity (i.e., a nap) alternating with 160 min of wakefulness (Fig. 1). The first cycle started 120 min after wake up. Duration of wakefulness in the last cycle was restricted to 40 min in order to ensure a start

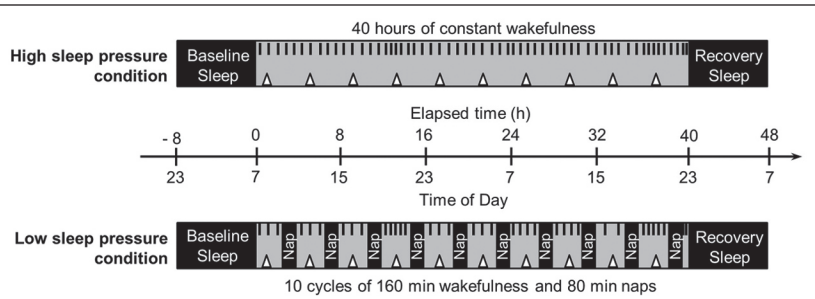

Figure 1. Schematic illustration of laboratory study. The high sleep pressure condition consisted of 40 h of constant wakefulness (gray), whereas the low sleep pressure condition comprised 10 short sleep-wake cycles, each encompassing 160 min of wakefulness (gray) alternating with 80 min of naps (black) to keep the homeostatic sleep pressure at low levels. Both conditions were preceded and followed by 8 h of sleep (black). Subjective sleepiness, well-being, and salivary melatonin (black short lines), as well as psychomotor vigilance task and n-back performance (triangles), were assessed during scheduled wakefulness.

of the recovery night at habitual sleep time. This procedure has already been used repeatedly to measure the effect of low (nap protocol) versus high (SD protocol) homeostatic sleep pressure levels at many circadian phases (e.g., Birchler-Pedross et al., 2009; Cajochen et al., 2001; Knoblauch et al., 2002; Sagaspe et al., 2012). Both conditions were controlled with regard to light influence (illuminance < 8 lux during wakefulness and 0 lux during sleep), caloric intake (standardized meals every 4 h), and body posture (semirecumbent position during scheduled wakefulness and recumbent during naps) to minimize potential masking effects on the sleep-wake regulatory system. Participants were not allowed to stand up except for regularly scheduled bathroom visits and did not have any indications of time of day. Social interaction was restricted to communications with study helpers. Note that EEG was monitored over the course of both protocols and functional magnetic resonance imaging (fMRI) data were collected at 5 specific time points in both conditions. These data will be published elsewhere.

Melatonin

Salivary melatonin was collected throughout the entire 40 hours, with an average sampling rate of 53 min starting 20 min after wake up. Sampling rates dynamically changed with circadian phase, such that during daytime, when no melatonin secretion was expected, sampling rates were lower (~60 min) as compared to the evening hours and nighttime (~45 min), when we aimed at tracking changes in melatonin secretion. A sampling rate of 30 min on average, as reported in other studies (i.e., Cajochen et al., 2001), was not implemented due to test bouts and naps longer than 30 min. Melatonin levels were analyzed by a direct double-antibody radioimmunoassay (validated by gas chromatography–mass spectroscopy with an analytical least detectable dose of 0.65 pm/ml; Bühlmann Laboratory, Schönenbuch, Switzerland). For estimation of amplitude, dim-light melatonin onset (DLMO), dim-light melatonin offset (DLMoff), and phase angle, a bimodal skewed baseline cosine function was fitted to raw values as described in Kolodyazhniy et al. (2012). Amplitude was defined as the maximum difference of the fitted waveform to its baseline. DLMO and DLMoff were defined as the times when the melatonin level crossed 50% of the maximum at the rising and falling limbs of the curve, respectively (Benloucif et al., 2008). The phase angle was calculated as the difference between the wake-up time and DLMO. The DLMO served as a marker of circadian phase position (Lewy and Sack, 1989), and the phase angle of entrainment indicated the relationship between the timing of the circadian clock and an environmental time cue (Duffy and Wright, 2005).

Self-Evaluation of Sleepiness and Well-Being

Subjective sleepiness was assessed at regular intervals (mean length of sampling interval: 65 min) by the Karolinska Sleepiness Scale (KSS; Akerstedt and Gillberg, 1990), the Karolinska Sleepiness Symptom Checklist (KSScl; Birchler-Pedross et al., 2009; Bromundt et al., 2013), and a visual analogue scale (VAS) ranging from 0 (*extremely awake*) to 100 (*extremely tired*). Assessments were more frequent when dynamic changes in sleepiness were expected and occurred less often during daytime. Values collected in the SD condition were excluded from analysis if no corresponding value in the nap condition was available (i.e., at times when napping was scheduled). The protocol encompassed an assessment of subjective sleepiness immediately after awakening from each nap. Here, we report values of a composite score ((KSS + KSSCL + (VAS / 10)) / 3) (Bromundt et al., 2013) collapsed into 4-h bins following the duration of 1 short sleep-wake cycle (160 min of wakefulness and an 80-min nap) in the nap protocol.

Together with subjective sleepiness, subjective well-being was measured by means of 3 VASs, each with a range from 0 to 100, assessing tension (ranging from *extremely relaxed* to *extremely tense*), physical comfort (ranging from *extremely comfortable* to *extremely uncomfortable*), and mood (ranging from *in very bad mood* to *in very good mood*). A mean of these scales (as calculated by ($VAS_{tension}$ + $VAS_{physical\ comfort}$ + (100 – VAS_{mood})) / 3) served as an indicator of well-being (Birchler-Pedross et al., 2009) and was pooled for analysis to 4-h bins.

Neurobehavioral Performance

After 1 hour of wakefulness, neurobehavioral performance was assessed by a modified version of the PVT (Dinges and Powell, 1985) and the n-back working memory paradigm. Both tests were repeatedly administered every 4 h, every other time in an MRI scanner.

During the 10-min PVT, participants were instructed to press a response button as fast as possible as soon as a millisecond counter appeared on the computer screen, which was displayed at random intervals with an interstimulus interval of 2 to 10 seconds. Feedback was provided by displaying the reaction time (RT) for 1 sec following the response. The dependent variables were median RTs, the fastest 10% and slowest 10% of RTs, as well as the number of lapses (RT > 500 msec, transformed by $\sqrt{x} + \sqrt{x + 1}$ according to Kuna et al. [2012] to stabilize variances), which were *z*-transformed due to different testing environments (every other session in the MRI scanner with a different response keypad).

The n-back consisted of the visual presentation of a series of consonants. Participants were asked to decide and indicate by differential button presses, whether the consonant depicted is the same as n trials before (target) or whether this is not the case. The task lasted for approximately 20 min and comprised a 3-back and a 0-back version. Five different variants of the task were presented throughout the protocol in a pseudo-randomized order. Each variant consisted of 9 blocks of a 3-back version and 5 blocks of the 0-back version, presented in a randomized order, each comprising 30 stimuli thereof 10 targets. Each stimulus was presented for 1.5 seconds with an interstimulus interval of 0.5 seconds.

A training session in the evening before the study ensured that participants were able to reach 70% of correct responses in the 3-back to prevent effects due to baseline differences in comprehension and transfer of instructions. One participant, however, performed 3 interquartile ranges lower than the 25th percentile during the entire course of the first condition such that performance values of this person were excluded from analyses as outliers. Hit targets (true positive) and missed targets (false negative) were analyzed as output measures in both the 3-back and the 0-back. Values reported represent *z*-standardized differences between 3-back and 0-back to account for baseline differences in basic attentional resources and to target working memory storage capacities.

Statistical Analysis

If not stated otherwise, statistical analyses were performed with SAS 9.3 software (SAS Institute, Cary, USA), using a mixed-model analysis of variance for repeated measures, with the factors Genotype (G/A and G/G genotype), Condition (SD and nap), and Time (11 bins in case of subjective scales and 9 bins when analyzing performance). Contrasts were calculated with the LSMEANS statement. Degrees of freedom of all *p*-values are based on an approximation described by Kenward and Roger (1997). The *p*-values of multiple post hoc comparisons were adjusted according to the false discovery rate procedure (Curran-Everett, 2000).

RESULTS

Salivary Melatonin

Irrespective of the sleep pressure condition, phase angle differed significantly between G/A- and G/G-allele carriers ($F_{1,22} = 4.34$; $p = 0.049$) with a 53 ± 13 min (mean ± standard error [SE]) wider phase angle in participants with the G/A genotype compared to homozygous participants. Concomitantly, DLMO appeared by trend to occur 48 min later in G/A- compared to G/G-allele carriers (at 10:42 p.m. ± 2 min; $F_{1,22} = 4.14$; $p = 0.054$) (Fig. 2A). Moreover, the experimental conditions affected the timing of the melatonin increase, such that, overall, phase angle was 24 ± 14 min (SE) narrower in the nap compared to the SD condition ($F_{1,22} = 6.79$; $p = 0.016$). Likewise, the DLMO was detected 24 min earlier (at 10:06 p.m. ± 12 min [SE]) in the nap protocol compared to total SD ($F_{1,22} = 6.85$; $p = 0.016$). Based on these results, all other parameters were adjusted to each participant's DLMO to compare data acquired at similar circadian phases.

The amplitude of melatonin as well as DLMoff did not differ significantly among genotypes or conditions nor did the analysis point to a significant interaction between genotype and condition ($p_{all} > 0.1$).

Self-Evaluation of Sleepiness and Well-Being

As expected, participants felt sleepier during SD compared to the nap protocol (mean ± SE: SD: 5.08 ± 0.15; nap: 3.99 ± 0.10) (Table 2). Furthermore, sleepiness displayed a circadian pattern modulated by the sleep pressure condition (Table 2): Subjective sleepiness increased during the biological night with a higher peak during SD compared to the nap protocol. Moreover, the subsequent decrease in the morning hours during the second day was weakened when participants were sleep deprived compared to when they were asked to nap regularly (Fig. 2B). As depicted in Figure 3A, the influence of genotype on the overall time course in subjective sleepiness

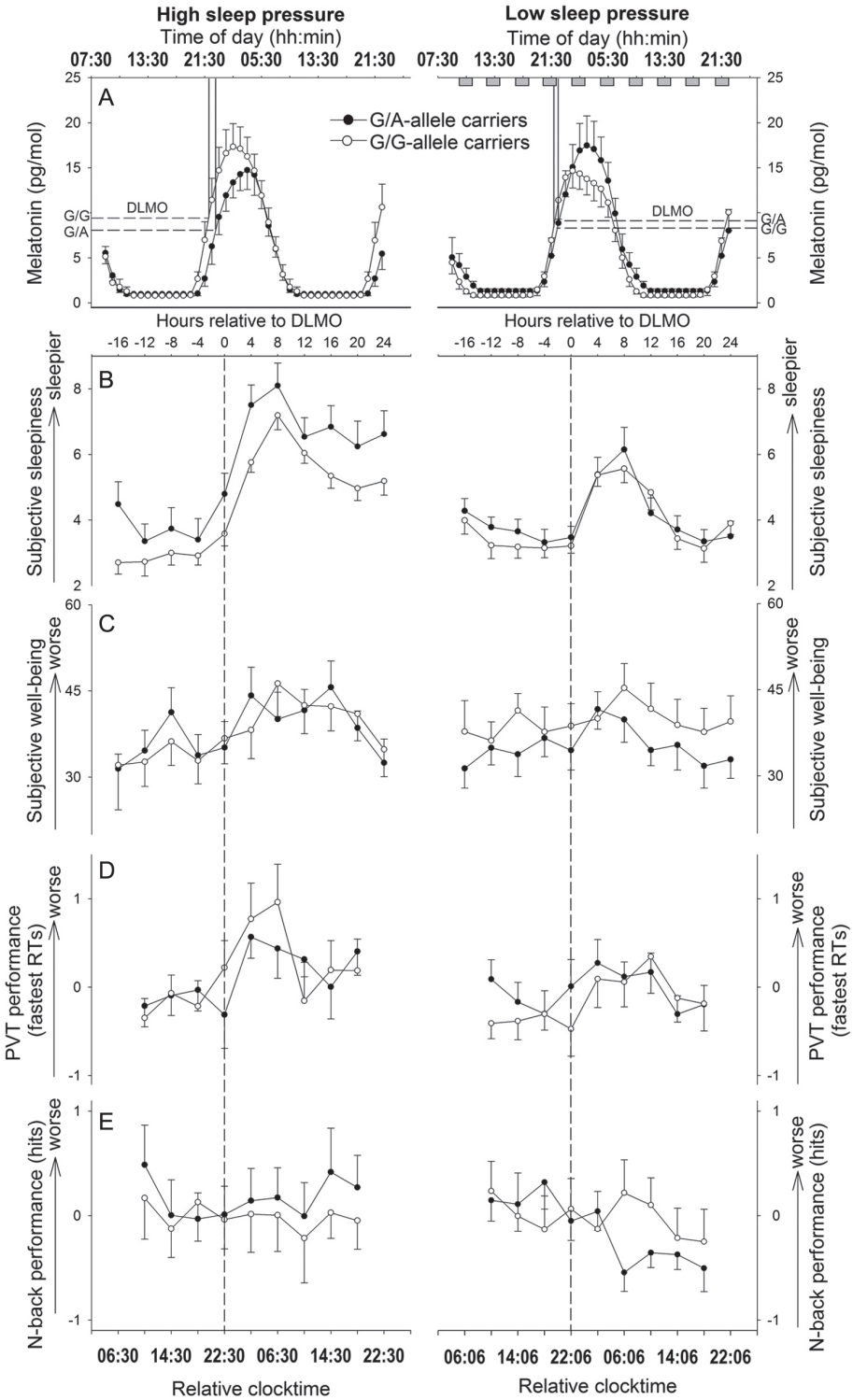

Figure 2. Time courses of dependent variables (means and standard errors) during high (left panel) and low (right panel) sleep pressure conditions in G/A- (filled dots) and G/G-allele carriers (open dots). (A) Time course of melatonin: dashed lines indicate the dim-light melatonin onset (DLMO) per genotype as calculated by 50% of the maximum. Gray rectangles during the low sleep pressure condition represent 10 nap sleep episodes each of 80 min. (B) and (C) Time courses of subjective sleepiness and subjective well-being (assessed earliest 30 min after waking up from scheduled sleep), respectively, both plotted relative to the DLMO. (D) and (E) Neurobehavioral performance profiles (*z*-values) of peak performance in vigilant attention (10% fastest RTs in PVT; [D]) and working memory (hit targets, and the difference between 3-back and 0-back; [E]) plotted relative to DLMO.

depended on the sleep pressure condition (Table 2), such that G/A-allele carriers indicated higher sleepiness during SD compared to G/G-allele carriers (p = 0.033), whereas the genotypes did not significantly differ during the nap protocol (p = 0.736).

In general, subjective well-being was better during high compared to low sleep pressure conditions (mean ± SE: SD: 36.97 ± 0.86; nap: 38.75 ± 0.80) (Table 2) and modulated by circadian phase (Table 2). Participants showed an overall decrease in well-being during nighttime in both protocols, which remained at low levels until the evening hours of the second day. Genotype-dependent effects appeared in interaction with the sleep pressure manipulation (Table 2), such that G/G-allele carriers felt worse in the nap condition compared to SD (p < 0.001; mean ± SE: nap: 41.16 ± 1.22, SD: 37.06 ± 1.33), while G/A-allele carriers' well-being did not differ between conditions (p = 0.640; mean ± SE: nap: 36.38 ± 1.00, SD: 36.89 ± 1.10).

The result of reduced well-being during the nap condition compared to SD, which was specifically pronounced in G/G-allele carriers, is contrary to what has been reported previously (Birchler-Pedross et al., 2009). To explore a

Table 2. Statistical results of subjective sleepiness and well-being.

Effect	Subjective sleepiness (all values assessed)	Well-being (all values assessed)	Well-being (excluding values assessed within 30 min after awakening)
Condition	**$F_{1,452}$ = 104.30 p < 0.001**	**$F_{1,452}$ = 7.37 p = 0.007**	$F_{1,444}$ = 0.52 P = 0.470
Time	**$F_{10,452}$ = 48.14 P < 0.001**	**$F_{10,452}$ = 4.99 p < 0.001**	**$F_{10,444}$ = 5.9 p < 0.001**
Genotype	$F_{1,22}$ = 1.78 p = 0.196	$F_{1,22}$ = 0.31 p = 0.584	$F_{1,22.1}$ = 0.22 p = 0.640
Condition × Time	**$F_{10,452}$ = 11.48 p < 0.001**	$F_{10,452}$ = 1.74 p = 0.069	**$F_{10,444}$ = 1.95 p = 0.037**
Condition × Genotype	**$F_{10,452}$ = 20.31 p < 0.001**	**$F_{1,452}$ = 11.37 p < 0.001**	**$F_{1,444}$ = 9.6 p = 0.002**
Time × Genotype	$F_{10,452}$ = 0.74 p = 0.687	$F_{10,452}$ = 1.33 p = 0.214	$F_{10,444}$ = 1.44 p = 0.158
Condition × Time × Genotype	$F_{10,452}$ = 0.79 p = 0.636	$F_{10,444}$ = 0.39 p = 0.949	$F_{10,444}$ = 0.49 p = 0.896

F-values, degrees of freedom, and *p*-values of a ProcMixed ANOVA. Significant results are printed in bold.

potential reason, we calculated a separate model only containing data collected earliest 30 min after each wake up, to exclude values possibly influenced by sleep inertia (Tassi and Muzet, 2000), that is, a "short period of confusion and degraded mood/performance immediately after awakening from sleep" (Naitoh et al., 1993, p. 110). Again, an overall circadian pattern with a trough during the biological night was observed that was particularly visible under SD (Table 2 and Fig. 2C). Moreover, we observed that specifically the well-being of G/A-allele carriers was significantly affected by the sleep pressure condition (Table 2). G/A-allele carriers indicated worsened well-being during SD compared to the nap condition (p = 0.007), while well-being did not significantly differ between conditions in the group of G/G-allele carriers (p = 0.097; Fig. 3B).

Neurobehavioral Performance: Vigilant Attention

Performance in the PVT, as assessed by median RTs, the 10% slowest RTs, the 10% fastest RTs, and lapses, was overall attenuated by SD (see Suppl. Table S1 for mean ± SE, Table 3 for statistics, and Fig. 4A for the fastest RTs) and revealed a circadian pattern with performance decrements during nighttime (see Table 3 for statistics; for the main effect of time in the fastest 10% RTs, see Suppl. Fig. S2; split by genotype and condition shown in Fig. 2D). These performance deteriorations did not fully recover during the second day, particularly during SD (interaction of Time × Condition; Table 3). The impact of genotype became apparent specifically in the fastest RTs and was modulated by sleep pressure as well as circadian phase (Table 3): Specifically, at the end of the biological night, G/G-allele carriers performed better during the nap protocol compared to SD (p < 0.001), whereas optimal performance of participants with the G/A genotype did not differ significantly at any time between sleep pressure conditions.

Neurobehavioral Performance: Working Memory Capacity

During SD, accuracy was lower as compared to the nap protocol (fewer hit targets and more missed targets; see Suppl. Table S3 for mean ± SE; and see Table 3 for statistics). Furthermore, performance was worse (fewer hit targets) at the beginning of both protocols, specifically during the first test compared to results achieved toward the end (Table 3 and Fig. 2E). The genotype-dependent influence was modulated by the experimental condition (Table 3): During the nap protocol, G/A-allele carriers performed better compared to SD (more hit targets: p = 0.002, Fig. 4B; fewer missed targets: p < 0.001, mean ± SE: nap: –0.13 ± 0.07, SD: 0.16 ± 0.11), while G/G-allele carriers' performance did not vary significantly according to the sleep pressure condition (hit targets: p = 0.795, Fig. 4B; missed targets: p = 0.623, mean ± SE: nap: –0.02 ± 0.11, SD: –0.01 ± 0.11).

DISCUSSION

In this study we investigated the time course of vulnerability to variations in sleep pressure based on genetic differences, associated with changes in ADA activity. In G/A-allele carriers, exhibiting reduced ADA activity (Battistuzzi et al., 1981; Riksen et al., 2008), changes in sleep pressure levels became apparent in subjective sleepiness and well-being as well as in working memory performance. Optimal vigilance performance at the end of the night, however, appeared to be a sensitive indicator for variations in sleep pressure in G/G-allele carriers. Our data suggest that the implication of ADA on neurobehavioral susceptibility to modulations of sleep-wake history depends on the investigated cognitive task and on circadian phase. They highlight the importance of a multimethodological approach applied during the entire circadian cycle when aiming at characterizing trait-like interindividual differences in vulnerability to sleep manipulation.

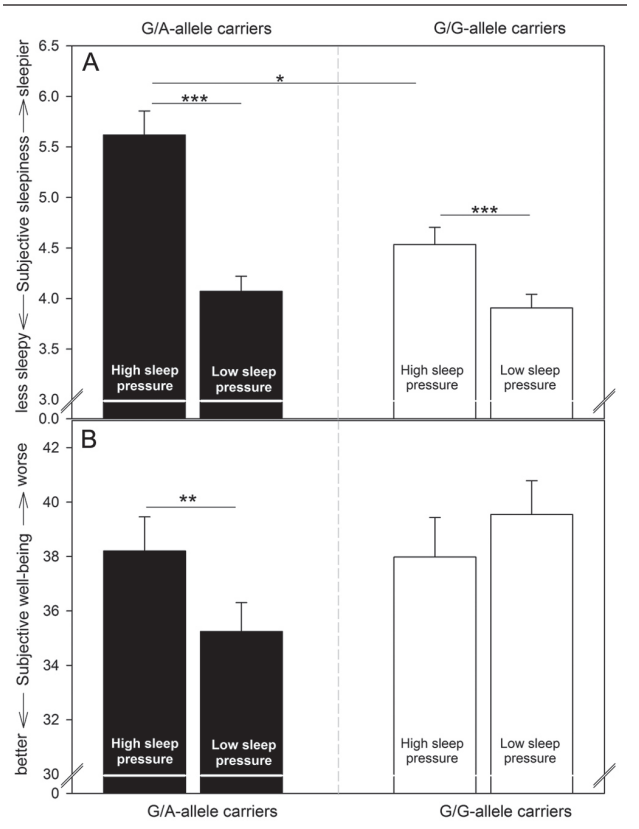

Figure 3. Means and standard errors of subjective sleepiness and subjective well-being per genotype and condition. (A) Under high sleep pressure conditions, G/A-allele carriers indicated significantly higher sleepiness compared to G/G-allele carriers. All participants felt sleepier during high compared to low sleep pressure (nap protocol). (B) Subjective well-being was significantly worse in participants with the G/A genotype during the high compared to the low sleep pressure condition, while G/G-allele carriers' well-being did not differ significantly between conditions. Note that the values plotted were assessed earliest 30 min after waking up after scheduled naps during the low sleep pressure condition and at corresponding times during sleep deprivation. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

It has been suggested that higher adenosine levels due to reduced ADA activity in heterozygous individuals contribute to higher sleep pressure levels in this genotype (Bachmann et al., 2012; Landolt, 2008; Retey et al., 2005). At the level of subjective sleepiness, we could confirm a higher impact of homeostatic sleep pressure under sleep loss in G/A-allele carriers (Bachmann et al., 2012). Importantly, differences between genotypes were no longer present when experimentally reducing sleep pressure, further indicating an implication of the *ADA* polymorphism in sleep regulation. Subjective well-being values, corrected for possible influences of the awakening process, also mirrored potentially higher sleep pressure levels in the G/A genotype through impaired well-being during SD (Birchler-Pedross et al., 2009). This result indicates that specifically G/A-allele carriers benefit in well-being from the reduction of sleep pressure by multiple naps. Importantly, condition-driven but also genotype-dependent differences in subjective well-being strongly depended on the temporal distance between the assessment of well-being and the last awakening from a scheduled sleep episode: If one includes values measured shortly after waking up, well-being appeared to be dampened during low compared to high sleep pressure, specifically in participants with the G/G genotype. Overall, such an effect might have been induced by a genotype-specific reaction to the repetitive disruption of sleep and wakefulness during the circadian cycle. This is inherent to the nap protocol and has the potential to affect well-being, especially if investigated immediately after awakening. It remains to be elucidated whether the negative impact of a close awakening on well-being could be traced back to potential genotype-specific changes in sleep inertia (assessed usually through cognitive performance), sleep characteristics prior to awakening (Tassi and Muzet, 2000), or other factors systematically appearing closely to waking up, such as stress due to the awakening process.

As suggested, self-evaluation of sleepiness and mood showed similar sleep loss–related trait-like patterns. By adopting a factor analytic approach, Van Dongen and colleagues (2004) revealed that these subjective measures do not inevitably resemble systematic interindividual differences in cognitive processing capabilities or vigilance during SD. For vigilant attention, we observed that homozygous participants showed increased performance in the fast RT domain at the end of the biological night under low compared to high sleep pressure conditions. Fastest RTs reflect peak performance in sustained attention, which is phasically delivered above and beyond baseline levels based on the ability to enhance the recruitment of attentional resources (Drummond et al., 2005). The negative impact of acute SD on vigilance is usually most likely observed in slowest RTs and lapses, reflecting momentary task disengagement and attentional failures (Lim and Dinges, 2008). These measures showed the well-known circadian and homeostatic pattern in our study, but they were not affected by genotype under high sleep pressure as has been previously reported, albeit with ambiguous results (lapses: Bachmann et al., 2012; Kuna et al., 2012). Intriguingly, fast RTs have been recently reported to be more likely influenced by chronic SD (Basner and Dinges, 2011), which amplifies the detrimental effects of acute sleep loss on attentional performance (Cohen et al., 2010). This kind of interaction between short- and long-term homeostatic processes might influence attentional

Table 3. Statistical results of psychomotor vigilance task (PVT) and n-back performance.

Effect	Median (PVT)	Fastest 10% (PVT)	Slowest 10% (PVT)	Lapses (PVT)	Hit targets (n-back)	Missed targets (n-back)
Condition	**$F_{1,375} = 102.71$ $p < 0.001$**	**$F_{1,374} = 14.69$ $p < 0.001$**	**$F_{1,374} = 82.64$ $p < 0.001$**	**$F_{1,375} = 143.64$ $p < 0.001$**	**$F_{1,351} = 6.07$ $p = 0.014$**	**$F_{1,351} = 6.05$ $p = 0.014$**
Time	**$F_{8,375} = 20.37$ $p < 0.001$**	**$F_{8,374} = 7.68$ $p < 0.001$**	**$F_{8,374} = 13.99$ $p < 0.001$**	**$F_{8,375} = 21.23$ $p < 0.001$**	**$F_{8,351} = 3.57$ $p < 0.001$**	$F_{8,351} = 1.73$ $p = 0.090$
Genotype	$F_{1,22} = 0.15$ $p = 0.705$	$F_{1,22} = 0.02$ $p = 0.898$	$F_{1,22} = 0.31$ $p = 0.586$	$F_{1,22} = 0.06$ $p = 0.803$	$F_{1,21} = 0.00$ $p = 0.974$	$F_{1,21} = 0.00$ $p = 0.954$
Condition × Time	**$F_{8,375} = 3.09$ $p = 0.002$**	$F_{8,374} = 1.71$ $p = 0.095$	**$F_{8,374} = 2.41$ $p = 0.015$**	**$F_{8,375} = 6.96$ $p < 0.001$**	$F_{8,351} = 1.39$ $p = 0.198$	$F_{8,351} = 1.20$ $p = 0.295$
Condition × Genotype	$F_{1,375} = 0.08$ $p = 0.773$	$F_{1,374} = 1.97$ $p = 0.161$	$F_{1,374} = 0.04$ $p = 0.843$	$F_{1,375} = 0.26$ $p = 0.614$	**$F_{1,351} = 4.42$ $p = 0.036$**	**$F_{1,351} = 6.54$ $p = 0.011$**
Time × Genotype	$F_{8,375} = 0.80$ $p = 0.604$	$F_{1,374} = 0.54$ $p = 0.823$	$F_{8,374} = 0.57$ $p = 0.802$	$F_{8,375} = 0.57$ $p = 0.799$	$F_{8,351} = 0.72$ $p = 0.671$	$F_{8,351} = 0.57$ $p = 0.799$
Condition × Time × Genotype	$F_{8,375} = 1.07$ $p = 0.385$	**$F_{8,374} = 1.99$ $p = 0.047$**	$F_{8,374} = 1.11$ $p = 0.354$	$F_{8,375} = 0.78$ $p = 0.619$	$F_{8,351} = 1.82$ $p = 0.072$	$F_{8,351} = 1.48$ $p = 0.162$

F-values, degrees of freedom, and *p*-values of a ProcMixed ANOVA. Significant results are printed in bold.

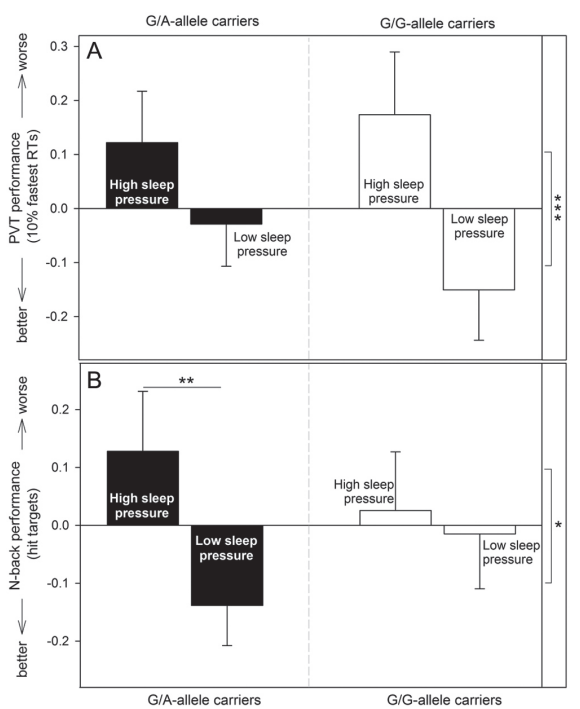

Figure 4. Neurobehavioral performance (means and standard errors of *z*-standardized values) per genotype and condition. (A) Peak performance in vigilant attention, as indicated by the 10% fastest reaction times in the psychomotor vigilance task, differed significantly between conditions. This difference was not, however, modulated by genotype independent of circadian phase. (B) Overall, working memory performance (hit targets, difference between 3-back and 0-back) was worse during high compared to low sleep pressure and was modulated by genotype: G/A-allele carriers performed significantly worse during high compared to low sleep pressure conditions, while performance of G/G-allele carriers did not differ according to condition. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

networks underlying peak performance in a genotype-specific manner.

It has to be emphasized that G/G genotype–dependent attentional performance modulation specifically occurred at the end of the biological night when comparing high with low sleep pressure conditions. The markedness of the typical circadian performance trough at this time of the day has been shown to be dependent on time spent awake (Dijk et al., 1992; Wyatt et al., 1999). In this perspective, our result points toward a differential interaction of circadian and homeostatic influences according to the *ADA* polymorphism. The polymorphism was also associated with differences in circadian phase angle, suggesting that the circadian timing system is differentially modulated in G/A- compared to G/G-allele carriers. With regard to the similar sleep-wake times of the 2 genotype groups, these differences might mirror a shift in circadian phase position reliably estimated from salivary melatonin samples (Benloucif et al., 2008). Circadian phase is influenced by several zeitgebers, such as light (Zeitzer et al., 2000), food (Feillet, 2010), motor activity (Escames et al., 2012), or sleep per se (Danilenko et al., 2003; Wyatt et al., 1999). Importantly, all these influences were kept constant between genotype groups. Therefore they cannot account for the later phase position of G/A-allele carriers, which is reminiscent of the phase delay induced by moderately heightened sleep pressure during partial SD (Lo et al., 2012). With regard to the G/A genotype, it is thus tempting to speculate that circadian factors adapt to a habitually higher level of sleep pressure to ensure consolidated periods of wakefulness of the same quality and length as in G/G-allele carriers. Importantly, we adjusted for genotype-dependent differences in circadian regulation in all neurobehavioral measures so that they do not confound the results in these variables.

The n-back task was originally designed to study working memory performance, even though its construct validity as pure working memory measure has been criticized (Kane et al., 2007; Jaeggi et al., 2010). Successful completion of the task also requires other cognitive abilities, such as sustained attention. The cognitive domains challenged during the 2 tasks investigated in the present study thus are not mutually exclusive, even though they tap mainly into different cognitive domains. The analyzed data indicate

genotype-dependent differences between the 2 tasks regarding the susceptibility to sleep pressure variations: In contrast to what we observed in PVT peak performance, an enhanced reactivity of G/A-allele carriers to manipulations of sleep pressure was measured in n-back performance. The latter pattern of susceptibility to both high and low sleep pressure levels mirrors our findings in subjective sleepiness and well-being, and is in accordance with the suggested pronounced reactivity of G/A-allele carriers to sleep loss (Bachmann et al., 2012). Moreover, it indicates a genotype-dependent beneficial potential of napping in the ability to temporarily store and manipulate information. Indeed, while sleep in general (Kuriyama et al., 2008; Steenari et al., 2003) as well as slow wave sleep in particular (Scullin et al., 2012) have been associated with working memory improvements in accuracy and span, respectively, it remains to be investigated whether genotype-dependent variations in nap sleep modulate the stronger modulation in working memory storage performance of G/A-allele carriers secondary to sleep-wake manipulation.

Importantly, we solely detected genotype-specific performance effects by comparing 2 conditions, in which we experimentally induced high and low homeostatic sleep pressure levels. Indeed, with such a systematic homeostatic state manipulation, our protocol might be more sensitive to unravel mechanisms for neurobehavioral susceptibility to sleep manipulation than using SD protocols only.

Studies comparing cerebral correlates underlying performance in a sleep-deprived state with the ones during rested baseline conditions revealed both task-related blood-oxygen-level dependent activity decreases as well as compensatory mechanisms resulting in activity increases (Chee and Chuah, 2008). The task-specific cognitive domain, task complexity, and interindividual differences in vulnerability to sleep loss have been ranked as factors accounting for the observed discrepancies (Chee and Chuah, 2008). Whether and to what extent the task- and genotype-specific modulations at the behavioral level observed here can be mirrored at the cerebral level remains to be explored.

Our results suggest that a genetically enhanced susceptibility to sleep loss does not become uniformly apparent among cognitive tasks. Such tasks require a differential implication of arousal- and cognition-related brain areas, the successful recruitment of which might depend on the specific individual trait. The data further implicate that the detrimental effects of a trait-like endogenously heightened sleep pressure might be counteracted by nap sleep. Moreover, they underline (Van Dongen et al., 2012) the importance of considering the circadian timing system when assessing interindividual vulnerability to sleep pressure manipulation, entailing a comparison of dependent variables assessed at the same circadian phase. Otherwise sleep loss–related effects on cognitive performance might be underestimated or even ignored.

Limitations of the Study

The individuals participating in our study were young, healthy, and free of any sleep complaints. Together with the small sample size, these strict selection criteria might reduce the generalizability of our study results to the general population. Through this selection process and the highly controlled study routine regarding external influences on circadian and sleep homeostatic processes, however, we aimed to provide an undistorted view of the impact of the *ADA* polymorphism on circadian and homeostatic regulation mechanisms. In real-life situations, this influence might be counteracted on a behavioral level, such that the transfer of the present results to less controlled or noncontrolled conditions might be done cautiously.

Furthermore, while our nap protocol allows the investigation of waking functions under low sleep pressure during the entire circadian cycle, it does not allow for the investigation throughout a complete waking period, which classically covers 16 h of wakefulness. Likewise, compared to the more effortful forced desynchrony paradigm, sleep does not occur in a consolidated fashion during an entire 8-h period but is fragmented during day- and nighttime, such that ultradian processes, for example, cannot take place (Schmidt et al., 2007). Nevertheless, combined with total SD, the applied paradigm appears suitable to gain important insights into the mechanisms governing interindividual modulations in response to homeostatic sleep challenges during the course of the circadian cycle.

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CONFLICT OF INTEREST STATEMENT

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article. The work was supported by the Swiss National Foundation #310030_130689, the L. & Th. La Roche-Stiftung, and the Niklaus und Bertha Burckhardt-Bürgin-Stiftung.

NOTE

Supplementary material for this article is available on the journal's website at http://jbr.sagepub.com/supplemental.

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Supplementary Online Material

Insights into Behavioral Vulnerability to Differential Sleep Pressure and Circadian Phase from a Functional *ADA* Polymorphism

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METHODS

Recruitment

Approximately 610 volunteers, aged between 20 and 35 years, filled in a set of questionnaires inquiring demographic information and evaluating health, sleep quality (Pittsburgh Sleep Quality Index, PSQI, Buysse et al., 1989), chronotype (Munich Chronotype Questionnaire, Roenneberg et al., 2003; and Morningness-eveningness questionnaire, Horne and Östberg, 1976), as well as depressive symptoms (Beck Depression Inventory, BDI-II, Beck et al., 1996). In a next step, approximately 520 healthy caucasian volunteers with good subjective sleep quality (PSQI $\leq$ 5), subjective habitual sleep duration of 8 $\pm$ 1 hour, and no symptoms of clinical depression (BDI-II < 9) were asked to provide saliva for genotyping.

Procedure of Genotyping

Salivary DNA was extracted using the Oragene^{TM} DNA Collection Kit applying the standard procedures (DNA Genotek Inc., Ontario, Canada; http://www.dnagenotek.com/ROW/support/protocols.html). Polymerase Chain Reaction served for amplification of DNA (50 cycles, annealing temperature 61°C) with the primers 5'-GGCGCACGAGGGCACCAT-3' (forward) and 5'-GCTGGGCCCCGCTAAGC-3' (reverse). Genotyping of the SNP rs73598374 was performed by means of the pyrosequencing method on a PyroMark ^{TM} System (Biotage, Uppsala, Sweden) with the primer 5'-CGCTCACTTTGGGCT-3'.

RESULTS

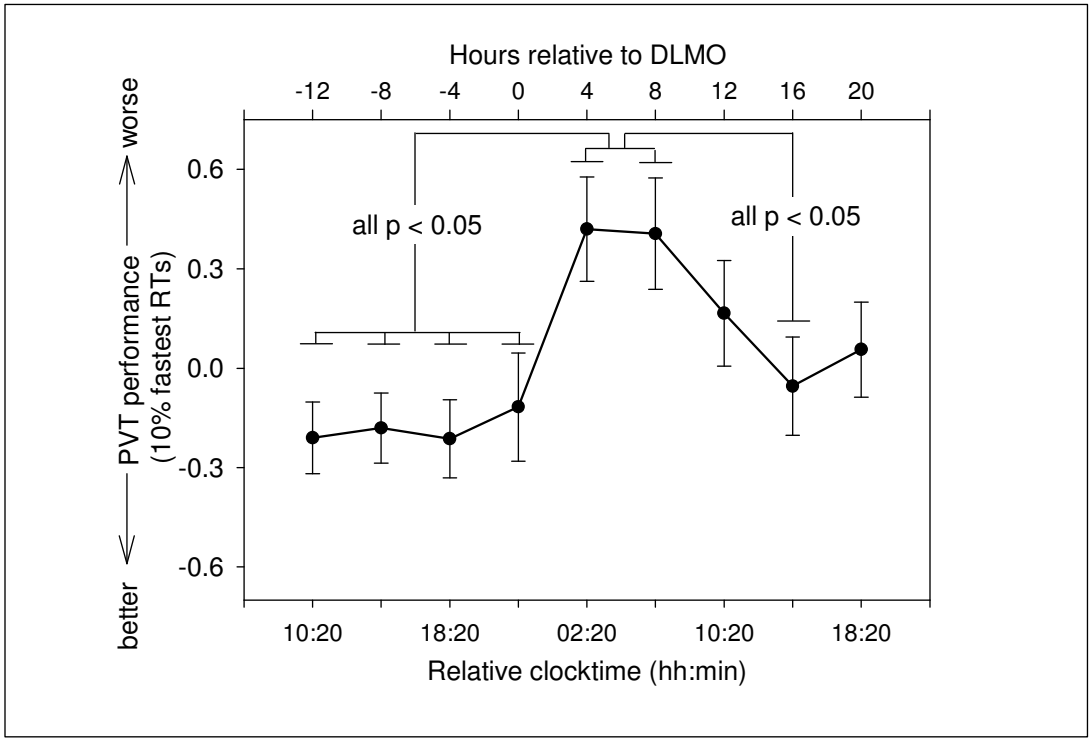
Description of PVT performance

Table S1. PVT performance per condition.

Performance measure	Nap condition	Sleep deprivation
Median RT	-0.208 ± 0.064	0.291 ± 0.069
Fastest 10% RT	-0.089 ± 0.061	0.148 ± 0.075
Slowest 10% RT	-0.2191 ± 0.069	0.3221 ± 0.059
Lapses	-0.322 ± 0.051	0.400 ± 0.076

Means ± standard errors of z-transformed values. PVT = psychomotor vigilance task; RT = reaction time.

Figure S2. PVT performance over time.



Averaged over both conditions and genotypes, PVT peak performance (depicted are means and standard errors of 10% fastest RTs) displayed a clear circadian pattern with worse performance during the night and the morning hours. *P*-values refer to post-hoc comparisons, following a ProcMixed ANOVA for repeated measurements (with the factors Time, Condition and Genotype) and were corrected for multiple comparisons. PVT = psychomotor vigilance task; RT = reaction time.

Description of n-back performance

Table S3. N-back performance per condition.

Performance measure	Nap condition	Sleep deprivation
Hit targets	0.090 ± 0.063	-0.060 ± 0.075
Missed targets	-0.073 ± 0.064	0.072 ± 0.075

Means ± standard errors of z-transformed ratios (difference between 3-back and 0-back).

4.2 Original research paper 2

The Circadian Regulation of Sleep: Impact of a Functional ADA-Polymorphism and Its Association to Working Memory Improvements

Reichert, C. F., Maire, M., Gabel, V., Hofstetter, M., Viola, A. U., Kolodyazhniy, V., Strobel, W., Goetz, T., Bachmann, V., Landolt, H.-P., Cajochen, C. & Schmidt, C. (2014). *PLoS One, 9*(12), e113734. doi: 10.1371/journal.pone.0113734.

PLOS ONE

RESEARCH ARTICLE

The Circadian Regulation of Sleep: Impact of a Functional ADA-Polymorphism and Its Association to Working Memory Improvements

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Abstract

Sleep is regulated in a time-of-day dependent manner and profits working memory. However, the impact of the circadian timing system as well as contributions of specific sleep properties to this beneficial effect remains largely unexplored. Moreover, it is unclear to which extent inter-individual differences in sleep-wake regulation depend on circadian phase and modulate the association between sleep and working memory. Here, sleep electroencephalography (EEG) was recorded during a 40-h multiple nap protocol, and working memory performance was assessed by the n-back task 10 times before and after each scheduled nap sleep episode. Twenty-four participants were genotyped regarding a functional polymorphism in adenosine deaminase (rs73598374, 12 G/A-, 12 G/G-allele carriers), previously associated with differences in sleep-wake regulation. Our results indicate that genotype-driven differences in sleep depend on circadian phase: heterozygous participants were awake longer and slept less at the end of the biological day, while they exhibited longer non rapid eye movement (NREM) sleep and slow wave sleep concomitant with reduced power between 8–16 Hz at the end of the biological night. Slow wave sleep and NREM sleep delta EEG activity covaried positively with overall working memory performance, independent of circadian phase and genotype. Moreover, REM sleep duration benefitted working memory particularly when occurring in the early morning hours and specifically in

heterozygous individuals. Even though based on a small sample size and thus requiring replication, our results suggest genotype-dependent differences in circadian sleep regulation. They further indicate that REM sleep, being under strong circadian control, boosts working memory performance according to genotype in a time-of-day dependent manner. Finally, our data provide first evidence that slow wave sleep and NREM sleep delta activity, majorly regulated by sleep homeostatic mechanisms, is linked to working memory independent of the timing of the sleep episode within the 24-h cycle.

Introduction

The quantity and quality of sleep majorly depends on its timing. During the biological night (i.e., during phases of melatonin secretion), the human circadian pacemaker facilitates sleep initiation and preservation, while it actively promotes wakefulness during the biological day [1, 2]. Circadian wake promotion is paradoxically strongest at the end of a biological day [1], allowing the achievement of a consolidated wake period, despite homeostatic sleep pressure levels accumulating towards the end of the day [3]. In comparison, maximal circadian sleep propensity is observed in the early morning hours in order to prevent early awakenings, when sleep pressure has mostly dissipated during night-time sleep [4]. The combined action of circadian and sleep homeostatic mechanisms consequently allows the maintenance of sleep and wakefulness at appropriate times of the day [1, 3, 5].

Specific sleep features are differentially influenced by circadian and homeostatic mechanisms. For instance, while rapid eye movement (REM) sleep is strongly modulated by circadian phase [6], electroencephalographic (EEG) frequencies in the delta range are rather independent of time of day, but predominantly modulated by prior sleep time, mirroring sleep homeostatic processes [2]. Additionally, the overall regulation of the sleep-wake cycle by circadian and homeostatic factors exhibits large and stable inter-individual differences, which can partially be traced back to genetic variations such as the c.22G>A polymorphism (rs73598374) located in the gene encoding adenosine deaminase (ADA; [7, 8]). This polymorphism acts on sleep-wake regulation most likely through genotype-specific differences in the ADA-dependent metabolization of adenosine [9–13], which is involved in the regulation of sleep homeostasis [14]. Carriers of the G/A-allele, associated to a lower enzymatic activity of ADA [10, 13], show a higher homeostatic non rapid eye movement (NREM) sleep pressure, as indicated by higher night-time EEG activity in the slow wave and delta range, longer slow wave sleep (SWS) duration, and higher sleep efficiency [9, 12, 15, 16]. However, circadian contributions to the genotype-specific patterns in sleep structure and intensity remain unclear. Interestingly, we recently gathered first evidence that the circadian timing system varies according to the *ADA*

polymorphism, since G/A-allele carriers exhibited a later onset of melatonin secretion [17], mirroring a shift in the opening of the gate for sleep [18].

Importantly, the dynamic interaction between homeostatic and circadian factors impacts not only on the timing of sleep and wakefulness, but also modulates a range of cognitive functions, among them working memory (WM) performance [19, 20]. The concept of WM refers to the temporary storage and manipulation of information. Previous investigations suggest improvements of executive aspects of WM performance, for instance in monitoring and manipulation of information held online, by training [21] as well as positive effects of night-time sleep [22, 23]. Moreover, we recently observed increased WM performance during a multiple nap compared to sleep deprivation protocol, specifically driven by heterozygous carriers of the *ADA* polymorphism [17]. However, it is unknown whether inter-individual differences in sleep-wake regulation can modulate the beneficial effect of sleep on WM and which specific sleep features contribute to sleep-dependent performance improvements. Also, it is unclear whether sleep-dependent benefits on WM depend on time of day, such that the advantageous effects occur only or most pronounced when sleep is expressed at a specific circadian phase, as shown for sequence learning and simple addition tasks [24, 25].

In the present investigation, a 40-h multiple nap protocol, similarly applied in prior studies (e.g., [26–28]), served to investigate circadian contributions under low sleep pressure levels to human sleep and waking functions with respect to the *ADA* polymorphism. We recently published data on behavioural effects of this genetic variation in response to different sleep pressure conditions (40-h sleep deprivation vs. the here reported 40 h of multiple napping). Working memory performance of G/A-allele carriers was more affected by sleep pressure manipulation than performance of G/G allele carriers. Here, we focus on the nap sleep protocol to investigate if characteristics over the circadian cycle are also differentially modulated by the *ADA* polymorphism, and whether they potentially associate to the reported genotype-dependent sensitivity to sleep pressure manipulation in working memory performance [17]. Concretely, we examined first if nap sleep, regularly scheduled along the circadian cycle, differs between G/A- and G/G-allele carriers under conditions of low sleep pressure. Sleep homeostatic and circadian mechanisms are inevitably linked such that a change of the state or dynamics on the one side entails a difference in the regulation in the other process (e.g., [19, 20, 29]). Considering the previously shown differences between genotypes in mainly homeostatic sleep features during night-time, we explored whether the circadian sleep-wake regulation might have adapted to these trait-like variations according to the *ADA* polymorphism. As circadian wake and sleep promotion is maximal at the end of the day and night, respectively, we assumed genotype-specific differences most likely to be detected during these crucial times of day. In a next step, we aimed at investigating the influence of nap sleep on WM performance, which was assessed before and after each of the scheduled naps. We explored which specific nap sleep properties act on WM performance and whether this is differentially expressed according to time of day

and genotype. Based on prior evidence of a circadian modulation in the beneficial effect of sleep on cognition [24, 25], we hypothesized that sleep will boost WM performance in a time-of-day dependent manner, especially in case of sleep features being under strong circadian control (e.g., REM sleep duration).

Our data provide first evidence for a more distinct circadian modulation of nap sleep in G/A- compared to G/G-allele carriers. Further, WM performance benefits from REM sleep duration, observed particularly in the early morning during its circadian peak time, were more pronounced in heterozygous compared to homozygous individuals. In comparison, independent of time of day and genotype, WM performance improvements were positively associated to the amount of NREM delta power, a sleep feature mainly under sleep homeostatic control.

Materials and Methods

1.1 Participants

As described earlier [17], 24 healthy young participants (12 G/A- and 12 G/G-allele carriers) out of 610 genotyped volunteers were willing to take part in the study. All participants were between 20 and 35 years old, healthy, non-smokers and free from depressive symptoms (Beck Depression Inventory [30], BDI-II <9). Exclusion criteria comprised transmeridian flights within three months before participation in the study, shift work, drug consumption or current medication (except contraceptives) and a history of prior psychiatric or sleep disorders. All participants slept habitually 8 ± 1 h, stated a good subjective sleep quality (Pittsburgh Sleep Quality Index [31], PSQI$\leq$5, see Table 1 for *M* and *SD* per genotype) and were medically screened by a physician before inclusion into the study. A screening night served to exclude sleep disorders and to habituate participants to the laboratory conditions. All women were tested for pregnancy before the laboratory part of the study and were required to participate during the luteal phase of their menstrual cycle (2 G/A- and 1 G/G-allele carriers) unless they were taking hormonal contraceptives. The genotype groups did not differ according to age, body mass index, subjective sleep quality, daytime sleepiness, chronotype and timing of sleep before and during study participation ($p_{all}>.10$; for *M* and *SD* see Table 1).

1.2 Genotyping

The procedure of genotyping has been described in detail in Reichert et al. (2014) [17].

1.3 Protocol and Procedure

The study was approved by the local ethics committee (Ethikkommission beider Basel) and performed according to the declaration of Helsinki. All participants gave written informed consent prior to study admission.

Table 1. Demographic data and questionnaire scores (*M* and *SD*) split by genotype.

Sample characteristics	G/A-allele carriers	G/G-allele carriers	*p*
N (f, m)	12 (8, 4)	12 (8, 4)	1.00
Age (y)	24.33 (3.9)	24.75 (2.5)	.76
BMI (kg/m2)	21.80 (2.9)	21.60 (2.0)	.79
PSQI	3.58 (1.2)	2.83 (1.1)	.12
ESS	4.46 (2.8)	4.29 (2.0)	.87
MEQ	54.80 (9.7)	57.60 (10.8)	.51
MCTQ Sleep Duration	7.92 (0.6)	7.87 (0.7)	.82
MCTQ MSF sc	4.34 (1.1)	4.26 (1.0)	.84
MCTQ MSF sac	7.29 (2.4)	7.62 (2.7)	.75
Wake Time (hh:min) during study	07:08 (57 min)	07:13 (57 min)	.83

Notes. F= female; m= male; y= years; BMI= Body Mass Index, PSQI=Pittsburgh Sleep Quality Index, ESS= Epworth Sleepiness Scale, MEQ= Morningness-Eveningness Questionnaire, MCTQ= Munich Chronotype Questionnaire, MSF sc= Mid sleep free days sleep corrected, MSF sac= Mid sleep free days sleep and age corrected. *P*-values were derived from χ2-(gender ratio) and t-tests (all other variables).

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Before the laboratory part started, participants were asked to maintain a fixed sleep-wake cycle for one week (8 h $\pm$ 30 min time in bed during night-time, no naps allowed) in order to control for circadian misalignment and accumulation of sleep pressure during the week. Sleep-wake times were derived from a 3-week actimetry field study and if required, adapted to the participants' professional obligations. Actigraphical recordings served to verify compliance to the regimen. Furthermore, participants were instructed to abstain from alcohol and caffeine during this week in order to control for effects of these substances on sleep and waking functions [32–34].

As reported previously [17], we implemented a randomized controlled within-subject design with two conditions, a nap and a sleep deprivation condition. Here, we mainly focus on data collected during the nap condition. The nap condition started with an 8-h baseline sleep episode. Following sleep, 120 min after regular wake-time, a repetitive short-day-cycle protocol started, with each cycle consisting of 160 min of wakefulness alternating with 80-min naps. After 40 h (encompassing 10 cycles), at regular bed time, the laboratory part ended with an 8-h recovery night (Figure 1). During wakefulness light was kept below 8 lux and body posture was semi-recumbent except for regularly scheduled bathroom visits. Meals were standardized and administered every 4 h (with a *SD* of 14 min). No indications of time of day were given. Social interaction was restricted to communication with study assistants. During scheduled sleep (at 0 lux), participants were asked to sleep if possible or to wait otherwise in darkness and recumbent position until the scheduled sleep episode has passed.

Beside the nap condition, a 40-h sleep deprivation was implemented in a randomized controlled order, separated by minimum 7 days from the nap condition. The sleep deprivation protocol was equal to the nap condition, except that no naps were scheduled [17]. Data of the sleep deprivation condition will be reported at the level of WM accuracy in order to verify that performance

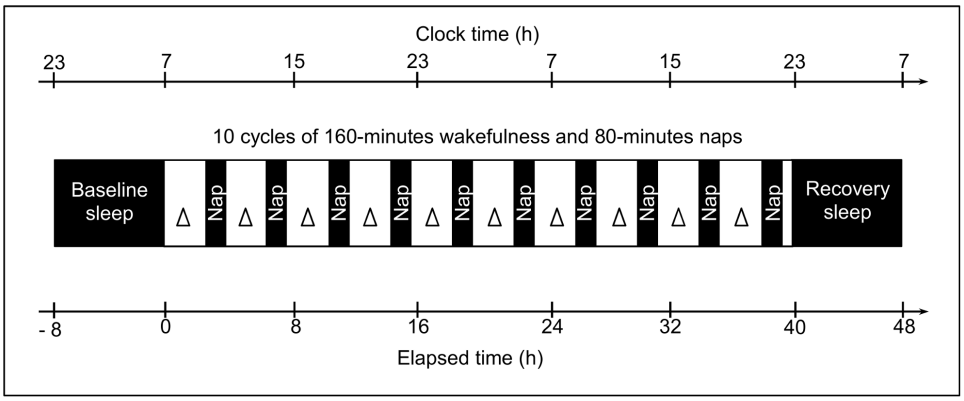

Figure 1. Schematic illustration of the laboratory part of the study. Following a 8-h baseline night, ten short sleep-wake cycles were scheduled over 40 h, each consisting of 160 min of wakefulness (white) under dim-light (<8 lux) and a 80-minutes nap (black bars, 0 lux). N-Back performance was assessed every 4 h (triangles) together with subjective effort, starting 1 h after waking up from the baseline night.

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improvements occur specifically when participants were allowed to nap and do not solely reflect overall practice effects occurring with task repetition.

1.4 Melatonin

In order to determine circadian phase separately for each participant, salivary melatonin was collected with an average sampling rate of 60 min and analysed as previously reported [17]. Here, we focused on group comparisons of the dim-light melatonin onset (DLMO) and phase angle during the nap protocol. For definitions of DLMO and phase angle please see Reichert et al. (2014).

1.5 Nap Sleep

During the laboratory part of the study, polysomnographic signals (F3, FZ, F4, C3, CZ, C4, PZ, O1, Oz and O2 EEG derivations, two electrooculographic, two electromyographic and two electrocardiographic derivations) were recorded continuously with sintered MRI compatible Ag/AgCl ring electrodes with a 15 kOhm resistor (EasyCap GmbH, Germany) and V-Amp digital sleep recorders (Brain Products GmbH, Germany). All signals were sampled at 500 Hz and filtered online by applying a notch filter (50 Hz). Visual scoring of sleep stages was facilitated by filtering out frequencies below 0.1 Hz (high pass) and above 20 Hz (low pass) offline. Scoring of nap sleep was done according to standard criteria [35] by experienced staff blind to the genotype of the corresponding participant. Each file was scored by one scorer, and the number of files analysed by one scorer was balanced according to the genotype. Sleep latencies to stage 1, stage 2, and REM sleep were defined as time elapsed until the first occurrence of a respective epoch and analysed separately. All sleep latencies were log transformed before statistical analysis to achieve normal distribution. Slow wave sleep (SWS) was

considered as sum of sleep stages 3 and 4, non rapid eye movement (NREM) sleep as sum of stages 2, 3, and 4. Sleep efficiency was calculated as percentage of total sleep time (TST, sum of sleep stages 1, 2, SWS and REM sleep) per nap. The timing of nap sleep was adjusted according to the DLMO of each participant and pooled to 4 h bins.

For consecutive 20-sec epochs, EEG power was calculated for artefact-free 4 sec epochs and averaged, using a fast Fourier transform with Hamming window. The resulting 0.25-Hz frequency resolution was analysed between 0.5 and 32 Hz. Here, we report EEG power spectra during NREM sleep (sleep stage 2, 3 and 4). A mixed model ANOVA with the factors genotype (G/A- and G/G-allele carriers), time (10 bins of 4 h) and hemisphere (left vs. right side derivations) did not reveal any significant interaction between genotype and hemisphere ($p_{all}>0.45$). Thus, EEG spectra were collapsed along the anterior-posterior axis resulting in one value for each of the frontal, central, parietal, and occipital sites. In order to investigate the time course of sleep and wakefulness within the naps, specifically the distribution of SWS as an indicator for the dynamics of sleep pressure, nap-sleep was analysed per 20-min interval in each sleep episode. We particularly focused on the time course of sleep characteristics within the naps scheduled in the late evening (from 9:00 to 10:20 p.m. on average) and early morning (from 5:00 to 6:20 a.m. on average), encompassing maximal circadian drive for wakefulness and sleep respectively. As an indicator for sleep structure during the so-called wake-maintenance zone [5] in the late evening, a mean was computed of the two naps scheduled to 14 h and 38 h after regular wake-up time [26, 36]. This mean was compared with the nap starting 22 h after usual waking time in the early morning, that is, when the circadian sleep tendency is supposed to be strongest [1, 4].

1.6 N-back Task

Starting 60 min after waking up, participants were asked to perform a visual verbal n-back task, which was repeatedly administered every 4 h (i.e., 1 h before each nap), every other session in a magnetic resonance imaging scanner. The task lasted approximately 20 min and consisted of the visual presentation of 14 blocks of 30 consonants each (1.5 sec presentation time for each consonant, 0.5 sec inter-stimulus interval) on a computer screen. The volunteer's challenge in the n-back task is to decide and to indicate by a button press whether the letter presented is the same as n trials before. During each session subjects performed 9 blocks of 3-back and 5 blocks of 0-back-tests presented in a randomized order, each separated by a pause with a randomly generated duration of 10–20 sec during which a fixation cross was displayed on the screen. The order of the consonants per block and the number of targets per block was fixed (10 targets). The same block was not repeated within a session and appeared maximally 2 times over the course of the study, separated with at least 20 h in between. Performance was calculated by subtracting false alarms from hit targets (hit targets – false alarms) in order to measure the accuracy of the responses [37].

The n-back has been shown to be a useful measure specifically of executive aspects of WM as it requires permanent updating and manipulation of information [38, 39]. Other processes, such as inhibitory control, familiarity- and recognition-based discrimination and attentional processes are implicated in performance as well [40, 41]. In the 0-back, participants were asked to react to a specific consonant with a button press, such that they were not required to keep and manipulate information in WM, but still need to decide and to react correctly to the target stimulus. Following the methodology of subtraction [38], we report difference values (3-back – 0-back) of accuracy in order to account for basic attentional resources and inhibitory control, referring to this measure as WM accuracy. WM accuracy was adjusted according to the DLMO of each participant and collapsed into 4 h bins. For quantification of improvements from before to after sleep, difference values were calculated by subtracting WM accuracy values assessed before a nap from those acquired after the nap (after-before).

In the evening before the study, participants were trained in n-back-performance until they reached 70% of correct responses in the 3-back version of the task in order to prevent effects due to baseline differences in comprehension and transfer of instructions. Nonetheless, one heterozygous participant performed 3 interquartile ranges below the 25th percentile during the entire course of the protocol. This performance was considered as an extreme value [42] and excluded from all analyses. Additionally, when quantifying performance changes from before to after a sleep opportunity in the late morning hours, the value of a homozygous participant was located 2 interquartile ranges above the 75th percentile, and thus excluded as an extreme value for the respective analysis, too [42].

1.7 Subjective Effort

After each test bout, consisting of the n-back task followed by a 10-min vigilance test (modified version of the psychomotor vigilance task [43]), subjective effort was assessed by means of visual analogue scales. Participants were asked to indicate on three separate scales ranging each from 0 (*little*) to 100 (*much*) how much they had to endeavor and to concentrate during task performance, as well as to what extent the tasks were tiresome. Means calculated over these scales were adjusted to DLMO. In order to quantify changes in subjective effort from before to after sleep, values assessed before a nap were subtracted from those acquired after the nap (after-before).

1.8 Statistics

Statistical analyses were performed with SAS 9.3 software (SAS Institute, Cary, USA) using t-tests and mixed-model analyses of variance for repeated measures. T-tests for independent groups were used to evaluate differences between genotypes in the timing of melatonin. The general mixed model for analysis of sleep structure included the factor ''genotype'' (G/A-genotype and G/G-

genotype), ''time'' (10 bins of 4 h), and ''interval'' (4 intervals of 20 min within each nap). The factor interval was not included for sleep latency analyses. For analysis of EEG power during NREM sleep, the mixed model for repeated measurements included the factors ''genotype'' (G/A-genotype and G/G-genotype), ''time'' (10 bins of 4 h) and ''derivation'' (frontal, central, parietal and occipital derivations). We did not include a factor ''interval'' due to a frequent lack of NREM sleep within the first two intervals of a nap (no NREM sleep at the beginning of a sleep episode due to wakefulness [50%] or REM sleep [5%]). If analysis of mean values in the delta (0.5–5 Hz), theta (5–8 Hz), alpha (8–12 Hz), sigma (12–16 Hz), beta (16–25 Hz) and gamma range (25–32 Hz) disclosed significant results, each frequency bin of the regarding frequency range was afterwards investigated separately. Analyses of WM accuracy and subjective effort included the factors ''genotype'' (G/A-genotype and G/G-genotype) and ''time'' (10 bins of 4 h). Contrasts of all mixed model analysis were calculated with the LSMEANS statement. Degrees of freedom of *p*-values are based on an approximation described by Kenward and Roger [44], and multiple post hoc comparisons were adjusted according to the Tukey-Kramer method [45]. *P*-values reported are adjusted for multiple testing.

The statistical software package SPSS 19.0.0 (IBM Corp., Armonk, USA) was used for analyses of covariance (ANCOVAs) to investigate both the influence of sleep features per se (SWS, REM sleep, SL1, NREM EEG delta, alpha and sigma activity) as well as the impact of these sleep features according to genotype (interaction genotype x sleep) on changes in WM accuracy within one statistical model. The difference in WM accuracy (3-back-0-back) between the first and the last test session in the study was considered as a global performance improvement index, independent of time of day. Genotype was considered as independent variable, sleep stages and intensities as covariates. Additionally, subjective effort to perform the task was included as a covariate into the model, since a recent study indicates that subjective effort influences n-back performance after sleep manipulation [46].

Results

2.1 Melatonin

Mean values of DLMO and phase-angle (DLMO G/A-allele carriers 10:28 p.m.; DLMO G/G-allele carriers 09:43 p.m.; phase-angle G/A-allele carriers: 15 h and 20 min; phase-angle G/G-allele carriers: 14 h and 30 min) did not differ significantly between genotypes (DLMO: $t_{[11]}=1.76$; $p=0.09$; phase-angle: $t_{[11]}=1.89$; $p=0.07$), but yielded trend levels. Thus, as mentioned above, the timing of all repeated measurements was adjusted individually according to the DLMO of each participant.

2.2 Nap Sleep: Visual Scorings

Results revealed that the proportion of wakefulness and of sleep stages per 80-min sleep opportunity varied as a function of the 24-h cycle (for wakefulness, stage 1, stage 2, SWS, REM sleep, NREM sleep, TST, sleep efficiency and movements $Fs_{[9,>844]}>2.0$, $p_{all}<0.05$; sleep latency to stage 1, to stage 2 and to REM sleep $Fs_{[9,>195]}>19.5$, $p_{all}<0.001$), exemplarily depicted for sleep efficiency in Figure 2A. Further, the occurrence of wakefulness and sleep stages depended significantly on time elapsed within a nap (interval) such that wakefulness and sleep stage 1 occurred more likely at the beginning, while deeper sleep stages, movements and REM sleep were more likely at the end of a nap ($Fs_{[3,842]}>5.6$, $p_{all}<0.001$). This pattern was modulated by circadian phase: Considering the first half of a nap, the duration of wakefulness increased over the course of the biological day and comparably dropped as soon as passing into the biological night ($F_{[27,842]}=2.4$, $p<0.01$), while stage 1 showed a reverse pattern ($F_{[27,842]}=3.8$, $p<0.001$). The increase of deeper sleep stages towards the end of the nap was most pronounced in the first half of the biological night ($Fs_{[27,842]}>2.5$, $p_{all}<0.001$), while REM sleep increased especially in the morning of the first experimental day (i.e., the day following baseline sleep) and during night-time ($F_{[27,842]}>3.0$, $p<0.001$). Across all nap opportunities, genotype did not significantly impact on visual sleep scorings ($p_{all}>0.51$ except for REM sleep $F_{[1,22.3]}=3.5$, $p=0.08$). However, a significant interaction between genotype and circadian phase in sleep stage 1 ($F_{[9,843]}=3.5$; $p<0.001$) indicated that G/A-allele carriers showed a shorter duration of stage 1 sleep during the nap in the late evening (from 9:00 to 10:20 p.m.) close to the DLMO compared to G/G-homozygotes ($p=0.02$; Figure 2B).

In a next analysis (see methods), we aimed at contrasting sleep structure assessed exclusively during maximal circadian drive for wakefulness and sleep respectively, that is during naps scheduled to the late evening (from 9:00 to 10:20 p.m.) and early morning hours (from 5:00 to 6:20 a.m.), respectively [1]. As expected, participants spent more time awake, initiated sleep later and slept correspondingly less during naps in the late evening compared to the nap in the early morning (for wakefulness, stage 1, stage 2, SWS, REM sleep, NREM sleep, TST, movements and sleep efficiency, $Fs_{[1,154]}>6.3$, $p_{all}<0.05$; for sleep latency to stage1, to stage 2 and to REM sleep $Fs_{[1,22]}>45.7$, $p_{all}<0.001$). Overall sleep occurred generally more likely at the end of a nap and was modulated by circadian phase, such that sleep appeared later within naps in the late evening compared to the early morning (for wakefulness, stage 2, SWS, REM sleep, NREM sleep, TST movements and sleep efficiency effects of interval $Fs_{[3,154]}>3.8$, $p_{all}<0.05$; for wakefulness, stage1, stage 2, SWS, REM sleep, NREM sleep, TST and sleep efficiency all effects time $\times$ interval $Fs_{[3,154]}>3.8$, $p_{all}<0.05$). Importantly, as depicted in Figure 2B and 2C, genotype-dependent influences on sleep structure were modulated by circadian phase (wakefulness $F_{[1,154]}=5.5$; $p=0.02$; stage 1 $F_{[1,154]}=10.8$; $p=0.001$; TST $F_{[1,154]}=5.6$; $p=0.02$; sleep efficiency $F_{[1,154]}=5.6$; $p=0.02$): In the late evening G/A-allele carriers spent more time awake ($p=0.04$)

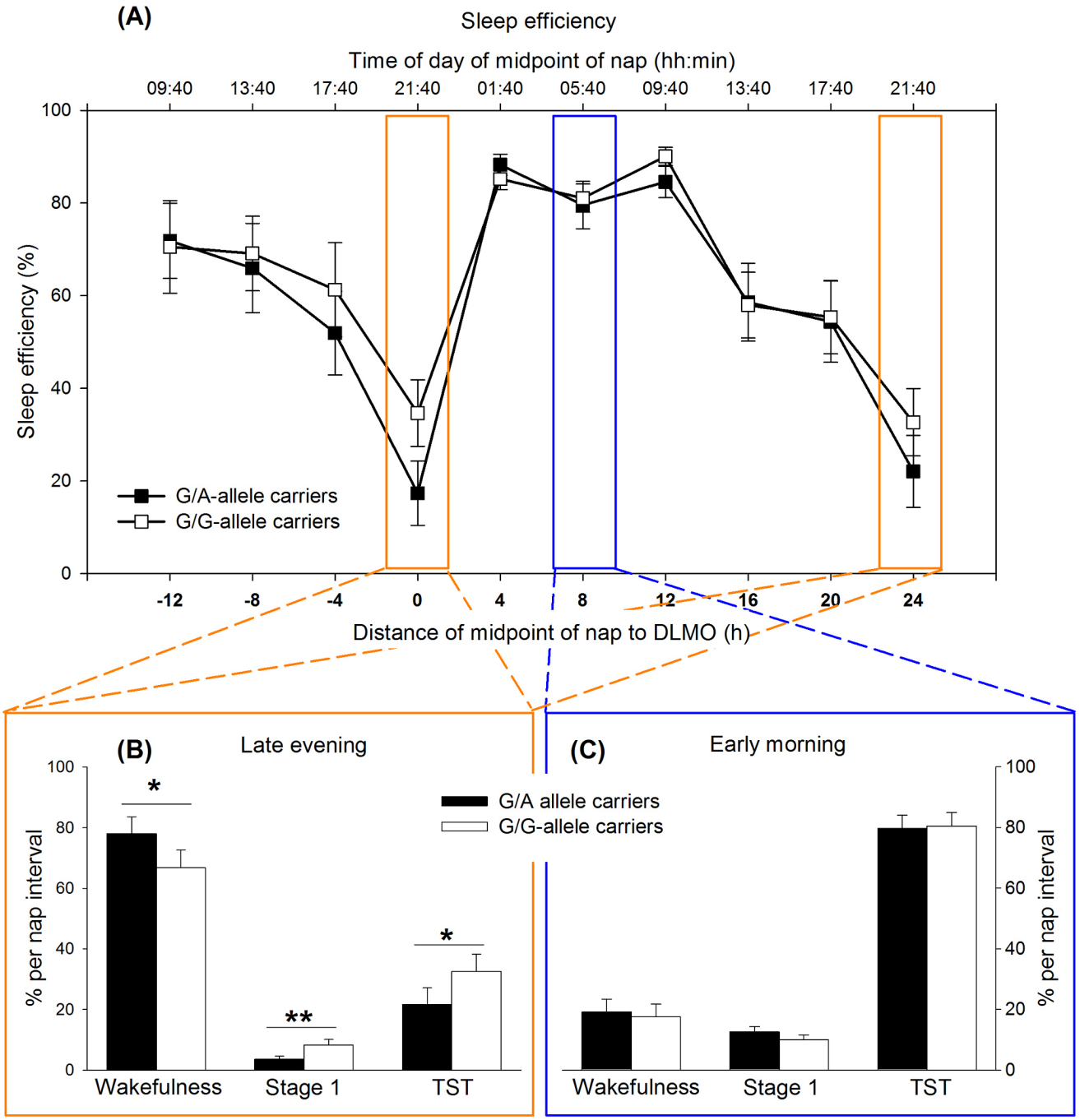

Figure 2. Sleep and wakefulness along the circadian cycle according to genotype. (A) Sleep efficiency was calculated by (sum of stage 1, 2, SWS and REM sleep)/(nap duration)*100. Sleep efficiency of G/A-allele carriers (black squares) and G/G-allele carriers (white squares) displayed a similar circadian pattern with low values in the late evening hours and high values during the biological night. (B) In the late evening hours, during highest circadian wake drive, the duration of wakefulness, stage 1 and total sleep time (TST) was modulated by genotype, while (C) genotypes did not differ in these variables during highest circadian sleep promotion (early morning). * $p<0.05$; ** $p<0.01$.

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and slept less ($p_{all}<0.05$) compared to participants with the G/G-genotype. This was by trend mirrored in less NREM sleep of G/A- compared to G/G-allele carriers at the end of the nap in the late evening ($p=0.06$), while the duration of NREM sleep ($p=0.03$) and in particular SWS ($p=0.001$) was longer in G/A- compared to G/G-allele carriers at the end of the nap in the early morning (interactions genotype × time × interval: NREM sleep: $F_{[3,154]}=2.7$; $p<0.05$; SWS $F_{[3,154]}=3.1$; $p=0.03$).

2.3 Nap Sleep: Spectral Analysis

The well-known circadian phase and derivation-dependent modulations in EEG activity were evident over the entire power spectrum. The variations of EEG power along the circadian cycle are illustrated in Figure 3 as deviations from the mean over time per genotype. As depicted, a genotype-dependent impact on specific frequency bands became evident according to circadian phase (Table 2). Spectral EEG power in the delta range of G/G allele carriers (specifically between 0.5–2.5 Hz) dropped significantly ($p=0.003$) from the early (5:00 to 6:20 p.m.) to the late evening hours (9:00 to 10:20 p.m.), and increased again ($p<0.0001$) when passing into the biological night (nap scheduled to 1:00 to 2:20 a.m.). This pattern was not present in G/A-allele carriers. Furthermore, EEG delta power (particularly in the range of 1.25–2.5 Hz) increased significantly ($p=0.01$) from the early (5:00 to 6:20 p.m.) to the late morning (9:00 to 10:20 a.m.) in G/A-, but not in G/G-allele carriers ($p=0.99$).

Dependent on circadian phase, genotype groups differed as well with regard to alpha power (Table 2, Figure 3). Only G/A-allele carriers showed a decrease in activity ($p=0.002$), specifically between 8.5 and 12 Hz, in the early morning hours (assessed between 5:00 and 6:20 a.m.), which recovered afterwards in the late morning (9:00 to 10:20 a.m.; $p=0.0006$).

Similarly, the influence of genotype on EEG power in the sigma range was modulated by circadian phase (Table 2; particularly between 12–12.75 Hz and 13.25–14.75 Hz) with a G/A-genotype-specific decline in the early morning (12–12.75; 13.25–13.75 Hz; 5:00 to 6:20 a.m.), followed by an increase during the late morning (12–12.25 Hz, 14.5–14.75 Hz; 9:00 to 10:20 a.m.). Additionally, analysis disclosed that the genotype-specific influence in the sigma power range (particularly between 11.75–16.5 Hz) differed according to derivation. However, post hoc comparisons did not reach significance after correction for multiple comparisons.

The EEG theta, beta and gamma activity did not significantly vary according to genotype ($p_{all}> 0.14$).

2.4 N-back Performance and Subjective Effort

Figure 4 depicts the genotype-specific time courses of n-back accuracy throughout the 40-h nap protocol separately for 3- (Figure 4A) and 0-back (Figure 4C) in order to illustrate the evolution of accuracy under high compared

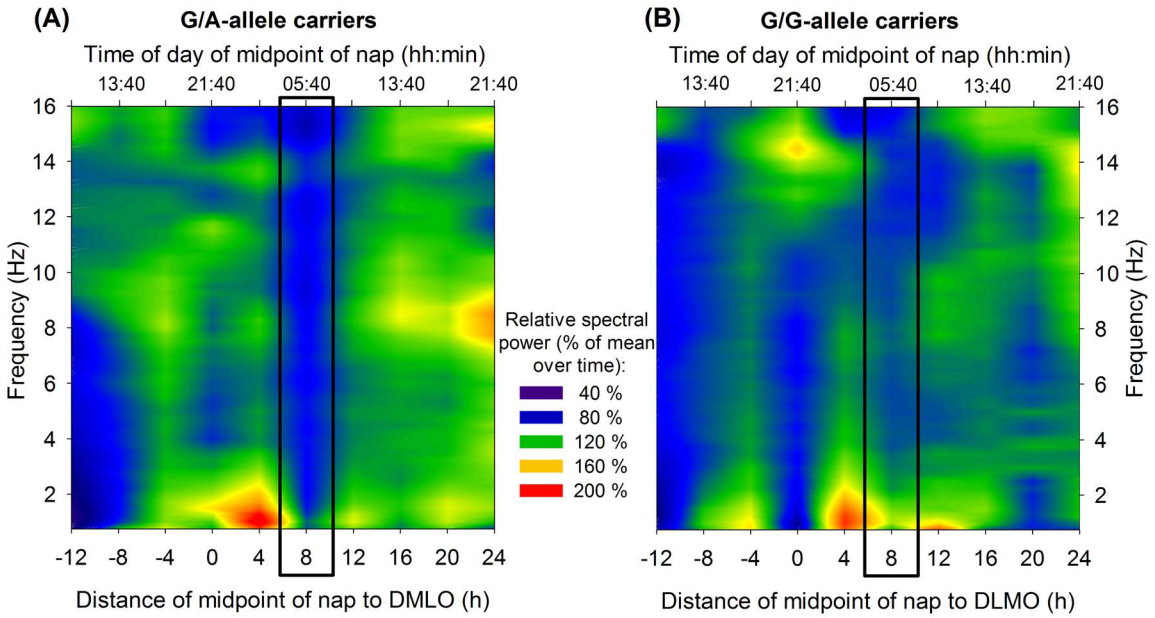

Figure 3. Relative EEG power density per genotype. Relative EEG power density is depicted as deviation from mean over time (i.e., over all naps) per genotype. Blue colours mirror relative decreases in EEG power density compared to the mean over time; green, yellow and red colours indicate relative increases of EEG power density compared to the mean over time. During the early morning hours (i.e., during the nap scheduled to 5:20–6:00 a.m.), highlighted by black boxes, G/A-allele carriers showed a relative decrease specifically in the range of 8–16 Hz (A), which was not present in G/G-allele carriers (B).

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to minimum working memory load. As mentioned (see methods), we calculated a difference ratio (3-back-0-back) of the depicted accuracy to account for variations in basic attentional resources and refer to this ratio as WM accuracy. WM accuracy values improved over time ($F_{[9,183]}=10.14$; $p<0.0001$) similarly in both genotypes (genotype × time: $F_{[9,183]}=1.75$; $p=0.08$; post-hoc tests $p_{all}>0.6$ after corrections for multiple comparisons): Participants performed significantly better during the last compared to the first session ($p<0.0001$). Importantly, such an increase in WM accuracy was not observed during 40 h of constant wakefulness

Table 2. Effects of genotype, time and derivation on delta, alpha, and sigma power.

Effect	Delta power	Alpha power	Sigma power
Genotype	F[1,22.3]=0.2 p=0.70	F[1,22.1]=0.2 p=0.72	F[1,22.1]=2.5 p=0.13
Time	**F[9,704]=25.4 p<0.0001**	**F[9,703]=8.1 p<0.0001**	**F[9,703]=6.3 p<0.0001**
Derivation	**F[3,702]=198.8 p<0.0001**	**F[3,702]=120.8 p<0.0001**	**F[3,702]=327.0 p<0.0001**
Genotype × time	**F[9,704]=2.1 p=0.03**	**F[9,703]=2.4 p=0.01**	**F[9,703]=2.3 p=0.01**
Genotype × derivation	F[3,702]=0.6 p=0.64	F[3,702]=0.3 p=0.88	**F[3,702]=10.3 p<0.0001**
Time × derivation	F[27,702]=0.4 p=1.0	F[27,702]=1.0 p=0.45	F[27,702]=0.4 p=1.0
Genotype × time × derivation	F[27,702]=0.1 p=1.0	F[27,702]=0.2 p=1.0	F[27,702]=0.1 p=1.0

Notes. Delta range: 0.5–5 Hz; alpha range: 8–12 Hz; sigma range: 12–16 Hz. F-values, degrees of freedom and p-values are derived from a ProcMixed ANOVA. Significant effects are printed in bold.

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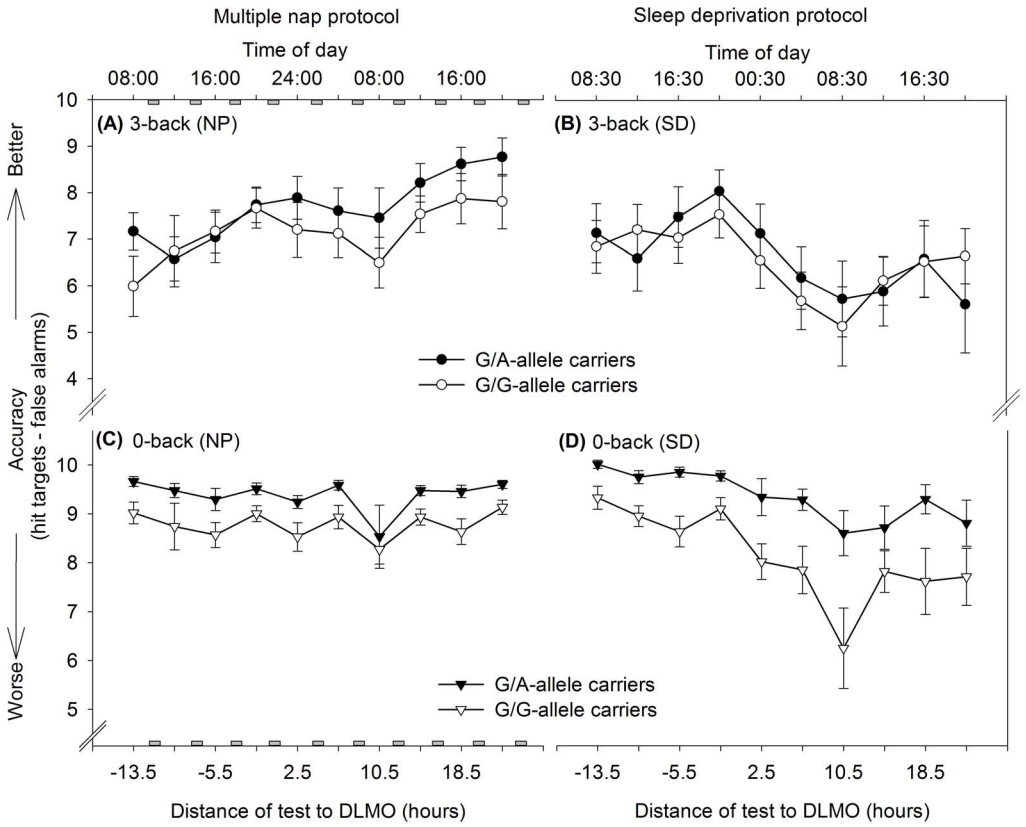

Figure 4. Accuracy patterns over time according to sleep pressure condition and genotype, separately for 3-back (upper panels) and 0-back (lower panels). Accuracy was calculated by a difference ratio (hit targets – false alarms). Grey rectangles indicate scheduled nap sleep episodes. In the 3-back task, accuracy improved from the first to the last test in the nap condition (NP, [A], $F[9,183]=11.66$, $p<0.0001$; post hoc $p<0.0001$), while the first and the last test did not significantly differ during sleep deprivation (SD, [B], $F[9,184]=8.84$, $p<0.0001$, post hoc $p>0.1$). When working memory load was set to a minimum in the 0-back task (lower panels), accuracy remained stable from the first to the last test in the nap condition ([C], $F[9,183]=3.65$, $p=0.0003$; post hoc $p>0.1$), but decreased significantly during sleep deprivation ([D], $F[9,183]=3.65$, $p=0.0003$; post hoc $p=0.01$). G/A-allele carriers performed constantly at a higher level in the 0-back version compared to G/G-allele carriers ([C], $F[1, 21]=8.17$, $p=0.009$), indicating differences in basic attentional resources between genotypes during the nap condition.

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neither under high (3-back, Figure 4B) nor minimum working memory load (0-back, Figure 4B). This result indicates that improvements in WM accuracy from the first to the last session were dependent on the reduction of sleep pressure by nap sleep and do not simply reflect general practice effects due to repetitive task administration. Sleep-dependent consolidation processes of working-memory related skills might associate to the benefits in WM accuracy observed after multiple napping.

Subjective effort brought up during task completion can influence n-back performance after sleep manipulation [46]. Thus, we investigated this measure in parallel to working memory accuracy. Subjective effort changed over time ($F_{[9,186]}=6.05$; $p<0.0001$) exhibiting a circadian pattern: Participants perceived performance as less effortful during tasks scheduled in the evening (at 8:00 p.m.) of the first day compared to the tests scheduled before ($p_{all}<0.05$). Afterwards subjective effort increased ($p_{all}<0.001$) and stayed stable during the biological

night. Starting around lunch time (12:00 a.m.) of the second day, participants indicated again task performance as less exhausting ($p_{all}<0.05$). No genotype-dependent modulation was observed for this measure.

2.5 Relation between Nap Sleep, N-back Performance and Subjective Effort

In a final step, we explored whether the observed nap sleep-dependent improvements in WM accuracy from the first to the last test could be linked to specific sleep features collapsed over all circadian phases. We observed a positive impact of SWS ($F_{[1,7]}=11.46$, $p=0.01$) and NREM sleep EEG delta power ($F_{[1,7]}=17.28$, $p=0.004$) on WM accuracy improvements, such that a longer duration of SWS and a higher delta power was associated with greater WM accuracy benefits. Furthermore, the effect of REM sleep duration appeared to be modulated by genotype ($F_{[1,7]}=37.16$, $p<0.001$), indicating a positive influence of REM sleep duration on WM accuracy improvements in G/A-, but not in G/G-allele carriers. Analyses of all other frequency bands and sleep stages did not indicate an association with WM accuracy improvements ($p_{all}>0.05$).

In the light of the strong circadian regulation of REM sleep duration (e.g., [20]), we considered in a next step if the genotype-dependent impact of REM sleep duration on WM accuracy improvements is dependent on circadian phase. To do so, performance changes were quantified as difference ratios from before to after nap sleep episodes for those naps with a reliable REM sleep duration >5 min (mean of midpoints of excluded naps at the first day at 5:40 p.m. and 9:40 p.m., at the second day at 1:40 p.m. and 5:40 p.m.). For each of the remaining times of assessment, one ANCOVA was calculated aiming at a combined investigation of both the influence of REM sleep duration and its interaction with genotype on WM accuracy improvements, at the same time controlling for subjective effort. Results were adjusted for multiple comparisons according to the false discovery rate procedure [47]. This approach revealed that only REM sleep duration at the end of the biological night (5:00 a.m. to 6:20 a.m.) seems to affect subsequent WM improvement with a longer duration associated with higher performance increases ($F_{[1,18]}=6.3$; $p=0.02$; does not reach significance level when corrected for multiple comparisons). Importantly, this relationship was modulated by genotype ($F_{[1,18]}=9.0$; $p=0.008$) such that the beneficial effect of REM sleep duration on WM accuracy was more pronounced in G/A-allele carriers compared to G/G-allele carriers (Figure 5).

Discussion

Our study suggests that the circadian regulation of sleep differs according to the *ADA* polymorphism, with the most prominent group differences during maximal circadian wake and sleep promotion. In parallel, results indicate that WM improvements depend on specific sleep characteristics. EEG delta power during

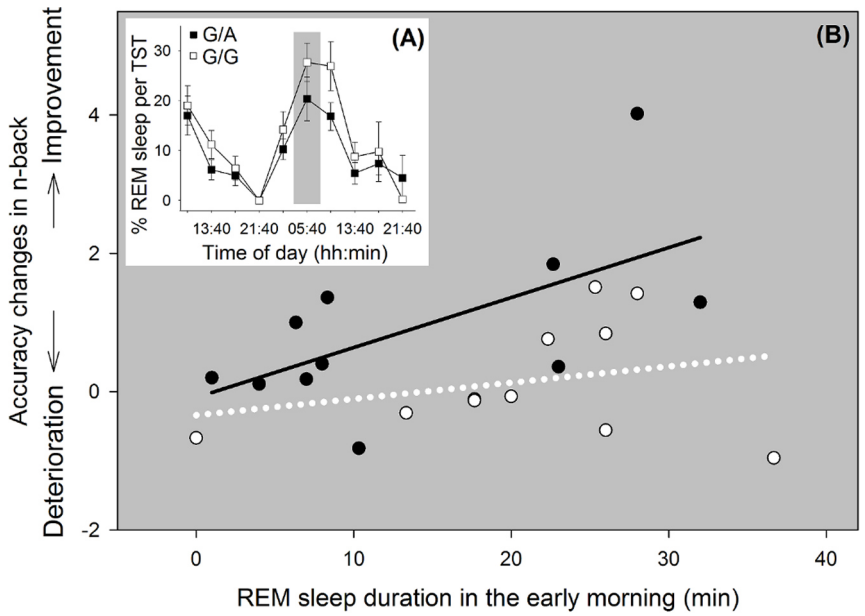

Figure 5. Association between REM sleep duration in the early morning and performance changes per genotype. (A) A strong circadian modulation in the proportion of REM sleep per nap was visible in both genotype groups (effect of time $F[9,844]=18.38$, $p<0.001$; effect of genotype $F[1,22.3]=3.45$, $p=0.08$; interaction time × genotype $F[9,844]=0.96$, $p=0.46$) with a peak duration in the morning hours. The grey rectangle indicates the nap in the early morning (midpoint of nap: 5:40 a.m.), in which REM sleep duration was positively related to improvements in WM accuracy. (B) Performance changes are expressed as difference ratio (after nap sleep – before) in WM accuracy (3-back – 0-back). Overall, REM sleep duration in the early morning hours (5:20–6:00 a.m.) is positively related to improvements in WM accuracy ($p=0.02$). The strength of this relation depends on genotype ($p=0.008$) and is more pronounced in G/A-allele carriers (black solid line) compared to G/G-allele carriers (white dotted line).

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NREM sleep as well as SWS was associated with WM accuracy independent of circadian phase and genotype. Positive effects of REM sleep duration appear particularly when it is expressed during its 'natural' circadian time window and seem to be more beneficial for G/A-allele carriers, presenting a more distinct circadian modulation in sleep structure and intensity.

3.1 Inter-individual differences in the circadian regulation of sleep

The *ADA* polymorphism has previously been associated with differences in sleep pressure levels, as indicated by genotype-dependent variations in subjective and behavioral variables as well as sleep during night-time [9, 12, 17]. The stronger behavioural vulnerability of G/A-allele carriers in response to sleep pressure manipulation we recently published [17] might at least partially be explained by the here observed differential circadian sleep regulation, as both sleep pressure and circadian processes tightly interact to produce consolidated sleep and wake bouts.

The wake-maintenance zone or 'forbidden zone for sleep' [48] reflects maximal circadian drive for wakefulness opposing high sleep pressure levels at the end of a regular waking day [1, 3]. At this time window, we detected higher amounts of wakefulness and shorter sleep duration in G/A-allele carriers, speaking in favour of a stronger circadian wake promotion in this genotype. The here observed more pronounced circadian arousal expression in heterozygous individuals might contribute to the previously reported improved working memory during multiple napping as compared to sleep deprivation [17]. The stronger wake promoting signal in the G/A-genotype might have evolved in order to oppose higher sleep pressure levels as reported under normal and high sleep pressure conditions [9, 12, 17]. Indeed, a differential circadian sleep-wake regulation according to the amount of accumulated sleep pressure has already been shown previously [19, 20, 29, 49]. Animal studies demonstrating a diurnal pattern of ADA activity in the rats' sleep-wake regulatory brain areas, such as the ventrolateral preoptic nucleus (VLPO) and the basal forebrain [50], suggest potential target sites at which ADA modulates circadian sleep-wake regulation. Note that it could be argued as well that genotype-dependent differences to initiate sleep at the end of the day may be attributed to a concomitant shift in the timing of melatonin [17], since the latter has been shown to play an important role in opening the gate for sleep [51, 52]. Nonetheless, by adjusting the analysis of sleep to DLMOs, we tried to control for this factor.

A genotype-specific pattern in the evening hours was as well observed in NREM sleep power in the low delta range. While G/A-allele carriers remained stable from the early to the late evening, G/G-allele carriers displayed a significant reduction in NREM sleep delta power during this time frame. At a first glance, this finding stays in contrast to the above discussed indications of a stronger circadian wake promotion in heterozygous individuals and seems to be in line with earlier reports of a higher SWA in G/A- compared to G/G-allele carriers [9, 12]. Note however that NREM EEG data could only be analysed from those participants who initiated NREM sleep during this time of strongest circadian wake promotion. Interestingly, these were by trend less G/A- (n=4) than G/G-allele carriers (n=9, χ^2 test one-sided: $p=0.05$). Considering the low sample size of G/A-allele carriers (n=4), an interpretation at this level appears thus doubtful.

Besides promoting wakefulness during biological daytime, the circadian clock is also involved in sleep consolidation, which appears as particularly important in the early morning when sleep pressure has mostly dissipated under entrained conditions [1, 4, 53]. At the end of the nap in the early morning, particularly G/A-allele carriers maintained high levels of NREM sleep and SWS under low sleep pressure conditions. Concomitantly, they exhibited a pronounced decrease in sigma activity, which has previously been observed in recovery sleep after sleep deprivation [54, 55]. Beside sleep-homeostatic influences, sigma activity exhibits a strong circadian regulation [2]. In this perspective the data may point towards a trait-like, G/A-genotype-specific increased strength of the interplay between homeostatic and circadian sleep-wake regulatory factors.

Genotype-dependent differences at the end of the biological night were also detected in NREM sleep alpha activity. Alpha activity bursts during NREM sleep have been associated with cortical arousal [56]. Within this perspective, the G/A-genotype-specific alpha decrease suggests a genotype-dependent mechanism to prevent the interference of arousals for the achievement of a consolidated sleep period, even under conditions of low sleep pressure. However, together with a dominant vagal activity during NREM sleep, lower alpha activity has also been proposed to mirror processes of worse sleep maintenance during NREM sleep [57], such that the observed decrease in alpha activity of G/A-allele carriers at the end of the night might be associated to a decline in sleep maintenance. In the same perspective, alpha activity increases have been detected in recovery sleep following sleep deprivation [55]. Within this framework, a reduction of alpha power would paradoxically indicate a reduced sleep pressure in the G/A-genotype. Note however that in our protocol the state of the sleep homeostat was kept low by multiple naps. Under these conditions the homeostatic function of alpha activity [2] remains virtually unexplored.

By the implementation of multiple short sleep-wake cycles we aimed at specifically investigating the circadian regulation of sleep according to genotype under low sleep pressure conditions. Previous studies report genotype-dependent differences in SWS and SWA assessed during consolidated night-time sleep periods following intervals of 16 h [9, 12] or 40 h [9] of continuous wakefulness. However, by multiple napping we were able to assess the initiation of sleep as well as its structure and intensity in dependence of circadian phase. Sleep might be considered as a highly sensitive measure to unravel differences circadian sleep-wake regulation. Nonetheless, future studies should focus on the replication of genotype-specific differences in circadian regulation of sleep as similarly done for consolidated night-sleep episodes [15, 16].

3.2 Sleep-related ameliorations in WM performance

Previously, it has been shown that WM generally profits from sleep [23] and from low compared to high sleep pressure levels [17]. Here, we observed that both EEG NREM delta power and SWS promotes WM accuracy independent of circadian phase. NREM sleep delta power and SWS are conceptually linked and mirror mainly sleep homeostatic mechanisms [2, 20] while exhibiting a rather weak impact of circadian rhythmicity [2]. The homeostatic function of delta power has been linked to local modifications occurring at the synaptic level during cognitive challenges while awake [58]. In studies investigating the domain of visuomotor learning, enhanced delta power has been associated with prior mechanisms of encoding as well as with post-sleep benefits [59].

The circadian peak of REM sleep duration, mediated by the suprachiasmatic nuclei and their connections to orexin-containing neurons [60], occurs under entrained conditions in the early morning [6]. Our data indicate that REM sleep duration positively influences WM improvements, especially when occurring within this particular time. This highlights the impact of circadian processes on

sleep-related cognitive performance modulation under low sleep pressure levels [24, 25], and suggests a possible circadian influence in the domain of working memory.

In the animal domain, Smith proposed a ''paradoxical sleep window'', suggesting REM sleep to be specifically involved in memory formation (e.g., place learning or shuttle avoidance) at particular discrete time intervals [61]. The administration of cholinergic and dopaminergic antagonists [62] during such a window has been shown to impair memory formation. High cholinergic activity during REM sleep [63] has been associated with REM sleep-dependent memory consolidation in procedural learning [64]. In parallel, reduced acetylcholine levels in the prefrontal cortex impair WM performance [65]. In the light of changes in the dopaminergic system following WM performance trainings [66], the REM sleep-specific increase in dopaminergic activity [59] might additionally play a role in REM sleep-associated improvements of WM. This is supported by the observation that Parkinsonian patients under dopaminergic medication improved over night in WM span, compared to patients without dopaminergic medication [67].

Finally, our data reveal that the REM sleep benefits in WM performance is modulated by inter-individual differences in sleep regulation. This post-hoc observed result suggests that G/A-allele carriers appeared to be more sensitive for the association between REM sleep and WM performance. A higher sensitivity for circadian mechanisms underlying the beneficial effect of REM sleep on WM is plausible. Genotype-dependent differences in the adenosinergic system might impact on cholinergic and dopaminergic mechanisms [68, 69] potentially implicated in REM sleep-dependent benefits on WM performance.

Note that WM capacity, that is the maximum number of information that can be kept in WM, is classically considered as limited and fixed to a small number of items [70]. Improvements in n-back performance, as observed in the current study, do most probably not concern WM capacity, but reflect ameliorations in the executive aspects of WM. Such benefits in monitoring and manipulation of information held online as well as inhibition processes have been reported earlier [21] and might mirror changes in task-specific strategies. Whether these strategies can be generalized to other cognitive challenges of executive processes as reported for adaptive n-back task versions [21] remains to be elucidated.

This study is the first to demonstrate genotype-specific inter-individual differences in the circadian regulation of nap sleep and its association with working memory performance. However, regarding the sample size the result should be considered as preliminary as long as not being replicated by independent observations. Nonetheless, the data suggest the consideration of circadian mechanisms when investigating sleep-dependent performance improvements.

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Author Contributions

Conceived and designed the experiments: CFR MM CC CS. Performed the experiments: CFR MM VG TG CS. Analyzed the data: CFR CC CS. Contributed reagents/materials/analysis tools: MH AUV VK WS VB HPL. Wrote the paper: CFR MM CC CS.

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TITLE: Time of Day Matters: Circadian Modulation of Sleep loss-related Changes in Cognitive Brain Functions

ABBREVIATED TITLE: Circadian variations in sleep loss-related cognitive brain functions

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ABSTRACT:

Sleep loss-related detrimental effects on neurobehavioral functions are particularly harmful at night. However, once wakefulness is further extended into daytime, cognitive performance has the potential to recover again, putatively due to the release of circadian wake promoting mechanisms. Importantly, the impact of such mechanisms on sleep loss-related cognitive brain function remains virtually unexplored. Here, BOLD activity underlying successful working memory performance was quantified in 31 participants during peaks and troughs of circadian arousal promotion respectively both in a 40-h sleep deprivation (SD) and a 40-h multiple nap-protocol (NP, keeping sleep pressure low). As expected, performance was worse during SD compared to NP, particularly at night, but recovered again during the following day. Intriguingly, BOLD activity very similarly decreased under SD compared to NP in a widespread cortical network at night, while an additional extension of wakefulness during the following day was not accompanied by further activity declines. Furthermore, task-related postero-lateral hypothalamic BOLD activity in the evening not only covaried with a typical marker of circadian wake-promotion (reduced nap sleep efficiency in the evening), but was also associated to the ability to perform well under sleep loss during daytime. During night time, activity in this region was reduced according to the individual's homeostatic sleep pressure built-up, as quantified by the NREM sleep delta rebound in response to sleep loss. These results strongly indicate an important role of hypothalamic structures for the integration of circadian and sleep homeostatic mechanisms to control for human neurobehavioral functions under challenging sleep loss conditions.

INTRODUCTION

Sleep loss-related decrements in neurobehavioral performance vary according to time of day (Schmidt et al., 2007). When kept awake, performance deteriorations are most prominent towards the end of the biological night, while being attenuated during the subsequent day even though

wakefulness is further extended (e.g., Dijk et al., 1992; Cajochen et al., 1999). This daytime stabilization in cognitive performance is presumably supported by circadian wake promoting mechanisms (Cajochen et al., 2004), counteracting the detrimental impact of continuously rising sleep pressure throughout wakefulness (Edgar et al., 1993; Dijk and Edgar, 1999).

The impact of circadian phase on waking quality crucially depends on sleep homeostatic mechanisms (Dijk and Franken, 2005). Thus, circadian-related performance decrements during the biological night are exacerbated with increasing homeostatic sleep pressure levels (Dijk et al., 1992; Wyatt et al., 1999; Wyatt et al., 2004). On the other hand, circadian arousal promotion in the late evening is diminished under increasing sleep pressure (Wyatt et al., 1999; Wyatt et al., 2004). As circadian wake-promotion originates in hypothalamic areas projecting to brainstem regions (Aston-Jones, 2005; Saper, 2013), such circadian opposing mechanisms might rely on subcortical input. Accordingly, higher sleep pressure levels under normal waking conditions have been associated with lower attention-related hypothalamic BOLD activity (Schmidt et al., 2009).

Sleep loss-related cerebral correlates of performance have been extensively investigated in the domain of working memory (WM; Chee and Chuah, 2008). Performance declines after sleep deprivation (SD) have often been linked to activity decreases in a fronto-parieto-occipital network (Thomas et al., 2000; Chee and Choo, 2004; Habeck et al., 2004; Choo et al., 2005; Mu et al., 2005b; Chee et al., 2006 but see also Lythe et al., 2012), while compensatory increases in frontal, anterior cingulate and thalamic regions were associated to the maintenance of performance under SD (Chee and Choo, 2004; Habeck et al., 2004; Choo et al., 2005; Mu et al., 2005a). Importantly, even though clearly affecting behavioural outputs, the impact of circadian phase on cerebral correlates of performance during sleep loss has not been systematically investigated.

Therefore, we assessed BOLD activity at several times throughout 40 h of continuous wakefulness (i.e., SD, Figure 1a). We expected that the biological night induces typical sleep loss-related BOLD activity decreases underlying WM performance. After a night of a sleep loss, during the following day, we assumed circadian arousal signals to prevent further sleep loss-related declines. In order to control for day-night differences per se, a 40-h nap condition was added (Figure 1B), allowing for the investigation of BOLD activity at the same critical times, but without rising sleep pressure. Furthermore we linked the assessed BOLD activity modulations during WM performance to classical markers of circadian wake promotion and accumulated homeostatic sleep pressure. These were operationalized as nap sleep efficiency in the evening and the difference in NREM sleep delta power between baseline and recovery sleep, respectively.We assumed that hypothalamic regions play a key role in the integration of these circadian and sleep homeostatic signals (Schmidt et al., 2009) and co-determine WM performance.

METHODS

Participants

Volunteers were recruited via advertisements in the internet. Questionnaires served to include young (20-35 years), healthy non-smokers indicating a good subjective sleep quality (Pittsburgh Sleep Quality Index [PSQI] ≤ 5; Buysse et al., 1989), a habitual sleep duration of 8 ± 1 h, and no symptoms of clinical depression (Beck Depression Inventory [BDI-II] < 9; Beck et al., 1996). Exclusion criteria comprised flights, passing more than 3 time zones within three months before study participation, shift work, drug consumption or current medication (except contraceptives) and a history of prior psychiatric or sleep disorders.

Overall, 31 participants (14 male, 17 female) took part in the laboratory study (see Table 1 for demographic description). In order to control for a potential effect of genetic vulnerability to SD, we also genotyped participants with regard to specific polymorphisms in the genes encoding for PERIOD3 (rs57875989; 15 $PER3^{5/5}$, 16 $PER3^{4/4}$) and adenosine deaminase (rs73598374; 12 G/A-, and 19 G/G-allele carriers; frequency within the sample between both genotypes *n.s.*, χ^2=.21; Viola et al., 2007; Bachmann et al., 2012). All participants were medically screened by the physician in charge, underwent a toxicological check (Drug-Screen-Multi 6, Nal von minden, Germany) and spent a habituation night in the laboratory in order to exclude sleep disorders and to familiarize participants to the laboratory conditions. Female volunteers were tested for pregnancy and participated, if not taking oral contraceptives, during the luteal phase of their menstrual cycle.

Procedure

The study was approved by the local ethics committee (Ethikkommission Beider Basel) and performed according to the Declaration of Helsinki. All study volunteers gave written informed consent before participation.

We carried out a randomized controlled within-subjects design with two 40-h conditions (Figure 1). In the SD condition, participants were asked to stay awake for the entire 40-h episode, and wakefulness was verified by continuous electroencephalography (EEG) recordings. In the nap (NP) condition, we scheduled 10 short sleep-wake cycles, each consisting of 160 min of wakefulness and 80 min of a napping opportunity. By scheduling regularly naps, we aimed at inducing low homeostatic sleep pressure levels throughout the course of the protocol. The combination of a high and low sleep pressure condition has been successfully applied in a similar manner in earlier studies (e.g., Cajochen et al., 2001; Sagaspe et al., 2012) to investigate the impact of differential sleep pressure levels at different circadian phases.

Both conditions, the SD and NP, were separated by a minimum of seven days and were preceded by one week of a fixed sleep-wake cycle (8 h sleep per day, no napping allowed) in order to control for sleep pressure levels and circadian misalignment. Compliance to the regimen was verified by wrist-actimetry recordings. Participants' scheduled wake- and sleep times were adapted individually to their usual preferences, and fixed during the 7 days prior entering the laboratory as well as during the SD and NP protocol. Baseline and recovery-nights, each of 8 h, preceded and followed each laboratory condition. Over the course of both protocols, light was dimmed to < 8 lux during wakefulness (and 0 lux during napping), meal intake was regularly scheduled (snacks every 4 h) and body posture was controlled (semi-recumbent during wakefulness, recumbent during naps and functional magnetic resonance imaging (fMRI) sessions) in order to control for potential masking effects. Participants were allowed to get up only for regularly scheduled bath room visits (equally distributed through both protocols) and did not have any time of day indication. They were allowed to read, watch documentaries or play dice-games. Social activities were restricted to communication with the study helpers.

In regular intervals we assessed subjective sleepiness and well-being, attentional vigilance, WM performance as well as underlying cerebral correlates, along with salivary melatonin. Here, we focus on BOLD activity patterns of WM performance during both the NP and SD condition in the late evening hours of the first evening (13 h after scheduled wake-up from the baseline-night, on average at 8 p.m., day1), at the end of the night (21 h after scheduled wake-up, at 4 a.m., night), and in the late evening of the second day (37 h after scheduled wake-up, again at 8 p.m. day2). As illustrated in Figure 1, these time windows are particularly crucial for either circadian wake and/or sleep promotion. In the late evening hours shortly before habitual bedtime, in the so-called wake-maintenance zone (Strogatz et al., 1987), the circadian wake promoting signal is strongest (Dijk and Czeisler, 1994). Please note that the scanning sessions of 30 of 31 participants in the late evening (day1 and day2) took place before individuals' dim light melatonin onset (DLMO, defined as 50% of the maximum, according to (Reichert et al., 2014)). The DLMO has been associated to the opening of the gate for sleep and might be considered as marker of the end of the wake-maintenance zone. During the second circadian window in the late night, it has been proposed that the circadian system strongly facilitates sleep (Dijk and Czeisler, 1994). Thus, we were able to investigate cerebral correlates of WM during high circadian wake and sleep promotion both systematically under low and high sleep pressure levels (see Figure 1 for an illustration).

N-back paradigm

WM performance was assessed by a n-back task of about 20 min every four hours, starting one hour after waking-up from the baseline night (Figure 1). Every other time, participants performed the

task in a functional magnetic resonance imaging scanner in order to assess cerebral correlates. The n-back task consisted of the visual presentation of a series of letters and participants are asked to decide and indicate by a button-press whether the letter presented is the same as n trials before. We implemented 9 blocks of a 3-back (high WM load), and 5 blocks of a 0-back condition (no WM load). In the latter, participants were instructed to press a button as soon as a specific letter appeared. Per block, a series of 30 letters comprising ten targets was presented, each for 1.5 sec with an inter-stimulus interval of 500 ms. Blocks were separated by a break of a randomly generated interval of 10-20 sec, during which a fixation cross was displayed on the screen.

In order to prevent baseline differences in comprehension and transfer of instructions, participants were trained in 3-back performance until they reached a level of 70 % of correct responses in the evening before the study. Nonetheless, one participant performed three interquartile ranges below the 25^{th} percentile during the entire course of the nap condition. This performance was considered as extreme value (Leonhart, 2004) and excluded from further analyses.

Statistical analysis of n-back performance

Hit targets in the 3-back condition, corresponding to the number of true positive answers or correct identifications of a letter presented three trials before were considered as the dependent variable. The time course of hit targets assessed after 13h (day1), 21h (night) and 37h (day2) elapsed time in the protocols (see yellow arrows in Figure 1) was analysed with a general mixed model by the SAS 9.3 software (SAS Institute, Cary, USA). The model included the factor “condition” (NP vs. SD) and “time” (day1, night, day2). Contrasts were calculated with the LSMEANS statement. Degrees of freedom of p-values were based on an approximation described by Kenward and Roger (Kenward and Roger, 1997), and multiple post hoc comparisons were adjusted according to the Tukey-Kramer method (Hayter, 1984).

Sleep EEG

Polysomnographic signals (F3, FZ, F4, C3, CZ, C4, PZ, O1, Oz and O2 EEG derivations, two electrooculographic, two electromyographic and two electrocardiographic derivations) were recorded with sintered MRI compatible Ag/AgCl ring electrodes with a 15 kOhm resistor (EasyCap GmbH, Germany) and V-Amp digital sleep recorders (Brain Products GmbH, Germany). All signals were sampled at 500 Hz and filtered online by applying a notch filter (50 Hz). Frequencies < 0.1 Hz and > 20 Hz were filtered out offline (bandpass filter, butterworth type, third order, slope -60dB/decade) in order to achieve a better visual scoring of sleep stages. Manual sleep stage scoring of the nap sleep episodes was done according to standard criteria (Rechtschaffen and Kales, 1968) by experienced staff. Sleep efficiency was calculated as the sum of the duration of all sleep stages (stage

1, 2, 3, 4 and REM) per nap duration (80 min). Non-rapid-eye-movement (NREM) sleep was considered as the sum of sleep stages 2, 3 and 4.

Calculation of EEG delta (0.7-4 Hz) power density during NREM in baseline and recovery nights was based on an automatic scoring algorithm (ASEEGA, Version 1.3, Physip, France, accordance rate with manual scorings 82.9%, (Berthomier et al., 2007)). EEG power of the central derivation (CZ-PZ) was calculated using a fast Fourier transform with Hanning window for consecutive 30-sec epochs after an artefact automatic rejection step. As an approximation of accumulated sleep pressure levels following SD, we calculated the difference of NREM sleep delta power density between the recovery night and the baseline night assessed in the SD protocol (Borbely and Achermann, 1999; Cajochen et al., 2001). The difference of one participant was two interquartile ranges below the 25^{th} percentile and was as an extreme value excluded from the analysis.

Assessment of fMRI data

Functional MRI images were assessed with a 3 Tesla MR Scanner (MAGNETOM Verio, Siemens Healthcare) using a standard twelve-channel head coil. Multislice T2*-weighted fMRI images were acquired with a gradient echo-planar sequence applying axial slice orientation (32 slices; voxel size: 3 x 3 x 3 mm³ with 17% interslice gap; matrix size 76 x 76 x 32; repetition time = 2200 ms; echo time = 32 ms; flip angle = 82°). For anatomical reference, structural T1-weighted images were obtained with a magnetization-prepared rapid gradient echo (MPRAGE) sequence (repetition time = 2000 ms, echo time = 3.37 ms, flip angle = 8°, field of view = 25.6 cm, matrix size = 25.6 x 25.6 x 17.6 cm^3, voxel size = 1 x 1 x 1 mm^3). 176 contiguous axial slices covering the entire brain were assessed in sagittal diraction. Due to technical problems out of 186 datasets, nine datasets were lost (two of them assessed during NP-night, one during SD-night, three during NP-day2, and three during SD-day2).

Statistical analysis of fMRI data

For analyses we implemented SPM8 (http://www.fil.ion.ucl.ac.uk) in MATLAB 12. In order to minimize the residual sum of square between the first and subsequent images, functional scans were realigned with iterative rigid body transformations. Following normalization to the Montreal Neurological Institute (MNI) EPI template (third-degree spline interpolation; voxel size 2 x 2 x 2 mm3), scans were spatially smoothed with a Gaussian kernel with full width at half maximum (FWHM) of 8 mm. Within a two-step analysis brain responses were first modeled for each subject at each voxel using a GLM to account for intra-individual variance. For each condition, the model included five regressors representing events associated to behavioral task performance events (true positive, true negative, false positive, and false negative responses as well as events where no

response was recorded). To account for effects of time on task, we additionally included for each event type (true positive, true negative, false positive, false negative and missing responses) a first order polynomial regressor. For each event type, we modeled the expected change in the blood oxygen level-dependent signal by a canonical hemodynamic response function. Six regressors derived from realignment and a constant vector were as well included in the model and considered as variables of no interest.

Statistical inferences were performed at a threshold of $p = 0.05$ after correction for multiple comparison over the entire brain (family-wise-error, FWE-correction). Around a-priori defined locations of interest, corrections for multiple comparisons were applied on small spherical volumes (radius 10 mm in cortical regions, radius 8 mm in hypothalamic regions according to (Schmidt et al., 2009)). We expected activity differences in regions implicated in active verbal n-back performance at rested wakefulness (Owen et al., 2005) and in sleep deprived states (Choo et al., 2005; Vandewalle et al., 2009) as well as in regions shown to be deactivated during task performance (Tomasi et al., 2006; Laird et al., 2009). With regard to the potential impact of circadian wake-promoting mechanisms, we additionally focused on hypothalamic regions (Schmidt et al., 2009) as well as the locus coeruleus (Schmidt et al., 2009).

Correlates of true positive answers (hit targets) during task performance were estimated using linear contrasts. First, we assessed the main effect of condition (NP>SD) in order to investigate decreases in brain activity due to the impact of sleep pressure independent of time of day. We studied thus the influence of SD in a manner highly controlled for variations in circadian phase, as every assessment during SD has a counterpart in time of day assessed during NP, under low sleep pressure. In a second step, we aimed at disentangling which of these sleep pressure-related effects (NP>SD) can be attributed to a rise of sleep pressure specifically from day- to the nighttime [contrast SD-day1 (13h awake) > SD-night (21 h awake)] or to a rise of sleep pressure from night- to daytime [contrast SD-night (21h awake) > SD-night (37 h awake)]. To do so, we applied a conjunction analysis ($p=0.001$) with the contrast reflecting the main effect of condition and the contrast assessing decreases from day1 to night [(NP>SD) $\cap$ (SD-day1>SD-night)] and from night to day2 [(NP>SD) $\cap$ (SD-night > SD-day2)] under SD, respectively. Parameter estimates were extracted for those regions revealing significant activity changes between our sessions of interest. Multivariate analysis of variance for repeated measures was applied to investigate the general time course of these estimates over the three assessment times (day1, night, day2), using the statistical software package SPSS 19.0.0 (IBM Corp., Armonk, USA)

Similarly to the procedure investigating decreases in activity due to sleep loss (NP>SD), we also tested whether brain activity increases from SD to NP (contrast SD>NP) might be attributed to higher activity during nighttime as compared to the first evening [(SD>NP) $\cap$ (SD-night>SD-day1)] or to

higher activity during daytime under high sleep pressure as compared to nighttime [(SD>NP) ∩ (SD-day2>SD-night)].

Finally, due to the implementation of NP, we were also able to investigate which effects emerging during a change in circadian phase in SD can be traced back to a change in circadian phase per se, that means independent of the rising sleep pressure levels. To assess this, we applied a conjunction analysis ($p<0.001$) with the contrasts assessing changes in brain activity according to circadian phase under SD and NP [(NP>SD) ∩ (SD-day1>SD-night) ∩ (NP-day1>NP-night)].

Analyses of covariance

In a next step, we investigated whether a hypothalamic region, suggested to be differentially modulated according to sleep pressure and time of day (Schmidt et al., 2009), is associated to the strength of circadian wake promotion. The impact of circadian wake promoting mechanisms on sleep-wake regulation is mirrored in problems to initiate and maintain sleep during daytime, specifically in the late evening shortly before habitual bedtime (Dijk and Czeisler, 1994). Sleep efficiency values of the NP protocol in the late evening of day1 were thus considered as an approximation of the strength of circadian wake promotion. At the group level they were integrated as covariate in the model investigating brain activity in the evening, for NP and SD. We report small-volume corrected p-values [coordinate of (Schmidt et al., 2009)], which have been controlled for the accumulation of type 1 errors arising from two analysis (NP and SD separately) according to Bonferroni (Leonhart, 2004).

We also aimed at linking task-related BOLD activity to a classical marker of sleep homeostatic pressure (Schmidt et al., 2009). The sleep homeostatic challenge during extended wakefulness is commonly reflected in higher delta power in the following sleep episode as compared to baseline levels (Figure 1). Thus, the difference of NREM sleep EEG delta power between the recovery und the baseline night in the SD protocol was used as marker of accumulated homeostatic sleep pressure, and termed NREM sleep delta rebound. It was integrated at the group level as a covariate of interest in the model targeting brain activity at different times of day (day1, night, day2) under SD. We report small-volume corrected p-values [coordinate of (Schmidt et al., 2009)] of these three analysis (SD-day1, SD-night, SD-day2 separately) which were Bonferroni-corrected in order to control for false positive results (Leonhart, 2004).

Relation of wake-promoting regions to performance

Circadian sleep-wake promotion has earlier been associated to nighttime troughs in performance during SD as well as to the recovery of performance as soon as passing into daytime (Cajochen et al., 2004). We were specifically interested in whether regions associated at the BOLD activity level to circadian sleep-wake promotion and to sleep pressure accumulation are linked to the observed WM performance time course.

Therefore, we calculated a) performance differences from SD-day1 to nighttime and grouped participants based on a median split according to the extent of the performance decrease. We investigated by a two-sided t-test whether these groups differ in hypothalamic BOLD activity at night. Coordinates to extract hypothalamic BOLD activity were chosen from the region shown to be dampened at night the higher the sleep pressure increase.

Furthermore, we calculated b) performance differences from SD-day1 (after 13 h of wakefulness) to SD-day2 (after 37 h of wakefulness; same circadian phase) and grouped participants based on a median split in a group with slightly decreasing and a group with stable or even increasing performance at day2. *P*-levels of the two sample t-tests (for groups a) and b), respectively) were corrected for multiple comparisons according to Bonferroni (Leonhart, 2004).

RESULTS

Sleep loss-related decreases in WM performance are most pronounced at night

Generally, performance was worse under sleep loss (SD), compared to the NP condition ($F(1,139)=25.82$; $p<0.001$) and during day- compared to nighttime ($F(2,139)=14.58$; $p<0.001$). As expected, post-hoc tests of the interaction between condition and time ($F(2,138)=6.42$; $p<0.002$) indicated that performance specifically differed between conditions during nighttime ($p<0.0001$) and during the second evening (day2, $p<0.001$). Figure 2 illustrates that decreases in performance during the night under SD were recovered during the second evening ($p<0.001$) even though wakefulness was further extended.

Sleep loss-related BOLD activity modulations depend on circadian phase

In line with earlier studies (e.g., Choo et al., 2005; Vandewalle et al., 2009), sleep loss-related activity decreases (NP > SD) were observed in a network comprising frontal, paracentral, parietal, temporal as well as fusiform and occipital regions (NP> SD; see Table 2, Figure 3A).

Interestingly, conjunction analyses revealed that sleep loss-related BOLD activity decreases could be attributed to a transition from day- to nighttime (Table 2; [NP>SD] ∩ [SD-Day1>SD-night]) in

frontal, paracentral, parietal and temporal regions (Figure 3B), while none of the general sleep loss-related activity decreases could significantly be ascribed to decreases from night to day2 (i.e., ([NP>SD] ∩ [SD-Night >SD-day2], Table 2). A multivariate analysis of the respective parameter estimates underlined this pattern (Figure 3B) by a main effect of time (p < 0.01, Huynh-Feldt corrected) with a post hoc structure (repeated contrasts) indicating a significant change from day1 to nighttime (p < 0.001), but no further significant variation from night to daytime (p > .35). In a next step, we aimed at targeting cerebral activity patterns contributing to stable or even increased performance levels under high sleep pressure at daytime. Even though significant differences at the group level were not observed from nighttime to day2 accounting for the general sleep loss-related declines, differences at the individual level still occur. Indeed, a multiple regression analysis revealed that the individual ability to increase BOLD activity in the temporal lobe (x=48, y=-68, z=6), from nighttime to day2 was positively linked to the ability to perform after 37 h of wakefulness (T = 2.29 β=-.62, p=0.03).

Importantly, the observed changes in BOLD activity levels from day-to nighttime could not be traced back to a change in circadian phase per se. With the exception of the right middle frontal gyrus (x=22, y=44, z=20), none of the observed regions (i.e. prefrontal, paracentral, parietal and temporal) decreased activity from day-to nighttime, also under low sleep pressure conditions (Table 2; conjunction analysis between NP and SD condition, see methods)

Finally, we tested whether task- or arousal-related regions increased activity from SD to NP. A anterior hypothalamic region showed higher BOLD activity under high, compared to low sleep pressure conditions (SD>NP; see Table 2). Conjunction analyses did not indicate that this sleep pressure effect depends on changes in circadian phase.

<u>Circadian wake-promotion, homeostatic sleep pressure and task-related BOLD activity</u>

Covariance analyses were performed to relate the assessed BOLD activity modulations occurring under sleep loss to known markers of circadian wake-promotion (sleep efficiency during the nap in the late evening) and sleep homeostatic pressure (NREM sleep delta rebound in response to sleep loss). Sleep efficiency in the late evening of day1 was negatively associated with activity in a postero-lateral hypothalamic region (Figure 4A) at the same time of day in the SD condition (i.e., after 13 h of wakefulness; x=4, y=-12, z=-10; s.v.c. p<0.01; Figure 4C). Concomitantly, the NREM sleep delta rebound was negatively linked to postero-lateral hypothalamic activity under sleep loss during nighttime (x=10, y=-6, z=-8; s.v.c. p<0.01; Figure 4D). A conjunction analysis (p<0.001) revealed that this region overlapped with the hypothalamic region significantly linked to sleep efficiency (Figure 4A).

Furthermore, activity in this hypothalamic region was linked to performance. Those participants showing less pronounced sleep loss-related performance decrease at night exhibited higher hypothalamic BOLD activity at night (t(28)=-2.11, *p*=.044, n.s. after bonferroni correction). A higher BOLD activity during daytime was observed in participants with stable or increasing performance levels from day1 to day2 (Figure 4B, t(29)=-2.05, *p*=.049)

DISCUSSION

Our results suggest, that the coincidence of high sleep pressure levels with circadian arousal reduction at night, is particularly harmful both at the level of cerebral activity and behavioral performance. Further, the data indicate that the circadian modulation of arousal and the associated cognitive performance profiles is mediated by dorso-lateral hypothalamic structures in a sleep pressure-dependent manner. BOLD activity in this region not only covaried with a typical maker for circadian wake-promotion (i.e., sleep efficiency in the late evening), but was also positively associated to the ability to perform well under high sleep pressure at daytime. On the other hand at night, BOLD activity in this region correlated negatively with the individual accumulation of sleep pressure under SD, as indexed by the sleep EEG delta activity rebound after SD. The postero-lateral hypothalamus appears thus as an interface converging circadian and sleep homeostatic signals related to daytime WM performance under conditions of sleep loss.

Sleep loss-related changes from day- to nighttime

We observed typical sleep loss-related BOLD activity decreases specifically when wakefulness was extended from day- to nighttime in a set of regions associated to WM performance, such as ventro-lateral prefrontal and superior parietal areas (Owen et al., 2005). Such a decline neither occurred during a passage from day to night under low sleep pressure, nor during a further extension of wakefulness from 21 h to 37 h throughout the following day. While similar activity decreases after a night of sleep loss have been reported previously (e.g., Habeck et al., 2004; Mu et al., 2005b; Chee et al., 2006; Lim et al., 2007; Lythe et al., 2012), our data show for the first time that circadian phase majorly affects the impact of sleep loss on cerebral correlates of WM with serious consequences at the behavioral level. The results indicate that sleep loss-related BOLD activity decreases can be traced back to the combined impact of both high sleep pressure and circadian arousal reduction at night, and thus parallel the striking decreases observed in nighttime performance under sleep loss. Alternatively, the reported activity decreases might appear as soon as passing a certain threshold of sleep pressure, which might have been reached here during the passage from day to night. However, earlier studies controlling for this confound indicate a differential impact of sleep pressure according to time-of day at the behavioral level (Dijk et al., 1992; Wyatt et al., 2004; Grady et al., 2010).

Sleep loss-related changes from night-to-daytime

In line with earlier reports (e.g., Graw et al., 2004; Blatter et al., 2005; Sagaspe et al., 2012), we observed behavioral performance increases from night to daytime under sleep loss. As similarly reported by Chee and colleagues (Chee et al., 2006), we did not observe any significant sleep loss-related declines in performance underlying cerebral activity, even though wakefulness was further extended. Nevertheless, our data indicate that the individual ability to recruit the right middle temporal lobe in a stable or even increased manner from night to daytime predicts performance at daytime after 37 h of wakefulness. Accordingly, right middle temporal regions have earlier been shown to be involved in verbal WM performance (Tomasi et al., 2006; Vandewalle et al., 2009), and positively associated to performance in a verbal learning task under sleep deprived conditions (Drummond et al., 2000). Our results suggest that such a beneficial effect might depend on circadian phase and its associated wake promotion allowing the maintenance in task-related BOLD activity when passing from night-to daytime under extended wakefulness.

Sleep loss-related effects independent of circadian phase

Concomitant to task-related cortical BOLD activity decreases under sleep loss, we observed increases in activity irrespective of time of day in a bilateral anterior hypothalamic region, compatible with the suprachiasmatic area (posterior part). More specifically, the activity increases were evident as reduced deactivations in SD compared to NP, pointing generally to a reduced anterior hypothalamic activity during task-performance as compared to baseline comparison levels (i.e., non-task-engaged behaviors). Previously, it was observed that increased sleep pressure was associated to higher BOLD activity in the suprachiasmatic area during vigilance performance (Schmidt et al., 2009). Also, in the animal domain it has been shown that electrical activity in the suprachiasmatic nuclei varies according to sleep pressure: Activity was reduced under high sleep pressure, recorded in freely moving rats not engaged in any specific behavior (Deboer et al., 2007). Our results fit into this frame, as it might be speculated that sleep pressure influences human 'baseline' BOLD activity levels, assessed in the present study as activity during phases of no engagement in a specific task-related response. This could potentially explain that we observed a reduced negative activity difference (i.e., a deactivation) between active task performance and baseline levels in SD compared to NP. As prior studies, investigating the impact of sleep loss on cognition-related brain activity, did not report a similar pattern, our observation might be due to low sleep pressure levels in NP, a condition which has been exclusively implemented in the present study to properly control for circadian phase effects. Unusual low levels of sleep pressure during NP appear thus to be associated with a strong

deactivation of anterior hypothalamic areas during performance, potentially due to sleep pressure-related changes in baseline levels.

Hypothalamic integration of circadian and homeostatic processes to control performance under sleep deprived conditions

The assessment of sleep-features over the 24-h cycle, and during baseline, and recovery nights allowed us to investigate cerebral correlates of both circadian wake promotion and differential sleep pressure levels underlying successful task performance. We focused on hypothalamic areas, as these have been suggested to integrate circadian and homeostatic signals (Deboer et al., 2003; Deboer et al., 2007; Schmidt et al., 2009). Our analysis revealed postero-lateral parts of the hypothalamus to be crucially involved in the mediation of wake-promotion The observed region is located at the lateral border of the postero-lateral hypothalamus (adjacent to the medial and anterior part of the substantia nigra). We think that this region is compatible with the generation site for the neuropeptide orexin/hypocretin in the lateral hypothalamus, crucially involved in stabilizing behavioral states including wakefulness (Inutsuka and Yamanaka, 2013; Saper, 2013; Saper et al., 2005; Saper, 2013), specifically during the active phase (Belle et al., 2014). In line with evidence from the animal domain (Deadwyler et al., 2007), our data suggest an impact of this region on behavioral performance, such that those individuals with a higher activity are less susceptible to the influence of high sleep pressure during daytime. Interestingly, evidence suggests that orexin/hypocretin levels vary according to both circadian rhythmicity originating in the suprachiasmatic nuclei and homeostatic sleep pressure (Zeitzer et al., 2003; Deboer et al., 2004; Zeitzer et al., 2007). In animals, downregulation of the hypothalamic orexingeric system during the biological night induces SWS (Tsunematsu et al., 2011) and increases NREM sleep delta activity (Cerri et al., 2014). In line with this, postero-lateral hypothalamic BOLD activity in our study was reduced according to the individual's delta-rebound in response to sleep loss. These data point to the interaction of circadian sleep-wake-promotion and sleep homeostatic mechanisms in the observed postero-lateral hypothalamic region, which influences behavioral performance (Dijk and Franken, 2005). As we restricted our analysis to hypothalamic regions of interest, future studies might elucidate a network involved in the transmission of the combined circadian and sleep homeostatic information to cortical regions underlying behavioral performance modulation.

Conclusion

Overall the data support a combined action of circadian and homeostatic mechanisms on brain activity patterns. They underline an important role of hypothalamic structures in the integration of the differential states of sleep-wake-regulating processes during WM performance. According to our

results, circadian phase and its associated arousal promotion need to be taken into account when investigating sleep loss-related brain activity correlates of cognitive performance.

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FIGRUE LEGENDS:

FIGURE 1. Schematic illustration of laboratory study and the effects of sleep pressure manipulation. A) The high sleep pressure condition consisted of 40 h of constant wakefulness. Subjective sleepiness (orange line) increased as soon passing into the biological night (as assessed by salivary melatonin, black dotted line), and remained at stable, high levels during the following biological day. N-back performance (dark red line) decreased during the biological night, recovering as soon as passing into the biological day. Sleep pressure built-up during sleep deprivation is depicted as increase in relative NREM sleep delta power density during recovery sleep compared to baseline levels. Yellow arrows indicate times when cerebral correlates of n-back performance were analysed, referred to as day1, night and day2 in the text. B) The low sleep pressure condition comprised ten short sleep-wake cycles, each encompassing 160 min of wakefulness alternating with 80 min of naps, in which the circadian course of sleep efficiency was measured (light green bars, calculated as percentage of sleep time per rest time). Subjective sleepiness and n-back performance follow a circadian course as well under these low sleep pressure conditions, the latter indicated by stable levels of relative NREM sleep delta power density in baseline and recovery sleep.

FIGURE 2. Performance according to condition and circadian phase. The interaction of condition and circadian phase became evident as significant performance decreases at night under sleep deprivation (SD), followed by increases during the following day. Even though increasing, performance did not fully recover under SD at day 2 and significantly differed from performance levels assessed at the second day during the nap protocol (NP). *: $p< 0.05$.

FIGURE 3. Change in BOLD-activity according to condition and time of day. A) BOLD activity generally decreased under high sleep pressure, depicted here as the mean of (negative and positive) BOLD activity in prefrontal, paracentral, parietal, and temporal regions (depicted in B; glass brain). To better visualize the similarity to the course of performance, we plotted the mean arbitrarily +1. In SD, a multivariate analysis of variance revealed a main effect of time ($p<0.001$) with post-hoc contrasts indicating a decrease of BOLD activity from day1 to nighttime (corresponding to 13h and 21h of wakefulness, respectively) as well as from day1 to day 2 (corresponding to 13 h and 37 h of wakefulness, respectively).*: $p<0.05$; **$p<0.001$

FIGURE 4. Hypothalamic BOLD activity differences and relation to sleep efficiency, delta-rebound and performance. (A) BOLD activity covarying with sleep efficiency in the late evening (light blue) overlaps with BOLD activity covarying with the rebound in NREM sleep delta power density after SD (blue) during the night. (B) Activity in the overlapping hypothalamic region during SD in the late evening was significantly lower in participants, who decreased performance from the first to the

second evening under SD [13 hours (day1) vs 37 hours (day2) of wakefulness] compared to participants able to show stable or increasing performance levels. *: $p<0.05$. (C) Correlation of postero-lateral hypothalamic activity assessed at day1 in SD with sleep efficiency assessed at day1 in the late evening nap during NP. (D) Correlation of postero-lateral activity assessed during nighttime with NREM delta power density rebound. The latter was quantified by a difference of the mean NREM power delta power density during the recovery night after SD (SDRN) and the mean NREM sleep delta power density assessed during the baseline night before SD (SDBL).

TABLE LEGENDS:

TABLE 1. Demographic information. PSQI =Pittsburgh Sleep Quality Index (Buysse et al., 1989); BDI=Becks Depression Inventory (Beck et al., 1996); BMI = Body Mass Index; ESS = Epworth Sleepiness Scale (Johns, 1992); MEQ= Morningness-eveningness Questionnaire (Horne and Östberg, 1976); MCTQ =Munich Chronotype Questionnaire (Roenneberg et al., 2003); MSF sc = Mid sleep free days sleep corrected.

TABLE 2. Differences in task-related BOLD activity during true positive answers. Coordinates (x, y, z) are expressed in mm in the Montreal Neurological Institute (MNI) space. P_{FWE}: p-value after family wise error correction. p_{svc}: p-value after correction for multiple comparisons over small volumes of interest taken from the literature. Ref. = references for coordinates.

682 **TABLES**

683 **Table1.**

Variable	*M*	*SD*
Age (years)	24.68	3.31
PSQI	3.13	1.18
BDI	1.87	2.23
BMI	22.21	2.49
Wake time (h:min)	07:10	51.52
ESS	4.21	2.46
MEQ	55.29	9.74
MCTQ MSFsc	7.50	2.46

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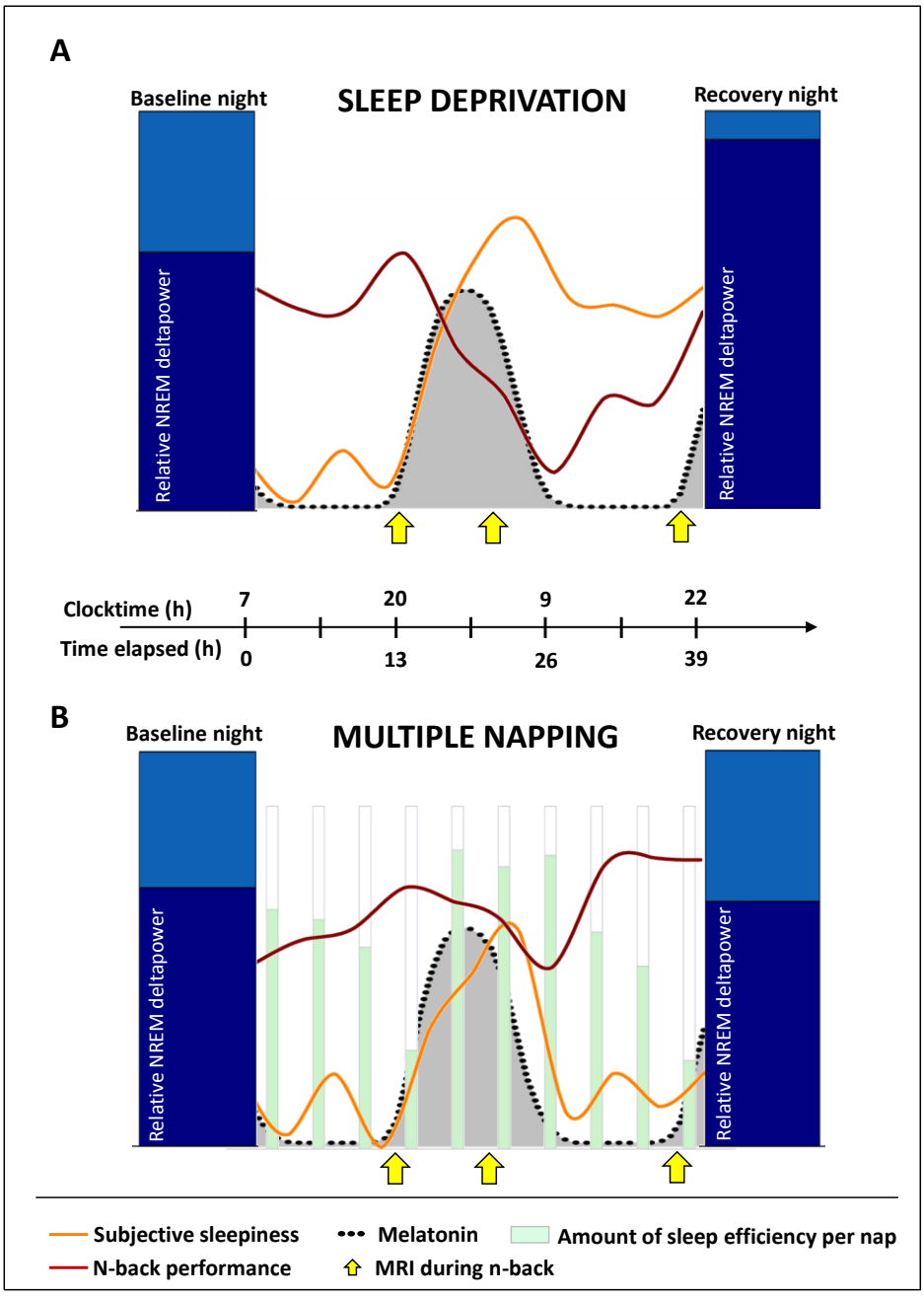
Table 2.

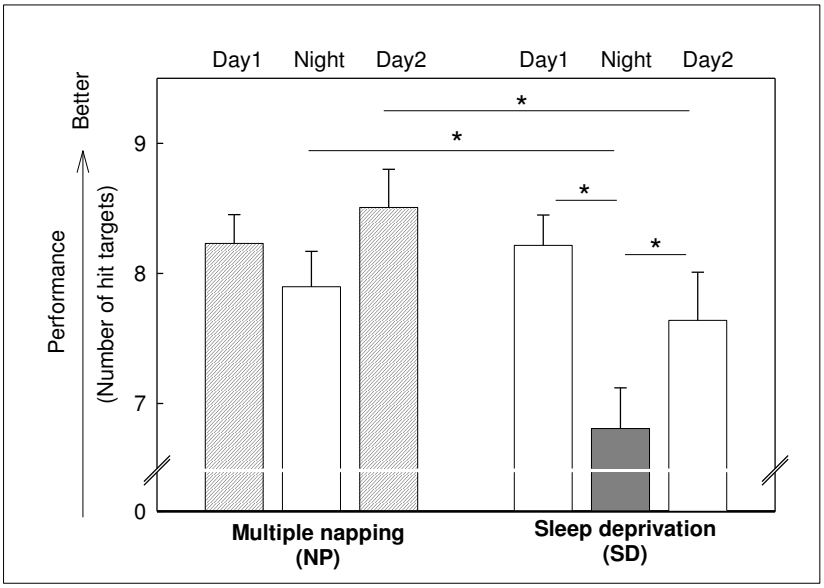
								NP>SD ∩ SD04vsSD06					NP>SD ∩ SD04vsSD06 ∩ NP04>NP06				NP>SD ∩ SD06vsSD10
NP>SD																	
Brain area	size	p_{FWE}	p_{svc}	Ref.	x	y	z	p_{svc}	Ref.	x	y	z	p_{svc}	Ref.	x	y	z	
Ventrolateral prefrontal	405		0.001	40	-58	6	10	0.024	40	-54	10	6						
			0.011	40	-42	8	6											
Middle frontal gyrus	94		0.006	51	36	14	16											
	142		0.021	15	22	46	10								22	44	20	
Middle frontal/precentral	122		0.016	53	-44	-8	56											
Anterior cingulate cortex	24		0.026	53	14	26	36											
Precentral gyrus	697		0.027	51	-24	-24	70											
			0.01	40	8	2	68	0.012	40	8	0	66						
	122		0.018	40	54	-4	42											
Paracentral gyrus	127		0.018	51	-8	-46	72	0.027	51	-8	-46	72						
Superior parietal lobe	239		0.027	51	-26	-46	50											
	95		0.018	51	26	-54	60	0.018	51	26	-56	60						
Inferior parietal lobe	99		0.021	34	56	-22	20											
Posterior parietal lobe	387		0.026	40	12	-76	42	0.026	40	10	-76	42						
Precuneus	162		0.014	51	-20	-72	38											
Middle temporal lobe	3177	*		34	46	-58	4	0.01	34	48	-68	6						
Middle temporal lobe		*		34	40	-62	10											
Dorsal cingulate cortex /medial premotor cortex	152		0.006	40	-6	6	38											
Fusiform gyrus	687		0.002	51	-44	-68	-4			-48	-72	-4						
Parahippocampal gyrus	403		0.027	51	-24	-42	-24			-28	-36	-28						
Middle occipital lobe	687		0.021	15	-26	-76	10											
Middle occipital lobe			0.027	15	-20	-84	14											
Medial lateral cerebellum			0.004	40	34	-48	-26			46	-44	-20						
SD > NP																		
Hypothalamic area	65		0.001	48	2	0	-10											

FIGURES

Figure 1.



692 **Figure 2.**



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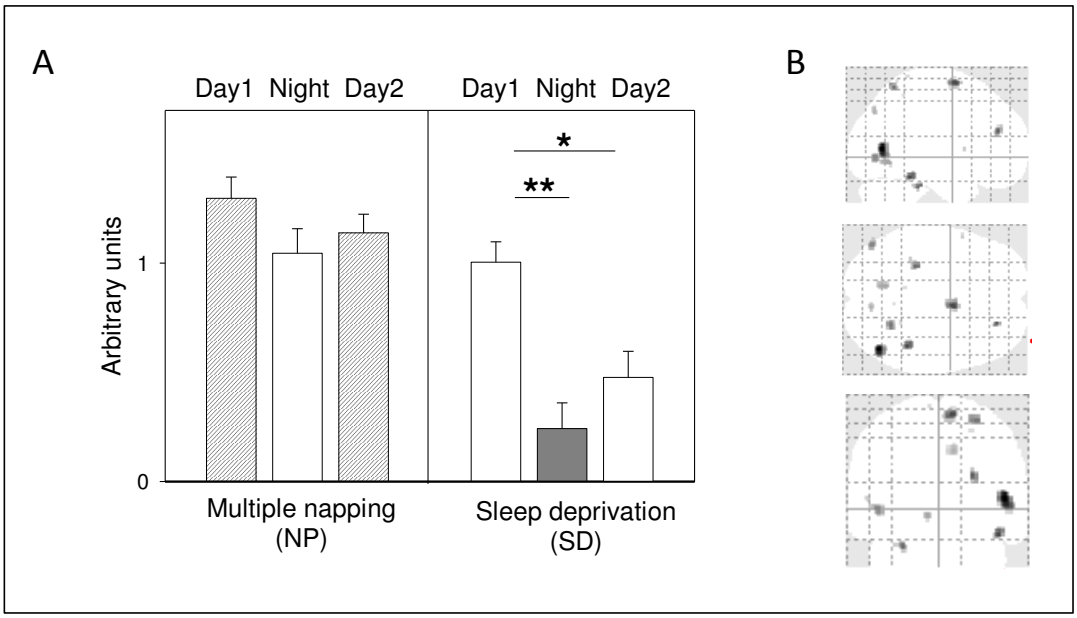
695 **Figure 3.**



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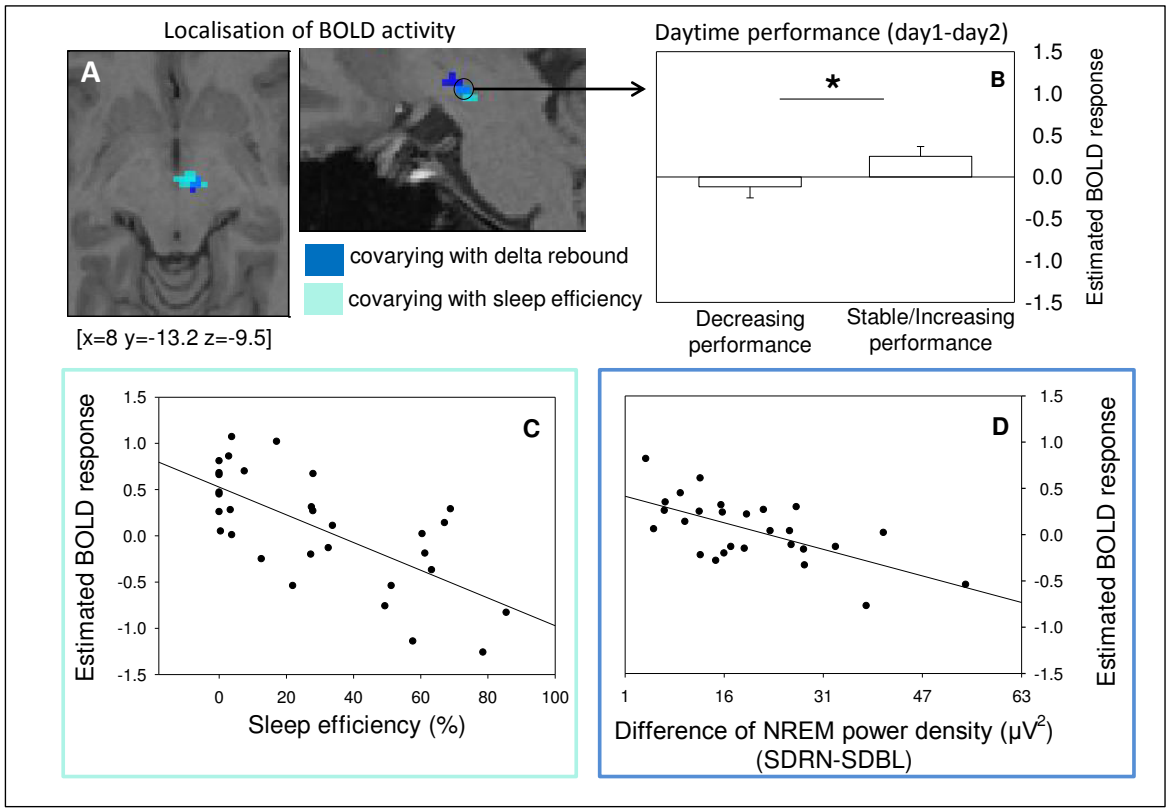
699 **Figure 4.**



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5. Discussion

The present data strongly emphasize the interaction of sleep homeostatic and circadian mechanisms for the regulation of sleep and waking functions. They provide extensive insights about the impact of sleep pressure on WM and how it is modulated by circadian sleep-wake promotion. Our results indicate that the detrimental consequences of high sleep pressure on executive WM performance are modulated by adenosinergic mechanisms. Furthermore, we observed that long-term adenosinergic variations are associated with alterations in circadian processes. A longer phase-angle in melatonin secretion, associated to a longer biological day, and a reduced evening nap-sleep efficiency was found in G/A-allele carriers with a lower ADA activity compared to G/G-allele carriers. This indicates that the circadian system copes with lower ADA activity and presumably higher sleep pressure levels (Bachmann et al., 2012) during daytime by extending and strengthening the influence of circadian arousal promotion. Such mechanisms have been proposed to consolidate wakefulness against the impact of homeostatic sleep need (Dijk et al., 1992; Edgar et al., 1993). Accordingly, we observed during daytime that the influence of high sleep pressure on WM performance is counteracted by circadian arousal promoting mechanisms, which we localized in hypothalamic areas by the analysis of BOLD activity. Finally, our data suggest that cognitive coping mechanisms according to the ADA-genotype are differentially efficient depending on cognitive domain.

In the following, it will be first discussed how our data contribute to the current understanding of sleep-wake regulatory differences according to the ADA-polymorphism, addressing the interactive crosstalk of sleep pressure and circadian phase. Afterwards, the cerebral mechanisms underlying the impact of this interaction on WM will be outlined. Finally, we will point out some limitations of the study, which at the same time bear future perspectives on how to advance the investigation of sleep –wake regulation and its impact on WM functions.

5.1 Differences in sleep - homeostatic mechanisms or differential sleep-wake switch?

Former studies reported differences in sleep pressure levels according to the ADA-polymorphism as indexed by classical sleep homeostatic markers such as NREM sleep EEG delta power (Bachmann et al., 2012; Mazzotti et al., 2012; Retey et al., 2005). Our results question for the first time whether differences in sleep between G/A- and G/G-allele carriers are of a purely sleep homeostatic nature. As summarized below, they rather point to genotype-dependent alterations in the transition between sleep and wakefulness, which might be due to both changes in circadian arousal promoting strength and in the adenosinergic tone.

Genotype-specific differences in evening sleep efficiency mirror variations in circadian wake promoting strength (Dijk & Czeisler, 1994; Dijk et al., 1992). Circadian wake promotion arises from

hypothalamic areas and their projections to the ascending arousal system (Aston-Jones, 2005; Saper, 2013b). During awakening (i.e., the transition from sleep to wakefulness), a rapid activation of the arousal system precedes the decrease of sleep inertia (Balkin et al., 2002), which is a "short period of confusion and degraded mood/performance immediately after awakening from sleep" (Naitoh, Kelly, & Babkoff, 1993, p. 110). Interestingly, G/G-allele carriers suffer more from sleep-inertia (Reichert, Maire, Gabel, Viola, et al., 2014), indicated by a worse well-being immediately after napping (see supplemental Figure 1). An effect of awakening from a different sleep stage (Tassi & Muzet, 2000) on this pattern appears unlikely (differences between groups in sleep stages during the last ten minutes of nap episodes: p_{all}<0.1). This sleep-stage-independent difference in awakening between genotypes points to a more efficient arousal-promotion of G/A-allele carriers after a nap sleep episode. Future studies should specify this assumption by investigating cognitive performance (Lovato & Lack, 2010) or thermophysiological variations (Krauchi, Cajochen, & Wirz-Justice, 2005) after awakening according to the ADA-polymorphism.

Once wakefulness is initiated, orexin-containing neurons in the lateral hypothalamus stabilize the state of wakefulness and prevent sudden transitions to sleep (Inutsuka & Yamanaka, 2013; Saper et al., 2005). Independent of genotype, we observed lateral hypothalamic BOLD activity to be negatively linked to the individuals' evening sleep efficiency. In other words, the higher the activity in the lateral hypothalamic region, the less likely was sleep initiated and maintained in the evening. The observed lower evening sleep efficiency of G/A-allele carriers points thus to a better ability to maintain wakefulness, which might prevent them to fall asleep, specifically shortly before habitual bedtime. Interestingly, a consolidation of wakefulness allows a continuous rise in homeostatic sleep need, and thus can co-determine the level of NREM sleep delta power, once sleep is initiated. In this perspective, a better consolidation of wakefulness in G/A-allele carriers can contribute to a higher NREM sleep delta power at the beginning of a sleep episode, as we observed similarly in the evening nap.

The G/A-genotype has earlier been associated to a higher intensity of NREM sleep delta power both in baseline and recovery nights from SD (Bachmann et al., 2012; Mazzotti et al., 2012; Retey et al., 2005). However, in our analysis of nocturnal baseline and recovery sleep episodes, we could not replicate this finding (supplemental Figure2 for NREM delta power). Consistent results might be hampered by small sample sizes and differences between studies, for instance in terms of light influence (Chellappa et al., 2013), a confound for which we controlled.

Even though we did not observe clear-cut sleep homeostatic differences in consolidated night-sleep episodes, waking functions of G/A-allele carriers particularly benefited from a reduction of sleep pressure compared to constant wakefulness. In contrast to physiological sleep recordings, these measures can be consciously influenced by participants. Still, at the physiological level we

observed genotype-dependent variations, but these were rather evident in circadian than homeostatic markers of sleep-wake regulation. Interestingly, these circadian differences between genotypes can be reconciled within the assumption of a higher sleep pressure in the G/A-genotype, as well be explained in the next section.

5.2. Differences in circadian variations between genotypes

Accumulating evidence underlines that circadian variations change according to the level of sleep pressure. Within this context, a later circadian phase has been observed in response to partial (Lo et al., 2012; Rogers & Dinges, 2008) as well as in response to total SD (Cajochen et al., 2003). While the underlying mechanisms are not yet entirely uncovered, the observation of a later phase in melatonin levels of the G/A-genotype suggests an implication of adenosinergic mechanisms. This is in line with evidence from the animal domain: Adenosine agonists (Elliott, Todd Weber, & Rea, 2001; Sigworth & Rea, 2003; Watanabe et al., 1996), but also SD per se (van Diepen et al., 2014) reduce circadian phase delays and activity in the SCN, which controls the timing of melatonin secretion by the pineal gland (Pevet & Challet, 2011) .

As a result of the higher adenosinergic tone in G/A-allele carriers and its association to a higher sleep pressure, a strengthening of the influence of daytime wake-promoting mechanisms may have occurred. The latter serve to oppose sleep pressure (Dijk & Czeisler, 1994; Dijk & Edgar, 1999) in order to maintain wakefulness in the evening. Interestingly, reduced evening nap sleep efficiency further suggests an enhanced strength of circadian arousal promotion in G/A-allele carriers. Such a mutual adaptation of sleep-wake regulating processes has been observed in a reversed direction during aging: In elderly, a circadian phase-advance (e.g., Dijk, Duffy, Riel, Shanahan, & Czeisler, 1999 but see Cajochen, Munch, Knoblauch, Blatter, & Wirz-Justice, 2006) coincides with an attenuated wake-promotion strength (Munch et al., 2005) and a reduced homeostatic need for sleep (summarized in Schmidt, Peigneux, & Cajochen, 2012).

However, the potential genotype-dependent difference in evening wake-promotion, as assessed by the EEG, was neither mirrored at the subjective level, nor in WM or vigilance performance at this time of day. This mirrors the high sensitivity of sleep EEG assessments for circadian arousal peaks in the late evening, which are commonly not reflected at a neurobehavioral level under low sleep pressure conditions (Frey et al., 2012; Maire et al., 2014; Sagaspe et al., 2012). Therefore, a detection of genotype-dependent differences in arousal promotion specifically in the evening at the subjective or behavioural level is rather unlikely.

Independent of time of day, we observed a benefit in well-being in G/A-allele carriers during the nap protocol compared to SD. This observation cannot be traced back to general differences in a genotype-specific reduction of sleep pressure during napping (i.e., a higher NREM sleep delta power

independent of time of day). Thus, the pattern might rather mirror the positive influence of a stronger circadian wake promotion in the G/A-genotype.

In sum, these observations underscore the importance of a multi-methodological assessment of sleep-wake regulatory mechanisms. They strongly suggest that the interaction of homeostatic and circadian sleep-wake regulatory processes allows for adptations to cope with chronic changes in ADA activity. Regarding the common practice of sleep restriction in our society (Banks & Dinges, 2007), future studies might focus on the interplay between variations of the adenosinergic tone and compensatory increases in arousal promoting strength under these conditions. Also, the results strongly suggest a consideration of circadian phase changes and arousal promoting mechanisms when investigating consequences of pharmacologically altered adenosine levels (e.g., by caffeine consumption).

5.3 The regulation of working memory performance

One of the main aims of the current thesis was to characterize state- and trait-like circadian and sleep-wake homeostatic alterations in WM performance. In the following, the underlying cerebral underpinnings will be first discussed from a state-like perspective. Afterwards, we outline which mechanisms might mediate the trait-like impact of the ADA-genotype.

5.3.1 Working memory according to sleep pressure and circadian phase

As expected WM performance deteriorated under high compared to low sleep pressure levels. This sleep homeostatic modulation was corroborated by the analysis of nap sleep in the low sleep pressure protocol, indicating a positive link between WM performance and NREM sleep delta power, a classical sleep homeostatic marker. A potential use-dependent increase of NREM sleep delta power due to WM performance, as similarly shown for other cognitive domains (summarized in Rasch & Born, 2013), remains to be elucidated. Together, the observed results suggest that a negative impact of homeostatic sleep need on behavioural WM performance becomes evident as soon as a certain sleep pressure level is reached. As outlined below, such a level most likely depends on time of day.

It has been assumed that the impact of sleep pressure on WM is mainly mediated by prefrontal regions and their continuous challenge during wakefulness (Harrison & Horne, 2000). In addition, accumulating evidence underscores the importance of parietal and occipital areas in the modulation of WM performance during sleep loss (Chee & Thomas, 2013). Accordingly, we observed sleep loss-related activity decreases in a fronto-parieto-occipital network during WM performance. Importantly, these declines occurred specifically during a change from day- to nighttime, but were not observed after a further rise of sleep pressure during the following day. This underlies the observed classical

nighttime declines in behavioural performance under sleep loss, which stabilize during daytime even though wakefulness is further extended.

Postero-lateral hypothalamic activity was associated to performance under sleep loss, both to the steep decrease during nighttime (supplemental Figure 3) and to its stabilization during the following day under extended wakefulness. Bearing in mind the limited spatial resolution of fMRI data, we speculate that the observed region encompasses the origin of the orexinergic arousal promoting system in the lateral hypothalamus (Saper, 2013b). This system has not only been linked to active circadian wake-promotion (Zeitzer et al., 2003), but also to the duration of wakefulness, or in other words to sleep homeostatic mechanisms (Deboer et al., 2004; Zeitzer, Buckmaster, Lyons, & Mignot, 2007). Its potential role in performance has earlier been demonstrated in primates by a reduction of sleep loss induced performance deficits following orexin administration (Deadwyler, Porrino, Siegel, & Hampson, 2007). Our data suggest that human pharmacological research might focus on specifying the role of orexin in the enhancement of WM performance under conditions of sleep loss.

In the present context, it has to be kept in mind that napping represents a simple way to reduce sleep pressure and benefit WM performance. Our data indicate that such benefits are not solely related to a reduction of sleep pressure but also to an active promoting role of specific sleep features in WM. Our insights into the relationship between sleep and WM performance might refine future research on n-back trainings and their enhancements of fluid intelligence (Au et al., 2014).

It is important to note that our analyses at the cerebral level do not allow differentiating between several aspects of WM performance, such as guiding attention, holding information active or suppressing interference. Accordingly, an open question remains whether changes in cerebral correlates of WM performance rely on underlying alertness variations or whether they specifically relate to particular higher order WM aspects (Lim & Dinges, 2010). The observed associations of lateral hypothalamic activity to performance indicate an important role of subcortical arousal promotion, strongly associated to alertness (Aston-Jones, 2005). In parallel, we observed activity differences from day to night solely in cortical areas, suggesting higher order cognitive functions to be affected, such as maintaining information or preventing interference (Irlbacher et al., 2014; Sander et al., 2012). The analysis at present thus only allows for the conclusion that a combined effect of both subcortical and cortical activity reductions underlies the steep decrease of WM performance under high sleep pressure at night.

5.3.2 The ADA-genotype and its influence on working memory

Interestingly, we observed that the ADA-polymorphism modulates sleep pressure-dependent effects on executive aspects of WM. The data thus suggest an implication of adenosinergic

mechanisms in the regulation of executive aspects of WM according to the state of sleep pressure. The application of caffeine has previously not been associated to enhanced WM performance when studied during a normal waking day (Klaassen et al., 2013; Koppelstaetter et al., 2008; Wyatt et al., 2004). According to our results behavioural effects of a blockade of adenosine, and also the effect of napping, might be more likely observed under conditions of a high adenosinergic tone and a concomitant high sleep pressure level. Likewise, caffeine enhanced performance in several executive functions when administered after 64 h of SD (Wesensten et al., 2005). Interestingly, animal studies revealed that the stimulation of adenosinergic A_1 receptors inhibits orexinergic hypothalamic neurons (Liu & Gao, 2007; Rai et al., 2010; Thakkar et al., 2008; Thakkar, Winston, & McCarley, 2002). Enhancing performance under high sleep pressure by A_1 receptor antagonists might thus also involve orexinergic pathways.

In line with earlier findings (Bachmann et al., 2012), our data do not support that genotype-specific behavioural responses to high sleep pressure depend on circadian phase, neither in WM performance nor in other measures such as vigilance, subjective sleepiness, or well-being. However, we assume that genotypes cope at a cerebral level differentially with high sleep pressure in a time of day-dependent manner. First analyses indeed point into this direction. We will briefly outline the results in the following section, as they represent an exciting mid-term perspective, which allows linking the above described trait-and state-like impact of sleep pressure on WM performance and underlying cerebral correlates.

5.3.2.1 Outlook: Cerebral correlates of sleep loss-related WM performance declines: Impact of the ADA-polymorphism

Our analyses were inspired by two observations. 1.) Circadian mechanisms influence sleep loss-related BOLD activity decreases underlying WM performance (chapter 4.3). 2.) The ADA-polymorphism impacts on circadian and sleep homeostatic processes and affects WM performance (chapter 4.1 and 4.2). Therefore, we investigated whether sleep loss-related BOLD activity during WM performance differs between G/A- and G/G-allele carriers according to time of day[1]. In brief, the results indicate that the genotypes differed in their cerebral coping mechanisms in response to sleep loss in a time of day-dependent manner.

Sleep loss-related activity decreases from day- to nighttime were pronounced in G/A- compared to G/G-allele carriers, and observed in parietal, cingulate and parahippocampal regions (see

[1] The according methodological and statistical descriptions can be found in the supplemental material of this thesis (section entitled “Analyses of cerebral correlates according to genotype”). Basically, it parallels the analysis conducted in the third manuscript. The focus is on statistical comparisons designed to specifically contrast G/A- and G/G-allele carriers. Please note that the results of these comparisons are not mandatorily associated to patterns observed over the entire group.

supplemental Table 1). These decreases were mostly evident as enhanced deactivations from day- to nighttime in the G/A-genotype. Interestingly, deactivations in these regions have been associated to an inhibition of potential neural distracting processes, which serves to optimize performance (Tomasi, Ernst, Caparelli, & Chang, 2006). Therefore, we assume that pronounced cortical deactivation patterns of G/A-allele carriers during a night of sleep loss mirror a compensatory mechanism to cope with lower ADA activity and associated higher sleep pressure levels (Bachmann et al., 2012). When wakefulness was extended from night- to daytime, G/A-allele carriers did not recruit the described cortical compensatory network anymore. Also, no other striking differences between genotypes were observed, neither in WM performance networks nor in subcortical arousal promoting regions. Importantly, motivational effort brought up for task performance under high sleep pressure varied similarly in both genotypes (p>0.1).

In general, this time of day-dependent cortical compensation of G/A-allele carriers might have hampered the observation of a genotype-specific circadian WM performance pattern under high sleep pressure. Future analyses should focus on genotype-dependent differences in cerebral correlates of vigilance performance. This might allow investigating whether genotype-specific cerebral coping mechanisms prevent to detect differences in vigilance performance at a behavioural level. Within this context, the assessment of cerebral correlates opens new perspectives to characterize mechanisms of resilience in response to altered adenosinergic modulations of sleep pressure.

5.4 Limitations and future directions

Our study was well designed to assess the classical circadian and homeostatic variations in several variables at different levels of behaviour and physiology (Cajochen et al., 2001; Dijk & Czeisler, 1994; Dijk et al., 1992; Graw et al., 2004; Sagaspe et al., 2012; Wyatt et al., 1999). However, given the ultradian pattern of NREM-REM cycles (Borbély & Acherman, 2005), it might be argued that we induced a selective REM SD in the low sleep pressure condition by fragmenting sleep into short 80 min episodes. Yet, evidence is limited for an impact of REM SD on behaviour and physiology (Horne, 2013). An impact of sleep fragmentation on cognitive functioning can however not be excluded. It has, however, mostly shown in conjunction with reduced total sleep time (Reynolds & Banks, 2010), which does not apply for the multiple nap condition.

The investigated polymorphism appears as a parsimonious tool to vary long-term enzymatic activity of ADA in a non-invasive way. Yet only under reserve, we can infer the adenosine levels per se, as these are regulated by a range of different enzymes and receptors (Landolt, 2008). Nonetheless, an inference seems highly relevant given the predominant role of adenosine in the

regulation of sleep pressure (Porkka-Heiskanen, 2013). A systematic control of factors implicated in the reuptake and degradation of adenosine would strongly enhance the significance of our results. Also, future research investigating the local distribution of ADA in the human brain will lead to new perspectives concerning the interpretation of our present data.

A further limitation of our study concerns the sample size and related statistical power, not sufficient to detect small, but systematic effects. However, we reduced error variance by strict inclusion criteria during recruitment and by a constant control of variables impinging on sleep-wake regulation during the laboratory study. On the other hand, these conditions limit the generalizability of our results to other populations and real-life situations. Yet, they might serve to develop hypotheses to be proven in field studies with larger sample sizes.

From a methodological point of view, it is further important to note that the n-back task has been criticised as a measure of WM regarding both its reliability and validity (Jaeggi et al., 2010). Also, it has been argued that successful performance in this task is confounded by familiarity based responses (Kane et al., 2007). The number of so-called lure-trials (Kane et al., 2007), inducing such familiarity based responses, was however controlled in our study between conditions and genotypes. Nevertheless, it appears as an intriguing research question, to investigate the impact of sleep pressure, circadian phase and genotype on familiarity based responses as well as on their underlying cerebral correlates.

Currently, the analyses of cerebral correlates do not allow differentiating the impact of sleep pressure on several subcomponents of WM performance. A more fine grained inspection of cerebral activity underlying performance during high (3-back) in comparison to low (0-back) load can lead to further insights specifically concerning executive aspects of WM. Also, the analysis of cerebral activity underlying incorrect responses can contribute to identify networks mediating WM errors. Finally, at present, the question remains open how sleep pressure and circadian phase impact on cerebral activity underlying vigilance performance. With regard to the genotype-dependent modulations in a typical marker of circadian wake-promotion, the analysis of cerebral correlates of vigilance performance can generate an intriguing perspective on subcortical arousal promoting mechanisms (Schmidt et al., 2009) according to changes in adenosinergic mechanisms.

Overall, our data suggest a flexible system regulating sleep and wakefulness, which copes with long-term changes of internal origin in an adaptive manner. Future studies might focus on similar coping mechanisms in response to several external influences, such as regular caffeine consumption or chronic sleep restriction. This might offer promising insights into the difference between acute and chronic effects of a blockade of adenosine in the circadian regulation of arousal and its impact on behaviour and physiology.

5.5 Summary

In brief, our data suggest the following:

- WM performance is strongly influenced by sleep pressure
- This influence is modulated by circadian phase, the ADA-polymorphism (rs73598374), and the interaction between these two factors
- The modulation of WM according to sleep pressure and circadian phase putatively involves adenosinergic and lateral-hypothalamic orexinergic pathways
- Future research should focus on adaptive coping mechanisms or general alterations of the circadian system in response to chronic changes in the adenosinergic tone and in sleep pressure levels. This might also involve a change in WM performance.

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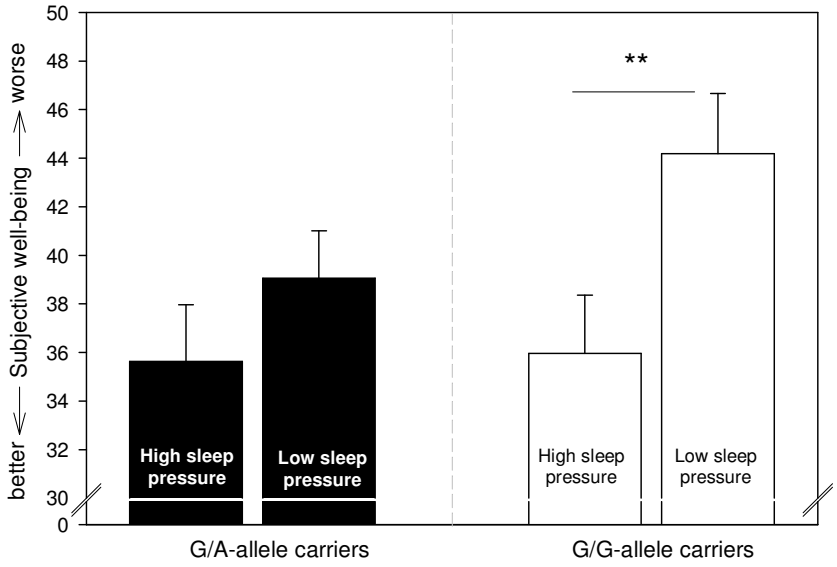
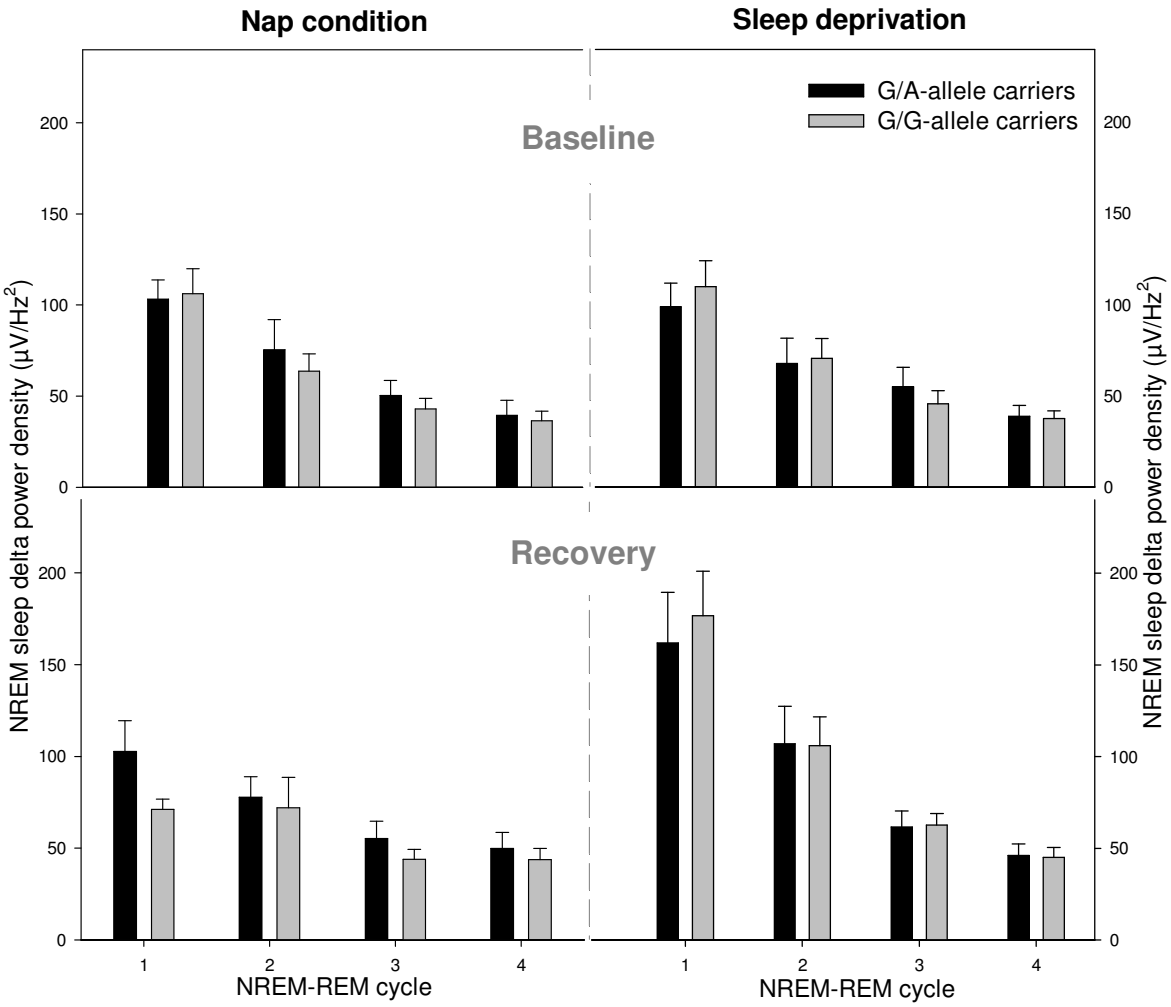
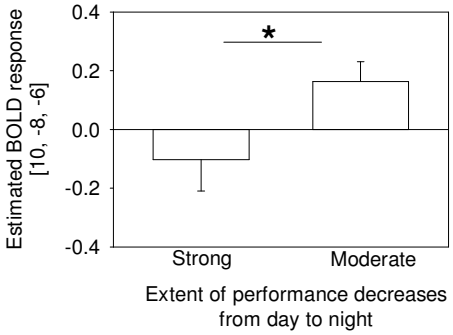
7. Supplemental material



Supplemental Figure 1. Well-being in the first 30 min after a nap sleep episode per genotype. As in (Birchler-Pedross et al., 2009), well-being was assessed by 3 visual analogue scales assessing tension (ranging from *extremely relaxed* to *extremely tense*), physical comfort (ranging from *extremely comfortable* to *extremely uncomfortable*), and mood (ranging from *in very bad mood* to *in very good mood*). We calculated a mean of these values, assessed during the first 30 minutes after the end of each scheduled nap sleep episode and at corresponding times during sleep deprivation. While G/A-allele carriers did not significantly differ between high sleep pressure (sleep deprivation) and low sleep pressure (multiple napping; $p>0.1$), well-being was significantly worse in G/G-allele carriers during low compared to high sleep pressure. A general mixed model was calculated for each genotype with the factors time (three levels of first day, night and second day) and condition (high vs low sleep pressure). **:$p<0.001$



Supplemental Figure 2. NREM delta power during night sleep per genotype. A general mixed model was calculated with the factors condition (Nap vs. sleep deprivation), night (baseline vs. recovery), cycle (first, second third and fourth NREM-cycle) and genotype (G/A- vs. G/G-allele carriers). NREM sleep delta power decreased in all nights from the first to the last cycle (p<0.001), irrespective of genotype and condition. In the recovery night after sleep deprivation, it was significantly enhanced (condition*night p<0.001), particularly in the first cycle (condition*night*cycle p<0.001). All effects including the factor genotype were not significant (p<0.1).



Supplemental Figure 3. Postero-lateral hypothalamic BOLD activity according to performance decrease from day to night. A t-test for independent groups revealed that BOLD activity was significantly reduced in participants who decreased in performance from day to night to a larger extent compared to participants with a less pronounced decrease. Groups are based on the median decrease in performance from day to night (difference in hit targets of 3-back). *: $p<0.05$

Supplemental information: Analyses of cerebral correlates according to genotype

METHODS: As in the third manuscript "Time of Day Matters: Circadian Modulation of Sleep loss-related Changes in Cognitive Brain Functions", BOLD activity was analysed assessed during hit targets in the 3-back task. Also, we focused on data assessed in NP and SD during crucial circadian time windows, i.e., the late evening (i.e., during strong circadian wake-promotion (Dijk & Czeisler, 1994)) and the end of the night (i.e., during strong circadian sleep promotion (Dijk & Czeisler, 1994). During SD, these time windows correspond to 13 h (late evening, day1), 21 h (night) and 37 h of wakefulness (late evening, day2). The assessment and the design of the analysis is identical to the one described in the third manuscript, with an additional second level analysis comparing the two genotype groups.

Based on a) a higher vulnerability to sleep pressure variations and high sleep pressure (Bachmann et al., 2012; Reichert, Maire, Gabel, Viola, et al., 2014; Retey et al., 2005) and b) a stronger circadian variation in the G/A-genotype (Reichert, Maire, Gabel, Hofstetter, et al., 2014), we assumed pronounced activity decreases from day- to nighttime during SD in G/A- compared to G/G-allele carriers (contrast SD-day1>SD-night GA>GG and GG>GA, respectively). To test, which of these differences can be traced back to a change in circadian phase per se, we applied a conjunction analysis with data assessed during NP at the same times of day ([SD-day1>SD-night GA>GG]∩ [NP-day1>NP-night GA>GG]). In a final step we explored BOLD activity patterns which differed between genotypes, due to an extension of wakefulness from night to daytime (corresponding to 21 h and 37 h of continuous wakefulness, respectively, contrast SD-night>SD-day2 GA>GG). Again, in a next step we applied a conjunction analysis with data assessed during NP at the same times of day in order to test which differences under SD can be traced back to a change in circadian phase per se ([SD-night>SD-day2 GA>GG]∩ [NP-night>NP-day2 GA>GG]).

Generally, we expected differences to occur in regions implicated in active verbal n-back performance at rested wakefulness (Owen et al., 2005) and sleep deprived states (Choo et al., 2005; Vandewalle et al., 2009) as well as in regions shown to be deactivated during verbal n-back performance (Tomasi et al., 2006). Corrections for multiple comparisons were applied on small spherical volumes with a radius of 10 mm.

RESULTS: To analyze performance, a mixed model of variance for repeated measures was calculated [including a factor condition (NP, SD), time (day1, night, day2), and genotype (G/A and G/G-allele carriers)]. Performance decreased under high sleep pressure, particularly at night, but recovered during daytime (condition $p<0.05$, time $p<0.05$; condition x time $p<0.05$). This pattern was similar in both genotypes ($p_{all}>0.1$). Note that we focused here on correct positive answers during the 3-back task, assessed in the late evenings and early morning.

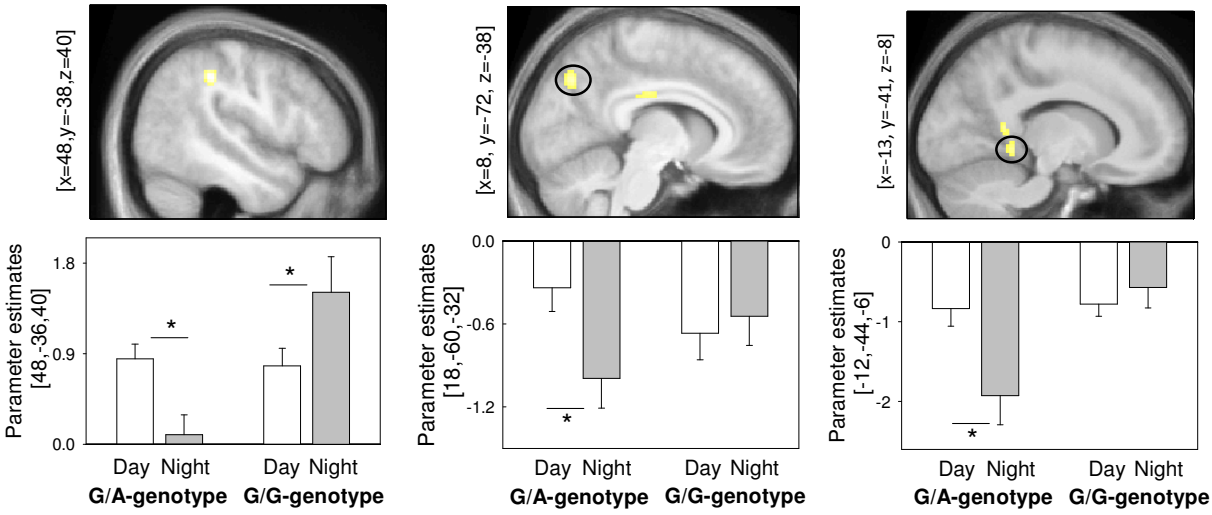
During SD, exclusively G/A-allele carriers showed decreases in activity from day- to nighttime in a set of parietal and occipital regions compared to G/G-allele carriers (Table 1, supplemental Figure 4). In contrast, solely G/G-allele carriers increased activity under the same conditions (Table 1). These

genotype-dependent modulations were not significantly driven by a circadian phase changes from day to night ($p_{uncorrected}$ >0.001).

During a further extension of wakefulness from night to daytime, analysis revealed solely an increase in BOLD activity of G/A-allele carriers in a parahippocampal area (x=-10, y=-44, z=-8; and x=-20, y=-36, z=-16), in which G/G-allele decreased significantly (SD-night>SD-day2 GG>GA, p<0.001). These genotype-dependent modulations were not significantly driven by a circadian phase changes from day to night ($p_{uncorrected}$ >0.001).

Brain area	size	s.v.c.	Reference	x	y	z	G/A: day1>night	GG: night>day1	Night: GG>GA
middle frontal gyrus	8	0.006	Choo et al., 2005	28	44	10	x	x	
postcentral gyrus	41	0.004	Vandewalle et al., 2009	48	-36	40	x	x	
posterior cingulate gyrus	18	0.01	Vandewalle et al., 2009	6	-20	28	x	x	x
Precuneus	87	0.012	Tomasi et al., 2006	12	-68	34	x		
		0.009	Tomasi et al., 2006	18	-60	32	x		
	3	0.017	Tomasi et al., 2006	-16	-66	28	x	x	x
Calcarine sulcus	3	0.013	Tomasi et al., 2006	-14	-48	10		x	x
Parahippocampal gyrus	19	0.022	Tomasi et al., 2006	-12	-44	-6	x		x

Supplemental Table 1. Differences in task-related BOLD activity between genotypes. Coordinates (x, y, z) are expressed in mm in the Montreal Neurological Institute (MNI) space. Size: Number of voxels significantly modulated. p_{svc}: p-value after correction for multiple comparisons over small volumes of interest, taken from the literature. Reference: References for coordinates. Crosses indicate a significant difference in parameter estimates (p<0.05), tested by 2-sided t-tests. Note that parameter estimates did not indicate significant difference between genotypes during day 1, or higher values in G/A- compared to G/G-allele carriers at night.



Supplemental Figure 4. Genotype-specific modulations of cerebral activity during sleep deprivation. G/A-allele carriers exhibited pronounced decreases in activity from day to night compared to G/G-allele carriers, exemplary depicted for the poscentral gyrus (left), the precuneus (middle) and the parahippocampal gyrus (right panel).

Curriculum Vitae

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- Grant of the Freiwillige Akademische Gesellschaft Basel, 02/2014.
- Travel grant of the European Sleep Research Society (ESRS) for a scientific stay at the Cyclotron Research Centre, Liège, 02/2014.
- Grant of the University of Basel, Niklaus und Bertha Burckhardt-Bürgin-Stiftung, Basel, 04/2013.
- Travel grant of the University of Basel for the congress of the Deutsche Gesellschaft für Schlafforschung und Schlafmedizin (DGSM), Berlin, 12/2012
- Travel Fellowship of the Swiss Society for Sleep Research and Chronobiology (SSSSC) for the Congress of the European Sleep Research Society (ESRS), Paris, 09/2012.
- Travel Award from Stockgrand Ltd.for the congress of the European Biological Rhythm Society (EBRS), Oxford 08/ 2011.

Publications

First authorships

- Reichert, C.F., Maire, M., Gabel, V., Viola, A.U., Strobel, W., Götz,, Scheffler K., Klarhöfer M., Berthomier, C., Strobel W., Cajochen C., Schmidt C.: Time of Day Matters: Circadian Modulation of Sleep loss-related Changes in Cognitive Brain Functions. Submitted.
- Reichert, C.F., Maire, M., Gabel, V., Viola, A.U., Strobel, W., Götz, T., Landolt, H.P., Bachmann, V., Cajochen, C., Schmidt, C. (2014). The circadian regulation of sleep: impact of a functional ADA-polymorphism and its association to working memory improvements. PLoS ONE 9(12).
- Reichert, C.F., Maire, M., Gabel, V., Viola, A.U., Strobel, W., Götz, T., Landolt, H.P., Bachmann, V., Cajochen, C., Schmidt, C. (2014). Behavioral vulnerability to differential sleep pressure conditions depends on cognitive domain and circadian phase: Insights from a functional ADA polymorphism. *Journal of Biological Rhythms,* 29(2), 119-130.
- Maire, M.*, Reichert, C.F.*, Schmidt, C. (2013). Sleep-wake rhythms and cognition. *Journal of Cognitive and Behavioral Psychotherapies,* 13(1), 133-170.

Co-authorships

- Gabel V., Maire M., Reichert C., Chellappa S.L., Schmidt C., Hommes V., Cajochen C., Viola A.U. (2014). Dawn simulation light impacts on different cognitive domains under sleep restriction levels. *Behavioral Brain Research*. In press.
- Chellappa S.L., Viola A.U., Schmidt C., Bachmann V., Gabel V., Maire M., Reichert C.F., Valomon A., Landolt H.P., Cajochen C. (2014). Light modulation of human sleep depends on a polymorphism in the clock gene Period3. *Behavioral Brain Research*, 271, 23-29.
- Maire, M., Reichert, C.F., Gabel, V., Viola, A.U., Krebs, J., Strobel, W., Landolt, H.P., Bachmann, V., Cajochen, C., Schmidt, C. (2014). Time-on-task decrement in vigilance is modulated by inter-individual vulnerability to homeostatic sleep pressure manipulation. *Frontiers in Behavioral Neuroscience,* 8, 1-10.
- Maire, M., Reichert, C.F., Gabel, V., Viola, A.U., Strobel, W., Krebs, J., Landolt, H.P., Bachmann, V., Cajochen, C., Schmidt, C. (2013). Sleep ability mediates individual differences in the vulnerability to sleep loss: evidence from a PER3 polymorphism. *Cortex,*52,47-59.
- Gabel V, Maire M, Reichert C, Chellappa SL, Schmidt C, Hommes V, Viola AU, Cajochen C (2013). Effects of artificial dawn and morning blue light on daytime cognitive performance, well-being, cortisol and melatonin levels. *Chronobiology International,* 30(8), 988-997.
- Chellappa SL, Viola AU, Schmidt C, Bachmann V, Gabel V, Maire M, Reichert CF, Valomon A, Götz T, Landolt HP, Cajochen C. (2012). Human melatonin and alerting response to blue-enriched light depend on a polymorphism in the clock gene PER3. *J Clin Endocrinol Metab*, 97(3), 433-437.

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Conference Abstracts

First authorships

- Reichert C.F., Maire M., Gabel V., Hofstetter, M., Viola A.U., Kolodyazhniy V., Strobel W., Götz, T., Bachmann V., Landolt H.-P., Cajochen C., Schmidt C. (2014). Schlaf-Wach-Regulation und Arbeitsgedächtnisleistung - Einfluss eines funktionalen ADA-Polymorphismus und der zirkadianen Phase. 22. Jahrestagung der Deutschen Gesellschaft für Schlafforschung und Schlafmedizin e.V.(DGSM). Köln. *Somnologie*, Volume 18, Supplement1.
- Reichert C.F., Maire M., Gabel V., Viola A.U., Scheffler K., Klarhöfer M., Strobel W., Cajochen C., Schmidt C. (2014). Impact of circadian phase, sleep pressure and an ADA polymorphism on cerebral correlates of working memory performance. 22nd Congress of the European Sleep Research Society (ESRS). Tallin. *Journal of Sleep Research*, Volume 23, Supplement S1.
- Reichert C.F., Maire, M., Gabel V., Viola A.U., Scheffler K., Klarhöfer M., Strobel W., Cajochen C., Schmidt C. (2014). Impact of circadian phase and prior wakefulness on cognition-related cerebral activity in humans. 22nd congress of the European Sleep Research Society, Tallinn. *Journal of Sleep Research*, Volume 23, Supplement S1.
- Reichert C.F., Maire M., Gabel V., Viola A.U., Kolodyazhniy V., Strobel W., Götz, T., Bachmann V., Landolt H.-P., Cajochen C., Schmidt C. (2014). Association between sleep and working memory: Contributions of circadian phase and an *ADA*-polymorphism. Jahrestagung der Schweizerischen Gesellschaft für Schlafforschung, Schlafmedizin und Chronobiologie (SSSSC). Luzern.
- Reichert C.F., Maire, M. (2014). Inter-individual differences in human sleep-wake regulation: Evidence from ADA- and PER3- polymorphisms. Colloquium. Current Topics in Sleep and Chronobiology Research, Institute of Pharmacology & Toxicology, University of Zürich. Zürich.
- Reichert C.F., Maire M., Gabel V., Viola A.U., Kolodyazhniy V., Strobel W., Bachmann V., Landolt H.-P., Holst S., Cajochen C., Schmidt C.(2013). Inter-individual differences in subjective sleepiness, working memory and nap sleep: Influences of a genetic variation of adenosine deaminase. Jahrestagung der Schweizerischen Gesellschaft für Schlafforschung, Schlafmedizin und Chronobiologie (SSSSC). Aarau.
- Reichert C.F., Maire M., Gabel V., Krebs J., Valomon A., Strobel W., Viola A.U., Bachmann V., Landolt H.-P., Cajochen C., Schmidt C. (2012). Der Einfluss einer genetischen Variation der Adenosindesaminase auf die Anfälligkeit für Schlafdruck ist tageszeitabhängig. 20. Jahrestagung der Deutschen Gesellschaft für Schlafforschung und Schlafmedizin e.V.(DGSM). Berlin. *Somnologie*, S1.
- Reichert C.F., Maire M., Gabel V., Krebs J., Valomon A., Strobel W., Viola A.U., Bachmann V., Landolt H.-P., Cajochen C., Schmidt C. (2012). Influence of a genetic variation of adenosine deaminase on individual susceptibility to variations in sleep pressure. 21st Congress of the European Sleep Research Society (ESRS). Paris. *Journal of Sleep Research*, Volume 21, Supplement S1.
- Reichert C.F., Maire M., Gabel V., Valomon A., Viola A.U., Bachmann V., Landolt H.P., Cajochen C., Schmidt C. (2012): Subjective sleepiness under differential sleep pressure conditions depends on a genetic variation of adenosine deaminase. Annual Meeting of the Swiss Society of Sleep Research, Sleep Medicine and Chronobiology (SSSSC). Zürich
- Reichert, C.F., Schmidt, C., Viola, A.U., Wallach, D., Kräuchi, K., Cajochen, C. (2011). Motor skill learning under sustained wakefulness: Influence of age, sleep pressure and time of day. XII Congress of the European Biological Rhythm Society (EBRS). Oxford.
- Reichert C.F., Viola A.U., Wallach D., Kräuchi, K., Cajochen C. (2011): Time dependency of motor sequence learning - time alive, time awake and time of day. Annual Meeting of the Swiss Society of Sleep Research, Sleep Medicine and Chronobiology (SGSSC). St.Gallen.
- Reichert, C.F., Viola, A., Schmidt, C., Kräuchi, K., Cajochen, C. (2011). Circadian and homeostatic influences on sequence learning are affected by age. Annual Meeting of the Swiss Society of Neuroscience (SSN). Basel.
- Reichert, C.F., Degen, B., Schulz, A., Dürsteler-MacFarland, K., Schächinger, H. (2011). The PASAT induces stress in opioid-dependent individuals. Annual Meeting of the Swiss Society of Addiction Medicine (SSAM). Zürich.

- Reichert, C.F., Degen, B., Schulz, A., Dürsteler-MacFarland, K., Schächinger, H. (2010). Der PASAT zur Stressinduktion bei Opiatabhängigkeit. Kongress der Deutschen Psychiatrie, Psychotherapie und Nervenheilkunde (DGPPN). Berlin.
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- Reichert, C., Schulz, A., Schoene, L., Bruns, C., Schächinger, H. (2008). Effect of stimulus intensity and early vs. late cardiac cycle phase on acoustic startle. Magdeburg. In: Herrmann, C.S. & Sabel, B.A. (Ed.). Tagungsband Psychologie und Gehirn 2008 (S. 131). Calbe: GCC.

Co-authorships

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Declaration by candidate

I hereby declare that I have independently carried out the PhD-thesis entitled “The impact of sleep pressure, circadian phase and an ADA-polymorphism on working memory: a behavioral, electrophysiological, neuro-imaging approach”. This thesis consists of original research articles that have been written in collaboration with the listed co-authors and have been published in or submitted to peer-reviewed journals. All references were cited accordingly, and only indicated resources have been used.

Date:

Signature: